

**Veterinary work in the Netherlands, 1953 : lectures, delivered to the participants of the Intra-European T. A. training course on livestock disease control, May 18-June 12, 1953.**

**Contributors**

Netherlands. Veeartsenijkundig Staatstoezicht.

**Publication/Creation**

[’s-Gravenhage] : Staatsdrukkerij Uitgeverijbedrijf, 1954.

**Persistent URL**

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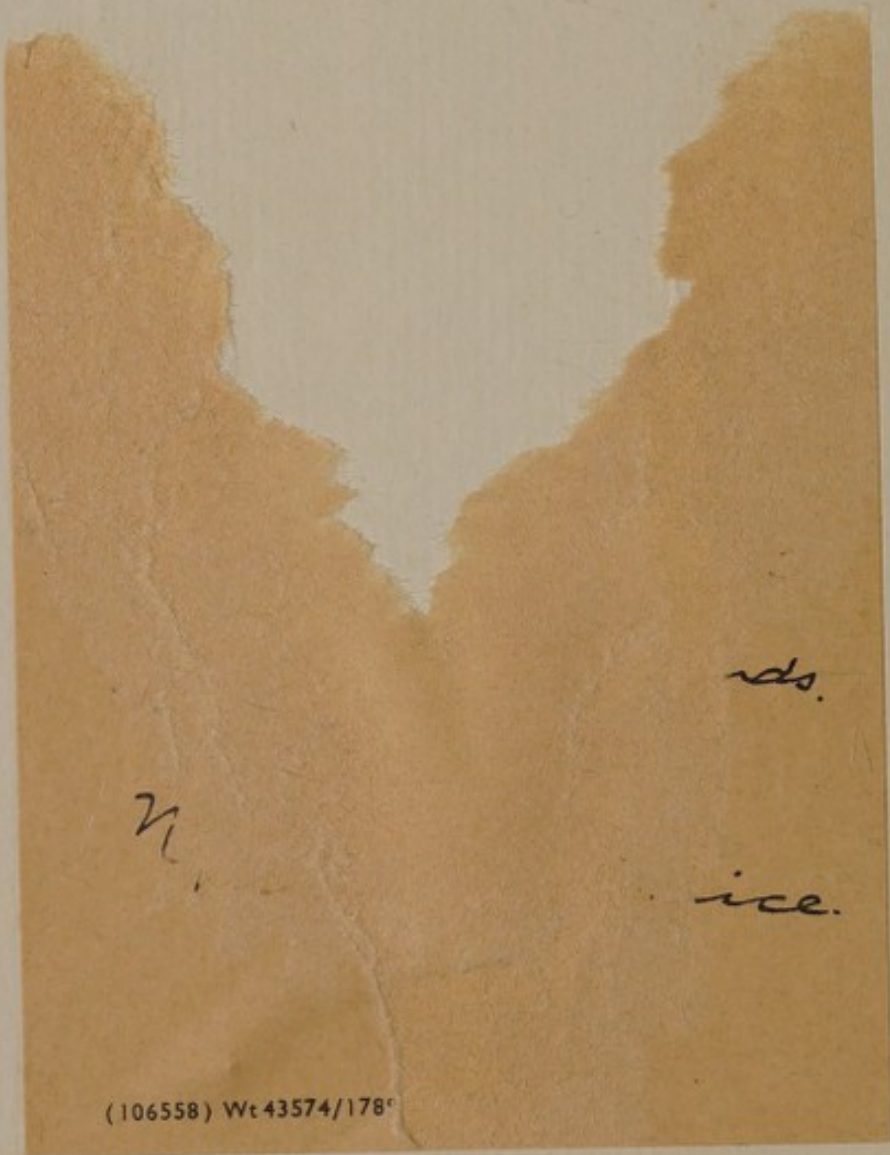


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VETERINARY  
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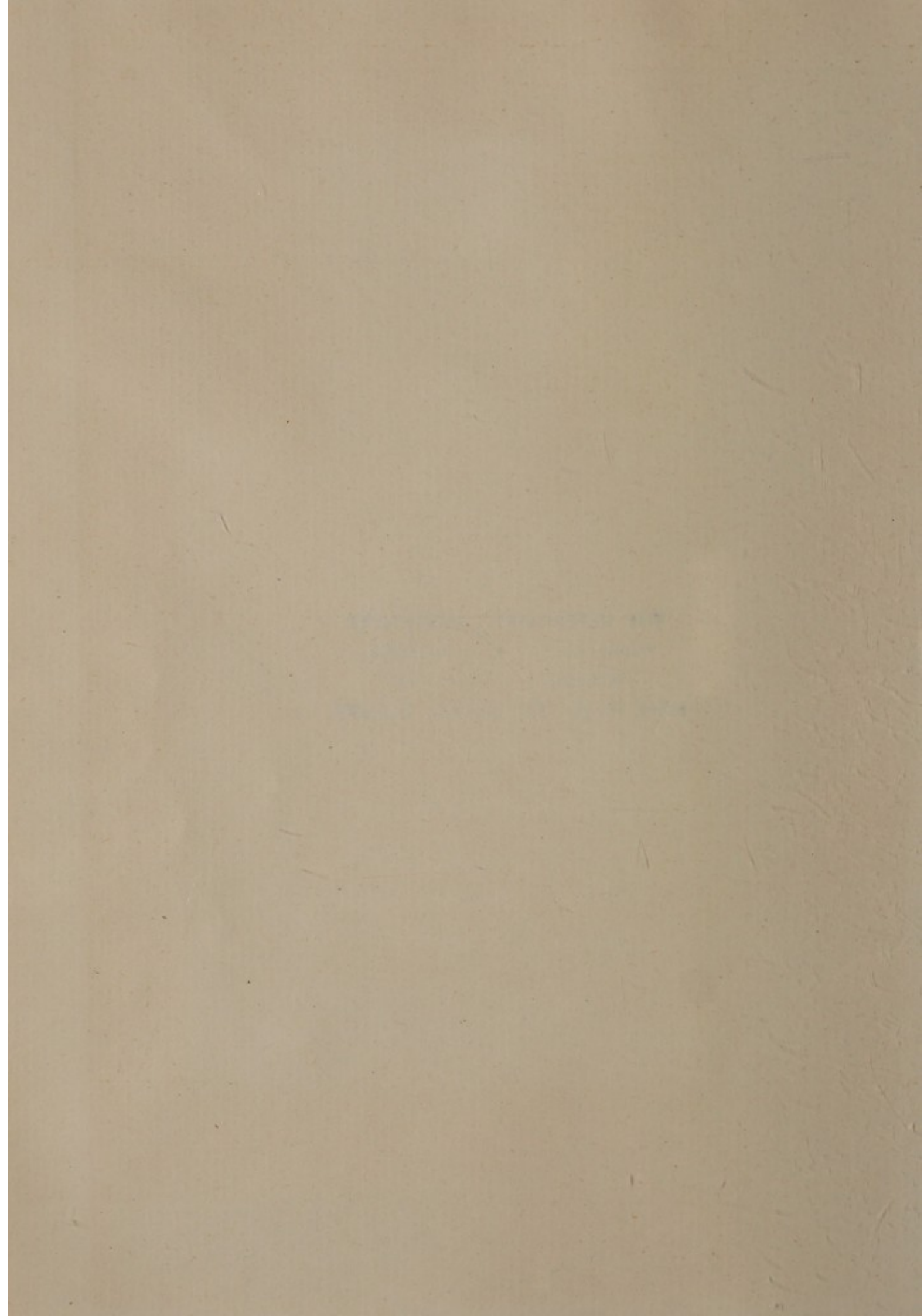


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VETERINARY WORK IN THE NETHERLANDS, 1953

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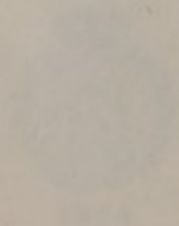
REPORT PREPARED FOR THE PARTICIPANTS OF THE  
INTERNATIONAL TRAINING COURSE  
ON PASTORAL DISEASE CONTROL

MAY 11 - JUNE 12 1953

REPORT BY  
DR. J. H. VAN DER  
KAMMEN, VETERINARY  
OFFICER

1953

U.S. GOVERNMENT PRINTING OFFICE



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# VETERINARY WORK IN THE NETHERLANDS, 1953

LECTURES, DELIVERED TO THE PARTICIPANTS OF THE  
INTRA-EUROPEAN T.A. TRAINING COURSE  
ON LIVESTOCK DISEASE CONTROL

MAY 18 - JUNE 12, 1953

STAATSDRUKKERIJ



UITGEVERIJBEDRIJF

1954



VETERINARY WORK  
IN THE NETHERLANDS, 1953

FACTORS DELIVERED TO THE PARTICIPANTS OF THE  
INTRA-EUROPEAN TRAINING COURSE  
ON LIVESTOCK DISEASE CONTROL

MAY 15 - JUNE 15 1953

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## PREFACE

Under the auspices of the M.S.A. (now called F.O.A.) an international course on Animal Diseases was held at the Hague in 1953. This course was organized by the Veterinary Service of the Ministry of Agriculture, Fisheries and Food.

Thirty experts from Austria, England, France, Western Germany, Greece, Italy, Luxemburg, Portugal, Turkey and Yugoslavia participated in this course.

The numerous lectures, which have been fully included in this book may together give a review of the veterinary work in the Netherlands for the year 1953.

May this publication contribute towards the strengthening of old ties and establishment of new relations in the friendly sphere, which characterized this first international course in Western Europe.

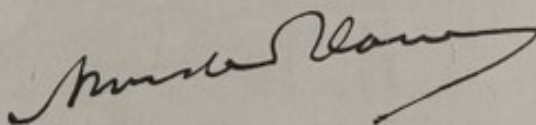
In fact, many veterinary problems of paramount importance for production increase require close international cooperation.

I am convinced that cooperation in the „technical” field may considerably contribute towards better mutual understanding of the peoples.

In addition, it may promote the unification of the countries in the political and economic spheres.

In this sense I gladly give this book my recommendation.

THE DIRECTOR GENERAL OF AGRICULTURE





*Participants Intra-European T.A. Training Course on Livestock Disease Control, May 18 - June 12, 1953*

## INTRODUCTION

The lectures included in this book were all of them delivered at the international course on veterinary science, which was organized in the Netherlands in 1953.

This course was held at the International Academic Institute in The Hague from May 18 - June 12, 1953 under the auspices of the Marshall organization. It was opened by the interim Minister of Agriculture, Mr C. STAF in the presence of many authorities.

On this day expositions were given of the agriculture of the Netherlands, the organization of the Veterinary Service in the Netherlands and the education of veterinarians.

This course was attended by 30 representatives from Great Britain, Luxemburg, Germany, Austria, France, Portugal, Italy, Yugoslavia, Greece and Turkey, all of whom hold leading positions in their country in the field of the control of diseases in animals.

The course was organized by the Veterinary Service with the enthusiastic co-operation of all Dutch veterinary authorities.

The idea was to give an insight into the veterinary activities in the Netherlands and to use this information for exchanging views which might, in the end, contribute to an increase of the production through a more effective control of animal diseases in Western European countries and a closer international co-operation.

The programme was in tune with this objective and included different problems each of which was disposed of in one day. In the morning lectures were given on organization and financing, research and the practical application of the results, followed by the showing of various films dealing with the subject concerned, while in the afternoon there was an exchange of views, which made it clear that also on an international level the workshop method is strongly recommended and in fact was enthusiastically participated in by all members.

In this way the following subjects were dealt with: Foot-and-mouth disease, tuberculosis, brucellosis, sterility, ox warble-fly, swine diseases, poultry diseases, zoonoses, milk, meat inspection and hygiene in the manufacture of meat products.

Half of the time available for the course was reserved for making excursions, which enabled the participants to see in practice what had theoretically been dealt with beforehand.

In one of the conference rooms an exhibition had been organized of pathologic-anatomic preparations and literature.

Linguistic difficulties were avoided by the presence of 8 interpreters who performed their duties eminently.

It was remarkable to see how the members of this motley company within only a few days made friends with all their fellow members. Not only were they mutually interested in all veterinary affairs, but they formed a group where humour reigned supreme, for which, besides the very interesting and excellently composed lectures, the great hospitality received from everybody was mainly responsible.

On June 12th the course was closed, in the presence of many, by the Director-General of Agriculture, Mr VAN DE PLASSCHE, who expressed his thanks to the organizers and other co-operators, while the participants from each country expressed their admiration of the level of the veterinary science in the Netherlands and their gratitude for the hospitality received here.

It is, therefore, beyond doubt that the objective of the course, set forth above, has fully been realised and that it was an outstanding success in every respect.

*The Hague, September 1954*

THE CHIEF VETERINARY OFFICER  
IN THE NETHERLANDS,

J. M. VAN DEN BORN

# A SURVEY OF DUTCH AGRICULTURE

IR J. M. A. PENDERS

*Inspector for Agricultural Advisory Services at  
the Ministry of Agriculture, Fisheries and Food*

The serious European agricultural depression at the end of the last century, induced by the large scale overseas imports of arable products from the 'New World', had a far reaching effect on the development of agriculture in the Netherlands. This country adapted itself to the changed conditions by alterations in the customary modes of husbandry. Arable produce was gradually but largely converted on the farm into more valuable animal products.

Another trend of the development was the expanded production of horticultural products to meet the increased demand of foreign markets. This greater demand was due to the development of manufacturing industries in neighbouring countries and their need for imports to balance the manufactured exports. On account of that trend the smaller holdings were afforded an opportunity of intensification.

Production of some arable products, as for instance cereals and oilseeds, is insufficient to meet home demands. About two thirds of the total demand for bread grains is met by imports, and of the total quantity of coarse grain required, more than one third is imported. Of the home grown agricultural produce, one third is exported and two thirds of the total exports are intended for West European countries. Of the typical converted and specific commodities, a much larger fraction is exported viz. 90 per cent of the flowering bulbs, 66 per cent of the cheese, 60 per cent of the butter and about 50 per cent of all vegetables, fruit and eggs.

Since the turn of the century the Dutch farmer has specialised more and more in production for marketing abroad and has become more dependent upon the conditions prevailing in such markets. In general the Netherlands adopted a free trade policy affording to her agriculture and horticulture a certain rate of resisting power and it fostered a rational intensification in farming.

On account of the agricultural depression in the thirties and the financial confusion since 1945 the free trade ideas were, from sheer necessity, pushed to the background. Now that the post-war period of scarcity has passed and Europe has recovered with the support of Marshall-aid, the free trade of commodities may begin again and, through the European Payments Union, the problem of monetary intercourse may be solved.



By imposing a rigid control on produce intended for export under supervision of the Government, its quality is guaranteed to foreign purchasers. Agriculture has also shown a vigorous development. This resulted from greater co-operation among farmers and has thereby created conditions favourable for farmers and growers to keep the markets better under control. It has also exerted a beneficial influence on the quality of produce offered for sale.

As to the purchase market, 60 per cent of all feeding stuffs and fertilizers are bought co-operatively. With regard to the sales-market, 100 per cent of the vegetables and 70 per cent of the fruit are sold at co-operative producers' auction markets, and three quarters of the milk intended for processing is sent to co-operative dairy factories. Half of all eggs are sold by co-operative marketing societies.

With regard to agricultural credit, one local co-operative farmers' credit bank of the Raiffeisen pattern is available for every 2,000 ha of cultivated land. By the mutual creation of funds for vegetables, dairy produce, seed and ware potatoes, endeavours are made to prevent excessive drops in prices of the products involved. In that way a dislocation of marketing conditions both at home and abroad is prevented as much as possible.

The yields of most crops per unit of area in the Netherlands are among the highest in the world, being for instance on the average, per hectare, 25 tons for potatoes, 40 tons for sugarbeets, 3.5 tons for wheat and 3 tons starch equivalent for grassland.

The use of fertilizers, per unit of area, in the Netherlands is higher than in any other country of the world, being on the average about 50 kg each of N,  $P_2O_5$  and  $K_2O$  and 150 kg CaO per ha. It has increased gradually on the grassland. The application of nitrogen has surpassed the prewar level by about 100 per cent, being at the present 60 kg N per ha on an average.

The value of livestock products are two thirds of the value of gross realized agricultural output. In accordance about three quarters of the total agricultural area is utilised for grassland and forage cropping to feed the herd.

One of the foremost tasks of Dutch farming is to economize considerably on the costs of imported feeding stuffs. This is done by intensification of grassland and arable fodder cropping and by improved conservation and utilisation of home grown feeding stuffs. This has helped to meet the shortage of the postwar supply of feeding stuffs from abroad and of foreign currency, and particularly of dollars.

While feed imports before the war exceeded 20 per cent of the total feed consumption of 6,200 tons starch equivalent, it has been reduced to less than 15 per cent of the total feed consumption of 6,600 tons starch equivalent existing at present. Farmers expenditure on feeding stuffs as percentage of value of gross realized output was 18 per cent in 1951 as compared with 25 per cent before the war.

Feed production from the grasslands dominates, exceeding 40 per cent of the total consumption of feed units. More than half of the total agricultural land is covered with grass, of which only a relatively small, however increasing part consists of leys.

The production level of grassland has been increasing rapidly since the war, amount-

ing to nearly 3 tons of starch equivalent per hectare at present as compared with 2.5 tons before the war. This is especially due to:

1. larger dressings of nitrogen – they have been doubled per unit of area;
2. improved grazing methods – the number of electric wire fences for more intensive rationed grazing amounting to nearly 70,000 as compared with nil before the war;
3. better conservation methods for the meadowcrop as hay, silage, and artificially dried grass. For example the use of the tripod in haymaking is expanding rapidly.

As to forage crops much attention is paid to fodderbeets, fodder potatoes, leguminous crops and maize; the area covered with maize being at present already three times the pre-war area.

While the average dairy cow used about 400 kgs of concentrates per year before the war, this amount has been reduced to 200 kgs at present as a result of pasture and meadow crop improvement and the inclusion of other home grown seeds.

For the fattening of pigs, steamed fodder potatoes are increasingly replacing cereals.

Of as great importance as increase in the productivity of the soil, is an increase in returns from livestock. During the last fifty years the yields of crops have increased by an average annual rate of 1.5 per cent, but also the returns from livestock have increased considerably.

The average egg yield per hen has doubled since 1900, being at present 175 eggs annually.

The average milk yield per cow is now 3,750 litres containing 3.5 per cent butterfat, the steady rise being hampered slightly by the t.b.c. eradication scheme during the last years. The milk yields of more than 50 per cent of all dairy cows are now recorded. Artificial insemination has developed considerably since the war and is now applied to about 30 per cent of the total number of cows and heifers. In co-operation with the farmers organizations and the Government, a five years t.b.c. eradication scheme has been started in 1951 with extremely good results up to now.

The total number of cattle in the country is nearly 3 million, of which 1.5 million are dairy cows. Pigs total nearly 2 million and poultry nearly 25 million. The number of sheep, mainly of the *Texel breed*, is nearly 400,000. This means that per ha of agricultural land nearly 2.5 animal units are kept, and fed for 85 per cent from home grown feeding stuffs.

The total milk yield has already exceeded pre-war production and is now over 5,500 million litres a year, about two fifths being intended for liquid consumption, i.e. 200 litres per capita per year. The production of eggs is over 2,000 millions, about half of which are exported.

Beef production is about 175,000 tons at present, which has been increased slightly during the last years by the t.b.c. eradication scheme, in operation since 1951. There is no special race or husbandry of beef cattle in the Netherlands, beef production being a side line of milk production and also carried out on some arable farms to utilize the offal of the arable produce. There are three races of cattle, i.e. the *black-and-white cattle* to which nearly three quarters of the total cattle herd belongs, the *red-and-*

*white Maas-Rijn-IJssel cattle*, to which nearly one quarter belongs and a minority of the so-called *Groninger zwartblaar*.

Production of pork and fatbacks amounts to about 250,000 tons annually. There are two races of pigs; *the Dutch Landrace* for the manufacturing of Wiltshire bacon and *the Great Yorkshire breed*, a heavier slaughter pig.

Full attention is being devoted to land improvement and reclamation work and to consolidation of farms. About  $\frac{1}{3}$  of the agricultural area is in need of reallocation. These works are very essential in a country like the Netherlands, with a population more dense than any other country in the world. There are 312 capita per square kilometer, and the population is increasing more rapidly than any other country in Europe, e.g.  $1\frac{1}{2}$  per cent annually.

There is a serious shortage of tillable land and annually 2,500 ha must be sacrificed to housing, manufacturing industries and traffic. So in the course of 8 years an area equal to that of the reclaimed Wieringermeer in the Zuyderzee is lost in consequence.

Almost three quarters of the total area of the Netherlands is agricultural land. The area under forests is now only 7 per cent of the total area, and it can supply only one fifth of the home need of timber. This area cannot be further reduced. Only 7 per cent of the total area is waste land and of this only 50,000 ha could still be reclaimed.

By reclamation of heaths and peat, by the drainage of the IJsselmeerpolders and enclosures within the coastline, it is estimated that in total 10 per cent can be added to the present area of cultivated land. The addition will have to be acquired mainly from the sea, the hereditary enemy of the Netherlands, which they must continuously conquer. Of the 5 designed IJsselmeerpolders, two have been enclosed and reclaimed in the last 20 years i.e. the Wieringermeer and the North-Eastern Polder of respectively 20,000 and 40,000 ha. The average elevation of over  $\frac{1}{5}$  of the Netherlands is about 1 m under sea-level and 40 per cent of the area would be inundated by high tides if there were no dikes to prevent it. By the flood disaster early in 1953 more than 100,000 hectares in the South-West of the country were inundated.

Agriculture in the Netherlands is confronted with the task of feeding the population of the future and this implies that its production must be increased by  $1\frac{1}{2}$  per cent annually. As the total cultivated area is nearly 2.5 million ha and the population is more than 10 million, four people are to be fed by the produce yielded from each ha of agricultural land. In 1948 exports and imports in the agricultural sector were nearly balanced but since that year exports have exceeded imports steadily. Agriculture and fisheries supply nearly 40 per cent of total Dutch exports.

Nearly 20 per cent of the total population is employed in agriculture. In a round figure there are 200,000 farmers in the Netherlands (mostly livestock farmers) and 30,000 gardeners. The latter occupy only 4 per cent of the total agricultural area. In addition there are about 100,000 regular agricultural workers.

Approximately 80 per cent of the holdings are smaller than 12.5 ha and the total of these cover half of the total agricultural area. The average size of all holdings is about 9.5 ha.

Family operated farms, on which no hired labour is used, are predominant in the country. The average number of the agricultural population per holding is 8, the highest in Europe. About half of the farms are rented, the other half occupied by the owners themselves.

Horticulture is a very specialized occupation and is localized in certain districts. It occupies only 6 per cent of the total cultivated area, but the value of horticultural products is nearly 15 per cent of the total agricultural output and exports amount to 20 per cent of the total exports of agricultural commodities.

The more purely arable, larger farms are found on sea clay and in the peat colonies. The purely pastoral farms occur mainly in the western and northern part of the country.

According to number and total area, the mixed farms, particularly the smaller mixed farms on sandy soils of an average size of 8 ha are predominant in the country and they give rise to the chief problem to be accounted for in the agricultural policy of the Netherlands. They are continuously drawing the full attention of the agricultural advisory service.

The rapidly increasing population creates a population tension in rural districts, which, considering the limited area of cultivated soil available on the smaller holdings, can only be eased by industrialization and emigration. About 40,000 new workers apply for work at the labour market each year.

Since the last war, mechanisation of farm operations has strongly developed, though the use of motors is confined to certain limits by the small average size of the holdings. Tractors number at present more than 25,000, six times the pre-war number.

The number of horses in consequence has strongly declined during the last years, being at present 240,000. That is nearly 100,000 less than the pre-war number.

Co-operative machinery pools have increased in number since the war, especially in districts of small holdings. The number of milking machines is increasing since the war, numbering at present 5,000. The total need has been estimated at 20,000.

Since the Dutch farmers and growers have started to produce more marketable crops, particularly for the worldmarket, it has become more essential to be able to offer produce of high quality at competitive prices by reducing the production costs. This was already realized by a Governmental Committee, which reported in the eighties of the last century, that more attention should be devoted to intensive agricultural education, agricultural research and advisory work. The Committee was of the opinion that by these means a foundation could be laid for rational agricultural production based particularly on converted and specialized commodities, which would give better results than might be attainable by high import duties at the frontier.

The services cited were established at the expense of the Exchequer and they expanded gradually, especially after the last war.

The first *agricultural school* was opened in 1876. In 1877 the first experimental station was founded, particularly adapted to control work and research. In 1890 the first agricultural teachers were appointed by the Government on the pattern of the

'Wanderlehrer' in Germany. They were the predecessors of the agricultural advisers of to-day.

Right from the beginning, the advisers have been charged with tuition in addition to their advisory task and have also rendered assistance to research.

Agricultural research, education and extension, which are related very closely to each other, are financed nearly wholly from the State budget, in approximately equal amounts of about 10 million guilders annually; the total sum being nearly 1 per cent of the total gross value of agricultural production.

To get an idea of the intensiveness of agricultural education it may be noted that about 50,000 pupils annually attend agricultural educational institutes at the various levels. For the sake of comparison it may be noted that in the Netherlands there are in total about 230,000 farmers and gardeners.

As to *agricultural extension* it may be noted that there is one regional adviser for every 400-500 farmers. The State advisers are mainly concerned with advisory work in technical matters and farm economics. General economics and social matters are the concern of the farmers' organisations. Advisory work in home economics and with regard to farm youth is also mainly dealt with by the voluntary farm women's and rural youth organizations.

Co-operation of farmers organizations with the Government is intensive and extending gradually. In this connection attention may be drawn to the provincial health services for livestock founded after the war by the farmers organizations in co-operation with the Government, and to the associations for managerial instruction, to which belongs nearly 20 per cent of the farmers.

A National Agricultural Advisory Council, consisting of representatives of the Government and the Farmers Organization, has been founded. Provincial councils will be established during this year.

As to *agricultural research* (about 20 per cent of the funds being provided by private bodies), the co-ordination is based on the Applied Physical Research (Organization) Act 1930 and it is conducted under the auspices of the Applied Physical Research Institution (T.N.O.), an organization in which both the Government and the various industries participate. The agricultural branch of this organization has in its governing committee representatives of the Government, of agricultural science, and of the farmers organizations.

As to the educational institutes in agriculture, part of them belong to and are governed by the private farmers or farm women's organizations, however all are supervised by the Government.

The director-general of agriculture at the Ministry of Agriculture, Food and Fisheries is co-ordinating agricultural education, research and extension.

Finally I should like to draw attention to the fact that European farmers have tended to concentrate on the production of commodities with a high unit price and in particular on animal products. Animal production constitutes by far the greatest part of gross realized agricultural output, amounting in all to between 60 and 80 percent of the value of the total agricultural production, whereas in Southern European

countries the percentage is somewhat lower, however still ranging between 30 and 40 percent of the total output. Imports of animal products from outside Europe as percentage of the total consumption, therefore only amount to about 7 percent for eggs and dairy products, and to about 10 percent for meat. In spite however of the efforts made after the war to produce more home-grown feedingstuffs, imports of the latter still amount to 1,000 million dollars yearly, widening the dollar gap existing in Europe

E. J. A. G. ...  
Director of the ...

The Veterinary Service in the Netherlands is known in the Department of Veterinary Affairs under the Ministry of Agriculture, Fisheries and Food. It is headed by the Director of the Veterinary Service who has supervision over:

- a. the Veterinary Director
- b. the Inspector-General

One of their primary tasks is to carry out all matters relating to the control of animal products for which organized control is necessary. This includes the control of meat and meat products, eggs and egg products, milk and milk products, and other animal products. The control of these products is carried out by the Inspector-General and the Veterinary Director. The Inspector-General is responsible for the control of the products and the Veterinary Director is responsible for the control of the animals.

The Inspector-General is also responsible for the control of the products and the Veterinary Director is responsible for the control of the animals. The Inspector-General is also responsible for the control of the products and the Veterinary Director is responsible for the control of the animals.

As far as this is required, they are assisted in their work by assistant-inspectors. Moreover, a number of 'overseers of the Veterinary Service' are added to these inspectors; they are especially commissioned to carry out verification work and to trace irregularities or non-compliance with the regulations. The Inspector-General is also responsible for the control of the products and the Veterinary Director is responsible for the control of the animals.

It will be clear that a major part of the daily routine cannot be performed by the Inspector or his assistant-inspector personally. Therefore, he has under his command so-called deputy inspectors who carry out their duties, as such, by order of the Inspector. Such deputy inspectors are not full-time officers. By far the greater part of their

# ORGANIZATION OF THE VETERINARY SERVICE IN THE NETHERLANDS

E. J. A. A. QUAEDVLIEG

*Director of the Veterinary Service*

The Veterinary Service in the Netherlands is known as the Department for Veterinary Affairs under the Ministry of Agriculture, Fisheries and Food.

It is headed by the Director of the Veterinary Service, who has supervision over:

a. the Assistant Director;

b. six Inspectors in general service.

One of them is particularly in charge of all affairs relating to combating tuberculosis in cattle and other animal diseases for which organized combat is considered necessary, e.g., the ox warble-fly. One of them is especially commissioned to deal with all matters relating to the inspection, etc., of meat and meat products destined for export. The third deals in particular with zoonoses. The remaining three attend to affairs regarding the inspection of meat for home consumption.

c. eleven Inspectors of the Veterinary Service, each of whom acts as head of a district assigned to him. They are commissioned, each in his own district, to perform any duties relating to the implementation and the supervision of the observance of all provisions laid down in the respective Acts and Orders such as the Cattle Act, Bird Disease Act, Act Governing the Practice of Animal Medicine, the Tuberculosis Act, Destruction Order, etc., etc.

As far as this is required, they are assisted in their work by assistant-inspectors.

Moreover, a number of 'overseers of the Veterinary Service' are added to these inspectors; they are especially commissioned to carry out verification work and to trace irregularities.

The latter officials are not veterinary surgeons, but they have received special training for the fulfilment of their task. They do their work under the supervision of the Inspector, who is responsible for their activities.

It will be clear that a major part of the daily routine cannot be performed by the Inspector or his assistant-inspector personally. Therefore, he has under his command so-called deputy inspectors who carry out their duties, as such, by order of the Inspector.

Such deputy inspectors are not full-time officers. By far the greater part of them are



*Dutch scenery*

veterinary practitioners and/or directors of a cattle and meat inspection service. Thus, for instance, inspection at the importation and exportation of cattle is frequently effected also by such deputies together with the Inspectors themselves.

Inspection of meat and meat products, also entrusted to the Veterinary Service, is performed by the following officials belonging to this Service:

1. State Inspectors in official service.
2. State Inspectors in special service. They are mainly directors of Abattoirs or directors of Inspection Services in municipalities where packing-houses and/or meat-processing works are established.



Both categories of Inspectors are aided by Overseers of the Veterinary Service, who have limited inspecting powers.

Finally, the organization of the Veterinary Service embraces the following State Institutions:

- the State Serum Institute at Rotterdam and
- the State Institute for Veterinary Research at Amsterdam.

In order to accomplish its task to the best of its ability, the Veterinary Service maintains close contact and co-operation with a great number of organizations, official and non-official services, etc., etc., both at home and abroad.

#### *A. At home*

The Veterinary Service is represented through its Director or in the latter's name by one of the Inspectors of the Veterinary Service in:

1. the Agricultural Organization, Institute for Applied Physical Research ('T.N.O.');
2. the Central Health Committee for Animals;
3. the Board of the Provincial Health Service for Animals;
4. the Public Health Board;
5. the Advisory Board of the Institute for Preventive Medicine;
6. the Ox Warble-Fly Committee;
7. the Central Committee for Artificial Insemination;
8. the Committee for Destruction Affairs;
9. the Committee for International Public Health Affairs;
10. the Bacon Inspection Association;
11. the Committee for the Meat Products Inspection Office;
12. the Serum and Vaccine Committee;
13. the Meat Products and Inspection Office, etc.

#### *B. Abroad*

The Veterinary Service is represented there through its Director in:

1. the 'Office International des Epizooties' (International Epizootics Office) at Paris as permanent delegate of the Netherlands Government;
2. the Commission on Agriculture of the F.A.O. Committee;
3. the Benelux Committee for Meat Hygiene;
4. the Working Party Animal Health of the O.E.E.C.;
5. the Foot-and-Mouth Disease Committee of the 'Office International'.

The task of the Veterinary Service is laid down in the so-called Cattle Act.

This Act provides that veterinary supervision exercised by the State shall comprise the following sections:

- a.* Care of the general state of health of live-stock.
- b.* Prevention and combat of infectious cattle diseases.

- c. Prevention and combat of rabies in cats and dogs.
- d. Inspection of cattle and meat intended for export.
- e. Whatever the Crown may provide also to be covered by such supervision.

In particular the provisions a) and e) offer the Veterinary Service a possibility of extending its activities, if necessary, over a much wider field than that of infectious cattle diseases. It is clear enough that the category b), although constituting only part of the task, must be considered to be highly important. At present this importance is still more outstanding than it was formerly, now that the 'international' aspect in regard to the fight against infectious diseases is coming more and more to the fore and carries much weight.

It is now generally realized that international co-operation in this field is imperative, but within the scope of the present lecture I cannot go into further details on this point.

Although opinions on the line to be taken or the methods to be adopted in combating some infectious diseases may be divided here and there, it is generally agreed – a standpoint also defended by the Netherlands Veterinary Service – that tuberculosis, processing infected meat, etc., cannot be effectively counteracted by police measures only, but that the best method consists in organized combat of such diseases and practices.

More detailed exposés on this question will be given later.

#### *The State Serum Institute at Rotterdam*

The object of the State Serum Institute has been formulated as follows:

Making more recent veterinary data, particularly on infectious animal diseases, directly subservient to agriculture.

Within this scope, the above Institute supplies information on animal hygiene; it carries out investigations into unknown causes of diseases of cattle, poultry, etc., as well as research on milk and milk products, on drinking water, on water used in dairying, etc. The Institute prepares sera and vaccines both for preventive and curative treatment of animals, and also preparations for the diagnosis of diseases.

In addition to various other sections it also comprises an ambulatory section. The Institute remains at the disposal of veterinary surgeons; whenever this is required, it makes local investigations and assists the veterinary surgeon by word and deed.

#### *The State Institute for Veterinary Research*

The arrangement of this Institute is similar to that of the State Serum Institute, but the former directs its activities towards the research, etc., of virus diseases and the preparation of vaccines to prevent and to combat these diseases.

Owing to the Foot-and-Mouth Disease threat, these last few years the above Institute has had to deal particularly with the preparation of the well-known vaccine against that disease. Further information on this point will be supplied by its Director, Dr FRENKEL.

Both Institutes are in urgent need of room, but extension is not possible where they

are located now. We are now preparing the creation of a large new Institute in the centre of this country in the immediate vicinity of the Veterinary Faculty.

### *Cattle Markets*

It is a well-known fact that cattle markets can play an important part in the spreading of virus. Therefore, it stands to reason that, with a view to preventing and combating infectious diseases, the Veterinary Service exercises special supervision of cattle markets. It is prohibited to hold cattle markets unless the Municipal Corporation has established a bye-law governing them. This requires the approval of the Crown. It is provided that particular regulations must be incorporated in this bye-law. One of the principal regulations states that, before being admitted to the market, all beasts must be subjected to a veterinary examination on arrival (foot-and-mouth disease, ox warble-fly, etc.).

At present only such cattle are admitted to markets as carry a certificate of vaccination against foot-and-mouth disease.

In the Netherlands there are already a great number of markets where only tuberculosis-free animals may be admitted.

### *Artificial Insemination*

Artificial insemination has developed to a fairly considerable extent in the Netherlands. During the war a marked decline could be observed, but this has now been entirely offset. The advantages of artificial insemination, both from a point of view of combating animal diseases and from a cattle-breeding standpoint, are generally known so that it will not be necessary to go into these matters at greater length.

The Minister of Agriculture, Fisheries and Food has instituted a central committee which also includes the Director of the Veterinary Service. In each province, a provincial committee has been set up.

Insemination is carried out by inseminators specially trained for this work, which they do under the supervision of veterinary surgeons.

### *Combating of the Ox Warble-Fly*

This takes place on the strength of statutory provisions. The supervision of the observance of these provisions is entrusted to the officials of the Veterinary Service.

It is prohibited by law to send cattle out to pastures and markets, etc., if they are infected with warbles.

Already before the statutory provisions had been issued, interested parties had taken up the fight against this pest.

Those interested parties, namely cattle breeders, butchers, leather dealers, cattle dealers, etc., set up a committee which still exists and which spends the funds placed at its disposal for purposes of propaganda and grants subsidies to organizations treating the infested animals.

In the Netherlands this treatment is organized by the provincial health services.

### *Milk products*

The officials of the Veterinary Service also exercise supervision of the observance of the legal provisions in regard to the preparation and transport of milk products, particularly skimmed milk and whey. This is with a view to the possibility of infectious diseases being spread by means of the consumption of these products as fodder.

### *Import, Export and Transit of Cattle and Cattle Products*

In the Netherlands there is a prohibition of import, export, and transit of cattle and cattle products. Although there are general dispensations from this prohibition and, moreover, special exemptions may be granted, this system has ensured full control by the Veterinary Service of the transportation of cattle in both directions across our frontiers.

By imposing conditions for importation, e.g., as regards means of conveyance, quarantine, etc., full control of importation by the Veterinary Service is ensured as well.

All the cattle destined for export have also been brought under control and are only released for export after examination by, or on behalf of, the Inspectors of the Veterinary Service.

At this inspection and examination it is also ascertained whether the requirements of the importing country have been duly met, for instance, if cattle are free from tuberculosis, Abortus Bang, vaccinated against foot-and-mouth disease, etc.

### *Bird Disease Act*

Until a few years ago, in the Netherlands there was only an act specifying measures that might be taken to prevent poultry diseases.

Measures to combat infectious diseases were not incorporated in it.

When the Newcastle disease broke out in France and Belgium, this old act was superseded by a new one which makes it possible to adopt very rigorous measures, for instance, a prohibition of transporting poultry, holding markets, killing animals on infected farms, etc.

This new act is very closely related to the Cattle Act, i.e. the act to combat infectious diseases as a whole.

At the invasion of the Newcastle disease in the Netherlands full benefits were derived from these rigorous measures, but in addition to such police measures endeavours have been made to combat the disease by preventive inoculation with 'dead' vaccine and, locally, by way of trial with 'living' vaccine. The results are very satisfactory, particularly those obtained with the 'living' vaccine. It would be advisable to adopt the system on a large scale but at present this cannot be done because of agreements entered into with the 'Office International des Epizooties' at Paris.

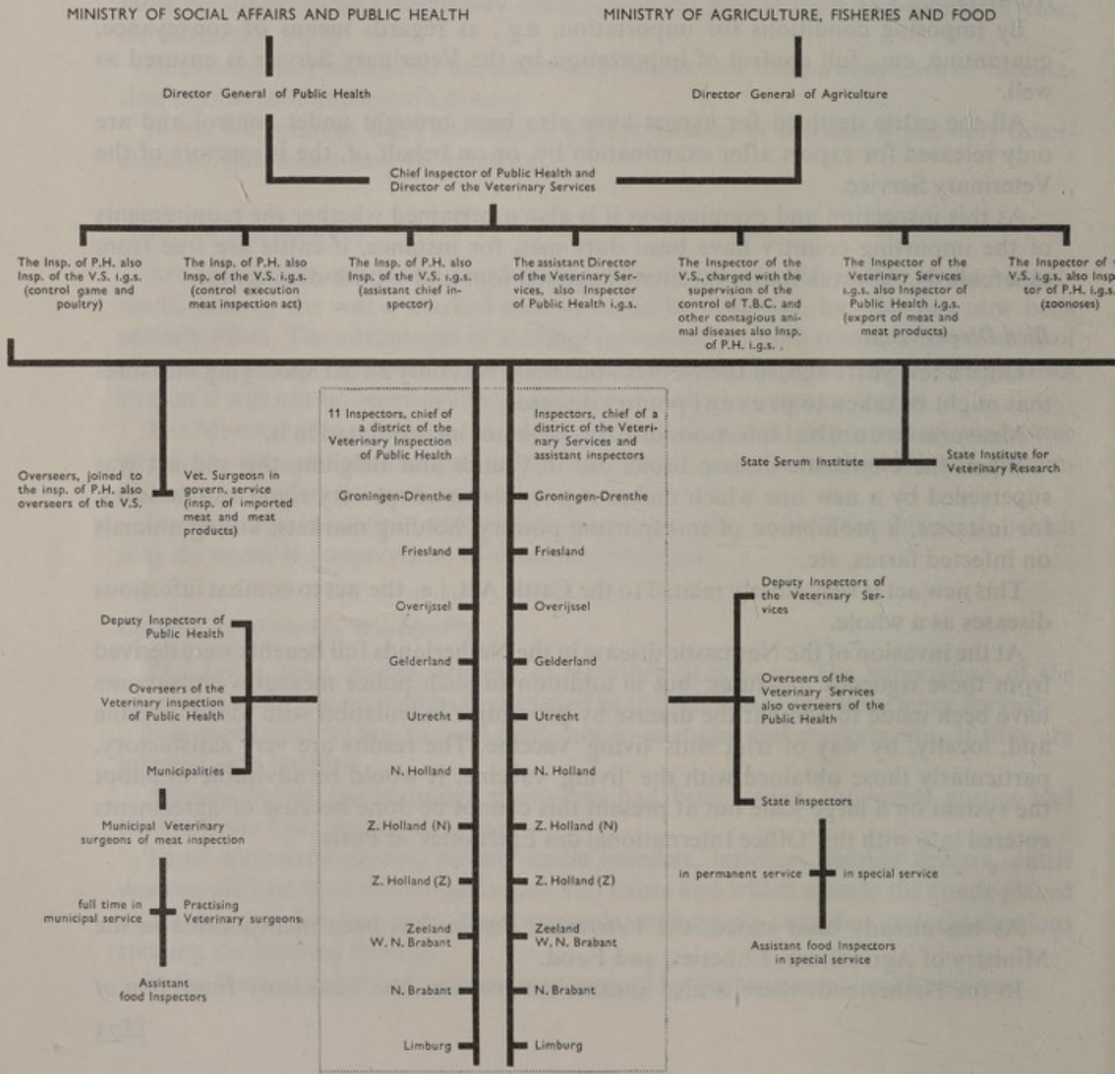
As has already been stated, the *Veterinary Service* has been incorporated in the Ministry of Agriculture, Fisheries, and Food.

In the Netherlands there is also another service, viz., the *Veterinary Inspection of*

*Public Health* which, in keeping with other services dealing with inspection and examination of food, is incorporated in the Ministry of Social Affairs and Public Health.

Therefore, essentially there are two Veterinary Services, but they are so closely interrelated that they employ the same officials. Thus, the Director of the Veterinary Service is at the same time Chief Inspector of Public Health and both the Inspectors of the Veterinary Service in general service and the District Head Inspectors are also Inspectors of Public Health.

The Chief Inspector of Public Health has two Inspectors under his command who are particularly commissioned to deal with the Meat Inspection Act, a third Inspector being specially entrusted with the preparatory work of drafting an act on game and poultry inspection.



# EDUCATION OF VETERINARY SURGEONS IN THE NETHERLANDS

C. F. VAN OIJEN

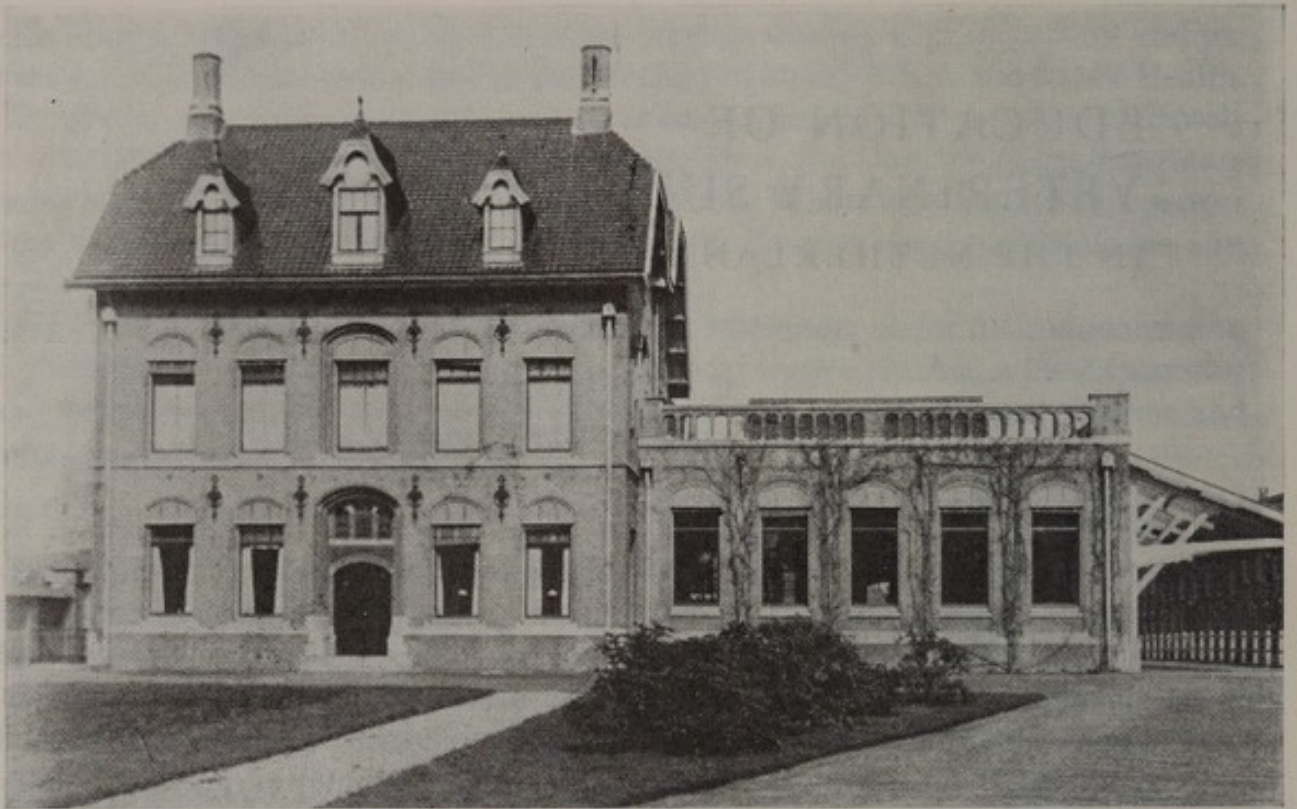
*Professor at the Veterinary Faculty  
of the University of Utrecht*

The purpose of this course is: the study of the organization and operation of effective livestock disease control. It is clear that no effective eradication or control of disease among livestock can be executed without the leading support of allround and able veterinary surgeons, be they practitioners or officers of any organization. Moreover the organizers of this course widened their interest to many other parts of applied veterinary science, such as animal husbandry, artificial insemination, meat and dairy hygiene, the menace of animal diseases for man, and the support it can give to many other branches of medical science. So a brief survey of the education of veterinary surgeons in the Netherlands fits very well in the scheme of these lectures.

But it is a pity to say, you cannot *bring up* allround and able veterinary surgeons. Such can only *ripen in practice*, after having received a sound scientific and technical base for further study and observation. It is the aim of the professors and lecturers of the veterinary Faculty at Utrecht to provide every young veterinary surgeon with so much knowledge of and skill in the different branches of veterinary science, that he can develop himself to become an able and honorable member of the profession.

Veterinary education started in the Netherlands about 130 years ago, in the year 1821. In Europe the Veterinary School at Utrecht is not one of the oldest teaching centres of this kind. It emerged soon after the French occupation and whilst Belgium and Holland were still united under the reign of King William the First, who took an ardent interest in its foundation. It was located in the outskirts of the town, in an old factory, with some acres of surrounding land. This old main building and an adjacent manor are still part of the faculty buildings.

In these years the staff consisted of 3 professors and 3 lecturers, none of the former being a veterinary surgeon; there were some 24 to 40 students. The school was run on the funds collected by a levy on livestock destined for the combating of infectious diseases in animals. Most of the students paid no fee at all, they lived in the college of the school. We will not study the ups and downs of the first 50 years of this school. Some of the teachers were also professors in the Medical Faculty of the ancient University of Utrecht. At the end of this period there were some veterinary surgeons as able and honoured teachers. The course lasted 4 years. There was only one (final) examination.



*Government University of Utrecht. Veterinary Faculty.*

There were several reasons for the depletion of the school about 1850.

*First*, the funds mentioned above ran out, the Government had to bring the cost of the school into its own budget, the students fee was raised considerably.

*Second*. The 'qualified-veterinary surgeons', as they were called officially, could hardly make a living because of the unlimited number of 'Quacks' operating in the country. The former had to be subsidized by state, provincial, and municipal governments, to hold their own. In 1871 a law regulating the right to practice as a veterinary surgeon reached the statute-book; the former pupils of the school had a better chance for a decent living and since that time the school has a greater number of students.

In the history of the school there have been *three main re-organizations*. The first occurred about the year 1900, when the qualifications of the pupils entering the school were raised nearly to the same level as for these students entering the University.

The obligation to live in a college under the supervision of junior members of the staff was abolished. I should like to draw your attention to this last point, as it is one of the educational principles cherished in this country for the training of those young men, who afterwards will have to assume a leading position. Here the students live in rooms and boarding houses and have to steer their way of living at their own responsibility. I admit, that perhaps in this way the spirit of belonging to a special corporation is not so well evoked as by living in colleges, but, on the other hand, when they enter actual life as veterinary surgeons they already know how to manage.

Most teachers in this country prefer this way of preparing for the actual life as a leading member of society.

About that time the staff consisted of 8 professors, 3 lecturers, and 3 junior assistants. The number of students was about 70.

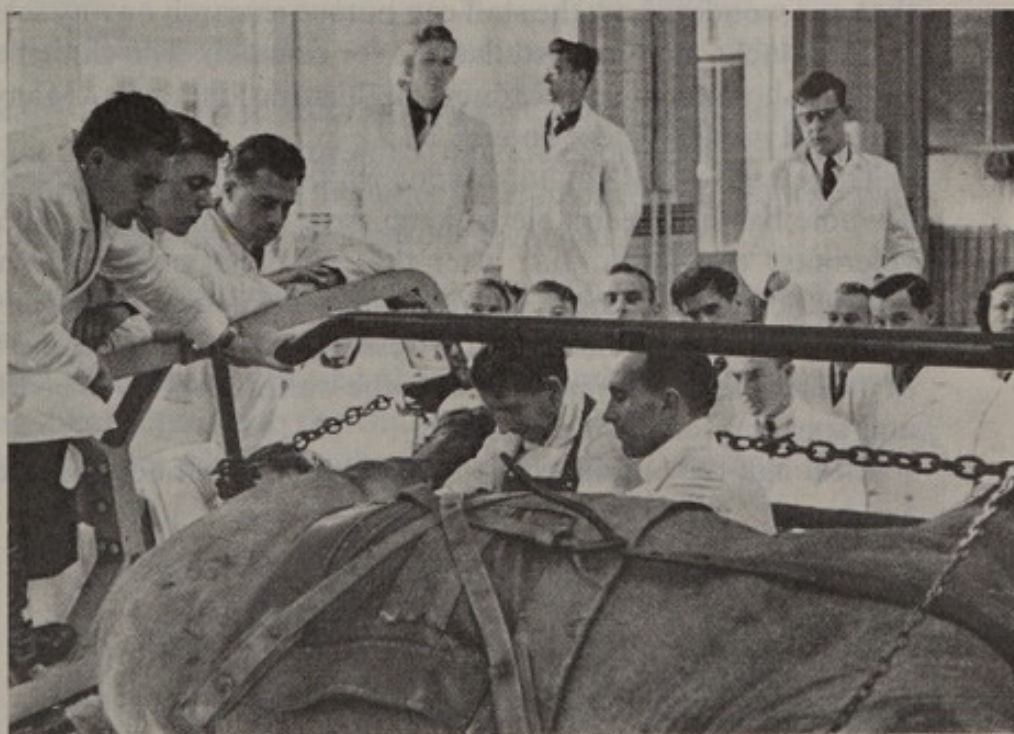
The second reorganization occurred in 1917, when the Veterinary School at Utrecht was promoted by law to the grade of 'Veterinary College', being a solitary institution, belonging to the Ministry of Agriculture, and having the same status as the Technical College at Delft and the Agricultural College at Wageningen.

The solemn opening of the Veterinary College took place on March the 16th 1918, while the first world war was still raging around the Netherland frontiers. The staff consisted at that time of 12 full-time and 2 half-time professors. None of these is at this time any more in function. There were about 20 lecturers and 10 junior-assistants. About 6 of them are still on the staff as professor or lecturer. So the scientific staff had been augmented to about 50 persons, the number of students had risen to about 200.

Under the direction of the Ministry of Agriculture every omen pointed to the happy development of all the necessary departments, manned with a staff of able scientists and teachers and provided with the necessary implements to fulfil the task of research and education of such veterinary surgeons as the Netherlands, a stockbreeding country of the first order, needs so very badly. Moreover in the Dominions overseas, at that time, there was a nearly endless task to tackle in the tropics.

In some years before and during this period many departments were reorganized and fine new buildings built. I should like to mention here the new buildings for the Surgical department, the department for pathological anatomy and general pathology, the department for anatomy, histology and embryology and the clinics for small animals (dogs, cats, fowls etc). This was a prosperous time for our country, but very soon the economic position declined and it was urged that all Ministerial departments should economize. That was the beginning of the third and, until this day, last reorganization of our institution.

In the year 1923 the idea was aired that both the centre for veterinary teaching and research and the University of Utrecht would prosper by close collaboration. For this



*Faculty Utrecht.  
Surgical clinic.*



purpose the existing Veterinary College had only to be incorporated as Faculty number 6 in the ancient University.

The whole veterinary profession in this country and especially the members of the scientific staff of the College stood at a crossroad. They realized that the promotion of the veterinary research and education centre to the level of a University-Scientific Institute would act for the benefit of the social standing of the veterinary corps. The school would be fully independent from agrarian domination and the staff would be able to fulfil their task on purely scientific lines. But they realized also, that the Ministry of education had a very extensive ground to cover, so that the funds for this special small part of its task would perhaps be minimized. Moreover, the veterinary section of education might lose the interest of the Department of Agriculture as this Ministry felt the need of research centres under its own direction.

Failing this incorporation, the state of the veterinary school could be reduced to that of a technical school, which would have been detrimental to the development of veterinary science in the Netherlands and the social standing of veterinary surgeons.

So, after due consideration, in 1925 the dice fell to the choice of incorporation of the Veterinary College in the realm of the University and now, after about 30 years we can say, that this choice has been beneficial for the veterinary corps as a whole. Veterinary officers are ranked in the same sections as medical and other scientists in the governmental and municipal services. The reception in University circles was and is wholehearted and led to scientific collaboration between different departments of the ancient University and the new Faculty.

But on the material side of the matter, there are many deceptions. There have been some augmentations to the staff and here and there slight ameliorations to the existing buildings have been executed, but we must state with great emphasis that in this third part of a century there has not been erected a single new building for any of the many departments which need it so urgently. Speaking of the material aids and appliances, the Utrecht Faculty cannot be mentioned any more as a leading centre of veterinary research and education.

The department of physiology was housed in some rooms of the clinic for small animals and in some huts constructed in the twenties of this century. The departments for infectious diseases and for tropical diseases are equipped in such a scanty way, that one wonders how the staff can pursue research on any scale. The clinic for internal diseases lacks modern installations for research. The clinic for obstetrics and gynaecology is housed in some almost prehistoric buildings. Many other departments are cramped in insufficient rooms.

There are several sound reasons why, since 1925, the Netherlands Government as a whole has not been able to keep pace with the requirements of modern scientific veterinary education. Just after that year the great economic crisis started, which reduced the Government's budget and our Faculty had to pay its toll in the reduction of the staff and in the funds for research. Then there came the uneasy pre-war time at the end of the thirties and finally the war of 1940-1945 nearly exhausted the material and spiritual forces of the country.

Although the buildings of the Faculty were not destroyed or bombed, at the end of

the war they were nearly depleted of modern scientific equipment. I should like to pay a tribute of gratitude to all corporations and government departments in the Neutral and Allied countries, who have wholeheartedly helped to restore our library and scientific installations, so that we could carry on in some way immediately after the liberation from the German occupation in 1945.

But after some rather prosperous years, the ogre of cutting down the subsidies is raging again. As you will hear, there are some promising aspects for the next year in regard to the panel of teachers, but we have not the staff and the materials to develop research on an adequate scale.

Having given a short survey of the embryonic development, the flowering youth, and the sorrowful coming of age of the veterinary education centre at Utrecht, I should like to draw a picture of the actual state of the teaching.

First of all, let us mention that the number of professors is still 12 as in 1918.

*Chairs in the Veterinary Faculty of the Utrecht-University*

Anatomy, Embryology . . . . .	Dr. H. A. Meyling
Physiology . . . . .	Dr. R. Romijn*
Physiological and pathological Chemistry . . . . .	Dr. L. Seekles*
Genetics, Animal Breeding and Hygiene . . . . .	Dr. G. M. van der Plank
Bacteriology, Immunology and Infectious Diseases . . . . .	Dr. J. Jansen
General Pathology and pathological Anatomy . . . . .	J. H. ten Thije
Protozoic and tropical Diseases . . . . .	Dr F. C. Kraneveld
Internal Diseases (large domestic animals) . . . . .	Dr. J. A. Beijers
Surgical Diseases (large domestic animals) . . . . .	Dr. S. R. Numans
Diseases of small domestic animals; Pharmacology . . . . .	Dr. G. B. H. Teunissen
Obstetrics and Gynaecology . . . . .	Dr. F. C. van der Kaaij
Meat- and dairy Hygiene . . . . .	C. F. van Oijen

*Lecturers in the Veterinary Faculty*

Agricultural Economics . . . . .	Ir. J. Wind*
Applied Meat Inspection . . . . .	Dr C. de Graaf
Genetics, Breeding and Hygiene of Poultry . . . . .	Dr. W. K. Hirschfeld*
Histology . . . . .	W. H. Schultze
Ophthalmology . . . . .	Dr. H. Veenendaal
Parasitology and parasitical Diseases . . . . .	D. Swierstra

Those marked with \* are not veterinary surgeons.

In the budget for next year is foreseen the addition of a professor in pharmacology to this list, and the promotion of two of the lecturers to the state of professor. Besides the 6 lecturers, there is a scientific staff of about 35 senior and junior assistants, who partake in the teaching and research. There is a numerous technical staff.

The number of students has risen to about 600. For every one of them the complete study takes 6 years at the very least, most of them will stay for 6½ to 7 years. These students are only admitted after having passed the final examination of a licensed 'secondary-school', giving them entrance to the University.

The teaching of science (physics, chemistry, botany and zoology) in these schools

should give them a sound base for the study of these subjects in their first year. These lessons are given by the professors and lecturers in the Faculty of Science in this University. They follow these highly scientific lectures and practical courses, with the medical students, the young dentists, pharmacists and biologists, so in these courses there is little opportunity to treat those subjects that are of special interest for veterinary students. It is a wish of the Faculty, that they should be completed in this respect by lectures from special teachers. On the other hand I think it a great privilege for the mental training of these young men to listen for one year in their life to scientists of European or world fame.

As can be seen in the scheme (pag. 31), the curriculum is split up into five periods or 'years', some of them lasting three half years. At the end of each one, the students have to pass an examination before entering the courses for the next one. As an introduction to the veterinary study, they receive in the first year some lessons in anatomy, cytology and physiology. At the end of this year they pass an examination in physics, physical chemistry, chemistry, botany and zoology and their knowledge in those subjects at that time, in the main, reaches to that of a bachelor of science in many other countries.

Then they commence the second period of the curriculum which takes one and a half years. This time is devoted to anatomy, embryology, histology, cytology, physiology and physiological chemistry. They study a special chapter in botany about nutritive and poisonous plants and about plants used in pharmaceuticals. They start with the study of zootechnics and animal husbandry (genetics, hygiene, horse shoeing, nutrition etc.).

I shall not go into details of the teaching of each one of these subjects. The Faculty intends to issue a programme in English, French, and German containing all information and will be very glad to send it to anybody interested in this matter.

After this period they pass a second examination and are now entitled to the indication 'candidaat in de veedartsnijckunde' (bachelor of veterinary science).

The third period has a duration of one year and may be called the 'pre-clinical teaching'. The students get theoretical and practical lessons in investigation methods used in the internal and surgical clinics and in those for small domestic animals. They begin to study pathological chemistry, bacteriology and immunology, parasitology and obstetrics, general pathology and general therapeutics. They receive theoretical lessons in surgery and dairy hygiene. They finish the theoretical instruction in genetics, nutrition and animal hygiene. In the second part of this year they tentatively enter the clinics and gain their first contact with the actual observation of diseased animals and clinical methods.

After passing the third examination they come into the fourth period which will take, as the second one, three half years. It was the aim of the Faculty to finish, as far as possible, the theoretical instruction in most of the departments at the end of the second half year, so that the students in the third one could devote themselves to actual work in the clinics, in the laboratories, and in the dissection room. They might

# VETERINARY FACULTY

SCHEME OF THE CURRICULUM IN RELATION TO THE DIFFERENT BRANCHES OF APPLIED VETERINARY SCIENCE

Examinations	Half Years	Clinical Veterinary Science										Hygiene	Science Ingeneral	Animal Husbandry Zootechnics	Propeduse
Final Ex	12	Intern. A. Surgical Clinics	Internal Clinics Large	Obstetric Clinics	Surgical Clinics	Vet Government Control Forensic Vet Science	Surgical Large Domestic Animal	Obstetric Clinics	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	11	Practical Pharmacy	Small Domestic Animal	Domestic Animal	Large Domestic Animal										
4th Ex	10	Toxicology Pharmacology and General Therapeutics	Internal Diseases	Infectious Diseases	Parasitic Diseases	Protozoal and Tropical Diseases	Surgical Diseases	Obstetrics and Gynecology	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	9														
3d Ex	8	Toxicology Pharmacology and General Therapeutics	Internal Diseases	Infectious Diseases	Parasitic Diseases	Protozoal and Tropical Diseases	Surgical Diseases	Obstetrics and Gynecology	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	7														
2d Ex	6	Toxicology Pharmacology and General Therapeutics	Internal Diseases	Infectious Diseases	Parasitic Diseases	Protozoal and Tropical Diseases	Surgical Diseases	Obstetrics and Gynecology	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	5														
1th Ex	4	Toxicology Pharmacology and General Therapeutics	Internal Diseases	Infectious Diseases	Parasitic Diseases	Protozoal and Tropical Diseases	Surgical Diseases	Obstetrics and Gynecology	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	3														
1th Ex	2	Toxicology Pharmacology and General Therapeutics	Internal Diseases	Infectious Diseases	Parasitic Diseases	Protozoal and Tropical Diseases	Surgical Diseases	Obstetrics and Gynecology	Meat and Dairy Hygiene	General Pathology	Animal Husbandry Zootechnics	Theoretical and Practical Instructions	Propeduse	Theoretical and Practical Instructions	Theoretical and Practical Instructions
	1														

get an opportunity to study a special subject, just to train them for autonomous scientific investigation. But the inflation of new discoveries and principles in nearly all branches of veterinary science puts a serious bar to this intention. Much of the time of the last half year must be used for theoretical and practical courses.

In the whole period of three half years they receive the bulk of the lessons in surgery and internal diseases both in large and small domestic animals, in pathological anatomy and pathological histology, in obstetrics and gynaecology, in infectious, parasitic, protozoic and tropical diseases, in animal husbandry and nutrition and in meat-hygiene. There are special courses in ophthalmology, radiography and in the dispensary. To be sure, a good deal of the time is devoted to the post-mortem room and most of all to the clinics.

At the end of this fourth period the students pass their fourth examination. (They are then called 'doctorandus' in veterinary science). But they still have two aims to fulfil before their education is finished.

First, after about another year, they must pass the final veterinary examination. It entitles them to be called 'veearts' or synonymous 'dierenarts' (veterinary surgeon) and gives them the legal right to practise as a veterinary surgeon.

In this last year, there are only a few theoretical lessons to attend (meat and dairy hygiene, forensic veterinary science, veterinary government control, agricultural economics). Most of the time the students work as so called 'co-assistants' in the clinics, the laboratories and the abattoir. They treat diseased animals, perform obstetrical duties, establish bacteriological diagnoses under the supervision of the senior members of the staff.

The theoretical and practical training as a veterinary surgeon being spread over the last three periods of the curriculum, an impression will be given about the time spent in each one of these three divisions in the schemes at page 33.

They cover the whole ground of the teaching fairly well, but it was impossible to bring into these drawings the time spent by the students in the final year in the ambulatory services of the different clinics. At all hours, by day and night, they start, under the guidance of one of the senior assistants, to give veterinary advice in the area served by our clinics. They assist at deliveries, they observe and treat diseased animals under the conditions of common veterinary practice. They partake in the systematic eradication of tuberculosis among some 15,000 cattle. In the area around Utrecht, with a radius of about 10 kilometers the ambulatory services attend to some 9,000 calls a year.

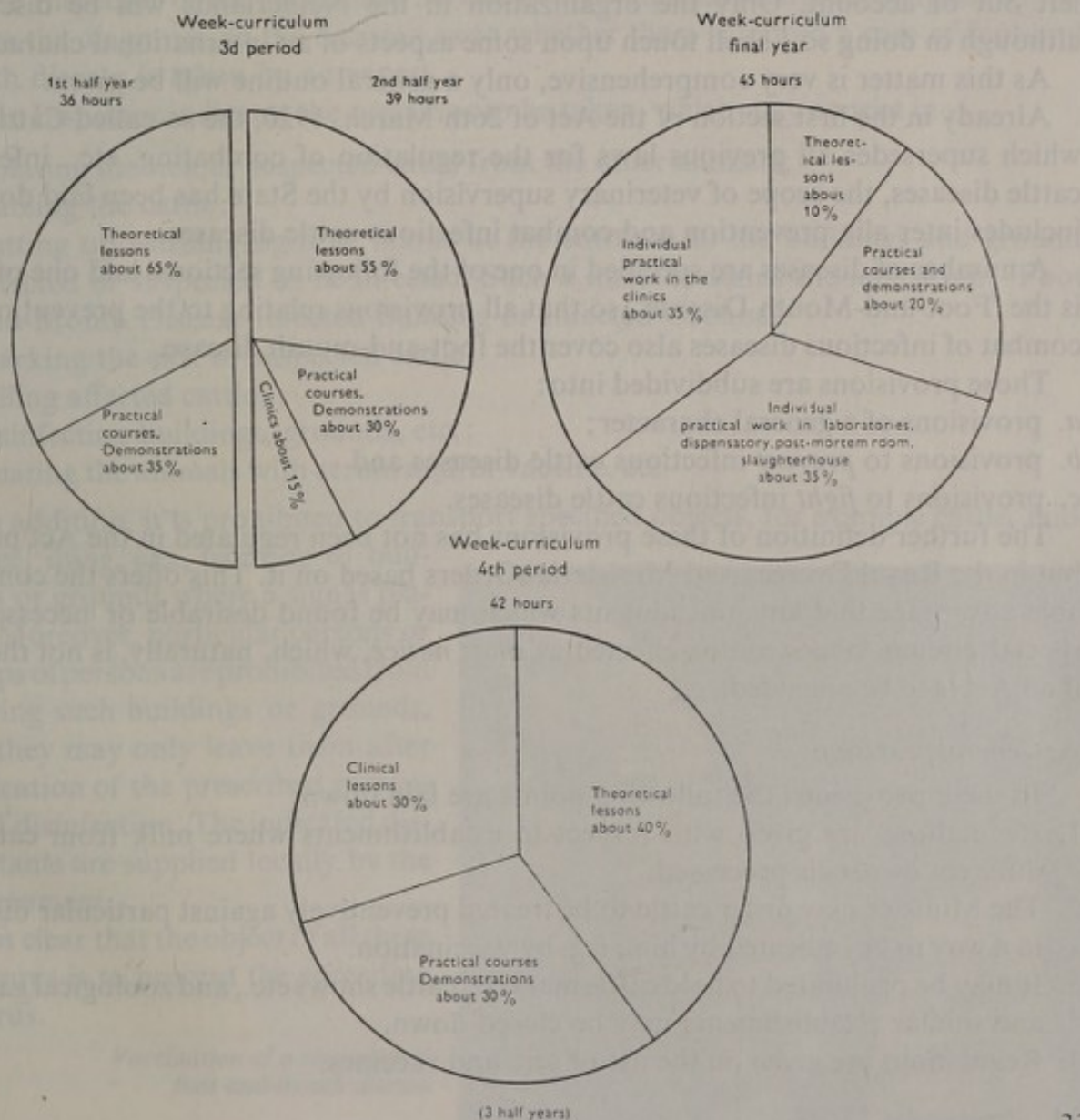
Besides the practical courses in obstetrics with the phantom, the students must have been present at about 50 deliveries of cattle or horses before entering for the final examination. Part of those should be abnormal or difficult deliveries, which they have to perform under the guidance of a senior assistant.

We must finally consider the second aim of a doctorandus in veterinary science. This one is not compulsory. Some of them, after passing the final examination, compose a scientific research on a special subject under the guidance of one or two of the professors and write a thesis. That may take them more than one or two years,

while they are making their own living as a practitioner, as a meat-inspector, or as a scientist in any laboratory of veterinary science. After a solemn colloquium they are promoted to the rank of 'doctor in veterinary science'.

I should like to finish by saying that the members of our Faculty do not presume that at present in our Institutions all is well in the best of all worlds. I regret to say that the accommodation and equipment threaten to hamper in years to come the training of such able and allround veterinary surgeons as may be needed in the intrinsic and effective eradication of diseases among livestock. It will need a joint effort of all Ministries and parties concerned to restore our Faculty to the prosperous development of the beginning of this century.

In the meantime the staff of the Faculty will try to master the substantial difficulties, so that the spirit of research and teaching may conquer and that – as in many times before – it can be said, that the veterinary Faculty at Utrecht – sub pondera crescent – will fulfil its task.



# FOOT-AND-MOUTH DISEASE

E. J. A. A. QUAEDVLIEG

*Director of the Veterinary Service*

## ORGANIZATION AND RESULTS OBTAINED IN THE NETHERLANDS

Numerous problems in regard to the combating of foot-and-mouth disease will be left out of account. Only the organization in the Netherlands will be discussed, although in doing so I shall touch upon some aspects of an international character.

As this matter is very comprehensive, only a general outline will be given.

Already in the first section of the Act of 26th March, 1920, the so-called Cattle Act, which supersedes all previous laws for the regulation of combating, etc., infectious cattle diseases, the scope of veterinary supervision by the State has been laid down. It includes inter alia prevention and combat infectious cattle diseases.

A number of diseases are specified in one of the following sections, and one of them is the 'Foot-and-Mouth Disease', so that all provisions relating to the prevention and combat of infectious diseases also cover the foot-and-mouth disease.

Those provisions are subdivided into:

- a. provisions of a general character;
- b. provisions to *prevent* infectious cattle diseases and
- c. provisions to *fight* infectious cattle diseases.

The further definition of these provisions has not been regulated in the Act proper, but in the Royal Decrees and Ministerial Orders based on it. This offers the considerable advantage that any amendments which may be found desirable or necessary in special circumstances can be effected at *short notice*, which, naturally, is not the case if an Act is to be amended.

### A. *General provisions*

In these provisions the following points are laid down:

1. Regulations are given with respect to establishments where milk from cattle of different owners is processed.
2. The Minister may order cattle to be treated preventively against particular diseases in a way to be indicated by him, e.g. by vaccination.
3. It may be prohibited to hold cattle markets, cattle shows etc., and zoological gardens and similar establishments may be closed down.
4. Regulations are given on the use of sera and vaccines.

### B. Provisions to prevent infectious cattle disease

These provisions open the possibility of prohibiting importation and transit – or of allowing them only subject to particular conditions – of cattle, meat, skins, milk, dung, fodder, etc., and, generally, of objects by which virus may be communicated.

### C. Provisions to combat infectious diseases

They contain inter alia definitions for:

1. sick cattle;
2. suspected cattle.

Every owner, breeder or herdsman of cattle showing symptoms of an infectious disease, in this instance foot-and-mouth disease, is obliged to declare this at once. The same obligation rests with every veterinary surgeon.

The obligation to make this declaration already exists if *symptoms* of an infectious disease are observed.

The Inspector of the Veterinary Service or the veterinary surgeon assigned by him makes the diagnosis, so the decision as to whether there is indeed a case of foot-and-mouth disease is taken by an expert.

The Inspector indicates the measures to be taken, which may consist in:

- a. isolating the sick or suspected cattle from the other animals;
- b. stabling the cattle;
- c. putting up warning signs or marks at the entrance to the buildings and grounds infected or suspected to be infected. Such a mark contains the indication: 'Foot-and-Mouth Disease Infected Building or Infected Ground'.
- d. marking the sick or suspected cattle;
- e. killing affected cattle;
- f. disinfecting buildings, grounds, etc.;
- g. treating the animals with serum and/or vaccine, etc.

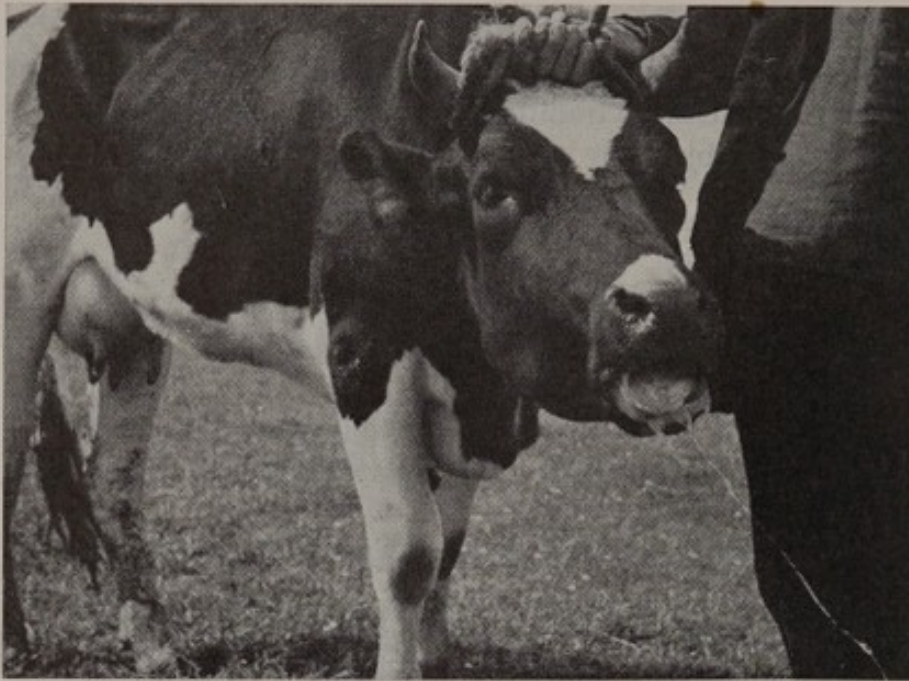
In addition, it is prohibited to transport specified objects, for example cattle, milk, fodder, dung, etc., from or to buildings or grounds where a sign is placed. Moreover, particular persons or groups of persons are prohibited from entering such buildings or grounds, and they may only leave them after application of the prescribed measures of disinfection. The indicated disinfectants are supplied locally by the Government.

It is clear that the object of all these measures is to prevent the spreading of virus.

*Vaccination of a cow against foot-and-mouth disease*







*Holland is making an all-out effort to prevent the occurrence of foot-and-mouth disease among her cattle. The animal shown above is suffering from this dreaded disease.*

The obligatory declaration, the isolation of the infected farm, the transport prohibition, etc., all aim at avoiding this.

Particularly by killing of cattle on infected farms – stamping out – is it intended to destroy the source of the virus. After slaughtering the animals susceptible to the disease and disinfection of the buildings and grounds the greatest danger of communication of the virus from those places has been largely obviated, but to those who know how extremely difficult is an appropriate disinfection of cowsheds and especially pastures it will be obvious that such disinfections should not be overestimated.

Although the above measures of an hygienic character should no doubt be considered to be of value, provided that they are applied rigorously, the results obtained diverge to a considerable extent in the various countries.

In countries like the Netherlands, where most intensive cattle movements occur, where the density of cattle is enormous, where existing usages in cattle-breeding and cattle-trade must be taken into account, where the soil is flat, with many ditches and a very great number of pasture birds – all conditions favourable to the spreading of virus – the system of stamping-out, despite all police measures and the like, i.e. apart from the vaccination now available, has failed repeatedly. In other countries where for instance geographical conditions not only counteract communication, but even form an obstacle to quick spreading, the situation is quite different.

It would mean a misunderstanding and conniving at reality to feel that combating foot-and-mouth disease in our days could still be considered as a national problem. The occurrence of foot-and-mouth disease in a country of Europe constitutes the danger that this disease may spread into any other country.

Since 1939, the *Office International des Epizooties* at Paris, in which 60 countries are represented through their experts, has adopted numerous resolutions on combating foot-and-mouth disease, culminating in the following:

1. recommendation to proceed to stamping-out *wherever circumstances permit it*;
2. application of police measures with the greatest possible rigour;
3. admission to vaccination only of such vaccines as have been prepared and approved in accordance with the directives of the Office International des Epizooties by State Institutions or at any rate under the supervision of the State.

With regard to these recommendations there is general unanimity, except on the application of the stamping-out system. On this point there are clearly two different opinions. Some are in favour of it, others oppose it, at any rate if this question is put in its generality. For example, it has been found repeatedly that Switzerland and England back up this system, whereas other countries adopt a markedly negative attitude in this respect. During the recent epizootic in Europe, which is now strongly on the decline, neither Germany nor Italy or France, Belgium, the Netherlands or Denmark have considered handling the stamping-out weapon.

Apart from the fact that mass-killing would not be justified from an economic point of view – the remedy would no doubt be far worse than the evil – I wonder how this system could be put into practice technically if every day several hundreds of new cases are declared. This is a sheer impossibility!

Formely, several efforts in this respect were made in the Netherlands, but they resulted in as many failures. Nevertheless, we can accept the stamping-out in exceptional cases as an expedient and we do apply it at present.

Of course, the question will arise what exceptional cases are meant here.

Well, as such I would consider a few initial cases encountered in a country, *provided* that a high percentage of the cattle has been subjected to vaccination, and, under the same circumstances, the cases that 'straggle behind' in an epizootic largely brought under control by vaccination.

It is felt here that even if police measures are rigorously applied, the adoption of the



*The symptoms of foot-and-mouth disease are evident*

stamping-out system without vaccination of the live-stock up to a high percentage must lead to disillusion in this country as well as in others where similar conditions prevail.

As already stated, we have repeatedly experienced this in actual practice, so that we think we may establish comparisons on that account. That is why, on the strength of the experience gained, in the Netherlands the stress has been laid on *prevention* of the disease by preventive vaccination.

The very best method to be adopted here would be to come to a general compulsory inoculation of all the cattle. Generally, Netherlands cattle-breeders are rather averse to obligations imposed on them, but in practice they have seen with their own eyes what excellent results were obtained by preventive vaccination, so that it has not taken much trouble to have over 90% of the Netherlands cattle vaccinated on a voluntary basis in the past months.

This result is also due to the organization of vaccination, to which the animal health services as well as the veterinary surgeons have lent their full co-operation.

If it is true that, to a considerable extent, these vaccinations have taken place *entirely* on a voluntary basis, partly the prohibition of the transport of non-vaccinated animals has proved to be an enormous stimulus in this respect.

As a matter of fact, the supply to markets, shows, etc., briefly the transport of cattle, is prohibited unless the animals have been vaccinated against foot-and-mouth disease and, moreover, come from a fully vaccinated farm. Only in special cases can an exemption from this prohibition be granted by, or on behalf of, the Director of the Veterinary Service, subject to conditions to be established by him. Therefore, any non-vaccinated animals are practically 'blocked'.

With reference to some graphs indicating the development of foot-and-mouth disease epizootics in our country since 1930, it will be shown what results have been obtained with this system in the Netherlands. It will be left to the reader to make comparisons between the results obtained with the various systems.

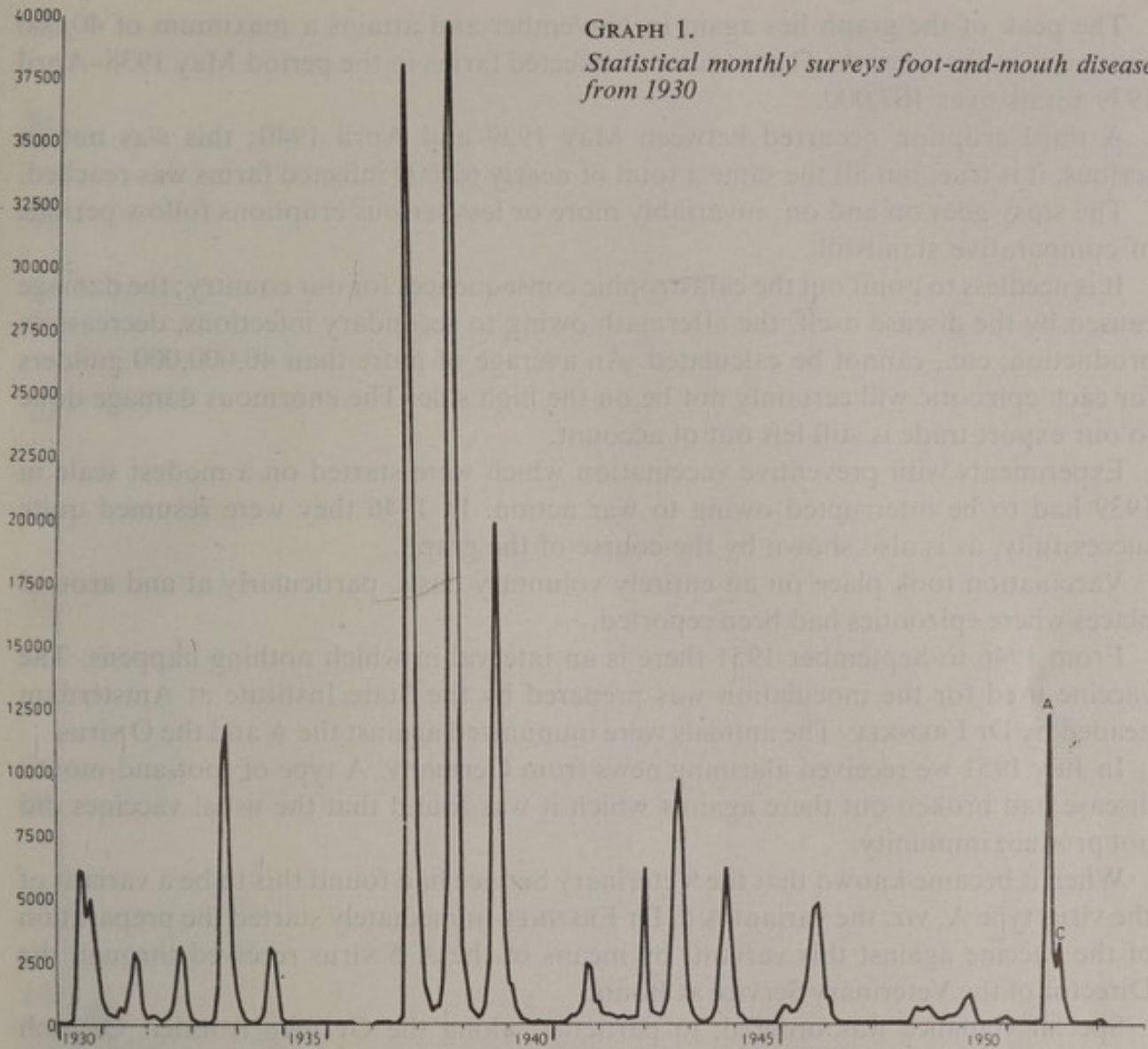
Among other things it appears that the assertion advanced by some people, viz. that also in the event of an ordinary epizootic the graph recedes strongly after the peak has been reached is not untrue in itself, but that it is incorrect to identify this decline with the marked decreases encountered if general vaccination is resorted to. The graphs will prove this error and demonstrate that properly speaking two quantities are compared which are essentially based on one cause but which, for the rest, offer no ground for comparison at all.

This will be illustrated in greater detail later on.

At any rate, it cannot be denied that the damage and losses caused by foot-and-mouth disease among vaccinated cattle constitute only a *fraction* of those resulting from a foot-and-mouth disease eruption among non-vaccinated live-stock.

In my opinion, in view of the circumstances prevailing in this country, in combating foot-and-mouth disease, the Netherlands is right to lay the stress on live-stock vaccination accompanied with rigorous enforcement of hygienic and police measures, such as the prohibition of transport of non-vaccinated animals, and also to accept (and to apply) the stamping-out system only in exceptional cases as referred to.

GRAPH 1.  
*Statistical monthly surveys foot-and-mouth disease  
 from 1930*



Moreover, another drawback, not to be underestimated and hardly to be calculated, of the application of the stamping-out system in its generality or on a large scale will no doubt consist in the irreparable loss of extremely valuable pedigree cattle farms, to the development of which whole generations of families have devoted their knowledge and care.

The graphs indicate the course of the foot-and-mouth disease epizootics that have occurred in the Netherlands from 1930 up to the present.

Graph no.1 shows how in the past our country was repeatedly infested by foot-and-mouth disease epizootics, the most extensive of which were encountered in the period of July 1937–May 1938. During that period, particularly in October and November 1937, there were 37,000–38,000 newly infected farms. The total number of infected farms was then over 102,000.

A few months later, when the number of new cases had declined to 500 monthly, suddenly another rapid extension was observed, which was even more serious than the previous one.

The peak of the graph lies again in November and attains a maximum of 40,000 new cases in that month. The number of infected farms in the period May 1938–April 1939 totals over 107,000.

A third eruption occurred between May 1939 and April 1940; this was not so serious, it is true, but all the same a total of nearly 60,000 infected farms was reached.

The story goes on and on; invariably more or less serious eruptions follow periods of comparative standstill.

It is needless to point out the catastrophic consequences for our country; the damage caused by the disease itself, the aftermath owing to secondary infections, decrease in production, etc., cannot be calculated. An average of more than 40,000,000 guilders for each epizootic will certainly not be on the high side. The enormous damage done to our export trade is still left out of account.

Experiments with preventive vaccination which were started on a modest scale in 1939 had to be interrupted owing to war action. In 1946 they were resumed quite successfully, as is also shown by the course of the graph.

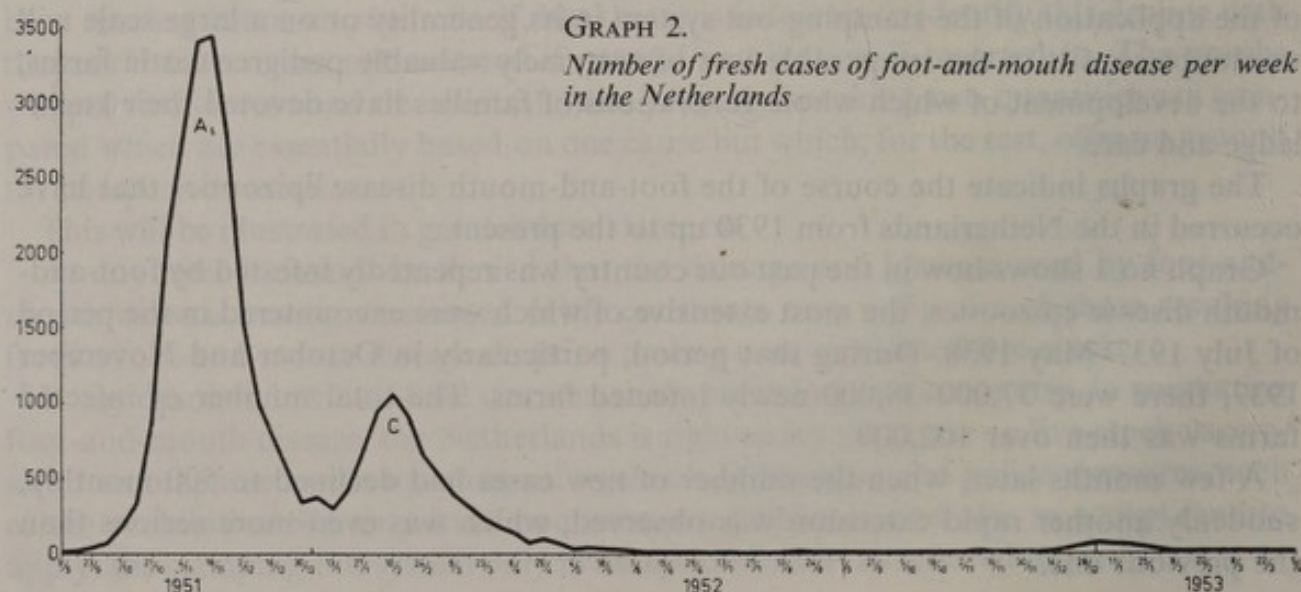
Vaccination took place on an entirely voluntary basis, particularly at and around places where epizootics had been reported.

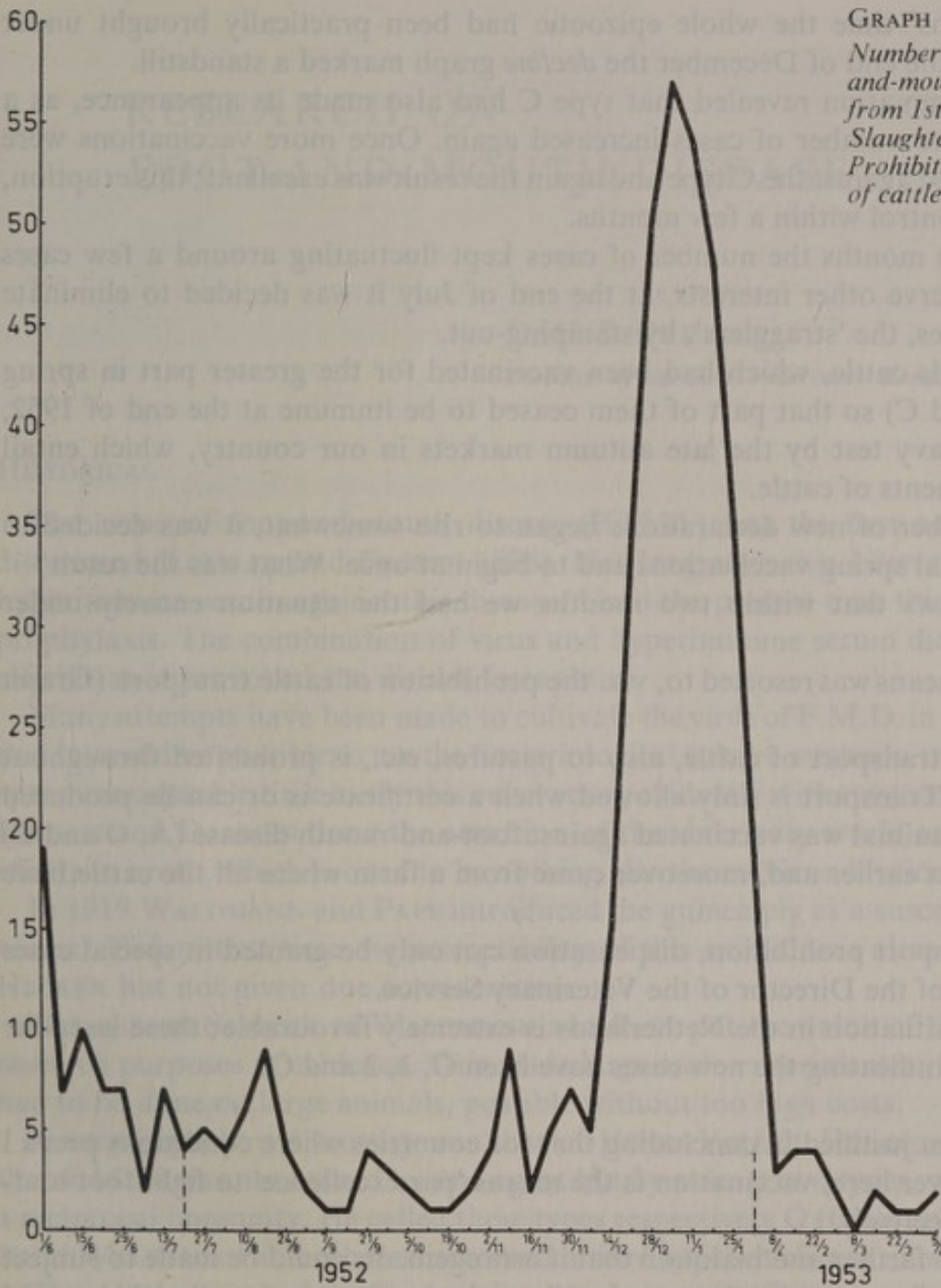
From 1946 to September 1951 there is an interval in which nothing happens. The vaccine used for the inoculation was prepared by the State Institute at Amsterdam headed by Dr FRENKEL. The animals were immunized against the A and the O virus.

In July 1951 we received alarming news from Germany. A type of foot-and-mouth disease had broken out there against which it was found that the usual vaccines did not produce immunity.

When it became known that the Veterinary Service had found this to be a variant of the virus type A, viz. the variant A 5, Dr FRENKEL immediately started the preparation of the vaccine against this variant, by means of the A 5 virus received through the Director of the Veterinary Service at Bonn.

Special vigilance was ordered, in particular along the German frontier. Of each case of foot-and-mouth disease encountered the type was established and in the middle of September the variant A 5 was also found in the Netherlands.





**GRAPH 3.**  
*Number of fresh cases of foot-and-mouth disease per week from 1st June 1952.*  
*Slaughtering July 15th, 1952.*  
*Prohibition of the movement of cattle. Febr. 1st, 1953.*

Elimination of this former type by stamping-out might have been considered, but how could this have been realized from a technical point of view alone, when taking into account that already in the first week the number of newly infected farms was 50, in the second week 150, in the third 350 and in the fourth already over 1,000, etc.! Vaccination has been resorted to. The imminence of the danger caused an increase in the demand for vaccinations to such an extent that it was almost impossible to prepare sufficient quantities of vaccine to keep pace with those vaccinations.

Graph no.2 shows the result of these large-scale vaccinations. After six weeks the peak had been attained, the extension had been checked and a rapid decrease set in.

In three months' time the whole epizootic had been practically brought under control, when at the end of December the *decline* graph marked a standstill.

A detailed examination revealed that type C had also made its appearance, as a result of which the number of cases increased again. Once more vaccinations were made, now directed against the C type and again the result was excellent; this eruption, too, was under control within a few months.

When for some months the number of cases kept fluctuating around a few cases a week, also to serve other interests, at the end of July it was decided to eliminate those isolated cases, the 'stragglers', by stamping-out.

The Netherlands cattle, which had been vaccinated for the greater part in spring (against A, O and C) so that part of them ceased to be immune at the end of 1952, were put to a heavy test by the late autumn markets in our country, which entail enormous movements of cattle.

When the number of new declarations began to rise somewhat, it was decided to anticipate the usual spring vaccinations and to begin at once. What was the result?

The graph shows that within two months we had the situation entirely under control!

Then another means was resorted to, viz. the prohibition of cattle transport. (Graph no.3).

At present the transport of cattle, also to pastures, etc., is prohibited throughout the Netherlands. Transport is only allowed when a certificate is or can be produced showing that the animal was vaccinated against foot-and-mouth disease (A, O and C) at least a fortnight earlier and, moreover came from a farm where all the cattle have been vaccinated.

From this transport prohibition, dispensation can only be granted in special cases by, or on behalf, of the Director of the Veterinary Service.

At present the situation in the Netherlands is extremely favourable; these last four weeks the figures indicating the new cases have been O, 1, 2 and O.

I think that I am justified in concluding that for countries where conditions prevail similar to those over here, vaccination is the means 'par excellence' to fight foot-and-mouth disease effectively.

I would even go farther and maintain that if arrangements could be made to subject the whole *live-stock* to vaccination, *nationally* and even internationally, this would provide a means of wiping out this dreaded disease, which would mean an enormous increase in food production.

# RESEARCH ON FOOT-AND-MOUTH DISEASE

Dr H. S. FRENKEL

*Director of the Gov. Veterinary Research Institute*

## HISTORICAL

The virus of foot-and-mouth disease (F.M.D.) was the first animal virus to be discovered (LÖFFLER and FROSCH 1898). Not long after this LÖFFLER introduced the hyperimmune serum against this disease. Until the present time this serum is used in prophylaxis. The combination of virus and hyperimmune serum did not prove to be reliable as it provoked the disease (seraphtin).

Many attempts have been made to cultivate the virus of F.M.D. in vitro. In the belief that it would be possible to use the bacteriological culture methods, many investigators spent considerable time to try this cultivation, evidently without success. Even in 1924 FROSCH and DAHMEN drew the attention of the veterinary world to their cultivation efforts in broth, which proved to be nothing else than a virus dilution phenomenon.

In 1919 WALDMANN and PAPE introduced the guinea pig as a susceptible laboratory animal. Before that time the susceptibility of this animal was already recognized by HECKER but not given due consideration.

The systematic work of WALDMANN and PAPE put the value of the guinea pig for research purposes in evidence. This made large scale experimentation, which thus far had to be done on large animals, possible without too high costs.

A very important discovery was that of HENRI VALLEE. He introduced the notion that F.M.D. was not caused by one agent but by at least two types, which did not give a reciprocal immunity. He called these types respectively O (Oise) and A (Allemagne). Later WALDMANN and others discovered a third type which was named type C.

The development of the tissue culture technique by CARREL and his school, which provided us with a tool for virus cultivation in vitro, made it possible for many investigators to try to cultivate viruses. The general notion that viruses can only be propagated in living susceptible cells put a short end to the attempts to cultivate viruses in the various bacteriological media.

The first to grow the virus of F.M.D. were MAITLAND and MAITLAND and almost simultaneously, HECKE. They succeeded in obtaining multiplication of this virus in embryonic tissue of the guinea pig. However, the antigenic power of this culture virus was not satisfactory. WALDMANN explained this by stating that it was not possible to use this virus in vaccination unless the animal had passed through the disease. In this case it could enhance immunity.



The first F.M. virus that could provoke F.M.D. in cattle was cultivated by FRENKEL and VAN WAVEREN. By following arbitrarily the tissue culture methods of CARREL and the MAITLANDS they were successful in this cultivation in explanted skin tissue of bovine, ovine and swine embryos.

Meanwhile, SCHMIDT found that aluminium hydroxyde in colloidal form offered a very useful substrate for the absorption of the F.M. virus.

He developed the basis for the F.M. vaccine now almost universally in use. Before this very important discovery, VALLEE had proved that the action of formaldehyde on the F.M. virus could destroy its pathogenic power, without damaging very much its antigenicity. WALDMANN very luckily combined these two important facts and issued an aluminium hydroxyde formalised virus as a vaccine. This proved to be very efficient. These vaccines were prepared with so-called 'native' virus.

A native virus is prepared in the living susceptible animal. In the case of F.M.D. this virus is grown by injecting susceptible cows intradermo-lingually in numerous spots. After 24 hours, when many confluent vesicles have developed, the animal is slaughtered and the virus gathered from the tongue.

The cultivation of F.M.D. virus in vitro was continued. The number of embryos not being sufficient, another source of susceptible tissue had to be found. FRENKEL found that epithelial tissue of the normal bovine tongue was very satisfactory. First, a method for laboratory scale production was worked out, later a large scale virus production technique was developed.

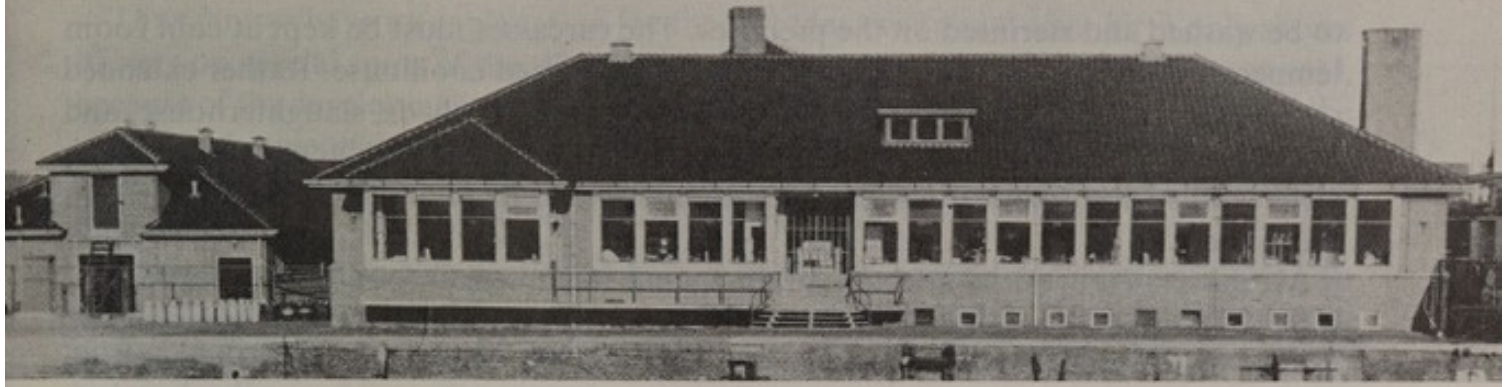
The application of HENDERSON'S technique for the titration of F.M. virus in bovines has proved to be of very high value. Together with the quantitative complement fixation test, it forms an indispensable step in the preparation of standardized vaccines.

#### RESEARCH IN HOLLAND

Research in Holland started in 1904 as a part of the task of the State Serum Institute in Rotterdam. Most of this work has been done with the aim of preparing a potent hyperimmune serum. Indeed, this serum gave much satisfaction when applied as a prophylactic in adult but more especially in very young animals.

Most of the research work had a bearing on disinfectants. Furthermore, many of the findings in other countries could be repeated and confirmed. After CIUCA, LOURENS was the first to obtain positive results with the complement fixation test as a diagnostic method. He also pointed out that the meat of carcasses of animals which had been killed during generalised F.M.D. sterilized itself by the formation of lactic acid within 24 to 48 hours, thus confirming the results of English investigators.

In 1932, the State Veterinary Research Institute, which was mainly established for research on F.M.D., commenced its activities. A number of investigations have been made from which we mention research on: the possibility to provoke F.M.D. in the brains of guinea pigs, rabbits, white mice, calves and hogs; intratesticular inoculations with F.M. virus in rabbits and guinea pigs; propagation of F.M. virus in the embryonated hen's egg; F.M. virus in artificially provoked vesicles in swine; the cultivation of



*The Government Veterinary Research Institute*

F.M. virus in non-pathogenic microorganisms; the precipitation of F.M. virus with alcohol and ether, etc.

The most important work done in the Institute, however, is the cultivation in vitro of the F.M. virus.

Before giving some details on this subject, it seems desirable to describe the method of obtaining virus for vaccine preparation as it is still being used routinely in most other countries.

In order to get as much virus as possible for the preparation of vaccine, susceptible bovines are injected intradermo-lingually with virus on numerous spots. These animals have to be fully susceptible in order to produce an abundant quantity of virus.

Animals that were recently diseased or vaccinated may offer quite a considerable resistance to artificial infection. In periods of epizootics this might be a great impediment to virus formation. Not infrequently, in countries where F.M.D. has been widespread, is this difficulty very evident. Sometimes, somewhere between 40 and 60 % of the animals do not react to this very heavy infection or just show a very poor vesicle formation.

Twenty-four hours after the multiple tongue inoculation, the animals show an abundant vesicle formation. After slaughtering, the tongues are taken to the laboratory and the virus is gathered. The virus material consists of the blisterwalls, the lymph and the scraped off tissue beneath the blisters. This material is the crude virus from which the pure virus suspension is obtained by centrifugation in a serum separator and filtration through asbestos pads.

The preparation of a tri-valent vaccine or three monovalent vaccines demands about 1,000 animals per week. This is in a country with a cattle population of about 2.5 million, where these animals have to be vaccinated once a year. It is unnecessary to say that this very large number of animals requires large shed accommodation for the three different types. These sheds have to be strictly isolated, not only from the surroundings, but also from each other. For the slaughtering of the cattle an equally isolated slaughterhouse is necessary. It must be large enough for the slaughtering of a thousand animals a week on a total vaccination programme of 2.5 million cattle. Consequently this has to be quite a large building. The staff must be dressed in clothes

to be washed and sterilised on the premises. The carcasses must be kept at cool room temperature for 24–48 hours. This requires a large sized coolhouse. Rather extended sanitary installations are required for the staff working in sheds, slaughterhouse, and coolhouse. With all these carefully applied regulations, still the danger of spreading virus with such a tremendous virus development, and its high infective power, is not imaginary.

Moreover, the artificial infection of the tongue in numerous spots is a very cruel one. After 24 hours most of the animals suffer enormously. Very often the tongue is hanging out of the mouth and is very swollen. They cannot eat and hardly drink and, in consequence, lose in condition. Besides these symptoms, the tongue gets infected with numerous kinds of microorganisms which mix with the lymph and the tissue. In countries where tuberculosis is present in a high percentage among the cattle population, the chance of mixing tubercle bacilli with the crude virus material is not imaginary. The consequence of this fact may be that the likelihood cannot always be excluded that viable tubercle bacilli may enter into the vaccine, because we cannot expect that the action of formaldehyde in such a diluted form as is used in the vaccine preparation, will kill these microorganisms. The use of asbestos filter mats does not always guarantee the bacteriological sterility of the virus. All this depends on the quality of these filter mats.

As the work started in the State Veterinary Research Institute the main purpose was the cultivation of F.M. virus, with the aim of finding a method for practical virus production *in vitro*. On the occasion of the 9th International Veterinary Congress, 1909, held in Scheveningen (Holland), VALLEE in his report on F.M.D. made the statement that a rational campaign against F.M.D. was widely dependent on the possibility of cultivating the virus of F.M.D. on a large scale. This prophetic statement was the starting point, as we agreed fully with VALLEE'S opinion.

At first we tried to cultivate the causal agent in fibroblast cultures of chicken embryos, evidently without positive results. Later on we made efforts to grow the virus in embryonated hen's eggs. We could not obtain better results than a survival of the virus for some days. Meanwhile MAITLAND and MAITLAND, and HECKE, cultivated guinea pig adapted virus in embryonic guinea pig tissue. This had no practical value.

The only possibility which was considered successful was the propagation in tissue of a naturally susceptible animal. This was the reason why we started our work with bovine embryos and especially with the skin tissue.

Foeti of bovines were taken under aseptic precaution from the normal uterus after slaughter of the animal. The skin was removed and minced, then mixed with nutrient medium which was mostly a combination of calf plasma and Tyrode solution. This was then grown at 37 °C in the incubator. We found that 2–3 days' incubation was the best time for virus formation. Our tissue cultures showed growth, first, of fibroblasts, then of epithelial cells.

It is interesting to know that the whole skin could be used for this purpose and that it was not necessary to grow the F.M. virus in what is known as the predilection spots. On the contrary, we even rarely used them in our cultures.

Thus far, we have no explanation for the fact that the animal after birth only shows blisters on special spots of the skin. It seems that the skin after the birth of the animal, because of the development of hair, has another chemical constitution which makes the virus development almost impossible. In how far this has a bearing on hair formation or perhaps on a special type of keratin formation is a problem to be tackled by biochemists.

It might be that in this particular behavior of F.M. virus an indication is given to the way in which the problem of virus action has to be tackled. It might then give us more information as to which parts of the virus molecule are responsible for the cell lesions leading to blister formation.

This part of the F.M. virology, which is of eminent importance, is still more or less completely unknown, and is one of the subjects on the programme of research of our institute.

We succeeded in growing continuous passages of virus from 1934 to 1940, when the second world war put an end to regular research work.

Meanwhile it was found that not only very fresh foeti yielded a good virus culture but also foeti in utero, kept for eight days in the refrigerator after having been treated as mentioned above, gave satisfactory cultures of tissues and virus.

This important fact did not restrict us to the local slaughterhouse for the obtaining of foeti, but opened the possibility of using this material from other slaughterhouses. We will see later on what the significance was of this fact for the obtaining of viable tissue for virus cultivation, even from abroad.

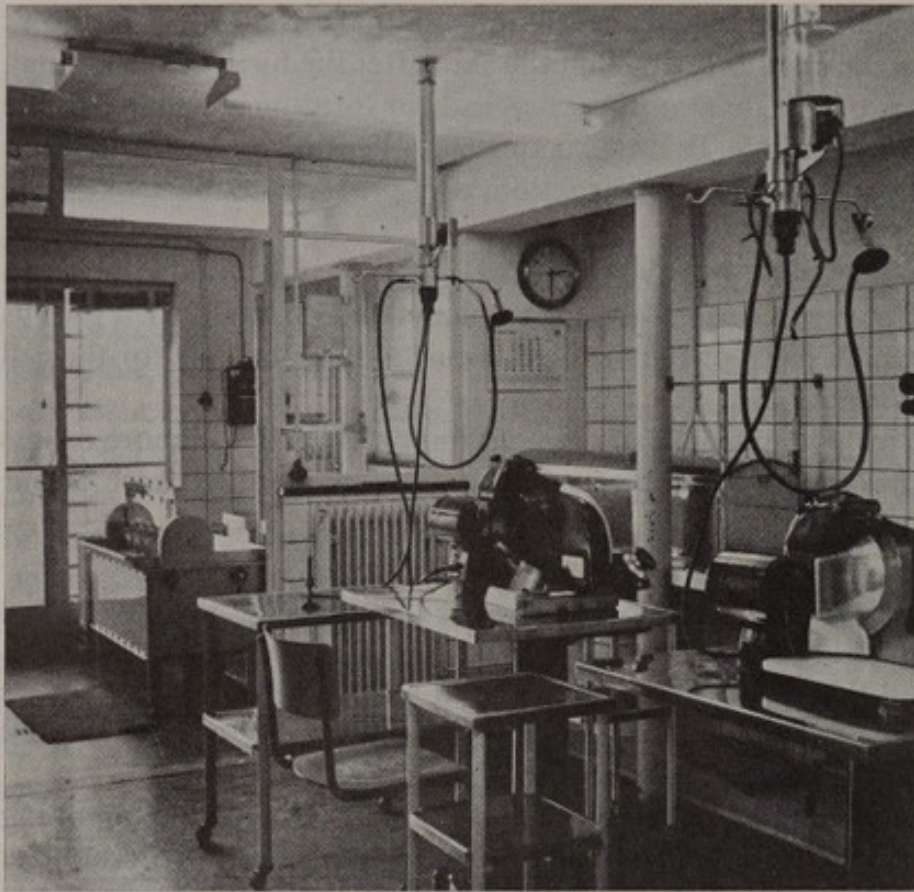
However, given the fact that bovine foeti are relatively rarely found in the slaughterhouses of our country, our attention fell on foeti of ovines and also of swine. With the skin tissue of these species virus could also be cultivated.

As the litters of swine usually consist of quite an important number of embryos we played with the idea of having a large swine raising plant in which we could produce numerous embryos for our purpose. These could be collected at a convenient time and of the size we wished.

However we knew from our histological studies of embryonic skin, that the quantity of epithelial cells is not very large, especially if no hair formation is present. As the quantity of virus production is closely related to the number of epithelial cells available, it was believed that another source of epithelial cells had to be found, which could provide us more regularly and abundantly with susceptible epithelium.

Two sources were investigated, viz., the matrix of the horns and that of the claws. A technique was worked out to uncover the horn and claw matrix in an aseptic way. With this epithelial tissue brought into culture it was possible to grow F.M. virus but its titre was lower than that of the embryonic skin.

It seemed more promising to use the normal bovine tongue. Already in 1938 we attempted to use this organ for our purpose. The technical difficulties however were so great that we were unsuccessful. It seemed to be impossible to reach the deeper layers of the mucous membrane without bacterial infection.



*Laboratories for the disinfection of cattle tongues and cutting off of epithelium intended for culture "in vitro" of foot-and-mouth disease virus*

Fortunately, this difficulty was eased by the introduction of the invaluable antibiotics, which permitted less aseptic precautions. The problem of using normal bovine tongues was again tackled.

This time the tongue was fixed at the tip in a clamp after having been thoroughly washed with luke warm water and disinfected with 70% ethyl alcohol. The skin of the tip was loosened from the underlayer. After having been incised on both sides of the tongue, the loosened part of the mucous membrane was fixed in a special metal clamp and rolled down to the base of the tongue. The remaining part of the epithelium on the tongue, which is essentially sterile, could be cut off by sterile safety razor blades and collected. The inner layer of the removed part could be kept sterile by using a sterile cellophane paperstrip when it was rolled down with the clamp. This inner layer was also collected and used in the culture. The tissue material thus collected in a small amount of sterile Tyrode solution was clipped by hand with scissors and brought together with virus and 125–250 ml of nutrient medium in a flask. The total amount of tissue from one tongue being about 8–10 grams, about 5000 units of penicillin and 5 cc of streptomycin solution were added. The mixture was aerated by carbogen, a gas mixture consisting of 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

After 24 hours' incubation at 37 °C, the virus was collected by pouring off the nutrient medium and grinding the epithelium particles with sterile quartz sand in a mortar. Virus was shown to have grown in this surviving tissue by titration on tongues of Irish cattle that were imported for this special purpose. (Irish cattle are very sus-

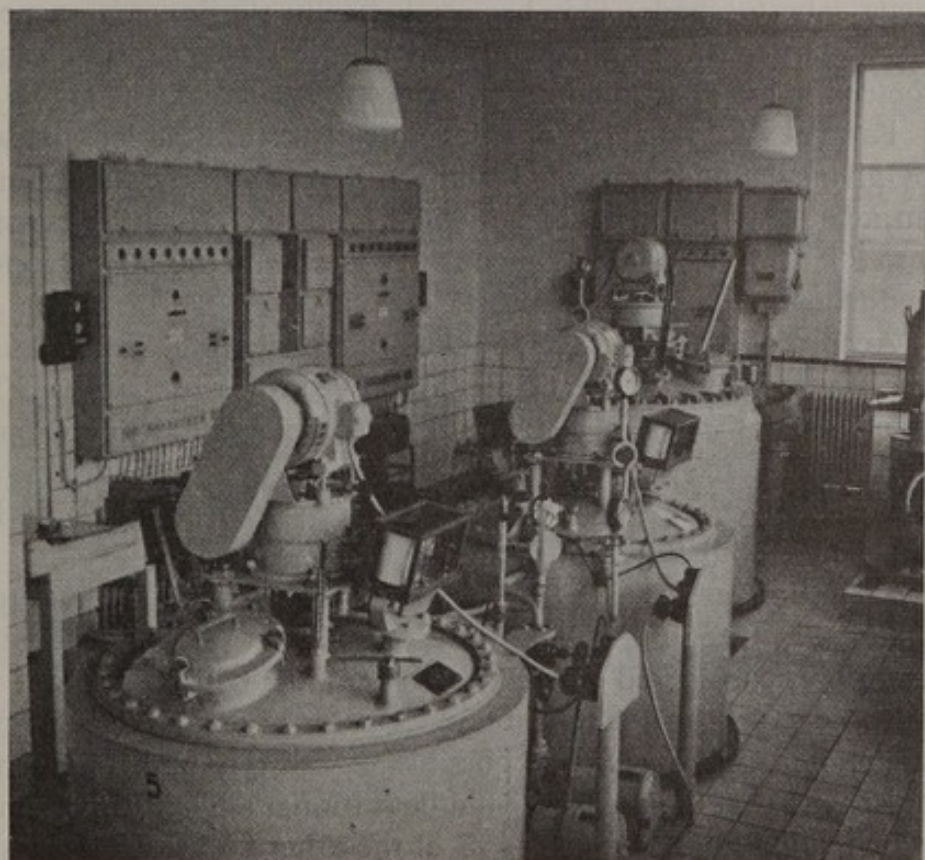
ceptible to F.M.D. because of the fact that this country has been free from this disease for a great number of years).

In the beginning, we used as a liquid nutrient medium, Baker's fluid. However, as this fluid contains homologous serum, not always of the same composition, we used amino acids instead of serum in order to have practically a standardized nutrient medium.

The titre of this 'artificial' virus varied from 300,000 to 1,000,000. Vaccine prepared according to the SCHMIDT-WALDMANN method proved to be satisfactory. However, the work involved from the collection of the epithelial tissue to the composing of the culture was very great and would render its use for practical purposes almost impossible.

In the solution of this problem, the antibiotics were again of great advantage. Though we used them for a considerable time, we still believed that the utmost asepsis had to be maintained in the handling of the tissue. We noted however that we could be more liberal, as we observed that a piece of epithelial tissue which had fallen on the floor could still be used in a culture without doing harm to the bacteriological sterility. This does not mean that we can abolish the asepsis.

We introduced a machine which is normally used for slicing fish. This was very useful. After it was found that cutting of the mucous membrane of the tongue presented a difficulty, we fixed this organ on a cylinder, stretching it so that enough resistance was offered to the knife. The tongue was tightly fixed around this cylinder, washed with luke warm water, and disinfected with 70 % alcohol. This was all done by hand.



*Autoclaves for the preparation of absorbate vaccine against foot-and-mouth disease*

The mucous membrane was then cut off with the slicing machine and the tissue sheets collected in nutrient medium to which penicillin and streptomycin, or chloromycetin alone, were added.

Now the epithelial sheets had to be minced. For this purpose, a clipping machine has been constructed. With this it was possible to mince the epithelium of four tongues at a time. This machine is electrically driven and has three scissors, consequently, with one apparatus the tissue of 12 tongues can be minced very uniformly in 12 minutes.

Instead of using glassware for the cultivation, we took stainless steel containers, first of one litre size. Later we used tanks with a 60-litre volume in which the tissue of 40-60 tongues could be grown. As a matter of fact this large quantity of tissue must be stirred in order to make respiration possible. For this purpose two Chamberland (3L5) candles were mounted on a T-shaped stainless steel tube, through which carbogen was blown. A motor on the lid of the tank rotated the tube. This in turn caused the candles to revolve and stir the tissue suspension. At the same time the gas mixture was sterilized by the Chamberland candles. The pressure of the gas was kept at 1.5 atmospheres.

The tanks were kept in an incubator room at 37 °C for 20 hours. Then the virus had to be collected. This was done by decanting the liquid nutrient medium through a gauze sieve, squeezing the tissue through rollers of porphyry, and re-suspending the pressed out virus in the original nutrient medium. This suspension was centrifuged in an Alpha Laval Separator. The crude virus thus obtained was filtered through E.K.S. Seitz asbestos pads. This bacteriologically sterile virus suspension was immediately titrated by means of the quantitative complement-fixation test, using an electrophotometer for the exact reading of the haemolysis.

Parallel with this type of titration we also use the HENDERSON method of titration, by giving 10 injections of a dilution  $10^{-5}$ , and an equal number of a dilution  $10^{-6}$ , intradermally in the tongue of two Irish cattle. Together consequently 20 inj. of  $10^{-5}$  and 20 of  $10^{-6}$ . With these data we determine the 50% endpoint according to Reed and Munch.

According to the titre of the thus-obtained virus, a calculation is made of the number of litres of vaccine to be prepared. We always prepare a 2.5% vaccine on a basis of a virus which titres 1:2,000,000. If for example, our virus has only a titre of 1:1,000,000 and we have 150 litres of virus, we consider these 150 litres as being only 75 litres. To make a 2.5% vaccine, we can produce 75,000:25, equals 3,000 litres. For this quantity we use the epithelium of about 500 tongues.

As one 60 litre culture tank can hold only about 40 tongue epithelia, we need for the cultivation of 500 epithelia more than 12 tanks. This is again troublesome. It was, therefore, considered worth while to try the cultivation in a very big tank. For this purpose we chose a 600 litre tank thus far in use for vaccine preparation. With some slight modifications it was possible to use this tank. A stirring device was already present so that the candles could be stationary. The cultivation in this very large tank, which could hold as much as 12 of the other tanks, was very successful. We observed

that generally the titre of the virus from this tank was remarkably higher than that of the virus from the 60-litre tanks.

Instead of Chamberland candles, we now use a stainless steel tube in which very fine holes are drilled. Previously the gas mixture for the respiration had to be sterilized through a large sterile cotton filter. We believe that this has a still more favorable influence on the virus formation because of the fact that there is less foam formation than when using Chamberland candles. In the last case, we always see a rather thick layer of foam lying on the surface of the nutrient medium. This might be a hindrance to the respiration of the tissue.

With the perforated steel tubing, hardly any foam is produced and the whole room above the liquid surface can be considered as an oxygen carbon-dioxide bubble.

We recently constructed a machine for disinfection of the tongue surface which disinfects the tongues much better than by hand. The principle is that a set of feathering brushes, in which 70% alcohol is dripping, moves in tight contact with the tongue surface to and fro. The time and the flow of alcohol is automatically regulated.

Fout-and-mouth disease virus can also be cultivated in surviving epithelium of swine tongues. These tongues, however, being relatively small and the mucous membrane much thinner than that of the bovine tongue, are not so suitable for the purpose of a practical virus production.

Calf tongues can be used, but are comparable in their usefulness with swine tongues.

Sheep tongues are of practically no use.

From April 1, 1952 till April 1, 1953, a total of 160,000 litres of vaccine have been produced exclusively with in-vitro cultivated virus. Much research is going on concerning the improvement of the virus production in the artificial culture and of the potency of the vaccine. Also investigations are carried out on the possibility of reducing the dosage of the vaccine without diminishing the results of the immunization.

The reduction of the dosage would not only be of great importance for the practitioners but also would lower significantly the costs for storage and transportation in the country itself as well as to other countries which would like to order vaccines from the producing countries.

As these international transportations are usually made by air, the importance for an international campaign against F.M.D. is evident. We are convinced that the production of the F.M.D. virus in vitro, as long as no better means of this production is known, should be taken up by every country that wishes to prepare its own vaccine. It is less dangerous and much more economic than the original method of virus production. All countries concerned should carry out investigations on the possibility of reducing the dosage materially.



# HOW CAN WE ERADICATE FOOT-AND-MOUTH DISEASE IN PRACTICE?

J. M. DIJKSTRA

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We have means at our disposal for this purpose which can be divided into two groups. The first group refers to the means which decrease the virus or hinder it from coming into contact with susceptible animals, while the second group combines those means which increase the resistance-power of the cattle.

Various combinations can be applied as seems to be required.

## 1. MEANS WHICH CONCERN THE VIRUS

The most thorough of these is the slaughter and destruction of all sick animals and those suspected of being infected, supplemented by extensive disinfection of the ground. By these methods the increase and possible variation of the virus is prevented, while the virus that has already been produced is destroyed. If this is consistently and thoroughly done it is a very good method but it is attended with great difficulties. This system depends very much on the co-operation of the cattle owners whose cattle are infected by the disease. A very quick notification is the essential condition for success.

Now follows the great difficulty: the cattle owners are willing to co-operate as long as their own cattle are not suffering from the disease, but as soon as their animals fall victims they are convinced that the disease cannot be held back by these means. Interest diminishes greatly in this case. Thus the system requires a high development and morale among cattle owners.

There are, however, further difficulties. The disinfection can never be executed so thoroughly that all virus already produced is destroyed. One does not always know all the places where virus is to be found and so one must consider the fact that the virus remaining behind may be the cause of fresh infections. Probably these infections have already taken place by the time of the slaughter.

The 'stamping out' must therefore be reinforced by other measures to try to prevent new infections by any remaining virus and, if these infections have already taken place, to keep the results of them in control. Otherwise we may get new centres of virus production.

In England, where they have had great experience with 'stamping out', the attempt is made to circumvent these difficulties by stopping the transport of cattle in large

circles round the infected herds. At the same time, the movement of people within a narrower circle is more or less restricted and made subject to disinfection. The slaughtered animals are not used as food, but are destroyed.

With this system the animals in the surrounding districts remain untreated and so they keep their susceptibility in full measure. In England this is considered as an advantage because, owing to that fact, a mild form of the virus still gives clearly perceptible symptoms of the disease.

An important factor with this system is the distance between the infected and the uninfected countries, because the smaller the distance, the easier an infection from abroad may be brought about. It is therefore of great interest to the country that applies the 'stamping out' that the surrounding countries remain free from the disease.

No one needs be astonished that the United States of America has given technical and financial help to Mexico to suppress foot-and-mouth disease. A continuous focus of foot-and-mouth disease in Mexico would doubtlessly have been the cause of outbreaks of infection in the United States. The fact that the initial method of 'stamping out' failed in Mexico and has been replaced by another method (in which the main feature is vaccination) is of no significance.

Seen from this point of view, it is of great importance for England and Ireland that the continent of Western Europe be kept free from foot-and-mouth disease. Thus, the application of the 'stamping out' method becomes much simpler and much less expensive for these two countries.

The advantage of great distance to the infected countries is also enjoyed by Norway, Sweden, and Finland, all of which often apply the 'stamping out' system. This is demonstrated very clearly by the fact that Sweden, for instance, has temporarily suspended the use of this system in the extreme southwest part of the country, a section which is separated from Denmark only by the narrow Sound.

Switzerland, which is quite surrounded by countries frequently infected, is apparently in a very disadvantageous position for the application of the 'stamping out' system. However, the difficulty of access offers fewer contact possibilities than does a more flat one. They have not always succeeded in keeping the country free from infection. The narrow Canton of Geneva, for instance, almost completely surrounded by French territory, has sometimes become infected.

It is of the utmost importance, specially for Switzerland because of its central position in Western Europe, that the disease be vigorously eradicated in the surrounding countries. It is therefore no accident that a spokesman from this particular country pleaded for the application of the 'stamping out' system among the surrounding countries.

The Swiss version of the 'stamping out' system deviates somewhat from the English one. In Switzerland it is customary to kill the animals and to use the meat for consumption on the grounds that the virus in the meat has been destroyed after rigor mortis. The animals are taken to the slaughterhouses (in vans especially delegated for that purpose) where they are killed. Thus the slaughter does not take place on the farm itself. In Sweden this method is also known.

In Switzerland they try to prevent further extension by raising the resistance power of the animals which are kept within a fixed distance from the focus. At first this was done by means of serum injections, in late years by vaccinations. Whereas England, after killing and disinfecting, puts the stress on stopping the transport of all cattle, Switzerland considers at the same time the increasing of resistance.

The success of the 'stamping out' system depends also on the virulence of the virus. A very virulent virus is far more difficult to destroy than a mild one. At the same time, success is strongly influenced by the susceptibility of the whole herd. This, for instance, is dependent on the density of the cattle population (the number of animals per 1000 hectares), or on the situation of the farms with regard to each other or to the trade. Essentially, these are more or less the possibilities of contact. Many possibilities of contact make a herd vulnerable and endanger the application of the 'stamping out' system.

In the years from 1912-1918 the 'stamping out' system was applied in Holland. In the years up to 1917 it was very successful in regard to mild virus but with the malignant virus of 1918 it failed completely. Continuation of the system in the Netherlands would be equivalent to committing economic suicide.

The other means, which can be applied to lessen the virus or prevent its coming in contact with susceptible animals, are much less effective than the 'stamping out' system. When the 'stamping out' system leads to failure, one cannot expect that less drastic measures will affect the course of a virulent virus. With a mild virus it is perhaps possible to obtain some result, however, it always remains doubtful.

The duty of notification holds good as a simple means when combined with the isolation of the cattle at the farm during the period in which chances of infection are still expected. Most countries take 3 or 4 weeks for this, others take a longer time. For instance Switzerland formerly took 8 months in connection with the danger of carriers.

A little more effect can be expected from a command to stable the animals, but even if all these measures are backed up by extensive disinfection of both sheds and animals, by permanent supervision, by prohibition of marketing and prohibition of meetings, etc., it always remains very doubtful if much can be done with this in practice.

The successes that have sometimes been attained with this moderate system in the case of a mild, very slow, petering-out local epizootic have often been called 'the flogging of a dead horse'.

## 2. MEANS WHICH RELATE TO THE RESISTANCE POWER OF THE WHOLE STOCK OF CATTLE

The thesis that the resistance power of the cattlestock is the total sum of the resistance power of the separate components can be considered over and done with. There are factors other than the individual resistance that play a very important part. Probably one of the most important is the density of the cattle, because the contact possibilities are greatly effected by this.

Practice has taught us that foot-and-mouth disease spreads itself much more quickly in regions devoted solely to cattle-raising than in regions where cattle-breeding

is combined with agriculture. The fact is that, to the degree that agriculture increases, the rapidity of foot-and-mouth disease epizootic decreases.

In former centuries the cattle plague compelled cattle owners to change over to agriculture. With regard to foot-and-mouth disease this has not been considered up to now and it is not feasible for a country to apply this means of enhancing the resistance power of the cattle stock against foot-and-mouth disease.

An alternation of meadows with woods or with wasteland is probably of much more significance than alternation with cultivated land.

Another important factor which is difficult to change is the average size of the herds. The greater the herd the sooner an infection shows itself.

The ennobling of the cattle stock, which is closely connected with efforts to increase production, undoubtedly promotes the susceptibility to diseases. With regard to foot-and-mouth disease this can hardly be put forth as an argument for a return to nature.

All these are factors which greatly affect the course of an epizootic but which cannot be altered in practice without changing the basis of a country's economy.

*The injections with serum from highly immune animals or from animals which have recovered from a natural attack (convalescents) have served for many years to raise the resistance power of the individual.*

In conjunction with the Swiss 'stamping out' system all cattle in the neighborhood of a slaughtered herd were treated (so-called Ring inoculation) to prevent the cases which otherwise are so often a sequence.

In Germany it was combined with other methods and was much used between 1920 and 1940 to protect the cattle taken to the market against infection. The prevention of these market infections may be of essential significance in affecting the course of an epizootic, especially when the virus is comparatively mild.

This method is also much applied in The Netherlands, especially with young animals and with highly productive animals. When the disease has attacked the herd, the great difficulty with all these methods of application is the short duration of the effect since an early reinjection is necessary when contact possibilities with the virus remain.

It remains an individual treatment to give a certain animal or at most a certain herd of cattle an increased resistance for a very short time (certainly not more than 10 days). However it can be a welcome complementary treatment to other measures of eradication which, by themselves, could not achieve the required result, for instance, the 'stamping out' system or isolation.

*The vaccination with adsorbate vaccine, applied in Germany in 1938 for the first time to any significant degree, has created quite different conditions in respect of these matters. The immunity attained with this is much more reliable than that with serum and the effect lasts considerably longer. An immunity of 6 to 8 months can be expected.*

At first there was the difficulty that the manufacture of the vaccine could not take place 'ad libitum'. The raw material, the tongue epithelium, had to be collected from an artificially infected animal and this required a great number of susceptible animals.

Owing to this, there was never enough vaccine available so that the whole cattle stock of a country could be vaccinated when the disease was threatening. The Veterinary Services had to divide the vaccine available and direct it to places where it was most needed. At an outbreak of the disease it could render great service when used for extensive vaccinations round these cases. The vaccine material can then be used in support of campaigns based on other measures, for instance, the 'stamping out' system or isolation.

The greatest difficulty with this system is that the immunity only fully develops after a fortnight. The objection to the serum treatment (that outbreaks developed 10 days or more after the treatment) is now replaced by the objection that they now occur in the first 10 days. This objection occurred rather frequently because vaccinations had been performed *just in the neighbourhood* of the outbreaks. When the vaccinations were performed as far as vaccination material was available at the request of the owners in regions without foot-and-mouth disease, these outbreaks did not occur.

As more vaccine became available these vaccinations became more frequent in uninfected districts. This was greatly promoted when Dr H. S. FRENKEL succeeded in enhancing the production of the vaccine by his method of cultivation on an artificial medium.

In the Netherlands especially, it were the owners of animals selected for production exterior qualities, namely the breeders, who had these vaccinations done annually. These voluntary vaccinations could however not prevent the country being infected now and then with foot-and-mouth disease. Still they had brought an essential change in the situation.

Before the vaccination the situation was, as a rule, such that the whole cattle stock was susceptible on the appearance of a new invasion, but now the susceptible herds were alternated with immune ones. The contact of the cattle herds that were still susceptible was therefore much less narrow. Owing to this, a situation arose in the Netherlands in the meadow districts which was to be compared with the situation where the meadows are separated by arable land. Therefore the disease increased much less quickly. Because of this there was much more opportunity to execute ring-vaccinations round the focus. The epizootic acquired a more lingering character and became much milder in form.

Of late years a triple vaccine (A.O.C.) has been used more and more.

At this stage the Dutch government decided to avail themselves anew of the means which, before the vaccination, was bound to fail in the Netherlands, namely the 'stamping out' system. They were conscious indeed of the fact that this would be a very strong stimulus for voluntary vaccination. This seemed to be true indeed, but not at all sufficient to obtain a vaccination of the whole cattle stock.

A new invasion came in December 1952 from the south. This reached its limit at about the turn of the year with more than 50 new cases per week. These foci were all stamped out. The course of the disease proved convincingly that the disease could be suppressed in the Netherlands with the 'stamping out' system completed by vaccination. This vaccination was executed as generally as possible, without having been made

obligatory. One should consider how to obtain a still more general vaccination so that the number of victims and the cost of the 'stamping out' system would be limited.

For this purpose a prohibition of transport has been issued on cattle for which no voucher has been issued that the animals originated from a farm herd totally vaccinated within a period of 2 months before the meadow period. All animals transported to market now must be provided with a certificate that they originate from a herd that has been totally vaccinated with a triple vaccine (A.O.C.).

This leaves room for the farmers' organization, which also works for the eradication of tuberculosis, namely the Health Services for Animals, to organize vaccination. This vaccination is executed by the practicing Veterinary Surgeons who report the vaccinations done by them to the centers of the Health Services. If the cattle owner declines to vaccinate his cattle then the Veterinary surgeon mentions this to the farmers' organization. The latter then will try to persuade the cattle owner.

If a cattle owner wants to send an animal to market, or to sell in another way, he can get a voucher that it is vaccinated against foot-and-mouth disease just as he gets a voucher that this herd and animal are free from tuberculosis. The buyer must deliver this voucher to the administration office of *his own* Health Service, so that it is always possible to check that only vaccinated animals are added to a herd that has been totally vaccinated.

If a cattle owner does not wish his cattle to be vaccinated he cannot get a vaccination voucher when he wants to transport an animal to market. If he still attempts to market the animal he exposes himself to the danger that his animal will be seized because the checking of these certificates takes place before arrival at the market or at a random test of the market by officials of the Government Veterinary Service.

This measure has been the cause of the attainment of a very complete vaccination of the Dutch cattle stock, approaching 100 %.

#### *What is now the best method to eradicate foot-and-mouth disease?*

The best method would be:

1. the six-month vaccination of the whole cattle stock with a superior vaccine combined with
2. a stamping out of the sick herds,
3. an extensive disinfection of the infected grounds,
4. a prohibition of transport in a large circle round a focus.

This is, however, not feasible in all countries and would have to be reinforced when a new strain of virus showed itself. For instance, no one would plead for a six-month vaccination in North America. For the United States and Canada the 'stamping out' system is now sufficient.

England and Ireland have always opposed vaccination because they think that a yearly vaccination is more costly than the system of 'stamping out' that is now followed. If the Western European countries will come to a yearly vaccination the eradication will become much easier for England and vaccination less necessary.

Switzerland prefers ring vaccination to complete her 'stamping out' system. On the

other hand the Netherlands has the vaccination system, still insufficient, completed by the 'stamping out' system and a checking of cattle transport.

Thus there are numerous differences based on differences in cattle farming in the various countries and also in the geographic position of these countries. Every land has to build up its own system for itself on three pillars: destruction of the virus; checking of cattle transport, and raising the immunity; in other words by the 'stamping out' system with subsequent disinfections, the prohibition of transport, and vaccination.

The perfection with which these have to be executed differs for every country. A complete knowledge of local circumstances is necessary, at the same time a knowledge of the methods used abroad and results reached there are all necessary for the establishment of a definite plan of campaign. However the system to be followed can never be found by accepting, without thought, a system from a foreign country.

# THE DUTCH FIVE YEARS' SCHEME FOR THE ERADICATION OF BOVINE TUBERCULOSIS

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## INTRODUCTORY

The area of the Netherlands covering 12,850 square miles consists for 36 percent of grassland, on which 2.6 million head of cattle are kept (mainly valuable productive animals).

The cattle are mainly kept for the production of milk, which is the basis of an important industry, not only highly contributive to the feeding of the home population (10,000,000), but also representing a considerable part of the exports of the country.

The control of animal diseases in a country such as ours with a human overpopulation and dense livestock population is of outstanding importance to public health and represents an indispensable element in any programme to increase agricultural production. Control of animal diseases in the Netherlands is a particularly difficult problem to contend with.

When one makes a tour of the Netherlands it is as though one drives through one enormous pasture, where, apparently not separated from each other, innumerable numbers of cattle are grazing.

In addition to this a special Dutch problem is the almost constant movement of cattle from one region to another. This is due to the fact that most of the cattle breeding is done in the North and East of the country, and the cattle are then eventually brought to the West where less breeding is done, but where they are producing liquid milk for the larger cities.

Thus an eradication scheme will have to be carried out very thoroughly and every cattle farmer will have to assume his share in the attempt to make regional control a feasible proposition. Laggards cannot be tolerated as they jeopardize the attempts of the willing. The administration must be a very exact one, a trustworthy identification and registration of cattle is one of the prime requirements.

In order to safeguard the very keen trade in cattle a thorough certification scheme will have to be carried out. The cattle will have to be examined regularly according to uniformly directed lines and must be performed by men trusted by the cattle farmers. These are the veterinary surgeons.

As the cattle farmer is in fact the actual combatant, it is essential to educate him so that he may have the conviction of his task.



In addition to extensive oral enlightenment, guidance by the press, radio, and films, the enlistment of the cattle farmers and their organizations has proved to be a very effective expedient in this connection.

During the past few years, therefore, endeavours have been made in the Netherlands to familiarize the farmers as much as possible with the conception of eradication of bovine tuberculosis and its utility.

#### ERADICATION OF TUBERCULOSIS

Following the example given by the province of Friesland, eleven regional Animal Health Services have been established in the Netherlands and accredited by the Minister of Agriculture.

They have been incorporated as autonomous institutions with a board consisting of representatives of the farmers' organizations and expert advisers.

Also the Controllers of the Veterinary Service are members of the Animal Health Service in the regions with which they are concerned.

The executive power is conferred on a director-veterinary surgeon who acts as the deputy controller of the Veterinary Services and who has some veterinary collaborators. Every health service has a laboratory of its own and keeps central records of the cattle in their region (province).

The Animal Health Services get the annually required money out of a fund formed by a levy of 5 cents per 100 kg delivered milk. This is supplemented by a levy per cow, to be paid by the farmer. From this money all the costs of the Health Services are paid, and also the annual inspection done by the veterinary surgeons of the cattle in their districts.

The Boards of the Animal Health Services develop the regulations which the cattle farmers have to observe. These regulations are in the first instance judged by a National Committee of Farmers' Organizations of which the director of the Veterinary Service is a member. Subsequently, the Committee discusses them with the Veterinary Service. The Director of the Veterinary Service then submits the regulations for the approval of the Minister of Agriculture. In this way it has been possible to co-ordinate the plan for the eradication of tuberculosis in a national scheme with regional conditions being taken into account to a considerable degree. The activities of the Animal Health Services are supervised by the Veterinary Service through a special section charged with carrying out this task.

Up to a few years ago the participation in the tuberculosis eradication scheme was voluntary, but today the laggards are compelled by Law to join the Health Services and to take in the programme for the eradication of bovine tuberculosis. When they refuse to do so, they are no longer permitted to keep cattle on the farm. This was the result of pressure that was brought to bear by the farmers' organizations. In 1949 all cattle were involved in the examination.

#### EXAMINATION

The cattle are all examined once in the winter of each year, while they are indoors, by a practising veterinary surgeon under the supervision of the Health Service con-

cerned. They are subjected to the single test – an intradermal test with P.P.D. tuberculin, prepared at the State Serum Institute at Rotterdam, in the side of the neck of all cattle. It has been prohibited by law to apply other tuberculin.

When any unaccountable response is shown in herds free from t.b. the comparative tests are judged according to uniformly defined lines as directed by the State Serum Institute. These are founded on comprehensive research. The director of the State Serum Institute will tell more of the P.P.D. tuberculin test.

The reacting animals are subsequently examined for open tuberculosis.

#### RECORDING

All cattle are identified on each farm by an earmark and a sketch and then registered.

The results of the examinations are entered in the register on the lists concerned. Based on these records a cattle farmer is entitled to a certificate when selling an attested animal; a *white certificate* when on two inspections the herd proved to be t.b. free; *white with a green line* when the herd proved to be t.b. free only at one inspection; and a *red certificate* in case a t.b. free animal is sold from a herd that is still infected. The certificates are valid for ten days.

The white certificates are therefore a better guarantee to the purchaser than the red ones as the seller does not receive a white certificate until he has agreed to take back the animal if another examination at the farm of the purchaser proves that it responds to the tuberculin test. This however must be done within 16 days after the sale, otherwise the seller is not liable to take back the animal.

#### RESULTS OF THE EXAMINATIONS

In the annual examination in the winter '49/'50 all cattle were tested for the first time.

In this respect of the 2.6 million cattle approximately 400,000 animals responded to the test, i.e. about 16 percent.

In the cattle breeding region in the East of the country this percentage was 11, except in Friesland and in a substantial part of Drente, where the cattle were free from t.b. In the West of the country, however, the number of tubercular animals was about 40 percent of the total. Of 220,000 farms in the Netherlands 62.5 percent proved to be free from bovine tuberculosis.

These figures show clearly that despite a voluntary control carried out over a large number of years, bovine tuberculosis was still a menace to public health, to cattle farming, and to exports of dairy produce.

Nothing but substantial financial support would open the way for the slaughtering of all responding animals in order to create improvements in the prevailing conditions.

#### FIVE YEAR PLAN

In May 1951 the five year plan for eradication of bovine tuberculosis commenced; it intends to bring all reacting animals to the slaughter-house within five years. That was the result of the basic proposal in 1949 to the Minister by the farmers' organizations that they were prepared to suggest to the cattle farmers the raising of a fund of 10 million guilders over five years, by means of a levy of 25 cents per 100 kgs of milk

delivered from the farms, if the Government would be prepared to contribute a similar amount. In the province of Zeeland where more beef-cattle is held, a levy of 4 guilders per cow was proposed.

The Minister discussed this proposal with the Marshall-aid organization and the latter was immediately prepared to grant 50 million guilders from the local currency fund for this purpose. Together with the money from the levies a total amount of 100 million guilders became available during the five years for the eradication of bovine tuberculosis. From this fund, administered jointly by the farmers' organizations and the Government, slaughtering of cattle responding to the tuberculin test is subsidized. This indemnity is granted as slaughter bounties and paid to a cattle farmer for any reacting animal that is slaughtered. No bounty is given, when the farmer sells his reacting animal. A bounty will only be granted to a farmer for those responding animals which were already owned at the time the scheme came into operation, or which are purchased with an available certificate, or are born on the farm and are infected later.

No bounty will be granted for the slaughter of animals, brought in without a certificate.

The bounty amounts to about 150 guilders per animal, but the grant can be changed every half year, if thought desirable. The bounty is not immediately paid after the slaughter of a reacting animal, but later when the farmer has declared that all reactors have been removed from his herd and a tuberculin test six weeks later has proved that his entire herd is free from reactors.

If new outbreaks occur in herds already free from tuberculosis or on farms that have just been cleared of responding cattle, then the reacting animals must be slaughtered within 20 days after having been traced and the owner will receive a compensation equal to 90 % of the difference between the slaughter value and the commercial value of the animal in question. Here the comparative test is of much importance.

From the fund of 100 million guilders are also paid the expenses issuing from the implementation of the scheme. These costs amount to some 5 percent of the contributions. *All cattle farmers* contribute to this 100 million guilders fund, whether their herds are already free from t.b. or not.

It was anticipated that the financial pressure brought to bear would be an impulse to the slaughtering of responding animals on a large scale.

In view of the difficulties involved in the quick tracing of all animals suffering from open tuberculosis and effective isolation of responding animals, this was considered the only proper method to arrive actually at an eradication of tuberculosis.

Furthermore it was considered necessary to stimulate even more the co-operation of cattle farmers with infected herds by bringing extra pecuniary pressure to bear upon them. For that reason an extra levy of 55 cents per 100 kgs was put on milk deliveries from these farms.

The extra levy, however, is not of necessity definitely forfeited by these cattle owners.

It is booked in the name of each cattle farmer concerned, as the extra levies will be repaid to these who have freed their herd of tuberculosis in time.

If a farmer omits to do so, then the money saved on his behalf will be forfeited.

The time within which a cattle farmer is obliged to free his herd from tuberculosis,

in order to get back his money, is determined in accordance with the rate of animals responding to the test at the time the scheme was incepted, that is at the so called 'gauge-date' and the following schedule applies here:

Herds with a rate of responding animals of	0-10	percent shall be t.b. free within	1 year(s)
" " " " " " " "	10-20	" " " " " "	2 "
" " " " " " " "	20-30	" " " " " "	3 "
" " " " " " " "	30-50	" " " " " "	4 "
" " " " " " " "	50-100	" " " " " "	5 "

This schedule applies to all cattle owners with t.b. infected herds, irrespective of the district in the country. Therefore all cattle are taken into consideration in the scheme. If a cattle farmer does what is expected of him he will consequently be paid the slaughter bounties due to him together with the aggregate of the extra levies booked in his name.

It should be noted here that a cattle owner will never receive a higher amount in slaughter bounties, than is proportional to the number of responding animals in his herd at the time of the inception of the scheme.

By this provision speculators who in anticipation of the 5-year-plan purchased responding animals, have been headed off and at the same time cattle farmers are encouraged to take measures against the spread of tuberculosis within their herds by isolation of responding animals, etc.

The eradication of bovine tuberculosis in the Netherlands is spread over a period of five years.

From a veterinary-hygienic point of view a shorter period would have been more opportune, but for economic reasons it was not feasible to adopt such a course, the main reason being that there would be adverse interference with the husbandry.

Both milk and meat production must, in spite of the 5-year scheme, be kept on an even keel.

The problem of national eradication is therefore two-sided: on the one hand the slaughtering, and on the other hand the substitution of t.b. free animals for infected ones. The latter side is obviously the main aspect of eradication of bovine tuberculosis.

The prospects for the swift extermination of tuberculosis amongst the cattle in the breeding regions in the East and the North of the country are favourable, as at the time the scheme was introduced the rate of responding animals was considerably lower than in the west of the country, in the vicinity of the large towns.

Steps are being taken in the breeding regions to free large areas that are very much under the influence of dairy factories, from tuberculosis within a short time.

Cattle farmers with heavily infected herds who still had plenty of time available for reaching the target are afforded extra financial aid to accelerate total eradication. The supply of commercial cattle from the East to the West contains an ever declining rate of animals responding to the tuberculin test.

The gradually growing supply of t.b. free cattle is a challenge to the farmers in the West of the country, with heavily infected herds, in freeing them from the t.b. scourge and therefore the hope is well founded that the 5-year scheme will be implemented without many adversaries.

As a final attempt to realise the 5-year scheme, apart from the financial measures

referred to, a bill has been enacted by Parliament conferring powers upon the Government to compel the reluctant farmers to do their duty.

It is, for instance now possible to prohibit farmers in specially appointed districts keeping cattle responding to the tuberculin test and measures can be taken in regard to transport, markets, shows, etc., to safeguard the results already attained.

The scheme as a whole is publicized by a comprehensive action amongst farmers at meetings, by the press and radio. A new film of the Ministry of Agriculture on eradication of bovine tuberculosis, particularly intended to arouse confidence in the tests amongst farmers, but at the same time showing an instructive character as regards the measures to be taken on the farms, will occupy a prominent place amongst the attempts to achieve the final aim.

The 5-year scheme was incepted in May 1951 and therefore it is possible to tell something about the experience gained:

At this moment already 200,000 reacting animals (of the total number of 400,000 present at the beginning of the plan) are slaughtered.

Of the total of approximately 80,000 infected herds some 40,000 are now already freed from tuberculosis.

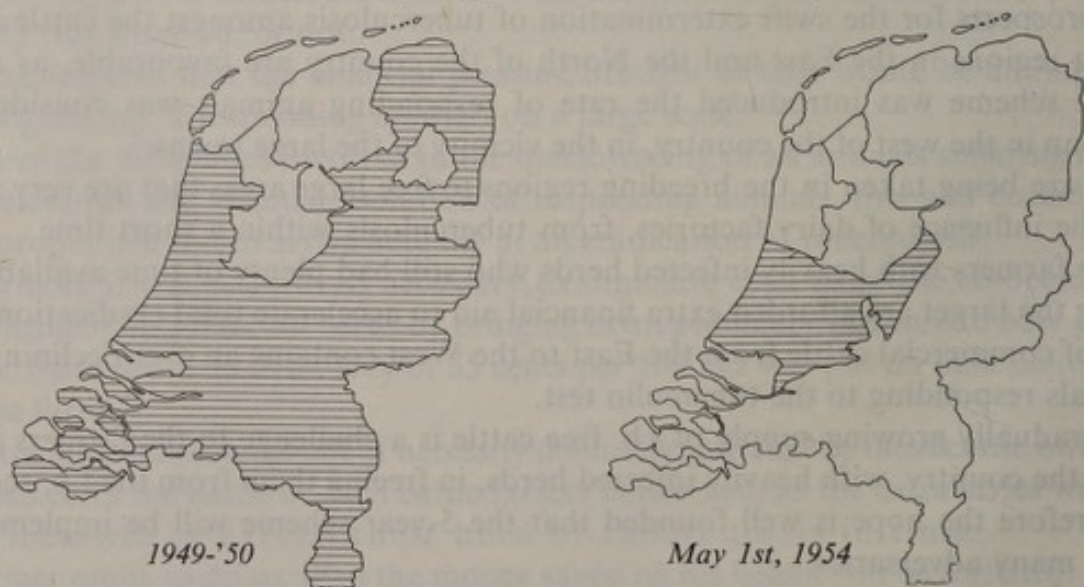
Only a few hundred farms have been late in achieving the aim in view.

On the other hand 16,000 other herds have been thoroughly culled of responding animals, though according to the scheme the period set for them has not yet elapsed.

Substantial parts of the country are already free from bovine tuberculosis and we are quite justified in anticipating that all important breeding regions will be free on November 1, 1953. After this has been achieved the main attack on the difficult regions in the West of the country must be launched.

The co-operation of the cattle farmers has been very much better than was expected.

All bodies concerned with the scheme, both farmers' organizations and official bodies, are enthusiastically co-operating and we feel justified in hoping that the efforts of the farmers and the veterinary surgeons will lead to complete success.



*The shaded part was not yet free from tuberculosis*

# THE TUBERCULIN TEST IN CATTLE WITH PPD

G. M. VAN WAVEREN

*Director of the State Serum Institute, Rotterdam*

In modern schemes of eradication of tuberculosis among cattle, the tuberculin test is of fundamental importance as the chosen method for the detection of infected animals which must be segregated from the herd.

Since KOCH prepared his Old Tuberculin, much work has been done by scientific workers in the laboratories and much experience has been gained in the field to improve the diagnosis and to standardize the technique of its application.

Although remarkable progress has been obtained in different countries with the aid of Old Tuberculin, the shortcomings of this preparation are well-known. The potency of the different batches of OT can fluctuate between wide ranges as a result of variation in bacterial growth on the glycerine-broth. Mostly these tuberculins are of low potency.

On the other hand, the presence of concentrated residues of the culture medium in the final preparation (especially glycerine and broth-proteins) may be the cause of false positive reactions in healthy animals.

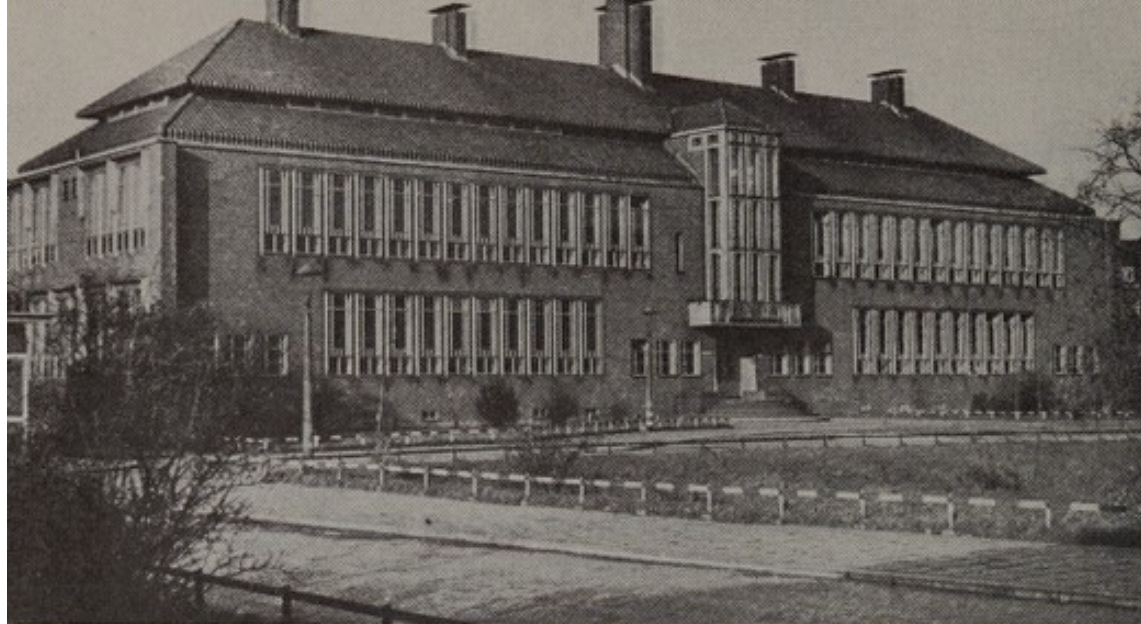
As a very important improvement we report the introduction of synthetic media in the routine production of tuberculin, media which are composed of exactly the same chemically known constituents and which guarantee an optimal growth of the Mycobacterium and a high yield of active principle.

This principle was concentrated in the same way as OT, namely by heating and evaporation.

A very potent product was obtained, known as HCSM tuberculin (Heat Concentrated Synthetic Medium Tuberculin) and is in use in the United States of America, Canada, Australia, Switzerland and, in former years, in England and the Netherlands.

As one of the best HCSM-tuberculins we consider that prepared by the United States Bureau of Animal Industry, in which are used three human strains (C, PN, DT), grown on the synthetic medium of DORSET-HENLEY. This medium is free from proteins, but contains the aminoacid asparagine in a high concentration as a rich source of nitrogen, glycerine and glyucose as carbon-producers for the Mycobacterium and some mineral-salts to provide growth.

This tuberculin still contains the decomposition residues of the artificial medium but these impurities prove to be of smaller influence than those in the Old Tuberculin.



Main building Government Serum Institute

In our experience, the concentration of active principle in this newer tuberculin could, vary up to 100 %.

Standardization is not yet extensive. It is the great merit of the American investigator Miss FLORENCE SEIBERT, that she demonstrated that the Active principle of tuberculin is of a protein-nature. In a synthetic medium, originally free from protein, appear during the growth of Mycobacteria proteins as a result of enzymatic autolysis of the bacteria. These proteins were precipitated with the ordinary precipitation methods (for example by half saturation with ammonium sulfate), washed and purified by means of ultrafiltration, and gave all the specific reactions of tuberculin. Tuberculin-filtrates freed from protein do not give the required reactions.

It was found that chemical determination of protein in synthetic medium tuberculin issues served as accurate measures of potency, far more precisely than could be done by biological testing.

GREEN, at the Veterinary Laboratory at Weybridge, England, simplified SEIBERT'S method for preparing purified protein derivative (PPD) tuberculin and succeeded in manufacturing a practically pure tuberculo-protein on a large scale.

Briefly, his production-procedure is as follows:

From propagation flasks pellicles of three human strains (C, DT, PN) are seeded onto the surface of a synthetic medium containing 0.18 per cent dipotassium phosphate, 0.09 per cent sodium citrate, 0.15 per cent magnesium sulphate, 0.03 per cent ferric citrate, 1.4 per cent aparagine, 1 per cent glyucose, and 10 per cent glycerine.

The growth period is extended over 10 weeks. After steaming the cultures, the liquid is filtered through copper gauze and pressed filter-paper-pulp. The filtrate is transferred to calibrated bottles and one ninth of its volume of 40 per cent trichloroacetic-acid solution added. Total protein is allowed to form a sediment for several hours in glass cylinders and the supernatant solution sucked off. The sediment sludge is rinsed with dilute trichloroacetic acid (1 per cent), centrifuged and again washed with concentrated salt solution (5 per cent NaCl). For practical issues of tuberculin, the wet precipitate of purified protein derivative is dissolved in alkaline phosphate solution (m/15  $\text{Na}_2\text{HPO}_4$ ) and sufficient M/15 NaOH is added to bring it to about pH 6.8. With a certain quantity of antibacterial fluid (50 per cent glycerol, 2.5 per cent phenol and 2.5 per cent sodium chloride) the tuberculo-

protein is dissolved in about 1/10th of the original culture filtrate volume and the pH adjusted to 7.0.

The solution is freed from higher molecular weight material of lower potency by being passed a few times through a Sharples super centrifuge.

The protein nitrogen of the centrifuged concentrate is then determined and the PPD content calculated.

The concentrate is stable more or less indefinitely in cold storage and kept until required for dilution to issue strength of 1.5 mg per ml. The diluting fluid is the five times lower concentrated anti-bacterial fluid.

The finished PPD tuberculin is then passed through asbestos sterilizing filters and bottled under aseptic conditions.

Although the product is always made in the same way from the same strains and the analytical check on quantity of protein is accurate to  $\pm 1$  per cent, there is always the possibility that a potency variation may occur and, therefore, to satisfy the veterinary surgeons with a biological cross-check, tests are carried out on sensitized guinea pigs and on cattle. In no single case during many years has the biological test contradicted the chemical test. The guinea pig test has an accuracy of  $\pm 40$  per cent and therefore fails to reveal differences of lesser magnitude in material already standardized to  $\pm 1$  per cent by chemical analysis.

By studying the complete prescription of the production of Weybridge PPD, it is impressive how carefully and uniformly these tuberculins are fabricated. Neither this, nor the physical way to purify and the chemical method to standardize the products, means that the preparation of PPD tuberculins is very complicated and expensive; on the contrary the utmost economy and efficiency are practised. For example, the wastage of  $\pm 50$  per cent of the active principle which occurs during the concentration of HCSM tuberculin (oxydation of proteins during the forced evaporation) is avoided in the chemical process of protein-gathering.

It was with this promising tuberculin that we started investigations in the Netherlands five years ago.

The mammalian PPD, human type, 1.5 mg protein/ml, that is  $\frac{3}{4}$  OT, was applied in a uniform technique, injection into the center of the neck of cattle in the deeper layers of the skin. It has been proved that this area is of extraordinary sensitivity, far more pronounced than the sides of the body and the caudal fold. The dose of tuberculin was exactly 0.1 ml, and to standardize this we have developed from the dental syringe that has to be loaded with closed ampullas, a tuberculin-syringe for veterinary use with an automatic dosage of 0.1 ml, a uniform needle with a diameter of 0.8 mm, a free part of 4 mm and a short bevel. The closed ampullas contain 1.7 ml of tuberculin; contamination of the contents before and during the tests is excluded.

A thorough investigation into the reliability of the single intradermal test with PPD tuberculin, human type, was carried out by us on about 700 *ordinary slaughter-cattle*. They were tested after a two days rest and the test-results read during the following four days. Afterwards a very minute dissection of the organs and lymph glands took place and, in the absence of macroscopic tuberculous lesions, a biological test of 5 different groups of lymph glands (head-, lung-, intestinal- and liver-, mammary- and meat lymph glands) was performed by inoculation of 5 groups of 4 guinea pigs.

The careful post-mortem examination gave a yield of 15 per cent more tuberculous animals (with one or two minute lesions) than could have been detected by ordinary



meatinspection. The biological test gave an additional 4 per cent. It will be clear from these figures that only experiments with very careful and profound dissection complemented with biological tests are of value in investigating the reliability of the tuberculin test. In all our experiments to be mentioned here we have paid attention to this procedure.

The results of the tuberculin-tests on the slaughtercattle, in which circumscribed swellings of 2 mm or less at 72 hours after inoculation are considered negative, and swellings of 4 mm or more positive, are briefly given in the following tabulation:

TABLE 1. Single intradermal test with PPD (human type) in 706 slaughter-cattle

	Tuberculin-test		
	Positive	Doubtful	Negative
542 tuberculous cattle . . . . .	523 (96.5 %)	7 (1.3 %)	12 (2.2 %)
164 tuberculosis-free cattle . . . . .	25 (15.2 %)	20(12.3 %)	119 (72.5 %)

It may be concluded that the single intradermal test with PPD tuberculin (human type, 1.5 mg. per ml.) gives very satisfactory results in detecting tuberculosis in individual animals. We may expect even better results in the herd-tests for which the tuberculin-test is meant.

But the question of the aspecific and no-visible-lesion reactors resolves itself in the group of tuberculosis-free animals that shows a percentage of false-positive reactions of 15.2. In the herd-tests with PPD in single intradermal tests over large tuberculosis-free areas in the Netherlands, the problem of aspecific reactors amounts to about 5 per cent. For the greater part, this is due to skin lesions and in some regions to avian tuberculosis.

The economy of this problem forced us to look for more specific tuberculin.

Our attention was drawn to the bovine type tuberculin, that was recently prepared by the Veterinary Laboratory at Weybridge, a very tuberculogenic bovine strain, AN5.

This strain can be cultivated very well on the synthetic medium of DORSET-HENLEY on which it gives a high yield of proteins, nearly as high as the human strains.

With PPD from this bovine strain, we could confirm the English information that on avian sensitized guinea pigs this bovine PPD was three times less active than human PPD and, on bovine sensitized guinea pigs of similar activity; so this bovine PPD is more specific.

An orientation-experiment was organized in the field and several hundred cattle from clean and infected herds were simultaneously tested with bovine and human PPD tuberculins of equal protein strength (1.5 mg. per ml.) on the left and right sides of the neck.

The average figures obtained in healthy cattle were 3.3 mm. human reaction and 1.8 mm. bovine reaction, in infected animals 9 mm. human reaction and 8.9 mm. bovine reaction.

The type of swelling with bovine PPD in tuberculous animals was distinctly more edematous, so typical.

The impression was gained that the bovine PPD gave far less rise to aspecific reactions and was very potent in detecting tuberculosis.

Very careful research was done at the institute on about 50 head of cattle that were selected at the annual tuberculin-test in the field because of their unexpected positive reactions.

The animals were retested with bovine and human PPD and after the slaughtering minutely examined; in negative cases the biological test with guinea pigs was made. The average figures obtained in tuberculosis-free animals without processes of aspecific infection were: 3.3 mm. human reaction and 2.2 mm. bovine reaction.

In tuberculosis-free animals with skin lesions: 3.9 mm. human reaction and 2.5 mm. bovine reaction.

In tuberculous animals, 4.2 mm. human reaction and 5.3 mm. bovine reaction.

From these figures it is clear that the bovine PPD is at least as potent in detecting bovine tuberculosis as is the human PPD and considerably more specific. In clean herds it gives a far smaller chance of positive reactions.

After these experiences, we thought it an advantage to replace the human PPD by bovine PPD. Since the start of the Five Year Tuberculosis Eradication Programme in the Netherlands (1950) the tuberculin test has been carried out with bovine type PPD.

The sensitivity of paratuberculosis-animals to this type of tuberculin was very small and mostly insignificant; cattle in which we isolated *Mycobacteria* of the avian type by guinea pig inoculation reacted from 2 to 6 mm.

In cleaned areas there remained the need of a retest of the positive reactors that still represented an amount of a few percent.

To demonstrate the existence of aspecific sensitivity in these animals, we used the comparative intradermal test with avian and bovine type PPD tuberculins.

The techniques of this test were exactly the same as in England namely injection of 0.1 ml of avian PPD in the middle third of the neck at a distance of 10 cm. from the crest and the same dose of bovine PPD at 12.5 cm. below the spot of the avian tuberculin. The strength of the avian PPD was 0.4 mg. protein per ml, that of the mammalian tuberculins 1.5 mg. In our experience no animal may be retested within a period of at least six weeks after the single test, as retesting within 3-7-10 or even 30 days gives unreliable results.

Readings were made after 72 hours and the reactions interpreted after a somewhat more rigorous scheme than the official English one (details are given at the end of this paper).

The comparative test is applied at the institute on about 50 head of cattle with a known, mostly safe, herd history. Bovine and human PPD are used simultaneously in these test. After slaughtering, a very minute search for lesions took place and biological investigation of the lymph glands.

Below are the results of the various tuberculin tests.

19 tuberculosis-free cattle without skin lesions

	positive	doubtful	aspecific	negative
single test-human PPD . . . . .	6	6	—	7
single test-bovine PPD . . . . .	4	5	—	10
comp. test-human PPD . . . . .	2	6	4	7
comp. test-bovine PPD . . . . .	1	3	5	10

10 tuberculosis-free cattle with skin lesions

	positive	doubtful	aspecific	negative
single test-human PPD . . . . .	5	3	—	2
single test-bovine PPD . . . . .	1	4	—	5
comp. test-human PPD . . . . .	2	3	3	2
comp. test-bovine PPD . . . . .	0	2	3	5

17 tuberculous cattle

	positive	doubtful	aspecific	negative
single test-human PPD . . . . .	12	1	—	4
single test-bovine PPD . . . . .	13	3	—	1
comp. test-human PPD . . . . .	9	3	1	4
comp. test-bovine PPD . . . . .	10	4	2	1

It may be learned from this table that, in all of the three groups of animals, the replacement of human PPD by bovine PPD in the single as well as in the comparative test shows a significant improvement.

It may be noticed that the comparative test is not the chosen method to detect tuberculosis in cattle.

This was also proved by us in large slaughterhouse experiments on more than 1000 head of cattle of unknown origin.

The eradication must be commenced with the single intradermal test and the comparative test must be restricted to cleaned herds that are protected against contamination.

Under these conditions the intradermal tuberculin test has been executed in The Netherlands for four years with exactly the same technique of application, reading, and interpretation, and with onetype (PPD) of official tuberculin.

In practice, experience has been gained on about 10,000,000 single tests and nearly 200,000 comparative tests.

The best approbation for our single test is the fortunate progress in tuberculosis eradication. The supplement of the comparative test in justified cases means a clarification of about 90 per cent of the number of aspecific reactors.

Aspecific reactors, declared free from tuberculosis on the ground of the comparative

test, appear only exceptionally to be infected. In general, an aspecific skin lesion reactor will become a negative reactor within one year.

It is with emphasis that we recommend the single intradermal tuberculin test with application at the center of the neck as a very sensitive method for the tracing of tuberculosis in cattle. PPD tuberculins, prepared after the prescription of Green, and especially the bovine type PPD, have to be considered at the moment as the most specific tuberculin, that can be standardized very precisely.

For reasons of economy the comparative test with avian and mammalian PPD tuberculin is of great value in clean herds (and only in these).

#### EXECUTION AND INTERPRETATION OF THE TUBERCULIN TESTS

##### *Single intradermal test*

Tuberculin: bovine PPD, 1.5 mg. protein per ml.

Dose: 0.1 ml.

Application: into the deeper layers of the skin at the center of the neck, depilated by cutting.

Reading: after 72 hours with calipers and with annotation of type of swelling.

Interpretation: circumscribed swellings up to and including 2 mm. are considered negative; swellings of 4 mm. or more positive, any swelling showing edema should also be regarded as positive.

Retests: not sooner than after 6 weeks and preferably on the other side of the neck.

##### *Comparative test*

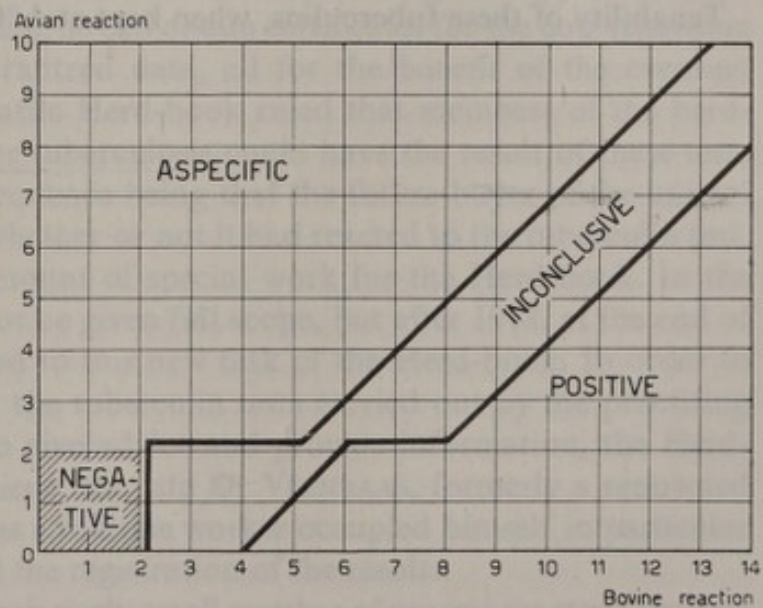
Only to be used in cleaned herds with a good history.

Tuberculins: bovine PPD, 1.5 mg. protein per ml; avian PPD 0.4 mg. protein per ml.

Doses: of both tuberculins 0.1 ml.

Application: in the middle third of one side of the neck; avian PPD four inches below the crest and the bovine PPD five inches from the site of the avian PPD on a line parallel with the line of the shoulder.

Reading: after 72 hours with calipers and with annotation of type of swelling.



For interpretation see page 72

*Interpretation*

Avian reaction	Bovine reaction	Decision
not exceeding 2 mm	not exceeding 2 mm	free of tuberculosis
more than 2 mm	smaller than avian reaction	aspecific sensibilization
not exceeding 2 mm	excess of bovine reaction not exceeding the avian reaction by more than 4 mm	inconclusive
not exceeding 2 mm	excess of bovine reaction exceeding the avian reaction by more than 4 mm	bovine tuberculosis
more than 2 mm	bovine reaction exceeds the avian reaction by at the utmost 3 mm	aspecific sensibilization
more than 2 mm	bovine reaction exceeds the avian reaction by 3 to 6 mm	inconclusive
more than 2 mm	bovine reaction exceeds the avian reaction by more than 6 mm	bovine tuberculosis
	if the bovine reaction is oedematous	strongly suspected, segregation and retest

*Restriction*

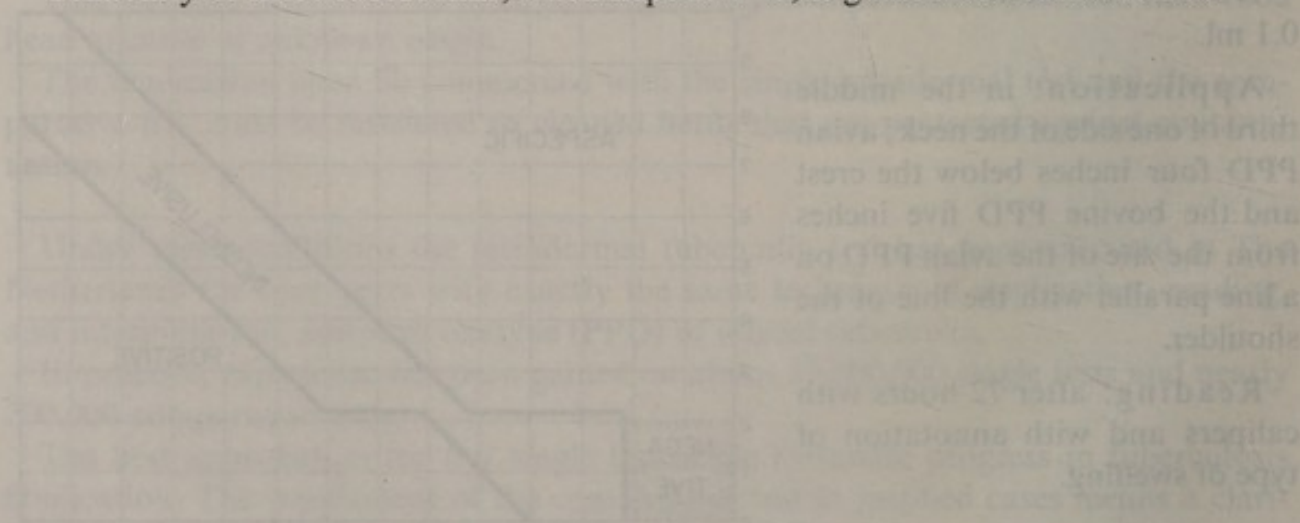
If, in a herd, one or more animals show pronounced positive bovine reactions or are highly suspected, because of other reasons, of suffering from tuberculosis, every animal on which the bovine reaction exceeds the avian reaction has to be considered as infected with bovine tuberculosis and all other cattle in which the bovine reaction is doubtful or positive has to be retested.

Aspecific reactors may be retained in the herd; they are not considered as non-reactors, and are provisionally left out for certificates of health.

Retests: not sooner than after six weeks, and preferably on the other side of the neck.

In the official test, only PPD tuberculins of the State Serum Institute are allowed. These tuberculins are delivered in dental ampules of 1.7 ml. that fit the modified dental syringe with automatic dosage and standard needles.

Tenability of these tuberculins, when kept at 4 °C, is guaranteed for 9 months.



# THE TASK OF ORGANIZED AGRICULTURE AND THE PROVINCIAL HEALTH SERVICES FOR ANIMALS IN TUBERCULOSIS CONTROL AMONG CATTLE

L. P. DE VRIES

*Secretary of the Health Committee for Animals*

Something will be told about the task of organized agriculture and the provincial health services for animals in the control of tuberculosis.

I will give a review of the origin of these health services and their organization.

I will begin with a short introduction from the past and for that purpose take you to the province of Friesland, a cattle-rearing district par excellence, and the area in which the cradle of the black and white Holland-Friesian cattle stood.

I go back to the year 1913; a form of expression of organized agriculture is the Pedigree Herd-book for cattle, and in that year the Friesian Cattle Herd-book had become quite extensive. In those days a certain interest began to be taken in the control of tuberculosis, in the sense that a number of stock-holders had their cattle tested with the help of subcutaneous injections, followed by recording the course of the temperature curve.

The Friesian Herd-book is, in particular, an institution which gives a guarantee; if a farmer offers a herd-book cow for sale, he can obtain certificates for the cow regarding pedigree and production with guaranteed data, all for the benefit of the eventual buyer. Then in 1913 the Friesian Cattle Herd-book ruled that members of the herd-book who had their herds tested for tuberculosis could have the result of these tests entered in the herd-book, the consequence being that the future buyer of the animal concerned could also be informed whether or not it had reacted to the tuberculin test. This naturally entailed a certain amount of special work for the Herd-book. In the first world war this activity could not be given full scope, but after 1918, at the end of the war, more attention was directed to this new task of the Herd-book. In order to enable control to be exercised over the tuberculin tests carried out by the practising veterinary surgeons, and further, to give advice and provide information, the Herd-book appointed an adviser for hygiene, the late Dr VEENBAAS, formerly a renowned practising veterinary surgeon, who as a fulltime worker occupied himself in particular with the control of tuberculosis and the registration of the results.

In the beginning there were just a relatively small number of prominent stock-owners

who had their cattle tuberculin-tested but, as you know, Friesland exports large numbers of cattle to foreign countries, and it soon became known that a cow with a t.b. free certificate realised a considerably higher selling price than a cow without such a certificate. This encouraged more herd-book farmers to have their cattle tested. But on the other hand the tests caused great disappointment to many cattle-owners; often many animals unexpectedly reacted positively. The Herd-book was therefore faced with two things; an increase in the number of cattle owners who wished to have their herds tested, and the creation of many problems which called for a solution.

The milk was and is still prepared in Friesland in dairy factories, 82 of which are co-operative ones. The skim-milk, buttermilk and whey of these factories goes back to the farmer out of the mixed quantity. Infectious germs from a cow of one of the members of the dairy factory may therefore become a source of infection for the cows of another member. It may therefore be of great importance for the control of tuberculosis to establish contact with the dairy factory. When this contact has once been established, it affords further advantages, especially in the case of the co-operative factories, where a good opportunity to discuss the control of diseases is presented at the meeting of the members of the factory.

It need not therefore cause surprise to hear that the Friesian Herd-book authorities proceeded to talk things over with the Union of Co-operative Dairy Factories in Friesland. This led to an important decision: both organizations would co-operate to control those diseases which could be eradicated in organized form, in particular tuberculosis. Further, special attention was to be directed to sterility (in these days already an important problem) and contagious abortion.

They therefore set themselves a common task and to enable this task to be carried out the *Health Service for Cattle in Friesland* was established. A committee of management was set up for the service, consisting of delegates from the Herd-book organization and the Union of Co-operative Dairy Factories. The adviser for hygiene became its director.

A laboratory was made available to him, with an analyst, a clerk and a bottle-washer. Everything for the time being was on a modest scale although efficient. But as regards organization, things were done on a generous scale. The object of the service was described thus: 'the furtherance of the state of health of the cattle and a good yield of milk.'

It was laid down as a fundamental principle that the individual who conscientiously made efforts to raise the standard of and to keep his stock up to the mark may not only not be hampered in his efforts by the other herds of a particular cattle community, but that conditions should be so created on all farms that he could find support in them.

On the grounds of this principle, opportunity was given to co-operative dairy factories as well as to associations of milk suppliers of private dairy factories to join the service. Working regionally, the ultimate objective was to have the whole of Friesland join the service so that, profiting from the registration, the service could be placed

*Main building Provincial Health Service for animals in North Brabant*

on an entirely new basis, better than had previously been the case. Thus the danger of contamination, effected through the cattle trade, would be considerably reduced.

The whole system was based on voluntary co-operation. By rendering good service, efforts were to be made to include the greatest possible number of farmers under the care of the health service.

As regards the technical side, regulations were drawn up for the members (these were, therefore, the co-operative dairy factories or the associations of cattle-owners of private dairy factories) concerning the control of tuberculosis; these mem-

bers thereby undertook to see that their members (these were, therefore, the farmers) kept to the rules laid down in the regulations.

By a series of tests, they had already satisfied themselves that subcutaneous tuberculin testing with its laborious taking of temperatures could be superseded by the ophthalmic test. It was prescribed that the test on the farm should be carried out by the local veterinary surgeon; in the early days the dropping of the tuberculin in the eye was done by lay helpers who had been specially trained by the Health Service for Cattle. Later, when the number of veterinary surgeons had increased, the use of lay helpers was dropped altogether. The veterinary surgeons are 'allowed' by the health service to carry out the test. They are subject to control by the health service.

The method was to tuberculin test the herds of cattle at least once a year; clinically to test the animals which had reacted for the occurrence of open tuberculosis (the examination of sputum, excreta and secreta could take place in the laboratory of the health service); further the breeding of t.b.-free calves was promoted. Farmers were advised to isolate the reactors from those animals which were free from the disease. Of course the theory soon found favour that the animals which had reacted could best be isolated from the herd by expelling them from that herd, also by selling them. That brings us to a tender point: does not the farmer who markets his reactors spread





tuberculosis among other people's cattle? Would it therefore not be desirable to mark all the reactors? There has been a great conflict of opinion on this point in The Netherlands; Friesland has always adopted the view-point that the marking of such animals is an evil thing and that the man who wishes to buy t.b.-free animals can do so by buying cows with certificates showing that they are free from tuberculosis. Finally, those who support the Friesian view-point have been declared to be in the right.

It will be seen, therefore, that the Friesian health service has never lost sight of its original objective, viz. accurate registration of the tests carried out on the animals and making available certificates concerning their state of health.

Immediately after its establishment in 1919, 7 dairy factories joined the service; in the second year there were 14. Up to the outbreak of the war in 1940 constant expansion of the work of the health service for animals was to be observed. In 1939, 81 co-operative dairy factories had joined the service (the private dairy factories had then temporarily created their own health service). In the winter of 1939-'40, 231,678 cows were tested on 11,102 farms; the reaction percentage (averaging 35 in 1919) had dropped to 6.2. The number of farms free from t.b. was 8,016 and here and there people began to talk of making free from the disease all the cattle belonging to the members of a dairy factory. And so, the favourable development of voluntary control of t.b. – without Government aid – was to be seen. The entire cost was borne voluntarily by the farmers, an almost ideal state of things, attainable in Friesland because there the stock-breeder stands high in his class, and the export trade constitutes a powerful incentive to eradicate tuberculosis.

Since as far back as 1905, the Government has occupied itself with the furtherance of the control of tuberculosis among cattle. In 1927, financial support was promised to associations of cattle-owners who had undertaken to control t.b. in accordance with certain regulations. Although such associations were formed in all the provinces, the results were not of such a nature that they gave satisfaction; in any case they were less satisfactory than those achieved in Friesland.

Ways and means were devised to found health services for animals in the other provinces, modelled on the Friesian example. Under the direction of the State Veterinary Service, representatives of all provinces were summoned in 1939 and 1940, before the world war, to consult together on the matter. Now there is a great difference between Friesland and the majority of the other provinces; in the first place stock-farming in those provinces has not, generally speaking, the exclusive significance it has in Friesland. In the second place the tie with the dairy-factories is in a certain number of cases not so strong, or there are no dairy factories, because the milk is used for direct consumption in the large towns, a factor which makes it more difficult to set up self-acting organizations. The idea occurred of finding something to encourage the farmers to set to work themselves. In The Netherlands we have the *Dairy Trade Board*, a body which in accordance with its object extends its activities to everything having to do with milk.

At the beginning of the war, the Government ordered that all drinking milk should

be standardized to contain  $2\frac{1}{2}$  % fat. The Dairy Trade Board was and still is charged with giving effect to the measure. All milk intended for direct consumption had to be and must still be delivered to standardization plants. The Dairy Trade Board now controls the administration of all dairy factories and standardization plants and therefore knows exactly how much milk The Netherlands farmers deliver. The Government then decreed that the dairy factories and standardization plants should deduct from the payments made to the farmers 5 cents (about 1 penny) per 100 kilogrammes of milk delivered for the control of diseases among cattle. The Netherlands produces nearly 4.5 thousand million kilogrammes of milk per annum, therefore, the levy for the control of diseases among cattle amounts to about 2,250,000 guilders (£ 225,000) per year. The knowledge that every stock-keeper already pays a certain amount for the control of diseases among his cattle, was the cunningly devised means for stimulating the founding of provincial health services for animals in every province.

During the war, the further establishment of health services was held in abeyance. When the war was over one of the first things with which the State Veterinary Service occupied itself was making preparations for setting up those provincial health services for animals.

The difference between the old Friesian health service and the health services in the other provinces, as the Government was proposing to establish them everywhere, was that the former was purely a farmers' organization, while the new ones were to be more subject to Government control.

Immediately after the war an entirely new agricultural organization made its appearance; the employers as well as the workers engaged in agriculture united in the *Netherlands Federation of Agriculture* which proposed to undertake all kinds of tasks. As one of the first items on its programme it put the control of diseases among cattle; and it adopted the standpoint that not the Government but the Federation itself was the proper body to watch over control of diseases and that this Federation should have a hand in setting up the provincial health services for animals. Agreement on this point was reached with the Minister of Agriculture, Fisheries and Food.

This gave rise to the situation as we now know it. A small committee appointed by the Federation of Agriculture, of which the Director of the State Veterinary Service was also a member, founded the first health services. When 7 of these had been founded (great eagerness was displayed in founding them), the Federation of Agriculture formed its permanent organ for these health services, namely the *Central Health Committee for Animals*, an administrative committee consisting of a representative of each of the health services and a number of technical advisers, including the Director of the State Veterinary Service. The Federation of Agriculture appointed the President and the Secretary of the Health Committee. In 1946 each province had its provincial health service for animals.

In a number of these the Friesian system was followed for the internal method of work, namely in this sense, that the dairy factories play a considerable part in the activities of the health services; in places where there are no dairy factories or where those who deliver their milk to the factories are not sufficiently united, the farmers are directly associated with the health services.

Each health service has a building with a laboratory and offices. The committee of management consists of cattle-keepers from the various groups in the provinces, to which are added the Provincial Inspector of the Veterinary Service and a few other persons (the stock-raising adviser, the dairy adviser) as advisers. The technical management is in the hands of a director – a veterinary surgeon – who is assisted in his work by a staff of employees. The regulations for the control of diseases, in the first place of tuberculosis, are laid down by this committee, in connection with which, of course, account is taken of conditions prevailing in the province. The regulations for tuberculosis control require the approval of the Minister of Agriculture, Fisheries and Food, before they become generally operative. Membership of the health service was voluntary at first; when 80 to 90 % of the farmers had joined voluntarily, the committee requested the Minister of Agriculture, Fisheries and Food to make membership of the health services compulsory. Since 1950 membership of the health service has been made obligatory for every stock-keeper; accordingly all cows in The Netherlands are now tested for tuberculosis at least once a year.

The committees of management of the health services meet at stated times, for instance once a month and at these meetings every possible issue relating to the control of diseases is discussed. The Health Committee for Animals meets every month, when they discuss the national problems, and, in addition, the directors of the eleven provincial health services gather at least once a month, usually twice monthly, to discuss technical matters.

In the provinces information relating to problems and lines of policy for the control of diseases is regularly communicated to the dairy factories or local associations of cattle-keepers, as the case may be, so that discussions on these matters may also take place in decentralised groups of cattle-keepers.

From the money raised by the levy of 5 cents per 100 kilogrammes of milk, each health service receives the amount due to it in accordance with a scheme framed by the Central Health Committee for Animals and approved by the Minister; these funds serve for the maintenance of the service and for contributions toward the cost of the tests for tuberculosis.

In this way a system has been evolved by which the control of tuberculosis among cattle is a matter in which the farmers take a lively interest. True, in contrast with the old Friesian health service, they are under the watchful eye of the Minister, but on the other hand the latter ensures that the levy of 5 cents per 100 kilogrammes of milk is effected and that all cattle-keepers are compelled to join the health service of their province.

Direct co-ordination between the health services (for example, the drafting of regulations for matters which must be prescribed in the same way everywhere, such as those for the issue of certificates, certain regulations for dealers), is maintained by the Central Health Committee for Animals.

In this way the control of tuberculosis has indeed become a matter in which the cattle-keepers take a lively interest; this became apparent at the moment when the cattle-keepers said it was a good thing that their cattle were tested for tuberculosis

every year, but that still more should be done; that in addition to the levy of 5 cents per 100 kilogrammes of milk they were prepared to pay a levy of 25 cents per 100 kilogrammes for 5 years, to promote the freeing of the whole Netherlands stock of cattle from t.b. in a short time. At the same time the wish was expressed that the government should vote a similar amount, because the control of tuberculosis is not only in the interest of the farmers but of the whole population. As you have already been informed, this five-year plan was initiated; the outcome of its operation will show that organized agriculture has not only understood its task in the control of tuberculosis, but is also carrying it out efficiently.

# CONTROL OF TUBERCULOSIS AMONG CATTLE IN THE PROVINCE OF NORTH HOLLAND

D. REMPT

*Director of the Animal Health Service in the  
Province of North Holland*

The province of North Holland numbers about 12,500 farmers with about 200,000 head of cattle. In the north and center, we find the stock-rearing areas with the co-operative and private dairy factories where the milk is made into butter, cheese and other products.

In the south, the milk is not delivered to dairy factories. Here live the farmers who supply milk to the large towns, Amsterdam, Haarlem etc.; the milk is therefore sold as such.

The consumption area extends still further into the provinces of Utrecht and South Holland, for the large towns of Utrecht, The Hague, and Rotterdam.

In this consumption area, little breeding is done but there is always a brisk trade in cattle. For the milk supply, milk cows are drawn from the periphery of the breeding areas.

In the autumn of 1946 the Animal Health Service in North Holland was founded and on 1st December 1946 I entered its service as Director. The control of tuberculosis among cattle was then still based on voluntary lines. Of the 200,000 cows some 130,000 were tested in the winter of 1947-1948.

On 11th September 1947 tuberculosis control was made obligatory by law in our province. That meant therefore that in the winter of 1947/1948 all cows had to be tested.

In the breeding area this was done very smoothly, but in the consumption areas the farmers were not so accommodating, owing to the fact that these farmers traded very much. The reaction percentage was always very high, much higher than in the breeding area. They were therefore by no means combat-minded and did not believe that they would ever get rid of the disease.

A great deal of talking was necessary, talking time and again with the farmers. They had to get confidence in the Health Service. We repeatedly gave lectures and showed films, and after the lectures opportunity was given to ask questions.

We then always had to have the courage to be honest. Biological reactions are never 100% successful. Tell the farmer this and he appreciates it, because at meetings you always hear of the exceptional cases. If you admit that this is possible, the critic is silenced. If you don't admit it, you arouse opposition.

## T.B. RESEARCH

The Animal Health Service in North Holland is established at Alkmaar where we have our head office.

There we have:

1. The Administration, employing 15 persons.
2. The laboratory with a veterinary surgeon at its head and 6 laboratory assistants.
3. The outside service with two veterinary surgeons, one for the control of T.B. and other diseases; one for sterility, A.I., nutrition, deficiencies etc. In addition 7 laymen (surveyors) work there.

They exercise control and also give advice to the farmers at home and at the markets.

42 practising veterinary surgeons co-operate in carrying out the annual herd testing.

Each veterinary surgeon has an average of 5000 animals in his practice, but the number varies between 2000 and up to as many as 14,000 to 15,000 animals.

Students help in the large practices during the winter months.

## ADMINISTRATION

In order to have better contact with our stock-keepers our province is divided into 44 associations.

In the north and centre these are the dairy factories and in the consumption area, the Amsterdam, Haarlem, and 's-Gravenland associations. These associations constitute the intermediaries between the cattle-keepers and the Health Service. Concurrently with the revenues from the 5 cent levy on the milk, the cattle-keepers also pay an annual contribution per animal. The associations collect these annual contributions and hand them over to the Health Service. In addition they occasionally hold meetings with their members to discuss certain problems of T.B. control.

To ensure efficient administration it is necessary that all cows should be registered.

In our province we have a system of double registration, viz.

1. All cows are sketched (both from the left and right side).
2. In addition, the non-herd-book cattle have a tin disk in the right ear, bearing serial letters and numbers.

The sketches are made in duplicate, one for the farmer and a duplicate for the Health Service. The particulars of the ear disc are also noted on the sketches. The sketches themselves are numbered serially and in addition there is a letter before the number. This letter indicates the province. For North Holland it is the letter G.

The sketches are filed per Association and alphabetically per stock-keeper, so that in a very short time we can turn up the sketches of a given farmer in our central office.

On behalf of the annual herd-testing we prepare a shed list of each farm. For that purpose we use the tuberculosis statement of the previous year. Newly-bought cows are added, as well as the calves born in that year.

On the shed list, therefore, all the cows present are mentioned, stating the number of their sketches, name of the cow, ear disc number or herd-book number, and year of birth. It is also noted whether they were free or not in the previous year.

This registration is done by laymen called shed administrators. These are frequently

young farmers who cannot get a farm, also inseminators who, in summer, work on an A.I. station.

They are in the employ of the Health Service and are paid by us. Every winter we have about 40 to 50 of them in temporary employment.

At our central office at Alkmaar we have a file for each stock-keeper. In this file the tuberculin lists of each year are kept. In addition we keep in this file all the details concerning the T.B. of the herd.

The herd-testings take place in the winter when the cows are in the sheds. The veterinary surgeons usually begin their work about the 15th of November, since they must have finished it on 15th of February at the latest.

They tuberculin test, using the intradermal method in the middle of the neck.

Before the second world war we used the ophthalmic method, i.e. the tuberculin applied on the conjunctiva.

Tuberculin applied twice and two check-ups was the method. That meant 4 visits to every farm and it took up a lot of time. For mass work this is also too expensive.

After that we changed to the intradermal method using the tail fold. In those days we injected tuberculin and checked up 72 hours later.

Every diffuse painful swelling was considered positive.

Finally, four years ago we changed to the intradermal tuberculin injections into the neck. The middle of the neck is very sensitive to reaction.

Let us say that the practising veterinary surgeon is now planning to start tuberculin testing because the cows in his practice are in the sheds.

The shed administrator sees to it that the tuberculin lists are brought up to date. He checks the sketches and ear discs and registers the new cows and calves, and then takes the tuberculin lists to the veterinary surgeon.

The veterinary surgeon then starts tuberculin testing on the farms in accordance with the single test. Besides doing his ordinary work he can tuberculin test 500 to 600 cows a week.

When, on a certain day, he has tuberculin-injected cattle on several farms, he sends a report (the so-called yellow card) to the health Service the same evening.

We can then, if we wish to do so, check up on these farms the third day to see whether the veterinary surgeon has done his job well. We do this regularly sometimes retesting the herds as well.

Moreover, we know in the office at any time how far the veterinary surgeon has progressed with the tuberculin tests in the area of his practice. This is because we have previously sent such a blank yellow card to the veterinary surgeon to be completed by him for each of his clients. We know how many clients every veterinary surgeon has and can therefore, by simply counting the cards received, see how many farms have been tested.

The third day the veterinary surgeon returns to the farm to make a check.

Each diffuse oedematous swelling is considered positive.

If the swelling is not diffuse but more circumscribed, then he must make measurements. Swellings of 2 to 4 mm are doubtful and larger ones positive.



*Tuberculination*

It makes a great difference on what farms reactions are found.

Alas! tuberculin testing with our P.P.D. bovine tuberculin is not 100 % reliable.

If the veterinary surgeon discovers new reactions on an infected farm (a farm with reactors) he then regards the new ones as positive reactions. But if he finds reactions on a farm which was formerly tuberculosis-free, he is very cautious with his decision.

If he is convinced it will quite probably not be tuberculosis, he will decide to make a comparative test. He makes this comparative test 6 weeks later with avian and bovine tuberculin. There is then the possibility that it will prove to be a non-specific reactor. It is also possible that both swellings are so small that the conclusion is no tuberculosis.

It may also be doubtful or positive.

In Holland we frequently contact skin lesions, especially with cows 4 to 5 years old, and these skin lesions often give a non-specific reaction.

Moreover, there are still various other causes which may give rise to non-specific reaction. The comparative test is indispensable and is a splendid means of arriving at a wellfounded decision.

When checking-up on the third day, the veterinary surgeon has the list of tuberculin-tested cattle with him. This is a list in quadruplicate. He needs only fill in +, — or ± and then give one list to the farmer, one list to the association; one list to the Health service; one list he keeps himself.

It is a rule that he sends the statement to the Health Service within a week after the



test. By means of the yellow cards received we can check whether the veterinary surgeon keeps to this rule.

The Director of the Health Service decides whether a comparative test shall be made.

If the veterinary surgeon finds reactors on a farm he must carry out a clinical examination of these reactors. In particular he should pay attention to affections of the womb and udder. If necessary he collects samples of pus and/or milk.

The sputum test is made by our service. Our cow shed administrators collect sputum from all reactors and, in various sheds with many reactors, also from the non-reactors, especially from the old non-reacting cows.

Three years ago we carried out only microscopic examinations. We then still had 50,000 reactors and found about 350 open lesions. After that we began cultivating sputum on a special culture medium: First the Assen 61, later the Assen 126 of our colleague Dr STONEBRINK from the town of Assen.

Whith this Stonebrink 126 we had optimum growth in 17-20 days. We have slightly modified the composition of the Stonebrink 126.

Two years ago, by cultivating a part of the sputum samples, we found 823 open lesions among 50,000 reactors.

Last year the number was 2264, because we then cultivated all sputa. There were well over 34,000 samples from about 40,000 reactors.

The composition of the Stonebrink 126 is as follows:

FORMULA STONEBRINK 126

A. Salt-mixture		B. Egg-mixture . . . . .	200,0 ml
Pyruvic acid	} . . . . . 1,0 gr	C. Dye-mixture	
5 n NaOH until pH 6,5			Crystal violet . . . . .
KH <sub>2</sub> PO <sub>4</sub>	} . . . . . 0,5 gr	Malachite green . . . . .	200 mg
Na <sub>2</sub> HPO <sub>4</sub> until pH 6,5			Water to make . . . . .
Water to make . . . . .	.75,0 ml		

In our modified Stonebrink 126 there is no crystal violet.

Mycobacterium tuberculosis grows much better when there is no crystal violet in the culture medium. Therefore our culture media are green-coloured and not violetblue.

Microscopic examination requires much time and many employees. Since we started cultivating we need 50 % fewer employees. We can deal with about 400 sputa daily. Our cow shed administrators send us the sputum in 0,5 N.H<sub>2</sub> SO<sub>4</sub>.

This is shaken and centrifuged and then inoculated into the Stonebrink 126. Two laboratory assistants are busy with this the whole day.

As we ourselves make arrangements for taking samples of sputum we can make sure that they are regularly sent to the laboratory.

If the cultivation is positive, we notice a characteristic growth in the case of bovine tuberculosis. It rarely happens that we then have to inject cavia.

Cows with open tuberculosis must be slaughtered within 14 days. The Health Service conveys these cows to a slaughter-house.

We receive a report of the section, in duplicate, one copy being for the veterinary surgeon. The cattle-keeper is paid the slaughter value and in addition a premium of f 150 (£ 15).

Normal milk cows cost from f 750 to f 1000 each (£ 75 to £ 100).

When slaughtered an animal with an open lesion brings about f 400 to f 600. To that the premium of f 150 is to be added. The cattle-keeper nearly always suffers a loss and this acts as a stimulus for him to get his shed T.B. free as soon as possible.

Further, every month we forward to the Medical Inspector of Public Health a list with the addresses of the stockholders on whose farms we have found open lesions. This Inspector distributes these particulars among the consultation centers. The stock-keepers are then invited to have themselves, their families, and employees X-rayed, as cattle tuberculosis is dangerous for man, and especially for children. Psychologically this has an extraordinary effect and our cattle-keepers now consequently show that they clearly realise the dangers of bovine tuberculosis.

It is often the best cow of the herd which suffers from open tuberculosis and sometimes the cattle-keeper cannot believe it. We then always invite him to come to the slaughter-house and satisfy himself that this is the case.

We sometimes take 4 or 5 open lesions at one time from a shed with many reactors (for example 20 milk cows, of which 12 react), but occasionally 6, 7 or 8.

Owing to the fact that we find so many open lesions we are able to satisfy the farmers more easily that they must isolate them. Isolation of reactors and free animals in the shed and in the pasture is very important! Moreover, they must give their young cattle t.b.-free cattle-fodder.

#### CERTIFICATES

Reactors are therefore low quality animals and consequently cows which are t.b.-free are more valuable. We have therefore introduced certificates for the t.b.-free cows. These are certificates which give particulars of the cow.

We recognize three kinds of herds.

1. *Reaction herds.* In these herds reactors are still present. Red certificates are issued for the free animals from reaction herds. These certificates are valid for 10 days. The last herd tuberculin test may not have taken place less than 8 weeks ago. If it is longer ago than 8 weeks, the veterinary surgeon must first tuberculin test the cow before issuing a red certificate.
2. *Reaction-free herds.* A cattle-keeper with a reaction herd sells his reactors. After he has sold the last reactor he must still wait 6 weeks. Tuberculin-testing may then be carried out again. If then there are no new reactors, the herd is called reaction-free.

White-green certificates are issued for cows from a reaction-free herd.

These certificates are valid for 10 days and the last tuberculin test must not have taken place more than eight weeks ago.

3. *T.B.-free herds.* A herd which has become reaction-free cannot be t.b.-free before the expiration of 6 months thereafter. For this a tuberculin test is necessary, at which time no reactors may be found.

White certificates may be issued for tuberculosis-free cows. These certificates are also valid for 10 days.

# North Holland Animal Health Service

## BOVINE TUBERCULOSIS CONTROL WHITE CERTIFICATE

This certificate has been issued in conformity with the rules and regulations laid down for the whole of the Netherlands by the Animal Health Service Committee of the Netherlands Agricultural Society in behalf of the accredited animal described below.

SKETCH TO BE ATTACHED HERE

Name of keeper .....	Name of animal .....
Residence .....	Sex: male/female .....
Address .....	Year of birth .....
An. Health Serv. Reg. Nr. or can nr. ....	Sketch                      Series                      Nr. ....
Veterinary Surgeon .....	Number of eartag .....
Dairy Factory .....	Number in herd-book .....
Association .....	Number in Reg. of Young Cattle .....
Date of issue of this certificate ..... 19.....	Breeders' Soc. Nr. ....
(in letters)	Other distinguishing marks .....

This certificate is valid till and inclusive of ..... 19.....	Latest date of tuberculin-test of the animal ..... 19.....
(in letters)	(in letters)
Issued by .....	(signature)

### FOOT- AND- MOUTH DISEASE

Latest date of vaccination of this animal

..... 19..... \*)  
(in letters)

Issued by .....

(signature)

\*) Completion of this line means that the animal is one of a herd vaccinated for foot-and-mouth disease.

THE NORTH HOLLAND ANIMAL HEALTH  
SERVICE

D. Rempt

Director.

Warning to buyers: **THIS CERTIFICATE IS OF NO VALUE, UNLESS PROVIDED WITH SKETCH.**

The certificate printed and completed overleaf is considered to have its full value for the Animal Health Service only during the period of ten days mentioned overleaf. This form is and remains the property of the Animal Health Service mentioned overleaf. Any regional Health Service within whose district this certificate is found, is entitled to withdraw it if such withdrawal should be considered necessary. Any change, modification or falsification of this form and/or the completed certificate is liable to punishment under section 225 of the Criminal Code. Any one reprinting this form is liable to punishment under section 31 of the act of 23 September 1912 (published in Government Gazette Paper nr. 308).

## TO BE COMPLETED BY

### THE RECEIVER OF THE ANIMAL

Date of admission .....

to the cattle stock of: .....

Name .....

Residence .....

Address .....

An. Health Service Reg. Nr. or can nr. ....

Dairy Factory .....

Veterinary Surgeon .....

### THE REGISTRAR

Date of surrender of this certificate .....

19.....

(signature)

Remarks, if any, to be added exclusively by the registrar to whom the certificate has been surrendered.

According to the prescriptions of the Animal Health Service of which the receiver of the animal is a member, this certificate has to be completed and surrendered within three days after admission of the animal to the new cattle stock.

The cows with white certificates have the greatest value on the market. Those with white-green ones a somewhat lower, and the cows with red certificates have the least value.

In order to protect the buyers as much as possible we have ruled that the sketch of the cow must be attached to the certificate. The farmer who wants to sell a 'free' cow goes with the sketch to the veterinary surgeon.

The veterinary surgeon issues the certificates and enters the particulars of the sketch (number of the sketch; number, name and date of birth of the cow, name of the owner) on the certificate. In addition he also mentions the name of the association or dairy factory on the certificate, the date of tuberculin-testing, and date of issue. The buyer can therefore check whether the sketch and the certificate really relate to the cow he wishes to buy. Falsifications of the certificates are punishable. The certificates are only valid for 10 days in order to prevent the cow going from market to market and increasing the chance of infection.

The cattle-keeper who buys a cow with a certificate and sketch must hand in the certificate within 5 days to his veterinary surgeon. The sketch remains with the stockholder. The veterinary surgeon makes a note of the new cow on the tuberculin-list of the farmer and sends the certificate to the Health Service. The latter puts the certificate away in the cattle-keeper's file.

Both the cattle-dealers and the farmers had to become gradually used to dealing with cows with certificates. This took at least 1½ to 2 years.

Things are now so far that they are used to the certificates and have confidence in them. This is very important for purposes of control.

#### FIVE-YEAR PLAN

As you already know, we launched a five-year plan in The Netherlands on 21st May 1951.

For the whole of The Netherlands a sum of about f 100,000,000 (10 million pounds) has been made available. Of this amount North Holland receives about f 11,000,000 (1,100,000 pounds).

This money is mostly spent to further the slaughtering of reactors. In our province we pay a subsidy of f 150 for each slaughtered reactor. Not all reactors receive the subsidy:

1. Only those which were present on the farm on May 1st, 1950 or
2. those which were then free and later became reactors, or
3. those which were born after May 1st, 1950 and have become reactors on the farm, or
4. those bought with a valid white or white-green certificate and afterwards have become reactors.

As soon as the herd has become reaction-free the farmer receives his slaughter premiums.

It is possible that later on when tuberculin-testing is carried out on this reaction-free or perhaps already t.b.-free farm, new reactions will occur. We call these cases reverses. These reverses must be sold within 20 days after they have been detected. If the stockowner has them slaughtered, he can get a slaughter premium of at least f 100 and a maximum of f 250. The number of premiums per herd is unlimited for these reactors.

This plan has now been in operation for nearly 2 years and it is working very well. On May 1st, 1951, 3,692 of the 12,471 farms with a total of 56,084 cows were tuberculosis-free.

There were 1,545 farms with 23,413 reaction-free animals. Total 5,237 farms with 79,497 t.b.-free cows.

On May 1st, 1952 there were 5,356 tuberculosis-free farms with 89,073 cows and 1737 reaction-free ones with 24,399 cows. Total: 7093 farms with 113,472 t.b.-free cows.

The cattle-owners are convinced of the great importance of fighting t.b.

For many stock-owners it is very difficult and expensive to isolate their animals. Isolation in the cowshed is often difficult in our sheds with their two rows of cows. In the pasture, too, isolation gives rise to difficulties; shortage of personnel, narrow ditches between the pastures of the different farmers etc.

Consequently there are numbers of stock-owners who sell all reactors at once and then buy free cows to replace them. They often do this long before the time when they should be free.

This mainly happens in the breeding area, thus in the districts of the dairy factories in the north and center of our province.

The return to healthy conditions was therefore effected much quicker there than we had expected. In the summer we had talks with the factory boards. We proposed to them altering the 5-year plan into a 3-year plan.

According to the provisions of the regulations of our plan we may make extra slaughter premiums available in case a factory or a district accelerates the restoration of healthy conditions. There are slaughter premiums left from farmers who had already made themselves free after May 1st, 1950 and before May 21st 1951.

If a dairy factory decides to make itself free in 3 years, all the slaughter premiums from May 1st, 1950 may be counted together. We therefore then regard the factory as being one farmer. We know how many slaughter premiums have already been paid and therefore also know how many there are still available. These can be used to assist farmers who on May 1st, 1950 had only a few reactors and who later got many of them. The paying of a larger number of slaughter premiums makes the 3-year plan attractive.

We have now got so far that all the dairy factories in the center and the north of North Holland will make themselves free in 3 years. On November 1st, 1953 the last reactors must have been cleared off, and after May 1st, 1954 the factories may not process any milk from farms where there are still any reactors. At the same time it will be forbidden to keep reactors in the whole of this area after May 1st, 1954. This will prevent the category of graziers, who do not supply milk, from keeping reactors. On 1st of May 1954 we then hope to have a complete area free with 9605 farms and 156,818 cows.

The part of the province below the North Sea Canal is still left with 2719 farms and 45,599 cows. Of these 45,599 cows, 20,589 still reacted on May 1st, 1952. The average reaction percentage here is 45 %.

This area is much more difficult to make free owing to the high reaction percentage of many farms. These farmers will do well to sell all their cows, including the free ones and start with a new free herd. Isolation is difficult here, both in winter and in summer.

Furthermore, there is a category of farmers who are not at all interested in the fight against t.b. These are the farmers near the large towns. At any moment they may receive notice that they have to evacuate their farms owing to towns extension. And they don't know where they will go. In The Netherlands we have too many farmers and too few farms.

Emigration affords the young farmers or the families with many children a good chance. It is and remains a problem.

But in this area, too, below the North Sea Canal, there is a nucleus of willing farmers. There are several of them who have restored their whole farms to healthy conditions all at once. There are also some who have achieved this via isolation or who are trying to do so.

Plans are being devised to accelerate by one year the restoration of this district, or perhaps parts of it, to healthy conditions.

What has been told about our province also holds good for the other parts of our country. Every province has its own problems but the total impression is that we in The Netherlands will certainly succeed in eradicating bovine tuberculosis within 5 years.

# ORGANIZATION OF THE FIGHT AGAINST BRUCELLOSIS BY THE PROVINCIAL ANIMAL HEALTH SERVICES

L. P. DE VRIES

*Secretary of the Health Committee for Animals*

I told something about the origin of the Provincial Animal Health Services and the way in which the scheme has been evolved from the voluntary scheme for the control of tuberculosis, by which each stock-holder is obliged by law to submit to the regulations for the control of t.b. These regulations are framed by the provincial health services, co-ordinated by the central animal health committee, and only become operative after they have been approved by the Minister of Agriculture, Fisheries and Food. This is in accordance with the provisions of the act for the control of tuberculosis.

In addition to the control of tuberculosis, which therefore has a statutory basis, the provincial health service occupies itself with the organization of the control of other diseases; this then takes place on an entirely voluntary basis.

It is almost self-evident that one of the most important diseases with which the health services have to deal is *brucellosis bovis*, or the abortion Bang.

In our country this disease is very rife. One might take up the view that this disease can be adequately controlled with the aid of vaccination with Strain 19. But we fairly often see breakdowns of immunity after vaccination. The health services have therefore devised a scheme which goes much farther, and it is primarily about this scheme that some particulars will be given. In this scheme the same system is actually applied as with the eradication of tuberculosis. It is determined what cows in a herd are to be regarded as reactors in respect of abortion. The herd can then be made abortion-free by the removal of the reactors or by segregating them. It is not possible to fight against two diseases in this way at the same time. Hence, the system of eradication of abortion is only applied in t.b.-free areas. A stockholder, who applies for this scheme of eradication, at the same time submits to the relevant regulations.

The health service charges the stock-holder who has joined the scheme a certain amount per cow per annum. Only this stipulation has been made, that the health service only accepts stock-owners for this scheme if at least 75 % of the stock-owners in a certain area or of a certain dairy factory apply. This rule has been made because abortion is so contagious. It is only to be fought in a community, in an association of stock-holders.

The regulations to which the stock-owner submits by applying for the control are as follows:



At least once per 3 months a sample of milk taken from the milk cans is tested by the health service according to the A.B.R. method (ring-test). The milk is usually put into cans of 40 litres on the farms; consequently milk from more than one cow is contained in one can. The test is sufficiently accurate to be positive if only a part of the mixed milk in a milk can comes from an abortion-positive cow.

If in any of the samples taken from the milk cans from a certain herd, a positive A.B.R. ring-test is found, the stock-owner can have the milk from all the milk-cows tested in the laboratory of the provincial health service. This is therefore an individual test per cow. In this way the infected cows are distinguished from the non-infected ones; the former can be segregated from the latter or removed from the herd. It is left to the stock-holder's own discretion whether or not he will do this under the scheme.

If the samples from the milk cans, taken in three successive quarters of a year are negative, according to the A.B.R. method (ring-test), the health service will at the request of the stock-owner test a blood sample of all the cows in the herd. If then the titre of all the blood samples is negative, the herd can be regarded as abortion-free.

The stock-owner who has joined the scheme for the control of abortion must at once segregate every cow which aborted or which shows symptoms indicating an approaching abortion, from the other cattle for at least 4 weeks after the abortion.

The aborted foetus and the afterbirth (fetal membranes) must be destroyed if they have not been sent for examination to the laboratory of the provincial health service for animals, in accordance with the regulations of that health service. The cows of which it is known that the milk or the blood gives a positive reaction, must shortly before and 4 weeks after parturition be kept segregated from the other cows.

Moreover, the stock-holder may have the young cattle vaccinated against abortion at the ages of 6 to 10 months with a vaccine approved by the health service (the Health Service in the province of Drente has made this vaccination compulsory).

Older cows may also be vaccinated if, in the opinion of the health service, the situation in the herd warrants this. Cows that have calved too early or from which the afterbirth did not come normally are not allowed to be served by a bull before three months after the parturition.

As told before a herd, after a negative blood test following the negative reaction of the samples from the milk cans in three successive quarters of a year, may be regarded as abortion-free.

This herd is registered by the provincial health service. For cattle in herds which have been registered as abortion-free, an abortion-free certificate will be issued on request in accordance with the regulations of the provincial health service. This is issued provided the herd is also t.b.-free. In practice this is so applied that a note is made on the t.b.-free certificate that the animal described on that certificate is abortion-free and that it comes from an abortion-free herd.

As regards the herds entered in the register of the abortion-free herds, the following should be noted. As a result of the regulations for control of tuberculosis, all the animals of all the herds, including a sketch of each animal, are exactly known to the

administration of the provincial health service. If a herd is to continue to be regarded as t.b.-free, only cows with a t.b.-free certificate may be added to the herd.

As soon as any cow is added to the herd, the t.b.-free certificate must be sent at once to the administration of the provincial health service.

If the herd is to remain on the register for abortion-free herds then only animals may be added to the herd which are provided with a t.b.-free certificate, on which at the same time it is noted that they are abortion-free and come from an abortion-free herd.

If a herd has been certified abortion-free, then the rule remains in force that every three months samples from the contents of the milk cans must be tested in the laboratory of the health service. If a sample is found to be positive the herd will be removed from the list of the abortion-free herds. The herd will also be removed from the list whenever, in any other way, an abortion is detected.

Moreover, there is a stipulation that cows in an abortion-free herd may only be served by bulls from abortion-free herds.

A herd removed from the list can be replaced on it if the samples from the milk cans, taken in three successive quarters of a year, and a subsequent test of the blood of all the animals, were negative.

The provincial health services have thus provided the opportunity in areas which are t.b.-free to arrive at abortion-free herds in accordance with a definite scheme. In carrying out this task there are to be distinguished:

1. Provision for the testing of milk and blood. The health service takes the sample of milk from the milkcans at the dairy factory when the milk is delivered there. An apparatus has been made to enable the samples to be taken quickly. The samples of milk from the individual cows are taken on the farm. This too is done by the health service. The samples of milk are tested in the laboratory of the health service by means of the ring test. The samples of blood are taken by the practising veterinary surgeons. The farmer pays the fees for taking the samples of blood directly to the veterinary surgeon.

The blood is subjected to an agglutination test in the laboratory of the health service. A titre of 1:50 is counted as positive (this can be deviated from if, in the case of a heifer which has been vaccinated a short time before, the titre is higher).

2. The provincial health service keeps a record of the results of the A.B.R. tests, both of the samples from the milk cans and the samples from the individual milk cows, and also of the results of the agglutination tests of the blood.

Further, a record is kept of the animals (heifers) vaccinated with strain 19.

3. The provincial health service keeps a list of the herds which may be regarded as abortion-free.

4. For cows in abortion-free herds the service, on request, issues abortion-free certificates (endorsement on the t.b.-free certificate).

The health services are convinced that the fight against abortion is an extremely difficult task if carried out in the way described. In those parts of the country in which

a start has been made, the health services did not start them before exhaustive discussions had been held both in meetings of members of the dairy factories and of members of associations corresponding to them.

The farmers have discovered that it has been possible to bring the fight against tuberculosis to a satisfactory finish; this gives them confidence in undertaking the fight against abortion. They have learned that the eradication of tuberculosis can only succeed if they afford their full co-operation, based on knowledge of the disease and the measures to be taken. Armed with this knowledge, the farmers in the areas which have become t.b.-free do not shrink from submitting to the onerous demands which have to be made to become abortion-free as well.

And what do the provincial health services do in places where the fight against abortion cannot be taken up in accordance with the abovementioned scheme? This, of course, still applies to the greater part of our country. In those places as much propaganda as possible is made for the vaccination of the herds, which means that the young animals are vaccinated with Strain 19 at the ages of 6 to 10 months.

When the whole of The Netherlands has become t.b.-free, and when the scheme described above has proved that it is possible to render areas abortion-free, it may then be contemplated enforcing the scheme for the whole of our country.

But we are not yet so far; we are really still in the experimental stage. We are, however, convinced that in certain districts the scheme has a good chance of success. This will be achieved by intense co-operation between the provincial health services for animals and the farmers, by involving the dairy plants, and perhaps other associations of farmers.

# BRUCELLOSIS

## VACCINE PRODUCTION AND DIAGNOSIS

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### VACCINE-PRODUCTION

In 1946 the *attenuated Brucella Bang strain 19* has been introduced in our country as vaccine against infectious abortion of cattle. The favourable properties of this strain are: immunizing potency, it maintains its attenuated form so does not revert to a virulent phase, it does not induce abortion when not applied too late in gestation. Experiments have established that only large doses administered intravenously can provoke abortion, but the organisms never spread in the herd and the milk does not contain strain 19 bacilli. When calves are vaccinated at the age of 6-8 months, the agglutinins disappear in the course of 1 year. On account of all these excellent properties, strain 19 has become popular in the greater part of the world. During the first 3 years the vaccine prepared with this strain consisted of a broth-culture of 4 days. The disadvantage of this vaccine was that the number of living organisms practically did not exceed 500 millions per 1 m.l. We tried to compensate this by inoculating twice, respectively 10 and 20 ml., with a 3 week interval. If the broth-cultures were not immediately used by the practitioners, a great number of the organisms were killed by shaking during transport and high temperatures in summer. As only living *Brucellae* have immunizing properties, this loss involves a lower potency of the vaccine. Further a contamination of the vaccine easily occurs, when out of the same vial a dose is drawn off several times a day. This contamination can cause oedema, phlegmons and abscesses at the site of inoculation. Further a dissociation of the active smooth germs into the inactive rough forms in broth-media occurs.

Three years ago another technique of vaccine preparation was introduced in our country, namely the *lyophilized or freeze-dried vaccine*. This consists of pure smooth cultures of strain 19 on potatoe-infusion-agar that grow for 2 days at 37 °C, because this is the period of highest viability of the organisms. These are washed off the agar medium in the Roux's flasks and collected as a thick suspension in a flask. Careful control tests are carried out in order to establish the purity of the product and the number of living organisms.

Sterile ampullas are filled with several doses of vaccine for 1, 5, 10 or more cattle and then rapidly

frozen and dried at a temperature of  $-20^{\circ}\text{C}$ . Then follows a subsequent dehydration by phosphorus pentoxide,  $\text{P}_2\text{O}_5$ , in a desiccator and finally the ampullas are sealed under vacuum. The counting of the surviving organisms in this freeze-dried final product enables us to establish their percentage compared with the number in the concentrated suspension before lyophilization.

To each ampulla a vial, filled with sterile distilled water, is delivered by means of which the dried vaccine can be emulsified and be prepared for use in a few seconds. The single dose for cattle is a suspension of 0,4 ml dried organisms in 5 ml of distilled water.

The advantages of the freeze-dried vaccine over that of the former broth-cultures are many. The number of surviving organisms, that can be standardized to a certain degree, is approximately  $60-80 \times 10^9$  per ml, consequently 5 times the number in 20 ml of the broth-vaccine. In freeze-dried state the strain remains in the original smooth-form, can be stored for an almost unlimited period, provided it is kept in a dark and cool place. Contamination, if possible, is not so serious because the few airborne organisms cannot multiply in a dried condition and, when the practitioners carefully prevent infections by using sterile syringes, the application of the vaccine cannot cause any complications.

The number of strain 19 organisms needed to confer a solid immunity to cattle is not known, but generally in different countries a technique is followed by which the greatest possible yield is reached. In this respect the process of freeze-drying means an extraordinary improvement in our methods of immunizing against Brucellosis.

#### DISAPPOINTMENTS WITH THE APPLICATION OF STRAIN 19 VACCINE

Many of the complaints concerning the efficacy of strain 19 vaccine date from the first years of its use, when people were not yet acquainted with its properties. The greater part of the criticism refers to abortion. But in many cases vaccinations took place during an abortion-storm and a number of cattle were already infected. In the herds, injudicious replacements of cattle can undo the favourable results of the inoculation. Another unjustified complaint was, that vaccination would impair the fertility. LAWSON has conclusively proved that this view was not correct and that the conception-rate increased by immunization against Brucellosis. Other objections of less importance were: fever, in many cases as high as  $42^{\circ}\text{C}$ , decrease of the milk yield, that especially at the end of the lactation period can remain disturbed until the termination of the gestation, considerable oedema at the site of inoculation. According to KARSTEN the latter is due to allergic conditions of cattle in infected herds. The greater number of these swellings is caused by infections as a result of a careless inoculation technique.

Insufficient immunity and overwhelming by virulent infections is also a rather frequent complaint. But how many farmers think that after vaccination of their cattle all further precautions to prevent the infection have become superfluous. We must emphasize that vaccination is only a support in the combating of Brucellosis, but is in itself by no means effective to withstand a serious virulent infection.

#### BLOOD REACTIONS AFTER VACCINATION WITH STRAIN 19

The presence of agglutinins in the serum of cattle treated with strain 19 vaccine can be very inconvenient, especially when this concerns animals that are used for

breeding purposes and that must be eliminated in case of Brucella-infection. Long experiments have shown us that the most suitable time to vaccinate cattle is at the age of 6-8 months, not because then the best resistance is created, but because the appearance of the agglutinins in the blood is of a temporary nature so that the blood-titres are negative again one year after treatment, in more than 90 % of the cases, and certainly at the age of maturity. If inoculated later, i.e. at the age of 1 year, the agglutinins may persist for a much longer time, sometimes for several years, in the blood of heifers. Normal blood titers become positive 6-17 days after vaccination and also the agglutination test of milk whey and the A.B.R. reaction are positive for approximately 3 months due to a diffusion of the reactive bodies from the blood into the milk. Some calves react slowly and positive blood titers become traceable after 3 weeks whereas 5 per cent of the young animals do not show any titre at all.

STABLEFORTH noticed that, when heifers have lost their vaccination titres, the agglutinins may reappear at the time of the first parturition but only for a short time.

We have no absolutely reliable method to differentiate vaccine- and infection titres. As is mentioned before, the first appear 6-17 days after inoculation. Suppose a heifer may have a persistent titre of 1:100, then revaccination causes a higher index, i.e. 1:400 within 6-17 days in case the initial titre had been the result of a previous treatment. Should the first titre be due to a virulent infection, then an injection of strain 19 results in a slow increase of the titre that lasts for 1 month or longer. VAN DRIMMELEN has found another method of differential diagnosis of infected and vaccinated bovine reactors, namely by applying the A.B.R. milk reaction. In either case the milk can react positively with the A.B.R. test but a vaccine-reaction only lasts 3 months after treatment and disappears in the fourth month.

VAN DRIMMELEN made serial dilutions of the milk of the suspected cow in normal milk and after adding 1 drop of coloured antigen the tubes were placed into the incubator for 50 minutes. In the case of a virulent infection the A.B.R. reaction is positive in at least 3 tubes, but when cows have previously been vaccinated, their milk shows a ring in the first tube only, the distinction of which decreases and disappears in the third to fourth month, whereas the blood titre may remain high.

#### SERO DIAGNOSTIC

For this purpose the sero-agglutination reaction is chiefly used, which must be differentiated in the rapid test that is performed on the heated glassplate, and the tube test. The first method, although not used in many other countries, is very popular in the Netherlands. We need for it a rocking case, in which two electric bulbs allow a moderate heating of the glassplate that is ruled off into inch squares with a glass cutter or diamond. The advantage of the rapid method over the tube-agglutination test is that the results can be recorded within some minutes.

#### TUBE-AGGLUTINATION

Therefore, generally, two methods are used. The first is that in a series of tubes 0.1 ml of serum dilutions 1:5, 1:10, 1:20 etc. is introduced to which 0.9 ml of antigen is added, so final serum dilutions 1:50, 1:100, 1:200 etc are reached.

Another method is that in the first of a series of tubes 0.9 ml of physiological saline is put and in each of the following tubes 0.5 ml of saline. In the first is added 0.1 ml of serum so that we get there in 1 ml serum dilution 1:10. 0.5 ml of this is transferred to the second tube in which, after mixing thoroughly, 1 ml of a serum 1:20 is reached. By transferring 0.5 ml of the serum dilutions to each following tube and adding the same amount of antigen to all tubes, final serum dilutions 1:20, 1:40, 1:80 etc. are performed all in a quantity of 1 ml. The tubes are placed in the incubator for 24 hours at a temperature of 37 °C and the results are here after immediately recorded. A complete agglutination is characterized by an antigen-sediment and an almost waterclear supernatant liquid column.

#### PLATE AGGLUTINATION OR RAPID TEST

For this method serum amounts of 0.08, 0.04, 0.02 and 0.01 ml are pipetted into the squares of the glass plate, to these equal amounts of 0.05 ml concentrated antigen are added and, after the liquids have been mixed, the electric bulbs switched on. By rocking the illuminated case and the moderate heating of the plate, the agglutination-reaction is accelerated. The optimum time for this reaction is indefinite, but the results are recorded as soon as the border of the fluid begins to dry. A positive reaction is complete when in a waterclear liquid white flocculations appear.

The above-mentioned amounts of serum diluted in 0.05 ml of concentrated antigen represent the equivalents of serum dilutions respectively 1:25, 1:50, 1:100, 1:200 used in the tube test.

#### INTERPRETATION OF THE RESULTS OF THE AGGLUTINATION TEST

In our system of interpretation the titer 1:100 is the minimum positive titer, 1:50 is doubtful and the serum test is repeated after 2 weeks. 1:25 on the plate is negative and due to presence of normal agglutinins.

#### THE ANTIGEN AND ITS OPACITY

Strain 19 is used as antigen on account of its low virulence, its slight tendency to dissociation by which it is easily kept in smooth form. Smoothness is the first essential for a reliable antigen. For production of antigen 3 days old growth on dextrose-glycerine-agar is used because, although the yield is less than on potatoe-agar, its sensitivity is far more reliable than when cultivated on the latter medium. For tube test antigen a bacilli suspension in 0.5 per cent phenol-physiological saline is prepared with an opacity that matches tube no 2 of McFarlands or Brown's nephelometer. The density of the plate test antigen is 75 times that of the former one.

When a new batch of antigen has been prepared, its concentration is approximately adjusted to that of the two antigens of the former batch. By the rapid and tube agglutination the titres of samples of dried sera are determined in such a way, that those found with the plate test must coincide with those recorded by the tube test. So of each serum sample 4 titres are found that must be identical.

By varying the opacity of the new antigens, their sensitivity can be adjusted to that of the former ones. When this is obtained, there is no reason for preference of one of

the two agglutination methods, but as the rapid test is an empirical one and the tube test is submitted to certain general rules, the latter is used in all cases of greater importance, i.e., the export, whereas the rapid test is applied to the routine examinations.

#### SOME PHYSICO-CHEMICAL VIEWS OF THE AGGLUTINATION PROCESS

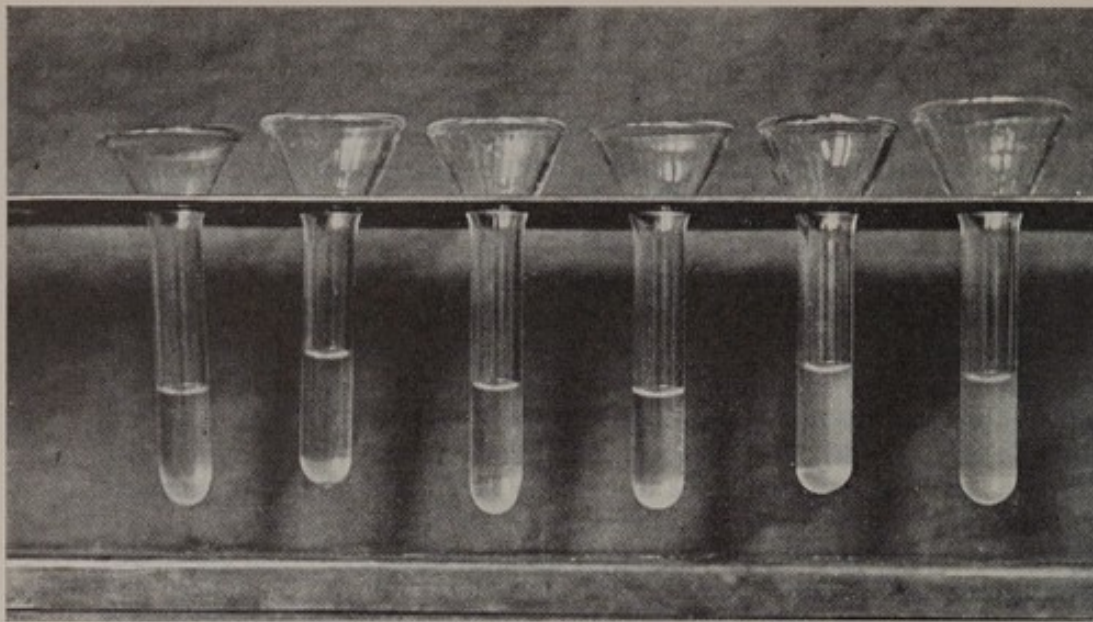
An absorption of agglutinins by organisms is not to be compared with a chemical combination. The way in which agglutinins are absorbed is a very complicated process. According to EISENBERG and VOLK, in the higher serum dilutions proportionally more agglutinins are absorbed than in the lower ones by a definite antigen-opacity. In a serum dilution 1 : 300-1 : 10,000 all agglutinins are absorbed, in a serum dilution 1 : 10 and 1 : 50, respectively containing 2000 and 400 agglutinin units, only 1500 and 340 units are absorbed irrespective the titre of the serum. This failure in complete absorption of the agglutinins appears in those serum dilutions where the dubious titres occur somewhere about 1 : 20-1 : 100. The degree of absorption does not take place in proportion to the opacity of the antigen. When, of a serum dilution that contains 20,000 agglutinin-units, by an antigen of a definite opacity, 11,000 units are absorbed, then a 6 times higher antigen-concentration only absorbs 13,000 units and a ten fold concentration absorbs 15,000 units. Consequently, an antigen with a high opacity in proportion to its concentration has relatively less absorptive capacity than a thin antigen which is agglutinable with a smaller amount of agglutinin-units, in other terms the sensitivity of an antigen decreases according to its concentration. With an antigen of higher concentration we can for example find a titre 1 : 50, whereas a reduced opacity indicates a titre 1 : 100 of the same serum. After all the agglutinability of an antigen is not constant, so the whole agglutination-reaction is unstable, and we may say that any serum dilution needs its special antigen-opacity in order to attain complete agglutinin absorption.

We do not prefer concentrated antigens, especially not for the tube test, because in the higher serum dilutions the agglutinin absorption is complete and of course the negative titres do not yield any difficulties. But the point at issue is the lower serum dilutions 1 : 20-1 : 100 where the doubtful titres are found. With a 5 times higher antigen concentration perhaps only 25 per cent more agglutinins are absorbed, however, still this is incomplete, but the fluid remains turbid so the interpretation is difficult. On the other side, when the antigen is too thin we get even agglutination with normal agglutinins which can be confusing.

To determine the correct opacity of antigens used in different countries STABLEFORTH has prepared samples of dried standard serum. Of this serum, resuspended in 1 ml of water, the titre is defined with the antigen used in the country in question. STABLEFORTH has calculated that the minimum positive titre of any serum has to be  $\frac{1}{10}$  of the titre of his standard serum found with the antigen of that country. The titre of that standard serum determined by our agglutination method was 1 : 800, so our minimum positive titre should be 1 : 80 instead of 1 : 100 according to our regulation. But carried out with our antigen according to the British method, the agglutination yielded a titre 1 : 1000, to the minimum titre 1 : 100 has been maintained for our serum tests.

We can say that we have adjusted our system of sero-agglutination more or less to a standard suggested by STABLEFORTH. That prevents us from losing any contact with general conceptions about the serological examination of *Brucella* Bang. But this does not effect the possible introduction a uniform system by which it is possible to differentiate with absolute security the infected from the non-infected animals. When all the positive and negative cases are detected, there remain in every system a certain number of doubtful reactors the handling of which cannot be standardized. In our system the doubtful titre is 1:50 and by repeating the serum test 2 weeks after the first examination, it can be established whether the titre persists or is liable to variation. In the first instance we assume that the reaction results from normal agglutinins and such animals are recorded negative. In the case where the titre increases in not-vaccinated cattle, they are positive. There is a theoretical possibility of a decreasing





*Brucellosis.*  
*Tube agglutination*  
*of serum.*

titre i.e. a declining vaccination-titre or in a cow that is becoming a 'ceased reactor', but this does not take place in a noticeable degree in 2 weeks.

The statement, that the decision whether a cow is infected or not cannot be left only to a laboratory-examination, but that also other factors referring to an animal in question must be considered, has proved to be correct.

#### THE ABORTUS BANG RINGTEST OF THE MILK

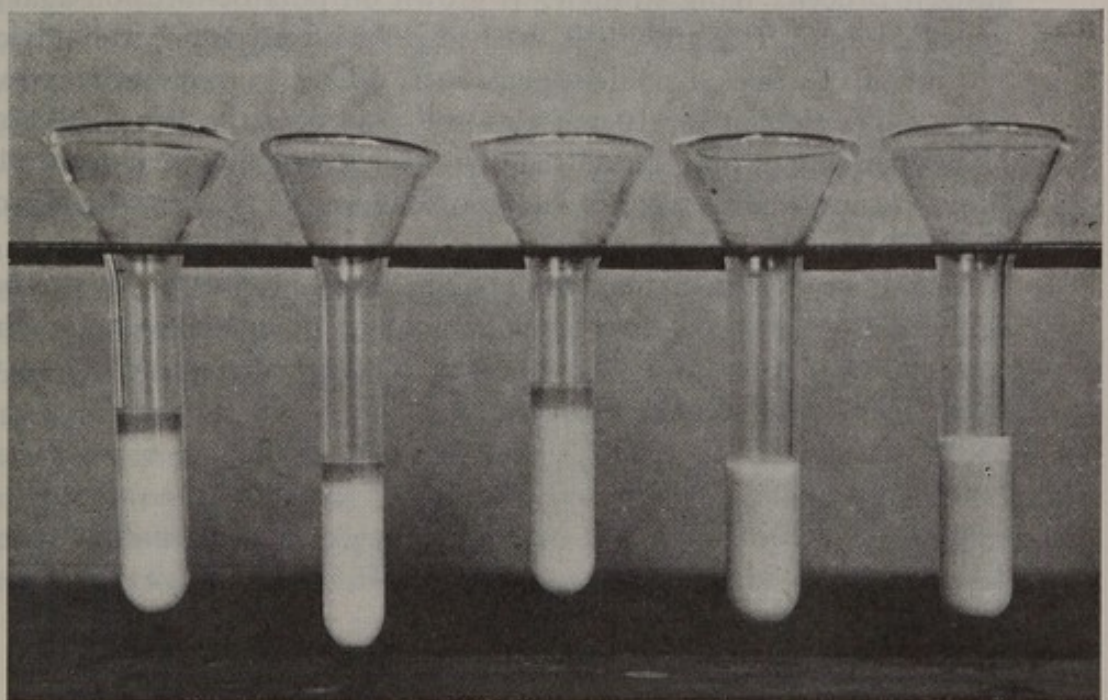
This method of detecting brucellosis in cattle has become known almost throughout the world. The milk test is performed in this way: to 1 ml of milk 1 drop of coloured antigen is added, and after mixing this sample is placed for half an hour at 37 °C. In case of infection the cream layer contains the coloured bacilli of the antigen and has got a clear tinge, whereas the milk column has discoloured more or less. In negative milk the cream remains white and the milk is coloured. In literature we find on the one hand a high appreciation of this ringtest on account of 95–99 per cent agreement with the results of the serum agglutination-test (KAPLAN), on the other hand indications of little estimation because 25–30 per cent of serum and milk tests yielded divergent results, i.e. more positive A.B.R. reactions than serum titres. When the cattle at the time of testing are in milk production or when the examination of the milk is repeated with 1–3 months interval in order to catch all the animals during their production, the agreement exceeds 90 per cent. In Denmark the A.B.R. test is reported to be positive in 82 per cent of the reactor herds.

The statement, that the A.B.R. would be more appropriate for pooled milk than for individual milk, must be defined more fully. Some people understand by pooled milk, that of a whole herd mixed in the vessels of a factory, but according to others it is the contents of a can in which the milk of 4–5 cows is transported to the factory. As a matter of fact, the A.B.R. is so sensitive that, when pooled milk contains for a small part milk of infected cows, the reaction can be positive. According to the experience of lactologists, a dilution of positive milk of one or two cows in milk of a great number of non-infected cattle is not favourable for a reliable A.B.R. reaction. But the latter

can be more distinct when carried out with the milk from a small number of animals in case the positive milk has a low percentage of fat. For the reaction depends on the amount and the condition of the cream. In pasteurized milk the heating has destroyed the structure of the fat globules and the A.B.R. reaction cannot be accomplished. A sample of positive individual milk can show an incomplete A.B.R. reaction when the percentage of fat is low and adding some cream of normal milk will be sufficient to create a distinct positive ring. Besides lack of cream, there are some other reasons that influence the A.B.R. test i.e. mastitis milk can inhibit this reaction or *Brucella* negative milk can create nonspecific reactions, for negative milk does not react, provided it contains a low number of organisms. Further normal colostrum can show a positive ring during the first one or two days. And finally the incubation time, in which a reliable A.B.R. reaction has to take place, must not be too long.

There are two conceptions of the nature of the A.B.R. test. The original idea was that the milk serum of infected cattle contained agglutinins either formed in the udder or derived from the blood by diffusion. These milk-agglutinins, brought into contact with *Brucella*-antigen, cause flocculation of the organisms, and the cream, rising to the surface of the milk column, takes along these clumps by way of a sifting action. Owing to this, the milk column turns white whereas the antigen concentrates in the creamlayer and this takes a vivid colour. In negative milk the non-agglutinated bacilli remain in suspension and slip through the interglobular spaces, the milkcolumn is coloured but the cream is white.

This conception must be revised. We know that the fat globules are covered by a haptogen membrane of globulins that prevents them from confluating. But agglutinins are also globulins and these are absorbed in the same way on the fat globules. So only a part of the agglutinins are dissolved in the milk serum, the greater part is concentrated on the fat corpuscles. In case of *Brucella*-positive milk the bacilli are agglutinated on the latter and transported to the surface. The optimum temperature, that favours this process, is 37 °C, irrespective of whether it is reached in a waterbath or in an incubator.



*Brucellosis.*  
*A.B.R. milk test.*

The main principle of the A.B.R. reaction is that the surface-tension of the fat globules is the cause of the absorption and the smaller these globules are the greater is their surface-tension.

Consequently, when only traces of agglutinins are found in the milk, the smaller fat globules especially can absorb them and the bigger ones fail to do that. Also normal agglutinins can occur in such low concentration that they cannot be detected by the milk whey-agglutination, but they can be concentrated on the surface of the small fat globules and not on the larger ones. A great number of non-specific reactions can be provoked by allowing the smaller fat globules, that have absorbed normal agglutinins and react to the contact with antigen, to rise to the surface after the optimum time. This time is  $\frac{1}{2}$  hour at 37 °C and the results are recorded immediately after the milk samples leave the incubator.

These findings make it clear how positive A.B.R. reactions can be associated with negative serum titres and even with negative wheyagglutinations. Our lowest dilutions of serum and whey in the tube agglutination are respectively 1:50 and 1:10, because lower titres are recorded as negative. But there may occur traces of agglutinins in the blood that are not detected in serum dilutions 1:50 and, after being excreted in the milk, also give negative whey-reactions in dilution 1:10, but these traces are absorbed and concentrated on the surface of the fat globules with the highest surface-tension, i.e. the smaller ones. So positive A.B.R. reactions may occur, although they appear behind the time whereas the whey- and blood titers were supposed to be negative.

According to WOOD, when spreaders secrete the *Brucella* organisms in the milk, they are concentrated in the cream 50-100 times as much as in the milk serum. We now understand that most of these bacilli are agglutinated on the fat globules.

In association with some provincial health services comparative A.B.R. tests are carried out with the original violet-antigen, coloured with haematoxylin, and the new red preparation of BENDTSEN for the staining of which triphenyltetrazolium chloride is used. The results of both these tests were identical. For the preparation of Bendtsen's antigen *Brucella* Bang strain 19 is grown on potatoe-infusion-agar in Roux's flasks for three days. The growth is washed off with glycerin-physiological saline and to 500 ml of the thick bacilli-suspension is added 1 gramme of tetrazolium chloride. After being shaken for 10 minutes and placed in the incubator for 3-4 hours, the bacilli have taken on a blood-red colour. Then phenol is added and the cell-concentration is adjusted to 5 per cent. According to this prescription a product is obtained of absolute uniformity.

It is to be expected, that this antigen, used in comparative tests with the serum agglutination, will yield a higher percentage of agreement than the original violet antigen, as this is a very complicated product, the sensitivity of which will show divergences in different countries.

# THE ERADICATION OF BANG'S DISEASE IN PRACTICE

P. SJOLLEMA

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For the eradication of Bang's disease the same systems can be applied as with the eradication of most other infectious animal diseases. Two systems are especially relevant here.

In the first place it can be stated, after examination, which is a sick animal and which a healthy one. On the basis of this knowledge further attempts can be made, with the help of hygienic measures, to get a herd free from disease. This is the system as it is applied for the eradication of tuberculosis.

In the second place, one can try to make the herd immune by vaccinations. This system is now mostly applied when eradicating foot-and-mouth disease.

When eradicating T.B. we depend entirely on the first method and a combination of the first and second method is quite impossible, but with the eradication of Bang's disease the situation is somewhat different. Here a combination of the 2 systems is indeed possible. This means, on the one hand, an advantage but, on the other hand, there are also disadvantages; we shall see this later.

A comparison with the struggle against T.B. teaches us, however, still another fact which must be thoroughly considered in the fight against infectious abortion. T.B. does not transmit itself easily from one farm to the other when no cattle transport takes place. Because of this, a herd can be made T.B. free, and remain so, while the surroundings are still infected.

The spread of infectious abortion is, however, much more easily achieved from one herd to another. No cattle transport is necessary for this. In infected surroundings, one frequently sees a free herd fall a victim to infection with *Br. abortus* when no new supply of cattle has come to the farm.

This is a frequent phenomenon, especially in a region that is densely populated with cattle. The Province of Friesland with an area of 323,400 hectares, for instance, has about 400,000 head of cattle. This is 1.25 beasts per hectare. Further, the fact must be considered that this province is very low lying and that the divisions between the pastures are mostly formed by ditches and small canals filled with water. It will be obvious that the dispersion of this disease from one farm to the other is easily affected in such a district even without cattle transport. From this it follows that the eradication of the disease is no easy task. But it will also be obvious that the eradication must be

done in an organized way. The position of the individual cattle-owner is too weak. The fight must be a regional one. The farmers in a certain district must be induced to co-operate in some sort of organisation.

This can best be done by means of the local cattle-breeders organisations. In Friesland this can be done in a most efficient way by means of the dairy factories which are linked with the cattle owners. In this province  $\frac{4}{5}$  of the cattle owners are members of a co-operative dairy factory and the rest are affiliated to a private dairy factory. It does not often occur that the farmers change factories. Thus the factories have a permanent group of cattle-owners who deliver milk to them. Because of this the dairy factory must be considered as a local unit of farmers. This unit is very accustomed to working in groups to combat disease. (The eradication of T.B., foot-and-mouth disease, and warble fly is arranged for in this province by these local units).

In organizing the fight against Bang's disease the example of Denmark has been especially followed. In Friesland, where T.B. was mastered in 1950, the organized combat of Bang's disease was started in 1951.

The participation of the farmers in this combat is only possible in conjunction with a dairy factory. As the combat must be a regional one, at least 75 % of the cattle-owners of a dairy factory must be willing to participate in the struggle to eradicate before a factory can be affiliated.

In general the interest for this struggle against the disease appears to be very great and within 2 years  $\frac{3}{4}$  of the dairy factories have already joined. This participation takes place on a purely voluntary basis.

The purpose of it is to impart to the farmers an understanding of the possibilities of the methods of eradication applied. Because of that they will be willing later on to give co-operation to more extensive measures; measures which must lead in the long run to making free a whole district. Experience with T.B. eradication has taught that it is only possible to make great progress when the full co-operation of the farmers has been obtained. This can only be done little by little, when the advantages and possibilities have been made clear to them. All this demands, one way and another, time and pains from the Health Services. Many introductory speeches must be delivered at factory meetings, many visits must be paid to farms where there are difficulties, etc.

Before discussing the process of organized eradication it seems to be very useful to dwell first on the methods being applied in the laboratory for the examinations on Bang's disease.

The methods for finding Br. abortus and the serological methods for the demonstration of anti-bodies must be mentioned.

#### EXAMINATION OF THE FOETUS, PLACENTA, DISCHARGE, ETC.

When a cow has aborted, the foetus, the placenta, or the discharge can be sent for immediate identification of brucellosis.

When, as is the case in Friesland, the laboratory is in the centre of a province rich in cattle and to which daily carrier services travel, the aborted foetus is the best

material for the immediate demonstration of brucellosis. Of this foetus, the stomach or the bowel contents are examined.

If the material must be sent by post, a piece of placenta is more appropriate. Also some discharge (for preference obtained before abortion) can be sent in such cases. The great disadvantage of placenta or discharge is that this material is usually contaminated with other bacteria. Placentas that are not fresh are much less suitable or even quite unsuitable for examination.

The examination of the material takes place by microscopic examination of stained smears and by the setting up of cultures.

*Smears* are made from the stomach or bowel contents of the foetus, possibly from material of the placenta or discharge. These smears can be stained according to the method of HANSEN. By using two staining materials a contrast stain is obtained. For the preparation of the first stain 50 cc of 0.04 % KOH are added to 15 cc saturated alcoholic methylene blue solution. The smear is stained with this for 2 min. and then rinsed. After that it is stained with 1¼ % watery solution of safranine (Geigy) for a period of 10 seconds. *Br. abortus* has a blue color in these smears and lies mostly in little clumps on a red background. In many positive cases, especially with the foetii sent, it is not necessary to make a culture.

*Cultures* are made on a culture medium of Difco-tryptone glucose extract agar, to which is added Poviet extract, glycerine and glucose. For holding back undesired bacteria gentian violet (Geigy), 1 to 250,000 is added. The prepared plates are incubated for at least 3 days at a temperature of 37–38 °C in a 10 % CO<sub>2</sub> atmosphere.

Placenta and discharge only lend themselves for this latter examination when they are fresh and unsoiled.

In 1952 764 foeti or placenta were examined. Among these brucellosis was found 256 times (further; vibrio foetus, among other things, was cultured 34 times and *coryn. pyogenes* 28 times).

In 261 negative cases, it was possible to examine afterwards the blood or milk of the aborting cow. 45 times (17 %) there was a positive milk or blood reaction for brucellosis. Therefore we can say that in about 15 % of the abortions caused by *Br. abortus* we have not detected the germ in the foetus or placenta.

## SEROLOGICAL EXAMINATION

### a. *Blood serum examination*

Only the agglutination test is used. This is the simplest to apply for mass examination. The agglutination test is carried out in 2 ways; in the form of the tube-test and as the plate test.

The tube-test is carried out in the dilutions 1:25, 1:50, 1:100, 1:200. The antigen used is the same as mentioned by Dr DE MOULIN. For the technique also reference can be made to his writings. The examination takes 18–24 hrs. Reactions in the dilution 1:50 are considered doubtful; those in dilution 1:100 and higher, as positive.

By preparing a much thicker antigen, the agglutination test can be executed as a plate test. By this, the result of the examination is obtained in a couple of minutes.

With a certain preparation of antigen and way of execution the plate-test can be of quite equal merit to the tube-test. Because of this it can replace the tube-test for the greater part.

With the ordinary examination, within the framework of the eradication, the plate-test is therefore always set up first. When this gives a negative or clearly positive result no tube-test is done. The tube-test is therefore only applied when the plate-test gives a doubtful or weak positive result. The result of the tube-test is then decisive.

b. *A.B.R. test*

For the execution of this test, together with the preparation of antigen, one can refer to what Dr DE MOULIN has stated. In our laboratory the antigen described by BRUHN is always used. With this haemotoxylin is used as colouring material. The preparation of this antigen is more laborious than that of BENDTSEN where the bacteria are vitally coloured with triphenylterazolium chloride. But the first antigen gives reactions which remain a longer time at the same level. When using this antigen it is not necessary to read the reactions immediately after the samples have been removed from the incubator. There is some freedom of time to read the reactions. This is an advantage when some thousands of samples must be examined every day.

The A.B.R. test is executed in the first place with so-called cansamples. These are samples taken from the milk cans as they are delivered to the dairy factory. From this, an insight can be gained into the question whether infected cows occur on the herd supplying the milk.

For this purpose can-samples are much more suitable than a sample from the tip-basin of the weighing machine. In this case only one sample of the whole herd is obtained. For big herds there is, with a tip-basin sample, a chance that there will be too strong a dilution, and with small herds a chance that one sample infects the other. With can-samples the dilution is not so great, so that in practice the infected herds can be pointed out with sufficient accuracy, even if the herd has only one reacting cow.

If one wants to trace further the reacting cows of an infected herd, milk-samples can be taken separately from the cows and examined according to the A.B.R. method. Also it is possible to take blood-samples from cows for serological examination. This is, however, more cumbersome and more expensive than the first. Besides, the milk examination can be repeated easily. The individual milk examination gives very satisfactory results, therefore it can replace for the greater part the blood examination in the affected herds.

With the A.B.R. method – especially when it concerns the milk of individual cows – the possibility of wrong indications must be considered. For instance, milk originating from a mastitis-case gives a positive A.B.R. reaction, this may also be the case with milk from cows which are ending the lactation period and with milk from cows that have just calved.

By similar causes, can-samples may also sometimes give doubtful or weak positive results. In cases where the can sample examination of brucellosis-free herds gives doubtful A.B.R. reactions, it is necessary therefore first to instigate a closer examination

before drawing conclusions. With that closer examination the attention can also be given to possible abnormalities of the milk (mastitis, etc.).

It can be said that none of the diagnostic methods mentioned here are infallible. In general a positive result is convincing, a negative one is not. This latter case is especially true of blood-serum and milk examination in an acutely infected herd. Therefore these examination methods do not show in the right way the extent of the infection in acutely affected herds.

#### IMMUNISATION METHODS

Not much will be said about this subject. I want to refer to what Dr DE MOULIN has stated about it.

In connection with the application of vaccination in the practice of the organised eradication, however, it is important to dwell on some points.

When a vaccination in a case of Bang's disease is spoken of we mean the vaccination done at about 6-8 months of age. Vaccination of older animals is only applied in emergency cases; for instance in cases where a short time before a single infection took place in a herd that has been brucellosis free up to that time. In such cases there is a chance that by vaccinating the adult cows (also the pregnant one) with Strain 19 a useful immunity is aroused.

Vaccination of the young animals has been applied in the province of Friesland for 30 years. At first the strains used for the vaccine had been cultivated in the laboratory. The vaccination was mostly done at the age of 10-12 months. During the last 15 years the vaccination has been done at the age of 6-8 months using the well-known American Strain 19.

Experience in practice has taught that the immunity obtained is not absolute even in the first year after vaccination. Immunity-breaches are sometimes observed. A further result of vaccination is that the blood of the animals, in the first period after vaccination, shows positive results with the serological examination. At sales when the buyer requires a favourable blood-test (for instance on export) these blood reactions may be a disadvantage. These serological reactions have mostly disappeared after the animals have calved once. Yet some troubles are experienced in the blood-test of herds where calfhood vaccination is applied from the reactions with 2 years old cows. The A.B.R. test, however, seldom gives a reaction in these cases.

In spite of these difficulties we deem it wise that, in surroundings where Bang's disease occurs rather frequently, the young animals should be vaccinated against this disease; also on farms free from disease because the vaccination gives protection. The animals in clean herds in infected surroundings need this protection just as much as the animals in an infected herd.

Vaccinations, however, may never lead to the neglect of hygienic measures. A danger of this certainly exists among cattle owners. This danger must be considered as a disadvantage of vaccination. It is a good deal of trouble to teach the farmers that for the eradication of Bang's disease the hygienic measures are the most important ones and that vaccination is only a means of helping.





*Livestock Market at Leeuwarden*

#### THE SETTING UP AND THE PROCESS OF THE ORGANISED ERADICATION OF BANG'S DISEASE IN FRIESLAND

A dairy factory can take part when 75 % of the cattle owners wish to participate in the combat of Bang's disease.

Four times a year samples are taken from the cans of the farms allied to this dairy factory and are sent to the laboratory of the Health Service.

Every cattle-owner can, if his herd appears to be infected, send separate samples from each cow for the A.B.R. test. This forwarding is done by the factory. He can, of course, also have blood samples sent in, but the practice confines itself more to the milk, which is sent frequently if necessary.

If a farmer has received a favourable result from his can samples three times in succession he can have a blood-test made of all his cows by his veterinary surgeon. Should the result be favourable then his herd is registered as a Br. abortus-free herd.

A farmer who has a Br. abortus-free herd can obtain a certificate for his cows that any cow in question originates from a free herd. For cows from herds not registered as Br. abortus-free no certificates are issued by the Health Service.

The data of the tests are communicated to the dairy factories who issue the certifi-

cates. The Service keeps a central record on cards of the tests done and other relevant data, if any.

It is left to the cattle owner to decide whether or not he wants to have his young cows vaccinated. As a matter of fact the Health Service makes vigorous propaganda for the calfhood vaccination. For herds free from Bang's disease, this is also the case. Only in districts known to be quite free from Bang's disease (for instance, the islands) is vaccination omitted.

The recommendations to a cattle owner are as follows:

1. Isolation of every aborting cow for 4 weeks.
2. The rendering harmless of the aborted foetus and the foetal membranes.
3. Isolation of cows which are known to have given a positive blood or milk reaction; this isolation lasts 4 weeks beginning from the point of time when they showed symptoms of the partus.
4. The cows showing a reaction with blood or milk must always be milked last.
5. Disinfection takes place with chlorine bleach lye, chloride of lime or creoline. The foetal membranes are buried.

These measures are prescribed in this way because the following must be considered as sources of infection:

- a. The aborting cow;
- b. The infected cow that calves at full term;
- c. The infected cow that excretes the bacteria with the milk.

They are mentioned in order of importance. In this connection a possible infection via the bull has not been considered. He may be an infectious factor only if he himself has been infected (orchitis, vesiculitis).

When a cattle owner has a registered Br. abortus-free herd the following regulations are relevant:

- a. Notification must be made of every case of abortion to the Health Service and the material for examination must be sent there;
- b. Only cows from registered Br. abortus-free herds may be added to the herd;
- c. When a farmer has a bull he may only admit cows to service which are free from Bang's disease; when he goes with his cows to a bull of another farm that farm must also be free from Bang's disease;
- d. He may not take his cows to common pastures unless these pastures are completely Br. abortus-free.

The stipulations for Br. abortus-free herds are, in contrast to the above stipulations, compulsory. The reason for this is the fact that certificates are issued for these herds and the basis on which these certificates are issued must be firm. Freedom here cannot be left in every respect to the cattle-owner.

Once more it may be observed that in Br. abortus-free herds the vaccinating of young cattle is indeed allowed. This is in contrast to Denmark where a herd is not registered as Br. abortus-free as long as vaccinations are performed.

The reason why this point of view is held here is that vaccination is essential for the

combat of this disease in rather strongly infected surroundings. It can be further stated that vaccination with Strain 19 is not dangerous; no disease is developed by it. Application of the vaccine in Br. abortus-free herds need not be a difficulty and in view of the eradication of infectious abortion it can only be an advantage.

The eradication is based on regular examination. By reason of this the cattle-owner knows which cows are infected and which are healthy. By taking measures to isolate the infected cows he would safeguard the rest of his herd against further infection. In this case vaccination of the young animals may be a help. By taking care that the young cattle grow up free from disease, and by getting rid of infected cows, he can gradually build up a disease-free herd.

#### IN COMBATING THIS DISEASE THERE ARE, HOWEVER, A GREAT MANY DIFFICULTIES TO BE OVERCOME

The success of the fight depends mainly on good isolation of the sources of infection. This will be self-evident. For any veterinary surgeon, who has experience in practice, knows how much harm can be done in a herd by one cow that aborts. It is necessary for the aborting cow and the infected cow which carries a calf to full-term to be isolated before the respective abortion or calving. In summer when the cattle are in the pasture this isolation is feasible. They can be kept inside for 4 weeks. It is however very difficult to persuade all cattle owners to take this easy measure.

In winter – the period in which in this country most cows calve – isolation is much more difficult. On most farms there is not a separate place for sick animals. For effective eradication it is absolutely necessary that there is a so-called calving-byre where a number of animals can calve and remain isolated for some time. These 'calving-byres', or if necessary 'sick-byres' are totally missing in many parts of our country. This is especially the case in the cattle-rich districts where cattle-owning is the only branch of farming which is carried on. In these districts are fewer farm buildings available than in the districts where agriculture is also carried on. This is a great handicap in the business of eradication. In this case farmers in Denmark, where they have generally more farm buildings at their disposal, have more possibilities.

Because of this difficulty, the farmers are often makeshift with the isolation of affected cows; these are segregated to a certain part of the cow-shed. This segregation may be further completed by putting up a partition. We are very pleased when there is a good opportunity of isolating a single aborting cow.

A factor which must also be considered, in herds where cows of high value are bred, is that infected cows which represent a high breeding-value will not quickly be removed from the herd. This is especially true when such animals do not abort.

The fact of the great density of cattle in many parts of our country should be added to this. This is a factor which greatly facilitates the spread of the disease from one farm to another.

At the same time, the common pastures for the young cattle play, in some districts, an important part in the spreading of the disease. It is true that the young cattle taken to these pastures are vaccinated but, since this vaccination does not give absolute immunity, these pastures repeatedly form a dangerous source of infection.

Finally the trade in milk-cattle must be mentioned. This is very lively in The Netherlands and, owing to this, infected cows frequently carry the infection to other farms.

As vaccination of the young cattle can only be considered as a means of help in the eradication of this disease we are dependent in our combat, in the first place, on the thoroughness with which the cattle-owner takes measures against the sources of infection.

From the above, I think it will be clear that in a district which is rich in cattle, where cattle-breeding is at a high level, the eradication of the disease is much more difficult than in a district which is poor in cattle, where cattle-breeding is at a comparatively low level.

There will be a much higher percentage of infected farms in a district rich in cattle than in a district poor in cattle. It is therefore very difficult to make good comparisons between different countries in the field of the eradication of Bang's disease as the circumstances may be so different.

#### DISCUSSION

As you will have noticed no financial stimuli are being used in the eradication system set up in our country. In the first place, this is not possible because the combat is not yet properly under way and owing to this fact it is not yet sufficiently known to what degree the herds are infected. In the second place, the full co-operation of the cattle-owners on a voluntary basis should first have been obtained. The possible results must be clearly pointed out before these stimuli can be contemplated. These stimuli must come from the cattle-owners themselves in one way or another – maybe a dairy factory, maybe through greater co-operation.

The eradication system which is set up is thus quite voluntary but still on a co-operative basis. The arrangement is twofold, that is to say the carrying-out of the system of eradication in the district combined with the driving power which may be provided by the local unions.

This system resembles that in Denmark. There they also began on a voluntary basis and the organization is also carried on in conjunction with a dairy factory.

In that country they had the advantage of being able to work out this project on a large scale during the years of the 2nd World War. In 1942 the eradication system was already in full swing. The progress of the affair can be referred to as an example of how further results can be achieved once the voluntary basis of co-operation has been gained.

Several of the smaller islands in Denmark are already brucellosis free, the island of Fünen with 20,000 herds is nearly free. When one considers that in 1942 there were about 25 % infected herds, then it appears that within a decade, with a well organised plan of eradication of Bang's disease, a great deal can be achieved.

In Friesland we are only in the first stage. In 1951 the eradication was organized and out of the 100 dairy factories  $\frac{3}{4}$  of the number have already joined voluntarily. With most dairy factories the percentage of farmers participating is from 80 % to 100 %, so that in 2 years more than 60 % of the herds have come under control in this province.

The intention is to persuade all factories to join within the next 2 years. Within the union of the dairy factories the participation will have to be raised from 75 % to 100 %. By enlightenment talks and propaganda we shall try to obtain results in the struggle against the disease. It is also speculated that support will be received because a demand will come for milk-cattle originating from Br. abortus-free herds. Only when the participation and co-operation in the effort of eradication has become general, and when visible success has been achieved, will the time come to consider further measures, measures to insure the factories freeing themselves from brucellosis.

To reach this end much will be demanded from the Health Services since in fighting the disease, numerous difficulties will have to be overcome, especially in the cattle-rich districts.

# STERILITY IN CATTLE

## (PRINCIPAL CONTAGIOUS CAUSES, DIAGNOSIS AND TREATMENT)

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Investigations which are concerned with the causes of sterility rise in importance if the influence of these causes on one or another factor of fertility becomes clearer.

The principal factors which determine the destiny of fecundation in the first place, might be summarized as follows:

Under influence of hormones which are excreted by hypophysis and testes, sperm-cells are formed by the tubuli seminiferi of the testis and are transported to the epididymis. There, after mixing with products of the accessory genital organs, they undergo a ripening process so that they will be ejaculated at last under influence of psychic stimuli.

Arriving in the female genital tract, these sperm-cells migrate via vagina, cervix, and uterus, to the oviduct. There the fecundation has to take place. The chance of fecundation will be as great as possible if an optimal number of vital sperm-cells has the opportunity to come into contact with the egg.

The growth, development, and release of the egg are under the influence of hormones. These are excreted by the hypophysis, the mutual relation of which will determine whether these processes will develop in a way which is normal or abnormal for the egg.

In addition to the development of the egg, an excretion of hormones takes place in the ovary. This prepares the genital tract for the attaching of the egg.

In general in cattle the release of the egg takes place between 12-15 hours after the termination of oestrus. At this moment it is important that the maximum number of vital sperm-cells should be present in the oviduct. This is also the reason why a service in the last part of oestrus has more chance of success than in the beginning.

When the egg is fecundated it is in the two cell state after about 48 hours and traverses the oviduct slowly, continuously dividing itself. It enters the uterus after 72-96 hours. Here it divides itself till morula form and attaches itself, by means of its membranes, to the uterus about 12 days post-oestrus.

So, during a relatively long time, the egg develops freely in the uterus cavity, and for its feeding depends on the yolk which it carries with it.

As for the question, in which way and to what degree an infectious agent can be considered as a cause of sterility, some conclusions result in considering the above-mentioned fundamentals of fertility.

So it is clear that a micro-organism, which gives rise to a destructive process in the testis or epididymis and in ovary or uterus – so that the sperm or egg-cell is destroyed – can be considered as a cause of the resulting sterility.

It is also expected that micro-organisms, because of inflammatory processes which they may cause in the accessory genital organs of the male or female animal, give rise to sterility. These forms of sterility are generally less distinct than the above mentioned ones.

Examples of these inflammations, caused by micro-organisms, are sufficiently known from practice.

The orchitis and epididymitis in consequence of *Brucellae*, *Cor. pyogenes* and streptococcal infections, are well-known. Also the endometritis and vaginitis caused by *Trich. foetus*, *Vibrio fetus*, *Cor. pyogenes*, staphylococci, streptococcine, *pseudomonas* and so on.

It strikes one, however, that many of the above-mentioned germs such as streptococci, staphylococci, *Cor. pyogenes*, *pseudomonas*, as well as the pleuro-pneumonia-like organisms, although considered in many cases as the cause of sporadic or so-called herd sterility, are often to be reckoned as the normal inhabitants of the genital tract. They may occur in sperm and vaginal mucus, for example in equal quantities as found in the sterile animal.

For explanation of these cases one often resorts to a difference in virulence of the several germs. From our own experience however, it is known, that in cases of sporadic and herd-sterility, bacteria cultivated in pure culture from the genital tract of the female animal do not generally interfere with the reproductive capacity of the animal after their injection into the genital tract of the normal (even pregnant) animal.

The success of an infection, however, is not only dependent on the character of the micro-organism, but also on the resistance of the individual, in this case on the genital tract, whose resistance is highly influenced by the functional capacity of this organ.

It has been proved that the pregnant uterus is very sensitive to infection by *Brucella abortus*, which readily causes an endometritis in this stage. The non-pregnant uterus, on the contrary, is highly resistant to this infection and we can see, consequently, that the *Brucellae* disappear from the uterus in the case of a normal involution process.

*Cor. pyogenes* can often be cultivated in pure cultures from the inflammatory products of purulent endometritis. They are, however, sometimes also found in the apparently normal uterus and cervix.

From the investigation of the antitoxin contents of the blood it has been proved that there are more *pyogenes* infections in the cow than merely purulent inflammations caused by them.

It seems that the *pyogenes* bacteria do not fully develop their pathogenic properties until they are found in tissues which do not properly function.

Well known are the mastitis of the non-active gland, the pneumonia of the pre-

mature-calf, the tissues which do not show a pyogenes infection until they are injured by blow or push, and the pyogenes infection in connection with a *retentio secundinarum*.

The favourable results which are observed following treatment with hormones in certain endometrites are to be ascribed to the fact that the functional condition of the genital tract is brought into normal channels, through which the resistance of these tissues is also raised.

The fact that the functional condition of the genital apparatus may be decisive for the course of an infection, might form the explanation for the phenomenon that certain micro-organisms, which are found in the genital tract, are to be considered sometimes as originators of inflammatory processes and in other cases as innocent saprophytes.

In the investigation of sterility we only have at our disposal the methods which can approximately tell us something of the functional condition of the genital tract. This is indeed the reason why cases of sporadic and so-called herd-sterility are often so differently accessible for investigation and why the significance of bacteria in their aetiology is not always well understood.

If, by clinical or bacteriological investigation, the presumption is justified that bacteria play a certain role in these cases of sterility, it will be important to treat according to these lines. For this purpose the several antibiotics have been proved to be of great value.

It is demonstrated in the Artificial Insemination practice, for example, that addition of, for instance 500 I.U. penicillin and/or 500 I.U. streptomycin to the sperm of certain problem bulls caused a sharp increase in the fecundation, although apparently no pathogenic germs could be isolated from this semen.

In treatments of several endometrites and vaginitis in which streptococci or staphylococci were demonstrated, a mixture of 200,000 I.U. penicillin and 100,000 I.U. streptomycin proved to be very favourable. On the contrary, the treatment of *Brucellae* and *Cor. pyogenes* infections of the genital tract still form an obstacle which is difficult to conquer.

In those cases in which their aggressive power is less dependent on the abnormal functional conditions which may prevail in the genital tract, the influence of micro-organisms on the fertility will be easier to estimate.

Similar cases of sterility may be observed in several herds if these micro-organisms are spread by the bull.

Several forms of so-called enzootic sterility are described by various investigators. Well known are, for example, the investigations of WEBSTER who described a sterility occurring in Australia caused by streptococci. In Holland the *Vibrio fetus* and *Trichomonas fetus infection* are of particular importance.

The course of the *Vibrio fetus infection* often presents itself in the following manner:

A bull is introduced into a normal farm where until recently the fecundation of the cows did not provide any difficulties. This bull does not show abnormalities of the genital tract and no abnormalities can be found in the sperm. Nevertheless, the fecundation results of the bull are bad or less good than with previous bulls.



Several cows come back at irregular times after services. The same thing occurs at following services and also if the cows are first served by a unsuspected bull.

Some days after service, symptoms are often observed which point to the presence of a catarrhal vaginitis and cervicitis; these symptoms are not always equally clear, however.

This condition continues for 3–4 months, after which the animals gradually become pregnant.

Next year the same difficulties occur with the young animals which are served for the first time by the suspected bull. In general, no difficulties occur with the animals which have been served the previous year.

In the course of the first year, it already becomes clear that some animals – mostly some of those which became pregnant after the first service – abort. This is in consequence of a *Vibrio fetus* infection. On the other hand it is demonstrated that, when in a herd one or more cases of *Vibrio fetus* abortion occur, as a rule one can trace a history of sterility as mentioned above.

Besides, it appears that the blood serum of the cows which aborted generally agglutinates a *Vibrio fetus* antigen, i.e., up to a titre of 1:400.

One can also see that the serum of a few animals, which have been served by the suspected bull one or more months ago, sometimes agglutinates this antigen up to a rather high dilution very quickly.

The vaginal mucus of the served animals can be used. This material has to be prepared for this purpose in the proper way. Here one can find that the *Vibrio* antigens often agglutinated up to very high dilutions over a rather long period of time, (e.g., one year).

In the course of the last years it has been proved that the bull which infected the female genital tract with *Vibrio fetus* during service, has to be considered as the chief source of these *Vibrio* infections. This infection can, however, also be effected if the sperm of infected bulls is used for Artificial Insemination.

As far as is known, these vibrios do not trouble the bull at all. They are living as saprophytes, in particular on the mucous membrane of the prepuce. In the female, however, they cause a catarrhal endometritis and cervicitis which forms sufficient reason for harming the developing embryo which is not yet enveloped by its membrane.

These facts, which have been demonstrated for the first time in Holland during the last years, have attracted attention outside the Dutch frontiers.

The results of several investigations in foreign countries all point in the same direction and give evidence of the fact that, as in Holland, *Vibrio fetus* infection has to be considered as a wide-spread and important cause of the clinical picture of enzootic sterility.

Based on the Dutch laboratory and field observations, it has now been proved that in practice one has the use of several methods of research, with the aid of which one can diagnose more or less certain sterility caused by *Vibrio fetus*. As such, the following proved to be important:

1. The demonstration of an increased number of agglutinins in the blood serum, but still better the demonstration of agglutinins in the vaginal mucus.

2. The demonstration of *Vibrio fetus* in the vagina and cervix of cows which have been served by the bull, and particularly also in the uterus of cows which have been served recently.
3. The demonstration of *Vibrio fetus* in the sheath and sperm of the bull concerned.
4. This all in connection with the epizootic data and the clinical changes in cases of sterility.

During the last years these methods of investigation have been applied on a wide scale and proved to be of ample use in demonstrating the cause of diminished fertility.

Also measures such as substitution of the bull which served heifers, afterwards showing a positive mucus test, and treatment of cows (and in a limited scale also bulls which were carriers of *Vibrio fetus*) seemed to be very favourable for the resulting pregnancy percentage in many cases.

It must be said, however, that our present methods of investigation have their restrictions.

It became clear that in practice the demonstration of vibrios in sperm or prepuce-washings is not so easy. The many micro-organisms which occur in this milieu often overgrow the blood agar media which otherwise form an excellent medium for the vibrios, so that the relatively slow growing vibrios are difficult to discover. There is hope, however, that a recently developed medium which has given good results in laboratory tests may be of better use in practice.

In connection with the mucus test the following difficulty may arise: Now and again one meets cases of sterility which are reminiscent of a vibrio infection, although the mucus test proves to be negative. In these the character of the antigen used is of the highest importance for the success of the agglutination reaction.

The antigens used in practice are generally H-antigens. It has been proved, however, by the absorption tests of Castellani, that the H-antigens of the different vibrio strains do not all contain the same components.

So that, if one performs agglutinations in a herd where the animals have been infected by a strain which contains one or more components not present in the antigen used, one may expect difficulties in the mucus test and obtain negative reactions. These should prove to be positive when using a more capable antigen.

To prevent similar disillusionings, it will also be necessary to use an antigen which contains all those components which may be expected to be present in the different *Vibrio fetus* strains.

All this, however, demands an investigation of the antigenic structure of many *Vibrio fetus* strains. This has not been finished up to now.

Apart from these drawbacks to which the diagnosis of *Vibrio fetus* infection may give rise, it can be concluded that the above mentioned guides have proved to be of considerable use in practice in Holland during the last years.

Another cause of sterility in Holland which gives rise to the spread of enzootics is caused by the wellknown *Trichomonas foetus* infections.

The way of infection and clinical course strongly remind one of the vibrio infections.

Even clearer than in the last cases the bull proves to be *the* only source of infection for the female animal infected with Trichomonads.

There is no symptom which can be considered to be pathognomonic for the presence of infection. The degree of infection, sensitivity of the individual, and competing or secondary bacteria can strongly influence the clinical picture.

Still it is permissible to say that the following combination of symptoms is strongly suspicious:

1. Several cows come back after 3 weeks or after 6 or 9 weeks;
2. Abortion occurs, especially in the first part of pregnancy;
3. Cases of vaginitis, cervicitis and endometritis will frequently develop.

Often one gains the impression, after accurate observation, that a variable immunity develops during infection. One notices that during the beginning of an enzootic in a herd the disease often gives rise to repeated services after three weeks. Later on more cases of abortion occur in the first months of pregnancy, and eventually excretions, pyometrae, and cases in which animals are in calf in a normal time, dominate the picture.

The Trich. foetus infection is diagnosed with certainty by demonstrating the trichomonads.

As material for investigation the following can be considered:

1. Scrapings of penis or washings of prepuce;
2. Aborted foetus (contents of stomach, intestines, nasal mucus, and foetal membranes);
3. Material from vagina or uterus.

The time this last material is examined is of great importance for the results of the investigation. There is a characteristic fluctuation in the number of trichomonads in the vagina. The practical consequences of this are, that for examination the following are pre-eminently suitable:

1. Cows after being served once by a suspected bull, 12–20 days after service;
2. Cows, in which oestrus has already passed, some days before the next oestrus;
3. Excretions and pus from uteri of so-called dischargers and pyometras;
4. Vaginal mucus of animals which aborted quite a short time previously (48 hours).

It must be pointed out, however, that trichomonads are difficult to demonstrate in material of cows which were treated recently for a vaginitis or endometritis. The way in which material is obtained is not important if care is taken that it is not contaminated by faeces. In the faeces one sometimes find protozoa which resemble Trich. foetus in a very confusing way.

First of all the material is examined unstained as a direct examination. To begin with, one examines with a low magnification with a screwed down condensor, in order later to look under a higher power in suspected cases.

The most characteristic feature of the trichomonads is their movement; when they are motionless they are very difficult to distinguish or may not be seen at all. The kind of motion is different. In thin material they move quickly through the visual field in a whimsical way. In thicker material and at lower temperatures they move less quickly.

The form of the trichomonad varies from a round to lancet-form. Its length is somewhat larger than that of a leucocyte.

If the movements are not too quick the characteristic movement of the undulating membrane is clearly visible (this undulating membrane is typical of *Trich. foetus*).

Because trichomonads die soon, the material must be examined as soon as possible, preferably on the farm. If this is not possible then the sample must be stored at body-temperature. If cooling is inevitable then the sample has to be kept at 37 °C for some hours for the benefit of the examination.

At present the cultural examination is also significant in practice as a means of tracing the trichomonads. It is possible to cultivate trichomonads in pure culture from material which has been on the way for 1–2 days. For this purpose simple mediums can be used such as serum-broth or blood-broth to which penicillin or streptomycin has been added.

In diagnosing trichomonads one can also use methods based on the demonstration of specific antibodies, e.g., agglutinins in the blood-serum or vaginal mucus, and antibodies in the skin.

The eradication of infections can be pointed out as follows: the prevention of infection, the combat of the course of infection within the herd, and the treatment of the affected animals.

*The prevention of infection:* In purchasing a bull one should convince ones self of the mating results. On suspicion prepuce-washings are examined microscopically and culturally. In the case of negative findings experimental matings have to be done with maiden heifers (examination of these animals 10–20 days after service).

Regular control of the service-bulls.

Suspicious material on the farm, e.g., aborted foeti, should be eliminated.

A cow with abnormal discharge, or a cow, which has had vaginitis shortly after the last mating, ought not to be mated naturally.

Artificial insemination is an important aid in preventing infection.

*The control of the course of infection:* Stoppage of services. Selection of the uninfected suspected, and infected animals.

Uninfected are: nearly all non-served heifers.

Suspected are: all animals which have come back or have aborted, discharging animals, and all animals which were normally in calf by means of the bull concerned. In brief, all cows which have been mated by the infected bull. Examination microscopically and culturally at the proper times.

Infected are: besides the bull, all animals in which trichomonads have been demonstrated. Only unsuspected animals can be served by a bull which is considered not to be infected.

*Treatment:* of infected and suspected cows by irrigation of the uterus and vagina using the well-known principles with Lugol (1:2:200), chinosol (1:100) and such, till the trichomonads cannot be demonstrated anymore and until abnormal excretions

have disappeared. The treatment is successful in that trichomonads mostly are not demonstrated any longer after one or more irrigations. Also they often disappear without treatment sooner or later. A sexual rest of 3-4 months after treatment is sufficient for this purpose.

Not all animals will become pregnant again, however, because the infection lasted too long and irreparable changes occurred in the uterus.

In cases where the anoestrus lasted about 7 months, treatment does not seem justified if this anoestrus has not been caused by a seven-months pregnancy.

The treatment of the bull is uncertain. This is comprehensible if one considers that trichomonads live freely not only in the prepuce, but also in the mucus membranous crypts. Consequently only valuable bulls can be treated.

Several therapies are given, such as treatment with tryptoflavine ointment (0.5 per cent) or application of 3 per cent  $H_2O_2$  which is deposited into the prepuce under pressure. Relapses following the different treatments occur, however, rather often.

## ORGANIZATION OF THE RESEARCH ON STERILITY

J. H. TEN THJE

*Professor at the Veterinary Faculty of the University  
of Utrecht*

Something will be told about the 'organization of the research on sterility'. In our country we have much trouble with cases of contagious sterility in cattle. Our State Serum Institute and the Provincial Veterinary Health Services are quite able and well equipped to combat that damage. Great progress in this campaign is obtained by artificial insemination which is rapidly developing in our country.

Dr STEGENGA will give many particulars of the organization of artificial insemination in Holland.

Still, we have many cases, in a certain district or among the herds on certain farms, where there is wide-spread sterility, while the neighbouring farmers do not have that trouble or, at all events, not to such a degree. The result of the artificial insemination, i.e., the efficiency-number, is bad in comparison with the results obtained with the same semen on the neighbouring farms. When causes of a contagious nature cannot be found in the investigations of the common clinical routine-work, we have to look for other causes. These causes of disturbances in the reproduction may be spread somewhere throughout the large field of veterinary science where the investigations into the causes of sterility point to different areas. It is the opinion of Prof. VAN DER KAAJ, my colleague of veterinary obstetrics and gynaecology, that the research into sterility is too much for one man, even for one institute. This one man may be as able as possible, but at the same time he cannot be gynaecologist, histologist, biochemist, microbiologist and so on. That is the reason why Prof. VAN DER KAAJ for many years has encouraged the collaboration of several institutes, each of them well equipped and with their own scientific and technical staff, to solve the many problems of the different causes of sterility.

In that way in Holland the research work on sterility has developed as teamwork. As soon as methods have been proved to be worthwhile for clinical use they can be introduced into the clinic for gynaecology. These methods then can be practiced by the members of the clinical staff who have had their training in making use of these methods in the special institutes where they have been developed.

As far as the histological examination of the uterine mucous membrane and the bacteriological and virological control of the uterus, reliable methods have now passed from the level of research into the level of clinical routine work. The biochemical

investigations lead to remarkable results, but not yet to simple methods for clinical routine work.

The Dutch 'sterility teamwork' was completed last year with the co-operation of the protozoölogist and the specialist in the problems of feeding, soils, genetics and housing of our animals. From time to time we have a meeting where the gynaecologist is in the chair, to discuss problems of organization of our work. When results of investigations are to be delivered and are to be discussed we meet in a larger company with our co-workers and many other colleagues for whom our work is valuable.

The financial basis for this work has been provided by special grants from the Central National Council for Applied Scientific Research in the Netherlands. (A.T.A.-T.N.O.). Our supervising Veterinary Board of the Agriculture Branch of this Central National Council consists of an equal number of representatives of these organizations and the veterinary profession; the chairman is Mr QUAEVLIEG, director of the State Veterinary Services.

It goes without saying that the central place on the team is occupied by our gynaecologist, Prof. VAN DER KAAJ, the director of the clinic for obstetrics and gynaecology. He provides the cases of sterility from the herds in the districts around our town. As the research work is now spreading over our country, the State Serum Institute at Rotterdam and the Provincial Health Services will prove to be of value.

One of the main tasks for the gynaecologist is to work out better methods for the diagnosis of a slight inflammation in the uterine wall. For that purpose he claimed the aid of the histologist but, since I myself am the histologist, I will speak last of the contribution of my institute in this teamwork.

The next step is to have a better insight into histological pictures of the mucous membrane at several intervals during the uterine cycle and, in cases of sterility, it must be regarded as a matter of great importance if we can get an insight into hormonal disturbances. For that, the biochemist of our Faculty, Prof. SEEKLES, has for several years made investigations into hormonal research work. It is clear that, in those cases where the cause of sterility is still obscure, this form may be attributed to a disturbance of internal equilibrium in the animal body. First of all we think of a disturbance in the equilibrium of the hormones of the gonads and of the adrenal cortex. If we can get an insight into the nature of the hormonal disturbance in these forms of infertility, it is within the realm of possibility to restore fertility by an appropriately applied therapy or by prophylactic measures. Apart from this, especially in the case of cattle, this hormonal research work may yet lead to the following important result:

The relatively small number of calves that a cow can bring forth during the limited number of productive years has, hitherto, prevented a stringent selection being made in cows in the matter of fertility. Owing to this the care bestowed on the choice of bulls and the improvements in artificial insemination have frequently been made to no purpose. A profound knowledge of hormonal conditions in cattle may also be useful in examining young female animals as to their future fertility.

For practical reasons, Prof. SEEKLES and his co-workers restricted themselves in their research at first to the estimation of the 17-ketosteroids in the urine. One group

of those steroids, the androgens with a neutral reaction, have by means of chromatography been divided into 11 different groups. Some of these come from the adrenal cortex, others from the sexual glands, whereas the origin of still other fractions is as yet unknown. In this way an endeavour was made to get an insight into hormonal proportions in normal cattle and horses compared with animals with disturbed sexual functions.

The results of these investigations will be published at the International Veterinary Congress at Stockholm, summer 1953.

In the meantime, new research work will start soon concerning the estimation of estrogens and progesteron in the blood of cattle and horses.

You see that from this starting point a fair start into the research of the causes of sterility has been made by our biochemist.

It is clear that these investigations are still at the level of research work and it will take a good deal of laboratory work before routine-tests for clinical purposes will be forthcoming.

First of all I very much wish to compare the results of these hormonal spectra with the histological picture of the uterine wall, to seek a correlation in cases of difficulties in the nidation of the fertilized ovum.

Besides the histological and biochemical work, microbiological investigations have already been carried out in a way we can see as research work in sterility. This work has been done at the Faculty at Utrecht by Prof. JANSEN and his co-workers and at the State Serum Institute at Rotterdam. The latest success of the State Serum Institute has just been published by the assistant-director, Dr TERPSTRA. It is the finding of the pleuropneumonia-like bodies in cases of herd-sterility in cattle in the province of Friesland. Reliable methods have been worked out in taking samples from the uterine wall of the cow and of the mare, both for histological and microbiological examination. In regard to the microbiological examination, the work has been restricted to the bacterial examination using several bacterial culture-media in many hundreds of sterile cows and mares and, for the virological examination, use of the incubated egg.

In future the examination will also include spirochaetosis, rickettsiae, and pleuropneumonia-like bodies.

Based on the methods I just mentioned, much routine work had already been done for these investigations. This has been published by BRUS, KAMPELMACHER, VAN DER KAAJ, and myself at the Copenhagen Congress of 1952 and other places.

The technique of cultivation in the embryonated chicken egg belongs to the routine-methods, just as the haemagglutination test and the inhibition test. Experimental work will be done in several animals, in cattle, and so on. A small number of experiments in this direction have been carried out.

Till now, q-fever infection in the Dutch cattle has never been found. In several cases of herd sterility, micrococci have been isolated from the biopsies of the uterine mucous membrane, especially in those cows that showed a positive agglutination of brucella. In cases of sterility in the mare, streptococci have many times been found in bacteriological cultures from uterine biopsies.



Next to the bacteriologist and virologist, our protozoölogist, Prof. KRANEVELD, takes his part in the research work on sterility.

Though the damage caused by trichomoniasis in enzoötic sterility can effectively be decreased by making use of artificial insemination, still much attention is paid in research work to the different methods of microscopic and cultural diagnostics. The biological diagnostics, i.e., the tricine-test and different problems in the laboratory, are studied so as to work out better culture-media and to study the resistance of the trichomonads against low temperatures. This is to determine whether the semen of an infected bull, after having been cooled to a sufficiently low degree below zero, can be used without risks. Also the cycle of trichomonads still needs better insight. Is there a development in the epithelial cells? Do the parasites come into the circulating blood? What is the localization in the prepuce (BARTLETT)? What is the histological picture of the infected tissues? Is there any cyst formation?

Last but not least, there is the study of therapeutic measures that, in several aspects, may lead in other directions. All this work had been planned by our protozoölogist, Prof. KRANEVELD. He has already had experience in the routine work of the diagnosis, so as to distinguish the pathogenic trichomonads from the saprophytic ones in the grass and hay, as they are sometimes found in the materials received for examination.

There are several reasons, also, why the Zoötechnical Institute of our Faculty, under the direction of Prof. VAN DER PLANK, co-operates in the research work connected with diminished fertility.

First, to study the influence of feeding. Although our country is small, there are quite different conditions of management on the farms; the pH of the soil, minerals and trace-elements, differ to a certain extent on various farms.

As the importations of concentrated feeds are limited, most of the cows are fed the whole year round with products of the farm on which they are kept. Only in winter some concentrated feeds (about 2 kgs) are fed.

The meal-mixtures or cakes which are bought by the farmers contain 2% of a standardized mineral mixture. In spring and summer all cows are grazing on permanent pastures. There are several indications that on some farms the proper balance of the minerals and trace-elements needed is lacking.

This condition may have a direct influence on fertility but also an indirect one by reducing the resistance against bacterial infection. Therefore it is necessary to study all factors of feed composition, feed conservation (silage), and management of the cows, on farms where diminished fertility is predominant. In this respect not only the minerals are important but also the vitamin A and D content of the feeds. We find indications that in certain circumstances the vitamin A (or carotene) content of the feed is too low. We plan to analyze the grasses and winter-feeds (hay, silage, dried grass, concentrates, and eventually beets or mangolds).

Generally speaking, analysis of the soils is available; analysis of blood and urine of the cows will complete the picture.

There is no doubt that infertility is also a genetic problem. In swine-crossings we could demonstrate a difference between breeds, but, although in cows those differences

are less obvious, breeders know that fertility between cow families which are kept on the same farms will vary. The heritable characteristics between families vary more than within families; they influence the endocrine system and are also the basis of susceptibility for, or resistance against, infections.

It is therefore necessary to study the pedigrees of all cows in the herd and to calculate coefficients of in-breeding and relationship. Hypoplasia of the sexual glands has been shown to be inheritable. Special attention will be paid to this condition.

In our opinion, the comparison of the factors mentioned with those of neighbouring farms having high fertility-figures will bring the complicated problems nearer to their solution.

It is clear that in those cases giving negative results to the bacteriological and pathological-anatomical tests, breeding and nutrition are needing more attention. But even in positive cases, the factors of breeding and nutrition cannot be neglected.

Not long ago some correlation was found (Germany) between the construction of the cow-house, ventilation, temperature, and fertility.

At last I have come to the contribution of the Institute for Veterinary Pathological Anatomy of which I have the direction.

For many years a chronic endometritis has been considered as the principal cause of sterility in cattle. The gynaecologist, however, is many times handicapped in the diagnosis of a slight inflammation in those cases where no such distinct phenomena can be observed. For that purpose, he can assure himself of the aid of the histologist who, in many cases, could ascertain for his colleague, by means of a histological examination or small biopsies, whether there was the picture of an inflammation or not. This method had been worked out as a clinical routine-method by our colleague BRUS, who afterwards tested this method in one of our Provincial Health Services.

This method of examination has proved to be reliable in many aspects. In the clinic for gynaecology of Prof. VAN DER KAAAY, a biopsy is taken from every cow or mare that does not become pregnant, and in those cases where sterility is observed in animals without distinctly apparent abnormalities in the genital apparatus or the oestral cycle. When, by microscopic examination, an endometritis is present the animal is not to be served and, in case of artificial insemination, for the sake of having the best efficiency number, it is not allowed to be inseminated.

Experience showed us that, as long as an endometritis was present in the biopsy, pregnancy would not follow, though we know some rare exceptions. The data in the histological picture has some characteristics of an inflammation due to brucellosis. After having some experience we can also say whether the endometritis will be cured or whether it will take a long time, say 6 months. Such cases can be expelled from the dairy-herd for economic reasons.

When practising the biopsy method, combined with a bacteriological examination from the same biopsy, figures show that both methods are of nearly equal value in showing disturbances which the gynaecologist has to know. Only the positive data do not result from the same animals, so he cannot eliminate one of the methods.

Though the histologist knows his work is useful for the gynaecologist, he regrets

only one thing. It is the fact that in the clinic his diagnosis as routine work was needed before he was able, as a research worker, to study the histological basis of the changes that happen in the uterine wall during the normal uterine cycle. That is the reason why one should be very careful as to what pictures of the epithelium, both of the surface and of the uterine glands, and of the stroma, are within the limits of the cycle and what pictures are pathologic. Though we have literature on this point I believe that not everywhere in the world are these 'normal' pictures the same. To a certain degree they depend on the way in which cattle are kept. Does the cow live on green meadows during the greater part of the year, is there a bull in the herd, is she housed in a dark stable nearly the whole year (as we see in the Alps), is her only relation with a bull to be found in the artificial insemination? These problems may be of equal interest to the biochemist in his hormonal studies. For some years we have been studying these normal pictures of the uterine wall, including reconstructions of the uterine glands, in order to understand better the pictures of our microscopic slides. Also we are just beginning to study the histochemistry of the uterine wall with relation to phosphatase, acid polysaccharids, the interstitial cells belonging to the autonomic nervous system, and so on. We hope that within some years we shall be able to combine our results with those of our fellow-workers in the sterility-team, first of all with those of the biochemist, and to diagnose what is a mucous membrane fit for nidation of the fertilised egg and in what directions therapeutic measures, hormonal or others, have to be taken. Before reaching that milestone in the research on sterility there will be a great deal of work to do for us all.

# THE SIGNIFICANCE OF ARTIFICIAL INSEMINATION IN THE CONTROL OF STERILITY

Dr TH. STEGENGA

*Secretary Central Committee (Execution of  
Artificial Insemination)*

In broad lines artificial insemination<sup>1</sup> serves two purposes:

1. Control of sterility in cattle.
2. Improvement of the quality of cattle.

In some countries the control of sterility in cattle will be preminent, in other countries the quality improvement of cattle. There may even be great differences in the aims pursued in several parts of the same country.

It is evident that there will be justification, in some cases, for putting much emphasis on the control of sterility. Sometimes it may be the only target aimed at. Yet the *consequences to cattle breeding should never be entirely lost sight of.*

Conversely, the cattle breeding experts should also bear in mind in their efforts to achieve improvements in the national cattle herd that A.I. must comply with some rigid hygienic requirements.

In the Netherlands the foremost aim of A.I. has been to combat bullborne diseases of the genital tracts in cows. Very likely the same will apply to other countries in Western Europe.

As long as the main aim is the eradication of infections caused by service in either one or a few herds, the essential measures can be taken on the advice of practising veterinary surgeons. The owner will have to buy another, healthy bull. The veterinary surgeon will then inseminate the infected animals with semen of this bull.

In our country, and most probably also in other countries, however, the control of sterility has to be taken in hand on much broader lines. Therefore application of A.I. in the Netherlands is subject to a license from the Ministry of Agriculture. Such a license is only issued to Societies of cattle farmers. Individual farmers are never allowed to sell semen of the bulls they own.

With a few exceptions which are provided for, the A.I. Societies may only work for their members, and it is made obligatory for all members to have all their cows and heifers inseminated. Therefore on one and the same farm all cows are either artificially inseminated or naturally mated.

<sup>1</sup> I am restricting myself here to Artificial Insemination of cattle.

These provisions and other ones will be referred to further on. I just refer to them here as I want to emphasize that A.I. in this country is rigidly regulated. The provisions imposed are of extreme importance to the success of A.I., particularly with regard to the control of sterility.

It might be feared that such regulations could *impair the expansion of A.I.* in practical farming. This fear has proved to be groundless in this country. A.I. attained practical proportions after the second world-war and today already one-third of the national cattle herd is artificially inseminated. The number of inseminated cows in 1952 was 665,000.

*Too high a charge* for inseminations would most likely be a much more rigid drag on participation in A.I. than strict rules. It is of much importance that the costs be kept as low as possible. The most beautiful systems and methods are invaluable for practice if the price is too high and farmers cannot take part. The average charge in our country is about 10 to 11 guilders per cow per annum. The charges fluctuate between 5 and 17 guilders. It is especially in the interest of small farms that the charges are kept low, as in their case participation is even more essential than for large farms. A high charge would be more prohibitive to small holders than to big farmers.

When we are now going to deal with the actual subject 'The significance of artificial insemination in the control of sterility', it seems opportune to start with the following statement.

If it is the intention to keep sterility in cows under control through A.I. this task can only be discharged successfully if A.I. is applied in a correct manner. This implies that the cows must be inseminated at the right moment in a proper way with good quality semen, and that exact records are made of the manipulations applied. A proper system of registration of records is, therefore, essential. If A.I. is not carried out correctly, the result will be that neither diseased nor healthy animals will be fertilized in good time. It may be that some diseases are still combated as no contact is established between the male and female through coition. The sterility symptom, eventually inadequate fertility, is not eliminated, however.

If cows do not conceive by natural mating, the cause must be sought either with the cow or with the bull, or with both.

If cows do not conceive by artificial insemination it may also be due to a mistake made by the inseminator.

Personally I am convinced that the practice of artificial insemination in this country and elsewhere has already contributed a great deal to keeping diseases under control. I am also convinced that in several cases the inseminated females conceive too late or not at all due to technical mistakes made in insemination.

For that reason I believe it is opportune to accentuate the importance of applying the correct technique in insemination. It is not necessary however, to dwell upon the details of this technique. I will restrict myself to one general remark in this respect: *The success of A.I. depends a great deal on the suitability of the people who apply insemination.* In judging their suitability preparatory training may be important but is not a decisive consideration.

I also want to comment, that for achieving success it is essential that at least 3 bulls are available for the procurement of semen. If only one or two bulls are used for supplies, there is too big a risk that occasionally no good semen will be available.

I will now deal in some detail with the significance of artificial insemination in the control of diseases.

For this purpose we will devote our attention in the first place to the *direct* and secondly to the *indirect* significance of artificial insemination in the control of diseases.

I understand by *direct* control, the control of bull-borne diseases of the genital tracts. The most notorious today are undoubtedly trichomoniasis and vibrio foetus. It is well known that trichomoniasis is a scourge widely spread in Europe. Vibriosis is not so well known, but it is gradually being revealed that this disease occurs all over Western Europe and even in some parts to a considerable extent. Both diseases are transmitted from one animal to the other in natural mating.

Artificial Insemination does not incur mating and therefore a further spread of the diseases is excluded, *at least if the bulls used for artificial insemination are not infected, and provided that the essential hygiene precautions are observed with insemination.* It has been revealed over and over again that trichomonads can be present in the ejaculates of bulls. The same applies to vibrio fetus.

We have a few times encountered A.I. bulls contaminated with vibrio fetus. Almost all the cows inseminated with semen of these bulls became infected with vibrio fetus.

If the aim is to control these bull-borne diseases by A.I., it will be essential to use exclusively bulls that are free from these diseases and that are not, at the same time, used for natural mating.

I am well aware of the fact that the spread of vibrio fetus by semen can be kept under control to-day by the application of antibiotics. Yet there is no justification for the use of infected bulls for A.I. for the reason that it has not been proved beyond doubt that all germs of the disease can be killed.

Even if such were the case there would be no justification, in my opinion, for keeping infected bulls at an A.I. Station. It would not be right to do so as it is not at all certain that the germs would not be transferred from one bull to another with the collection of semen. It is for that reason that the only proper course would be, in my opinion, to use only bulls for artificial insemination that are free from the genital tract diseases known to us to-day.

*Not the antiseptic but the aseptic methods are acceptable to us:* In my opinion there is no justification for the making of concessions to breeding-technical interests in this respect.

Is it possible to make sufficiently certain whether a bull is free from vibrio fetus or trichomoniasis?

The Statutory provisions pertaining to A.I. in the Netherlands imply that any A.I. bull shall be submitted to a veterinary examination. The examinations are carried out by the Provincial Animal Health Services. At least one veterinary surgeon, specializing in matters concerning artificial insemination and sterility in livestock is attached to



*Artificial insemination.  
Vaginal method.*

each Provincial Health Service. He examines the A.I. bulls. The examination will reveal whether a bull is, or is highly suspected of being, infected with germs of a disease of the genital tract. If so, such a bull will not be used for A.I.

When examining a bull for such infections, they are subjected to clinical and microbiological tests, if necessary, complemented by clinical and microbiological tests of the cows they served. In most cases such tests suffice to ascertain whether a bull can be considered to be free from such infections.

I want to emphasize that, when investigating whether a bull is free from germs of diseases of the genital tract, complete data on the fertility of the bull cannot be dispensed with. Without the complete breeding history of a bull being available, it is impossible to pass a definite judgement. If, for example, a bull has been used in service for 3 years, the breeding history of only the last year will not suffice. Also the data recorded in the previous years must be controlled. This practice does not give rise to any difficulties in the Netherlands. Bull keepers are under obligation to keep exact records of all matings of the bull on behalf of the Herd Book Societies.

Thanks to the accurate examination carried out by the specialist-veterinary surgeons and the support afforded by the statutory provisions, very few bulls have of late been traced in the Netherlands which proved to be infected with genital tract diseases. If one wants to avert all risks it will be necessary to select bulls which have never been used for natural mating. Considered from the aspect of cattle breeding, it would by no means be justified to stress this point.

It is obvious that it sometimes happens that no judgement can be passed when older bulls have been examined. If a very good breeding bull is involved he is sometimes used for A.I. on trial for a number of months. During the period of trial special attention will, of course, be directed to the conception rate of this bull. It is of great interest to

watch the inseminated animals in order to ascertain both the number of animals that come in heat again and the length of time before they come in heat.

For preference some heifers will be inseminated with semen of these bulls, the former to be examined at a later date for infectious diseases of the genital tract.

If, during the trial period, it is revealed that a bull is infected with genital tract diseases, he is returned to the seller.

The experience, gained in this country with the examination of A.I. bulls, has been that not uncommonly the old bulls used for natural mating are infected. This is rather obvious if it is realized that such bulls have in several cases served annually 100 or more cows kept at a large number of farms.

It stands to reason that the examination of the bulls does not only include the presence or absence of infectious diseases of the genital tract. It also concerns the suitability of the bull for A.I. in general. (Semen quantity and quality, libido, general health etc.).

As has been told the official provisions in the Netherlands do not allow part of the cows of one farm to be naturally mated, whilst the other part is artificially inseminated.

From the very moment that a farmer joins an A.I. Society, none of his cows may be naturally mated in the future. The principle 'everything or nothing' is applied here.

You will understand that this provision is of outstanding importance in keeping the genital tract diseases under effective control. As long as some of the cows would be served by a bull, the menace of bull-borne diseases could not be eliminated.

Once it has been decided to control these diseases on a farm by means of A.I., it is of paramount importance to advise the farmers to have the likely infected animals treated before the first insemination or to postpone insemination for, say, two months. If this advice is not followed up, the results attained with A.I. will be disappointing at the outset and confidence in this method of control might be undermined thereby.

If the diseased animals are first given a chance to recover, it is possible to achieve satisfactory conception results right from the beginning.

Moreover, it is of importance that cows whose genital tracts show visible symptoms of inflammation are not inseminated. The owner should be instructed to have these animals treated. This method has been almost generally applied in the Netherlands.

This course was possible as insemination was carried out exclusively using the speculum during the first years and even to-day this method is generally applied. The technician therefore inspects the vagina and cervix before every insemination. If he notices any abnormalities with a cow, the owner is advised to have her treated.

The result has been that at the beginning the veterinary surgeons had to treat many animals for sterility. As soon as a Society has been active for a certain time quite different conditions prevail. The number of cows subjected to treatment for sterility will gradually become very small. This is conclusive proof of the high merits of artificial insemination in regard to keeping venereal diseases under control.

If the precautions are taken as recommended it is possible within about 6 months to eradicate the bull-borne genital tract diseases on affiliated farms by means of



artificial insemination. If such farms might return to natural mating, the risk of renewed infection will be very high in most cases. For that reason we advise the farmers to proceed with artificial insemination.

A brief digest of the *indirect significance* of artificial insemination in the control of sterility and stimulation of fecundity in cows will be given now.

A properly equipped A.I. station will continuously keep exact and detailed records of the conceptions. Such a course is not only desirable; it is vital. In the Netherlands the conception results of any ejaculate are meticulously recorded from day to day. In that way it is possible to determine the fertility of any bull at any time.

Most of the A.I. Societies take pride in being able to judge also the rates of successful conceptions effected by each technician employed.

Such records have proved to be of outstanding importance as one person attains much better results, under the same conditions, than another. Particularly the results produced by newly appointed technicians are frequently not quite so good as those attained by technicians with longer experience.

In the third place the conception results are judged per farm. The provisional data are based on non-returns. At the end of the year we determine the final conception rates. This is not done by rectal examination of the inseminated cows, but by inquiries made of the farmers. Such an inquiry is based on a record into which all the inseminated animals have been entered.

Partly on account of this extensive and exact recording of the conception results, we have added a great deal to our knowledge of sterility. In this way not only we, but also the farmers, are more emphatically reminded of these matters. Abnormalities are much sooner noticed and infected animals will be treated much sooner. As artificial insemination, provided it is being properly applied and the part played by the bull in general is revealed, precludes bull-borne infections definitely, the problem of sterility is going to show a much less complicated aspect. The causes of sterility not due to the bull come now much sooner and much more plainly to the fore.

Thanks to the extensive and accurate recording of conception results it has been possible to get a proper idea of the part played by infection with Brucellosis in the pathogeny of sterility. Various research workers have come to the conclusion that the conception rates on farms where contagious abortion occurs are some 5 percent lower than on farms free from this scourge. Also the effects of feeding and season on fecundity, can now be much better investigated. Especially with regard to this indirect mode of investigation, artificial insemination can render extremely useful service to the control of sterility in cattle.

As will be known perhaps, the inseminations in this country are almost exclusively performed by technicians. I want to point out here emphatically that the experience we have gained from the engagement of these laymen has been very favourable indeed. In this country we only allow these technicians to work under expert supervision. We feel it would not be justified to charge them with the full responsibility of A.I.

I want now to give some further particulars of the way the *expert supervision on the technical execution of A.I.* has been organised in this country. It occurs to me that

these particulars fit into the framework of this discussion as they are most closely related to the significance of A.I. in the control of sterility in cattle.

Each province in this country has a Provincial Animal Health Service. It has already been stated that all A.I. bulls must be previously examined by the veterinary surgeons attached to these Services. In addition to this, the task of the Health Services is a much more elaborate one:

According to the statutory provisions imposed, any A.I. Society in the Netherlands must submit itself to the supervision of the Health Service concerned with regard to the technical application of A.I. The Health Services have been made responsible for the proper application of A.I.

It is considered to be of vital interest that the supervision on proper application is exercised *daily*.

Due to the fact that there are several A.I. societies in each province of the Netherlands, the veterinary surgeon of a Health Service could not discharge such a supervisory task day after day. For that reason the Health Services have implicated the practising local veterinary surgeons in the supervision.

The instructions on the technique given to the veterinary surgeons in charge of local supervision, are approximately as follows:

The veterinary surgeon shall always be present when semen is collected and he shall test the fresh and diluted semen. He despatches once a fortnight some semen to the Health Service Laboratory concerned for a more elaborate test;

He controls regularly the conception results and keeps a regular supervision on the technicians while at work.

In most provinces an A.I. Society has to pay to the Health Service a certain fee per cow inseminated to meet the costs of veterinary supervision.

If this work has been assigned to the local veterinary surgeons, they are paid by the Provincial Health Service.

It sometimes happens that no local veterinary surgeons are available to carry out the regular control. In that case the supervision is exercised by the veterinary surgeons attached to the Health Services.

Some big A.I. Stations have appointed a full-time veterinary surgeon to direct the activities. There are very few big A.I. Stations in this country, however. Only 10 of the 160 A.I. Societies have to inseminate more than 10,000 cows per year.

For the predominantly small A.I. Societies the appointment of a veterinary surgeon as a director would be too costly.

Recapitulating, the technicians work under permanent supervision of veterinary surgeons. The practising veterinary surgeons work under the supervision and on the instruction of the specialists of the Health Services.

We are of the opinion that in this way a proper and hygienically justified application of A.I. is assured, both to the benefit of a thorough control of sterility and also to cattle breeding.

#### SUMMARY

A.I. is of outstanding importance for the control of infectious diseases of the genital

tract. To assure that this method will achieve success, only bulls must be used which are free from infection with germs of venereal diseases.

Furthermore, the achievement of success can be advanced as follows:

1. by the prohibition of natural mating of cows or heifers on farms affiliated to the A.I. Stations;
2. by either, treatment of the infected animals previous to starting with A.I., or allowing them a rest of say 2 months;
3. by not inseminating animals showing visible symptoms of inflammation of the genital tract before they have gone through treatment.

Indirectly A.I. can contribute a great deal to the eradication of sterility and stimulation of fecundity in cows. Due to A.I. our knowledge of the male genitals has been widely enlarged, whilst also sterility of the females has come more into the limelight. The latter part of this statement applies to the farmer whose attention can more easily be drawn to unsatisfactory conception results and who thereby becomes much more inclined in many cases to have his cows treated.

The exact and elaborate recording of conception results implies a considerable support in the study of the sterility problem. If A.I. is applied to keep sterility under control, it is of outstanding importance that the technique of insemination should be up to standard. With healthy cows, high conception rates can be anticipated.

A proper regulation of A.I. is absolutely essential, in particular as regards expert supervision on technical application and the organisation of the activities associated with A.I.

# COMBATING BOVINE HYPODERMOSIS OR THE OX WARBLE-FLY IN THE NETHERLANDS

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Bovine hypodermosis is an affection caused by the larvae of the ox warble-fly, a genus of a dipterous insect of the Oestridae family.

The evolution from egg to adult larva takes place on the body of the cattle. Almost every one of the Oestridae has its own host to which it is tied for the perpetuation of its kind.

Ox warble-flies are large flies of which the female lays its eggs on the hairs of ruminants in warm summer days (in the northern temperate zones from June till September; in southern regions in autumn). From those eggs larvae are developed which consist of twelve rings and which parasitize cattle for some time.

The nature of the soil as well as the climatic conditions favourable to the development of this fly are not yet known. Thus there are areas where, in spite of the importation of warble affected cattle, the larvae do not develop to the state of maturity and where hypodermosis does not spread.

Ox warble-flies occur in Europe, Asia, Africa and North-America. They require some ten months to reach the stage of adult insects. They have a rudimentary buccal apparatus, as a result of which their life is only short.

Two species of hypoderms are known, viz.: *Hypoderma bovis* and *Hypoderma lineatum*. The most remarkable feature is that these two species of Oestridae may parasitize together the same host and that they resemble each other to such an extent that it is not always easy to distinguish one from the other. On one point, however, there is a marked difference: the way in which they lay their eggs.

*Hypoderma bovis* is slightly larger than *Hypoderma lineatum* and reaches a length of some 15 millimetres, i.e., the size of a bee; *Hypoderma lineatum* is approximately 14 millimetres long. The two flies are covered with yellowish hairs behind the head; with *Hypoderma lineatum* these hairs are less close-set than with *Hypoderma bovis*, whilst the former has the back portion of its body covered alternatively with yellow, black and orange hairs.

Ox warble-flies cannot sting and are not haematophagous. Their buccal apparatus is so rudimentary that it is impossible for them to take any food during their short life of about four days. On warm days, mostly in June and July, these insects fly about in meadows and try to lay their eggs on the skin of ruminants. They lay their eggs, which

are oval and about 1 millimetre long, by means of an ovipositor; during this operation the eggs are stuck onto the hairs of the cattle.

The *Hypoderma bovis* remains on the skin for only a short time, each time fixing one egg to a hair root quite near the skin of the hind legs or the lower part of the chest. It repeats its attacks frequently and thus lays one hundred and more eggs on one beast.

The *Hypoderma lineatum* lays several eggs (ten to fifteen) in a row on one hair. It appears that this fly preferably attacks crouching animals and lays its eggs on the parts of the body which are in contact with the soil (such as the lower portion of the chest, the lower part of the flanks, the cubital region).

At first sight, *Hypoderma* eggs might be mistaken for eggs of lice. Eggs of cattle lice, however, are larger and coarser and are stuck onto the hairs up to about half their length. Moreover, they are not pediculate such as those of the ox warble-fly.

After three to six days a larva develops in the egg, leaves the egg, falls on the skin and penetrates through the canal of the orifice of the sebaceous gland across the skin into the subcutaneous connective tissue.

The migration of the larva, about half a millimetre long, through the body is different for the two species. The larvae of *Hypoderma bovis* migrate along the peripheral nerves via the epineural tissue toward the epidural adipous tissue of the medullary canal, where they end their development, whereas the larvae of *Hypoderma lineatum* migrate toward the thorax and the abdomen and partly toward the peri-esophageal connective tissue, where they arrive two months later to migrate after some time along the diaphragm toward the proximal extremity of the flanks and thence towards the dorsal and lumbar region.

After some time the larvae of *Hypoderma bovis* leave the epidural adipous tissue, move across the intervertebral openings and appear again under the skin of the dorsal, lumbar, and sacral region. With the back of their body bristling with small stinging appendices (spines) they make a little hole in the skin, secreting a strong dermolytic-toxin (hypodermotoxin). This hole is indispensable to aeration and to the excretion of the products of nutrition. During this stage of evolution of two and a half months, the larvae change their skin twice, which phenomenon is accompanied with a change of colour: from yellow to blackish.

From July till November, the larvae of *Hypoderma lineatum* live under the mucous membranes of the esophagus and between the head and the paunch. The larvae of *Hypoderma bovis* live in the rachidian canal from December till March. From January till July, August (in southern regions from July till February) the two species may be encountered in the subcutaneous connective tissue of the back, the croup, and the loins. Deviations from normal development may occur with larvae in different stages, for example, owing to encystation in the maxillary cavity or in the mucous membranes of the mouth and throat.

The larvae of *Hypoderma bovis* and *Hypoderma lineatum* have never been observed in the gastro-intestinal canal. Therefore it may be assumed that if they penetrate into it accidentally, they perish there, their remains being excreted with the dung. Ruminants may lick up the larvae, which are then stuck onto the mucous membranes of the buccal

cavity or of the pharynx, where they continue their development. However, they never reach full growth, but die before that stage.

After arriving under the skin, the larvae of the ox warble-fly continue their evolution. During this process, the skin swells and tumours or warbles are formed. These tumours normally attain the size of a pigeon egg, and they may develop into a phlegmon of the size of a fist as a result of inflammation and also of secondary infections by the action of purulent bacilli.

Most of the ox warble-fly larvae are encountered in the lumbar region because at that place they are best protected and least subject to muscular movements; moreover, the cattle cannot possibly reach those places.

In spring (May-June) the larvae have finished their development and leave the tumour by wriggling through the opening. At this operation first a small part of the body of the larva gets through; it swells and thus slowly pulls out the part wrapped by the skin. This operation is repeated several times, so that in a few minutes, sometimes even quicker, the larva liberates itself and finally leaves the back of the ox. By contractions rapidly following each other it moves on until it falls. If it falls on the floor of the cow-house, it seldom continues its evolution. If it falls in the meadow, it seeks a shady spot and in a few hours it is transformed into a pupa.

Within thirty-five to fifty days, the pupa develops into the ox warblefly, which sets itself free because the extremity of the pupa suddenly opens like a lid. The young hypoderm fly then frees itself from its case, cleans its wings which are still humid and flies away. Then follows copulation on the ground or on grass blades. The females swarm out, attack the ruminants, lay their eggs on the skin and the cycle commences again.

During their migration through the body, the Oestridae often cause inflammations. The larvae of *Hypoderma lineatum* often cause esophagitis, sometimes accompanied with an oedema and sanguinolent infiltrations, whilst the Oestridae of *Hypoderma bovis* may provoke paralyses as a result of lesions of the spinal cord.

The larvae migrating through the body may also be germ-carriers; thus several research workers have discovered the presence of colon bacilli and micrococci in the Oestridae.

In most cases, migration canals are infected, so that after slaughtering of the carrier, valuable parts of the meat must often be cut off and rejected.

Consequently, the damage done to skins is considerable. In 1947 in the Netherlands 434,000 oxen and 76,000 heifers were killed, of which nearly 30 %, i.e. 130,000 oxen and 25,000 heifers had skins infested with warbles, which involved a loss of over 700,000 guilders.

The loss of milk, meat etc. was 15-20 million guilders. After a few years combating the warble fly, these losses are reduced to less than the one third.

If we add to the above the losses caused by the lower milk yield, by the inferior quality of some pieces of the meat, and by the decrease in the production of fat, the total of the damage done in our country by the ox warble-fly may be estimated at some millions of guilders per annum. It is obvious, therefore, that an intensive fight against the ox warble-fly is an imperative necessity.

For several years experiments have been made with all kinds of methods and med-

icaments to destroy the ox hypoderm. Efforts to combat the flies by applying to the skin of the animals substances preventing the insects from laying their eggs on it have not been successful. The fight against these noxious insects must therefore aim at an interruption of the cycle of evolution of the larva to adult insect. The most appropriate time is when the larvae live in the subcutaneous connective tissue of the back and loins.

Two methods are known, viz.:

1. mechanical de-warbling;
2. chemical de-warbling.

#### 1. *Mechanical de-warbling*

The larvae that have reached the state of maturity are expelled from the swellings and destroyed. First, if need be, the orifice of the warble may be enlarged by means of a lancet and the oestrus may be pulled out, if necessary, by means of a hook. This operation must be carried out with care by experts, in order not to run the risk of crushing the larvae in the swellings, which may result in intoxication which, in serious cases, may entail the death of the ox or, in less serious cases, cause abscesses, phlegmonic inflammations, or urticaria phenomena.

Moreover, this method involves the drawback of taking much time and causing much pain to the beast, so that it is not suitable for large-scale application, which makes it almost impracticable.

#### 2. *Chemical de-warbling*

According to this method, dissolved parasiticides or ointments are applied; the medicament must penetrate through the respiratory orifice into the tumour to destroy the larvae there.

In order to be suitable for a large-scale fight against the warbles, the insecticide should meet the following conditions:

1. it should be cheap;
2. it should not be toxic and not cause any pain to the ox;
3. its application should be simple and easy.

In the course of years several medicaments have been tried out, mostly without much success. Ointments have been used containing parasiticides, which, when rubbed on the tumours, should close the respiratory orifice and thus suffocate the Oestridae.

Prior to the application of these prophylactics, the hairs should be cut at the places to be treated, the orifice of the tumours be mechanically enlarged and the ointment rubbed in with a brush for about one minute. Cutting the hairs became necessary because the layer of ointment was pushed back by the elasticity of the hairs, which counteracted the effect of the preparation.

This treatment took a long time, was fairly expensive and hardly suitable for large-scale application in the fight against the warblefly, and therefore it was given up in the Netherlands.

#### METHOD APPLIED IN THE NETHERLANDS

The method which is applied on a large scale in this country consists in brushing

the affected animals with a solution of rotenone-based products (Derris and Lonchocarpus powder containing 5% rotenone).

This treatment is based on the research work carried out in this field by Mr BAUDET and Mr DE BOER in 1933.

This brushing mechanically removes the crusts covering the stigmata in the tumours, as a result of which the insecticide can penetrate into the stigma and kill the Oestridae. For the application of this prophylactic method it is not necessary to cut the hairs; it will even be preferable not to do so, since the hairs retain longer the solution employed.

This method meets all the conditions set; it is not expensive (about 25 cents per beast), the treatment is simple and does not take much time, while the beasts endure this treatment fairly well, and it aims at an attenuation of itching.

Let us now summarize the excellent results obtained by this method.

As there are always backward cows, it follows that the treatment must be repeated several times.

With young beasts the first washing must be effected at the beginning of April, the second at the beginning of May. With older beasts, the last larvae develop about one month later, so that for those animals a third washing at the beginning of June is recommended.

In the years prior to 1940, Dutch breeders were quite ready to join in the fight against the ox warble-fly.

A committee to combat the ox warble-fly, consisting of representatives from the leather, meat and milk industries, made a great effort of propaganda and popularization to stimulate the fight.

Although in some areas people wholeheartedly shared in the efforts to wipe out the pest and the results obtained were sometimes remarkable, in general the efficiency of this campaign was low.

When it was found impossible in actual practice to arrive at really effective results in the fight against the ox hypoderm without the backing of legal measures, in 1942 an *Order on Ox Warble-Fly Prophylactics* was promulgated, which remained in force after the liberation of this country. By virtue of that Order, every stock breeder is obliged to kill every year before June 1st all the hypoderm Oestridae that exist on his cattle and that reveal their presence by tumours or other visible signs. Moreover, cattle are prohibited from grazing if they are affected by warbles, if they show visible signs revealing the presence of hypoderm larvae. Therefore, it is forbidden to take such cattle to markets, shows, etc., if these larvae have not been killed.

Under these legal provisions the fight against the ox warble-fly was organized and popularized energetically.

During the war, combating was difficult because of the scarcity of Derris powder, but in 1948 this product was again obtainable.

The fight is taken up by dairies and by local associations, all of them under the supervision of provincial health services and in some cases at present by those services themselves.



The Derris or Lonchocarpus powder, the rotenone content of which is checked beforehand, is placed at the disposal of those interested, by the health services.

The contribution made by the Committee for the fight against the ox warble-fly consists in subsidies, publication of pamphlets, as well as in a great propaganda effort made by press, radio and film.

The supervision on the observance of the regulations is in the hands of the Veterinary Service, which exercises strict control by inspecting pastures, cattle markets, cattle shows, etc. Stock breeders who take cattle to markets or show any beasts infested by living larvae of Oestridae have their names taken and are obliged to subject the beasts, on the spot and for their own account, to treatment with a solution of Derris powder and to rid them of the larvae.

This campaign, energetically organized as from 1948, has yielded excellent results. In the years preceding 1948, some 25 % of our livestock was infested by the ox warble-fly, but after an intensive struggle pursued for four years, this figure has now decreased in several provinces to less than 3 %.

When combating bovine hypodermosis, it is necessary to consider the flying capacity of the oestrus. Although according to some observers, the insect moves only over a distance of 50–100 metres, so that it is practically tied to the meadow where it was born, others feel that the flying range is much larger, up to as many as a few kilometres if the weather is favourable. Probably for this reason the results of the campaign in the frontier provinces – open to an invasion of ox warble-flies from abroad – are much less favourable than those obtained in the rest of the country.

If the fight is to result finally in the radical extermination of the ox warble-fly in this country, it must be taken up intensively in the neighbouring countries as well.

#### SUMMARY

In the Netherlands owners of cattle affected by warbles are under an obligation by law to treat the infested animals.

The transport, pasturing, sale, etc., of cattle carrying hypoderm larvae is prohibited by law.

It is possible to combat the ox warble-fly in every country in a simple and inexpensive way.

Washing with solutions of rotenone-based product is not expensive or offensive; this treatment is easy to carry out and may be recommended on the strength of the excellent results they have yielded.

Legal provisions relating to the fight against the ox warble-fly are essential.

To obtain effective and lasting results it would be necessary to ensure international co-operation.

#### MEASURES GOVERNING THE FIGHT AGAINST THE OX WARBLE-FLY

##### Order governing the prophylaxis of the ox warble-fly

Order of the Secretary General of the Ministry of Agriculture and Fisheries dated April 2nd, 1942, No 4842, Section III, Direction of Agriculture, Section No 77, as it has been amended by the

Decree of February 18th, 1944, Section No 36, which was suspended at the liberation of the country but became again operative provisionally in accordance with the provisions of the Royal Decree dated September 5th, 1945, S. No F. 162.

By virtue of the paragraph of Order No 23/1940 and in conformity with the provisions laid down in paragraphs 2 and 3 of Order No 3/1940 of the German High Commissioner for the Occupied Territory of the Netherlands, the following stipulations were made:

*Section 1.* In the present Order is understood by 'Ox Warble-Fly or Hypoderm' the large hypoderm fly (*Hypoderma bovis*) and the small hypoderm fly (*Hypoderma lineatum*).

*Section 2.* I. Every stock breeder is obliged to kill any larvae of ox warble-flies found on his cattle or the beasts entrusted to his care.

*Section 3.* I. It is prohibited for users of a meadow to send out to graze or to admit to that meadow any cattle that show warbles or other visible signs revealing the presence of hypoderm larvae.

*Section 4.* It is prohibited to take any cattle that show warbles to a cattle market, competition, show, auction or any place whatever where cattle are assembled.

*Section 5.* I. He who acts in contravention of the provisions of Sections 2, 3 or 4 shall be punished by imprisonment not exceeding one year or by a fine not exceeding 500 guilders.

II. These facts are considered to be criminal offences.

*Section 6.* If any of the offences referred to in Section 5 is committed by or by order of a juridical person, legal proceedings shall be instituted against him and a penalty be inflicted on him who gave order or actually perpetrated the prohibited offence or caused the non-observance.

*Section 7.* In addition to the officials mentioned in Section 141 of the Code of Criminal Procedure, the following officers shall be entrusted with the implementation of this Order:

- a. The officers of the Constabulary and the Municipal Police.
- b. The officers of the Veterinary Services.

*Section 8.* The officials in charge of detecting offences are authorized to enter any places where cattle may be encountered, if they think this necessary for a satisfactory accomplishment of their task. If admittance is refused to them, if necessary they may gain access to the places in question by force of arms.

*Section 9.* The officials referred to sub 2 of Section 7 are also authorized, if need be, to resort to force of arms to accomplish anything, or have this accomplished, that has been omitted in contravention of the provisions of Sections 2 and 4, the whole at the cost of the offender.

*Section 10.* I. The Director of the Veterinary Service may claim by warrant the costs resulting from the application of Section 9.

II. The warrant is served by a process-server at the cost of the offender and executed as specified in the Code of Civil Procedure in regard to judgments and authentic deeds.

III. Within a period of thirty days from the notification of the deed, opposition may be entered to the warrant by citation of the State.

IV. The opposition shall suspend the execution.

*Section 11.* I. The present Order shall take effect on the day following that of its promulgation.

II. It may be quoted as 'Order governing the Prophylaxis of the Ox Warble-Fly'.

# THE ERADICATION OF THE WARBLE-FLY IN THE NETHERLANDS IN PRACTICE

K. F. JOLING

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In 1946 the Animal Health Services were established in the Province of Utrecht. These organizations are farmers co-operatives. In the spring of 1947 the eradication of the warble fly in the province of Utrecht was organized for the first time by the Provincial Animal Health Service. This was done by having eradication measures carried out in all cattle by trained personnel.

The warble fly, *Hypoderma bovis* and *Hypoderma lineata*, harmful to cattle and troublesome and disadvantageous to the cattle farmer, lays its eggs in summer, as is generally supposed, on the hair of animals legs. The larvae that emerge from these eggs crawl into the hide and in March of the following spring they turn up under the skin of the back. In summer they emerge through holes they pierce in the hide. These larvae, that have matured in the hide, almost all come out at the same time from 9 to 10 a.m. during the fine summer weather. One month later, after pupation in the grass sod, the flies also emerge in groups from the larval skin, during the morning hours on warm days. This great number of warble flies, all 'born' on the same day, probably causes the phenomenon called 'birzen' by the cattle farmers in the Netherlands, which can be observed on warm summer days. This may be an explanation of the fertilization of the female fly. It is yet unknown where this takes place. Probably it is like the honey bee. The cattle are afraid of the fly and will, therefore, run from it. They recognize the sound and will try to run, so that injuries from barbed wire are very frequent, damaging the hides in addition to the damage caused by the larvae of the warble fly, and making them less suitable for tanning. The cattle will look for shade to escape from the fly. They are also known to wade into ditches.

The fly stays within a radius of 5 kms from the spot where it was born, and lives only 5 days, a relatively short span of life. While the larvae are present in the cattle for a long time and can easily be found, the fly is never seen.

Even before 1920 the extermination of the warble fly was advocated. At that time the action was based on voluntary participation. The recommended measures included pressing out the swelling and destroying the emerging larvae, cutting away the swelling, and the application of an ointment consisting of vaseline and 10 % paradichlorobenzol to the swelling after cutting away the hairs. A method used by farmers themselves with some success was rubbing the back with rancid, salt-containing fat, a household

waste product. Another less successful method was the application of used mineral oils; this action had little effect.

The cattle farmers are members of the Health Service. Membership in this Service is compulsory. The animal Health Service supervises the appointment of the personnel charged with carrying out the eradication. For this work farm boys are chosen or dairy workers or other persons used to handling animals and known to the local farmers. Compulsory eradication is based on the rules of the Health Service and on legal regulations. These regulations include the prohibition of bringing cattle with warble swellings to pastures and the obligation of killing as many larvae as possible if the cattle in the pastures are already infected.

The working method is as follows: In April all yearlings and heifers that have palpable swellings on their backs are treated. After one month, in May, they are treated again. Experience has shown that these two treatments practically free the animals from swellings, and that no new swellings appear in June. Some of these animals are treated while still in the shed, but by far the greater part are treated in the pasture. In June the older cattle (cows) are inspected and treated, if necessary, while at this time there is an opportunity to reinspect the heifers. In the older animals, the larvae appear at a later date than in the yearlings and heifers. Treatment in January or February has no result as only those larvae are killed that are already lodged under the skin and have pierced a small breathing hole through it. Through this opening, we think, the poisonous chemicals can penetrate. Possibly the chemicals penetrate the hide and kill the larvae by means of poisoned white blood cells.

In the Netherlands powdered derris or lonchocarpus root, suspended in water, is generally used for this purpose. This powder should have a rotenone content of at least 5%. Derris powder with a 10% rotenone content was not twice as effective. The root should be very finely pulverized and distributed in paper bags in quantities of 200 grams if the rotenone content is 5%, or of 150 grams if it is 10%. The contents of one bag are to be suspended in 5 litres of water. Extraction in hot water is not necessary. The powder can be suspended in cold water and used immediately. It is not advisable to treat animals in pasture in this way when it is raining. The liquid is applied to the back with a medium hardbrush made from cocoa-nut fiber combined with bamboo. This brush treatment should be carried out so that the openings in the skin are laid bare. It is necessary to remove the crust of larval secretion. Thick hair is not a disadvantage but an advantage as the liquid is retained better by the hairs.

The results of this method have proved to be very favourable, and are better than the application of ointment or any other method. By this simple and practicable method, and the farmers' co-operation, about 95% of the larvae are killed in time. A very few are not touched by the liquid because they do not occur on the back but on the neck or the rump and are not seen and washed. The larvae die within 5 days after treatment. The dead larvae dry up and within a month they fall from the skin.

The entire eradication procedure is directly supervised by the Health Service. This includes the personnel for treating the animals, the treatment itself, the liquid used, and inspection of the results. The Inspectors of the Veterinary Service have also the task of supervising the observance of the regulations. Cattle markets are very im-

portant in the Netherlands. These too are supervised in the province. It is forbidden to bring cattle with larvae to the markets. When necessary the animals are treated before they are admitted by the supervising veterinary surgeons. The farmers have to bear the expense.

Of the 150,000 head of cattle in the province of Utrecht, 30,000 are young animals. Adding the 10,000 head of older cattle that are infected with the warble fly, this means that 40,000 animals are treated each year. At present, the action has progressed so far that the number of swellings is not more than 5 % of what it was six years ago.

In some districts the action has had more success than in others. The success of this uniform and general action is especially due to the fact that the supervision and execution of the regulations was carried out by men appointed by the Animal Health Service.

The cost is estimated at 60 cents per treated animal in some districts, in others with a denser cattle stock, at 45 cents (this includes everything). Part of the expense (35 cents) is born by the cattle farmer, the other part by the Animal Health Service. A propaganda film has been made and is frequently shown. It stimulates the farmers to pay more attention to this disease.

Sporadic cases of poisoning caused by the treatment have occurred. One case was observed in which a cow, through licking other already treated animals, ingested so much powder that death followed after an hour with symptoms of paralysis. In light cases, only a flow of saliva and a decrease of appetite was observed. Pigs that drank of the liquid through carelessness of the men charged with the application, died. Ingestion of one quarter of a litre of the solution was followed by symptoms of paralysis and death.

If it is not possible for the Health Service to appoint the people who have to do the washing, this is done by the local dairy factories, whose inspectors do this work. They divide the area and appoint men to carry out the combative measures. These men may belong to the personnel of the dairy works, but usually this is not so.

The Utrecht Health Service has divided the whole province into districts. As a rule the same men are appointed as the year before. If they are not available, an advertisement is put in the local papers. Sometimes it is not possible to find a local man in each village. In this case two men are appointed in the next village, who will also carry on the work in the neighbouring village, assisted by their colleague in the village on the far side. From the Health Service they receive the powder in bags of 150 gr. with labels giving the contents and containing the warning 'poisonous for cattle'. If they should run out of powder, they can apply for more. Five litres of the solution are sufficient for fifteen animals. The bags of powder or the solution must not be handed over to the farmers. The farmers are quite willing to accept the responsibility, but the action might suffer through it, as in some cases farmers are known to have omitted the washing. The appointees have been ordered explicitly to wash the animals and to examine them personally for swellings. It is necessary that this is done by the appointee, for only in this way will it be certain that it is done everywhere on time. If

it should be done too late the larvae will have matured and dropped on the grass, where they will develop into flies. In this province in 1938, a voluntary action was taken. It was sponsored by some dairy factories which were suffering from this defect. The results at that time were poor. In this same area, in 1947, the farmers could only be persuaded with great difficulty to participate in a proper, general, combatant action by specially appointed men. They often preferred to take action themselves, and when the specially appointed man arrived to take combatant measures were likely to wish to put it off till some other time. The man should be reliable, not omit some animals that are difficult to catch or because the farmer is not interested. The work done should be done well. The washing need not take long: two minutes per animal is enough, but *then* the brushing should be done really vigorously. It is recommended, after brushing the animal, to dip the brush into the solution once more and to leave the back thoroughly wet.

Settlement of the amount to be paid by the farmers is by means of numbered receipts, carbon copies of which are sent to the Health Service. The appointee hands in the proceeds of the contributions from the farmers and receives a remuneration from the Health Service. He notes the names of those farmers who showed a lack of co-operation in catching the animals, and passes them on to the Health Service. At the second round, one month later, the farmer signs a list, adding the number of cattle, to show that these have been treated a second time by the officially appointed man. As a rule the farmers do not pay for the second round. At the first round he pays for two treatments. The appointee sends this list to the Health Service in order to get paid for the work he has done.

At the third round, when for the first time the older animals are treated, that is after another month (late in May-June), the farmers once more sign the list of the appointee and only pay for the older animals treated. In the province of Utrecht the farmer pays 25 cents.

It was found to be more just that the farmer should pay for the treated animals only, instead of an apportionment on all his animals, when only those with larvae are treated. At any time the appointee can report special cases to the Health Service or, in a particular district, to the inspectors of the dairy factories. These will then send assistants or go themselves to the farmers to inquire about this unwillingness to have their cattle washed by the appointee. As a rule, a talk will do the trick and the treatment can be applied there as well. If no agreement is reached the State Veterinary Inspection is notified, who will force the farmer to comply by drawing up a report of his refusal to take combatant measures. The fines imposed by the court in these cases vary considerably. It is, in fact, not possible for the judge to have a clear understanding of the mischief done by one farmer in refusing to cooperate. For he is the cause of new warble flies and will by his conduct create a reluctance in his neighbours to continue to fight the evil, as he, seemingly has no expense.

We must insist that the evil can only be combated satisfactorily when all farmers take part in it. It must be carried out by specially appointed men, so that the work is done properly and in time. They must not only call on the farmers, but keep a sharp

look-out in their district for herds of cattle on the common pasture, or grazing on pastures not under direct control of the neighbouring farmer. They must be skilful in handling cattle. It is much better to drive the cattle into a pen to be washed, than to have to catch them one by one in the pasture. The men to whom this task has been assigned must be assured in their work of the support of competent inspectors with the power to take severe measures against unwilling farmers, but who must also be able to talk things over. The inspectors should also give expert guidance and announce beforehand what is going to happen. The results will soon convince the farmers of the usefulness of the action and of the benefits to them. Once this stage has been reached, as it has been in the province of Utrecht, the combating of this evil becomes a fairly simple matter.

There is a distinct possibility of convincing the farmers of the use of the action by local co-operation of the farmers' clubs and local branches of the Health Service. The action may be furthered in this way because it can be said to remain in the hands of the farmers, although carried out by a specially appointed man and controlled by the Health Service. The passing of statutory enactments is in itself not sufficient. The combating of the evil should be made effective by practical measures.

At the entrance to markets, where cattle are examined for warble fly larvae, the Health Service has arranged that there shall always be some special washers to wash the cattle affected with warble fly larvae. These animals are not permitted on the markets until they have been treated. The washing is done irrespective of a report for illegal marketing. Apart from derris powder, other cures such as perfinita, an oil product and d.d.t. in solution or in powder were also experimented with. The results were less satisfactory. The procedure was also more laborious. Paradichlorbenzol in vaseline (10 %) is satisfactory, but the application requires more time, and is therefore more expensive. Other cures with a deadly effect, such as creoline, could be mentioned. Medicaments, to be injected into animals in order to kill the larva in its initial stage of development, were not used. Scientifically their effects are still doubtful.

*Conclusion:* A general action for the extermination of the warble fly carried out by especially trained personnel, with compulsory participation on the part of the farmers, supervision by the Animal Health Service and extra supervision (if necessary) by the State Veterinary Service, can have very good results in 5 years. The remedy used was powdered derris or lonchocarpus root suspended in water without addition of a detergent. 200 grams of the powder containing 5-8 % rotenone, or 150 grams containing 8-11 %, were suspended in 5 litres of water, and brushed out on the back, over the swellings, without removal of the hairs. If this method was followed in the spring (April-May), when the larvae are in the first stage of their development, 95 % of the larvae died. Young animals were treated in April and May, older animals in June and July.

Harmful symptoms after treatment included an abscess observed a few times at the site of the swelling, where the brush had damaged the larvae and infection had followed. There were also sporadic cases of poisoning through licking. A few times pigs were poisoned by the wash through carelessness of personnel.



In the province of Utrecht, the average cost for each treated animal is 55 cents. In future this figure will be higher, but the number of animals needing treatment is rapidly decreasing.

In 1947, an average of 25 swellings was found for every young animal in the province of Utrecht,

in 1948	15
1949	10
1950	5
1951	4
1952	2
1953	1,5 and many herds were entirely free from larvae.

In the province of Utrecht, where in 1947 38,700 animals of a total cattle population of 150,000 were treated, the treatment cost was fl 18,700. In the same province the cost in 1952, was fl 16,000, when 29,500 animals were treated one or two times.

# IMPORTANCE OF THE STOCK OF PIGS, BACON PRODUCTION, ORGANIZATION FOR CONTROLLING SWINE FEVER

H. VENEMA

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The Netherlands stock of pigs was following a rapid decrease during the second world war, but it was gradually restored as it became possible to import fodder from abroad.

At the May census in 1951 about 2,000,000 pigs were registered. This number of pigs is distributed over about 220,000 cattle farms, so that an average of 9 pigs per farm are kept. There are, however large pig fattening farms in the Netherlands where several hundreds of pigs are kept and, in addition to these, there are breeding farms. On the small farms, in particular pigs are kept on the lighter soils, while on the farms on the heavy clay soil, very few pigs are to be found.

Our stock of pigs is of great importance, in the first place for the supply of domestic meat and fat and, in the second place, for our export of meat and meat products. Since the Meat Inspection Act came into force we have at our disposal accurate statistics on the number of pigs slaughtered for the home market, including those concerning the animals registered for normal inspection and those intended for domestic slaughter (that is, for the use of the farmer's family).

Between 1923 and 1930, the number of animals normally inspected increased and came to an average of 1 million per year, while the number of animals killed for home consumption regularly decreased and came to an average of 43,000 per year.

Between 1930 and 1940, the number of normally inspected animals killed rose to 1,335,000 per year; the number of animals killed for domestic use fell to an average of 16,000 per year.

In the five war years, an average of 280,000 pigs per year were registered for inspection and the number of domestic killings rose to the formidable number of an average of 130,000 per year.

After the war the number of pigs killed in slaughter-houses increased regularly, and in the years 1951 and 1952 it was well over 2,000,000 per annum; on the other hand the number of domestic killings fell rapidly and in 1951 and 1952 came to an average of 40,000 per annum. We may therefore assume that about 2,000,000 pigs are now being killed for home consumption.

The Netherlands has well over 10,000,000 inhabitants, who in 1951 consumed a

quantity of 13.2 kilogrammes per head, that is a total of 132 million kilogrammes, produced by 2,000,000 pigs sold for slaughter; according to these figures the slaughtered pigs would only weigh an average of 60 kilogrammes each. But it may be assumed that this weight is considerably higher. Those parts not consumed in our own country consist of bacon, which as such, or melted down, is exported as pork fat, as well as a large part of the offal such as heads and trotters.

For years before the second world war, the Netherlands produced a large quantity of bacon intended for export to England. In the war years this immediately became impossible, but after the war the production of bacon and the export of this commodity gradually increased. In 1951 610,000 pigs were exported to England in the form of bacon; in 1952 the number was 700,000. The total production of fat pigs therefore came to 2,700,000 in 1952. The total value of exported pork amounted in 1951 to 63½ million guilders.

From the foregoing it may be seen that the stock of pigs in the Netherlands is of great economic importance, and that the maintenance in a healthy condition of this national asset lays great responsibility on the shoulders of the Veterinary Service.

#### ORGANIZATION OF THE FIGHT AGAINST SWINE FEVER

The prevention and control of contagious animal diseases is carried out in accordance with rules laid down in the so-called *Cattle Act*; on this Act are based a large number of regulations in the form of Royal Decrees and Ministerial Orders. It is not my task to discuss this Act and the decrees giving effect to it, but I will confine myself to those sections and decrees which relate to the contagious diseases of pigs, of which only swine fever is indicated as such. As regards the task allotted to the Veterinary Service, you have already read before, that this does not consist only in the prevention and control of the diseases set out in the Act, but that in the first place it comprises:

‘care for the general state of health of the stock of cattle’.

I thought it well just to refer to this point, so that you may not get the impression that the Veterinary Service has only to do with the diseases mentioned in Section 7 of the *Cattle Act*.

Although, beside swine fever, other contagious diseases of pigs also occur in the Netherlands, the fever is nevertheless the one disease which causes us great concern on account of its frequently virulent character, its rapid spread and the difficulty in recognizing it, the still imperfect preventive treatment, and the totally inadequate curative treatment. I will therefore only deal with swine fever and the regulations relating to it.

In Section 7 of the *Cattle Act*, a number of diseases are named to which the various Sections of the Act are applicable. Until 1937, swine fever was not named among them. This was intentionally omitted until that year because it was difficult to diagnose swine fever, so that the cattle keeper himself could by no means be expected to be able to recognise the disease and to report its occurrence. Before 1937 the provincial inspectors of the Veterinary Service regularly made mention of the occurrence of swine fever in their annual reports, but it is certain that only a part of the cases were recognised as such and brought to the notice of the Government. In any case we are

convinced that this ruinous disease constitutes a threat to our stock of pigs and that it is necessary to take measures for its prevention and control. It was therefore enacted by Royal Decree of 27 November 1936 that the regulations of Title III of the Cattle Act, with the exception of the provision of Section 37 of the Act, shall be applied to the prevention and control of swine fever. This Royal Decree became operative on 15 December, 1936, so that its results would only become observable in respect of 1937.

We will now, on broad lines, have to review the provisions laid down in Title III of the Cattle Act and see what measures thereof were adopted during the last epizootic.

In the first place the Minister of Agriculture, Fisheries and Food may impose on the cattle-keepers the obligation to have their pigs vaccinated. Up to the present day no use has been made of this possibility, although it has been contemplated, for instance, to impose this obligation in respect to the pigs taken to market, but the difficulties involved in checking the vaccination have, for the moment, restrained the Veterinary Service from advising the taking of this step.

Further, the Minister may, if in his opinion it is necessary in view of the danger of contagion, order the suspension of pig markets for the whole of the country or for certain parts of it and the closing of zoological gardens and similar establishments, and prohibit the holding of inspections, shows and sales, as well as the assembling of pigs in any similar way.

Rules are laid down with regard to the use of vaccines and also to prohibit pigs being vaccinated with living vaccines, unless the Inspector of the Veterinary Service has certified that he has no objection to this.

For the prevention of contagious cattle diseases and therefore of swine fever, the import of foreign cattle (pigs), and a number of products derived from cattle, is prohibited.

For special reasons the Minister of Agriculture may waive this prohibition and permit import and transit subject to the necessary precautions being taken against transmission and contagion. In general it is made a condition that when animals are imported a certificate shall be issued by an official veterinary authority stating that the animals concerned have been examined by a veterinary surgeon on the date of export, which examination must show that the animals were healthy and free from contagious diseases and that, in addition, these diseases do not occur on the farms of origin and have not occurred in the last 3 months, while within a radius of 25 kilometres these diseases may not have been prevalent.

On arrival at the frontier, the animals are subjected to a veterinary inspection by or on behalf of the Inspector of the Veterinary Service and, if found to be healthy and the prescribed conditions are fulfilled, these animals are kept in quarantine for another 3 weeks under the supervision of the Inspector.

Should pigs show symptoms of fever, their owner is obligated to report the fact at once to the local burgomaster. The latter at once notifies the Inspector of the Veterinary Service in the District, and at once takes the requisite provisional measures which consist in ordering the owner to isolate the suspected pigs and to put up a warning

notice bearing the words 'Swine Fever' at the entrance to the farm or the premises.

The Inspector of the Veterinary Service informs the burgomaster as soon as possible what measures he considers necessary to control the disease. In the case of swine fever these measures consist in:

- a. isolating diseased and suspected pigs;
- b. penning the diseased and suspected pigs;
- c. putting up warning notices;
- d. rendering innocuous diseased and suspected pigs which have been killed or have died;
- e. disinfecting buildings, lands, dung heaps, and movable objects;
- f. applying measures which have been designated as effective by science or practice.

It is forbidden to transport pigs from or to buildings or lands where a sign board has been put up. The burgomaster may grant exemption from this prohibition provided the Inspector of the Veterinary Service has no objection and, if necessary, on conditions to be laid down by this official.

The entrance to buildings or lands where a sign has been put up, and which may therefore be regarded as infected with swine fever, is prohibited, except for officials of the Veterinary Service and a few other specially named officials. When these officials leave the buildings or lands in question, they must first disinfect their footwear and hands with a solution of sodium hydrate. These measures are intended to prevent the spread of viruses.

The notice-boards and signs may only be removed by order of the burgomaster when the Inspector of the Veterinary Service has certified in writing that the disease on the farm concerned has ceased and that disinfection has taken place.

The rendering innocuous of diseased and suspected cattle which have been killed or have died is effected by destruction. In a Royal Decree detailed instructions are given regarding the manner of disinfection; I shall not go further into these here.

It is forbidden to transport diseased or suspected pigs, unless the burgomaster grants exemption from this prohibition on conditions to be prescribed by the Inspector of the Veterinary Service. This possibility is often made use of in order to give the owner of an infected herd of pigs an opportunity to sell his apparently still healthy pigs.

In such cases, it is laid down that these animals are conveyed under police supervision straight to a slaughter-house to be killed there as soon as possible. The transport of these animals to an export slaughter-house is prohibited in order to prevent the meat being allotted for export.

In order to prevent contagion, the Minister may prohibit the transport within or out of the Kingdom or to or within specified parts of the country, of a number of articles which may be carriers of virus such as, among others, pigs, meat, hides, brushes, hair, manure, and cattle fodder.

In the case of compulsory slaughter, which up to the present day has not been enforced, compensation is granted by the government after the animals have previously been valued.

The statutory measures outlined above in their entirety and individually, which may

be enforced under the provisions of the Cattle Act, have as their object the prevention of the spreading of contagion with swine fever and also the prevention of this disease being introduced from abroad.

Of the utmost importance is the compulsory reporting of each case of disease and suspected case of disease observed by the owner, and the subsequent isolation of the farm. The question remains to be answered whether the owner is in a position to recognise swine fever as such; this question will certainly be further gone into in one of the following lectures.

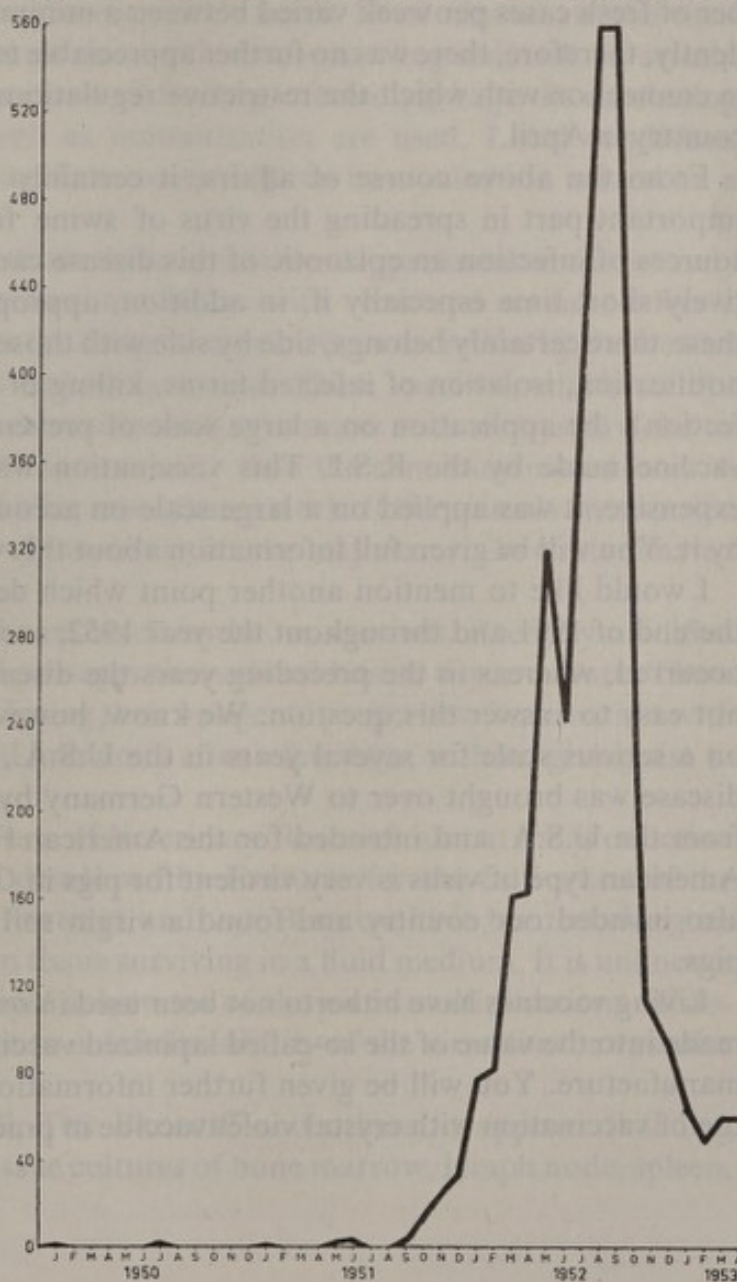
It is a well-known fact that markets constitute a source of infection and, viewed from the veterinary standpoint these sources of disease should be closed when any increase in the number of cases of disease commences. In this connection, however, account should be taken of the economic consequences of the closing of the markets.

The market system is highly developed in the Netherlands and, for large groups of people, it means their daily bread. If pig markets are closed down, a large number of these people are deprived of their income and some of them have to seek aid from social welfare organizations. Hence, before proceeding to close off these sources of infection, the pros and cons should be carefully weighed. This was the case in 1952. After the second world war only sporadic cases of swine fever occurred, viz:

in 1946	85 cases
in 1947	33 cases
in 1948	30 cases
in 1949	15 cases
in 1950	3 cases

spread over the whole country, without any tendency to increase.

In 1951 the total number of cases rose to 80; this increase became alarming and still more so when early in 1952 it persisted to a greater extent, namely, with 76 cases in January, 81 in



*Course of hog cholera 1950/53. Number of fresh cases per month.*

February, 169 in March and 177 in April. The Veterinary Service pressed for the closing of pig markets, but this proposal did not find favour in the trade and another method had first to be tried out. On the 30 April the country was divided into 3 zones, to and from which the transport of pigs was prohibited. This measure did not achieve the result expected; the number of fresh cases constantly increased and rose to 557 in the month of August. The losses sustained were heavy and many a pig farmer was reduced to poverty. Distress had then increased to such an extent that the pig farmers themselves were convinced that the strictest measures were necessary and, consequently, by Ministerial Order, the pig markets, except those for fat pigs, were suspended. The results were not wanting because a rapid fall in the number of cases set in; December ended with 92. From January 1st the measure could be discontinued in the east of the country, and on 30 January the markets were again permitted to open in the southern part of the country. The situation now remained stationary and the number of fresh cases per week varied between a minimum of 9 and a maximum of 21; evidently, therefore, there was no further appreciable tendency for the contagion to spread, in connection with which the restrictive regulations were lifted in the other part of the country in April.

From the above course of affairs, it certainly appears that the markets play an important part in spreading the virus of swine fever and that by closing off these sources of infection an epizootic of this disease can be quickly checked in a comparatively short time especially if, in addition, appropriate measures are applied and to these there certainly belongs, side by side with those previously referred to (compulsory notification, isolation of infected farms, killing of diseased and suspected pigs, disinfection), the application on a large scale of preventive vaccination with crystal violet vaccine made by the R.S.I. This vaccination was voluntary and, although rather expensive, it was applied on a large scale on account of the excellent results achieved by it. You will be given full information about this vaccination later.

I would like to mention another point which deals with the question why, toward the end of 1951 and throughout the year 1952, such a great extension of swine fever occurred, whereas in the preceding years the disease occurred only sporadically. It is not easy to answer this question. We know, however, that swine fever had occurred on a serious scale for several years in the U.S.A., and we may well assume that this disease was brought over to Western Germany by means of meat products exported from the U.S.A. and intended for the American Forces. It is not impossible that the American type of virus is very virulent for pigs in Germany, and that this type of virus also invaded our country and found a virgin soil in an extremely sensitive stock of pigs.

Living vaccines have hitherto not been used in our country. An enquiry is now being made into the value of the so-called lapinized vaccine which is considerably cheaper to manufacture. You will be given further information on this subject, as well as on the use of vaccination with crystal violet vaccine in practice.

# RESEARCH ON THE CULTIVATION IN VITRO OF THE HOG CHOLERA VIRUS

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Hog cholera may cause severe losses in susceptible herds. Dissemination of the virus is generally due to direct or indirect contact with virus-excreting animals. In certain regions new infections are often the result of feeding infected garbage. In combating the disease sanitary measures as well as immunization are used. Local conditions decide which is the most efficient way. In enzootic regions immunization is used on a large scale.

Until recently three different methods were available. Hyperimmune serum only protects for a few weeks. Simultaneous inoculation with virus and serum confers a solid and lasting immunity. However the danger exists of virus excretion by the treated animals. As a third method, inactivated virus is used. These vaccines, which are obtained by incubating the virus for some weeks with substances such as crystal violet or eucalyptol, mostly give a shorter lasting immunity than the simultaneous method. A sufficient protection is only obtained after more than a week. If the animals are exposed to heavy infections, a large number of immunity breaks may occur. On contaminated farms these vaccines are worthless.

These disadvantages stimulated the research for better vaccines. Recently modified virus strains were obtained by serial passages through rabbits or tissue culture. These had lost their pathogenicity without losing their antigenic properties. Vaccination with these strains is said to result in a solid immunity after a few days, without giving rise to the spread of virus.

The experiments at the State Institute of Veterinary Research aim at the production of a vaccine. Following the method developed by FRENKEL for the cultivation of the virus of foot and mouth disease, S. FRENKEL and VAN BEKKUM have been working on the cultivation of hog cholera virus in tissue surviving in a fluid medium. It is unknown which kinds of cells form the principal substrates of the virus multiplication. Histopathological changes, however, point to a definite affinity of the virus for the vascular endothelium.

From the experiments of HECKE<sup>1</sup>, TEN BROECK<sup>2</sup> and others, it appears that hog cholera virus can be propagated in tissue cultures of bone marrow, lymph node, spleen,

<sup>1</sup> *Zbl. Bact.* 126 (1932) 517.

<sup>2</sup> *J. Exp. Med.* 74 (1941) 427.



testis, and chorioid plexus of swine. The methods used by these workers, however, are not suitable for large scale virus production.

For our work, hog spleen was selected, as this material can easily be obtained aseptically and in large quantities. The tissue is sliced with a rotating knife and afterwards minced with machine-driven scissors. The investigations of a fluid medium, in which these particles survive for several days, are carried out with the use of the Warburg tissue respiration apparatus. The degree of survival is estimated by comparing the oxygen uptake of the tissue after the incubation in the medium which is to be tested, to that of the fresh tissue. In this way the total oxygen consumption of the cells is measured. It is presumed that a medium which allows maximum cell survival, will also permit maximum virus multiplication. It is possible, however, that the cells most suited to virus multiplication make other demands upon the medium than the other cells, which may form a far greater part of the tissue. Although this working hypothesis was only partly corroborated in later experiments, it served to obtain important results. The Warburg technique had to be used because a more direct method of judging the suitability of the medium for virus production could not be applied for practical reasons.

In the procedure we used, one component of the medium was varied at a time and the influence on the oxygen consumption studied. As a rule the tissue was incubated for four days, as both the incubation period in experimental animals and the results obtained by HECKE indicate that this period allows virus multiplication. To permit survival of the tissue during this period it was necessary to add homologous serum to the Tyrode solution originally used, aeration with oxygen and shaking of the cultures were also important improvements. For aeration, oxygen containing four to six percent carbon dioxide was used. Especially with reference to pH adjustment further experiments will decide if a slightly lower carbon dioxide content is not to be preferred. As a result of the aeration with the mixture now in use, a pH drop occurs within the first few hours (from 7.8 to 7.2). Later pH shifts, which are probably caused by metabolic processes, are much smaller. It seems probable that the first fall, especially causes damage to the tissue as soon as values below the optimum are reached. In the first twenty-four hours the oxygen consumption of the tissue in the cultures decreases about twenty-five percent. In the next three days the drop does not exceed another fifteen percent. Doubling the quantity of dextrose in the culture fluid did not improve oxygen consumption, although the cells metabolized more of this compound.

All experiments were carried out in one hundred or two hundred millilitres of culture fluid. Three to five percent of tissue were added. When a larger proportion of tissue was used survival was unfavourably affected. It is possible, however, that for a maximum virus yield in a certain quantity of fluid another proportion of tissue must be used.

To suppress bacterial contamination, antibiotics were added to all cultures. As a rule penicillin and streptomycin were used. This latter was employed in a concentration of fifty microgrammes per millilitre of fluid, as hundred microgrammes proved to be noxious to the tissue. In the cultivation of poliomyelitis and mumps viruses ENDERS<sup>1</sup> obtained favourable results when serum ultrafiltrate was used to replace serum in the culture fluid. By means of the Warburg apparatus we studied the effect of the replacement of hog serum by its own ultrafiltrate. This was obtained by filtration of serum through cellophane membranes under positive pressure. As the survival of the tissue in a medium containing ultrafiltrate was much diminished as compared to one containing serum, the effect on virus yield was not ascertained.

In cultivating the virus three to five grammes of tissue per hundred millilitres of fluid were used.

<sup>1</sup> *P.S.E.B.M.* 69, (1948) 124.  
*J. imm.* 69 (1952) 639-694.

The medium itself consisted of Tyrode solution, peptone, hog serum, and antibiotics. It will undoubtedly be possible to carry out the same process on a much larger scale. In the first cultures virulent serum was used as a source of virus. In further transfers the inoculum consisted of the supernate obtained by centrifugation of the homogenized tissue of the preceding culture. The virus strain used was received from Professor UBERTINI (Brescia) and was one used for simultaneous inoculation. No difficulties were encountered in adapting the virus to the cultures. Until now, more than fifty successive transfers have been carried out. In these passages the virus retained its full pathogenicity. In all cultures tested, the presence of the virus could be demonstrated by injecting susceptible pigs with culture fluid or suspended tissue.

Animals were obtained from farms known not to be contaminated with hog cholera. In addition they were kept in quarantine for at least a fortnight. Temperatures were taken twice a day. An autopsy was made on animals which had been severely ill. A bacteriological examination was carried out as a matter of routine. Brain sections were examined histologically.

In ninety-three percent of the cases a mononuclear encephalitis was found. Samples of tissue and serum used in the preparation of cultures have been tested and found free of hog cholera virus. This proves that no other virus than the original inoculum has been introduced into the cultures.

In the titrations, which were carried out on a moderate scale, one pig was always injected with successive ten-fold dilutions of tissue suspensions or culture fluid. Usually one-tenth of a milligramme was found to be virulent, sometimes one-hundredth of a milligramme proved to be sufficient for infection. From these experiments it appeared that no direct relation existed between the amount of virus which could be demonstrated in the tissue and the survival as measured with the tissue-respiration apparatus. The concentration of the virus in the culture fluid appeared to be about as high as that in the tissue. More than ninety percent of the virus is consequently liberated into the fluid. During the period of cultivation the quantity of virus increases about one thousand times.

At present the relation between the quantity of virus present in a culture and the duration of the incubation is being investigated. The first results suggest that after three days more virus is present than after four or five. During the first two days only small amounts of virus are found.

The use of pigs as experimental animals is both complicated and expensive. We have tried several different ways to demonstrate the presence of the virus.

*In the first place* spleen used for virus propagation was examined for histological changes as compared with tissue from uninfected but otherwise similar cultures. So far no clear-cut differences have been observed, cells from all sections showed the same regressive changes.

*In other experiments* the influence of the virus infection upon the survival of the cells, as measured with the Warburg apparatus, was determined. When the oxygen intake of an infected culture was compared with that of an uninfected control, no significant differences could be established.

As it was judged possible that the quantity of virus in these cultures was not sufficient to cause maximum cellular damage, other tests employing virulent serum were used. This serum was obtained from experimental pigs killed at an early stage of the infec-

tion at a time when the blood contains large amounts of virus. The survival in these cultures was compared with that in controls containing normal serum. Tissue from the infected cultures always proved to have the lower oxygen consumption of the two. However the difference showed a considerable variation. Consequently the method was not suited for virus titration.

*In a third series* of experiments oxygen intake of fresh tissue in the presence of normal serum was compared to that of the same tissue exposed to virulent serum. The purpose of these experiments was to keep the normally occurring regression at a minimum level. Here too the presence of the virus generally proved to be unfavourable for cell metabolism, but the inconstancy of the differences were still larger than in the series mentioned before.

It should be taken into account that in both cases sera from animals suffering from swine fever may differ from normal sera in respects other than their virus content. It has been impossible to eliminate this influence.

*Finally* we are attempting to demonstrate the presence of the virus by serological means. In different laboratories more knowledge of various serologic tests and their possibilities has recently been gained. So far their application to hog cholera has not been successful.

*Summarizing*, it can be said, that the method of cultivating hog cholera virus in serial passages in surviving tissue which has been developed in our institute, could be adapted to any desired scale. At present the quantities of virus obtained are not large enough to produce an inactivated vaccine on an commercial scale. If a non-pathogenic virus strain is used, the yield might be sufficient for practical use.

## RESEARCH AND VACCINE PRODUCTION IN SWINE DISEASES

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The title deals with a description of the research and production of vaccine produced for and directed against swine diseases in the interest of the very important production of pigs in Holland.

It here concerns the research done by the State Serum Institute at Rotterdam.

If we aim at getting a high production of meat from our pigs with a minimum of consumed food it is necessary to make conditions for the animals favourable as regards food, care, and the prevention of infectious diseases. For more than 20 years pigs' food has no longer been supplied empirically, but use has been made of scientifically composed rations, which appear to have very great value in preventing all kinds of production troubles and which deprive infectious diseases of their malignant character. It has not been found economical to provide large quantities of a cheap, plain food, as through this, especially with young animals, deficiencies as regards amino acids, vitamins, minerals (macro and micro-elements) easily develop.

With balanced feeding the growth of animals is regular, and sickness, such as the above, does not, or very seldom occurs.

But of late years it has been seen that it is quite possible to make pigs grow well without adding animal protein to the ration, if one adds small quantities of antibiotics such as aureomycin, procain, penicillin, or terramycin to the ration consisting of vegetable starch and protein. The addition of this is also said to further the prevention of disease. As our trials with this have not been extensive, it is only mentioned in passing.

The aim of our research is to gain more knowledge of the aetiology of the principal swine diseases to make prevention and therapy as efficient as possible. If, in this, we require the help of the food specialist, we shall accept it very gladly.

As the first disease to be taken into consideration I must mention *Erysipelas suis*, a disease which was formerly very frequent and of a very malignant character in Holland. In the years previous to 1933 I remember how greatly *Erysipelas suis* spread and was very malignant in my practice.

After 1933 epizootics can hardly be mentioned. Manifold cases of epizootics *did* occur, but the number of deaths with or without serum treatment decreased steadily.

I am inclined to believe that the greatly improved food of the animals has been a very favourable factor in this. The diagnosis presents few difficulties. One sees animals which have lost their appetite, are stiff in the hind legs and after a short or longer period develop urticaria. The body-temperature is very high, often higher than 42 °C. The postmortem examination of dead animals shows a picture of sepsis. Swollen parenchymatous organs are found from which *Erysipelothrix rhusiopathiae* can be cultivated in pure culture.

In many cases the bacilli (Gram positive slender rods) may be successfully demonstrated in smears of the kidney.

At present in Holland, it is generally so, that when Erysipelas occurs in pigs, the treatment of the sick animals with serum or penicillin as treatment of the herd is often sufficient. This method of treatment has been decided upon, too, by various practitioners as there is hardly any sense in passively immunizing the still healthy animals. But it is being decided more and more when epizootics occur to make the animals, which are not sick, actively immune. Thanks to active immunization methods developed during the last years – by means of avirulent erysipelas vaccines – it has become apparent to various practitioners that this method can be applied without difficulty.

As the occurrence of epizootics is largely bound up with predisposing moments, such as change of the weather, transportation, change of food, etc. it can hardly be reckoned that such treatment might be fatal if the circumstances have not changed.

As stated above during the last years some methods of vaccination have been developed which occur with avirulent vaccine as contrasted with the Lorenz-vaccination.

The Lorenz simultaneous vaccination takes place with serum plus virulent culture. Besides the fact that this is made with a virulent erysipelas culture, whereby the chance of vaccination erysipelas is very likely, it appears in practice that the disease through this is retained in a more or less degree on the premises. This vaccination, because of the need of serum, is also quite expensive.

The modern methods according to KONDO, STAUB and TRAUB are much cheaper, almost as efficient, and almost without risk of vaccination erysipelas.

The least good of these vaccinations is that according to KONDO. This vaccination has been in use in Holland since 1947.

#### PREPARATION OF VACCINE

The Kondo strain is cultivated in horse broth. After 16 to 24 hours incubation tryptoflavin is added up to  $\frac{1}{1000}$  per cent final concentration.

By the originally given dosage, 1–5 ml according to the weight of the pig to be vaccinated, too many breaks in the immunity occurred. When the dosage was increased (4–12 cc) the immunity seemed better. But there is not much faith in the Kondo vaccine in the Netherlands and consequently there is a decline in the turn over of this vaccine from year to year.

With the Kondo vaccination, as well as that according to STAUB and also TRAUB, there is the phenomenon of vaccination reaction (it may be called vaccination-erysipelas). Although the vaccines are entirely apathogen, in some cases within 14 days after vaccination one sees erysipelas appear. In my opinion this is here brought about by

the already formerly mentioned decrease in resistance of the animal through vaccination.

*The Staub vaccine* has been supplied in steadily increasing quantities to practitioners during the last years.

STAUB prepared this vaccine at the Pasteur Institute. He weakened an originally virulent strain by cultivating it in a very thin layer of broth which had been exposed over a large surface to the oxygen of the air at a temperature of 40 °C. The strain so weakened has proved to be constant. It is cultivated further in beef-broth. As adjuvans 0.2 per cent agar is added.

According to STAUB this vaccine is harmless for mice, rabbits and pigeons, it gives these animals 12–10–9 months immunity. The results with this vaccine with mice and pigs (both in the laboratory and also in practice) are very favourable.

A fortnight after vaccination with 0.1 cc Staub vaccine, 25 mice were infected intraperitoneally with a 100 times lethal dose of virulent erysipelas culture. All survived. Observation time 14 days. 12 control mice died within 2–4 days after infection.

Also tenfold passage through beef-broth or through horse serum + horse broth does not exercise any bad influence on the effect of the vaccine. Eleven mice vaccinated with vaccine prepared from a tenfold passage through beef-broth, and fourteen mice vaccinated with vaccine passed 10 times through horse-broth and serum, survived after being infected with a 100 times lethal dose. 13 pigeons were infected with 0.3 ml virulent erysipelas culture 14 days after intramuscular inoculation with 0.3 ml virulent erysipelas culture 14 days after intramuscular inoculation with 0.2 ml Staub vaccine. Two died. Eleven survived. Two control pigeons succumbed after 2 and 3 days.

In 1950 five practising veterinary surgeons were supplied with two kinds of Staub vaccine for application to pigs in practice.

In the first phase of the test the vaccine was cultivated in horsebroth plus horse-serum. There was a very luxuriant growth in this, much stronger than in beef-broth; this culture was used in the second phase of the test. In vaccinations with the vaccine grown from horse-broth and horse-serum, used in doses of  $\frac{1}{2}$  cc or 1 cc, there were cases of vaccination erysipelas in some practices. These were in a much milder degree in cases where vaccination had been done with vaccine prepared with beef-broth, and through this reverse the number of cases were reduced to 10 per cent of those with horse-broth plus serum.

In a total of 5076 pigs only two immunity breaks occurred in the most unfavourable case.

With vaccine prepared with horse-broth plus serum there was an average of 4 per cent vaccination-erysipelas, but with vaccine prepared with beefbroth, only 0.32 per cent. Later tests with larger numbers of pigs gave the following figures:

Total nr. of pigs vaccinated	22 388
Nr. of vaccination reaction cases	154 (0.7 per cent)
Nr. of break cases	13 (0.06 per cent)

The so-called vaccination reactions mean that, at the site of injection a big red spot develops, urticaria appear, the appetite declines, the temperature rises somewhat.

In 1950 it was obvious to us that 27 pigs developed urticaria one week after vaccination. This phenomenon disappeared without any therapy and without unfavourable results. If only a red spot appears on the place of injection, then this phenomenon is

of hardly any importance. The owner will be warned of this phenomenon and the unimportance of it pointed out.

*Traub's absorbed vaccine* is the third vaccine brought onto the market. It is an absorbed vaccine with  $Al(OH)_3$  killed by formalin. Definite erysipelas strains separate a soluble immunizing substance. The number of strains that do this, however, is small (from 2-5 per cent).

The preparation of the vaccines is bound by the following three main conditions:

1. One must have at ones disposal a strain which will separate immunizing substance in ample degree.
2. The culture must be grown excessively.
3. The exact quality of the  $Al(OH)_3$  is of great importance.

The correct strains were placed at our disposal, by among others Dr MASIC of Yugoslavia, Dr STABLEFORTH of Weybridge and Dr RISLAKKI of Finland.

An exceptionally strong growth was obtained with the following substratum formula:

Horse-broth:

10% horse serum	
2% pepton	pH 8
2% NaCl	the use of $\frac{1}{4}$ % lactose sometimes recommended
4% infused liver.	

With this addition the pH may decline too much through formation of lactic acid. So this is not done here.

The erysipelas bacteria are cultivated 2-6 days in this broth. Through this a strong growth takes place, so that a strong layer of bacteria is formed. The  $Al(OH)_3$  must have an ash-content of more or less 2 per cent and a pH of 8 to 9.

The preparation of the vaccine takes place now by adding 20 per cent  $Al(OH)_3$  to the well-grown culture. This mixture must be shaken for 45 minutes. Add formalin to final concentration 0.3 per cent, after which shake again for 15 minutes. Let the mixture stand some hours at room temperature. The supernatant liquid is then perfectly clear. Half of the total liquid is then siphoned off without stirring up the sunken  $Al(OH)_3$ . The remaining part consisting of killed culture plus  $Al(OH)_3$  is the desired vaccine. The end pH must not lie below 6. Control of eventually surviving germs must be negative. The dose per pig is 5 cc irrespective of age. Baby pigs are not vaccinated.

At experimental tests with the adsorbed vaccine the following facts were established:

Total number of vaccinated pigs	21780
Number of vaccination reaction	20 (0,09%)
Number of vaccination breaks	65 (0,3%)

Tests are made by the State Serum Institute to compare:

1. Lorenz simultaneous vaccination;
2. Staub vaccination;
3. Kondo vaccination;
4. Traub's adsorbed vaccine vaccination.

It appeared that the classical vaccination of LORENZ gave the best results (at least with these limited tests), for neither after the vaccination nor after the infection (after 24 days) were unfavourable results apparent.

The infection was applied intravenously with 5 cc broth-culture of virulent strain Amsterdam VIII. The immunity raised by STAUB is not less than that of LORENZ. After the vaccination, however, the temperature of the pigs rose somewhat.

Furthermore it appeared that the vaccination with Kondo vaccine and Traub vaccine brought no injurious effects with them, but the immunity was not so strongly developed as with the Lorenz and Staub vaccination. With the infection a mild phenomenon of erysipelas appeared after 24 days. With the controls there was a much higher temperature after the infection and 4 out of 5 developed severe urticaria, and one died through erysipelas.

Considering the very small cost of the Staub vaccine I deem it at the moment the vaccine of choice.

*Swine fever* (or Hog cholera) during late years has again become a severe threat to the Dutch pig herd. During the second World War it disappeared, presumably through the stoppage of trade and transport, and also perhaps through the lack of imports of pork from chronically infected countries such as East Europe, U.S.A. Only in the spring of 1951 did we again see swine fever appear. It was not long before the whole of Holland was infected via markets, public conveyances etc. The boar stations too played a big role in this.

I shall hardly need to go into the clinical and pathological anatomical phenomena, such as a post-mortem picture whereby hemorrhages dominate.

However, I wish to remark that in many cases it is impossible to make a diagnosis at an early stage, either with living or dead animals. According to American literature the living animal having a strong leucopenia (viz. below 9000 white blood cells per cc), gives a strong indication of swine fever. On our investigation regarding this assertion it seems that not the same value was attached to it for Dutch pigs as for American.

It *did* appear that pigs infected with very virulent American strains showed the phenomenon very clearly, but not so typically as we found described. If the diagnosis of pigs which have died does not seem clear, which often happens at an early stage, then we make an histological investigation of the central nervous system as an aid. As the swine fever virus is strongly endotheliotrophic we often see the first symptoms in the veins of the central nervous system, viz. thickening of the endothelial cells infiltrations around the wall of the small veins – monocytes in the perivascular lymph-crypts. We also see microglyosis, small and capillary haemorrhages and cell infiltrations in the soft meninges.

In consequence of the quick progress of the disease, a very early diagnosis is of very great importance, and everything must be put in motion to make an early diagnosis, which may prevent much damage – viz., by the quick slaughter of slaughter-animals and the treatment of young pigs which are still free from fever.

It is most important, however, that efforts are made in chronically infected premises or threatened premises to immunize the pigs by means of vaccination.

Our experiences with swine fever are not so great that I can and dare give our opinion as to the right way of combating this disease.

In connection with the fact that an infected animal which has shown signs of fever longer than 36 hours cannot be influenced therapeutically by serum with a big chance



of success, little can be expected from a therapeutical intervention. This has appeared from tests in our laboratories and in practice. For these tests we use the serum gained from hyper immunized pigs whereby  $\frac{1}{2}$  ml serum per kg pig must give 100 per cent protection against 1 ml virulent blood.

Preventive measures must be regarded as more efficient than therapeutic ones.

The measures which are at our service for this are:

1. The simultaneous vaccination;
2. Vaccination with chemically weakened virus;
3. Vaccination with rabbit adapted avirulent virus;
4. Vaccination by repeated passage of tissue culture weakened virus;
5. Vaccination by alternate tissue culture; rabbit and pigs passage weakened virus.

For Holland the simultaneous vaccination whereby serum and virulent virus are used, is hardly to be regarded as important, very certainly not on uninfected premises. Bringing a virulent virus to these premises is a method which in any case must be dissuaded. On infected premises it has sense, provided one is convinced that no virus carriers are formed. Regarding this, there is difference of opinion.

Vaccination with adapted virus is not much practised in Holland yet. The experimental stage here is not yet over. Although the reports over this vaccine are exceedingly favourable, we here are of the opinion that further investigation, also in practice, must be carried out before going over to the general use of these vaccines which must still be imported. We keep an eye on the possibility of sows, vaccinated with adapted virus in the first 30 days of gestation, farrowing misformed pigs without vitality, in small litters. Our experiments have shown this very clearly. I feel obliged to give a serious warning: 'Don't use rabbit adapted vaccines in breeding Stock'.

With regard to this, a connection between a history of swine fever and 'trembling pigs' is probable.

The mothers of these pigs seem to be infected in the first weeks of pregnancy, they survive, but at farrowing time they give birth to a litter of pigs which tremble continuously and die through emaciation.

In general, preventative vaccination is done with crystal violet vaccine on uninfected premises in Holland.

On infected premises the slaughtering method is followed.

Injection with crystal violet vaccine does not in general lend itself to use on infected premises, as the time in which a solid immunity may be expected covers at least 3 weeks – a period of time difficult to bridge over without infection.

The preparation of crystal violet vaccine is as follows: Healthy pigs of 100–125 kg living weight from unvaccinated sound premises are infected with one of the three selected strongly antigenic strains giving high value vaccine in contrast to the vaccines prepared from the Dutch strains. Six days after infection at the acme of the virus concentration in the blood of the sick pigs they are bled to death by heart puncture under anaesthesia. The blood is brought via the puncture-knife into a rubber tube in an aseptic way to the defibrination apparatus and defibrinized. After defibrination it is in proportion 4:1, mixed with aethylen glycol in which crystal violet 2% has been dissolved as bacteriostaticum.

This is followed by incubation for 14 days at 37 °C, in which space of time the virus becomes apathogenic although keeping the immunogenic characteristics.

After sterility, pathogenicity, and immunization tests have shown favourable results, it is given free for practice.

Previous to this, however, the vaccine charges of the 3 American strains are mixed to raise the polyvalence. The vaccine thus procured was in 1952 generally applied in Holland with favourable results. Although complaints were received over too little immunity, these were generally restricted to a minimum. The general impression was very favourable. The vaccine was of great use in the prevention of swine fever, especially at the big swill feeding premises in West-Holland. Attention must be paid, however, to animals bought for vaccination remaining free from infection 3 weeks following the vaccination. It is therefore a good custom to vaccinate the animals 3 weeks before they come to the formerly sound premises. Provoking the disease through vaccination with properly made vaccine has not occurred. The manifested immunity is solid and lasting.

After these remarks about swine fever and combat of this disease, a third important disease in Holland is called *Infectious cough of swine*. This very widely spread and continually spreading pigs' disease forms a very important sickness for the breeding and fattening trade, causing much damage.

Regarded economically, this disease very certainly comes before erysipelas and possibly also before swine fever. It is not a disease which is attended by an exceptionally high percentage of deaths, but the number of young pigs and older pigs too that are stunted in growth is very large, consequently the number of kg fodder required for 1 kg of growth is raised very considerably and the fattening period is greatly lengthened. This disease is also known in other countries.

The first to describe the symptoms was SHOPE, who already in 1931 in the U.S.A. described a pigs' disease which he called swine influenza. He declared that this disease was caused by a virus in co-operation with *Haemophilus influenza suis*. He discovered that the virus is the primary agent of the disease.

The serum of animals which have recovered can neutralize the virus in vitro. The virus neutralization already appears on the sixth day after infection. This virus has much in common with the human influenza virus type A.

In 1933 KOBE described a similar disease in Germany. This, however, differed considerably from SHOPE's influenza. In 1939 a pigs' disease in Korea was described by OCHI and NYAIRI, which was most like KOBE's Ferkelgrippe, clinically, pathologically and anatomically. Through nasal transmission in mice a virus was isolated that could be neutralized by the serum of a reconvalescent animal. Later on it was shown that this virus conformed with the human influenza virus A prime. In 1934 human influenza was successfully transmitted to pigs. *Haemophilus influenza* is necessary to obtain an extensive pneumonia.

In 1951 GULRAJANI and BEVERIDGE described a similar disease with English pigs. They called this picture of disease 'Infectious pneumonia in pigs'. It is still a question as to how far the Swedish, English and German pig diseases agree. We know a pig disease here in Holland which has the appearance of being similar. This disease also proceeds with lung symptoms while living, and when autopsy takes place pneumonia is shown, sometimes attended by pleurisy and pericarditis. Some twenty years ago

TERPSTRA succeeded in cultivating *Haemophilus* from these lungs. This experience makes me think that we may have to do with a virus here.

However, up to now, no-one has succeeded in demonstrating the virus or producing the disease in young non-coughing pigs using material which has been strained.

For some time we have tried to find ways of getting better acquainted with this injurious disease. For this purpose we have material sent in showing the symptoms transmitted intranasal or intratracheal to young healthy pigs. Up to the present we have not succeeded in making these animals sick or even feverish. Healthy young pigs have also been put on infected premises or put into sties with sick pigs. The results so far reached have not been such, either, that the alleged animals develop the disease promptly. So it is difficult to transmit the disease to healthy animals.

We now think as follows: Possibly the natural defences are strong enough to offer resistance to a mass-infection with the agent of this infectious pneumonia.

How will this be when these natural defences (hereditary and acquired) are eliminated?

A method has been worked out by us for rearing motherless pigs, and even for withholding the colostrum from them. Pigs born of healthy sows are received in sterilized paper bags at birth. The navels are bound and touched with iodine before the little pigs are put into sterilized cages in a room at a temperature of 28 °C.

Here, every little pig first receives 2½ mgr vitamine K and 25,000 I.U. vitamine A. Vitamine A is given in this dose for 3 days. They are fed the first 14 days from a bottle and teat with 'enriched' milk. The quality of the albumen must be raised to equal pig's milk. For the first day a whole egg (white and yolk) is added to every litre of milk and beaten in a Waring Blender. The milk is hygienically milked from healthy cows into sterilized buckets through which the germ standard is kept low. At the same time 1500 U. penicillin G is added to every litre of milk. It is not sterilized because with sterilized milk there is less chance of the little pigs' survival. Furthermore, to every litre of milk is added 10 grams of a vitamine B containing dried yeast 'Vital' and 400 I.U. vitamine D<sub>2</sub> and 5 cc salt mixture according to the perscription:

49.8 g FeSO <sub>4</sub>	7 aq.	} In 1 litre water
3.2 g CuSO <sub>4</sub>	5 aq.	
3.6 g MnCl <sub>3</sub>	4 aq.	
0.27 g KJ		

The pigs are fed 5 times the *1st day*, and once the 1st night with the enriched milk. The animals take about 50–60 g per time. The *2nd day* (after the 6th feeding) the white of the egg is left out (the yolk is still added to 1 litre of milk for 6 days) and there are 5 feeds a day. *After the 6th day* 4 times a day. The animals then take about 130–150 g a time. When later no egg is added a little baby pig meal is added. The quantity of it is gradually increased a little.

After 14 days (the animals are now accustomed to meal) the pigs are fed from little flat glass dishes. They learn this very quickly, with hardly any exception.

After 3 weeks they are put together under infra-red light in a sty, after which the rearing is practically finished. They stay in the sty together until they are used for infection tests. They are then fed 3 times a day out of an iron trough. The results of these infection tests are not yet fit for publication. Our hope for successful transmission tests with the agent of the infectious cough is greatly strengthened.

Besides the diseases already discussed, there are also the *diseases of the new born animals* to be examined.

It has struck us that so many little pigs die in the first week of life and have a pretty well uniform section picture, viz., degenerated parenchymatous organs, central liver necrosis.

Subcutaneous edema, edematous muscles and symptoms of anaemia are seen at the same time. That lack of vitamine A plays a role here is probable. With these little pigs an absence or low level of vitamine A of the liver is a rule.

As these baby pigs took the colostrum, which is apparent from the full little stomachs, it is supposed that the mother must have lacked vitamine A. With regard to this, improvement may perhaps be brought about by improvement of food.

Such a strong deficiency of the mothers, that eye-anomalies occur other than strictures of the Nervi optici of both sides, till now have not been seen here. With these little pigs *Esch. coli* is regularly cultivated from the parenchymatous organs.

The diagnosis colibacillosis seems too risky to me to be based on these experiences as primarily another deviation may be the cause of death.

Another disease, which, through its manifold appearances in Holland, caused the institution of extensive investigations, is the picture of a disease known as 'Edema of the Bowel'. As the name already tells us edematous symptoms are found here in the gut but principally in the fat of the colon convolute and the stomach wall. The subcutis of the head may be impregnated strongly too, whereby when living, besides other symptoms, the upper eye-lids are very much swollen. The diagnosis of this can generally be ascertained, especially as the animals show defects in co-ordination.

There is little clarity as to the nature of the sickness. It occurs chiefly with pigs of about 9 to 12 weeks old. The animals which are in excellent condition soon get ill, and nearly all the sick ones die.

Keeping them without any food for 2 days keeps the other pigs from dying. We have succeeded in transmitting the disease with the intravenous injection of centrifugate of the bowel-content of dead animals. Infection-experiments, others than this very rigorous injection, have never had a positive result. The application by stomach-tube of bowel content of animals, which have died through this disease, never gave any result. The possibility therefore that a toxine formed in the bowel is responsible for the appearance is hereby more improbable. Bacterial causes of filtrable agents are never shown in carcasses.

Other swine diseases have been studied by us, but I shall not go further into this.

# SWINE-FEVER CONTROL IN PRACTICE

E. RUTGERS

*District-Inspector of the Veterinary Service*

For the prevention and combating of diseases occurring in the Dutch pig stock, for the practice on the farms of the pig breeders and pig keepers using all vaccines, sera, and medicines which can be provided by science, the Netherlands can call upon about 600 practising veterinary surgeons.

They receive during their six year study at the State University at Utrecht, an excellent insight into the aetiology, the distinction, and the treatment of the infectious and non-infectious diseases of pigs which occur in our country. When the time comes for them to make their entry into society as veterinary surgeons it can rightly be expected that the prevention and combating of the diseases of pigs are in good hands.

Very soon after their entry into the country veterinary practice, apart from isolated cases of disease and those in connection with parturition, they are confronted with the following groups of diseases of pigs:

1. Diseases of sucklings.
2. Deficiency diseases.
3. Infectious diseases.

## 1. DISEASES OF SUCKLINGS

The diseases which attract special attention in the Netherlands because they occasionally lead to mass mortality, are:

### a. *Dying and withering of the baby pigs*

The causes of this are various. Hereditary factors and nutrition and maintenance influences are most important. These are often also accompanied by bacterial and virus infections or invasions of worms, accompanied by allergic symptoms.

The weak livability of the newly born baby pigs which die at, or a few days after, parturition may be due to underdevelopment in utero through incorrect nutrition of the sows, or through the presence of lethal factors in the baby pigs coming from the mother or father animal. This can be met through improved nutrition, especially protein nutrition, of the sows, or by the exclusion from breeding of the mother and father animals amongst whose offspring many cases of weak livability have occurred.

Baby pigs may wither or die during the period of a few days to some weeks after

birth. This may be accompanied by symptoms of oedema and symptoms of affected intestines, bronchi, and lungs, and must be attributed to the absence of minerals (e.g., iodine, iron and copper) or bacterial or virus infections. If it is not too late, treatment aimed at these indications can be applied.

b. *Enzootic inflammation of the liver*

This still rather frequently occurring disease of baby pigs develops acutely or chronically. In the acute form, the animals die within some days. All appetite is lacking, the skin is pale or yellowish. In the chronic form one sees disturbed appetite, emaciation, and slightly icteric discolouration of the skin. Usually they die after a few weeks. At dissection, inflammation of the liver and degeneration of the liver is shown.

The aetiology of these inflammations of the liver is not clear. It is not swine fever although the one is often mistaken for the other. Probably the disease belongs to the group of intoxications or diseases of metabolism. Therefore the treatment in practice is based on this and restricts itself to avoidance of too much feeding of cereals, cod liver oil, and oil-containing food.

c. *Piglet anaemia*

In the Netherlands this disease of the blood occurs mostly in the autumn and winter and involves paleness of skin and mucous membranes, weariness, heart weakness, and oedema of neck and abdomen.

It is still the cause of death of many piglets. Often the number of red blood corpuscles has decreased to half the amount. The cause lies in both the lack of sunlight and the absence of suitable cereal food, iron and copper salts. Therefore the treatment is based on the replacement of these shortages.

d. *Chorea* (Shivering of the young piglets)

This disease of newly-born piglets which occurs in many farms in the Netherlands is shown in the form of periods of shivering immediately after birth of all (or most of the) piglets of one litter. It is caused by hereditary factors but it can also be a result of certain shortages in the nutrition and maintenance of the sows, proteins, vitamin, and mineral shortages. The treatment is based on these causes.

## 2. DEFICIENCY DISEASES

Several deficiency diseases occur in the Dutch pig-stock. The diseases which most attract the attention of the practising veterinary surgeon are the following:

a. *Enzootic heart muscle degeneration, death from heart failure*

As a rule only well-fed young pigs and middle-aged fattening pigs are the victims of this disease, only seldom, old and thin pigs.

The animals come to the trough apparently completely healthy at feeding time but suddenly fall down and die during the hasty eating. This is sometimes preceded by loud squealing and some spasmodic movements. As well as during the meals these stroke-attacks are also observed after excitation and physical strain, e.g., after loading

into transport carts, during or shortly after transport, or simply during the chasing of pigs into sheds or farmyards.

At dissection, an apoplectic heart is found very similar to the heart degeneration found in foot-and-mouth disease after death.

Also repeatedly degenerative changes are found with symptoms of hyaline degeneration in the skeleton muscles.

The aetiology of this disease lies in the field of auto-intoxication and deficiency. Poisoning through products originating through an incorrect internal secretion of thyroids and suprarenal bodies, a shortage of animal protein in the food, and certainly also a shortage of sufficient exercise, lead to these apoplectic attacks with acute heart failure. Treatment, which is almost completely preventive is therefore based on this aetiology. This treatment consists of changes of food, administration of green fodder with sufficient vitamins A and B, and addition of animal protein, together with sufficient exercise in the open air.

In connection with the above-discussed acute heart failure, a remarkable deviation which we have observed since 1950 in the East and South of the Netherlands in slaughtered pigs, and almost exclusively in the pigs which are fattened by farmers for home consumption is mentioned.

In the Netherlands all slaughter animals and therefore also these so-called home-killed pigs, are examined alive and after slaughtering. In a rather large number of these home-killed pigs, symptoms of hyaline degeneration of back, ham, and shoulder muscles were found after slaughtering. The muscles were wet, flaccid, soft, discoloured by white and red stripes, and with well defined hydrops. Bacteriological examination and experiments in infecting other animals were negative.

During the life of these pigs nothing unusual had been noticed. Some points of great importance are: the breed, the way in which the pigs are kept, the stupefaction and speed of slaughtering.

Till now – and in the home-killing season 1952–1953 this abnormality has been observed less frequently than in the winter of 1950–1951 and 1951–1952 – this degeneration has only been observed after the slaughtering of pigs belonging to the 'landvarkens' breed, which was crossed with a relatively small number of Danish boars. It does not occur in the Yorkshire pig which is kept in the West Netherlands. Therefore a degeneration in the breed seems to play a part. This becomes more understandable since we have learned that also in Denmark much trouble is experienced through this disease.

In general in the East Netherlands, fattening pigs are not allowed exercise. Animal protein is still too often absent from the food.

These pigs are killed. With trouble and strain they are brought from their sheds to the place of slaughtering at the farm and there they are stupefied with a humane killer which is often not equal to the task.

The whole operation leads to a state of 'shock'. The animals and the muscles in question are in a more or less unstable state. This unstable state is a result of breed-nutrition-maintenance and the connected mistakes. The thyroids and the suprarenal bodies discharge an abnormally high quantity of their production during the agony

resulting from the 'shock' with the result that the unstable balance is upset and there is effusion of fluid between the muscle groups.

The meat of these pigs is quite harmless if it is eaten in a fresh condition. It is difficult to keep and cannot easily be made into meats. The latter is an important point for the farmers concerned. If canned there is a possibility that the products will develop too high a moisture content.

It seems most probable that the above-described abnormality rests aetiologically on the same base as the enzootic heart muscle degeneration and that the same preventive and curative measures must be taken.

#### b. *Rickets and osteomalacia*

A second deficiency disease which is of importance in the Netherlands is *rickets and osteomalacia*. This is a well known disease in young animals and is caused by deficiency of vitamins, especially vitamin D, which appears to exercise a certain influence on the calcium-phosphorus absorption by the body. A low calcium content in the food as well as a too low phosphorus diet will produce rickets, whilst the magnesium metabolism is strongly connected with that of calcium.

Shortage of the vitamins A, B and D co-operate in causing rickets. Vitamin A is the growth-promoting vitamin, its absence in the diet stunts the growth. Absence of vitamin D disturbs the calcium-phosphorus balance in the body. In that case calcium is withdrawn from the body (the skeleton) quicker than it is supplied. For the rest, these vitamins also seem to exercise an influence on the parathyroids. Therefore it is evident that rickets is produced by a whole series of factors including deficiency of vitamin D, calcium, phosphorus and magnesium, together with a total incapability of assimilating these factors into the body. This is in other words shortage of green fodder, sunshine or a badly composed diet, mineral deficiency etc.

In our Dutch pigs both rickets with bending of the long bones, and osteomalacia in which mainly the jaw bones are concerned, are observed.

*Vitamin shortages* play the main part in most of the deficiency diseases which still now and then cause mass losses of the pig stock in the Netherlands.

However it is seldom that only vitamins are absent. Usually there is also a mineral deficiency together with a badly balanced diet.

Vitamin A deficiency is shown, though sometimes not before the animals are already some months old, through lack of appetite, lethargy, dryness of the hairs, later restlessness and excitation, stiffness in the hind quarters, finally convulsions. The treatment is a matter of course by preventive administration of vitamin A-containing green fodder, e.g., yellow turnips.

Vitamin B deficiency is shown by lack of appetite, lethargy, loss of hair, vomiting, diarrhoea, pneumonia, convulsions. The preventive treatment is the administration of cereals, possibly brewers yeast.

Vitamin C deficiency leads to scurvy, loss of weight, bad tooth development. Administration of milk, fruits, and vegetables is indicated as a preventive measure.

Vitamin D deficiency leads to rickets and osteomalacia.

Vitamin E deficiency influences fertility. The vitamin can be supplied in the



form of vegetables, especially salad. For a long time the food rations which are composed and propagated officially in the Netherlands to be used for pigs have taken the above mentioned factors thoroughly into account.

### 3. INFECTIOUS DISEASES

In the Netherlands a distinction is drawn between scheduled and non-scheduled infectious diseases.

The scheduled diseases, in pursuance of the Diseases of Animals Law (the Cattle-law) of 1920 are:

Rinderpest, lung plague in cattle, foot-and-mouth disease, anthrax, rabies, mange, sheep-pox, glanders, swine fever, and foot-rot.

The Diseases of Animals Law gives the Minister of Agriculture the power to take all measures with regard to the combat of these diseases.

Only four of these diseases are of importance for pigs namely: Anthrax, Rabies, Foot-and-Mouth disease, and Swine fever.

a. *Anthrax* is a disease which rarely occurs in the Netherlands. In pigs it is usually noticed as throat anthrax, or after slaughtering in the form of lymph gland antrax of the mesentery.

Each case of anthrax must be reported immediately to the Mayor of the place where the animal is located. The veterinary surgeon who determines the disease has this obligation and also the obligation to notify immediately the District Inspector of the State Veterinary Service. The Inspector satisfies himself that the animal is really suffering from Anthrax (or has died, as is nearly always the case). Then he advises the Mayor of the measures which must be taken. These consist of: immediate transport of the body in a special Anthrax cart to the destructor where it is burned in a special furnace. The shed is disinfected and all the remaining animals are treated with Anthrax-serum manufactured at the State Serum Institute in Rotterdam. Depending on the weight, 50–100 cc of the special serum intended for pigs is injected, and if necessary it is injected several times.

b. *Rabies* has not occurred in the Netherlands for many years. Just now this disease seems to be spreading again in Germany amongst the wild animals, foxes, badgers, deer, and perhaps also wild boars. Therefore it is quite possible that we also will have to deal with the combating of rabies again. In that case, legal instruction will certainly be promulgated that all pigs suffering from the disease or suspected of having rabies will have to be killed immediately, and if found positive will have to be destroyed.

#### c. *Foot-and-Mouth disease*

Because this disease is 'scheduled' all positive and also suspected cases must, like Anthrax, be reported immediately to the Mayor. He has in turn to notify the Veterinary Inspector. Since the middle of 1952 all animals suffering or suspected of having foot-and-mouth disease have had to be killed. Animals which have died are transported to the destructor in a special way.

In pigs lameness is the main symptom and is usually accompanied by decreased appetite and high temperature. In the past more than one epidemic of foot-and-mouth

disease has led to mass mortality of young pigs. Almost the whole Dutch cattle stock is inoculated at least once a year preventively against the disease with a vaccine containing the components A, O and C. This is manufactured at the S.V.O.I. in Amsterdam. Because our starting-point is the supposition that pigs are infected by cattle, it is unusual to also inoculate the pigs. Before the measure of killing all infected cattle herds was made, pigs on a farm where foot-and-mouth disease occurred were usually treated with highly immune serum, specific for the type of virus present and in a quantity of 10 to 80 cc, depending upon the weight. If applied in time, the pig stock nearly always could be brought through the dangerous days. As already mentioned, since last year all pigs are killed if a case of foot-and-mouth disease occurs.

#### d. *Swine fever*

Since 27 November 1936, swine fever also belongs to the scheduled diseases. Therefore the Veterinary Inspector immediately receives a report of each suspected case of swine fever from both the Mayor and the Veterinary Surgeon concerned. The diagnosis is made in almost all cases by him personally. If at the farm, on the basis of the clinical picture, it is not possible to make a preliminary diagnosis of swine fever then one or more of the suspected animals can be sent to one of the laboratories or investigation institutes which have been established.

There it is nearly always possible to solve the problem by accurate dissection and histological examination of the brains. During the year 1952, when each provincial inspector often had to deal with 10 cases a day this last method had to be followed in many cases. At this time swine fever has already been reduced to a sporadically occurring case. The disease in this last epidemic was in a form which can often only be recognized with great difficulty, being lingering and easily mistaken for some disturbances in the feeding such as often occur in young pigs.

Under these circumstances you will not be surprised that the Inspector often had great trouble in persuading the owner to slaughter his whole pig stock, although this was the best way both for himself and also from the point of view of combating the disease. In the Netherlands in contrast to foot-and-mouth disease, it is not compulsory to have pigs killed if they are suffering from or suspected of having swine fever. This decision is left entirely to the owner. No compensation is awarded to him. However the best way to prevent further spreading of the disease is undoubtedly the immediate slaughtering of all pigs at the affected farms.

Naturally the affected farms are marked and isolated. All movement of pigs, pig manure, and rubbish, from one place to another is forbidden. Dead pigs are transported to the destructor in a special way. When the case is finished – either because all pigs have been slaughtered, or after they have all gradually died – the sties are disinfected by the State. This disinfection of all sties with 2 % sodium hydrate solution must be done very thoroughly because the chance of survival of the virus is considerable. The swine fever virus can remain infectious in the sties for a long time, e.g., under favourable circumstances, especially in a dried form, at least 2 to 3 months. Therefore the disinfection is always performed under the supervision of one of the officials of the State Veterinary Inspection.

In this connection, it is a safe measure to re-stock the disinfected sties with heavier pigs, e.g., of 70 kg in weight, and not to bring young piglets or sows with piglets immediately into these piggeries unless these animals have been satisfactorily inoculated.

From experience it is known to us that about 70 % of the swine fever infection is introduced via the pig trade through pigs newly bought in the markets. To help in the combat, the pig markets over most of the Netherlands have been closed, although of course this measure exercises an unfavourable influence on the prices. At this moment the disease has restricted itself to some sporadic cases and many markets have been re-opened.

A second source of infection is the so-called boar keeper. For keeping and exploiting a boar, a special permit is necessary and certain demands are made on the animals concerned. About 5,000 boars are in present use. Most of the farmers and certainly the smaller pig keepers have no boar. They take their sows when they are on heat to visit the boar. The boar keepers usually possess a number of pigsties where the sows concerned stay for a week. Naturally such a farm provides a good opportunity for the swine fever infection, especially as the early symptoms are so vague, as is the case in this epidemic.

To combat this way of spreading the infection, the boar keepers are obliged to have their boars inoculated preventively and to disinfect their sheds weekly.

They are also often visited and controlled by officials of the State Veterinary Inspection.

Further, preventive inoculation against swine fever is propagated in every possible way. This inoculation is quite voluntary and – apart from the boar keepers – no power is exercised. The practising veterinary surgeons do the inoculation almost exclusively with crystal violet vaccine. This is manufactured by the State Serum Institute in Rotterdam using one of the three American very virulent swine fever virus strains which are kept in this Serum Institute. These strains give an eminent vaccine which, before it is released for use, is mixed with vaccine charges from the three different American Strains in order to increase the polyvalency.

This vaccine is only used by veterinary surgeons on farms which are absolutely free from the disease and under the most favourable conditions.

These conditions are: – because the development of a reasonable immunity takes three weeks – that no immediate threat exists for the pigs which are to be inoculated, that any newly bought pigs are not imported during the first three weeks, and that care is taken that the chances of infection by people are reduced to a minimum. Also care must be taken that pigs suffering from disturbances in the feeding, or animals suffering from a disease, are not inoculated before they have recovered from their complaints.

If all these conditions are satisfied, inoculation with crystal violet vaccine gives a good immunity which is usually strong enough to withstand a heavy infection and to last about a year.

The vaccine can be administered to almost all animals which are on the farms, even to pregnant sows during the first half of pregnancy and to piglets 4 weeks old although, after the inoculation of suckling piglets, a re-inoculation after 2 months is desirable.

Unfortunately, the conditions for inoculation are not always favourable and then the great disadvantage becomes apparent, namely the length of time (3 weeks) necessary to obtain immunity. In fact we must be satisfied with the present crystal violet vaccine, but we look forward to the day when we shall have a vaccine which more approximates the ideal. This ideal is: development of immunity immediately or a short time after the inoculation, no danger for the animals which must be inoculated, and no spreading of virulent swine fever virus. During the year 1952, about 30 % of the Dutch pig stock was inoculated. As already mentioned, this has been voluntarily and exclusively with crystal violet vaccine. Therefore about 500,000 pigs were inoculated, most of which were breeding pigs. Breaks in the immunity occurred in such a small number that this can be ignored.

Finally, some words on the handling of the meat of pigs suffering from, or suspected of having, swine fever, which were slaughtered on our advice. In the first place it is forbidden to kill these pigs in slaughter houses where there is also slaughtering for export to foreign countries. They must be transported straight to the official district abattoir, escorted by police, where they are subject to examination before and after slaughter. In pursuance of the Dutch meat examination regulations, pigs suffering from swine fever are unfit for consumption if serious general abnormalities are present in the skeletal muscles or in the fat tissue and also if, on the grounds of the result of the bacteriological examination, it appears that the skeletal muscles contain germs or are considered to contain germs. In all remaining cases, the meat can be passed as fit for consumption under conditions of sterilisation.

This sterilisation is also performed at the district abattoirs under continuous supervision and is the responsibility of the meat examination service. The sterilised meat is sold to the public at decreased prices as such, or after being made into any meat products. However it may never be made into meats destined for export to foreign countries.

Among the non-scheduled infectious Cattle Diseases which are of importance for a short discussion because they can lead to mass mortality, must be reckoned: *tuberculosis, paratyphoid, influenza and streptococcic infections in pigs, and purples.*

1. Tuberculosis of the cattle stock is a disease which has been overcome in a great part of the Netherlands. Therefore the infection chances for pigs have strongly decreased. Where the disease is suspected, since curative treatment is useless special attention has to be paid to possible sources of infection, namely milk or milk products, tuberculous fowl, an infected sow, etc., and measures have to be taken to prevent further infection. If, after slaughtering, tuberculosis in pigs is found, the original source of infection is, if possible, also traced and rendered harmless.

2. Pig paratyphoid, a disease which mainly affects young pigs in the autumn and winter months, is caused by *Salmonella suipestifer*. This is the secondary cause of infection in swine fever and, in former days before the filtrable virus was discovered, it was supposed that this *Bacillus suipestifer* would be the real cause of swine fever.

This Salmonella is found in the contents of the intestines of normal healthy piglets and it is supposed that this can become virulent and make the animal ill if, through other causes, the resistance of the animal is decreased. The predisposing cause of paratyphoid infections may, in many cases, be mistakes in the nutrition or diet, vitamin deficiencies, damp housing, or addition of infected piglets to the stock.

In the Netherlands this disease is regularly combated by the practising veterinary surgeons using a vaccine manufactured in this country and prepared with strains of bacteria which occur in the Netherlands.

3. Swine Influenza and Piglets Influenza, both caused by filtrable virus, can cause considerable losses in the pig stock from time to time. This disease is shown by disturbances of the respiration with fever, polyarthritis, discolouration of the ears, lack of appetite, conjunctivitis etc. Predisposing causes are lying in bad housing and bad maintenance, mistakes in the diet, and vitamin deficiency.

4. Also acute streptococcic infections, occurring in newly born piglets, pigs of some weeks old, and older pigs belong in the Netherlands to the affections which cause serious losses in the pig stock. In newly born piglets, the disease progresses with symptoms which are also found in the 'dying and withering of baby pigs'. In pigs of some weeks old, the urticarial form is preponderant and sometimes a great part of the skin is covered with brown-red and black crusts, while a serious or purulent conjunctivitis is always present. In older pigs the disease gives more the impression of purples and more diplococci are found in blood and organs.

As already mentioned, there is much damage done to pig-keeping by these cocci infections. Against these infections, the veterinary surgeons often use, with much success, a vaccine prepared with strains of bacteria which occur in the Netherlands. A high standard of cleanliness in the pigsties is also demanded.

5. Last but not least, some views on Swine Erysipelas in the Dutch practice.

In the past, there has been a time when in certain parts of our country, especially the clay districts in the west, hardly one pig could grow up without becoming the victim of the Swine Erysipelas bacilli. In these districts the septicemic form of the disease came to the forefront and the losses to the pig stock were enormous.

In the east of the country the urticarial form nearly always occurred and cases of death were relatively an exception. During nearly 30 years of practice in the east of the Netherlands I very rarely observed the septicemic form. When in the beginning of the 20th Century, the simultaneous inoculation using the method of LORENTZ came into vogue, it was mainly the pig keepers in the clay districts who had this inoculation applied yearly and through this brought, more or less, a definite end to Swine Erysipelas.

With the progress of science it has been tried, in Europe and also in the Netherlands, to replace the LORENTZ simultaneous inoculation by other more simple inoculations.

Although the simultaneous inoculation has been able to hold its place for tens of years and undeniably has given good service, the number of cases of inoculation

erysipelas and breaks of immunity were such that the results were not considered completely satisfactory.

Moreover the living vaccine leads to distribution of infectious matter and spreading of the disease. Finally, a simpler and cheaper inoculation would certainly be preferable.

This has been found in the KONDO vaccine, the STAUB vaccine, and the Adsorbate vaccine, developed by TRAUB. These are applied in Dutch practice as well as the simultaneous inoculation. It has appeared that the classic LORENTZ inoculation gives the best results because symptoms of erysipelas occur neither after the inoculation nor after an eventual re-infection of the farm.

The inoculation described by STAUB is apparently satisfactory so far as immunity is concerned, but the disadvantage may arise that a week after the inoculation high temperature and decreased appetite occur, sometimes with slight symptoms of urticaria. These disappear again spontaneously after 2 days.

The inoculation with KONDO and adsorbate vaccine does not cause any undesirable reaction, however, the immunity does not develop as strongly as with the LORENTZ and the STAUB inoculation.

Finally it must be mentioned that the curative treatment of Swine Erysipelas has been changed since penicillin made an entry into the veterinary practice. One injection of 100,000 to 200,000 units of penicillin dissolved in water appears to be sufficient to bring about a quick recovery in the majority of cases of erysipelas. Most of the practising veterinary surgeons still also inject erysipelas serum.

# POULTRY-KEEPING IN THE NETHERLANDS IN CONNECTION WITH DISEASE ERADICATION

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By way of introduction to the subject of direct methods of eradication of poultry diseases in our country, some attention will be paid to the way in which intensive poultry keeping has developed its national importance and especially to the nature of the poultry farms, where necessary disease eradication is carried out. Further I shall pay some attention to a few subjects on preventive veterinary medicine relating to poultry disease, viz. breeding and keeping the fowls.

The figures below show at a glance the importance of poultry keeping as a national source of income:

	1930	1939	1949	1950	1951	1952
Poultry census; Month of May; Total number $\times$ 1000	24,637	32,805	20,265	23,443	25,335	23,802

Number of eggs produced totalling a value of 325 million glds.

Export - Value of slaughtered poultry: 25 million glds,

The great development of poultry keeping has gradually come about during the past 50 years. It is true that, at the beginning of this century, hens were kept on almost all farms, but feeding, tending, and housing were primitive.

The roosting place was usually in the cowhouse. During the day the animals roamed about the yard and for part of their food, especially that containing protein, depended on what they themselves happened to find.

Morning and evening grain was strewn, chiefly maize or barley. The greatest numbers of birds were found on small farms in sandy regions. The eggs were exchanged for groceries or sold on the market. Certain centres gradually became known for the steady increase of marketing eggs. This again gradually led to export. Barneveld in particular gained a name as a centre for egg trade. Later- on co-operative egg marketing took over a considerable part of the export. The greatest of these can now be found at Roermond.

Stimulated by 'fancy' breeding (exhibitions) and by information from other coun-

tries, poultry keeping was more systematically organised at the beginning of this century. Originally it was brought about by private enterprise, later by co-operation of the State, which at first, appointed one poultry breeding-advisor. Later followed more such appointments. Old native breeds were to be found in various provinces, but the winterproduction of these hens was inadequate.

Neither were the yields comparable with those of foreign breeds. This led to the import of birds from which a better yield could be expected. This was backed up by fancy breeding importations. Originally many Leghorns were imported from Italy.

The knowledge of poultry diseases was still in its infancy and occasionally substantial losses were caused by fowlpest and Klein's disease.

In 1917 a research centre was founded for the special study of breeding, the knowledge of various breeds, and the tending of poultry (including feeding). This centre was transferred to Beekbergen (near Apeldoorn) in 1921.

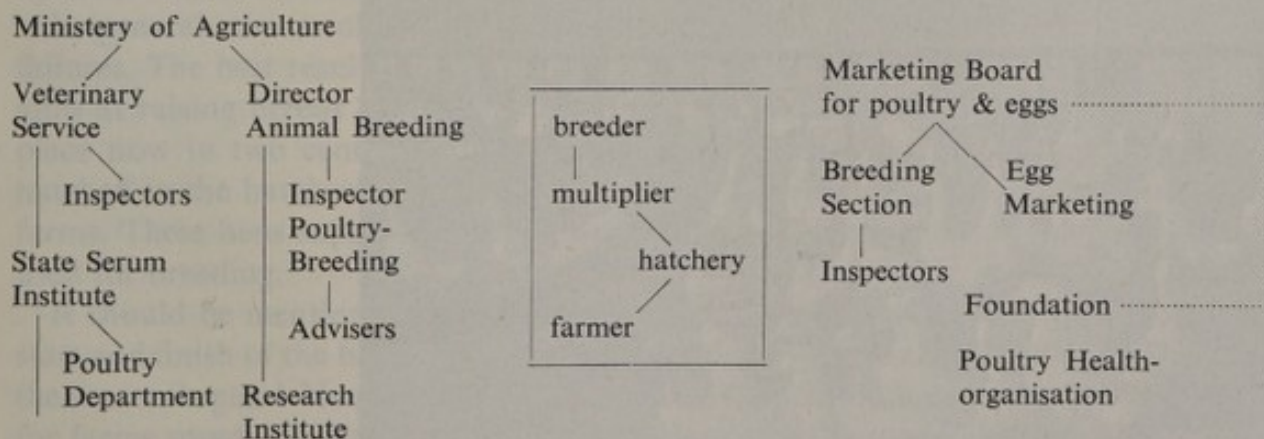
Breeding kept steadily on the increase thus entailing greater danger of spreading the various poultry diseases by infection. Systematic eradication of Salmonella pullorum and inoculation against Diphtheria and Fowl-pox started about 1920. These once dreaded diseases have ceased to be a problem. They are practically eliminated. Housing, feeding, and artificial incubation were greatly improved. Farmers obtained their chickens from breeding farms and later from big mechanical hatcheries.

As a divergence seemed to develop between the interests of the poultry farmers and the breeders, it was considered advisable, being partly brought on by the great agricultural crisis of 1930, to take measures that would serve the interests of both poultry farmers and breeders in one system.

This led to the establishment of the so-called breeding regulations with the specific purpose of improving the poultrystock by advising and instructing breeders in starting bookkeeping on a technical breeding basis.

This would ensure for poultry farmers the raising of better stock and a better market for organised breeders. The execution of this plan is still in the hands of the Marketing Board for Poultry and Eggs.

The official bodies, either advisory, supervisory, or those carrying out legal measures and Ministerial decrees, are shown in the following diagram:





The two departments of the Ministry of Agriculture which should be mentioned here are:

- a. The Animal Breeding Department
- b. The Veterinary Service.

The latter is well known. The Director, with the Inspectors for the provinces, carry out the statutory Enactments regarding the eradication of contagious poultry diseases.

Subordinate to the Director of the Animal Breeding Department is an Inspector, who is responsible for the sub-department of poultry breeding.

Together with six consulting experts, who in turn have some assistants at their disposal, this Inspector supplies information regarding breeding, feeding, and tending the animals. The State Institute for poultry breeding at Beekbergen also belongs to the Animal Breeding Department.

Apart from this State Department, there is also a semi-state institution, viz., the Marketing Board for Poultry and Eggs, in which sundry categories of interested persons are represented. Besides the Department for Egg-Trade there is a Commission for Breeding. The Chief Inspector, assisted by other Inspectors, sees that the instructions prescribed by the Marketing Board are duly observed.

They finally decide which animals are fit for breeding and which are not. The Health Service for Poultry is working in close co-operation with the Marketing Board.

Of this veterinary organization the Director, Mr. ROEPKE, can supply the best information.

The branches are divided into breeding centres, multipliers, and poultry farms. The running of the breeding centres requires a special permit from the Marketing Board.

Owners and installations must satisfy certain requirements before obtaining permits. Rearing and multiplier centres are subjected to permanent pullorum control. Breeding centres are also subject to trap-nest control. The hygiene must be approved, while accurate technical bookkeeping is another essential for being officially recognized.

The hatcheries must rear exclusively fowl of pure breeds. As the hatching is individual, the descent of the chickens is known. After hatching they are immediately



*Duck-farm*



*Dutch poultry-farm*

marked and registered. Usually the chickens are "sexed"; hens and cocks are separated.

During the rearing period the breeder makes a selection, guided by the conformation, colour of feathers, and vitality of the chickens. After 3 or 4 months, the birds are judged by the Inspector of the Marketing Board. The approved birds are then provided with a wing mark. They are then checked on their production. In the case of the winter yield (quantity and weight of eggs) being insufficient, the breeder is not permitted to use these animals for further breeding purposes. Finally follows the annual yield for judgement. The approved hens thus represent a repeatedly selected group.

The productiveness of a breeding centre depends to a great extent on the number of chickens that can be retained for breeding; the vitality of the chickens during rearing is the deciding factor. The multiplying centres obtain young hens and cocks from the breeding centres. As the name implies, here the breeding stock is increased. They are not subjected to the stringent demands which are imposed on breeding centres.

Originally the multipliers also used pure breeds, although hens and cocks were bought from different breeders. (Intra breed crossing; heterosis). Nowadays however the number which apply cross-breeding prevails. Cocks of light breed (*Exchequer Leghorn, White Leghorn, Brown Leghorn*) are crossed with hens of mid-heavy breed (*Rhode Island Red, New Hampshire* etc.). Especially after the world-war and inspired by the successes in U.S.A. these crossings have become popular.

In general, the results of this cross breeding are satisfactory apart from a few failures. The best results are obtained by using inbred animals. The advisory-service aims at raising breeds of limited variation. Extensive cross breeding tests are taking place now in two centres (Horst and Emmen). The eggs from the multipliers are hatched in the hatcheries and the hen chickens (mostly cross breeds) are sent to the farms. These hens supply the largest number of eggs for consumption. They are not used for breeding.

It should be mentioned here that each year (according to weather conditions) the start and finish of the breeding seasons are decided by the Marketing Board. This year the season began 1 November 1952 and ended 14 April 1953. An exception is allowed for farms supplying exclusively poultry for slaughter (North Holland-blues).

The hens on the farm are culled by selectors during the laying season, in order to remove the non-laying or low-producing birds. These selectors, who are men from the poultry industry, are trained to recognize at sight (by leg colour, feathers, consistency of abdominal skin, colour of eyes etc.) such non-laying or low-producing birds. These birds are removed.

On an average, in all branches of the industry, the annual yield is about 180 eggs per hen, the weight of each egg being over 57 grammes. This very satisfactory yield prompts breeders to pay full attention to the eradication of diseases and especially to the building up of resistance. The eradication of intestinal parasites claims particular attention.

Now we come to the important question of nutrition and breeding and their bearing on eradication of diseases in general. I hardly need bring forward here the fact that the trend of many bacterial or virus diseases is not influenced to any degree by the feeding condition of the birds or by their genetic composition. It would be uneconomical to try and breed fowls with great resistance to, say *Newcastle* disease.

There is however another virus disease, *Neurolymphomatosis* and it is possible to breed poultry families with high resistance to this virus or, on the other hand to breed families with increased susceptibility. As this disease can play great havoc on intensive poultry farms and so far no vaccination method has been discovered, other ways of eradication claim our attention. Before the World War, breeding tests had been made in my institute, but owing to difficult circumstances these tests were of a restricted kind. Before the material was completely lost, however, it appeared that, in spite of the limited number of animals, it was possible to raise families with increased or with diminished resistance.

After the war it appeared that HUTT, of Cornell University, had attained large-scale results. He raised strains in which the percentage of sick birds was limited to 10 % and others where 40 % of the animals perished. As HUTT experimented with a vast number of birds, it is obvious from his tests that there is a certain hereditary variability in resistance to neurolymphomatosis.

It would be as well for breeders in selecting breeding birds to bear this in mind. CARR, of the Poultry Research Centre at Edinburgh (*World's Poultry Science Journal* Oct.-Dec. 1952), raised some objections which had already been raised by others.

A strong argument against breeding toward resistance is that, on infected farms, the risk of the presence of virus-carriers is increased. Resistent fowls, either virus-carriers or not, can be of great value to individual breeders but, as CARR says, such an approach will not be likely to eliminate the disease.

To our regret we, so far, do not know of a reliable method of detecting virus carriers, consequently mass eradication does not yet promise many possibilities. CARR further points out the following: first, the fact or at any rate the great possibility, that a chicken may become infected at an early stage while the disease manifests itself later (beginning of laying period).

The number of sick birds is indeed greatest just before, or at the beginning of, the laying period, i.e., at an age of about six months. An attempt to bring about the

disease by spraying eggs for hatching, or the young birds with the virus, failed. CARR notes that this unfavourable effect may be due also to the proper care of the test birds.

Only injection of the virus can bring about results. The use of blood sucking insects is now being considered. The Poultry Research Centre has stated the great probability of virus being transferred by eggs of virus carriers. Finally it was found that breeding by one year old hens greatly reduced the risk.

In Edinburgh, the danger was reduced by 24/25. At the age of 1-1½ years the danger of virus from virus carriers seems to be greatly reduced. Although, as CARR declared, not by any means is all known about neurolymphomatosis. There is little objection to applying the two methods of eradication: rigorous insect destruction and breeding from only mature birds. Also for other reasons, in the Dutch breeding centres, the breeding from mature fowls prevails. Insect destruction could be intensified.

The multipliers must confine themselves to insect eradication. The breeder, in his selection, should pay due attention to the above-mentioned disease.

Next to the virus diseases, the intestinal parasites claim attention when rearing chickens. Prior to the application of sulfa-preparations and the knowledge of antibiotics, poultry breeders were aware of the fact that nutrition in the early stage was of great importance with regard to the damage caused by intestinal parasites (coccidia, nematodes, etc.).

Although certain diets (buttermilk or buttermilk powder) were very popular for a time, it finally appeared that eradication did not depend on one or a few specific acting nutriments, but rather that the right ratio of all components secured the best results. Neither was it unknown that a chance unfavourable change in surroundings (i.e., a drop in temperature) acted as a stimulant and in the second place brought about serious coccidiosis.

No doubt, the above causes have the same effect today, but we have now modern anti-parasitics, antibiotics, and a promising method (built-up litter) for fighting the parasite either in, or outside, the body of the fowl. The ratio between feeding and resistance against disease is not by any means clear. We are inclined to assume that optimal feeding secures the strongest resistance. Alas, we are not familiar with optimal feeding.

It is not merely an academic problem to decide what the rations should be for animals of a certain genotype and with a certain production. What is 'natural' feeding?

Although we know approximately the diet of the ancestors of our domestic animals we know little of the ratio of the various nutriments. These animals, led by instinct, carefully chose certain feeds. They kept changing their feeding grounds and probably varied their rations in such a way as to satisfy the demand for all nutriments.

The problem becomes more complicated when man increases production. The breeders, as a result, alter the genotype and the possibility arises of a greater demand for some nutriments. But even if we ignore this problem and with our present knowledge consider this food composition as the best optimal ration, the relation between ration and disease resistance is still rather uncertain.

It is a fact that various test animals are more susceptible to a certain virus when they are underfed.

Mice are susceptible to poliomyelitis virus when the ration is short of carbohydrates. (A. DELANUAY, *Notion du Terrain en Microbiologie à la lumière des découvertes récentes*. *Passé médicale* 59, 6 October 1951). Other examples are quoted showing that certain nutrients (amongst others, vitamins of the B. complex) should favourably affect the resistance against certain diseases.

There is the possibility, however, that in these tests the 'normal' rations were not well-balanced. These tests are of value only in cooperation with a bacteriologist or a virologist and an expert on nutrition.

A bull or a cow fed for a show is generally considered to be in very good condition regarding feeding, but it is doubtful whether the resistance of such animals is increased. Well-composed rations which are proved to raise the growth of young animals somewhat above the average, while not bringing about a fatty condition and keeping fertility above the average or somewhat higher, are in my opinion the best guarantee of a more than average resistance.

The normal feeding in our country has always been based on a compound mixture of meal. After the war, the Board for Animal Nutrition issued instructions for its composition. During this period the feed stuffs were strictly rationed and this continued even when the import of food increased.

The poultry farmer has thereby become familiar with serving compound rations supplied by either the cooperative or by the free market. A single example will make it clear that the idea is to procure a ration containing as far as possible all the necessary nutrients.

#### EXAMPLE

##### Meal for laying hens

Corn . . . . .	100	Crude protein . . . . .	20.4
Barley . . . . .	60	Crude fibre . . . . .	7.7
Oats . . . . .	110	Calculated starch value . . . . .	62.3
Milo corn . . . . .	100		
Peas . . . . .	50		
Soya bean oil meal cake . . . . .	70	Calcium . . . . .	1.5%
Sunflower seed oil cake . . . . .	50	Phosphor . . . . .	0.6%
Corn gluten feed . . . . .	50		
Wheat bran and middlings . . . . .	250		
Dried grassmeal . . . . .	50		
Fishmeal (60% prot.) . . . . .	75		
Vit. B. complex concentr. . . . .	10		
Vit. A. D. concentr. . . . .	1		
Minerals for poultry . . . . .	25		
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	1001		

Instructions are issued regarding meal fodder for hens and also for chicken fodder. The composition of mineral mixture is also definitely fixed.

Composition of mineral supplement for poultry

Ground limestone . . . . .	78
Iodinated salt . . . . .	20
Iron sulphate . . . . .	1,25
Copper sulphate . . . . .	0,15
Manganese sulphate . . . . .	0,85
	100

Even with such feeding, the risk of damage by parasites cannot be ignored. When, after the war the poultry stock rapidly recovered and poultry houses became more populated, the possibility of infection grew and the parasite problem (intestinal parasites in particular) promptly followed.

Sulfonamid preparations and a few other compounds made their appearance. They were later followed by antibiotics.

Apart from some disappointments, the breeder proved successful in mixing these drugs in the feeds. A few cases came to light where the keeper of a multiplying centre had regularly administered these drugs to chickens and young fowls. The breeding was excellent, but after the birds had been sold to poultry farmers it was discovered that disease and worm-infection occurred to a great extent. Obviously the fowls were not sufficiently immunised against infections and parasites.

In another case, sulfo-preparation had been regularly given to hens much troubled with worms. Though the general condition improved, production greatly declined.

In our country the application of antibiotics on a large scale is viewed with apprehension. If breeding farms and multipliers should begin with these drugs, it would be advisable for all poultry farmers to adopt their use. Even by mixing a certain amount with the meal fodder, the daily quantity administered would fluctuate because the ratio between meal fodder and grain varies in different centres.

Therefore, up to now, antibiotics have not been released and so are not included in the above mentioned rations. The administration of antibiotics is still a subject of discussion in various countries.

In the eradication of intestinal parasites and, in particular, of coccidiosis, two methods which have been tried in Holland in recent years, may become very important. It has become evident that by a proper lay-out of the hen-coops the runs become practically superfluous. It has also appeared that in many cases these very runs stimulate the danger of the breeding and spreading of parasites. As a result, on many farms of sufficient area, we find young hens housed in transferable coops (colony-houses).

In addition to the above, on many farms here, built-up litter is in use as is in the U.S.A. If this deep litter method is applied it is possible to keep the fowls housed all their lives.

The one and only objection to this method is the obtaining of the right application; this is an objection which can easily be overcome. In the mixture of peat-dust, fine woodchips or chopped straw transpositions must occur. This results in a rise of

temperature and the development of ammonia out of the organic substances of the faeces.

The oöcytes of coccidia are destroyed to a great extent and the hosts of various worms do not occur. The result is a substantial reduction in parasites. Again, the food of the fowls is improved by the introduction of various nutriments of which vitamin B-12 is the best known.

Whereas formerly young chickens were fed preferably on material, which had not been in contact with hens, it is now possible to keep them to advantage on litter which has been used, provided that it is managed in the proper way. The birds are probably slightly infected by this litter (e.g. with coccidia) and develop a certain resistance which makes this method a great asset in the eradication of coccidiosis.

From the above it appears that besides special legal measures together with the application of vaccines, a number of remedies which can be considered simple hygienic measures, can be of great importance in the eradication of diseases.

In the hatcheries, the disinfecting of the machines plays an important role. Formalin vapour (mixtures of  $KMnO_4$  and formalin) is generally used. The disinfecting of eggs to be hatched, with formalin vapour and with liquid formalin, has even been tried. Tests are being made to find out how far this treatment can adversely affect germination. Although this danger seems unimportant there are indications of the possibility of its occurrence.

# POULTRY DISEASES WITH SPECIAL REFERENCE TO NEWCASTLE DISEASE RESEARCH AND PRODUCTION OF VACCINE

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Since the intensification of poultry farming various infectious diseases have caused much damage to poultry.

The principal poultry diseases are:

Cecal and intestinal coccidiosis;

Intestinal parasites, cestodes, nematodes and trematodes;

The avian leukosis complex;

Fowl pox;

Newcastle disease;

Round heart disease;

Infectious coryza;

Paratyphoid infection of fowls (*S. pullorum*, *S. gallinarum*, *S. bareilly*);

Tuberculosis

Some of these diseases including *S. pullorum*, *S. gallinarum*, and tuberculosis, have virtually been eradicated through effective control. Other diseases, such as Fowl Pox and Newcastle Disease, may be adequately controlled. The researches on coccidiosis and intestinal parasites are steadily progressing, while the avian leukosis complex still constitutes a serious threat to poultry farming.

It is not possible within the scope of this survey to deal with all the diseases mentioned above and the relevant methods of control.

Besides, morbus pullorum, the avian leukosis complex, and the statutory measures for the control of Newcastle Disease, are treated elsewhere.

Therefore we shall confine ourselves to one subject: the vaccination against Newcastle Disease and the results obtained in the research on various vaccines.

It has been found that people and animals that have recovered from certain infectious diseases are, for a shorter or a longer time and sometimes for all their lives, immune to this disease. Striking examples of this are scarlet fever and measles in people and Newcastle Disease (ND) in chickens. Once a child has recovered from measles, it is all but certain that the disease will not be repeated.

The same is true of ND in chickens and many other infectious diseases. When the animal has survived the disease it becomes immune against its virus. Therefore, acting



on this experience animals are at any given moment inoculated with the virus of the disease in either a mitigated or an inactivated form. It is the aim to develop the disease deliberately in the animals in a very slight, often invisible, form so as to obtain the desired immunity. Such inoculations are carried out on a large scale against various animal diseases, such as foot-and-mouth disease, pox and diphtheria, red fever, swine pest.

Veterinary vaccines, especially ND vaccines should meet the following requirements:

1. Vaccination may not cause the spread of ND;
2. After vaccination chickens may not show undesired clinical symptoms;
3. Vaccination should give complete immunity against natural infection with ND for not less than one year;
4. In view of the extent of the Dutch poultry population one vaccination a year should be sufficient;
5. The producers of vaccines should be able to supply ND vaccines at reasonable prices and in sufficient quantity.

The ideal vaccine meeting all these requirements has not yet been found. So far, some 20 different vaccines have been dealt with in veterinary scientific publications. The preparers of these vaccines usually ascribe a good action to their products, but on careful examination in the laboratory and in practice the results of the vaccinations are usually disappointing.

The present vaccines fall into two classes:

1. inactivated vaccines;
2. live vaccines.

Of each group we have tested some eligible vaccines.

**Inactive vaccine: formol-inactivated aluminium hydroxide adsorbate vaccine**

The formol-inactivated aluminium hydroxide adsorbate vaccine was first made by the German research-worker TRAUB in 1941. This vaccine was based on the foot-and-mouth vaccine, which has given such good results in practice. The vaccine is prepared as follows:

Newcastle Disease virus (NV) is propagated on a large scale in a large number of chicken eggs which have been incubated for 10-12 days. After 2 or 3 days, when the virus has sufficiently multiplied, the contents of the eggs are collected and ground into an orange-coloured milky liquid in a reduction apparatus. This liquid is mixed with aluminium hydroxide which adsorbs the NV. Finally another chemical substance, formalin, is added to kill the virus in the liquid. This inactivated vaccine has been produced in quantity in the laboratories of the State Serum Institute and supplied for application in practice.

In our first experiments with this inactivated vaccine it appeared that in young chickens a single vaccination caused immunity against ND infection after 14 days. This immunity decreased sharply in the course of 7 weeks, and disappeared altogether in 14 weeks after vaccination.

In another experiment, White Leghorn cocks were vaccinated in the pectoral

muscle with 1 cu. cm inactivated vaccine. After 3 months the animals were infected per os with NV so as to produce a more natural infection than in the previous experiments. Half of the cocks survived the infection after showing serious clinical symptoms and the other animals, including the non-vaccinated control cocks, died.

This experimental result was confirmed in practice. The inactivated vaccine was applied on a large scale on poultry farms in the province of North Holland in the summer of 1950. In many chickens ND occurred 3 months after inoculation. The disease had a slow chronic course and few chickens died.

As a single vaccination in chickens gave only immunity for a short time, it was endeavoured to better the results by applying two vaccinations with inactivated vaccine.

Some three-month-old White Leghorn cocks were twice vaccinated with inactivated vaccine with an interval of 2 weeks. Each time 1 cu.cm vaccine was inoculated in the left pectoral muscle. After 2 weeks, this treatment was repeated in the right pectoral muscle. After both these vaccinations the cocks showed no abnormal or particular clinical symptoms.

Three months after the second vaccination the cocks were infected per os with NV. After the infection, 80 per cent of the twice vaccinated cocks showed at first sight no symptoms of ND, whereas the other 20 per cent showed slight symptoms of ND. The disease had a chronic course and was of a mild nature. This experiment showed that a double inoculation with inactivated vaccine with a two weeks' interval increases immunity in chickens and also that it extends the period of immunity to more than 3 months after the second vaccination.

It appeared, however, that the cocks that had at first sight shown no symptoms of ND excreted the NV together with their faeces. Healthy experimental chickens in which a small quantity of the faeces of the infected, apparently healthy cocks was administered per os, invariably died. The presence of NV in these faeces was shown by inoculating samples of faeces of the infected cocks into embryonating chicken eggs. In this experiment, it appeared that infection of cocks that have twice been vaccinated with inactivated vaccine, is followed by excretion of virus in the faeces until 33 days after infection, without the birds showing any clinical symptoms.

In a comparative experiment were included the original German formol-inactivated aluminium hydroxide adsorbate vaccine of TRAUB and the original English crystal violet-inactivated vaccine of DOYLE. This experiment showed that the action of the two last-named vaccines is not superior to that of the adsorbate vaccine made in the State Serum Institute in Rotterdam.

Summarizing, it may be said that the use of inactivated vaccines does not present immediate danger. The application of inactivated vaccine has three important disadvantages:

1. Even after double vaccination the resultant immunity against ND lasts only 3 or 4 months. For a reasonable result it is necessary to vaccinate the chickens 6 times a year.
2. When chickens vaccinated with inactivated vaccine are exposed to infection with

NV within 3 months after the last vaccination these animals excrete the NV in their faeces for 4 or 5 weeks without showing any clinical symptoms.

3. Inoculation with inactivated vaccine produces, indirectly, spreaders of ND that cannot be recognized and, on sale or removal, may cause fresh outbreaks of ND.

#### Live vaccine: Mukteswar vaccine

In India there was a violent outbreak of ND in poultry from 1930 to 1940. To control this epizootic the research workers in the veterinary institute at Mukteswar produced a live vaccine against ND.

NV was propagated in embryonating chicken eggs. The virus thus obtained was collected and injected into a fresh batch of embryonating chicken eggs. After 56 serial passages through eggs, so after 56 repeated inoculations, it appeared that the NV had lost much of its virulence whereas it had retained its immunizing properties. When chickens were vaccinated with this mitigated, live virus they first showed few, if any, symptoms and were immune against ND from the third day after vaccination. This vaccine is called Mukteswar vaccine after the place where it was first made.

At the State Serum Institute, the first experiments with live Mukteswar vaccine were carried out in January 1950.

A number of White Leghorn hens aged 9 months, were vaccinated with this vaccine. The birds showed serious clinical symptoms during 5–8 days after vaccination. One animal died and another became paralysed in both legs. The other animals were immune against a challenge exposure per os with highly virulent NV on the 13th day after vaccination.

In a second experiment it was found that chickens vaccinated with Mukteswar vaccine excreted the live, mitigated NV of the vaccine with their faeces during 2–16 days after vaccination.

On a poultry farm, more than 350 laying hens were vaccinated with Mukteswar vaccine. From 6–14 days after vaccination the animals showed serious clinical symptoms including bright green, thin faeces, little appetite, dullness and a bent position; 59 chickens, i.e., 16 per cent of the total, died or had to be killed on account of total paralysis. The egg production fell off considerably and remained far below normal for 4 or 5 months. The eggs laid from the 6th to the 9th day after vaccination contained live Mukteswar vaccine. The chickens were highly immune against ND. When, for instance, 8 months after vaccination the chickens were exposed to an infection per os with highly virulent NV, they did not show any symptoms, whereas non-vaccinated hens subjected to the same exposure invariably died of ND. The faeces did not contain NV, so that it had to be assumed that the virus was destroyed in the intestinal canal of the vaccinated hens.

In Belgium, live Mukteswar vaccine was used on several farms. The animals had intense reactions. Many chickens died after inoculation, showed obsessional movements with their heads, or became paralysed. According to recent information, in Belgium the view is held that inoculation with live vaccine is prohibitive for remunerative poultry farming. This refers to the inoculation with live Mukteswar vaccine, which causes serious symptoms and a prolonged, heavy fall in the egg production.

For the Netherlands the use of Mukteswar vaccine should be positively discouraged on account of its serious effects.

#### Live vaccine: Haifa vaccine

A series of experiments was carried out with KOMAROV's Haifa live vaccine. The virus in this vaccine was obtained by KOMAROV after serial passages through pigeons, ducklings, and duck-eggs. Excretion of the vaccine-virus occurred for 1-3 days following vaccination in 15-20 per cent of the vaccinated birds. The birds were partly immunized against a challenge exposure per os with virulent Newcastle virus three months after vaccination. The vaccinated birds excreted the virulent Newcastle virus up to 11 days after exposure; 90 per cent of the vaccinated birds did not show any clinical symptoms. The development of techniques for the production of this vaccine is still in progress (KOMAROV). Therefore, a definitive judgement must be reserved.

#### Live vaccine: Hitchner B<sub>1</sub> ND vaccine

The American research worker, HITCHNER, possessed in his laboratory a virus which was then considered to be the cause of a slightly dangerous poultry disease: infectious bronchitis. Afterwards it appeared that this virus was a particularly weak strain of NV, which could not even cause clinical symptoms in very susceptible day-old chicks, but gave a certain amount of immunity against ND to chicks and chickens when these animals were inoculated intranasally with it.

The experiments with the Hitchner B<sub>1</sub> ND vaccine in the Netherlands date from the spring of 1951 and include mainly chicks, young chickens and laying hens. So far, one-day chicks have stood this vaccination very well. Only sporadically after vaccination do slight respiration troubles occur which are, however, of short duration. The inoculation of healthy chicks does not cause mortality, not even in the most susceptible breeds such as the White Leghorn breed. The immunity against infection with ND lasts some 4 or 5 months. In practice, the highest degree of immunity was not reached before 10-14 days after vaccination. This immunity, however, is such that the animals that are infected per os after vaccination excrete the virulent virus with their faeces for some weeks. Within 4 or 5 months after vaccination the animals show no symptoms of ND, not even after intentional infection. Chickens of any age may, without any harm, be inoculated with the Hitchner B<sub>1</sub> ND vaccine. Laying hens sometimes show a slight decline in egg production, but this decline never exceeds 10 per cent of the normal production.

During 1 or 2 weeks after vaccination the chicks excrete very weak NV with their faeces. Contact-experiments have shown that non-inoculated chicks, coming into contact with these infected faeces, are not infected and do not develop immunity.

So the Hitchner B<sub>1</sub> ND vaccine may be considered harmless. In America this vaccine is successfully applied on a large scale. In the laboratory this vaccine can be produced quickly and cheaply.

If economic factors in the Netherlands allow preventive inoculation; the Hitchner B<sub>1</sub> vaccine may unreservedly be recommended, especially for one-day chicks which are to be fattened for slaughter at an early age. These fattening chicks are then immune

against ND for their lives. Pending further research on combined inoculations the use of the Hitchner B<sub>1</sub> ND vaccine for chicks intended to be reared into laying hens, should be judged with reserve.

#### Live vaccine: Beaudette vaccine

The American research worker, BEAUDETTE, screened 105 different NV strains, which were collected in natural outbreaks of ND in the U.S.A. The strains were classified according to virulence and immunizing capacity. Each strain was tested by inoculating it into 5 susceptible experimental chickens. The chickens were housed in a cage and observed for 21 days. When one or more chickens died or were paralysed, the strain applied was discarded as being too virulent.

After the first screening, 97 out of the original 105 ND strains were discarded, so that 8 strains remained for further examination. Each of these 8 strains was tested for virulence, in batches of approximately 50 susceptible experiment chickens, with the result that another 6 strains were rejected. Inoculation with the 2 remaining strains produced no immediate abnormal clinical symptoms, but some weeks after inoculation one batch showed symptoms of coccidiosis, so that the relevant strain also had to be rejected.

So out of 105 examined NV strains only one strain was weak enough to be used in practical experiments. On 20 farms, BEAUDETTE vaccinated more than 83,000 chickens of various ages. The mortality after vaccination was 1.82 per cent, including mortality caused by inflammation of the anterior air passages and by coccidiosis.

Some weeks after the first outbreak of ND in the Netherlands the laboratories of the State Serum Institute in Rotterdam were in possession of live Beaudette vaccine and since 1950 many experiments have been made with this vaccine, both in the laboratory and in practice.

Beaudette vaccine is produced from hen eggs that have been incubated for 12 or 13 days and which were 2 or 3 days previously inoculated with the Beaudette NV strain. The vaccine can be produced rather simply, cheaply and in thousands of doses a day.

The vaccine appeared to be specially suited for the vaccination of young healthy chickens from about 6 weeks old till laying-time. Sometimes chicks may be vaccinated when under 6 weeks old. This depends on their stage of development. Generally, 5-week-old chicks can stand vaccination if they are well-feathered and well-developed. On the other hand it may also occur that 6-week-old chicks are so poorly developed that they cannot yet bear vaccination well. In this case they should not be vaccinated before they are 7 or 8 weeks old. This vaccination causes a reaction. Between the 6th and the 10th day after vaccination the animals are somewhat dull and the feed consumption is below normal. As a rule light breeds such as White Leghorns show more reaction than heavier ones such as Rhode Islands and North-Holland Blues. In a vaccinated batch there will always be some victims. Such chickens get paralysed or lame in one or both legs, show a wry neck or obsessional movements with the head, or die after being ill for some days. In vaccinated batches the mortality or the para-

lysis figure varies from 0 to 3 per cent. On examination of the dead or paralysed chickens it usually appears that these animals had some complaint before vaccination, for instance nephritis, degeneration of the liver, visceral gout, etc.

Because of this, the birds would never have grown into productive animals anyway. So vaccination may also be considered a selection, preliminary to a final selection which is made from time to time on every well-managed farm.

If vaccinated young chickens are not perfectly healthy, but suffer for instance from latent coccidiosis or worm diseases, mortality after vaccination may even rise from 0-3 per cent to 10-20 per cent. In this case, the latent disease manifests itself after vaccination with fatal results. Therefore, the state of health of young chickens should be examined before vaccinating them with Beaudette vaccine.

Chicks under 5 weeks old should not be inoculated with Beaudette vaccine because the subsequent reaction is too strong. The younger the chicks the greater the mortality after vaccination. Day-old chicks die after 2 or 3 days when they are inoculated with live Beaudette vaccine.

Laying hens after vaccination show a rather heavy decline in egg production for 1-4 weeks, while, just as in the case of young hens, 0-3 per cent of the batch becomes paralysed or dies. The degree of the fall in egg production depends on the time of inoculation.

If the hens are just in lay, the decline in the egg production will be slight and will soon be offset by a subsequent rise. If the hens are half way through the laying period the loss in production is greater and there will be a longer productive decline varying from 2 to 4 weeks. Moulting may start according to the season. After this production recovers and continues longer than usual. If the hens are vaccinated at the end of the laying period, egg production is suspended till after moulting.

The immunity against ND, caused 2 or 3 days after vaccination with live Beaudette vaccine, is complete and lasts more than one year. In practice it may even be put at 2 or 3 years, so that, in Dutch conditions of poultry farming, the immunity may be considered lifelong.

As immunity begins 2 or 3 days after vaccination, the vaccine may also be successfully used with poultry already infected with ND. This may reduce mortality. The result depends on the number of animals already showing clinical symptoms before vaccination. If there are many such animals, there is little sense in vaccination, but if only a few animals are visibly infected with ND, live Beaudette vaccine may considerably reduce mortality.

Chicks hatched from eggs laid by chickens vaccinated with Beaudette vaccine are immune against ND during the first 3-5 weeks of their lives. The hen transmits the antibodies against ND into the yolk of the hatching egg. During the first few weeks the chicks still feed on the part of the yolk, which, at the time of their birth, is present in their abdominal cavity. When the yolk has been fully digested by the chicks also the antibodies against ND have been used up. The chick is then again normally susceptible to infection.

If chickens that have been vaccinated with live Beaudette vaccine are, say 9 months

after vaccination, exposed to an infection per os with highly virulent NV, the animals show no symptoms and, besides, do not excrete the virus with their faeces. The virus is destroyed in the intestinal canal of the hens. If non-vaccinated chickens are infected in the same way, they invariably excrete the virus with their faeces and die after the infection.

With two good assistants for catching the chickens a veterinary surgeon can vaccinate some 500 chickens per hour. After vaccination the chickens excrete the live Beaudette vaccine with their pharyngeal mucus and faeces for 5–21 days. The virus of this vaccine is also present in the eggs laid on the 7th–11th day after vaccination. This excretion of virus should be taken into account by Dutch poultry farmers who at a given time wish to have their young chickens vaccinated with live Beaudette vaccine.

On poultry farms the time when young chickens are the most suitable age for vaccination will generally coincide with the time when the laying hens are in full production. If at this time the young hens are vaccinated with Beaudette vaccine these animals will after vaccination excrete the weak vaccine virus with their faeces. The laying hens on the same farm will come into contact with this virus and take it in when feeding, which will reduce their egg production. This can, however, be avoided by inoculating the laying hens intranasally with Hitchner B<sub>1</sub> ND vaccine about a week before the vaccination of the young hens with Beaudette vaccine. This will give the laying hens sufficient immunity against Beaudette vaccine by the time it will be excreted by the young hens, so that the egg production will not be affected.

In foreign countries, especially in England, the view is held that vaccination against ND with live vaccines will promote in given areas the continuation and the spread of ND. It is advanced that poultry flocks vaccinated with live vaccine provide a constant source of infection. The results of such vaccinations in America, however, prove the contrary. In the U.S.A. preventive inoculations with live Hitchner vaccine and live Beaudette vaccine have kept the spread of ND under control.

Also experiments carried out in the Netherlands showed that inoculation with live vaccines does not cause a spread of the disease.

On a farm where 6 weeks previously the young hens were vaccinated with live Beaudette vaccine day-old chicks were introduced and allowed to walk freely about the yard and the houses of the vaccinated birds. The chicks showed no symptoms of ND. As already mentioned before, day-old chicks are very susceptible to live Beaudette vaccine and invariably die when brought into contact with it. In addition, we used for our experiment the most susceptible day-old chick, viz. White Leghorn. If it were true that a flock vaccinated with live Beaudette vaccine is a constant source of infection, White Leghorn chicks brought into contact with a flock vaccinated with live Beaudette vaccine would certainly die from infection. This has, however, never yet occurred on experimental farms, so that it may be assumed that vaccination with live Beaudette vaccine does not cause permanent sources of infection.

For the control of ND in the Netherlands, the inoculation with live Beaudette vaccine of young hens from 6 weeks old until they start laying is recommended.

TABLE I

## VACCINES AGAINST ND

Sort/Name	Age at which vaccination can be carried out	Reaction	Secretion of virus after vaccination	Immunity after vaccination	
<i>Inactivated vaccines</i>					
Formol-inactivated aluminium hydroxide adsorbate vaccine Rotterdam (State Serum Institute)				After single vaccination	After double vaccination
Formol-inactivated aluminium hydroxide adsorbate vaccine (Traub)	all ages	none	none	2 months	4 months
Crystalviolet-inactivated vaccine (Doyle)					
<i>Live vaccines</i>					
Mukteswar vaccine	2-6 months	serious up to 15% mortality	2-3 weeks	at least 1 year	
Haifa vaccine	2-6 months	slight	some days	3-5 months	
Hitchner B <sub>1</sub> vaccine	all ages	very slight	10-14 days	4-5 months	
Beaudette vaccine	6 weeks till laying period	slight, 0-3% mortality and paralysis	3 weeks	at least 1 year probably 2-3 years	

TABLE II

Virus secretion after infection	Immunity of chicks of vaccinated parents	Cost of production	Duration of establishment of maximum immunity after vaccination
3-5 weeks	none	high	14 days
none	4-5 weeks	very low	2-5 days
1½ weeks		very low	
2 weeks		low	10-14 days
none	4-5 weeks	very low	2-5 days



## SUMMARY AND CONCLUSIONS

Various vaccines against ND were tried out. The results of the investigations are given in table I. It appears that as yet no vaccine meets all the requirements of a good vaccine.

This is the reason why some poultry importing countries are opposed to the use of vaccines, the more so because the belief has spread that vaccination against ND would be a source of infection.

Though extensive investigations have proved that this is not the case, a poultry exporting country like the Netherlands has to comply with the requirements set by poultry importing countries. If one of these requirements should be the non-application of live vaccines against ND, this requirement has to be met. Otherwise valuable export may be lost.

If, however, for economic reasons preventive inoculation against ND is necessary, two live vaccines may be recommended:

1. Beaudette vaccine;
2. Hitchner B<sub>1</sub> vaccine.

Hitchner B<sub>1</sub> vaccine may be used for fattening chicks.

On egg producing farms the laying hens should first be vaccinated with Hitchner B<sub>1</sub> vaccine and about a week afterwards the young hens with Beaudette vaccine. In the next year only the young hens need be vaccinated with Beaudette vaccine, because the young hens of the previous year, now full-grown laying hens, are still immune against infection. So the vaccinations will have to be carried out in the months of May up to and including July.

When this system of vaccination has been applied for some years, most chicks will have inherited 4 or 5 weeks immunity after birth from their parents. At 6 weeks of age these chicks can then be vaccinated with Beaudette vaccine, so that there will be a susceptible period of only 1 or 2 weeks. In this way infection hazard is reduced to a minimum.

Still being investigated is the extent the vaccination of day-old chicks with Hitchner B<sub>1</sub> vaccine may be followed by a re-vaccination of the same animals at the age of 2-6 months with Beaudette vaccine. Provisional experimental results indicate that this combined vaccination does not produce such complete immunity as does single vaccination with Beaudette vaccine, so that, for the time being, this method cannot be recommended without reserve.

# ORGANIZATION, FINANCING AND WORKING METHODS OF THE POULTRY HEALTH SERVICE

W. J. ROEPKE

*Director of the Poultry Health Service*

Poultry keeping in the Netherlands has increased enormously during the last thirty years. Formerly there were only some fancy breeders and, on the farms, some birds of many breeds and crosses were kept. Their productivity was not sufficient to meet the demand and eggs had to be imported from other countries. After 1920, however, more attention was paid to the commercial side of poultry-keeping and special poultry farms and commercial hatcheries came into existence. Egg production increased and gradually an export of eggs and poultry products developed which soon became of considerable economic importance.

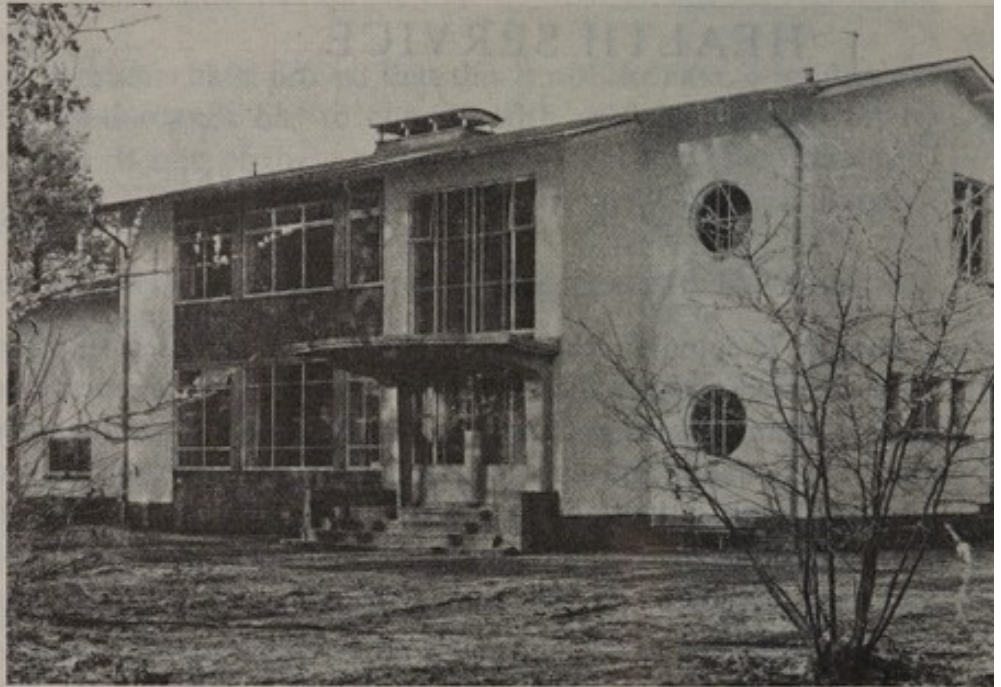
In 1933 the Poultry and Egg Marketing Board was set up with legal provisions affecting the extent of the poultry population, the production of eggs, and the marketing and improvement of the quality of all products. Pedigree breeders, producers of hatching eggs, and hatcheries, were licensed and breeding regulations were made, so that all fowls of the commercial egg producers had to be provided by accredited farms.

The advisory work and the education, the scientific research, and the control of pullorum disease, were taken in hand by the government and the Poultry and Egg Board. In the following years the entire poultry industry made rapid strides and became a very important part of our agriculture.

Because Holland is one of the most densely populated countries in the world, most of our farms are relatively small and so it is inevitable that each year many birds are raised on the same ground. Moreover the damp climate and the intensive traffic in poultry are factors which are liable to promote the spreading of disease. With the increasing number of birds it became necessary to pay more attention to the eradication and prevention of those plagues. However the knowledge and methods of combating disease among fowls had completely failed to keep pace with the rapid development of the poultry industry. Since poultry-keeping was formerly of minor importance in the country's economy, most of our veterinary surgeons generally devoted more time to other farm animals. Besides, the poultry farmers were regularly visited by agents of the feeding-stuff trade and other laymen who gave free advice but were not capable of doing the necessary diagnostic work and to indicate the right therapeutic and preventive measures. Expert veterinary consultants were

out of fashion and much quackery was the result. Although birds could be sent for post mortem examination to the State Serum Institute at Rotterdam and the Institute for Infectious Diseases at Utrecht, the farmers only occasionally made use of the possibilities offered by these institutes.

*Laboratory of the  
Health Service for  
Poultry at Soesterberg*



It became obvious that a special organization was necessary to improve this situation. So, after the war when the poultry population had to be rebuilt from a nucleus of high quality breeding fowl maintained during the German occupation, preparations for setting up a new organization were undertaken. Although the initial efforts were hesitant and tentative, the final result was the foundation in 1948 of the Poultry Health Service.

This organization has the status of a non-profit-making public body and is subsidized by the Poultry and Egg Board, with which it is closely connected. The funds of the Board are obtained by levies from the entire Dutch poultry industry, so that every poultry farmer participates in it.

In the board of the Health Service representatives of the Government (Veterinary Service) the Poultry and Egg Board, the Netherlands Federation of Agriculture, the largest poultry-keepers organizations and the Veterinary Medical Association are incorporated.

All accredited poultry farmers (breeders, producers of hatching eggs, and managers of commercial hatcheries) can call on the Health Service for assistance whenever it is needed.

All birds of the pedigree breeders and producers of hatching eggs are judged by the inspectors of the Poultry and Egg Board. These do the culling before the beginning of the hatching season. If too many birds have to be removed or suspicious symptoms are observed they can give orders for one or more birds to be sent forward for further

examination in our laboratory. The veterinary surgeons of the Health Service can visit the farms for a local examination or to discuss the problems and to give instructions. A report of the result of the examination is sent to the poultry farmer with advice on further prevention and treatment. All these services for the poultry farmers are free of charge. Copies of the report are sent to the local veterinary surgeon, the Poultry and Egg Board and the inspector for the area where the farm is situated. However no medicines or treatment are given, because this is the task of the local veterinary surgeon. In this way each of the authorities and individuals concerned receive an account of the cause of illness, which can be helpful in the preventive and remedial measures.

All the fowls reared in the Netherlands are supplied, directly or indirectly, by the 200 accredited pedigree breeders and 4,000 licensed producers of hatching eggs. These farms produce all the material required for the annual rejuvenation of the entire commercial poultry stock. It will be clear that the breeding hens and cocks, being the basis of the Dutch poultry population, must be as sound as possible. Their quality effects all other fowls and a high standard of health of these birds will benefit the entire poultry industry.

All attention on prevention and eradication of diseases must be concentrated in the first place on these farms. For this reason the Poultry Health Service's sphere of operations is confined to the farms of the accredited pedigree breeders and producers of hatching eggs. The owners of these farms, whose establishments are regularly visited



*Poultry farm inspection by the Health Service for Poultry*

by inspectors of the Poultry and Egg Board, have been instructed to send a few sample birds to the Poultry Health Service for examination in all cases of abnormal losses or other difficulties due to diseases. Besides this, they may send in every bird they wish to have examined.

Moreover the Health Service supervises all imports of breeding stock. These birds are examined before they are allowed on the farms and may not be used for breeding purposes until they are found free from transmissible diseases.

Imported hatching eggs must be incubated in a separate incubator and all chicks hatched from these eggs are kept under control until they are fullgrown.

In this way a general impression is obtained of the occurrence of all diseases, the standard of health on the farms, and the vitality of the progeny of different breeds and strains.

The Health Service has been at work now for some years and has obtained an insight into the necessary measures for combating and preventing disease on the farms of the breeders and producers of hatching eggs. Especially now that the organization has out-grown the stage of finding its feet, further steps can be taken with investigation work along pre-arranged lines.

The producers of hatching eggs obtain their basic material every year from the pedigree breeders as day-old chicks.

In practice it has been found that diseases and defects encountered among the stock of the hatching-egg producers can often be traced back to the breeding farms. It is possible that the clinical symptoms at the breeding farms are not so noticeable because the birds are kept under very favorable conditions. It frequently appears that the progeny of certain strains or individuals do not thrive in a different environment. Often susceptibility to disease is already detectable at the farms of the hatching egg producers before it shows itself on the corresponding breeding farm.

The hereditary aspect of susceptibility to diseases is a very important one. All the birds of our pedigree breeders actually originate from about 2,000 cocks and it is a well known fact that sometimes there will be a higher percentage of unsatisfactory birds among the progeny of one cock than among those of another.

The systematic examination of all unsatisfactory descendants of these cocks can be very important to estimate their breeding value and to detect their less desirable qualities before their progeny are distributed to other farms. Although, of course, some diseases are less dependant on hereditary factors, virtually most of the Health Service's activities should be concentrated on the breeding stock, and future work will therefore be oriented more and more in this direction.

The post-war years of reconstruction made establishment of the Health Service no easy matter but in 1952 a new laboratory was built with the most up to date equipment and a staff of competent personnel. The skill and devotion of this staff, which includes two veterinary surgeons with specialist training in poultry diseases, are entirely at the service of the Dutch poultry industry. With the high degree of development of our poultry-keeping it is of the greatest importance that the progress of the Poultry Health Service should keep abreast of that of the industry.

#### THE CONTROL OF PULLORUM DISEASE

*Bacillary white diarrhea* has been one of the greatest menaces of the entire poultry industry. The commercial hatcheries, with their large incubators where thousands of chicks were hatched at the same time, often suffered severe losses.

Particularly on these establishments, the spreading of the disease was a very serious problem.

The discovery by American workers that infected birds could be detected by means of an agglutination test was a very important one and must be regarded as the foundation work for the diagnostic procedures which are in use at the present.

Although control started on a voluntary basis with the testing of the breeding stock, not all poultry farmers participated in it and so losses continued to occur.

Accordingly the Poultry and Egg Marketing Board issued regulations in 1938 concerning the application of the blood test. Since then all the birds on the farms of the pedigree breeders and producers of hatching eggs have to be tested annually and the reacting specimens must be removed. All the hatching eggs must be from pullorum free birds.

Although formerly the tube test (serum agglutination) was also used, this method has now been completely replaced by the rapid whole blood test. The entire process of pullorum-control is now carried out under the supervision of the Poultry Health Service. All blood testing of the birds is done on the farms by the local veterinary surgeons who receive their instructions for this from the Health Service.

Most of the antigen used for the test is prepared by the State Serum Institute from selected strains of *Salmonella pullorum* bacilli. The antigen of other firms may be used also but only after it has been tested and approved.

Because all the Dutch poultry farmers get their material from the pedigree breeders and the hatching egg producers, the only eggs that may be used for hatching must be obtained from these farms. So these are the only ones that have to be tested and when they are pullorum-free all the other farms will become free of the disease.

If reactors are found on the farm of a pedigree breeder his entire stock must be retested three or four weeks later. The owner is not allowed to hatch any chicks until no more reactors are found. All reacting birds must be removed immediately from the farm and may not be used for breeding.



*Grading of eggs*

On a farm of a hatching egg producer no retesting of the entire stock is necessary unless the number of reactors is more than two percent.

If an abnormally high number of reacting birds is found on any farm, no hatching of eggs is allowed at all.

The results of the tests are reported to the Poultry Health Service and all positive reacting birds must be sent to the laboratory for further examination. Then the Health Service investigates the source of infection. An infection of the birds of a producer of hatching eggs may originate from the pedigree breeder's stock. All the breeding fowls are then retested by a veterinary surgeon of the Health Service.

Conversely, reactors on a breeding farm may make it necessary to retest all the birds of the hatching egg-producers whose stock has been obtained as day-old chicks from the infected breeding farm.

The annual testing of all breeding stock has proved to be a very successful method of control and the disease has become practically extinct in our country.

The number of reacting birds is now very small and we hope to be able to make all breeding stock entirely free from the disease within a few years. In the first place this must be achieved on the breeding farms, whereafter all the other farms will follow automatically.

In a country such as ours, where poultry-keeping has reached a high degree of development, it is of the greatest importance that we should get rid of this serious poultry disease.



# NEWCASTLE DISEASE

DR H. H. SCHOLTEN

*District-Inspector of the Veterinary Service*

In the preceding various poultry diseases are mentioned. Of these it is only Newcastle disease for which the Netherlands Government has laid down legal regulations and prescriptions. The *Bird Diseases Act* of 22 December 1949 has made it possible at any time to indicate a certain poultry disease as an infectious disease, if it is considered as a danger to the country's poultry stock.

So, when at the beginning of 1950 the first cases of Newcastle disease were reported, a ministerial order was issued on 20 Januari 1950 indicating this disease as an infectious bird disease according to the 1949 Bird Diseases Act.

Newcastle disease was introduced into this country from France via Belgium by poultry that had been smuggled into our country and the first cases were diagnosed at the beginning of 1950 in the southern part of the country, soon to be followed by more cases in another province, where fattened poultry had been brought from the south. This situation led to a ministerial order of 9 March 1950 prescribing that all poultry markets in this country should be suspended and prohibiting all mass inspections, shows, sales or any other form of bringing together of poultry. This order held good for the whole of the Netherlands.

Although by these control measures and the immediate slaughter of the entire stocks of infected farms, the disease was checked at first, we could not prevent Newcastle disease from spreading and after some time new cases were reported. As they were detected in a congested poultry district, the infected stocks were again killed at once and all poultry within a distance of 5 kilometres was vaccinated with formol-inactivated aluminium hydroxide adsorbate vaccine. Vaccination was started on the farms furthest away from the focus of the disease in order to prevent the attending veterinary surgeons transmitting the virus.

This control measure, which may be said to have yielded effective results, together with the prohibition of the sale of poultry, soon followed by a prohibition of the movement of poultry, prevented the disease from spreading to any considerable extent. The prohibition of the movement or sale of poultry was maintained for several months, until serious outbreaks had been checked. In November 1950 this prohibition was abandoned for a large part of the country now free from Newcastle disease.

In June 1951 it had been cancelled in the whole of the country, except for a narrow



strip along the Belgian frontier. However, poultry markets still proved to be continual sources for the spread of Newcastle disease virus, and the occasional outbreaks of the disease detected could almost always be traced back to these markets. As an extensive trade in fattened poultry is done via these markets, a permanent prohibition to sell poultry at markets would entail great economic difficulties. We found the following solution for the problem. By order of our Minister of Agriculture, Fisheries and Food dated 11 August 1952, all movement outside the frontier area just mentioned is prohibited for all live poultry with the exception of one-day old chickens and birds of which the tails have been cut. This prohibition does not apply to a poultry keeper conveying not more than thirty birds direct to another poultry keeper; so that it is still possible for a poultry farmer to obtain birds direct from a colleague; but he cannot buy them at a market or through a poultry dealer. Poultry dealers are only allowed to move birds with cropped tails and may keep them no longer than three full days, after which the birds have to be slaughtered. Everyone else having birds with cropped tails is liable to punishment. The buying and selling of fattened poultry at markets is still possible, but the chance of these birds passing into the hands of private poultry keepers has been all but eliminated.

These measures have been very effective in the control of Newcastle disease, though, of course, it occasionally occurs that unscrupulous poultry dealers do sell a number of birds with cut tails to private persons. Hence most of the outbreaks detected from time to time occur among these private stocks which, as a rule, are only small. The birds are killed at once and the pens are thoroughly disinfected. This has proved sufficient to keep the disease under control.

According to legal prescriptions in this country the owner, keeper or attendant of live stock who suspects his animals of having contracted an infectious disease is obliged to report this at once to the burgomaster of his place of residence. So if a poultry keeper should suspect any of his birds of being affected with Newcastle disease he is under legal obligation to report it to his burgomaster. Of course, in this country as, I suppose, will be the case in other European countries as well, there are always people who, either purposely or unwittingly, refrain from complying with this legal obligation. In such cases the virus has had every occasion to spread before any veterinary measures can be taken.

As soon as a burgomaster has a case of Newcastle disease reported to him, he will take provisional measures. He immediately informs the Veterinary Inspector of his district, gives orders for the stock to be isolated by means of a notice board and for the birds, dogs and cats to be kept strictly within the premises. He supplies sodium hydroxide as a disinfectant, prohibits the removal of birds, manure and eggs from the premises, and orders the stocks in the neighbourhood to be isolated as well.

The Veterinary Inspector or his substitute then personally inspects the farm and examines the situation on the premises. A general inspection of the stock, the behavior of the birds, examination of the faeces in the pens and local autopsy of one or more carcasses are as a rule sufficient to make a diagnosis. The most typical symptoms noticeable in this clinical examination are paralysis, gasping for air with wide open beaks, and thin grass-green faeces. In doubtful cases, however, some carcasses

or sick birds, under special precautionary measures, are sent for closer virologic and serologic examination to the State Serologic Institution. This is our scientific institution for official government veterinary control in the Netherlands.

Further an investigation is made into the way the stock was infected. Infection can take place along a variety of routes; so it is far from easy to detect its source. More than once it was found that persons who had been in contact with infected birds, farms or pens must have been the carriers. Unscrupulous poultry dealers are dangerous persons in this respect, as they have the most contacts. Also packing cases which had contained infected birds or eggs could occasionally be indicated as virus carriers. Very often the consumption of offal from private kitchens or poulterers' shops caused an outbreak of Newcastle disease.

Once Newcastle disease broke out among the five hens kept at a country house. On enquiry it appeared that a week before the owner had bought poultry offal for his dog. The dog had refused to eat them, the owner had chopped them up and fed them to his hens. A week later the birds showed perfect symptoms of Newcastle disease.

Another poultry keeper used to get kitchen refuse from several private households. The potato skins were given to his cattle, but bread and egg shells were macerated and crushed to be fed to his poultry. Now, egg shells, especially when not boiled, may carry particles of faeces containing the virus. When thrown into a dustbin, they can easily pollute the other eatables in the bin, such as bread. When this is used as poultry feed, the birds easily get infected. Especially during a period with quite a few outbreaks of Newcastle disease, it is no exception for eggs containing the virus, or carrying it on their shells, to be in circulation. More than once the source of infection proved to be offal from poulterers' shops given to pigs as food, for it often happens that the poultry on such farms take their share of the same food by eating from the same trough as the pigs.

Often an outbreak of Newcastle disease occurred among hens kept by ignorant private persons keeping them for the eggs or just as a hobby. They may, however, have obtained their birds, sometimes inferior or sick, from an unscrupulous dealer.

Once or twice it happened that during a period of continued dry, warm weather with the wind blowing in the same direction, the virus was spread over various farms lying in the direction the wind blew.

The control measures taken in this country as soon as Newcastle disease has been diagnosed will be mentioned now.

The burgomasters in this country have been charged with the administration of the legal prescriptions for the control of infectious diseases of cattle and poultry. The burgomaster in whose municipal area an outbreak of Newcastle disease has been detected is advised by the Veterinary Inspector to have all the surviving birds of an infected stock slaughtered. The value of these birds has first been fixed by an expert appointed by the burgomaster. The owner of birds that have to be killed on account of Newcastle disease among his stock, is in this country indemnified by the State for the expropriation of birds, feed, eggs and other objects considered for destruction. The burgomaster is also advised to have killed or dead birds destroyed in a destructor. All infected material which cannot be disinfected has likewise to be destroyed.

Pens, grounds, places to store manure, tools, etc. have to be disinfected with a 1% sodium hydroxide solution. Dogs and cats living on the premises have to be kept in confinement or tied up and a notice board has to be put up in a conspicuous place, clearly visible from the public road. Poultry in the neighbourhood of the infected

area have to be kept in their pens, and in the case of their having been in contact with the infected birds, they have to be slaughtered as well.

The burgomaster has to inform the owner or owners of any sick or suspected birds that they may not move, offer for sale, sell or deliver them. No birds may be moved from or to buildings or premises which have been publicly declared infected or suspected by the erection of a notice board to that effect. Admission to such buildings or premises is prohibited to persons or groups of persons indicated by our Minister of Agriculture, Fisheries and Food. Such persons are for instance peddlers, poultry and other dealers, etc.

Infected or suspected birds are killed and pens, premises, etc. are disinfected under supervision and according to instructions of an official of the Government Veterinary Service.

There are various ways of killing birds. The most humane way is the use of hydrogen cyanide gas. Gassing is carried out by a special staff appointed for the purpose. All the windows and apertures of the pens are carefully shut. Pens having window openings with wire-netting are closed by hanging sacks before them in order to prevent whirls if there should be a strong wind. A large sheet of paper, for instance, a paper foodbag cut open, is laid on the floor of the pen and the contents of a tin of 'singas' or 'cyclon B' are quickly strewn on it. 'Singas' is silicon dioxide containing hydrogen cyanide gas absorbed under pressure.

The person doing this work is provided with a gasmask. After strewing the 'singas', he leaves the pen as quickly as possible, shutting the door most carefully. After about one minute the birds get restless, but from one to three minutes later the pen is perfectly still, the dead birds lying scattered in it. There may be a single bird lying near an aperture in the wall which holds out just a little longer.

Where the pens are so large that the quantity of hydrogen cyanide gas needed would involve personal danger, the birds are driven to one part of the pen behind wire-netting. This part is then shut off from ceiling to floor by means of canvas; then singas is applied in the way mentioned above. Birds sitting on roosts or in their nests are also reached by the gas and soon die.

Five minutes after gassing has taken place, the doors and windows are opened again, of course with the necessary precautionary measures. The gas, if there should be any left in the pen, escapes and the pen can be entered again.

Great personal danger is attached to the application of hydrogen cyanide gas; therefore, this method cannot be applied in every case and in every place and it requires an expert staff and expert supervision.

Where it is impossible to apply this method, another way of killing the birds must be found. In order to avoid cruelty to the birds they are killed by a special staff under the supervision of an official of the Government Veterinary Service. The pen is carefully shut to prevent feathers and dust, and consequently, the virus, from spreading. The birds are caught and stunned by a knock on their heads.

The cervical medulla is then injured by a strong pull severing the cervical vertebrae.

After all birds have been killed, either by gas or otherwise, metal barrels are

brought into the pen, the dead birds are put into it together with as many collected feathers as possible, the barrels are closed and carried in a destructor van to the destructor where everything is burned.

After the carcasses have been removed, the pens are disinfected, which is an easy job when the pens have concrete floors. An ample quantity of a 1% sodium hydroxide solution is poured out on the uncleaned floor to prevent spread of dust and feathers. Then the rubbish is removed from the pen and heaped up on a spot previously indicated for the purpose; again an ample supply of sodium hydroxide is used for this heap. This dunghill is then covered with a thick layer of earth and must be left untouched for some months, after which period the virus in it is assumed to have been destroyed. This manure should preferably be used for land not likely to be entered by chickens.

When all the litter and manure has been removed the pens are cleaned by scraping and washing all dirt off the roosts, the manure boards, the laying nests, and the rest of the pen. Then the pens and everything in them are thoroughly washed by spraying them with a 1% sodium hydroxide solution.

Pens not provided with a concrete floor are much more difficult to disinfect. In such cases, the bottom of the pen is dug out to a depth of 8 inches. This earth and manure is heaped up and treated in the same way as mentioned above. The pens and everything in them are thoroughly cleaned and the bottom is filled up with a layer, 8 inches deep, of fresh earth or sand which is then rammed down. The grounds around the pens are amply sprinkled with a solution of sodium hydroxide.

Poultry runs are dug deep and an ample quantity of a sodium hydroxide solution is also poured over them.

All this work is carried out under the supervision of and according to the instructions given by an official of the Veterinary Service.

After the pens and the premises have been disinfected, the farm remains isolated for another three weeks, the owner is free again to do as he likes, though mostly he is advised not to buy any new birds for the next three months.

Then there is the case of eggs infected with Newcastle disease finding their way into an incubator. First the value of the eggs is fixed as the owner is to be indemnified by the state; then the eggs are destroyed at a destructor. The incubator is disinfected under the supervision of an official of the Veterinary Service. The best way to do this is to use a mixture of potassium permanganate and formalin. A plate containing some potassium permanganate is placed in the incubator and sprinkled with formalin. The mixture soon begins to effervesce, developing a large amount of vapour, so the incubator has to be closed very quickly. It is then kept going half an hour with closed valves at a temperature of  $99\frac{1}{2}$  °F.

The moisture content in the incubator should be between 55 and 60 degrees. After half an hour the valves are opened again, but the incubator is kept going a little longer to allow the formalin vapour to escape. Per cubic metre content of the incubator we use from 20 to 30 grammes potassium permanganate and from 30 to 50 c.c. formalin.

The mixture should be put in the incubator on a glazed dish, and not brought into contact with any of the metal parts, as these would be affected.

This method can also be applied for the disinfection of pens, provided all openings and narrow apertures can be completely shut, and provided it is done for more than half an hour.

Poultry markets were mentioned as dangerous sources of infection. Here less reliable poultry dealers try to dispose of poultry they have bought here, there and everywhere. These markets also sell fattened poultry destined for poulterers' shops. So many birds pass from hand to hand at these markets before reaching their destination, that it is no wonder that infection is very easily spread, and several outbreaks of Newcastle disease in poulterers' shops or among private urban stock could be traced back to these markets.

Crates used in the transportation of poultry are another source, when not or insufficiently cleaned. The birds conveyed in such cases do not show any symptoms of the disease until six or seven days later and soon some deaths will follow. In the meantime, the birds have arrived at their destination and usually their new owner is not familiar with the first symptoms of Newcastle disease. He thinks that these newly bought birds have caught cold during transport and will be all right again in a few days. Only when more birds fall ill and die, does he send for a veterinary surgeon or report the case to his burgomaster. However, the virus has had every opportunity to spread and make new victims.

Poulterers usually are more familiar with poultry diseases and immediately slaughter sick birds. But by selling the offal as pig food he provides another source of infection. Our State Veterinary Service exercises close supervision over poultry markets, and poulterers' shops are inspected by the veterinary staff at irregular intervals. When suspicious symptoms are detected, a closer investigation is made and suspected birds are seized. Poultry farms are inspected regularly in order to prevent farmers from hiding any outbreaks of the disease.

Factories slaughtering chickens for export are also regularly inspected. These chickens may not be bought at a market, but must be supplied direct by poultry farms or poultry rearing farms. Special delivery farms must be used to facilitate supervision.

Another potential source of infection are the large quantities of exotic birds and poultry regularly entering this country by airplane, a good business in such birds being done here. The Minister of Agriculture, Fisheries and Food, in order to prevent the introduction of infectious poultry diseases, has prohibited the importation and transit of a number of species of birds mentioned by name by him, and of their eggs and other products derived from these birds. For some of these birds, importation and transit are allowed on special conditions.

Birds which may be imported under certain conditions are: all kinds of poultry, pheasants, guinea-fowls, partridges, ducks, geese, turkeys, pigeons, parakeets and rice birds.

Anyone wishing to import such birds into the Netherlands has to apply for exemption from the import and transit prohibition order. If this is granted, the importer has to inform the Veterinary Inspector of the district of importation two full days before the date of importation. At the airport, the port, or the frontier station an

investigation is made into the health of the birds. If the result of this investigation is satisfactory, the birds are sent to a place of quarantine, where they are kept under supervision of the Veterinary Service for two or three weeks. Then they may be released.

In the unlikely event of our intensive legal precautionary measures failing to prevent a more or less serious spread of Newcastle disease in the Netherlands, it is my firm opinion, based on results gained from experiments made on a rather extensive scale, that we shall be able to exercise an effective control of the disease by applying live virus vaccination.

# PRINCIPAL ZONOSSES CO-OPERATION WITH MEDICAL HEALTH SERVICE

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## INTRODUCTION

The interest in zoonoses, by which we should understand diseases of man due to infections caused by animals carrying pathogenic microorganisms, whether the animal is ill or not, has greatly increased in the years after the late war.

This greater interest is not only caused by an increase in various countries of the different zoonoses, discovered in consequence of a more and more perfected research technique, but undoubtedly also in consequence of a closer co-operation between physician and veterinary surgeon.

Formerly the veterinary surgeon, when practising his profession, confined himself to rendering aid to the sick animal, by which, at the same time, financial loss for the owner was restricted or prevented. Nowadays, however, he is well aware of the fact that by combating disease in animals he contributes to the benefit of public health.

Not only in Holland but also in other countries, the importance of combating zoonoses is recognized more and more. This was expressed for example at the 'Seminar of zoonoses', held in Vienna in 1952 under the auspices of the W.H.O. and F.A.O. This meeting was attended by representatives of more than 20 countries. When discussing the papers read at this congress, stress was laid especially on 'fieldwork', which should go hand in hand with the laboratory and in which the medical man and the veterinary surgeon should complement each other.

In this connection, attention was also drawn to the co-operation between the Public Health Department and the Board of Agriculture, as zoonoses are not only of importance to Public Health but also to Food Supply. This is of course due to the great financial losses which diseases in animals may cause.

There are two ways in which animals may be the starting point for diseases in man: on the one hand, by direct contact with these animals or with products coming from them, such as hair, hides and wool, on the other hand by the consumption of foodstuffs that are prepared from them.

In the former case fall those people who, on account of their profession, come into daily contact with animals or their products and thus will principally run the risk of

being infected. In the latter case the whole population, as practically everybody consumes foodstuffs of animal origin.

It is impossible to give a survey of all zoonoses occurring in the Netherlands (already more than eighty are known in the world). A few diseases which have of late been the centre of interest in this country and with which the co-operation between medical and veterinary services is shown at the same time will be discussed.

#### SALMONELLA BAREILLY INFECTIONS IN MAN AND POULTRY

In Holland, *Salmonella bareilly* infections in man were observed for the first time in 1948. At the beginning we were in the dark about the source of infection, until in 1951 CLARENBURG and ROEPKE observed at several poultry farms in this country an extensive mortality among young chicks, from the organs and intestinal contents of which they could isolate *Salmonella bareilly*.

In view of the fact that in the years 1948–1951 the infections in man began to increase more and more, the possibility of a casual connection with infected chicks had to be considered.

In order to study this problem more closely and to bring it to a solution, the Ministry of Social Affairs and Public Health instituted in June 1951 a working group consisting of: DR A. CLARENBURG, Head of the Department of Zoonoses and Pathological Anatomy of the National Institute for Public Health at Utrecht, DR G. D. HEMMES, Medical Officer of Public Health at Utrecht and W. WAGENVOORT, Inspector of the Veterinary Service at the Hague.

The following can be said about the working method of this commission and about the results obtained:

##### *Method of investigation*

If in patients *Salmonella bareilly* was shown in the faeces or in other material, an epidemiological investigation was made for the purpose of finding sources of infection by foodstuffs.

If conditions gave rise to it, samples of material were taken for a further investigation on *Salmonella bareilly*.

If there was an indication of infection by poultry, an investigation was made at the farms and the necessary material for investigation gathered.

In the event of animals being suspected of infection on account of bacteriological or serological investigation, they were purchased for further investigation.

##### *Findings in man*

The morbidity of *Salmonella bareilly* among the population of this country has increased considerably from 1948 to the end of 1952.

This appears from the undermentioned table, in which a survey is given of the number of outbreaks during the years 1948–1952:

1948	5 from 3 provinces
1949	15 from 6 provinces
1950	14 from 7 provinces
1951	60 from 10 provinces
1952	more than 100 from 11 provinces



The vast majority of the patients had an acute gastroenteritis, a small part had a typhoid form.

Abscesses, chole-cystitis, orchitis and coxitis were observed as complications.

#### *Findings in poultry*

No exact data are known in Holland as regards the morbidity among poultry; it is assumed that the disease occurs throughout this country.

The clinical picture in the poultry is quite variable. In the adult chicken no clinical symptoms were observed, whereas newly born chickens may sometimes be seriously ill (poor appetite, diarrhoea, respiration-difficulties, poor growth). Especially under unfavourable circumstances, e.g. bad accommodation, transport, perhaps also as a consequence of sexing, the disease sets in in a serious degree. The mortality among groups of infected chicks varies from 0-100 per cent. Generally the mortality does not exceed 10-15 per cent. The breed, too, seems to influence the susceptibility. On a farm where Rhode Island Red and White Leghorn chicks were kept together, a considerable mortality set in among the White Leghorns, the animals of the former breed remaining healthy. Of the chicks that recover, a small percentage remains germ carriers (sometimes for several months).

In connection with cases of illness in man, 15 poultry farms were inspected. Samples of faeces and blood from the chickens were collected as well as eggs in so far as the birds which were laying.

On 4 farms *Salmonella bareilly* was found in the faeces. In none of 547 eggs was *Salmonella* bacteria shown in the contents. On one farm some eggs with dirty shells were also examined externally; in one case the presence of *Salmonella bareilly* on the shell was established.

The serological blood test was at first carried out with the rapid method on the slide with coloured antigen. With this method many non specific reactions were obtained. Therefore, the tube method was used later on. Of 920 blood samples that were investigated, 6 gave a positive and 15 a doubtfully positive reaction.

Also a farm was inspected in connection with an infection with *Salmonella bareilly* in a slaughtered pig. There were 62 chicks at this farm. The investigation of 6 mixed samples of faeces taken from the sty gave a negative result, whereas in one animal using the rapid agglutination method a positive result was obtained.

Twenty chicks in whose faeces *Salmonella bareilly* were found or in which a positive or doubtfully positive agglutination reaction of the bloodserum was observed, were purchased for further investigation. As a rule these birds were kept under observation for a month or longer, during which time the faeces, the blood serum, as well as the collected eggs were investigated. After that the birds were slaughtered and an exhaustive bacteriological investigation, particularly of the ovary, was made.

The result of this investigation was that in the serologically positive animals, but for one exception, no *Salmonella* bacteria could be demonstrated. The exception concerned a chicken from the farm to which the abovementioned infected pig belonged. With this chicken *Salmonella bareilly* was shown in the egg follicles exclusively.

This finding is of special importance, as herewith the possibility of a germinative infection of the eggs has been proved.

From the chickens purchased, more than 100 eggs were collected. In these *Salmonella* bacteria could not once be demonstrated.

It appears from the above that the serological blood test for tracing an infection does not give satisfactory results. In practice we need a specific antigen in order to get a rapid reaction on the slide.

#### *Findings in other animals*

In five infected families, in 1952, the faeces of the cat or of one or more dogs were found positive. It could not be ascertained whether the animal had been infected by man, or whether the members of the family had been infected by the animal.

In one case, however, the way of infection was clear. In that case the dog and the patients had eaten steamed mackerel. The source of infection of the mackerel could not be traced.

#### *Connection between infection in man and in poultry*

On questioning patients it was clear in many cases that they had been in contact with newly born chickens. As it had become known that *Salmonella* bareilly occurs among chickens, samples of faeces were taken from these chickens. In 14 cases *Salmonella* bareilly was successfully cultivated from these samples.

From the course of things it repeatedly appeared that the chickens had infected the patients. A reversed infection was not once made probable. Two patients with whom an infection by contact with organs of slaughtered poultry was considered possible, were examined. Two patients had eaten a raw egg shortly before they fell ill; it was obvious that they had eaten an infected egg, but we had no opportunity of showing this. Once a group of people fell ill at the same time; they had taken their meal together and the only dish that was suspected was cream, mixed with whipped egg white.

Several times an infection of *Salmonella* bareilly was obviously conveyed by food-stuffs, in which cases it was either certain or probable that man had infected the material (pastries, mackerel), or it could be proved that the infection had been conveyed through contact with manure (macaroni porridge, salad).

In some cases the infection could be shown by excreta-contact from man to man. In the majority of cases the source of infection could not be traced or there was no opportunity of making an investigation into it.

#### *Combating the infection in man and animal; Importance of combating*

On surveying the above we are first of all struck by the fact that the morbidity of *Salmonella* bareilly among the population has increased up to the end of 1952; likewise its spread and this not only among man, but also among animals. For man the main cause seems to be contact infection by poultry. That the egg is the source of contamination is most likely, but the chance of infection being caused by an egg must

so far be considered very small. The danger of other sources of infection (slaughtered poultry cats, dogs) is even smaller. In addition to causing human disease, *Salmonella bareilly* may result in financial losses to poultry farms.

#### *Spread of the infection among poultry*

The disease among poultry appears principally in young chicks, among which a serious infection not infrequently occurs. In adult chicks, on the other hand, no symptoms are observed, but germ carriers do occur now and then, and occasionally an infection of the ovary. With spread of the infection among chicks the following possibilities should be taken into account:

1. The hatching eggs have been faecally infected on the shell. During the process of hatching the shell is pecked at by the chicken.
2. Infection by contact with infected material in the hatching compartment of the incubator.
3. The penetration of *Salmonella bareilly* from the infected egg shell to the embryo. By several investigators great value is attached to this mode of infection. An experiment carried out in this connection by one of us did not support this view.
4. Germinative infection of the hatching eggs.
5. Conveyance of the infection by the sexer.
6. A few days after hatching, especially if the chickens are together in a very small area, they are infected by the faeces of a germ carrier.

#### *Measures of combating*

The combating of *Salmonella bareilly* should above all be focused on poultry. If this should be entirely successful there only remains a possibility of infection by a limited number of chronic human germ carriers.

Just as with *Morbus pullorum*, to which *Salmonella bareilly* bears resemblance, the combating should, according to CLARENBURG and ROEPKE (*Tijdschrift voor Diergeneeskunde* 1952, p. 174), principally be effected by:

- a. tracing and eliminating infected animals;
- b. disinfecting the incubators.

For tracing infected animals the serological blood test, as well as the bacteriological investigation of faeces and of sick or dead animals, are mainly considered.

We are not yet sufficiently informed about the value of the serological blood test. The fact is that the question arises whether a negative reaction precludes an infection and, on the other hand, whether a positive reaction is always a proof of infection. Further investigation will be necessary, aiming at finding a specific and quick acting antigen.

The disinfection of the incubator containing the hatching eggs is, as has been found in this country and abroad, a most effective measure for combating the infection when the chickens are born.

It stands to reason that in this way the germinative infection is not checked. It is, however, a question whether great value should be attached to this mode of infection; so far we have not succeeded in demonstrating *Salmonella bareilly* in eggs.

Formalin gas is generally recommended as a disinfectant. The good results attained therewith were described in a recent publication by J. E. WILSON (Vet. Record, 4 August 1951). Experiments made by us with different concentrations of formalin also gave a most satisfactory result. When using 26.5 ml. of formalin plus 17.5 g of potassium permanganate per cubic metre incubator contents, all *Salmonella* bacteria on artificially infected eggs were killed within half an hour. This is half the concentration which WILSON had used for his investigation.

Also in practice a few experiments were made in order to ascertain whether the disinfection of the filled incubator might exercise unfavourable influence on the hatching result. It appeared that even when disinfected three times (viz. on the 1st, 10th and 19th day of incubation), compared with non-disinfected control eggs, the viability of the embryo was not adversely affected.

According to literature, the hatching result would indeed be affected unfavourably if disinfection took place between 24 and 84 hours after setting.

In our opinion the disinfection of the incubators should preferably be carried out as follows:

1. Thorough cleansing and disinfection of the incubator before the beginning of the hatching season.
2. Disinfection of the hatching eggs just before incubation in a separate cabinet, to prevent disinfection taking place in the susceptible period or during the time when newly born chickens are already present in the incubator.
3. Regular cleansing and disinfection of the compartment in which the chickens are hatched, in connection with the possibility of infected chickens being born from eggs germinatively infected.

In addition to disinfection of the incubators, attention should be paid to the persons who handle disinfected hatching eggs or newly born chickens. It is desirable that these persons, and also the sexer, should have short cut nails and brush their hands with soap and hot water before beginning their work.

#### POX IN MAN AND CATTLE

In the literature of the last few years the occurrence of pox on teats and udders of is frequently reported. They are said to have been caused after members of the farmer's family have been vaccinated against smallpox, in which case it is assumed that the cow is infected by vaccine virus.

On the other hand it has appeared from observations on farms that cow pox occurs without there having been the slightest contact with vaccinated persons; these are cases of genuine cow pox.

According to recent investigations it may now be assumed that both occur in cattle and that, by way of cattle, man may be infected again by both types.

The vaccinia in man and the genuine cow pox in cattle are two different diseases,

which, however, cause the same symptoms on the teats of the cow and are indistinguishable clinically.

The differences existing between the two viruses can only be shown in the laboratory by inoculating the virus into the chorio-allantoic membrane of the embryonated eggs.

In cattle, pox usually causes a local affection of the udder and teats, mostly of a benign character. After an incubation period of about a week, characteristic lesions are observed on the teat skin, in the shape of pustules which have, after one or two days, the size of a pea. These pustules are of a yellow white to bluish red color, showing in the centre a small vesicle with clear contents. The clear contents become purulent and as soon as the scabs fall off they leave a deep scar behind. The process of recovery lasts about a fortnight unless, on account of trauma of the teat in consequence of milking, wounds are caused which are often infected secondarily.

In man, one or more characteristic pustules usually appear on the hand or the arm, mostly of a benign character. Sometimes, however, deep necrotizing ulcers begin to appear, by which the general condition is seriously affected.

It may be assumed that in this country the disease rather frequently occurs in cattle, but because of its mild character the veterinary surgeon is not consulted, so that many cases escape notice.

The Veterinary Officer of Public Health at Leeuwarden inspected from 1st September 1952 to 26th March 1953, 47 farms in Friesland where cases of disease resembling pox were observed in cattle.

An epidemiological investigation was made in co-operation with the Medical Officer of Public Health in order to trace the sources of infection, especially to find out whether there had been either any direct or indirect contact with vaccinated persons, or whether the cattle had been infected otherwise. Material obtained from the teats was sent to the National Veterinary Research Institute in Amsterdam, to decide on the type of virus.

From notes supplied by Mr DIJKSTRA it appeared that they were mostly cases of genuine cow pox. In a few cases vaccinia was diagnosed; herewith the connection with vaccinated persons could be proved.

A type case of vaccinia in cattle was recently demonstrated to me by Mr DIJKSTRA.

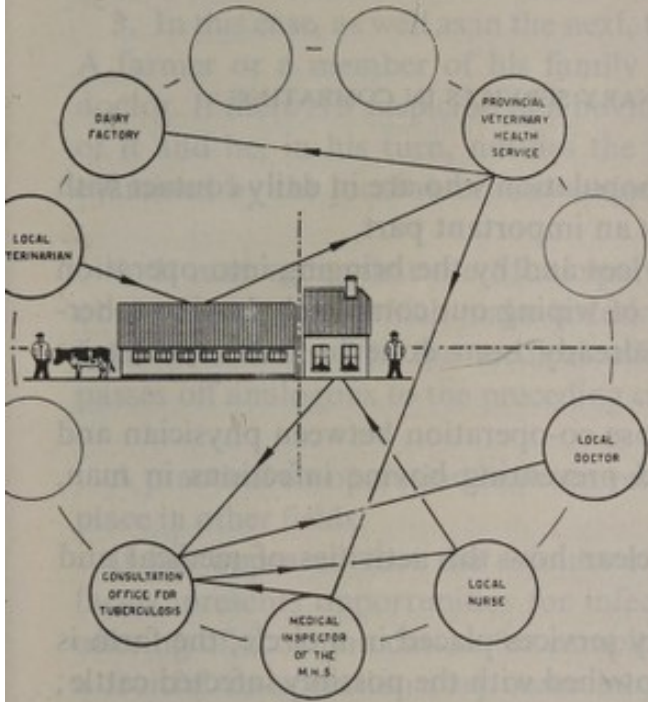
On a farm a 14 year old son of prospective emigrants milked the cows until 17th March 1953. The family emigrated on 20th March, after being vaccinated against smallpox on 3rd March 1953. Until 17th March the boy milked 5 cows. The first cow remained healthy, the second and third cow developed pox, the fourth was doubtful. After the boy's departure on March 17th, the farmer took over the milking of one sick cow, the other ones were taken over by a farm hand. Of the cows which the farm hand milked after the sick cow, three more developed pox. On 26th March, the farm hand had an extensive pox eczema on the arm with lymphangitis and swelling of glands. He was seriously ill and taken to hospital. On 7th April he was still in hospital, but was soon to be discharged. On 26th March the farmer had two small pox on the right hand forefinger.

On 1st April 1952 the remaining non infected cows were vaccinated on the perineum against pox. When visiting the farm on 7th April last I found that besides the six animals, there had been no fresh outbreaks.

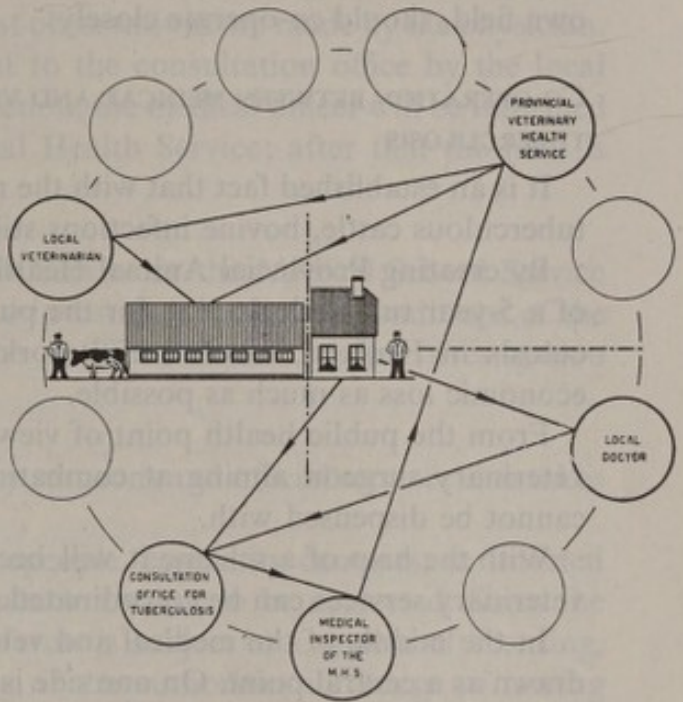
On 26th March material was sent to the National Veterinary Research Institute in Amsterdam, where the diagnosis vaccinia was confirmed.

**SCHEME OF COÖPERATION BETWEEN MEDICAL AND VETERINARY  
AUTHORITIES FOR THE ERADICATION OF TUBERCULOSIS.**

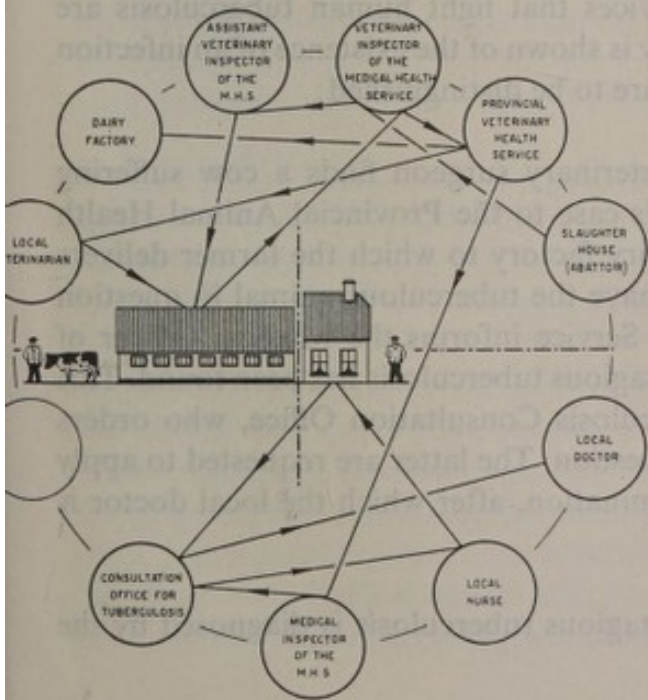
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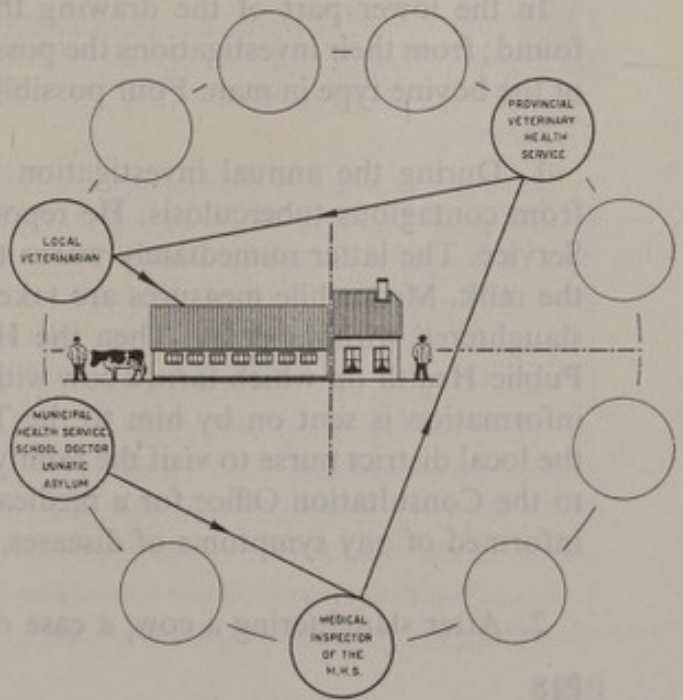
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It was generally advised to milk as much as possible wearing rubber gloves, or to brush the hands thoroughly with soda and soft soap and to milk the healthy cows first. It was further recommended to use globenicol ointment during milking.

It has clearly appeared from the above case that vaccinated persons may infect cattle and that man may again be infected by way of cattle.

The population will have to be enlightened on the measures that should be taken to prevent infection in cattle and man. Physician and veterinary surgeon, each in his own field, should co-operate closely.

#### CO-OPERATION BETWEEN MEDICAL AND VETERINARY SERVICES IN COMBATING TUBERCULOSIS

It is an established fact that with the rural population who are in daily contact with tuberculous cattle, bovine infections still play an important part.

By creating Provincial Animal Health Services and by the bringing into operation of a 5-year tuberculosis plan for the purpose of wiping out completely bovine tuberculosis in Holland, much useful work has already been done in order to restrict economic loss as much as possible.

From the public health point of view, a close co-operation between physician and veterinary surgeon aiming at combating and preventing bovine infections in man, cannot be dispensed with.

With the help of a scheme it will become clear how the activities of medical and veterinary services can be co-ordinated.

In the middle of the medical and veterinary services placed in a circle, the farm is drawn as a central point. On one side is the cowshed with the possibly infected cattle; on the other side the dwelling house of the possibly infected people.

In the upper part of the drawing one can see, beside the dairy factory, the veterinary services that take part in combating tuberculosis in cattle and by which valuable data are compiled.

In the lower part of the drawing the services that fight human tuberculosis are found; from their investigations the possibility is shown of the existence of an infection of the bovine type in man. Four possibilities are to be distinguished:

1. During the annual investigation the veterinary surgeon finds a cow suffering from contagious tuberculosis. He reports this case to the Provincial Animal Health Service. The latter immediately warns the dairy factory to which the farmer delivers the milk. Meanwhile measures are taken to have the tuberculous animal in question slaughtered without delay. Then the Health Service informs the Medical Officer of Public Health on which farm a cow with contagious tuberculosis has been found. This information is sent on by him to the Tuberculosis Consultation Office, who orders the local district nurse to visit the family in question. The latter are requested to apply to the Consultation Office for a medical examination, after which the local doctor is informed of any symptoms of diseases.

2. After slaughtering a cow, a case of contagious tuberculosis is diagnosed by the

Meat Inspection Service. The head of this Service reports this case to the Veterinary Officer of Public Health, who supervises the execution of the regulations under the Meat Inspection Act, mentioning particulars if any. This officer instructs his superintendant to collect the necessary data at the farm in question. The aforesaid officer sends these data on to the Provincial Animal Health Service. Further course of things as in case 1.

3. In this case, as well as in the next, the first observations are made by the physician. A farmer or a member of his family is sent to the consultation office by the local doctor. If there is a suspicion of a bovine infection, the medical officer will be informed of it and he, in his turn, notifies the Animal Health Service; after that the herd is examined by the local veterinary surgeon.

4. It may occur that the Municipal Health Service or the Medical School Service observe symptoms which make it desirable to investigate the health conditions of the herd on a farm. Via the medical officer the notification and further investigation passes off analogous to the preceding case.

A practical co-operation between veterinary surgeons and official services also takes place in other fields.

It has appeared that the sojourn of townspeople, especially campers, at infected farms, presents opportunities for infection which are not to be neglected. Since the offering of accommodation for camping on farms is subject to a system of licensing, a fruitful co-operation may come into being, if the authorities in charge of issuing these licences contact the Provincial Animal Service beforehand with a view to finding out whether the farm in question is free from tuberculosis. This is a very welcome measure from the view point of prevention.



# BACTERIAL FOOD-POISONING

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## INTRODUCTION

The examination of foodstuffs, which have caused disease in man, lies within the scope of the bacteriologically-trained veterinary surgeon. By far the greater part of these foodstuffs is of animal origin. It will, therefore, encounter little opposition that the veterinary surgeon, on account of his education and experience, is the right man for the interpretation of bacteriological findings.

As the symptoms of disease in man almost exclusively depend on the nature of the causal organisms, it is obvious to base the classification of food-poisonings on them. As far as this country is concerned we can distinguish the following groups of micro-organisms in this connection: *Salmonella*, *Shigella*, *Clostridium botulinum*, *staphylococci*, and *non-specific bacteria*. Of these only *Salmonella* and *Shigella* belong to the pathogenic germs proper, i.e., germs which multiply in the body and themselves cause diseases in man. *Cl. botulinum* and *staphylococci* cause symptoms of disease by the formation of toxins, whereas the group of non-specific germs most likely cause disease by the formation of poisonous products from the food-proteins (*food intoxications*).

The above groups will now be discussed. Attention will especially be paid to the *Salmonella* organisms.

### 1. SALMONELLA

The *Salmonella* group is a large genus of Gramnegative non-sporeforming bacteria which are characterized by certain biochemical and serological properties. More than 200 serologically different types are distinguished. The typing is of great importance for the epidemiology of outbreaks of food-poisoning as it enables the source of contamination to be ascertained.

Besides man, *Salmonella* also occurs in numerous species of animals. Not only do we find infections in slaughter-animals (cattle, horses, pigs, sheep and goats), but especially in birds. Of the latter, in this country chicks, ducks and pigeons are the most important. In addition to these, rodents (rats, mice, rabbits and guinea pigs), dogs and cats are also often infected by these bacteria.

There are *Salmonella* types which are adapted to the human body in such a way that they hardly ever occur in animals, e.g., *S. typhi* and *S. paratyphi B*. Reversely, some

Salmonella types almost exclusively occur in animals, e.g., *S. abortus equi* in horses and *S. pullorum* in chicks. *All Salmonella-types, however, are potential pathogens for man and animal.*

In general those Salmonella types that are autochthonous in man cause a clinical picture different from that caused by bacteria of animal origin. The former give rise to the so-called *typhoid form* of enteric fever, the latter to the so-called *gastro enteritic form*. Unlike the latter, the typhoid form is not only more serious but also gives rise to prolonged germ-carrying and to contact-infections. There are, however, many exceptions to this rule.

As a rule, poisoning only occurs if the food contains a great number of Salmonella organisms. It has frequently appeared that consumption of the contaminated food directly after preparation did not cause illness, whereas it did after the food had been kept under conditions favourable for the multiplication of the bacteria.

The *clinical picture* of Salmonella food-poisoning is characterized by gastro-enteritis. The symptoms are mainly nausea, vomiting, diarrhea, and fever. As a rule the Salmonella organisms are excreted for a short time only (1-2 weeks); contact infections do not occur. It must, however, be emphasized that there are many exceptions to this rule. The incubation-period is mostly 12 hours on an average; patients recover within 1-2 weeks; the mortality rate is very low (ca. 1 %).

It stands to reason that for tracing the source of infection an exact knowledge of the Salmonella types occurring in man and animal is of the greatest importance. During the last few years many investigations were made to this effect in the Department for Zoonoses and Pathological Anatomy of the National Public Health Institute, in which the *National Salmonella Centre* is established. In table 1 a survey is given of the Salmonella types identified in man and animal from 1946 up to and including 1952. It appears from this table that 41 serologically different types were found, of which 37 originated from man and 19 from animals. 22 Types were exclusively isolated from man and 4 from animals. (see table p. 193).

Numerous foodstuffs may give rise to food-poisoning due to a contamination with Salmonella. The most important are *meat and meat products*; further *eggs, spraydried eggs*, and *milk*, must be mentioned. These foodstuffs will now be discussed separately.

#### *a. Meat and meat products*

For a correct understanding we must distinguish with meat-poisoning, between an intravital and a postmortal infection of the meat. With intravital infection we have to deal with an infected slaughter-animal and consequently the causative agents originate from this animal. With post-mortal contamination on the other hand, which occurs after the animal has been slaughtered, the origin must be found elsewhere.

As a source of *intravital infection* the *primary Salmonellosis* of slaughter animals are of great importance. In this country these occur in most cases in *cattle*, both in adult animals and in calves. As a rule, they are caused by *S. dublin*; in various cases, however, by *S. typhi-murium*. It is of great significance that following this disease in adult cattle a considerable percentage of germ-carriers is found. These animals

TABLE 1. SALMONELLA-TYPE IN MAN AND ANIMALS IN THE NETHERLANDS IN 1946-1952

	Slaughter-animals							Rodents			Birds					Salmonella group				
	Man	Horse	Adult Cattle	Calf	Sheep	Pig	Dog	Cat	Guinea pig	Mouse	Rat	Pigeon	Chicken	Duck	Parrot		Parakeet	Turkey	Deer	Silverfox
<i>S. paratyphi A</i>	+																			A
<i>S. abortus equi</i>	+	+																		
<i>S. paratyphi B</i>	+					+	+		+											
<i>S. abortus bovis</i>			+																	
<i>S. saint paul</i>	+													+						
<i>S. reading</i>	+																			
<i>S. chester</i>	+																			
<i>S. san diego</i>	+																			B
<i>S. derby</i>	+																			
<i>S. typhi murium</i>	+	+	+	+		+			+	+	+	+		+	+	+				
<i>S. stanley</i>	+										+									
<i>S. bredeney</i>	+																			
<i>S. heidelberg</i>	+																			
<i>S. paratyphi C</i>	+																			
<i>S. cholerae suis</i>	+					+														
<i>S. montevideo</i>	+																			
<i>S. thompson</i>	+																			
<i>S. potsdam</i>	+																			
<i>S. oranienburg</i>	+																			
<i>S. virchow</i>	+																			
<i>S. bareilly</i>	+						+				+		+					+		C
<i>S. braederup</i>	+																			
<i>S. oregon</i>	+	+																		
<i>S. manhattan</i>	+																			
<i>S. newport</i>	+		+			+		+						+						
<i>S. kottbus</i>	+																			
<i>S. bovis</i>																				
<i>morbificans</i>	+		+		+	+	+		+	+										+
<i>S. duesseldorf</i>	+																			
<i>S. typhi</i>	+																			
<i>S. enteritidis</i>	+					+	+		+	+	+		+	+	+					
<i>S. blegdam</i>	+																			
<i>S. dublin</i>	+	+	+	+	+	+	+		+	+	+									+
<i>S. pullorum</i>													+							
<i>S. panama</i>	+																			
<i>S. anatum</i>	+		+										+	+						
<i>S. meleagridis</i>													+	+						
<i>S. london</i>	+																			
<i>S. give</i>	+					+														E
<i>S. weltevreden</i>	+																			
<i>S. newington</i>						+														
<i>S. senftenberg</i>	+						+						+	+			+			

harbour the *Salmonella* not only in the intestine but also in the internal organs and the muscles, especially in the liver and gall-bladder. As on inspection before slaughtering usually no symptoms of any disease are observed and after slaughtering pathological-anatomical lesions are often lacking, there is a danger of the meat of such animals being brought into consumption without a preliminary bacteriological examination. Experience has taught that the number of *Salmonella* organisms in the organs, but especially in the muscles of these animals, may be very small. If, however, meats are prepared from it, or if these products or the meat itself is placed under unfavourable conditions (e.g. at a high temperature) a considerable increase of these germs may occur, in consequence of which consumers may fall ill.

Another Salmonellosis is the infectious abortion in *horses* caused by *S. abortus equi*. This infection occurs occasionally in this country but has never given rise to meat-poisoning.

Finally the infection with *S. cholerae-suis* in *pigs* must be mentioned. This infection used to be rather frequent but has been observed very seldom for the last 15 years. It is a well-known fact that *S. cholerae-suis* may cause meat-poisoning.

In addition to primary Salmonellosis, the *Secondary Salmonellosis* of slaughter animals also play an important part in the occurrence of meat-poisonings. They are the Salmonellosis which occur in animals with mostly non-specific affections. Especially the animals slaughtered in emergency on account of a disease cause danger in this connection. Though difficult to prove, we must assume with these secondary Salmonellosis that *Salmonella* organisms, living saprophytically in the intestine or elsewhere (mesenteric lymph glands), give rise to the spreading of the organisms in the body as a consequence of a decreased resistance. Thus we have repeatedly been able to establish a *Salmonella*-infection in animals which had suffered from foot-and-mouth disease. Another indication of this is a meat-poisoning which we saw a couple of years ago after consumption of horse-meat originating from an animal that had broken a leg and was not slaughtered until 16 hours after the accident. An additional support for this assumption is that *Salmonella* organisms are occasionally found in the intestine of healthy slaughter animals and rather frequently in the mesenteric lymph glands of healthy pigs and cattle.

With meat-poisonings based on a *post-mortal infection* we must naturally think of man. In addition to this, attention should also be paid to a possible contamination by animals, especially by rodents (mice and rats). Salmonellosis frequently occur in these animals; as a rule they are caused by *S. typhi-murium* or *S. enteritidis*. The last few years we have frequently found infection with *S. bovis morbificans* in mice, a well-known causative agent of disease in man. Flies, too, may act as vectors of *Salmonellae*.

Let us now turn to the *meat-poisonings that were examined in the years 1949, 1950, 1951, and 1952*. They are summarized in table 2, while in table 3 a survey is given of the contaminated meats in connection with the *Salmonella* type found.

It appears from these tables that the number of meat-poisonings varies considerably in the years mentioned. No explanation can be given for this. Out of 19 cases 18 were caused by meat products (12 by sausage, 5 of which were liver-sausage, 3 by smoked beef, 2 by pork brawn, 1 by boiled ox-liver) and only 1 by fresh meat (horse-meat).

The comparatively great number of poisonings caused by meat products need not be surprising, seeing that during the process of preparation and storage a great increase of the Salmonella bacteria takes place.

TABLE 2. MEAT POISONINGS IN 1949-1952 IN THE NETHERLANDS

Date	Meats	Salmonella-type	Mode of contamination	Remarks
1949				
12-6	liver sausage	S. dublin	?	-
25-7	horse meat	S. typhi murium	intravital	liver +
Sept.	liver sausage	S. dublin	?	-
Sept.	smoked beef	S. dublin	?	-
Dec.	blood sausage, etc.	S. newport	?	approved pig; <i>private slaughter</i>
1950				
6-2	sausage	S. dublin	?	-
7-3	sausage, etc.	S. dublin	?	-
22-3	liver sausage	S. dublin	intravital	approved cow
23-3	liver sausage	S. oregon	?	non inspected pig; <i>private slaughter</i>
3-4	sausage	S. oregon	?	approved pig; <i>private slaughter</i>
7-4	sausage, etc.	S. paratyphi B	?	idem
25-4	boiled ox-liver	S. dublin	intravital	approved cow; portal lymph gland +
20-5	liver sausage	S. dublin	?	-
30-5	smoked beef	S. typhi murium	?	approved imported cow
24-10	pork brawn	S. paratyphi B	intravital	approved pig; <i>private slaughter</i> bone marrow +
31-10	smoked beef	S. typhi murium	?	-
1951				
15-6	sausage	S. bovis morbificans	?	approved bull
2-7	sausage, etc.	S. typhi murium	?	-
1952				
11-11	pork brawn and sausage	S. typhi murium	postmortal	approved pig; <i>private slaughter</i>

In 4 out of 18 meat-poisonings an intravital contamination was established; in the remaining cases the source of contamination remained unknown. Only once a postmortal contamination could be assumed, as in the meat and bone-marrow of the suspected pig no Salmonella was found (table 2, 1952). As regards the causal Salmonella types, S. dublin was found 8 times, S. typhi murium 5, S. paratyphi B 2, S. oregon 2, S. newport 1, and S. bovis morbificans 1.

TABLE 3. MEAT POISONINGS 1949-1952 IN THE NETHERLANDS

Kind of contaminated food	Number of meat-poisonings caused by:					
	S. dublin	S. typhi murium	S. paratyphi B	S. oregon	S. newport	S. bovis morbificans
Sausage	2	1	1	1	1	1
Liver-sausage	4			1		
Boiled ox-liver	1					
Smoked beef	1	2				
Pork brawn		1	1			
Horse meat		1				

*S. dublin* was identified in nearly half the number of cases. In our country this type frequently occurs in cattle, especially in young animals. It is also found in other species of animals, but only exceptionally. Consequently it is obvious that in cases of meat-poisoning caused by *S. dublin* we must first of all consider cattle as the possible source of contamination. Sick animals slaughtered in emergency on account of Salmonella infection are of little danger, because in these cases a bacteriological examination must always be made as required by the Meat Inspection Act. According to this Act the animal must be condemned if Salmonella is found in one or more organs. It is possible, however, that such animals are slaughtered illegally and consequently not offered for inspection.

Of greater importance in my opinion are the clinically healthy germ-carriers, which as mentioned above, occur rather frequently and in which after slaughtering no pathological-anatomical changes pointing to a sepsis are found. In consequence of this, no bacteriological examination is made and the animal is approved for consumption.

In 2 out of 8 cases of meat-poisoning caused by *S. dublin* (liver sausage and boiled ox-liver) an intravital infection was afterwards established. Both cases referred to healthy cows which were approved for consumption without bacteriological examination. These animals most probably belonged to the above mentioned group of germ-carriers. As in the remaining 6 cases, sick animals could not be found either, it is obvious, also in connection with the autochthonous occurrence of *S. dublin* in cattle, that here too the source of contamination must be sought in an animal which was latently infected.

Of the 5 meat-poisonings caused by *S. typhi murium*, an intravital infection was found in 1 case (horse meat). In this case Salmonella was cultivated from the liver of the animal after the consumers had fallen ill. As *S. typhi murium* occurs ubiquitously, it is difficult to point with any measure of certainty to the source of contamination in the remaining cases. In the first place we must think here of slaughtered animals and of man, in the second place of rats and mice. Also ducks' eggs must be considered. In this connection it is interesting that some years ago a meat-poisoning was examined which was caused by the addition of ducks' eggs in the preparation of liver sausage.

Meat poisonings caused by *S. paratyphi B* seldom occur. In 1950 two cases were

observed. They were caused by meats prepared from pigs slaughtered on the farm (private slaughter). One of these pigs had been ill for some days and was slaughtered on the day after recovery. From this pig *Salmonella* was cultivated from the bone-marrow. This was evidence of an intravital infection. Since *S. paratyphi B* is seldom found in animals, it is obvious that both pigs had been infected by human carriers.

The meat-poisonings caused by *S. oregon* and *S. newport* respectively, referred to healthy pigs slaughtered on the farm. The *Salmonella* types mentioned frequently occur in man so that the source of contamination might possibly be found in human carriers.

*S. bovis morbificans* is found rather frequently in man in this country. Besides, this type causes a fatal infection in rodents (mouse, guinea-pig). Therefore we must in the first place think of human germ-carriers and mice as sources of food-contamination.

When investigating the source of contamination in meat poisoning attention should not only be paid to man and animals, but also to *tools* which meat and meat products contact (e.g. chopping-blocks, mincing-machines). Several times we have succeeded in cultivating *Salmonellae* in scrapings of such materials.

#### b. Eggs

The incidence of *Salmonellosis* in birds (chicks, ducks, pigeons) is rather frequent. It is especially the young chicks that succumb in a more or less high percentage. It is quite different with adult animals which have a considerably greater resistance. From the public health point of view it is of special interest that it is not uncommon for the chicken ovary to become infected, thus leading to primary infection in the yolks.

We have examined about 7000 ducks' eggs; about 2% contained *Salmonella*-organisms internally. The eggs may also be contaminated on the outside of the shell by faeces.

So far no food-poisonings have been observed in this country which were caused by infected *hens'* eggs. The probable reason is that *S. pullorum* and *S. gallinarum* are little pathogenic for man. During the last few years, however, an infection in chicks caused by *S. bareilly* frequently occurs in this country. This organism has often given rise to infection in man. A connection with infected eggs has not yet been established. In one or two outbreaks of food-poisoning, however, this appeared to be most likely.

It is an altogether different case with *ducks'* eggs. These have already frequently caused outbreaks of food-poisoning. As a rule *S. typhi murium* or *S. enteritidis* was isolated, very seldom *S. saint paul*. In connection with the small number of *Salmonellae* in fresh ducks' eggs, the danger of consuming them is not so great. Far more serious is the danger when these eggs are used for the preparation of food which is not sufficiently heated to destroy the *Salmonellae* and in which this germ can multiply easily. In this connection we observed cases of food-poisoning after consumption of minced meat, pudding, mayonnaise, cakes, ice-cream, currant-bread.

Infected *pigeons'* eggs, too, may cause food-poisoning. This appeared from the examination of pudding prepared with pigeons' eggs. This pudding had given rise to 20 cases of illness. The infecting organism was *S. typhi murium*, the well-known causative agent of *Salmonellosis* in pigeons.

Though in this country infections after consumption of *geese-eggs* have not yet been observed, they should also be taken into account, according to foreign literature.

#### c. *Spray-dried eggs*

In imported spray-dried eggs various types of *Salmonella* are frequently found. In several countries food-poisonings were caused by them. The *Salmonella* organisms most probably originate from infected shells. It is known that birds are a considerable reservoir of *Salmonella*. In the Netherlands this is especially the case in ducks. On investigating 5000 samples of chicks' faeces *Salmonella* strains were isolated only twice (1 time *S. anatum* and 1 time *S. meleagridis*) whereas in 602 samples of ducks' faeces *Salmonellae* were isolated 63 times (10%). The following types were identified: *S. meleagridis* 18 times, *S. typhi murium* 17, *S. saint paul* 11, *S. anatum* 14, *S. newport* 1, *S. senftenberg* 1, and *S. ballerup* 1.

#### d. *Milk*

*Salmonella* epidemics are not only caused by contaminated milk, but also by products prepared from it (ice-cream). The contamination of the milk takes place either directly or indirectly by human carriers. It may also be caused by infected cattle. Bovine carriers of *S. dublin* must be considered in the first place. These animals are clinically healthy, have a normal milk-yield, but excrete with the faeces a great many *Salmonella* organisms. By these organisms the milk may easily be contaminated. In this country a fatal case of *S. dublin*-infection was observed in a farmer's child, which was caused by milk infected in this way.

Although uncommon, a mastitis caused by *S. dublin* does occur in cattle. Experience abroad has taught that in this connection numerous cases of illness in man may occur.

In cattle also infections with *S. paratyphi B*, originating from human germ-carriers, may occur. Twenty years ago a rather serious epidemic of paratyphoid fever was caused by milk thus infected.

## 2. SHIGELLA

Milk is a good nutrient medium for dysentery bacteria and therefore, after having been contaminated, it can easily give rise to many cases of illness. Contamination is usually caused by a human germ-carrier. Two mass infections of *Sh. sonnei* have of late been observed in this country, one of which had been caused by milk and cream, the other by cheese. In this case of cheese-poisoning 54 people from 16 families were taken ill, of whom a 72-year-old patient died. The incubation-period varied from 8½–51 hours. On the day on which the incriminated cheese had been made, several members of the cheesemaker's family suffered from diarrhea. The cheese was 37 days old on the day of consumption.

## 3. CLOSTRIDIUM BOTULINUM

*Cl. botulinum* is a spore-bearing, anaerobic bacillus, which is widely spread in nature, especially in the soil. The spore-stage has a strong resistance to heat. The organisms themselves are harmless; in food, however, they excrete powerful toxins (neuro-toxins). Even in very small quantities these toxins cause serious illness. Unlike



the spores, the toxins have only little resistance to heat. At 80°C they are already destroyed within a few minutes. On account of differences in the nature of these toxins we now distinguish 5 different types of bacilli, which are indicated by A, B, C, D and E respectively. Only the types A, B and E, have so far been recognized as a cause of intoxication.

Botulism is the most serious food-poisoning that we know. Not only on account of the long duration of the illness, but especially because the mortality-rate may be high. As a rule the incubation-period is about 24 hours, but may also amount to 3–4 days. The most important symptoms are vomiting, constipation, visual disturbances, thirst, and difficulties in swallowing due to decreased salivation. There is no fever, but sub-febrile temperature may occur. Death may occur in 1 day, but sometimes after a week. Complete recovery may commence after 6–8 months.

Botulism is rather rare in this country. In the last 15 years 8 cases were examined. The foods involved were smoked bacon 2 cases, salted smoked ham 2, liver- and blood sausage 1, meat-pie prepared from pigeon and pork 1, fish-sausage 1, and canned fish 1.

The cultural examination of the suspected samples mostly gave a negative result. In only 1 case (smoked ham) *Cl. botulinum* type B could be isolated. In 7 samples toxins of the B-type and in one (canned fish) toxins of the C-type could be demonstrated.

With the exception of the above sample of ham, all samples showed putrefaction due to bacterial spoilage.

Demonstration of the presence of toxins is very simple if the various anti-sera are available. A number of mice are injected intraperitoneally with an aqueous extract of the incriminated food, after which small groups of these mice are injected with the various anti-sera respectively. If botulinum toxin is present in the suspected food all mice will die, with the exception of those which received the homologous antiserum.

#### 4. STAPHYLOCOCCI

More than 90 per cent of the cases of food-poisoning in the United States are caused by staphylococci. These bacteria are of widespread occurrence, but are especially found in the nose, the throat, and on the skin. They may also be the causative agents of purulent inflammation. Not all staphylococci are able to cause food-poisonings. It has appeared from feeding experiments using culture-filtrates in human volunteers and monkeys, that only a few strains form toxins (enterotoxins). These strains are characterized by the following properties: formation of coagulase and hemolysins, fermentation of mannitol. Demonstrating staphylococci in suspected foodstuffs is, as such, no evidence of the symptoms of illness being caused by these bacteria.

In case of a food-poisoning by staphylococci symptoms of illness suddenly appear within 1–5 hours, 2½ hours on an average, after consumption of the contaminated food. Symptoms are violent vomiting and diarrhea. These symptoms usually vanish within a few hours and after 24–48 hours the patients have quite recovered. The death-rate is extremely low.

Especially milk and milk-products, together with dishes prepared with them, have given rise to this kind of poisonings. In such foodstuffs, which may be of perfectly normal appearance, numerous staphylococci can be demonstrated. It is not the live

germs that cause illness, but the toxins that are formed during the time in which the food is placed in a warm situation. These toxins are thermostable, i.e. they withstand boiling.

In 1951 and 1952, a total of 6 cases were examined by us, 3 of which had been caused by meat products (sausage) and 3 by steamed fish. With the exception of one sample of steamed fish, the suspected foodstuffs had a good appearance. However they all proved to contain a very large number of staphylococci with the characteristic properties previously mentioned.

#### 5. NON-SPECIFIC BACTERIA

The majority of outbreaks of food-poisoning in this country are caused by bacteria of this group. In the suspected food numerous bacteria belonging to different species are always present. We may find *Proteus* bacteria, coliform bacteria, streptococci and staphylococci, *Cl. perfringens* and various other aerobic- and anaerobic bacteria. The incriminated foodstuffs are as a rule in an initial stage of deterioration. This deterioration is often masked, because the foodstuffs may be fried, smoked or seasoned. It is caused by placing or carrying the foodstuffs in a warm temperature.

The symptoms (vomiting and diarrhea) appear soon after the consumption of the poisonous food; usually after 6-8 hours.

Very different foodstuffs may cause this type of poisoning; especially, however, we saw mass-poisonings after consumption of *fried and steamed fish, pea-soup, and boiled meat*. The cause usually appeared to be *ineffective cooling* after preparation.

In the Netherlands *cheese-poisonings* frequently occurred before the last World-War. The incubation-period was about 4 hours. The symptoms were vomiting and diarrhea. As a rule the patients had recovered after one or two days. The suspected cheese had a good appearance; pathogenic germs could not be found. The cheese-poisonings must probably also be classed in this category. The fact is that during milking serious hygienic mistakes are often made, so that the milk is contaminated with numerous bacteria; for example insufficiently cleaned and dried milk-pails. Especially if this milk is stored and processed at rather high temperatures is there ample opportunity for germs to increase and to form toxic products from the milk-proteins.

#### PROPHYLAXIS

The two essential aspects of the genesis of food-poisoning are:

- a. contamination of the food with the causative germs, and
- b. multiplication of the germs in the causative foodstuffs.

Prophylaxis is based on the prevention of either (a) or (b) or both.

##### a. *Prevention of contamination*

As far as *intravital infection in cattle* is concerned, the prevention must be ensured by a thorough veterinary inspection of the live and slaughtered animals. In all suspected cases such an inspection should include a bacteriological investigation. For this purpose the National Public Health Institute distributes polyvalent agglutinating *Salmonella O-serum*.

In order to minimize the risk of infection due to apparently healthy germs-carriers, it would be most desirable to institute compulsory notification of Salmonellosis in cattle.

The consequences of an *intravital* infection in ducks' eggs can only be prevented by 1. stamping all ducks' eggs with the indication: 'Ducks' egg; boil for 10 minutes', or by pasteurizing them; 2. by forbidding the use of raw (i.e. non-pasteurized) ducks' eggs in food industries, with the exception of rusk bakeries.

As far as infections due to *handling* are concerned, a high standard of hygiene is imperative. Hygiene involves: 1. personal hygiene of the staff (especially training in the habit of washing hands with soap and hot water after using toilet facilities and before starting work); 2. prevention of contamination of foodstuffs by rodents or insects; 3. prevention of contamination of foodstuffs by animal or human faecal matter or polluted water.

In this respect it must be emphasized that Salmonella-preparations used for eradication of rats and mice cause a great risk and should therefore be forbidden.

Since it has been demonstrated that enterotoxic staphylococci may be spread by skin infections and heavy colds, staff members suffering from such diseases should not be allowed to handle foodstuffs.

It is the authors' experience that dehydrated egg products often contain Salmonella organisms; it is therefore essential that such commodities should be checked bacteriologically before importing them.

#### b. *Prevention of multiplication*

The pre-eminent means of preventing the multiplication of bacteria in foodstuffs is their storage at sub-optimal temperatures. For this purpose temperatures below 10 °C generally give sufficient protection.

It is very important that foodstuffs that are heated during their preparation should be cooled as quickly as possible.

#### c. *Special measures*

For the prevention of botulism, hygiene in the preparation of non-acid foodstuffs may not be sufficient. If such foodstuffs must be stored before consumption, it is imperative either 1. to subject them to a time/temperature-combination which is lethal to spores of *Cl. botulinum*; or 2. to store them at temperatures below 10 °C; or 3. to boil them prior to consumption.

As appears from the foregoing our knowledge of the source of contamination, especially with meat-poisonings caused by Salmonella, is still far from complete. It stands to reason that in consequence of this an efficient control is hampered. An exhaustive investigation into the occurrence of Salmonella in man and in animal may give the necessary enlightenment. Of special importance in this respect is a close cooperation between the laboratory and the various Services of Public Health that are entrusted with the detection of the cause, and the taking of measures for the prevention and control of food-poisonings.

Finally I would draw the attention to the necessity of *international cooperation*, in such a way that the various countries exchange information on the occurrence and the causes of food-poisonings.

## ON COWPOX AND VACCINIA

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The cowpox and vaccinia problem still draws our attention, because of its great importance for humans and cattle and because of its economic interest.

Already in the time of JENNER and doubtless earlier, cowpox existed on the teats and udder of cows. Consequently in that period in Western Europe vaccination against smallpox did not yet exist. This is evident from the fact, that JENNER could not have made his fundamental discovery, of which we nowadays still have great benefit, without this knowledge.

Vaccinia has generally been considered to be variola virus that adapted itself to the bovine species. One can ask the question if cowpox is a bovine disease *sui generis* or a small pox virus unintentionally adapted to cattle. The answer on this question is very difficult to give because history gives us only insufficient information or none at all.

In connection with the great number of cases of encephalitis after vaccination, FRENKEL (1928-1929) investigated the occurrence of cowpox in the Netherlands. The aim of this was to obtain usable material for vaccination that, in opposition to vaccines in use in different countries, would not provoke encephalitis. For this purpose it was necessary to see if recent vaccinations of humans could be excluded in cases of so called natural cowpox. As far as could be stated - epidemiological investigations of this type very often are difficult and meet with resistance rather than with cooperation - there was no connection.

Though the relationship between these cow-infections and vaccinations of men against small-pox could not be demonstrated in our cases, it is not permissible to conclude that it would not exist. Such an infection can happen in many ways so that the persons who transmit the infection stay totally unaware of the role they have played.

Recent investigations have shown that there exist differences between cowpox and vaccinia. Mainly, cultivation experiments of the virus from both these diseases seem to have demonstrated differences between cowpox and vaccinia-virus. Especially these differences are displayed when these viruses are grown in the chorio-allantois of the 10-12 days hatched hen's eggs.

For more information we indicate the publication of DEKING<sup>1</sup> in which the main

<sup>1</sup> F. DEKING, Koepokken en vaccinia, *Tijdschr. v. Diergeneesk.* 75, 6 (1950).

differences are described. Vaccinia-virus gives proliferative necrotic lesions predominantly of the epithelium, whereas cowpox virus also multiplies in mesenchymal cells, especially in vascular endothelium cells. These differences show themselves distinctly in the pox developing on the membranes of the chick embryo. On these membranes, vaccinia causes 3 to 4 mm sized necrotic lesions around which, after 3 to 4 days, secondary pox are formed by dissemination; cowpox virus on the other hand, provokes small vivid pox (1 to 2 mm in size) which are usually very hemorrhagic and are rarely accompanied by secondary lesions.

How constant these differences are must be investigated in a large number of cases, though it seems that the experience already collected in England, as well as in our country, has a tendency to make this constancy very probable.

Histological examination of pox lesions caused by cowpox virus on the chorio-allantois of a chick embryo demonstrated a great number of large Guarnieri bodies (stained with phloxine-tartrazine). On the contrary, the pox caused by vaccinia virus contain only a small number of these inclusion bodies.

Again, if these differences would prove to be constant, there would be strong evidence to consider the cowpox and the vaccinia virus as two different viruses, though closely related.

VERLINDE recently noted that nearly always the cowpox lesions on the chorio-allantois show some non-haemorrhagic 'white' pox. These have some resemblance to vaccinia lesions though they are usually smaller in size.

DIJKSTRA<sup>1</sup> has found in his careful epidemiological study of cowpox infections on teats in Friesland, that mostly a relation existed between these infections and recent vaccination of humans. He observed that farmers who were going to emigrate and who had been vaccinated against small-pox, in the period of the development of the vaccination-pox, transmitted the infection to their milk cows.

In such cases, the infection spread quickly through the sheds where emigrants had worked. The clinical picture could not be distinguished from the so called spontaneous cowpox. Anyhow it can be accepted that many cases of 'cowpox' on teats and udders of cows are caused by retrovaccine. Also in many cases where the relationship between pox in cattle and vaccination of humans cannot easily or at all be demonstrated this may be true.

Clinically the diseases caused by these viruses are almost identical in humans as well as in cattle.

In connection with this the question arises whether the immunity developed after vaccination with cowpox virus also protects against infection with vaccinia-virus.

This question caused us to carry out experiments on 20 bovines, to which 5 control cows were added.

*First problem:* Are bovines, which have reacted positively to vaccination with vaccinia virus, immune against cowpox-virus?

<sup>1</sup> Oral communication

The animals used in this experiment were imported from Ireland; the vaccinia virus was obtained from the Vaccination Institute of Amsterdam<sup>2</sup> the cowpox-virus was a strain grown on the chorio-allantois of chick embryos. This cowpox strain was passed twice on the bovine skin before being used in the experiments.

Practically all the animals showed maximal pox lesions after cutaneous vaccination with vaccinia-virus on the perineum.

Of these 20 bovines, 16 have not reacted with pox-formation after intracutaneous infection with cowpox virus, which was applied next to the area where the vaccinia virus was administered. From the remaining 4 animals, 3 reacted feebly and 1 moderately.

Of these 20 bovines, 10 were cutaneously infected with cowpox virus. All of them were totally immune.

Five control animals reacted fully to cutaneous infection with cowpox virus on the perineal skin, whereas 2 controls, which were infected cutaneously on the skin of the teats, reacted fully with pox formation.

*Conclusion.* Dermal inoculation with vaccinia virus gives practically full protection against infection by cowpox virus, not only on the perineal, but also on the teat skin.

*Second problem:* Are cows that are purposely infected with cowpox virus immune against vaccinia virus?

For the answer to this question we used the five control animals that were infected with cowpox virus.

The result was that these animals had developed a maximal immunity against dermal infection with vaccinia virus.

*Third problem:* Are cows that reacted with pox lesions after infection with vaccinia-virus on the skin of the teats, immune to infection with cowpox virus on the perineal skin?

For this experiment we used the two control cows with an additional, fully susceptible, control-cow, to prove the viability of the cowpox virus used.

The result of this experiment was that cows having positively reacted to infection with vaccinia virus on the skin of the teats, were immune against infection with cowpox virus on the perineal skin.

*Fourth problem:* Are cows that have been dermally infected with vaccinia virus on the skin of the back, immune against dermal infection on the teats?

To answer this question, two Irish cows and one control cow were used.

From this experiment, it was shown that inoculation on a small surface of the dorsal skin gave such an immunity that a dermal infection with cowpox virus provoked no reaction whatsoever.

From all this we can see that, though differences exist between vaccinia- and cowpox-virus, they can hardly be observed in cows, especially not in the immune response.

According to the above mentioned experiments we can clearly see that by means

<sup>2</sup> We are greatly indebted to Dr A. VITRINGA (Director of the Vaccination Institute, Amsterdam) and Prof. Dr J. D. VERLINDE (Chief of the Bacteriological and Virological Department of the Netherlands Institute for Preventive Medicine, Leiden) for having given us the strains used in these experiments.

of the dermal vaccination with vaccinia virus of bovines a complete immunity against contamination with cowpox virus results and the reverse. However thusfar nothing can be said from these experiments about the duration of this mutual immunity (Lack of housing facilities was the reason the longevity of this mutual immunity could not be determined).

If we would like to vaccinate our livestock in order to protect men and bovines against pox, it is necessary to know more about the longevity of this immunity. However we can admit that with vaccination of the cattle population the main part of cowpox (the word 'cowpox' this time used for vaccinia- as well as for cowpox lesions) in men and cattle can be prevented; infections that can be of a serious character for both these species.

By regular vaccination of people who milk cows it is possible to restrict infection of cows on condition that, during the immunization period, contact with the milk cows is avoided. If it would be possible to milk all cows by machine it is likely that this source of infection could be excluded.

We believe that both vaccinia and cowpox virus infection are mainly occupational diseases in men and cows. These pox-forms are rarely seen in young bovines, in cows that are not milked, and in bulls.

Closely related to this problem is that of the vaccinia preparation for vaccination of men against small-pox.

We all know that JENNER, at the end of the 18th century, was among the first to use pox lesions of the teats as material for vaccination and that we still nowadays observe the same relationship between the lesions and accidental infections of men. In most countries we still make use of JENNER's fundamental discovery. But, though mankind has the great benefit of the vaccination against small-pox, some rare complications of this immunization tend to throw a shadow on this operation. Especially the encephalitis that, in rare cases, follows vaccination against small-pox is a very much feared complication. Unfortunately, we thusfar do not know what this connection between vaccination and encephalitis is. Some people believe that the virus is directly responsible for this condition, others are of the opinion that another virus is activated, again others think that it is an anaphylactic cerebral reaction; finally there are scientists who believe that the vaccinia virus, mostly collected on the skin of living animals, might contain substances which could be responsible. One could imagine that the coincidence of two or more factors in the vaccine and in the vaccinated person might give rise to cerebral reaction.

Besides the fact that a totally bacteriological sterile vaccine might offer some advantages over a vaccine which contains either dead or living microorganisms and also cell detritus, leucocytes, etc., the absence of these concomitant factors could be of importance regarding post vaccinal encephalitis.

All these considerations stimulated the research on the artificial cultivation of vaccinia virus.

In 1937, FRENKEL and VAN WAVEREN started their research on this cultivation using the embryonic skin of bovines.

They succeeded in cultivating, *in vitro* on surviving skin fragments, the vaccinia virus in 9 successive passages. The optimal time for cultivation seemed to be 6 to 7 days at 37° C. This virus was cultivated in Carrel and Kolle flasks in a medium consisting of minced fetal bovine skin, vaccinia virus, Tyrode solution, sodium phosphate buffer solution and heparinized calf plasma. The highest titre obtained in the 9th passage was 10<sup>-4</sup>. Thus it was proved that vaccinia virus could be cultivated *in vitro* in surviving tissue of the embryonic bovine skin.

These facts did not draw attention from other workers and further research had to wait until after the war, when FRENKEL started anew. This time we had the great benefit of the antibiotics, as these made filtration almost superfluous.

Some culture passages could be obtained, first on epithelial tissue of the adult bovine tongue in a liquid medium with a technique almost identical to that followed for the cultivation of foot- and mouth disease virus. Later the cultivation experiments were continued with the embryonic bovine and ovine skin, in collaboration with J. KAPSENBERG, M. D.

Before it can be known whether a vaccine cultivated in the above mentioned way has any advantages over the classic vaccine against small-pox, thousands of vaccinations must be done, especially on persons who have never been vaccinated.



## MILK

DR Y. M. KRAMER

*Director Milk Control Station, The Hague*

In order to get a good impression about dairying in this country it is necessary to go back into history. The soil that was reclaimed in the lower part of the Netherlands was especially fit for permanent pasture. So already in early times cows were raised and butter and cheese were made on the farms.

In the South of this lower part Gouda cheese was made, more Northward Edam cheese and in Friesland a special Friesian cheese with spices. Besides this production on the farms, of course, fluid milk was sold in towns and villages which were in that time still small. In the higher parts of the country in the East and South, the keeping of milk cows came a long time later. The use of fertilizers contributed very much thereto.

Around 1900 came the industrial manufacturing of dairy products. First small plants, later bigger ones. First only butter and cheese-factories and fluid milk plants, later bigger plants for the production of condensed milk, evaporated milk, milk powder and other milk products.

The results of this trend towards dairying have been that in the year 1952 there were 2,858,000 dairy cows in the Netherlands, of which 1,483,000 were in milk.

The average yield of these dairy cows in milk was 3750 litres, containing about 3.65% fat. These cows were held on 210,000 dairy farms, which means that the average Dutch farm has around 12 animals. In the lower Western and Northern part this average is higher; there many farmers have 30 to 40 dairy cows in milk, besides the young cattle. In the East and South the farms are, in general, smaller.

The total quantity of milk, produced by the farmers in 1952 amounted to 5,562,183,000 kg.

From this milk is used:

for direct consumption as fluid milk . . . . .	1,792,820,000 kg	32%
for cheesemaking . . . . .	1,418,821,000 kg	26%
for milk powder . . . . .	460,373,000 kg	8%
for condensed milk . . . . .	559,235,000 kg	10%
for butter and special products . . . . .	580,934,000 kg	10%
for use on farm and animal feeding . . . . .	750,000,000 kg	14%
	<hr/>	
	5,562,183,000 kg	

The dairy industry is the biggest industry of our country.

Some details about the quantity of dairy products which was produced during the past year are mentioned hereafter; at the same time, the quantities and percentages which went to outside markets are added.

	Production in kg	Exported in kg	Exported in %
Cheese . . . . .	145,089,000	78,071,000	53,8
Milk powder . . . . .	49,226,000	36,344,000	74,2
Condensed . . . . .	226,393,000	199,309,000	88,2
Butter . . . . .	73,240,000	49,921,000	68,4

The total value of these exportations amounted f 831,244,000,00 being 10.3 % of the total exportations of the Netherlands during that year.

The number of dairy plants, including fluid milk plants, was 568 in 1952. In these plants the above-mentioned quantity of milk was processed and the above-named quantity of products produced.

Of these plants 400 are co-operative and 168 are private industry.

After the dairy plants had been established many farmers quite soon stopped butter- and cheesemaking on their farms and passed on to deliver their milk to these plants. Nowadays there are still 5,000 farmers making cheese on their farms.

Some of them are doing this during the whole year, but most of them only from April to November when they have the disposal of a large quantity of milk. In the remaining months their herds either are producing only a small quantity of milk or are dry. At these farms nearly all the cows are calving in the months of February and March.

Till world war II many farmers were selling their milk directly to consumers and retail-dealers. These retailers in turn sold this milk raw for direct human consumption. Neither the farmer nor the retail-dealer took into account the fat content of the milk, in consequence whereof the same price was paid for milk with a low or a high fat content.

Owing to the differences between the fat content of the milk which the larger fluid milk plants also received, dishonest competition and other difficulties arose.

So, shortly before world war II, the government planned standardization in order to bring all fluid milk to the same fat content, either by withdrawing fat or by adding skimmed milk.

German war-economy accelerated these plans and fixed the fat content of fluid milk at 2.50 %. Therefore, it was necessary that all milk for direct human consumption was delivered to dairy-plants being able to standardize.

The consequence of this has been that since 1940 all the milk produced is delivered to the dairy-plants, excepting milk for cheesemaking on about 5,000 farms.

In our country the farmers deliver the milk daily; in the lower Western and Northern parts, from April to November, twice a day. You will readily understand that

this way of collecting milk greatly influences the problem of cooling the milk at the farms.

Since 1940 also, all milk is paid for on a basis of fat content. For this purpose an equal quantity of milk is collected daily in a sample-bottle with a preservative. Fortnightly the fat content of these samples is fixed and the milk price is wholly or partly based on that fat content.

It will be clear that this way of payment based on fat content has importantly influenced and is still influencing the breeding of cows with a high yield of fat.

It was pointed out already that the Netherlands are strongly dependent on foreign markets. This led to the fact that the government and dairy plants pay much attention to the products which are exported. In each province a dairy-adviser is charged with providing information to farmers and plants. He gives courses to farmers in milking and milk-hygiene, courses to butter- and cheesemakers, and advice to farmers and plants which have troubles. Moreover many plants have their own laboratories or connections with laboratories of their associations.

For research, the Netherlands have their college at Wageningen and laboratories at Hoorn and Leiden. Besides, an important research-center with experimental plant is now in construction in Bennekom.

To guarantee that exported butter is not falsified, there is a special butter law.

In addition the dairy manufacturers, being anxious to guarantee the genuineness of their products, constituted special associations such as butter control station, cheese control station and control institute for milk products.

Furthermore a special Dairy Quality Bureau was organized to guarantee the quality of the products exported.

Only some general information will be given about the manufacturing-methods of the dairy products.

In almost all dairy plants, small or large quantities of butter are made; in the fluid milk plants from the surplus milk, in the cheese factories from the fat that is not used in cheese with a lower fat content. The real buttermaking industry is in general situated in the East and South of the country.

Milk or cream for buttermaking has to be pasteurized. For ripening a starter is used. Only a few plants are making butter from sweet cream.

The butter law forbids addition of foreign fats into butter. The butter control stations, under governmental supervision, take a great number of samples of the produced butter to test fat content, moisture and absence of strange fats.

The Dairy Quality Bureau regularly grades the butter which is produced. The samples before grading are kept 10 to 12 days at 14° C.

So we try to guarantee real butter of high quality for our export.

Based on history, the industrial manufacturing of cheese is in general still located in the West and North, that is the lower part of the country. There live also the 5,000 cheesemaking farmers.

Originally only raw milk was used for cheesemaking. Later came the pasteurization of the cheese-milk in order to increase the production of cheese and to eliminate the

danger of one can of bad milk ruining all cheese from a whole cheese-vat. Therefore the destroyed bacteria are replaced by a starter, a culture of good bacteria.

This pasteurization of cheese-milk has made it possible to make cheese in the East and South where the milk is not so fit for cheesemaking from raw milk.

Of course the farm-made cheese is from raw milk and some cheese-factories are also trying to improve the quality by using raw milk.

The cheese control stations under governmental supervision very often sample the cheese to test fat content, moisture content and temperature of pasteurization.

The Dairy Quality Bureau examines all export-cheese in order to prevent cheese with defects from being sent abroad.

The Netherlands are manufacturing four varieties of cheese; *Gouda*, *Edam*, *Leiden* and *Frisian*.

Well-known markets are: Gouda, Alkmaar and Leeuwarden.

The different varieties are consumed rather soon after their production but they can be eaten too after a year's storage or even more. In consequence hereof it is possible to vary many times these different types.

For the production of *dried milk* we use the spray system and the roller system. In a spray powder plant the milk is precondensed to eliminate a part of the water and afterwards atomized and sprayed into a stream of heated air, which carries off the moisture. The milksolids fall down and are collected as a fine powder.

In a roller system milk is spread between two steam-heated rollers. Moisture passes into the air, the milk solids staying as a thin film on the rollers and are scraped off.

Good manufactured spray powder is totally soluble and mostly made of whole milk. Part of it is packed in small tins wherein the air is replaced by nitrogen gas to avoid oxidation of the milk fat.

Roller powder is not totally soluble and is more used for animal feeding. The powder plants are spread throughout the whole country.

The control station for milk products under governmental supervision is steadily collecting samples of the powder to test fat content, moisture content, total solids, and absence of foreign fat.

The Dairy Quality Bureau examines the powder before it is exported.

*Condensed milk* has always been an important product for export. The condensing factories are in general large and strongly mechanized. Many of them are favourably situated near shipping lines because they not only want milk but also tins, sugar and packing material and nearly the whole production is exported.

Evaporated milk is condensed milk sterilized at high temperature in tins to obtain a good keeping quality.

Sweetened condensed milk is condensed milk with nearly 40 % sugar (sucrose) added to prevent growth of bacteria. The greater part of this product is also exported in small tins.

The control station for milk products also regularly takes samples to test fat content and absence of foreign fat.

Since 1940 all *fluid milk* for human consumption is standardized at a milk-fat

content of 2.50 % and pasteurized. So it became necessary that all milk, formerly going directly and raw to the consumer, had now to pass a fluid milk plant.

In the Netherlands 38 % of the fluid milk is sold in bottles; of course the large cities are participating for the greater part herein. Paper containers are not used, these being too expensive.

Pasteurization is mostly carried on by means of plate heat exchangers. The phosphatase-test must be negative.

Many plants are pasteurizing at a temperature of 72 °C for 15 seconds; this system is known in the U.S.A. and Great Britain as H.T.S.T. = High Temperature Short Time Pasteurization.

There is a bacteriological standard for bottled milk. As to the bacterial count, 25,000 germs per ml. is the maximum, whereas bacillus coli may not be found in 1 ml.

The greater part of the fluid milk is delivered to the homes and only a small quantity is sold in the stores. Some milk plants have their own delivery men but by far the greater part of the fluid milk is sold by independent milk dealers. In the night-time and early in the morning the milk plants deliver the milk to these retail-dealers.

As a rule the fluid milk plants also manufacture pasteurized and sterilized cream, heavy and light cream, buttermilk, yoghurt, chocolate-milk and porridge. You will understand that our milk-plants have therefore a rather complicated character.

The War law or Food and Drug Act gives standards for fluid milk, bottled milk, and dairy products. This law also makes requirements as to personnel, localities, and stores.

For this purpose the Netherlands has been divided into districts, each with as centre a large city with a well equipped laboratory.

In the Food and Drug Act requirements are also made regarding the milk which the farmers deliver and the state of health of the herds.

To avoid difficulties with this governmental control, many plants passed on to the examination of the raw milk which they received from the farmers and of the milk and products which they delivered to delivery-men and retaildealers. In the Western part of the Netherlands, where the greater part of the fluid milk for human consumption is sold, many small fluid milk plants and many retail-dealers did not have at their disposal their own laboratory. Because of this fact they constituted associations, which established laboratories.

The present Milk control stations, are the result hereof; nowadays these Milk control stations control the milk of 18,000 farms, 80 fluid milk plants, and 4,600 retail-dealers.

The milk is sampled on arrival at the milk-plant, again sampled when delivered to the retail-dealer or delivery-man, and for the last time when sold to the consumer.

From the time it leaves the cow until it reaches the consumer the control is following the milk.

In the first years, the results of the examinations were sent to farmers, dairy plants, and retail-dealers, and at the same time the necessary advice was given in order to obtain better results. At first the results were satisfactory but later on improvement stopped!

Soon it was clear that only payment for quality could give better results.

As result of other measures in connection with the economic crisis of 1933, it was possible to introduce at that moment quality-payment for all the milk in the Western fluid milk area. All the plants and retail-dealers were obliged to join one of the 5 Milk control stations. From that time, these Milk control stations are sampling weekly the milk delivery by the farmers; morning and evening milk is examined in turn.

The following tests are carried out upon samples of all raw milk, received at the milk plants:

- the reductase test,
- the sediment test,
- the microscopic examination for mastitis streptococci,
- the smell test.

The standard of grading is as follows:

- Grade 1 good milk
- Grade 2 fair milk
- Grade 3 bad milk

At this moment the price-difference between grade 1 and grade 3 amounts to 1 cent per litre, that is 5 to 6 % of the milkprice.

Some time after this way of payment came into force it was clear that many farmers did not know how to obtain good milk. Therefore the milk control stations appointed fieldmen, who taught the farmers to produce grade 1 milk.

For this purpose a special information service or extension service was instituted in order to give help to the farmers.

Nowadays all the farms are inspected four times a year and during these visits the whole situation is noted on score-cards.

These score-cards are used as a basis for premiums varying from f 5,- to f 150,- each year.

Farms scoring below a certain point are barred from these premiums until the necessary improvements are made to bring their score up to standard.

It will be clear that these score-cards point out rather exactly the failings of the farmers. So the situation on the farm and the quality of the milk are readily brought together. This is a very important factor but in order to improve the milk supply, the general health of the cow must also make a contribution towards this aim.

In order to promote the eradication of cattle iseases and extra premium of  $\frac{1}{2}$  cent per litre is awarded to the farmer:

1. whose milk has many times grade 1.
2. whose farm-score is up to standard.
3. whose cows are free from tuberculosis.

From the beginning of the inspections of the dairy farms special attention was paid to:

- a. the care and cleanliness of cows.
- b. the cleanliness of teats and udders before milking.
- c. the rinsing and disinfection of milking tools.
- d. the cleanliness of sheds during the winter.

Moreover the Milk control stations give courses in good milking and machine-milking as well as providing help to farmers who have troubles.

The close relationship between this extension-service and the quality-payment with extra premiums has led within some years to big improvements.

Next to the above-mentioned activities the Milk control stations also give help and advice to the fluid milk plants and the retail-dealers. They continuously take samples of the fluid milk and dairy products, either for chemical analysis or for bacteriological examination or for both, and give help to these plants when the samples differ markedly from a good quality. They sample the fluid milk and dairy products in the retail stores and on delivery to the consumer.

Finally they give courses to the retail-dealers and inspect the premises of these milkmen.

All these activities are done under governmental supervision and have led, as already pointed out, to satisfactory improvements.

It is undoubtedly of great interest to know how far cattle diseases can be spread by the dairy industry.

In this respect only some dairy products such as buttermilk, skimmed milk, and whey, which the milk plants deliver back to the farmers for cattle-feed, play a role.

Buttermilk is safe because both the milk or cream which is used for buttermaking is first pasteurized.

As to skimmed milk, this product must have been pasteurized before delivery to the farmers. Many times employees of the Veterinary Services take samples of the delivered products on leaving the dairy plant.

In our opinion, cattle-diseases can only be spread by the dairy product whey when cheese-making is done by farmers on when farmers receive this whey from Cheese-plants where raw milk is used for cheese-making.

After this description of the dairy situation in the Netherlands still some factors which we consider very important for good milking and milk hygiene will be still mentioned. The first point is the *significance of good milking* for maintaining production of the milked cow and for avoiding mastitis.

It is known that a cow does not immediately give milk the moment the milking begins. She requires a short time to let her milk down. There is evidence which indicates that some nervous stimuli are responsible for the release of the milk from the udder. Some years ago Professor PETERSON at St. Paul, Minnesota, U.S.A. put forward a new theory regarding the mechanism of these stimuli and made it clear that they are inciting the hypophyse to administer oxytocin via the blood to the udder! As long as the administration of this hormone to the udder is maintained, the milk is forced out of the mammary glands.

In order to obtain sufficient secretion of the hormone the unusual must be avoided during milking and milking must be done rapidly and strongly without, however, treating the cow roughly.

If milking takes more than 10 minutes for our cows with high production the effectiveness of the hormone, that is essential for letting down milk, will be partly eliminated and incomplete milking will result.

Not only will all these points make it possible to increase both the milk yield and the milkfat, but they will also contribute toward prevention or eradication of streptococcic mastitis. I am impressed again and again by the fact that cows which are badly milked are more sensitive to infections than cows which are milked out as completely as possible. This fact is of great importance to hand milking and of still greater significance to machine milking.

There is also evidence which indicates that a close relationship exists between the application of tactual stimuli to the teats by the milker and the full yield of milk. By using milking-machines there is a very great chance that this relationship is lost, especially when the farmer aims too much at labour saving and too many cows are simultaneously milked.

In the Netherlands we are considering the milking-machine as an instrument which can do the heavy work of the milker, provided that the latter starts milking and milks the cow out after having removed the machine. This means in general that one milker ought to use only one milking-machine. Many farmers are sinning against this rule, in the Netherlands too. Nevertheless I am more and more convinced that this is the only way to maintain the high productive capacity of high-yielding cows and to prevent or to eradicate streptococcic mastitis.

Another problem which interests us considerably, is the *cleaning and disinfection of the utensils* on the farm.

Failure to clean and sterilize milk-tools is, in 80 to 90% of the cases, the most important factor responsible for a high bacterial count. The dairy plant can carry out the sterilizing of milk cans but the bacterial count of the milk depends furthermore on the proper cleaning and disinfection of pails and utensils on the farm. It is therefore of great importance that all milktools are vigorously rinsed immediately after milking is finished in order to remove loosely held milk-residues. If this rinsing is carried out at once and properly, much is won already.

As to the disinfection of milk-tools, the tendency is in progress to use more and more chlorine releasing chemicals which lead to a considerable decrease in bacterial counts. It cannot be emphasised too strongly that efficient cleaning or at least intensive rinsing immediately after milking must precede the use of these sterilizing agents as the proteins will bind the available chlorine and thus reduce the efficiency of this solution. Apart from that, we are still looking for a simple apparatus to sterilize the milk-tools at the farm.

What is said about the milk-tools in general is a great many times more important for the milking-machine with its complicated construction and rubber parts. For these machines it is still more necessary that immediately after milking enough clean water



is drawn through followed by disinfection or better sterilizing. That is just one of the problems with the milking-machine.

The following point is the *cooling of milk* on the farm.

The good keeping quality of the fluid milk in the U.S.A. is undoubtedly affected by the proper refrigeration on the farm and by the fact that raw milk delivered to the fluid milk plants at a temperature over 10° or 12°C is rejected by the buyer.

Farm refrigeration units are practically never used in this country. The initial cost and servicing required do not in practice make it possible for the small-scale producer to install a refrigerating plant.

It is generally recognized that active proliferation does not take place for a period of 4 hours after the milk leaves the udder, providing the initial number of bacteria is not too high. This is due to the bactericidal phase of the milk. Therefore we continuously aim at receiving milk at the dairy plants twice a day within these 4 hours. After arrival the milk then can be immediately cooled.

Finally brief mention may be made of a characteristic *flavour-fault* induced by direct sunlight or even normal daylight.

Oxidized flavour (oiliness, cardboard flavour) usually occurs in bottled milk which has been exposed a certain time to sunlight or daylight. We have experienced this characteristic flavour occurring in one bottle of milk which was exposed one hour only to daylight whereas this oxidized flavour did not appear in another bottle which was kept in a dark place.

We are therefore still studying ways in which this daylight problem may be solved and consequently the good keeping quality of our bottled milk may be maintained.

# ORGANIZATION OF MEAT INSPECTION IN THE NETHERLANDS

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## INTRODUCTION

When, in the early part of this century, the condemnation in England of meat exported from the Netherlands was discussed in our Parliament, the Minister concerned stated that 'the Government was considering the regulation of cattle and meat inspection as a whole.' It therefore seems to have been the original intention to regulate inspection at home and abroad in concert.

The outcome of the discussions was, however, that in 1907 statutory regulations were made only for the inspection of meat intended for export.

Inspection of meat for home consumption was only regulated by the so-called Meat Inspection Act of 25th July, 1919, which became effective on June 1st, 1922. I restrict myself essentially to this Act.

## ORGANIZATION SETTING UP INSPECTION SERVICES

Prior to 1922 several large municipalities had already organized a meat inspection service and the Netherlands had some 25 abattoirs, which were municipal property. It was obvious, therefore, that when in 1922 meat inspection became compulsory throughout the country, the existing state of affairs was taken as a starting point. The municipalities were entrusted with the implementation of the Meat Inspection Act, but in no respect was the autonomy of these authorities interfered with.

Considering that at present our country has 1013 municipalities – at the time the Meat Inspection Act became operative there were some 1100 – and that the area and density of population of these municipalities are widely divergent, there is no need to argue that it would be hardly practicable for each municipality to regulate meat inspection independently.

The Legislator had foreseen this difficulty, and to meet it had inserted in the Act a provision which opened the possibility of a common inspection service for various municipalities together.

If a number of municipalities cooperate for the implementation of the Meat Inspection Act, one of these municipalities will direct this action; it is then referred to as the 'central municipality', the others being the 'district municipalities'. The whole combination is known as the 'Inspection District'. The inspection officials who perform

meat inspection duties in a district are employed by the central municipality; they are authorized to carry out all activities in connection with the implementation of the Meat Inspection Act in the other district municipalities as well.

When the Act began to operate, municipalities had been grouped in 402 inspection districts. In many instances the formation of an inspection district did not entail any difficulties. In others, on the other hand, particularly in small up-country municipalities, autonomy was the first consideration. Such minor municipalities wanted to maintain their independence at all costs and were only willing to join a district if they were entrusted with the direction, i.e. if they could play the part of a central municipality.

In addition, when the Act became operative an urgent need for qualified inspection personnel chiefly of inspecting veterinary surgeons, was bound to make itself felt. The problem could only be solved by appointing the local veterinary practitioner in several municipalities as veterinary inspector and Head of the Service. This was also an obstacle to the formation of inspection districts.

Consequently, on the one hand the formation of inspection districts was unsatisfactory, on the other hand, in many meat inspection services inspection took place under the supervision of a veterinary practitioner.

Actually, if there are comparatively too many inspection districts, uniformity of inspection and, therefore, economy will often suffer.

It is not advisable that one and the same man should act as veterinary practitioner and Head of the Meat Inspection Service, a combination brought about under the stress of circumstances. The inspecting veterinary surgeon, Head of the Service, should be able to devote himself completely to his task as an inspector and not have his time taken up by his duties as a practitioner.

Both problems demanded a good solution to ensure the satisfactory implementation of the Meat Inspection Act.

Besides providing for voluntary cooperation between municipalities, the Act also provides for compulsory cooperation.

If consultation between the municipalities concerned has not led to any result, the Crown may designate municipalities which are to run the inspection service together. In that case the Crown also decides which of them shall act as the central municipality.

By mutual arrangement, or on high authority, the rights and obligations of the central and district municipalities are laid down in common regulations.

The Government will not remain passive in this procedure; on the contrary! On the one hand it tries to persuade the municipalities to be considered, to form districts of their own free will. On the other hand, if this does not produce the desired effect, the Government promotes compulsory cooperation to be ordered by the Crown.

It is logical that the most opportune moment should often be either for one action or the other, so that ideal conditions may sometimes be realized only after a great many years.

	Number of official Inspec- tion Services	Number of semi-official Inspection Services	Total
In 1922 . . . . .	55	347	402
June 1947, after 25 years of Meat Inspection Act	105	216	321
September 1952 . . . . .	127	144	271
May 1953 . . . . .	130	134	264
Target . . . . .	140-150	-	140-150

Above mentioned data will show how the pace was quickened in this respect, in particular after World War II.

Not only is the decrease in the number of inspection services (which means that more districts are formed) a conspicuous feature, but attention should also be called to the smaller number of semi-official inspection services (i.e. the combination of veterinary practitioner and veterinary inspector).

In my opinion, a satisfactory implementation of the Meat Inspection Act in this country will be best ensured by some 140-150 fully official inspection services.

#### ABATTOIRS, THE ORGANIZATION'S KEY-POINTS

Another, very important factor for the implementation of the Meat Inspection Act is the existence of abattoirs. In this respect, too, as far back as 1919 the Legislator proved to be a realist in the true sense of the word.

Although the implementation of the Act has been placed entirely in the hands of municipalities, the Government is willing, on conditions to be specified, to supply interest bearing cash advances to individual municipalities or to a combination of them, to enable them to establish abattoirs.

In the Explanatory Memorandum to the Bill in question it is rightly observed that for a satisfactory operation of meat inspection, abattoirs are of inestimable value.

The abattoir is the inspection service's domicile. It is the centre of the inspection district and it is advisable for all slaughtering in the district to be centralized there. Unfortunately such centralization has not yet materialized in all cases.

When leaving out of account the old slaughter-houses that were found in our country, the first abattoir may be said to date from the eighties (Rotterdam 1882, Amsterdam 1887). In 1924 the number of these establishments was 30; at present there are 83 public and 4 cooperative slaughter-houses.

It follows from the above that not every inspection service possesses a public or a cooperative slaughter-house. In inspection services where such an establishment is lacking, slaughtering is effected either at the butcher's or in larger private slaughter-houses, whether or not belonging to meat processing works.

If no public or cooperative slaughter-house exists in an inspection service it is important that there should be one or more emergency slaughter-houses within the district concerned. Their number should depend on the area of the district, but should be limited with a view to ensuring satisfactory supervision.

Emergency slaughter-houses should be establishments to be used only for finishing

off animals that died or were killed in cases of emergency. Such slaughter-houses should be municipal property.

The use of a private slaughter-house for finishing off animals that died or were killed in cases of emergency must be considered undesirable. The occurrence of anthrax, though this is fortunately sporadic in our country, fully justifies this conception. Spreading of anthrax bacilli must be prevented by all means wherever possible.

Moreover, from a general hygiene point of view it is not justifiable that animals that died or were killed in cases of emergency should be finished off on the same premises as animals brought in for inspection in the normal way.

#### INSPECTION OFFICIALS

The Inspection and re-inspection of meat animals and meat may only be entrusted in this country to persons who hold a veterinary surgeon's diploma. Inspection of meat animals and meat may also be entrusted, under the supervision and responsibility of an inspecting veterinary surgeon, to so-called assistant inspectors for cattle and meat inspection, in cases to be specified.

The competence of these 'laymen inspectors' is strictly limited and regulated by law.

In the event of inspection before slaughter, such an assistant inspector must not issue a slaughter permit if he observes anomalies on the beast that may affect its health.

After slaughter, he may perform inspections only if the organs, with the exception of stomachs and intestines, are still attached to the carcass by their natural tissues, if serious membranes have not been removed either partly or completely and if uterus or heart have not been opened, no incisions have been made into the beast and the organs have not been reduced or entirely or partly removed. Nor may he inspect an animal that has been entirely or partly inflated – except the lungs in case of slaughter according to Israelitish rites.

Inspection of animals that died and were killed in cases of emergency does not come within the assistant inspector's competence.

He is only permitted to perform the inspection if all organs and parts are sound, or if the following anomalies or changes are found:

- a. animal parasites which are not directly injurious to human health;
- b. anomalies of organs and parts which have not adversely affected the general condition of health nor the quality of the meat;
- c. local tumours;
- d. tuberculosis of one organ, or of one organ with corresponding lymph gland or of more than one organ, provided that the area is very limited and no miliary foci, or swelling or inflammation of lymph glands are found, or any emaciation is observed.

The training of assistant inspectors as defined by the Act has been regulated in detail by a Ministerial Order and comprises a three months' course, to be given at an abattoir by veterinary surgeons, designated for that purpose.

The age required for admission to such a course varies between 23 and 35 years.

Candidates must hold a butcher's certificate of proficiency and meet reasonable standards of general education. Courses are held whenever the need for assistant inspectors is felt. At the end of the course pupils are examined by a Board of Examiners whose members are veterinary surgeons and which is set up by the Minister of Social Affairs and Public Health; if examinees meet the requirements, the certificate of assistant inspector is issued to them.

#### FINANCING

After this brief outline of the organization of meat inspection in our country, the question may arise as to how these Government activities are financed.

It is obvious that municipalities entrusted with the implementation of the Meat Inspection Act must be able to obtain revenues to defray the expenses of implementation.

The Act provides in this connection that the cost of inspection, as far as it is not covered by the imposition of an inspection fee, shall be borne by the municipality. This not only means that municipalities are free to levy or not such an inspection fee, but that to a certain extent they are given a free hand to fix the amount of the inspection fee.

Inspection fees must not be so high that more than a reasonable profit is made by the meat inspection service.

As the organization of the various meat inspection services differs substantially, inspection fees are not the same everywhere. This is particularly striking if a reduction of the normal inspection fee is granted when a great number of meat animals are brought in for inspection by the same owner within a specified length of time.

If the Act had not provided that meat transported from one inspection service to the other should be subjected to another inspection to ascertain whether any changes had taken place – for which inspection the receiving municipality is allowed to levy an inspection fee – the disparity of inspection fees might have led to unrestricted competition among the various inspection services.

This so-called *receiving inspection fee* must not exceed the local inspection fee for slaughtering and inspecting.

Another cause for the disparity of inspection fees is the fairly different interpretation of the notion 'inspection fee' by the various inspection services.

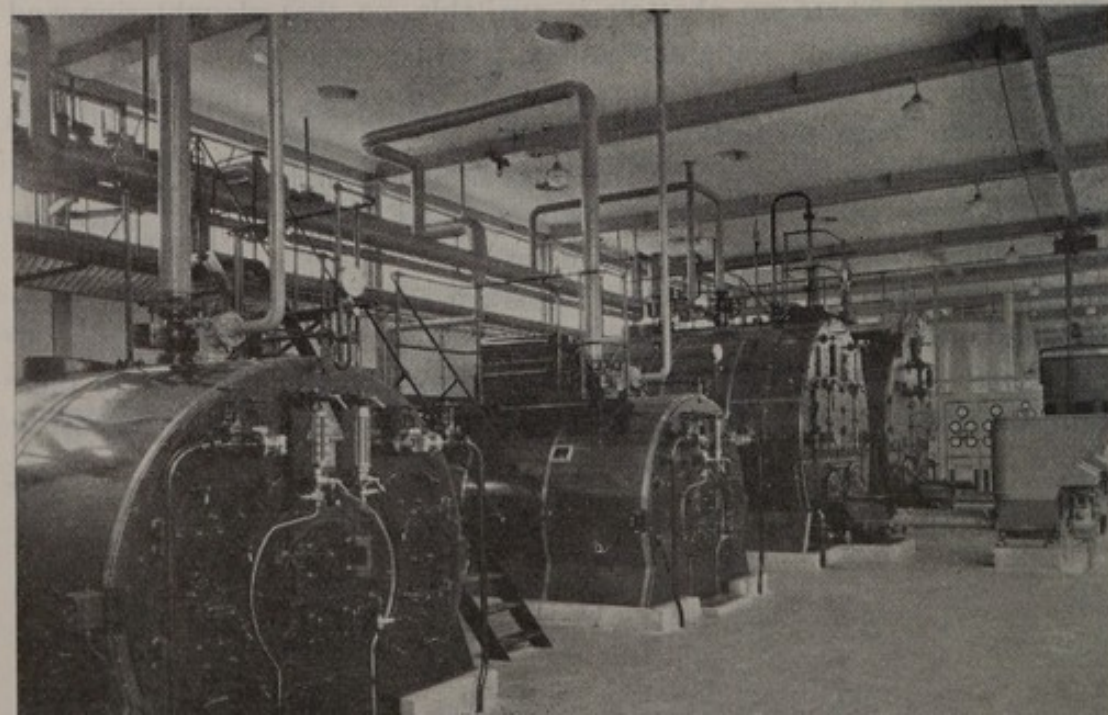
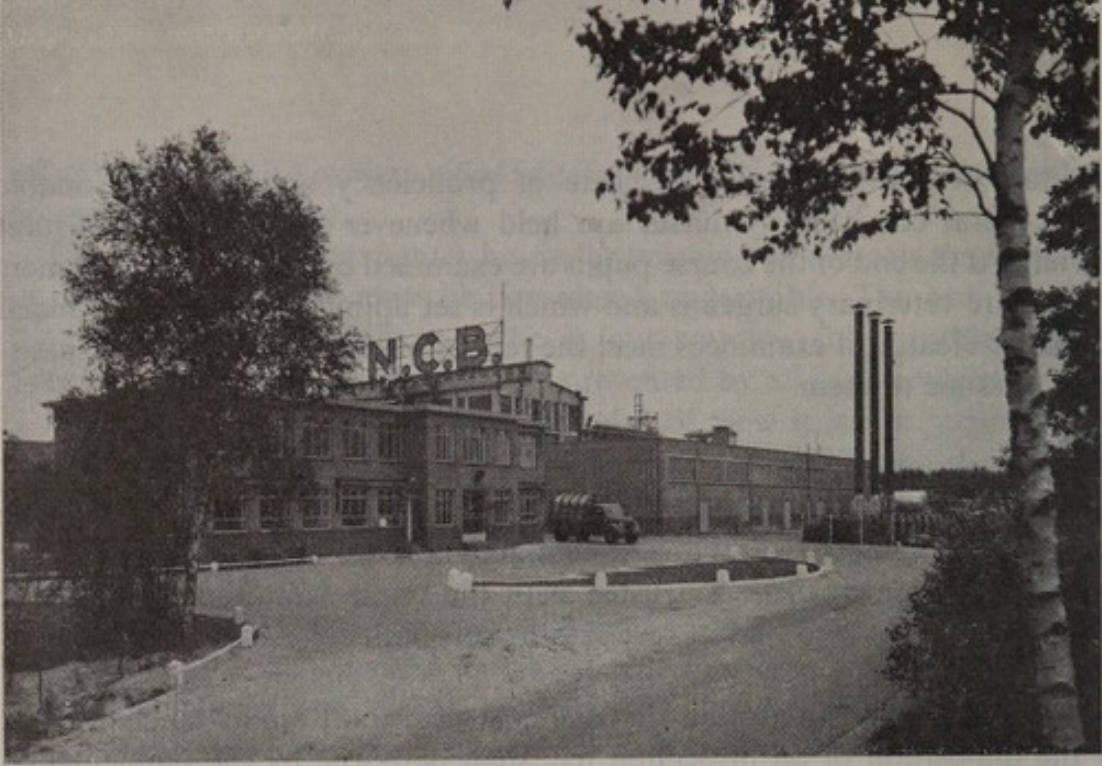
In inspection services where no abattoir is available, so that slaughtering and inspection take place in private slaughter-houses, the inspection fee is found in its simplest form, viz. the duty to be paid for inspection by the owner of the meat animal. This may be called the 'bare' inspection fee.

As a rule, in inspection services where slaughter and inspection take place in an abattoir, this 'bare' inspection fee is combined with the slaughter duty into one amount, which is then referred to as 'inspection fee'.

*Slaughter duty* is understood to be the sum paid for the provision of a slaughter house by the municipality.

In addition, some inspection services possess abattoirs where, to simplify their administration, the inspection fee also includes the remuneration for other accessory

*Carcass reduction  
plant at Son*



operations or uses. Thus, in some inspection services payment of the inspection fee also gives those interested the right to accommodate the meat animals for at most 24 hours before slaughter, to weigh the meat, to make use of the room where meat is hung for a certain length of time and to subject the meat to chilling.

It need hardly be said that all kinds of modifications varying between 'bare' inspection fee and inspection fee in a very ample sense, not only are imaginable, but do occur in actual practice.

Each municipality has its own inspection fee regulations. Usually one inspection service comprising several municipalities charges the same inspection fee. Therefore, also from a uniformity point of view, it is desirable to restrict the number of inspection services as much as possible.

#### INSPECTION

The question may now be raised as to what animals fall under the Meat Inspection Act, in what way the animals may be killed and how inspection of such animals takes place.

These subjects will be dealt with later on. Let it therefore suffice to say that the meat of the animals may be passed – whether or not on particular conditions – or condemned.

#### DESTRUCTION

The last phase of a well-organized inspection service consists in making unfit for human and animal consumption that which has been condemned. Except in a few areas in our country which are difficult to reach – seven islands, as well as a marshy district – this takes place exclusively by destruction, i.e. by passing the condemned substance through a destructor.

In the above areas, where exemption from the destruction regulations applies, the condemned substance is made unfit for consumption by mixing the cut-up meat with animal oil, lime, carbolic acid, creolin, saprol or similar products. Only after that treatment the condemned substance can be buried.

In our country there are 11 destructors. To each of them a clearly demarcated area of operation has been assigned, where the condemned substance must be collected for destruction on behalf of this establishment.

Destruction of condemned substance is considered to be of such primary importance that this subject has been regulated in a separate Order which has force of law.

The draft of the Destruction Act to supersede the said Order is in an advanced stage of preparation.

In Regulations, provisions have been made with regard to the arrangement and the method of operation of destructors, and the transport of the condemned substance to the destructor, particularly for meat from animals suffering from infectious cattle diseases such as anthrax, foot-and-mouth disease and the like.

Moreover, statutory provisions exist in regard to the temperature, pressure and time of the destruction process. In order to ensure a proper check on the temperature it has been provided that destruction vessels shall be fitted with a self-recording thermometer.



Supervision of the destructors has been entrusted to the Veterinary Inspection of Public Health. Samples are taken at regular intervals of the animal meal obtained and these samples are passed for examination to the State Institute of Public Health, in order to ascertain whether the treatment of the condemned substance has been effective and the animal meal can be released without any risk of infection.

A specially appointed Committee has the task of advising the Minister on all matters relating to destruction.

#### EXEMPTION FROM THE DESTRUCTION OBLIGATION IN SPECIAL CASES

Not all the condemned substance in municipalities which are under an obligation to join the destruction scheme is treated in a destructor. The Minister of Social Affairs and Public Health is authorized to designate municipalities from which, subject to particular conditions, condemned meat may be delivered to establishments to be specially indicated by him for feeding the animals kept there. It stands to reason that the condemned meat which zoological gardens, circuses, fox farms and the like may obtain must be harmless to the health of the animals fed with it. To prevent fraud, in addition to other measures taken, deep incisions are made into the meat concerned, on which an aqueous solution of methylene blue is poured.

Moreover, the possibility has been opened for working up certain condemned organs into pharmaceutical preparations in factories specially indicated for the purpose, and sometimes it is also possible to utilize the skin of condemned pigs for industrial purposes.

#### IMPORTANCE OF THE MEAT INSPECTION ACT FOR COMBATING ANIMAL DISEASES

If the question is asked whether the Meat Inspection Act is of any importance from the point of view of combating animal diseases, that question must be answered in the affirmative.

Generally speaking it may be stated that as a consequence of systematic inspection both infectious and parasitic animal diseases will be diagnosed, so that precautions can be taken on the one hand to prevent a virus from spreading, and on the other hand to interrupt a parasitic cycle.

With regard to meat animals that died and were killed in cases of emergency, which may be condemned without any further examination, the inspecting veterinary surgeon is obliged to satisfy himself that there is no question of any infectious cattle disease mentioned in the Cattle Act. In the event of sudden death, in particular with horned cattle, sheep and goats, to be able to preclude anthrax he will have to use a microscopic blood test.

Within the scope of our five-year plan to wipe out tuberculosis among horned cattle, the support of well-organized meat inspection could hardly be dispensed with.

Only by rigorous inspection of all meat animals can echinococcosis in our animals be converted into an historic memory. This point will be explained in greater detail.

When in 1922 the Meat Inspection Act came into force in this country, in the province of Friesland this parasitic disease had spread to such an extent among both men

and animals that for this province no exemption from inspection for home slaughter was granted.

In those times as much as 24 % of all sheep killed in Friesland were found to suffer from echinococcosis, but this figure has now been reduced to a fraction of one per cent.

During the war years, in part of the province of Gelderland, where inspection was not yet compulsory for home slaughter, echinococcosis had developed to an alarming extent.

In connection with the rationing of meat, at that time each pig was provided with a numbered ear mark, which made it possible always to trace the original live stock breeder by whom the pig had been fattened. When echinococcosis was encountered among pigs the track invariably led to the said province. By reconstruction of the case in that way it was often possible to find the dog, which, being the carrier of the sexually mature tape-worm, constituted the source of the virus. With co-operation of the owners some hundreds of suspected dogs were killed. In the intestinal canal of many of them thousands of little tape-worms were found.

What is not yet an accomplished fact for echinococcosis may actually be stated in regard to *cysticercus cellulosae* with pigs, an affection which has not been observed in this country for the last thirty years.

For horned cattle, however, the fight against *cysticercus inermis* is proceeding with unabated energy. In spite of this, the percentage remains practically constant. Several questions arise in this connection, of which the following are brought up:

Is even a scrupulous examination of the so-called predilection places sufficient to diagnose all cases of cysticercosis in horned cattle?

Is our knowledge of the cycle of this parasite sufficiently complete?

Do the medical authorities give sufficient care to the carrier of the sexually mature form, i.e. man?

#### REQUIREMENTS TO BE MET BY SLAUGHTER-HOUSES, MEAT SHOPS AND THE LIKE

The Netherlands Legislator has not restricted himself to laying down guiding principles for organization and financing, as well as for the method of inspection of meat animals, but he has also issued regulations to be met by establishments where meat is kept.

Certain requirements in regard to light and air supply, floors, walls, ceilings, connection to drinking water mains and the like must be met by slaughter-houses, meat shops, store-houses of meat, processed meat works and allied establishments.

The meat inspection services exercise regular supervision of the said establishments.

For processing meat into meat products and the use of other substances in such processes, regulations have been made.

#### SUPERVISION AND PENALTIES FOR INFRINGEMENTS

The supervision of compliance with the provisions of the Meat Inspection Act is entrusted to the Veterinary Chief Inspector and the Veterinary Inspectors of Public Health, who are appointed by the Crown. Moreover, the Veterinary Inspection acts

as adviser to the Minister of Social Affairs and Public Health in matters relating to the Meat Inspection Act and the Destruction Order.

Any infringements of prohibitions laid down in the Meat Inspection Act and the Destruction Order, the statutory provisions resulting from them as well as the Municipal By-laws governing the inspection service for cattle and meat, constitute economic offences, for which the penalty is specified in the Economic Offence Act.

#### IMPORTATION OF MEAT AND MEAT PRODUCTS FROM ABROAD

To give a general idea of the organization of meat inspection in the Netherlands the previous outline might suffice, but considerations of varying nature make it necessary to import meat and meat products from abroad. In respect to such imports protection has been sought in the following way.

The importation of meat from anywhere in the world is possible in this country, provided that the following conditions be met:

The meat must be imported in parts not smaller than quarters with the organs – except stomach and intestines – attached with their natural tissues to the carcass. The quarters, with the head and the tongue, which must also be available, must be marked in such a way that the animals can be reconstructed. No incisions must have been made into the quarters or the organs and no substances must have been added to the meat.

In order to prove that the animals supplying the meat have been examined the meat must be provided with a mark.

The killed animals thus imported must be accompanied by a document in which a legally certified veterinary surgeon in the country of origin truthfully declares that the animal supplying the meat was duly examined by him before slaughter, that during this examination no morbid symptoms were observed and that the animal was not killed in a case of emergency and did not die a natural death.

The said certificate must contain an impression of the mark stamped on the meat, and the signature of the veterinary surgeon concerned must be certified by the Head of the Municipality where the animal was examined.

It may be said that under this procedure the inspection before slaughter has been shifted to the country of origin and that only the inspection after slaughter takes place in this country. It might also be stated that the inspection before slaughter has been superseded by the veterinary certificate from the country of origin.

There is no need to argue that in view of the perishable nature of meat, this way of importing is of value only for countries situated in the immediate vicinity of ours.

However, if the Netherlands Government is convinced that the inspection in the country of origin offers sufficient guarantees, from such a country meat may be imported into the Netherlands in parts not smaller than quarters without organs, unless there is an express provision to the contrary. In that case the inspection after slaughter also takes place in the country of origin and in this country the meat is merely tested for its good quality, though a proper check is also made on the thoroughness of the inspection in the country of origin.

The State has reserved the latter examination for itself and it is effected exclusively

in 48 municipalities indicated for that purpose, which, in this respect, bear the name of 'first offices'.

The countries or parts of countries from which the Netherlands Government has approved the importation of *meat* without organs are the following:

Argentina, Australia (certain export slaughter-houses mentioned by name), Belgium, Brazil, Canada, Denmark, Eire, England (only the slaughter-houses of Aldgate and Islington in London), Finland (only the abattoirs at Abo, Helsingfors, Kuopio and Seinajoki), France (only the slaughter-houses of Bordeaux, Bressuire, Caen, Lyons (de la Mouche), Mulhouse, Nantes, Paris (la Villette and Vaugirard), La Roche sur Yon, Schiltigheim and Strasbourg), Hungary, New Zealand, Paraguay, Poland, Union of South Africa, United States of North-America and Uruguay.

From the above countries loose organs and fat may also be imported.

Importation of *meat products* into our country may only be made from countries specified for that purpose by the Minister of Social Affairs and Public Health. These countries are: Argentina, Australia (only products from the slaughter-houses indicated for meat), Belgium, Brazil, Canada, Czechoslovakia, Denmark, Eire, Finland, France, Germany, Hungary, Italy, Luxemburg, New Zealand, Paraguay, Poland, Switzerland, Union of South Africa, United States of North-America and Uruguay.

When imported into this country, each consignment of meat products must be accompanied by a veterinary certificate from the country of origin, containing in addition to an exact specification of the meat products, a statement to the effect that they originate entirely from animals which were inspected before and after slaughter by a veterinary surgeon and which, in the course of such an inspection, were found to be fully sound and fit for human consumption.

Moreover, the certificate must state that these meat products have been prepared and forwarded under hygienic conditions, do not contain any other preservatives but kitchen salt, nitre up to at most 0.2 %, sugar and uncoloured vinegar and no colouring matter or admixtures injurious to human health. A model of this certificate has been established by law.

In addition to the above, from the following countries or parts of countries salted or dried *intestines*, *bladders* and *rennet-stomachs* may be imported provided that they be accompanied by the prescribed certificate issued by an official veterinary surgeon who is at the same time director of an abattoir or inspecting veterinary surgeon of an export slaughter-house under Government supervision.

These countries are: Australia, Austria, Great-Britain, Northern Ireland, Norway, Rumania and Sweden, and also in the Union of Soviet Socialist Republics the towns of Leningrad and Moscow and in China the towns of Hankow, Shanghai, Tientsin and Tsingtao.

Inspection of the meat products imported is effected by Government officials in the above-mentioned 'first offices'.

#### INSPECTION OF MEAT AND MEAT PRODUCTS DESTINED FOR EXPORT

It will have been noted that in this country the inspection of meat and meat products destined for export had already been subjected to statutory provisions for 15

years when the Meat Inspection Act became operative. These regulations, which were originally laid down in the Act governing the inspection of meat for export, were taken up in the Cattle Act at a later date.

Therefore, the inspection of meat and meat products for export takes place on the strength of another Act than the inspection of meat for home consumption. This inspection, in which the requirements of the importing country are duly considered – since the Customer is King – is effected by Government officials, but exclusively in municipalities – at present 80 – which have joined the Government Inspection Service.

It goes without saying that the inspection of meat for export is performed in a way corresponding to that adopted for home consumption meat. If the meat is found to be fit for export, it is provided with a label, with the exception of pork for bacon preparation, which receives a stamped mark.

If unconditional or conditional approval can be given, meat not found fit for export is brought into home consumption on the strength of the provisions of the Meat Inspection Act.

If the meat is condemned, it is made unfit for human and animal consumption by destruction under the Destruction Order. Except for bacon, meat products offered for export are mainly prepared from meat inspected in accordance with the provisions of the Meat Inspection Act.

Inspection of meat products destined for export takes place under the provisions of the Cattle Act.

The foregoing explanation may make it clear that the inspection under the Cattle Act and that under the Meat Inspection Act are closely interrelated.

## MEAT INSPECTION IN PRACTICE

K. HOFSTRA

*Inspector of the Public Health Service*

### SOME REMARKS

Regulations for the inspection of slaughter animals and meat have been laid down in the Meat Inspection Act, which dates back to 1919 and became operative in 1922.

By slaughter animals are understood single-hoofed animals, cattle, sheep, goats and pigs. The possibility has been opened for about two years for the Act to be declared applicable, in full or in part, to other animals as well, but so far this has not been done.

All slaughter animals – dead or alive – are subjected to inspection, with the exception of the following categories:

1. *Still-born animals, foeti, dead single-hoofed animals and cattle younger than seven days, dead sheep, goats and pigs younger than thirty days.*

The consumer's aversion to the use of meat from the latter category of animals justifies the measure of ordering this substance to be made unfit for consumption, without previous inspection, by passing it through a destructor. Moreover, the meat from such animals proves to be a suitable breeding place for bacteria, so that after consumption of this meat the occurrence of cases of meat-poisoning would not be precluded.

2. *Slaughter animals intended for research.*

This exemption makes it possible to proceed to the dissection of meat animals in various institutions without the interference of meat inspection officials.

3. *Slaughter animals whose meat is destined for export.*

In view of the special demands, which are often made abroad and which should be met – for considerations of commercial policy – inspection in accordance with the provisions of the Meat Inspection Act cannot take place.

4. *So-called home slaughter.*

Until some years ago this category comprised a considerable amount of pigs, sheep and goats, which were killed at the owner's home and the meat of which was destined for consumption by the owner of the slaughtered animal.

When the Meat Inspection Act became operative, the original intention was also

to subject those animals to inspection, but opposition in Parliament, particularly from agricultural quarters, was so stubborn that an important concession in regard to this category of animals was found necessary if the Bill was to be adopted. In a new section of the Act the possibility was created to grant some municipalities exemption from the inspection of pigs, sheep and goats that had not died or were not killed in cases of emergency and whose meat was to be used exclusively by the inhabitants of the premises where the beast was slaughtered.

However, this arrangement did not apply to butchers and people, working in butcher's shops or in the meat processing industry.

In order to ensure as far as possible that only healthy animals – at any rate not seriously sick animals – could be slaughtered without inspection, it was provided that at least four days before a home slaughter, notice must be given to the appropriate authorities.

Several municipalities, however, never applied for this exemption because in this respect, too, the local authorities watched over the state of health of the rural population, whilst in other municipalities it was not granted because echinococcosis was very scattered. Thanks to organized meat inspection, under which organs infected with cysts were condemned and destroyed, infection of dogs was no longer possible, so that the development cycle of the parasite was interrupted.

The inspection of such animals, intended for consumption by their owners, repeatedly revealed tuberculosis – also with miliary foci – so that the assertion of the opponents of this inspection, viz. that the farmer kills only sound cattle for his own consumption, was found not to hold good.

From a public health point of view it was clearly unjustified to have the meat from a great number of beasts consumed without previous inspection. This insight has led to a systematic decline in the number of exemptions from home slaughter inspection, so that from 1st September, 1952, all home slaughtering – except those performed in a small area – have been subjected to inspection.

The Netherlands is fortunate enough to be a flat country and to have an excellent network of roads, so that even the remotest farms can be reached; the exception just referred to only applies to a marshy district without roads, and very sparsely populated on account of its inaccessibility.

Leaving this restriction out of account, all animals whose meat is destined for human consumption are now inspected under the Meat Inspection Act.

Except for such beasts as have died and were killed in cases of emergency, this inspection always comprises an examination before and after slaughter; naturally, for the former category only the inspection after slaughter has to be considered.

#### DECLARATION

Before the inspection of a slaughter animal can be made, the owner must declare his intention of slaughtering the animal. As in the Netherlands the implementation of the Meat Inspection Act is entrusted to the municipalities, declaration is made to a municipal office, mostly the Town Clerk's Office, which passes on this declaration to

the Head of the Meat Inspection Service. Only in municipalities where an abattoir is established is the declaration made at the office of this establishment direct. Both normal animals and such beasts as have died or were killed in cases of emergency must be declared.

Simultaneously with the declaration, as a rule payment of an inspection fee is made; moreover, municipalities, where an abattoir is used, levy a duty for the utilization of this establishment and the services rendered by the municipal personnel.

#### INSPECTION BEFORE SLAUGHTER

For living animals inspection before slaughter may now follow. This takes place in the abattoir, in the butcher's private slaughter-house or at the farm of the owner who wants to use the meat for his own consumption. Generally this inspection is performed at or near the place where the animal will be killed and at any rate in the same municipality. Thus the same person who performed this inspection is often likely to inspect the beast also after slaughter; in addition, long-distance transport-entailing the risk of accidents between the two inspections – is avoided.

During this inspection before slaughter, special attention is paid to symptoms of infectious animal diseases to which particular statutory provisions are applicable; for single-hoofed animals these are glanders and scabies, for the other animals especially foot-and-mouth disease. For pigs they also include swinefever and for sheep and goats, foot-rot and mange. In the course of years this inspection has clearly proved to be an important element in fighting these infectious diseases, since it has repeatedly occurred that the first cases of an incipient enzootic disease were revealed by this examination.

Secondly, it is established whether the animal shows diseases or anomalies likely to affect the soundness of the meat. In this examination an important point is the general impression made by the beast in regard to its state of health and nutrition; others are the condition of the natural openings of the body and the presence or absence of perturbations in the digestive and respiratory organs and the functioning of the heart. If the beast is presumed to be ill or if it arrives by car and cannot get up, the internal body temperature may be of outstanding importance.

Special attention must be given to lameness, arthritic and umbilical affections, udder inflammations and diarrhoea and, generally, inspectors must ascertain whether the animals are not overtired or overheated. As everybody will know, in the latter case slaughter must not be done at once, as the soundness and the keeping quality of the meat has then seriously deteriorated, bleeding often leaves much to be desired and the meat shows a dark colour with abnormal consistency.

After the inspection before slaughter, the owner receives a written slaughter permit and the animal is marked. For abattoirs, where supervision and checking by municipal officials may be considered sufficient and the possibility of proceeding to slaughter without previous inspection is precluded, the written permit may be replaced by verbal permission and marking of unconditionally admitted animals may be dispensed with.

Actually, the inspecting veterinary surgeon may make the slaughter permit condi-



tional on the fulfilment of particular conditions. If an infectious disease is presumed, it may be necessary that slaughtering should take place under supervision and that special precautions be taken, whilst overtired beasts must not be killed until they have been allowed to rest for a sufficient time.

The slaughter permit is issued for a period of twice twenty-four hours, which may be extended at most twice by the same period. This time has been made comparatively short, because in a longer period the condition of the beast might change to such an extent, that the inspection would lose its value.

The inspection before slaughter may also be performed by a lay-inspector instead of the inspecting veterinary surgeon, but this assistant must not grant permission for the slaughter, if he finds that the beast shows anomalies that may affect its state of health. Therefore, in such cases further inspection is effected by the veterinary surgeon.

After inspection, the animal is marked. All animals unconditionally admitted to slaughter – except pigs – receive a stamped mark on the skin, consisting of a circle with a capital G in it. At least two of these marks are stamped, mostly in blue, on the light coloured places of the skin. For pigs this way of marking is not practical, as after their death they are scalded in hot water, so that a mark, thus stamped on, would disappear.

These beasts are either provided with a metal earmark showing the letter G or with a tattooed mark containing the same letter printed into the skin and showing up after slaughter in the form of red dots. If a conditional slaughter permit is issued, the pig receives an earmark with the letter V, whilst other animals are marked on the loins with two red stripes crossing each other at right angles.

It will be clear forthwith that marking is necessary to establish with absolute certainty after slaughter that the beast in question was actually inspected before slaughter; therefore it is necessary for the skin to remain attached to the carcass at one point by its natural tissues when the beast has been slaughtered.

#### WAY OF SLAUGHTERING

After the above inspection, marking and issue of the permit, the owner of the beast may proceed to slaughter. After stunning, the beast is killed as rapidly as possible by bleeding. All meat animals must be stunned with the exception of animals whose meat is destined for consumption by Jewish persons and which, therefore, have their throats cut according to Israelitish rite. In The Netherlands such ritual slaughtering may only be performed in fourteen abattoirs assigned by the Minister of Social Affairs and Public Health. The number of animals intended for this purpose is determined by the number of Jews living in this country. The animals must be shackled and laid down with the required care and any harm to the head and horns must be prevented; the animals may only be laid down in the presence of the shohet, who must then immediately cut their throats.

Stunning is done by means of a so-called 'humane killer', a mask out of which – after the explosion of a gunpowder charge – a hollow spike is driven into the cerebrum. These last few years, however, tests have also been made to stun the animals by means

of electric current. Blows on the head, thrusts into the neck or blows on the neck are strictly prohibited.

If the butcher wishes to use the blood for consumption, it must be collected in clean preferably metallic dishes suitable for this purpose; the blood must then be kept with the beast until inspection begins. In order to keep the blood liquid use may be made of coagulation-preventing substances, such as phosphates, or it may be stirred until it has defibrinated. To prevent contamination of the blood, stirring may only be effected with clean objects; stirring by hand is forbidden. When, during slaughter, the gullet has been cut, the blood must not be used, as in that case contamination with the contents of the stomach is practically unavoidable.

In some slaughter-houses the blood may be worked up into blood plasma, which product is applied in the meat-processing industry.

When, after bleeding, the beast remains motionless, the butcher may proceed with the slaughter. However, with a view to infection by bacteria, inflation of the animal to facilitate removal of the skin is prohibited.

When slaughtering beasts, the butcher must work in clean clothing and use clean implements suitable for the purpose and, generally, do all he can to prevent contamination of the meat.

Pigs must be stripped of their bristles, other beasts of the skin, which remains attached to the carcass at one point. Until the inspection takes place, the organs remain attached to the body with their natural connective tissues, and the kidneys are loosened from the surrounding fat. Stomachs and intestines are kept with the animal and so are other parts, like the head and feet, in such a way that they cannot be exchanged with those of other animals. The animal must be cut in two longitudinally, so that the spine can be examined; if the inspecting veterinary surgeon is in agreement, calves, sheep, goats and sucking-pigs need not be split in this manner. With a view to the glanders test, with single hoofed animals the head must also be cut length-wise.

Animals that have died and were killed in cases of emergency must be slaughtered and brought in for inspection in the same way, unless the owner – supposing that the meat will be unfit anyhow and will therefore be condemned – does not want to finish off the beast. It can then be condemned without further examination by the inspecting veterinary surgeon, but the latter should ascertain carefully whether the animal has been suffering from an infectious cattle disease. As regards anthrax, particularly with cattle, sheep and goats that have died suddenly without specific symptoms, microscopic blood tests must be made. If suspected bacilli are encountered in the spread preparation, the diagnosis may be made by means of a supplementary bacteriological or serological examination. If an infectious animal disease is discovered, the carcass is confiscated, the means of transport disinfected, and a further examination made at the farm by officials of the Veterinary Service.

#### INSPECTION AFTER SLAUGHTER

As soon as the animal has been killed, inspection may follow, the inspector satisfying himself that the beast is still in the condition described before, that nothing has been

cut away of the meat or the organs and that no incisions, likely to affect the final decision on the inspection have been made.

Attention is paid to the external appearance of the meat and of the organs – which, for this purpose, are hung up on hooks or laid down on dissecting tables –, after which such organs with corresponding lymph glands are palpated and incised.

Particularly in view of tuberculosis the latter point is of outstanding importance. The heart is cut open on the left and on the right, so that the endocardium and the heart valves are clearly visible. With cattle, an incision parallel to the lower jaw is also made into the internal and external masticatory muscles, a deep incision is made into the heart septum and the gullet is split length-wise, so that the presence of *Cysticercus inermis* may be ascertained. Of single-hoofed animals, the nasopharynx, the larynx, the trachea and the lungs are tested for glanders after several incisions. For new-born calves special attention is paid to intestinal affections, umbilical inflammations and articular diseases, for which purpose the knee joints are always cut open to examine their contents.

With pigs in this country neither *Trichinella spiralis* nor *Cysticercus cellulosae* occur, so that no special test for those diseases is required.

It will be clear that the inspector must work systematically so that nothing may escape his notice. If anomalies are encountered during the inspection before slaughter, they should be given particular attention, even though no changes may be found immediately.

The inspecting veterinary surgeon should always take and handle the knife himself and wear such clothes that blood-staining need not be avoided.

In addition to a good insight into the pathologico-anatomical changes in the various organs and a vast knowledge of the diseases that may occur, capability to evaluate the quality of meat – i.e. of the muscular tissue, especially of beasts that died or were killed in cases of emergency – is of the utmost importance, since this is a great factor in commanding the respect of butchers as well as lay inspectors.

Further, the inspecting veterinary surgeon should always bear in mind that the decisions taken by him during inspection must be well-considered and justified, though resolute.

When a decision has to be made in a particular case, it is not always possible to give this immediately after inspection. There are numerous instances in which the laboratory has to lend a helping hand.

As a matter of fact, the object pursued by meat inspection is to bar from consumption any meat that may be injurious to public health. In so far as meat animals show anomalies that can be observed with the naked eye, such as cysticercosis and echinococcosis, the decision may be taken straight away. In the event of diseases caused by bacteria, this is not possible, so that expedients like incubators and microscopes must be resorted to, which causes some delay in the final decision.

It is not within the scope of this chapter to discuss all kinds of diseases from which slaughter animals may suffer, but a few of the most important infectious diseases will be briefly mentioned.

As is generally known, immediately after slaughter the meat of the killed animals is sterile. This knowledge justified the standpoint adopted in this country, viz. that if bacteria are encountered in meat – treated 'lege artis' – there is a bacteriaemia owing to which, during life or in agony, these bacteria were spread with the blood throughout the body.

The probability – or possibility – of the presence of a bacteriaemia is shown by the clinical picture during the inspection, if septic symptoms in organs are observed, such as splenic swelling, liver degeneration or kidney petechiae, or if before slaughter a high temperature of the body should be found. In all these cases, as well as with beasts that died or were killed in cases of emergency, a bacteriological examination is required.

#### LABORATORY

As a rule, this examination is made on the spleen and a piece of meat, the latter with surrounding fascia wherever this is possible. To prove the presence of Salmonella, a piece of liver and the gall bladder are also used. In minor municipalities these parts – well packed in sterile material – are sent to the State Institute for Public Health at Utrecht, situated in the centre of the country; the large municipalities all have a laboratory of their own at the abattoir, where examinations are made by the inspecting veterinary surgeon. The surface of the organs and the meat is sterilized by singeing with a blow torch, after which the material to be examined – taken from the centre of the organ or meat – is transferred with sterile grafting needles or knives to tubes, filled with agar or broth, where it is left to incubate at 37 °C for 24 hours.

Then the material is tested for development of bacteria, microscopic examination of a spread preparation and hanging drop being indispensable. The bacteriological examination must be performed with scrupulous care, because under our legal provisions the slaughter animal must be entirely condemned, if its meat is found to contain any bacteria, unless only swine-erisipelas bacilli are encountered, in which case the meat can be sterilized. This procedure can be adopted only for bacteria showing a distinct development within 1–2 days. Therefore, a different method must be applied for tuberculosis, since in artificial cultures tubercle bacilli grow so slowly, that the meat of the beasts might already have begun to perish before the final decision on the inspection could be given.

The dissection picture turns the scale in this case, the meat being considered to contain tubercle bacilli, if acute miliary tubercles are found in kidneys or meninges, or extensive miliary tuberculosis of the whole lung and exudative tuberculosis of one or more organs together with humid swelling or young, streaky, cheesy infiltrations of the lymph glands.

As with the naked eye it cannot always be ascertained whether the organs contain miliary tubercles or ordinary inflammation foci, a histological examination is often required, for which purpose coloured cuts of the organ in question must be made in the laboratory.

For the evaluation of the meat, acidity (pH) is also of outstanding importance.

With sick or tired beasts acid formation is sometimes insufficient, the meat showing a dark colour and its keeping quality being less satisfactory; moreover, it constitutes good material for the development of bacteria with which it is infected.

Therefore, in a great many cases it is extremely desirable that the pH of meat should be determined. This, too, is done in the laboratory by means of an electronic pH meter with direct indication, the electrodes being placed directly on the meat and the pH being read on a scale, or found by means of a colorimeter.

The pH, which varies normally between 5.8 and 6.1, in some instances may rise to above 7.0; in that case the meat can no longer be passed unconditionally.

Finally, if required, the boiling and frying test should be applied if the beast is presumed to have been treated during its life with substances giving off a penetrating odour, like turpentine or creolin, or if the beasts, especially pigs, were fed with fish offal, as a result of which the fat assumes a repugnant taste. Also in the event of yellow coloured meat only the laboratory can ascertain with full accuracy, whether the beast was suffering from icterus or whether the abnormal colour was due to the absorption of plant colouring matter with the food.

In connection with the fight against zoonoses, in all cases where *Salmonella* is encountered the culture is sent for further differentiation to the Netherlands *Salmonella* Centre at Utrecht.

#### VALUATION

When the meat has been subjected to an examination – if required, in detail – it is passed, passed conditionally or condemned. It is also possible that the slaughtered beast is passed, but that some parts or organs are condemned if local changes are encountered.

The statutory provisions governing these matters specify precisely what decision must be taken in any particular case.

If meat is passed conditionally, it may be frozen for 10 days at  $-10^{\circ}\text{C}$ , sterilized or sold retail under supervision.

Beef is frozen if the presence of a living *cysticercus* is ascertained. It has been found experimentally that any bladder worms still present in the meat, are definitely killed by freezing.

Sterilization may follow in cases where the presence of particular bacteria in meat is ascertained (swine erysipelas) or presumed (miliary tuberculosis) or in the event of a virus disease (swine fever).

After the bones and any affected or changed parts have been removed, the meat is steamed or boiled in small pieces – up to 2 kg each – for  $2\frac{1}{2}$  hours at  $100^{\circ}\text{C}$ , at the end of which period a thermometer inserted in the thickest pieces must indicate a temperature of at least  $90^{\circ}\text{C}$ . The fat of such beasts may be melted.

Finally, it is also possible to sell the meat – without any previous treatment – in pieces of at most 3 kg to the public direct, if there are doubts as to its keeping quality, so that the meat must be released for consumption as soon as possible.

All processes, required to make conditionally passed meat fit for human consump-

tion, are carried out under the constant supervision of an inspector; retail sale must not take place in the butcher shop, but is effected in a room designated by the burgo-master of the municipality.

If meat is condemned, it is made unfit for consumption in a destructor. Before transport to the destructor plant can be proceeded to – the substance is collected by vans of the destructor plant – the meat must be kept in such a way, that unauthorized people cannot take away any parts when the inspector is no longer able to exercise supervision personally.

To this end, in every slaughter house there should be one or more confiscation buckets with lids, which can be locked with a key. One key is kept by the inspector, the other by the driver of the destructor van.

By throwing the condemned meat into the confiscation bucket(s) and locking the latter, the inspector may consider his duty accomplished. In municipalities where the inspector makes use of a motor vehicle, he may take the confiscated substance with him and have it kept centrally in a store until the destructor van comes round to collect it.

#### MARKING

In order that the final decision taken on the inspection of meat may always be traced, after inspection the meat is provided with marks which are different for the various designations given to it. Thus, meat passed for consumption is provided with a rectangular mark stamped on all organs and on the principal muscular bundles and showing the name of the municipality where the meat has been inspected, a date and the word 'goedgekeurd' (passed).

To enable the inexperienced public to distinguish horse and goat meat from meat from other beasts, meat from the former category is provided with a red mark and meat from the other with a blue mark.

Good and distinct marking is of great importance, as the mark constitutes the only evidence for the butcher that the meat sold in his shop has been passed by the inspectors.

Though this terminates inspection in the limited sense of the word, the inspector's jurisdiction extends still further.

#### REPRESSIVE INSPECTION

When the meat has been passed, the owner must see that it remains sound. To ensure this, in the large slaughter houses it is mostly kept for some days in chill rooms at a temperature of 1–4 °C; besides, most butchers also have a refrigerator in their shops. The inspectors exercise supervision on meat keeping, for which purpose they inspect butcher shops; moreover, regulations have been made for the transportation of meat. Passed meat from abroad or from another municipality must be re-inspected in the receiving municipality to ascertain whether it is still perfectly sound. If any contamination or deterioration has occurred, the unsound meat is condemned.

Butcher shops, meat processing works and the like have to meet particular requirements in regard to cleanliness, light and air supply.

To make it possible that meat is confiscated in particular cases or that an investigation is carried out, police powers are vested in all meat inspectors, who can draw up proces-verbaux so that trespassers can be prosecuted.

#### RE-INSPECTION

To satisfy the sense of justice of the owner of the slaughter animal or meat, the possibility has been opened to require re-inspection of the beasts or meat condemned or conditionally passed, on the condition that the loser shall pay. Such re-inspection is then performed by two inspecting veterinary surgeons who have not taken part in the first inspection, one of them designated by the owner and the other one by the municipality concerned.

#### CONCLUSION

Thanks to the legal provisions on meat inspection, the Netherlands population feel convinced that they can consume meat without any risk of contracting a disease. Actually, the number of cases where meat poisoning occurs as a result of infection by meat from sick animals is very limited indeed, the few cases that have occurred are mostly attributable to infection of meat by persons carrying bacilli, who have not observed sufficient cleanliness when handling meat.

Meat poisoning may also take place after consumption of meat products manufactured without the observance of the required hygienic precautions.

Further, inspection has contributed largely to combating human diseases; trichinosis is no longer found in the Netherlands, whilst echinococcosis, though not yet wiped out, no longer constitutes a serious problem.

In conclusion, it may be stated that meat inspection services supply important data to the Veterinary Service, thus enabling the latter to take at short notice any measures, required with regard to infectious cattle diseases.

Also it will be clear, that the inspecting veterinary surgeon has an important task to accomplish in the interest of public health.

# THE MANUFACTURE OF MEAT PRODUCTS AND VETERINARY HYGIENE

DR S. G. ZWART

*Director of the Quality Control Bureau for  
Processed Meats*

The veterinary hygienic supervision of meat products is regulated by or in the Meat Inspection Act, Statute book 1919 No. 524, whereas in the Livestock Act, measures have been taken relating to the meat products intended for exportation.

If we consider the regulations of the Meat Inspection Act we see that they deal with:

1. establishments where meat products are prepared;
2. the way meat products are prepared.

As a matter of fact, offence against these regulations is punishable. The act states at the same time that it is an offence to sell, to offer for sale, to deliver, to give as a present, to transport, to have transported, or to have in stock for transportation, any meat products which have deteriorated or in any other way have become unfit for consumption.

As regards the establishments and premises where meat products are prepared it may be pointed out that the Royal Decree of 10 July 1926 Statute book No. 233 in conjunction with art. 19 of the Meat Inspection Act, gives regulations with which meat products factories have to comply. These are not only regulations for the meat products factories but also for other establishments which for purposes of trade are dealing with meat (slaughter-houses, butcher's shops, warehouses for meat etc.).

These requirements affect the amount of daylight, the ventilation, condition of floors, walls and ceiling, height of the premises, available space, water supply, paving of the ground and runways before the entrance to the premises, and the cleanliness of the runways, localities, and things which are to be found there.

Apart from these general regulations for meat products factories, there are the following particular requirements:

1. They must not be in direct and open connection with dwelling-rooms, butcher's shops, localities for processing intestines and other products of animal origin, with stores for offals, or with stables.
2. Matters which might taint meat or which might accelerate deterioration are not allowed to be found in meat products factories.
3. The staff engaged in processing meat must wear clean clothing and have clean hands.



4. A wash basin with soap and a clean towel must be available in each meat products factory.
5. No other activities than those relating to the processing of meat are allowed in these factories.

The Royal Decrees of September 13th 1924, Statute book 448, and of August 22nd 1938, Statute book 865, both based upon the Meat Inspection Act, give directions for the manufacture of meat products.

The former gives rules to prevent insanitary processing. The latter, which is also based upon the Commodities Act, mainly contains provisions on chemical matters.

Consequently, the supervision of the observance of these regulations and rules is in the first place designated to the officials mentioned in the Commodities Act.

The fact that these officials systematically exercised supervision had, at the time the Meat Inspection Act took effect, wrongly induced some meat inspection services to hold off the supervision on processed meats. This evil is now effectively settled.

I will not say anything against the value of an inspection on the composition of meat products from a chemical point of view but I should like to point out that from a view-point of protection of the public health the bacteriological nature of meat products is primary. Promotion of hygiene during the processing of meats is not only a question of keeping disease germs from these products but as a result these products will keep longer and also be more tasty. Though the meat inspection services have the aim of guarding the public health they are consequently usefully operating in the field of economy as well.

As regards the prescriptions of the Livestock Act the following may be said:

These instructions have the object of guaranteeing the importing country that sound products are received from the Netherlands which satisfy the requirements of the country of importation. Penal provisions are hardly necessary, for if the prescriptions are not complied with the exportation is not allowed.

Action is taken (art. 84 of the Livestock Act) if meat products not allowed for exportation are not handled as prescribed by law. At the beginning only articles for which veterinary certificates were required by foreign countries were subject to the export examination.

Not until 1951 was it decided that all meat products should be subject to export examination. (Some very unimportant exceptions left out of consideration).

Special attention is paid to the places where meat products for exportation are prepared. There is a tendency to set higher standards for these places than those given by the Meat Inspection Act. At the time being these higher requirements have not yet become part of the legal prescriptions but, probably this will not be long.

It is to be expected that among other things the following will be defined:

- a. The boilers must not be fitted up in spaces where there are uncanned meats.
- b. The tables on which the meat is boned or cut must be of stainless metal. If a wooden cutting-top is used this must be removable in order to be effectively cleaned.
- c. The receptacles in which meat or meat products are kept must be such that the contents cannot come in contact with water used for cleaning purposes.

- d. In the factory there must be more than one place to disinfect the knives and other utensils by means of steam.
- e. In the proximity of toilet rooms there must be sufficient washing accommodation with soap and a clean towel.
- f. Persons affected with contagious diseases, with open wounds, or skin-diseases, are not allowed to enter the factory.

Though the meat products industry in the Netherlands is on a high level, there is still a desire to improve things. Especially as regards the factories engaged in export.

It is realised, of course, that this cannot be achieved only by improving the regulations. An effective supervision by hygienists is essential. There is no lack of goodwill on the part of the managers of the factories. However, they cannot always supervise the work and the workmen often do not realise the hygienic importance of seemingly small neglects or incorrect dealings. Only the following will be mentioned about this: Washing the floor in a room where there is much meat. High piling of meat in transport containers so that part of the meat falls on the ground. Hams which are to be canned are left in the sun.

Again and again it is ascertained whether there is sufficient supervision. This is done by the Public Health inspectors who are at the same time inspectors of the Veterinary Service, together with the officials attached to them.

There are many first-class meat products factories in the Netherlands but we don't want to run the risk that the good name of the Dutch product will be injured by a poorly working factory. Therefore a continuous supervision by officials during the proceedings is prescribed for factories working for export.

This short summary would be very incomplete if there was not said something about what has been achieved in the Netherlands by private enterprise as regards the supervision of the quality of meat products intended for export.

The meat canners have instituted a foundation called Meat Products Quality-Control Bureau (V.K.B.).

The Trade Board for Livestock and Meat (B.V.V.) has made use of its authority to rule that meat products can only be exported by members of the V.K.B. The meat products have to comply with the demands made by the V.K.B. with the sanction of the B.V.V.

In the Board of the V.K.B. the Veterinary Service is represented. All factories preparing meat products for foreign countries have these systematically sampled by the officials of the V.K.B. Moreover the samples drawn by the officials of the Control Board of the Ministry of Agriculture, Fisheries and Food at the ports of exportation are sent to the V.K.B.

The samples are inspected by a committee which sits twice a week, at least, and which is formed by 4 meat packers, two veterinary surgeons and one chemist. Parcels which are rejected and which are still in the country are not allowed to be exported. If there is any uncertainty about the soundness of meat products of a manufacturer the latter is given notice that he shall only export meat products which are accompanied by a certificate of approval from the committee of inspection

The committee of inspection inspects according to the so-called 'standard-order'.

In this order, general requirements are given for all meat products as well as particular requirements with which specified products have to comply.

The general requirements mainly relate to:

1. the condition of the meat which is to be processed,
2. colouring agents,
3. preservatives,
4. starch and Feder figure,
5. keeping tests of canned meats,
6. condition of tins,
7. condition of packing material,
8. the marking of canned meats.

It might be interesting to learn something more about the keeping tests of canned meats. The more so as the inspection of the tenability involves both a complete examination of quality and an inspection of soundness from a veterinary point of view.

The prescriptions regarding the keeping tests are therefore given after consultation with the veterinary authorities and are at the same time in force for the examination of products intended for export under the Livestock Act.

The prescriptions are as follows:

I. *Tests of canned meats which are sold as being absolutely sterile:*

a. Intended for areas with a temperate climate:

To be incubated for 3 days at 37 °C and subsequently to be submitted to a bacteriological test on sterility at 37 °C.

b. Intended for areas with a tropical or sub-tropical climate: half of the samples to be incubated for 3 days at 37 °C and the other half for 3 days at 55 °C and to be subsequently submitted to a bacteriological test on sterility, respectively, at 37 °C and 55 °C.

II. *Tests of canned meats sold as 'commercially' sterile:*

a. Intended for areas with a temperate climate: after an incubation-period of 10 days at 37 °C the products shall not show any signs of deterioration.

b. Intended for areas with sub-tropical or tropical climate: after 3 weeks of incubation at 37 °C, the products shall not show any signs of deterioration.

III. *Semi-preserved meats:*

a. If the label bears the prescription 'keep cool' the products, if intended for countries with a temperate climate, must be kept for 20 days at 15 °C without showing any signs of deterioration and, if intended for a tropical area, for 10 days at 37 °C without showing any signs of decay.

b. If the label bears the prescription 'keep under refrigeration' the products must show no signs of decay after they have been kept for one day at room temperature (15–16°C) and subsequently for 10 days at 10 °C.

The manufacturer decides for himself to which category (either I, II or III) a product belongs. However, for certain products, the V.K.B. can prescribe to which category they shall belong.

Before the incubation test is performed, the tins have to be cooled down to at least room-temperature (15-16 °C).

The examination of tins which have been incubated shall not take place until the contents of the tins has been cooled down to room-temperature (15-16 °C).

Even if not a single tin of a parcel shows any visible abnormalities after incubation, a tin shall be opened in order to ascertain that the quality of its content is still up to standard.

As far as the chapter 'Condition of tins' is concerned, the following may be said: The filled tins must give the impression that they have been carefully handled by the processor.

They must be clean and free from rusty spots.

The inside of the tins shall be of such a nature that no complaints about corrosion are to be anticipated.

Before and after the prescribed incubation test, both lid and bottom of the tin shall have a concave appearance after having been cooled down at room temperature.

The tins must not leak, or be out of shape or overfilled. The tins shall be sealed under proper vacuum.

So-called 'loose tins' and 'flappers' shall not be exported.

In 1952 the V.K.B. rejected 98 parcels intended for exportation and in 63 cases it insisted on improvement of the quality of the product. As regards the weight of the content, in 39 instances excess weight of the content was ascertained whereas short-weight was ascertained in 13 instances.

Reasons for rejection were:

Different colour, odour, taste, appearance, or composition, dirty, insufficient curing, too large salt content, excessive waste.

I think, I have summarized this by giving a survey of the legal prescriptions on the inspection of meat products.

However, there is one subject to which I still have to pay attention. Of late it has correctly been pointed out in literature that the condition of the meat for processing purposes is very important. It has to be insured that meat is not being used from tired slaughter animals and that the meat does not become unfavourable in condition during transport from slaughterhouse to the meat products factory.

The first evil may be checked by supervision of the official in attendance in a factory and, as regards the second evil, the municipal regulations relating to the transportation of meat in the municipality and the government regulations relating to the transportation of meat from one municipality to another are applicable (Art. 62, Royal Decree of June 5th, 1920, Statute book S no. 825).

As the present time it is being considered in the Netherlands whether these prescriptions should not be more stringent and if it should not be prescribed that the transportation of hams to meat products factories should take place by cold storage.



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Event not a single tin of a parcel shows any visible abnormalities after incubation, it shall be opened in order to ascertain that the quality of its content is still up to standard.

As far as the chapter 'Condition of tin' is concerned, the following may be said:  
The filled tin must give the impression that they have been carefully handled by the processor. The tin must not show any signs of rust or corrosion on the inside. They must be clean and free from vermin. The tin must not be damaged. The inside of the tin shall be of such a nature that no complaints about contamination are to be anticipated.

Before and after the prescribed incubation test, both the bottom of the tin and the lid shall have a coarse appearance after having been cooled down to room temperature. The tin must not leak or be out of shape or deformed. The tin shall be sealed in under proper vacuum.

So-called 'loose tin' and 'lapped' shall not be exported.  
In 1952 the V.K.R. rejected 98 parcels intended for exportation and in 1953, 11 parcels. The reasons for rejection were:  
1. Inadequate weight of the product. As regards the weight of the content in 10 instances excess weight of the content was ascertained whereas short weight was ascertained in 13 instances.  
2. Defective tin, e.g. rust, holes, etc.  
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I think, I have summarized this by giving a survey of the legal provisions on the inspection of meat products.

However, there is one subject to which I still have to pay attention. Of this it has correctly been pointed out in literature that the condition of the meat for processing purposes is very important. It has to be assured that meat is not being used from tinned slaughter animals and that the meat does not become stale or rancid during transport from slaughterhouse to the meat products factory.

The first will be checked by supervision of the official in attendance in a factory and, as regards the second, the municipal regulations relating to the transportation of meat in the municipality and the Government regulations relating to the transportation of meat from one municipality to another are applicable (V.K.R. 62, Royal Decree of June 25th, 1920, Statute book 2 no. 822).  
As the present time is being considered in the Netherlands whether these provisions should not be made stringent and it is should not be provided that the transportation of tins in meat products factories should take place by cold storage.





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