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Publication/Creation

London : Wellcome Physiological Research Laboratories, [1914?]

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SOME FURTHER EXPERIMENTS IN
THE PREVENTION OF BOVINE
EPIZOOTIC ABORTION

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(Reprinted from "The Veterinary Journal," October, 1914)



From

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
BROCKWELL HALL
HERNE HILL
LONDON, S.E.



SOME FURTHER EXPERIMENTS IN THE PREVENTION OF BOVINE EPIZOOTIC ABORTION.

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FOLLOWING some laboratory experiments carried out with a view to preventing contagious abortion of cows, and a short series of field experiments, I read a brief paper before the Eastern Counties' Veterinary Society at Colchester. The results of these experiments were recorded, and it was pointed out that on account of their small number and the meagre data then available no definite conclusions should be drawn from the results. At the same time I appealed to the members present to afford me by their co-operation an opportunity of further investigating the prophylactic properties of a vaccine, the details of which were given. As a result of that appeal several practitioners volunteered to provide the clinical material and to test the properties of this vaccine. The preliminary report briefly outlined the results of the following experiments carried out with rabbits, goats, and cows.

RABBIT EXPERIMENTS.

Twenty-four full-grown, non-pregnant female rabbits received weekly injections of a saline suspension of *Bacillus abortus*, which had been killed by heating for one hour at 60° C. Three injections were given, the dose being 5, 10, and 25 thousand million organisms. From three to four weeks after the last injection the animals were served, and all were infected by means of a saline suspension of living organisms which had been grown for twenty-four hours on surface glycerine agar. All were infected forty-eight hours after coitus. Of the twenty-four animals six were infected by the intravenous injection of 0.5 c.c. of a rich suspension of living organisms and all aborted during the third week of pregnancy.

Nine were infected by the administration of 2 c.c. of the same suspension *per os* and all went full time and produced normal young, while of three normal control rabbits two aborted and one produced normal young. Nine protected rabbits were infected *per vaginam*, and of these eight produced normal young

while one aborted. Three normal control rabbits infected in a similar way all aborted.

Twenty adult non-pregnant female rabbits were infected twenty-four hours after coitus, ten *per os* and ten *per vaginam*. Eight from each of these two groups received subcutaneous injections of killed *B. abortus* in similar doses to the twenty-four rabbits originally protected. Four rabbits, two from each group, were kept as controls.

Of the eight rabbits infected *per os* all produced normal young, while of those infected *per vaginam* five produced normal young and three aborted.

Of the four control rabbits two were infected *per os* and two *per vaginam*. One of the two infected *per os* aborted and one produced normal young, while the two infected *per vaginam* aborted. The results obtained from these rabbit experiments were obviously not entirely a success, for, of the rabbits protected and subsequently infected *per vaginam*, while eight produced normal young one aborted; and of the three control rabbits infected *per os*, one produced normal young while two aborted. Of the twenty infected immediately after coitus and subsequently protected, while the eight infected *per os* all produced normal young, three of the eight infected *per vaginam* aborted. It would appear, therefore, that infection *per os* in the rabbit, even when a strain of the organism pathogenic for that animal is employed, is not entirely satisfactory. Intravenous infection, in spite of previous attempts at protection, produced abortion in every case.

These results were, however, sufficiently interesting to justify a continuation of the experiments with other animals.

GOAT EXPERIMENTS.

Goat 1.—One adult non-pregnant female goat received three subcutaneous injections of a saline suspension of killed *B. abortus* in doses of 50, 100, and 200 thousand million organisms at intervals of seven days. Seven weeks later she was served, and two months after coitus was infected *per vaginam*. She went full time and produced one normal young. The complement-fixation titre, which previous to the immunization equalled 0, was found to rise steadily during the injections, and to have reached the maximum one month after the last injection, then being equal to 0.0025. It then fell gradually and immediately before the animal

was infected was equal to 0.005. Four days after the injection of living material into the vagina it equalled 0.0001. Three weeks after the birth of the kid the complement-binding titre had again fallen to 0.005 and the agglutinating titre was 1 in 1,000.

Goat 2.—An adult female was protected in a similar manner. Two months after coitus she was infected *per os*. She went full time and produced two fully developed young, which were breathing when delivered, but owing to the length of time which elapsed during parturition (this being the first time that the animal had been bred from, and the kids large in proportion to the mother) subsequently died. The complement-binding titre showed in this case also a somewhat similar curve.

After the injections the titre was found to have risen from 0 to 0.0025. Two months later it had fallen to 0.01, and seven days after infection *per os* rose to 0.0025. Two months after parturition it had fallen to 0.2.

Goat 3.—An adult female was protected in a similar way to goats 1 and 2. Two months after coitus she was infected by an intravenous injection of living organisms, and forty-eight hours later she aborted.

Goat 4.—An adult female was employed as a control. One month after coitus she was infected *per vaginam* and aborted six weeks later.

Although the animals used in these experiments were few in number the results were encouraging. While the animal which was protected and subsequently infected by the intravenous injection of living organisms aborted, those infected by large doses by the ordinary channels of entry of the organism resisted infection and the control animal aborted. Four different strains of *B. abortus* were used for protection, and a fifth and different strain for purposes of infection. In no case was it found possible to obtain cultures of the organism from the uterus of protected animals which had produced young; but since it was not possible to recover the organism in every case from animals which had aborted, one was not able to attach much significance to this fact.

COW EXPERIMENTS.

These experiments were carried out on farms on which contagious abortion was known to exist, and the veterinary surgeons in attendance were kind enough to inject the material with

which I supplied them for the protection of the animals, and subsequently to furnish me with reports. My thanks are due to Mr. Warren, F.R.C.V.S., of Hadlow, who has helped to furnish me with many important facts concerning these experiments.

Farm 1.—The serum of eleven cows was tested by both complement-fixation and agglutination methods, and four were found to react, the titres being 0·02 and 1/25, 0·05 and 1/1,000, 0·1 and 1/300, and 0·02 and 1/500. Each of the cows received three subcutaneous injections of a saline suspension of killed *B. abortus*, the doses being 75, 150, and 300 thousand million organisms. One reactor was later slaughtered as she was in good condition, and the other three calved normally. At that time five of the seven non-reactors had calved normally and the remaining two had not then gone full time. Since then Mr. Warren has informed me that the two remaining cows have calved normally. In this case, therefore, normal calves have been obtained from reactors which had aborted and others which had not, and also from animals which gave a negative reaction to the combined serum tests.

Farm 2.—There were on this farm thirteen cows in all, eight old cows and five recent purchases. All the old cows had been on the farm two years, and the owner stated that new cows invariably aborted. Of the eight old cows, four had aborted the previous calf. All the old cows and one new one showed complement-binding titres to *B. abortus*.

As in the case of Farm 1, all the cows were protected and received similar doses. At the time of making my first communication eleven cows had calved normally and two had not then gone full time. Since then all the cows have calved normally, it being the first time to the owner's knowledge that such had occurred.

Through the kindness of Mr. Arthur Holl, M.R.C.V.S., New Buckenham, I have been able to carry out further experiments by attempting the immunization of two herds. The clinical diagnosis of epizootic abortion was confirmed by a series of complement-fixation and agglutination tests carried out with the blood from several animals in each of the herds, the first of which consisted of forty-eight animals and the second of one hundred animals. So far time only permits of a definite result

being obtained from the protection of the first of these herds, of which Mr. Holl reports as follows:—

“ A client, who owns a herd of forty-six cows and two bulls, had during the past two years greatly suffered from abortion in his cows. I was asked to go and look at these cows, and he showed me five cows which had recently aborted, one of these five having aborted three times in succession. I advised him to have the blood from these cows tested, to which he agreed. I sent the blood of these five cows to Mr. J. B. Buxton to be tested. I received a letter from him in the course of a day or two saying that the five samples of blood gave positive reactions to both agglutination and complement-fixation tests and showed very high titres in both. Mr. Buxton advised me to inject the herd with vaccine, which I did, and up to the present five cows have calved normally and not a single one has aborted. I have just started to protect another herd of cows, and shall be pleased to give the result later on. August 25, 1914.”

In these experiments a slight modification has been adopted in the method of immunization, the course being extended to five injections at intervals of one week, and increasing from a primary dose of 10 thousand million organisms in 10 c.c. to a final dose of 500 thousand million organisms in 25 c.c. of saline.

Similar experiments were carried out with a herd of seventy animals in conjunction with Mr. Ledger, M.R.C.V.S., of Redhill. Mr. Ledger reports that whereas, previous to the protection of the cows, the percentage of abortions had been very high and had entailed a serious loss to the owner, no abortions had occurred since the injection of the vaccine. More detailed results of experiments carried out with this and other herds will be furnished in a further communication at a later date. While the results obtained so far are eminently satisfactory, one must admit that the element of coincidence has not entirely been eliminated. It is, however, hardly to be expected that coincidence would account for the fact that on three farms controlled by three practitioners, the abortions should cease immediately after the course of immunization. The evidence would have been more conclusive had it been possible to have left some of the animals unprotected to act as controls, as in the case of the laboratory experiments, but owners are not anxious to risk the loss of

even one calf when there appears to be some hope of preventing it. The good results attending the experimental work may be ascribed partly to the fact that the disease is localized and of a subacute or chronic nature, thus rendering the effective elaboration of antibodies more marked than in conditions of a more acute nature, and also that very large doses of a polyvalent vaccine were employed. The dosage was based entirely upon experience gained in the immunization of other animals against various organisms for the production of specific anti-sera, and as a result of this and other experimental immunization one has been forced to the conclusion that in veterinary work, certainly, no definite dosage can be laid down for the effective elaboration of antibodies in more than one disease. Experience obtained from experimental work alone enables one to decide when and by what means the most efficient response on the part of the animal organisms can be induced, and it is only by obtaining the maximum efficiency that completely successful results can be produced. The enormous economical significance of a successful method of preventing contagious abortion among cows is obvious, and it is with a view to carrying out further research on the lines already adopted that I confidently make an appeal to members of the profession, in whose practices this disease occurs, to aid me by their co-operation in establishing the efficacy of this vaccine in the eradication of the complaint.

CONCLUSIONS.

From the foregoing experiments it would seem that there are reasonable grounds for assuming that it is possible to produce in susceptible animals a sufficiently high degree of immunity against *B. abortus* by means of suitable doses of a vaccine composed of killed organisms. The figures at present available are not sufficiently numerous to permit of a definite assertion being made. In view, however, of the many advantages which this method of protection possesses over that entailing the use of living organisms in which the element of risk of infection from the vaccine has to be taken into consideration,* further work in this direction is of the utmost importance.

A further communication supplying a more complete set of figures will be made at an early date.

* SURFACE, F. *American Veterinary Review*, September, 1913.



