Research into certain animal diseases / by T. Dalling, H.R. Allen and J.H. Mason.

Contributors

Dalling, Thomas.
Mason, J. H.
Allen, Hubert Raymond.
Wellcome Physiological Research Laboratories.

Publication/Creation

[Place of publication not identified] : [publisher not identified], [1925?]

Persistent URL

https://wellcomecollection.org/works/gfzzj8m9

Reprinted from "The Veterinary Record," July 11th, 1925.

Research into Certain Animal Diseases.*

By T. Dalling, H. R. Allen and J. H. Mason,
Wellcome Physiological Research Laboratories,
Beckenham.

Swine Erysipelas.

The method adopted in the production of an anti-swine erysipelas serum is to inject horses intravenously with dead cultures of *B. erysipelatis suis*, followed later by living cultures. The testing of its potency can be carried out with mice (the official German method), though we have, on the whole, got more consistent results with pigeons.

Table I. gives the results of testing on mice the protective power of some serum we have recently made. In the experiment a standard culture of the organism on agar was used which, when washed off and diluted suitably with normal saline solution, killed mice regularly in five days after the intravenous injection of 1 c.c.

Table 2 is a comparison of serum produced by one of our horses with two Continental official samples. In the experiments indicated, pigeons were used; the culture and serum were mixed and stood for an hour at room temperature before being injected intramuscularly. Readings were taken daily up to four days.

This test is the one widely used on the Continent; apparently the results obtained by this method in which the culture and serum are mixed and in which the bactericidal properties are given full play, gives results consistent with those obtained when the serum and culture are injected separately into the animal. (See Table I.).

TABLE I.

Serum.	*Dose.*	*Result.*	
Horse A.	0·1 c.c.	L	L
	0·075 c.c.	L	L
	0·05 c.c.	L	L
	0·025 c.c.	D4	D4
	0·01 c.c.	D2	D2
N.H.S.	0·1 c.c.	D2	D2
No serum	—	D2	D2

L = Lived.
D5 D4 = 1 mouse of pair died 5th day and the other mouse 4th day.
Serum intraperitoneally and same dose of culture intravenously given 24 hours later to all mice.

*Presented to the Central Veterinary Society at Langley Court, Beckenham, on June 11th 1925.

TABLE 2.

Serum.	*Dose Serum.*	*Culture.*	*Result.*
X	0·3 c.c.	500 M.L.D.	L
	0·2 c.c.	,,	L
	0·1 c.c.	,,	D4
Y	0·3 c.c.	,,	L
	0·2 c.c.	,,	D3
	0·1 c.c.	,,	D3
W.P.R.L.	0·3 c.c.	,,	L
	0·2 c.c.	,,	L
	0·1 c.c.	,,	D4
No serum	—	,,	D3
	—	100 M.L.D.	D4
	—	10 M.L.D.	D4

Pigeons used. Serum and culture mixed and injected intramuscularly.
L = Lived.
D4 = Pigeon died 4th day.

The Testing of B. Bronchisepticus Serum and Vaccine.

Because of the relative frequency with which *B. bronchisepticus* can be isolated during some outbreaks of canine distemper, in pure culture from the tissues of affected dogs, serum and vaccines made from strains of this organism have been used for curative and prophylactic purposes. Success has been claimed by some veterinary surgeons from their use in some outbreaks of distemper.

B. bronchisepticus Serum.

The serum is prepared by injecting horses with increasing doses of dead or living cultures of the organism—several strains being used. Guinea-pigs are used in testing the protective value of such serum. Table 3 represents an experiment to ascertain the smallest dose of serum protecting against a certain lethal dose of culture. To make certain that we were not testing merely for bactericidal properties in vitro, we injected serum in another experiment 24 hours before the culture. Table 4 seems to show that the serum was really anti-bacterial in the body.

Anti-bronchisepticus Vaccine.

Tables 5, 6, 7 show the evolution of the method now recommended for vaccinating dogs against *B. bronchisepticus* infection, *i.e.*, two doses of a killed vaccine followed by one dose of living culture. Table 5 shows that a high degree of protection was obtained after one dose of killed culture, five out of six animals surviving when tested a month later. Table 6 indicates that two doses of killed culture acted similarly while Table 7 sets forth the result got by adding a dose of a living culture; complete protection is evident fourteen days after the last dose of vaccine.

To show that the third dose, when a living culture is used, gives better results than killed culture, the experiment indicated in Table 8 was carried out. Complete protection was found in three weeks after the dose of living culture, while complete protection is not found until five weeks following the third dose of killed culture.

We do not wish to stress unduly this one experiment. This and other evidence we have is in favour of the third dose being a living culture. Until evidence accumulates that will convince us that a third dose of dead is as efficient as living, we think it wise to adopt the third dose of living culture.

TABLE 3.

Anti-bronchisepticus Serum (Horse A).		*Result.*
1·0 c.c.	4 c.c. culture B. bronchi-septicus	L L
0·5 c.c.		L L
0·25 c.c.		L L
0·1 c.c.		D D
Normal Horse Serum		
1·0 c.c.		D D
No serum	.....	D .....

L =Lived. D =Died.

Serum and Culture mixed and allowed to stand before intraperitoneal injection into guinea-pigs.

TABLE 4.

Serum.	*Culture.*	*Result.*
Anti-bronch. serum		
Horse A. 5 c.c.	5 c.c.	L
,,	4 c.c.	L
,,	3 c.c.	L
,,	2 c.c.	L
Normal Horse Serum		
5 c.c.	5 c.c.	D
,,	4 c.c.	D
,,	3 c.c.	D
,,	2 c.c.	L

All injections intraperitoneal: serum 24 hours before culture.

D =Died. L =Lived.

TABLE 5.

Inoculation.	*Test.*	*Result.*
0·25 c.c. Killed culture subcut.	4·0 c.c. living B. bronch. intra-peritoneally 4 weeks later.	L.L.L.L.L.D. P.M.T.
Control—	No Vaccine	D. P.M.T.

D =Died. L =Lived.

P.M.T. =Post mortem typical B. bronch. found.

TABLE 6.

1*st Inoc.*	2*nd Inoc.*	*Test.*	*Result.*
0·25 c.c.	0·5 c.c. killed culture subcut. 7 days later.	4 c.c. living B. bronch. intraperit. 3 weeks later.	L L L L L D. P.M.T.
Control—	No vaccine		D. P.M.T.

D =Died. L =Lived.

P.M.T. =Post-mortem typical B. bronch. found.

TABLE 7.

1*st Inoc.*	2*nd Inoc.*	3*rd Inoc.*	*Test.*	*Result.*
0·25 c.c. *Killed* cult. subcut.	0·5 c.c. *Killed* cult. subcut. 7 days later.	0·25 c.c. *Living* cult. subcut. 7 days later.	4·0 c.c. *Living* cult. intraperit. 14 days later	L L L L L L
Controls—No vaccine.				D.D. P.M.T.

L =Lived. D =Died.

P.M.T. =Post-mortem typical B. bronch. found.

Avian Diphtheria (Roup).

Avian Diphtheria is the term applied to a contagious disease in fowls presenting numerous symptoms, all or any of which may be present during an outbreak. Because of the possibility of more than one disease being included under the term and that each special

TABLE 8.

G.P.	1*st Inoculation.*	2*nd Inoculation.*	3*rd Inoculation.*	*Test.*	*Result.*
1	0·5 c.c. *Killed* cult. subcut.	1·0 c.c. *Killed* cult. subcut. 7 days later	0·5 c.c. *Living* cult. subcut. 7 days later	4·0 c.c. Living cult.	L
2					L
3					L
4			2·0 c.c. *Killed* cult. subcut. 7 days later	intraperit. 3 weeks later	D P.M.T. D P.M.T.
5	Control–No vaccine		2 doses as 1 and 2	As above 4 weeks later	L
6	2 doses as above				L
7			2 doses as 3 and 4		L
8					D P.M.T.
9					D P.M.T.
10	Control–No vaccine		2 doses as 1 and 2	As above 5 weeks later	L
11	2 doses as above				L
12			2 doses as 3 and 4		L
13					L
14					L
15					D P.M.T.
16	Control–No vaccine				

L =Lived. D =Died.

symptom may represent a different disease it was decided to attempt to reproduce the various symptoms in different fowls, using the same infecting agent. Scales from the combs of infected birds were emulsified in 50 per cent. glycerin in saline and strained through glass wool. Table 9 shows the results of applying this emulsion in various ways; it is interesting that intravenous inoculation produces the various symptoms associated with the disease. The question of natural infection was studied. Hens suffering from the natural disease were allowed to run with normal birds. Table 10 indicates that the disease we worked with was contagious.

Experimentally infected fowls were also penned with healthy birds which contracted the disease (Table 11). Samples of scales from infected hens were obtained from various sources. All were found to be infective though their degree of virulence varied. Table 12 indicates the results of testing one highly virulent sample.

Experiments were arranged to test whether birds which recovered from the natural disease were immune against artificial infection. The immunity was high (Table 13). Recovery from an attack conferred artificially by one strain conferred a high, but not complete immunity to massive infection by the same strain (Table 14) and by another strain (Table 15). These results suggest that there is one causal agent only producing avian diphtheria.

Many vaccines for the prevention of roup are available. Good results are claimed for some. We have experimented with a vaccine made according to the well known Beach's method.

Table 16 gives the results of one experiment where doses of virulent material infected vaccinated birds up to four weeks after vaccination, but not at six weeks.

Serum from immunised hens which received several inoculations of dead, and later, living infective material has been used experimentally for protective purposes.

TABLE 9.

Hen.	*Inoculation.*	*Result.*
7	Combs and wattles scarified and emulsion rubbed into comb	Comb + + Mouth + +
23	As 7	Comb + + Mouth + +
32	Emulsion rubbed into palate	Mouth + + Eye + + Nose + +
33	As 32	Mouth + + Eye + + Nose + +
0	1 c.c. intravenously ...	Comb + + + Wattle + + + Mouth + + + Eye + + + Nose + + +

+ + =lesions of moderate size.
+ + + =very large lesions.

We have done but few experiments up to now, but in two completed experiments, the serum of the immunised hens, when injected into normal birds has protected them against a subsequent inoculation of virulent material. Further work is in progress If the early experiments are confirmed, we may be able with immune serum to save a flock of hens in which roup has broken out We think that this serum is also curative, but we shall report later on this aspect of the work. Work is also in progress with filtered material.

TABLE 10.

Hens 10 and 31 put in contact with B hens (comb lesions); both contracted comb lesions in 14 days.
Hens 103 and 104 put in contact with G hens (mouth lesions); both contracted mouth lesions in 10 days.

TABLE 11.

Exp. infected Hen.	*Contact Hen.*	*Result.*
12 & 18 (Comb)	19	Comb +(14 days).
	29	Comb + +(9 days).
		Mouth + +(15 days).
34 & 200 (Comb)	14	Comb + +(8 days).
	500	Comb + +(8 days).
7 & 23 (Comb)	613	Mouth + + +(14 days).
		Nose + + +(14 days).
		Comb + + +(14 days).
32 & 33 (Mouth)	615	Nil.

+ =slight lesions.
+ + =moderately large lesions.
+ + + =very large lesions.
Nil. =No lesions.

TABLE 12.

1.—*Comb inoculations* (comb scarified and emulsion rubbed in).

Hen.	*Dilution of Emulsion.*	*Result.*
611	1—10	Comb, wattle & mouth + + +
610	1—100	Comb, wattle + + +
26	1—1,000	Comb, wattle + + +
102	1—10,000	Comb, wattle & mouth + + +
5	1—100,000	Comb + + +
607	1—1,000,000	Comb, wattle + +
605	1—10,000,000	Nil.
603	1—100,000,000	Nil.
601	1—1,000,000,000	Nil.

2.—*Intravenous Inoculations.*

612	1—10	Comb & wattle + + +
14	1—100	Mouth + + +
16	1—1,000	Nil.
6	1—10,000	Nil.
27	1—100,000	Nil.
606	1—1,000,000	Nil.
604	1—10,000,000	Nil.
602	1—1,00,000,000	Nil.
600	1—1,000,000,000	Nil.

+ + =moderately large lesions.
+ + + =very large lesions.

TABLE 13.

Hen.	*Nat. Infect.*	*Test.*	*Result.*
106		(6—8 months later)	Nil.
126			? Comb.
162	Comb ...	Scarified comb	Nil.
207		1/100 dil. H strain	Nil.
246	Mouth ...	rubbed in.	Nil.
354		Also 1 c.c. 1/100 dil.	Nil.
10	Comb ...	H strain i.v.	Nil.
31			Nil.
103	Mouth ...		? Comb.
104			? Comb.
	Controls 2		Comb + + + Mouth + +

+ + =moderately large lesions.
+ + + =very large lesions.
Nil. =No lesions.
? =doubtful lesions.

TABLE 14.

Hen.	*1st Infections.*	*Test.*	*Result*
4	Eye, mouth, nose	Full strength same strain rubbed into comb and 1 c.c. i.v.	Nil.
12	Comb ...		Mouth + +
13	Mouth, nose, comb		Nil.
14	Comb ...		Nil.
15	Nose, mouth, eye		Nil.
19	? comb ...		Comb + + +
28	Comb ...		Nil.
34	Comb ...		Nil.
200	Comb ...		Nil.
500	Comb ...		Nil.
	Controls 2		Mouth, comb, nose + + +

+ + =moderately large lesions.
+ + + =very large lesions.
Nil. =No lesions.

TABLE 15.

Hen.	*1st Injection.*	*2nd Injection.*	*Result.*
1—10	L. strain	H. strain	No infection.
11	(Control)	,,	Mouth, comb + +
12	(Control)	,,	Wattle, comb + +

Nil. =No lesions.
+ + =Moderate sized lesions.

Bacillary White Diarrhœa.

Bacillary White Diarrhœa of chicks is caused by *B. pullorum*, which can be isolated from the liver, heart blood and yoke sac of infected chicks. In one case we isolated it in pure culture from the lungs. Infection by mouth of chicks less than seven days old can easily be carried out (Table 17). Infection is also believed to be conveyed from the adult laying hen in diseased ova. We have isolated *B. pullorum* from apparently diseased ova on several occasions, but have not yet succeeded in obtaining the organism from the yokes of several dozen eggs laid by reacting hens; nor have we been able to isolate the causal agent from the yoke sac of any chick found dead in the shell or from any unfertile egg.

In several experiments we have been able to infect chicks with culture given by the mouth at all ages up to six days old, but not at eight or nine days or later. These experiments are to be repeated.

Attempts have been made to eliminate the carriers of " B.W.D." from flocks by carrying out agglutination tests with serum from all birds in the flock against *B. pullorum* and breeding only from the non-reactors. Good results have been reported from such a procedure. It was necessary to have many samples of blood sent from long distances by post, and many arrived contaminated and hæmolysed. We have got over this difficulty by the use of 5 per cent. boracic acid. 0·1 c.c. is placed in the blood collecting tube before being dispatched to the poultryman. The dilution of boric acid varies with the amount of blood drawn into the tube, from 1 per cent. to 0·02 per cent., neither of which concentration affects the suitability of the serum for the test.

It has been stated that the agglutination titre of reacting fowls varies according to the period of the year and whether the birds are in lay or not. We have tested weekly for twelve weeks the agglutination titre of sixteen birds. Though we found considerable variations from time to time, no obvious correlation between eggs laid per week and agglutination titre could be found.

The intradermic method of testing fowls, as described by Ward and Gallacher has been tried out and

TABLE 16.

Hen.	*1st Inoculation.*	*2nd Inoculation.*	*Interval.*	*Test.*	*Result.*
628	1 c.c. vaccine subcut.	1 c.c. vaccine subcut. 7 days later	2 weeks later	1/100 H i.v. and comb	Comb + +
629					Mouth + +
630			4 weeks later	1/100 H i.v. and comb	Comb +
631					Mouth +
632			6 weeks later	1/100 H i.v. and comb	Nil.
633					
Controls					
634	to 628 & 629	test only			Comb + + +
635	to 630 & 631				Mouth + +
636	to 632 & 633				

+ =slight lesions.
+ + =moderately large lesions.
+ + + =very large lesions.

results compared with those obtained by the agglutination test. Table 18 shows that of thirty-nine birds tested by both methods, sixteen gave positive results to both tests, seventeen were negative to both, three were doubtful to agglutination and positive to intradermic tests. One of these died and *B. pullorum* was isolated from the ovary. Two were doubtful to intradermic, but positive to agglutination tests, and one gave a positive agglutination and a negative intradermic test. Beaudette (*Journal of Immunology* Nov., 1923), has stated that the albumen of the egg of hens giving positive serum agglutination results, will also give a positive agglutination reaction. We think the so-called agglutination of the egg albumen is non-specific, for we have been able to demonstrate the same results by adding tap water, saline solution, etc. to the albumin instead of *B. pullorum* antigen. Furthermore, there is apparently no connection between the serum agglutination and the albumin agglutination tests.

TABLE 17.

Infection of chicks by B. pullorum given by mouth.

Dose.	*Result.*
1·0 c.c.	D 2 P.M.T.
0·1 c.c.	D 2 ,,
0·01 c.c.	D 3 ,,
0·001 c.c.	D15 ,,
0·0001 c.c.	D15 ,,
0·00001 c.c.	D 9 ,,

Day old chicks were used. Culture given by mouth.
D 2 =Died second day.
P.M.T. =B. pullorum recovered from tissues of chick showing diarrhœa during life.

TABLE 18.

Agglutination reaction.	*Intradermic reaction.*	*No. of Hens.*
+	+	16
−	−	17
±	+	3*
+	±	2
+	−	1

*One of these hens died: B. pullorum was isolated from the ovary.
+ =positive reaction.
− =negative ,,
± =doubtful ,,

TABLE 19.

Culture.	*Serum.*	*Result.*
0·1 c.c.	1·0 c.c. P	L
0·1 c.c.	0·5 c.c. P	L
0·25 c.c.	1·0 c.c. P	L
0·25 c.c.	0·5 c.c. P	D 3
0·1 c.c.	1·0 c.c. N	D 3
0·1 c.c.	0·5 c.c. N	D 3
0·25 c.c.	1·0 c.c. N	D 3
0·25 c.c.	1·0 c.c. N	D 3
0·1 c.c.	—	D 2
0·25 c.c.	—	D 2

P =Pullorum serum. N =Normal fowl serum.
Culture and serum inoculated same time, separately.
From the tissues of all dead chicks B. pullorum was obtained.

Experiments show that protection can be got by the use of an anti *B. pullorum* serum. We are investigating this point further and making supplies of anti-pullorum serum. Tables 19 and 20 show the results of two such experiments.

TABLE 20.

Culture.	*Serum.*	*Result.*
0·01 c.c.	0·5 c.c. P	D 3
0·01 c.c.	0·5 c.c. P	D 3
0·001 c.c.	0·5 c.c. P	L
0·001 c.c.	0·5 c.c. P	L
0·0001 c.c.	0·5 c.c. P	L
0·0001 c.c.	0·5 c.c. P	L
0·01 c.c.	0·5 c.c. N	D 3
0·001 c.c.	0·5 c.c. N	D 4
0·0001 c.c.	0·5 c.c. N	D 4
0·01 c.c.	—	D 2
0·001 c.c.	—	D 4
0·0001 c.c.	—	D 4

L =Lived. D 3 =Died 3rd day.
Serum given subcutaneously and culture by mouth at same time.

Blackleg and Braxy.

You will remember the description of the earlier work carried out here by Allen and Bosworth, described in the *Veterinary Journal*, September, 1924. These authors published tables showing the method of titration of *V. septique* toxin intravenously in mice, and the testing of the toxin against antitoxin and showed that guinea-pigs which had received an immunising mixture of *V. septique* toxin-antitoxin and *B. chauvœi* filtrate, later were immune and withstood lethal doses of *V. septique* or *B. chauvœi* culture. Since the publication of that paper we have slightly altered the method of preparation, with the object of reducing the local reaction, which is now very slight.

Some experiments in the field have been carried out and some are in progress now. Table 21 is the result of an experiment carried out in the North of England during the past winter, against braxy in sheep, each inoculated sheep having received two inoculations.

The protection conferred against braxy in the field seems to be satisfactory.

These experiments we owe entirely to Mr. Howie, M.R.C.V.S., Veterinary Advisor, Armstrong College, Newcastle, who arranged and supervised the whole of the field work and kept in contact throughout with the farmers concerned.

TABLE 21.

Sheep on Farms infected with Braxy: some inoculated with Blackleg Braxy prophylactic; approximately half sheep on each farm uninoculated.

Farm.	*Inoculated.*	*Inoc. dead.*	*%*	*Controls.*	*Controls dead.*	*%*
N	60	2	3·3	60	6	10
H	180	Nil.	—	120	7	5·8
H_2	20	Nil.	—	12	2	16·6
R	80	Nil.	—	80	14	17·5
B	70	Nil.	—	80	5	6·2
C	39	Nil.	—	12	6	50
L	100	Nil.	—	100	32	32
	549	2	0·37	464	72	15·5

Lamb Dysentery.

Lamb dysentery is a disease found affecting young lambs in the North of England and South of Scotland mainly, though it evidently is spreading Northwards and Southwards.

What appears to be a typical attack of the disease, with typical post-mortem picture, we have produced in several ways :—

(1) Feeding a mixture of *B. welchii* and *B. coli* (Table 22—note *B. coli* alone or *B. welchii* alone did not produce the condition) ; (6 occasions).

(2) *B. coli* injected intravenously followed in some hours by *B. welchii* per os (Table 23) ; (3 experiments).

(3) Intravenous injections of ground up ulcers from the intestine (Table 24) ; (4 experiments).

(4) Insertion of ulcers into the umbilicus (one experiment only).

We failed to produce the disease by feeding ground-up ulcers, organs and intestinal contents from infected lambs to healthy lambs (two experiments only).

Two methods of prevention have been carried out in the field (*a*) inoculation of pregnant ewes with antigens made from the organisms isolated from cases of the disease ; and (*b*) injection of corresponding sera into the young lambs. Better results have been obtained from the former method (see Tables 25 and 26).

Much field experimentation on the large scale must yet be done, but the results obtained are most hopeful. All the farms in Table 25 were under constant observation by Mr. Paul, M.R.C.V.S., without whose unlimited keenness and personal generous help, such a result could not have been got. We visited all these farms with Mr. Paul and saw owners and shepherds, and we are satisfied that the "controls" were real controls, exposed to every traceable influence affecting the inoculated ewes.

Table 27 indicates the results of the use of anti-serum on the young lambs on various farms. Further work along these lines is also planned.

TABLE 22.

Lambs	*Culture by mouth.*	*Result.*
1	50 c.c. B. coli +50 c.c. B. welchii	D 2 P.M.T.
2	20 c.c. B. coli +20 c.c. B. welchii	D 3 ,,
3	100 c.c. B. coli	L
4	100 c.c. B. welchii	L
5	40 c.c. B. coli	L
6	40 c.c. B. welchii	L

L =Lived. D 2 =Died on 2nd day.
P.M.T. =Ulcers in the bowel of lambs which, during life, had shown diarrhœa or dysentery.

TABLE 23.

Lambs	B. Coli (Injected i.v.)	B. welchii (by mouth).	*Result.*
1	2 c.c.	60 c.c.	D 2 P.M.T.
2	2 c.c.	50 c.c.	D 3 ,,
3	2 c.c.	50 c.c.	D 3 ,,
4	2 c.c.	—	L
5	—	50 c.c.	L

L =Lived. D 2 =Died 2nd day.
P.M.T. =Ulcers in the bowel of lambs which, during life, had shown diarrhœa or dysentery.

TABLE 24.

Lambs	*Inoculated i.v.*	*Result.*
1	1·0 c.c. ground up ulcer ...	D 2 P.M.T.
2	0·1 c.c. ,, ,, ,, ...	D 3 ,,
3	0·5 c.c. ,, ,, ,, ...	D 1 ,,
4	1·0 c.c. ,, ,, ,, ...	D 2 ,,
5	2 c.c. mucous membrane scrapings normal lambs bowel	L

L =Lived. D 2 =Died 2nd day.
P.M.T. =Ulcers in bowel of lamb which, during life, had shown diarrhœa or dysentery.

TABLE 25.

Farms we visited : controls in same flocks with inoculated : many post-mortem examinations. Daily records kept.

Farm.	*Lambs born from inoculated ewes.*	*Lambs died.*	%	*Lambs born from non-inoculated ewes*	*Lambs died.*	%
A	240	4	1·66	400	80	20·00
B	120	5	4·17	110	39	35·45
C	52	0	0	685	140	20·44
D	102	3	2·94	432	134	31·02
	514	12	2·4	1627	393	24·1

Ewes on Farms A and B had two inoculations.
Ewes on Farms C and D had one inoculation.

TABLE 26.

Controls partly in adjoining fields. Figures not rigidly kept : few post-mortem examinations done.

Farm.	*Lambs born from inoculated ewes.*	*Lambs died.*	%	*Lambs born from non-inoculated ewes*	*Lambs died.*	%
E	197	14	7·11	540	128	23·7
F	110	2	1·82	200	25	12·5
	307	16	5·2	740	153	20·7

Ewes on Farm E had one inoculation.
Ewes on Farm F had two inoculations.

TABLE 27.

Farm.	*Lambs inoculated.*	*Lambs died.*	%	*Lambs not inoculated.*	*Lambs died.*	%
A	80	35	43·75	70	47	67·14
B	140	10	7·14	95	67	70·53
C	500	19	3·80	200	25	12·5
	720	64	8·9	365	139	38·8

Lambs inoculated once as soon after birth as possible.