STUDIES ON CONTAGIOUS ABORTION

by

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INTRODUCTION

This thesis includes a review of the more important literature relating to contagious abortion or infectious abortion that was available, and particularly that dealing with experimental researches on the subject. In addition to this, a somewhat detailed report and discussion is given of certain phases of the experimental work on contagious abortion which has been carried out for the past three years or more by the Department of Veterinary Science of the Experiment Station, in cooperation with the Department of Dairy Husbandry; and in which work the writer has assisted in carrying out the experimental details. This research was instituted to develop more definite knowledge regarding the nature of contagious abortion; and particularly in regard to how the disease may be prevented and eradicated by handling the herd more intelligently.

In these researches the complement fixation test has been used for the detection of abortion infection, as it was regarded as being somewhat more delicate than other tests, and in the cases of mild infection, probably more reliable. In the large experience of this laboratory in the field work of testing numerous breeding herds, the complement fixation test has proven to be a satisfactory method; practically always checking with the clinical history of the animals tested. And, when once under way, the test gives but little or no trouble to the experienced technician. The writer believes that the following pages will show some substantial additions to the knowledge of contagious abortion, as the result of the experimental work done by his co-workers and himself.

HISTORICAL.

Before giving details and conclusions of the specific work, it will be well to give a brief review of the literature, especially that which presents experimental data.

Etiology.

The infectious nature of contagious abortion had been suspected for more than one hundred years, but it was not until the latter half of the nineteenth century that experimental proof of this fact was obtained when Frank, Lehnert and Brauer, between 1876 and 1880 succeeded in infecting healthy cows by introducing into their vaginas the discharges from aborting cows. In 1880, Nocard isolated a micro-coccus and a thick bacillus from the uterine exudate of an aborting cow but failed experimentally, to infect pregnant cows by inoculating them with pure cultures of these micro-organisms. In the summer of 1888 Paquin and Connaway¹ of the Missouri Station isolated a "diplo-coccus" from the placental scrapings of an aborting cow and were able to produce severe straining in a five months pregnant cow three days after an injection into the vagina of pure cultures of these micro-organisms. This straining was due to acute vaginitis as revealed at autopsy. The cow failed² to expel the foetus. Nearly ten years later, 1897, Bang of Copenhagen published the first account of his findings in investigating the bacteriology of the disease. He succeeded in isolating a small cocco-bacillus from the exudate found between the foetal membranes and the wall of the uterus of a cow showing signs of impending abortion. He was able

Notes:-References followed by the mark(X) are taken from abstracts in the Experiment Station Record.

1.Mo.State Bd. of Agr.Monthly Bul. Vol.3 (1904).

2.Jour. Comp Path. and Thera. 10(1897) No. 1 p.p. 125-149.

to grow this bacillus in pure culture and to cause abortion in cows experimentally infected with these cultures. He was again able to isolate the bacillus from the exudates associated with these experimentally induced abortions.

While it was recognized that the bacillus found by Bang was the real cause of the disease, it was not isolated in England until 1909 and in America in 1910. The failure on the part of bacteriologists to isolate the Bang bacillus is due largely to its peculiar habit of growth, especially as regards its behavior toward oxygen. In 1910, MacNeal and Kerr¹ published the results of their experiments in which they succeeded in isolating the bacillus from infected placentas of aborting cows. Subcutaneous injections of pure cultures of these micro-organisms caused abortion in guinea pigs and it was possible to recover the bacillus from the uteri and placentae of these animals following abortions. Since that time numerous investigators have been able to isolate and grow pure cultures of the Bang bacillus.

Diagnosis.

Early investigation in contagious abortion was greatly handicapped by lack of an accurate method of diagnosis. The early writers recognized two types of abortion disease, namely sporadic and infectious. The former type was recognized in herds where only an occasional abortion occurred. These isolated cases were regarded as being due to faulty feed, accident, or to some other disease. When abortion went

References:-

1. Jour. Infec. Diseases 7 (1910) No. 3 p.p. 469-475.

through an entire herd causing great and sustained losses year after year, the infectious type was recognized. This costly method of diagnosis was for many years the only sure sign. In 1897, Nelson¹ stated that there was no sure means of diagnosis and hence every case of abortion should be regarded as infectious until proven to be otherwise. Harger², in 1898, noted that the exudate from cows aborting from infection with the Bang bacillus was odorless, thus differing from ordinary metritis. The examination of this characteristic, catarrhal, uterine exudate was then used as a means of diagnosis. With the discovery of the Bang bacillus, the microscopic examination of the uterine exudates and the making of cultures from the same source often gave positive evidence of the true nature of the malady. While all of these methods gave a general idea of the condition of a herd, it was impossible to tell whether a given animal was infected until actual abortion took place.

When Widal³, in 1896, worked out the details of the agglutination reaction, and Wasserman⁴, in 1906, perfected the complement fixation test, a new field was opened for accurate diagnosis of abortion disease by means of blood tests. Moore⁵ states that while present in the laboratory of Sir John McFadyean during the summer of 1908, he found him using the complement fixation test for the diagnosis of abortion in cattle, and at that

References:-

1. N. Jer. Sta. Rpt. 1897 p.p. 248-250.
2. Jour. Comp. Med. and Vet. Archives 19(1898) No. 4 p.p. 226-231.
3. Bull. de la Soc. Med. des hopit. vi. 1896 (1912 p. 421) Hiss and Zinsser Text Book of Bacteriology
4. Deutsch Med. Wochenscher, 1906. (Bölduan Immune Sera 1908).
5. Rpt. N.Y. State Vet. College 1912-1913 p.p. 82-114.

time McFadyean was very enthusiastic in its favor. While the priority of the use of the complement fixation test probably belongs to McFadyean, the first published account of its use¹ is given by Holth of the laboratory of Jensen in Copenhagen, in 1909. Holth also found the agglutination reaction to be² of value in the diagnosis of the disease. Zwick, in 1910, found both tests to be reliable means of diagnosis.

In America, the first use of the complement fixation test for the diagnosis of abortion disease in cattle was reported³ by Larson in October 1911. He had become familiar with these reactions in the laboratories of Bang and Holth in Denmark. By means of an antigen prepared from a culture of the Bang bacillus received from Holth, he was able to get positive complement fixation reactions with the blood serum of aborting cows. This work indicated that the American and European abortion diseases were the same.

The extreme delicacy of the agglutination reaction, in certain cases, was shown by Brull who used this test alone in his⁴ diagnostic work. He found that the serum of cows that aborted from infection with the Bang bacillus, agglutinated in dilutions from 1-64 up to 1-16000. Serum from non-aborting cows never agglutinated in dilutions greater than 1-64 and in most cases in a much lower dilution. In 1912, McFadyean and Stockton⁵ reported that the tests had, in their estimation, passed

References:-

1. Berlin Tierarztl Wehnschr. 25 (1909) No. 37 p.p. 686-687.(X)
2. Ztschr. Immunitätsf. u. Exp. Ther. 11 (1910) No. 7 p. 810.
3. Jour. Infec. Diseases 10 (1912) No. 2 p.p. 178-185.
4. Berlin Tierarztl. Wehnschr. 27 (1911) No. 40 p.p. 721-727.(X)
5. Jour. Comp. Pathol. and Thera. 25 (1912) No. 1 p.p. 22-38.(X)

the probationary stage and should be adopted as reliable means of diagnosis.

¹
Wall reports the results of his work on nearly 1100 cattle in which he used both tests in diagnosis. He considers as a positive reaction, in either test, one obtained with .05 c.c. of the suspect's serum. A few of the bulls in the barns from which his samples were taken also gave a positive reaction to the tests. Klimmer and Wolff, in 1911, and Miessner, Schrieber and Hantsche in 1912, also reported the successful use of these reactions in diagnosing contagious abortion. Belfanti was able to get a positive complement fixation and agglutination reactions with the serum of aborting cows, using *Bacillus abortus* as an antigen. From these results he concluded that the abortion disease in Italy was also caused by the Bang bacillus.

In this country, the first extensive publication on the use of the complement fixation reaction for the diagnosis of contagious abortion is from the laboratory of Hadley and Beach of the Wisconsin Experiment Station. This publication, dealing with the technical use of the test, was published in June, 1912.

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1. Ztschr. Infek.u. Hyg.Haust.10(1911)Nos.1 p.p. 23-55. 2-3 p.p. 132-160.(X).
- 2.Handbuch der Serumtherapie und Serum diag. in der vet.med. Liepsic 1911 Vol.2.(X).
- 3.Berlin Tierarztl. Wehnschr. 28 (1912) No. 19 p.p. 316-317.(X)
- 4.Ibid.No. 45 p. 827.(X)
- 5.Inaug.Diss.Tierarztl.Hochsch.Dresden 1912 p.47.(X)
- 6.Ztsch. Infektionskrank.u.Hyg.Haust.12(1912)No.1 pp.1-25.(X)
- 7.Wis.Sta.Research Bul.24.

In the same month, Surface of the Kentucky Station published a bulletin in which he compares the two tests. He concludes that the complement fixation test is the best and most accurate means of diagnosis that has yet been devised. He believes that the agglutination test used alone is not always reliable. Following this work, many other investigators have had excellent results with either or both of these tests.

Of the other methods of diagnosing contagious abortion, the use of "abortin" has received the most attention. This material is essentially an extract of the *Bacillus abortus* used in a manner similar to the use of the tuberculin. Zwick, in 1910, reported that he was using such a test but gave no experimental data in regard to its use. Brull found that "abortin" gave unsatisfactory results as a diagnostic agent. McFadyean and Stockman, who seem to have done the greatest amount of experimental work on the use of this reagent, stated that their results were not sufficiently conclusive to cause them to recommend its use. In 1912, Schreiber, and independently, Belfanti, found it to be of doubtful value as a diagnostic agent. In this country, Giltner found "abortin" to have "narrow limitations" as a means of diagnosis. In a later publication, he states that although "abortin" has some value in diagnosis, the product needs refining

References:-

1. Kentucky Sta. Research But. 24.
2. Ztschr. Immunitätsf. u. Exp. Ther. 11, 3 (1910) No. 7 p. 810.
3. Berlin Tierarztl. Wchnschr. 27 (1911) No. 40 p. p. 721-727. (X)
4. Abstract Exp. Sta. Rec. 22 (1910) No. 6 p. p. 584-586.
5. Berlin Tierarztl. Wchnschr. 28 (1912) No. 45 p. 827. (X)
6. Ztsch. Infek. u. Hyg. Haust. 12 (1912) No. 1 p. p. 1-25. (X)
7. Amer. Vet. Rev. 42 (1912) No. 2 p. p. 145-156.
8. Jour. Amer. Vet. Med. Assoc. 49 (1916) No. 3 p. p. 320-339.

before best results can be expected.

Of other methods little work has been reported. Hantsche¹, in 1912, reported using the ophthalmic reaction in which he found that 52 per cent of cows known to be infected gave a positive reaction. Mohler and Traum² report another test used by Holth. He found that by injecting the exudate from an aborting cow into a rabbit, the rabbit's serum was made to give a noticeable increase of agglutinins and bacteriolytic amboceptors after an interval of seven days.

Transmission.

Numerous investigators including Bang, McFadyean and Stockman, Nowak, Holth and many others have been able to produce abortion, experimentally, in cows, mares, ewes, goats, rabbits, guinea pigs, bitches, monkeys, rats and mice. While this is possible experimentally, most of the evidence shows conclusively, that cattle are the only animals naturally susceptible to any great extent. Numerous channels of infection have been tried, but chiefly the sub-cutaneous, intravenous, vaginal and oral.

Transmission by the bull.

Following the work of Frank, Lehnert and Brauer, investigators commonly recognized the bull as the chief means of spreading the infection of abortion disease. This viewpoint is still held by many although several fairly conclusive experiments have proven that the bull is less commonly the cause of the spread of infection than other agents. Harger, as early as 1898, stated

References:-

1. Inaug. Diss. Tierarztl. Hochsch. Dresden, 1912 p. 47. (X)
2. U.S.D.A. Bur. An. Ind. Rpt. 1911 Cir. 216 p.p. 137-183.
3. Jour. Comp. Med. and Vet. Archives 19(1898) No. 4 p.p. 226-231. (X)

that the bull suffered a specific urethritis and from this focus, infected cows during copulation. Nelson also concluded from his experience that the bacilli entered the uterus simultaneously with the spermatazoa and set up an infection with the formation of the embryo. Bang's early work indicated that the bull was a major source of infection although he recognized cases that could not be traced to this source. A.E. Moore regarded the bull as a major source of infection and stated that he had known cases in which the bull had spread abortion to an entire neighborhood. Wilson of the Arizona Station, reported the spread of abortion from the college herd to nearby herds through the agency of the bull. He concluded that by the use of sanitary precautions at the time of service, this danger could be avoided. In the same year C.L. Beach of the Connecticut Station found that the bull was not the only means of spreading infection. In his observations, he noted that abortions continued where clean bulls from remote districts were used in service. Again, as late as 1915, Schroeder stated that the bull was liable to harbor and spread abortion infection. However, two years later he and Cotton stated that they were led to believe that the bull was not a very important factor in the spread of the disease. They were brought to this conclusion through failures to infect bulls, experimentally, or by natural exposure. Hadley and Lothe have done the great-

References:-

1. N.J. Sta. Rpt. 1897 No. 1 p.p. 248-260.
2. Jour. Comp. Pathol. and Thera. 10 (1897) No. 1. p.p. 125-149.
3. Amer. Vet. Rev. 28 (1904) No. 8 p.p. 743-747.
4. Ariz. Sta. Bul. 57 p.p. 279-283.
5. Conn. Storrs Sta. Rpt. 1907 p.p. 139-141.
6. Jour. Amer. Vet. Med. Assoc. 48 (1915) No. 3 p.p. 304-310.
7. Amer. Jour. Vet. Med. 12 (1917) No. 2 p.p. 73-78.
8. Jour. Amer. Vet. Med. Assoc. 50 (1916) n.s. 3 No. 2 p.p. 143-156.

est amount of experimental work toward solving this question. They conclude that it is possible for a bull to become infected with the abortion bacillus but that, in their experiments, bulls so infected were "incapable of spreading the disease to virgin, abortion-free heifers"; that bulls appear to have a sexual or individual immunity rendering them more resistant than cows and giving a more benign form of the disease. They further concluded that bulls had the ability to attenuate and thus render less virulent any abortion bacilli with which they may be infected. They were led to believe that cows usually acquired the disease through other sources than the genital system of the bull. These experiments as well as those of Schroeder and Cotton, are especially valuable in that they were checked by the use of serological tests on all animals in the experiments.

Vaginal Infection.

Other possibilities of infection through the vagina have¹ been investigated by various persons. McFadyean reported the results of the first experimental work conducted in great Britain. This work was conducted in 1887 by the Committee of the Highland and Agricultural Society of Scotland. They were able to infect, experimentally, cows and ewes by introducing into their vaginas contaminated cotton wool plugs and discharges² from aborting cows. A.E. Moore and MacFadyean have also suc-³ceeded in causing abortion in cows by introducing this natural

References:-

1. Jour. Royal Agr. Soc. England 62(1901)p.p. 96-112.
2. Amer. Vet. Rev. 28(1904) No. 8 p.p. 743-747.
3. Rpt. Dept, Com. Bd. Agr. and Fisheries Gt. Britain 1909 pt.1 (X)

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virus into the vagina. Bang was able to first establish the pathogenicity of the Bang bacillus by the intravaginal injection of pure cultures of these micro-organisms into pregnant cows.

Transmission Through Wounds.

The high percentage of cases in which the abortion disease can be experimentally transmitted through the blood stream has raised the question as to whether or not the disease can be naturally acquired through wounds. McFadyean² found that while experimental infection could be produced through subcutaneous injections with the Bang bacillus, the amount of infectious material required was so great as to exclude the danger of natural infection through this channel. Schroeder and Cotton³ found that subcutaneous injection with the Bang bacillus caused a passive production of antibodies but might or might not produce abortion. Mohler and Traum⁴ state that while it is possible to produce abortion through subcutaneous injection, this is purely an experimental method and should not be considered seriously as a natural means of infection.

Transmission Through the Respiratory and Alimentary Tracts.

The possibilities of infection through the respiratory and alimentary tracts have received considerable attention. In 1900, S.P.Smith⁵ in summing up the knowledge of the disease

References:-

1. Jour. Comp. Pathol. and Thera. 10(1897) No.1.p.p. 125-149.
2. Rpt. Dept. Com. Bd. Agr. and Fisherish Gt. Britain 1909 Pt.1 (X)
3. Amer. Jour. Vet. Med. 12 (1917) No.2 p.p. 73-78.
4. U.S. Dept. Agr. B.A.I. Rpt. 1911 p.p. 137-183.
5. Jour. Comp. Med. and Vet. Archives 21(1900)No.3 p.p.151-155.

to that time, stated that nothing was known concerning transmission of the disease through these channels. The following year ¹McFadyean stated that possibly infection occurred through these tracts, although no experiments to prove this had ever been tried.

As has been stated, Bang recognized in his early work that in some cases infection through the vagina could not explain abortion. He suggested that in such cases infection possibly took place through the digestive tract. Later he was able to prove this point by producing abortion ²in cows which were fed the virus. McFadyean, in 1909, stated that as a result of his experimental work and that of others, he was led to believe that the ingestion of the virus was more often the cause of in-³fection than any other natural means. Mohler and Traum state that "from the vast number of experimental abortions produced by introduction of virus through the mouth, particularly in animals such as rabbits, where the virus is introduced by tube and infection by vagina can be easily excluded, this means of infection must be given an important place, especially so until satisfactory experimental evidence is brought forward ⁴to the contrary." Moore and Fitch were able to produce abortion and to cause the production of antibodies in the blood of experimental animals by feeding pure cultures of the Bang bacillus. They conclude that "abortion can be produced by feed-

References:-

1. Jour. Royal Agr. Soc. England 62(1901)p.p. 96-112.
2. Rpt. Dept. Com. Bd. Agr. and Fisheries Gt. Britain 1909 pt.1 (X)
3. U.S. Dept. Agr. B.A.I. Rpt. 1911 Cir. 216 p.p. 137-183.
4. Rpt. N.Y. State Vet. College 1912-1913 p.p. 82-114.

ing the virus quite as readily as by intravenous inoculation." ¹
 Schroeder and Cotton suggest the possibility of abortion being transmitted from herd to herd through the feeding of raw, infected milk. This question of infected milk has opened a new field in which much experimental work has recently been done. ²
 As early as 1909, McFadyean stated that it was improbable that the Bang bacillus was eliminated in the milk of infected cows. ³ However, in 1912, the experiments of Schroeder and Cotton and of Mohler, reached the stage where it was possible to prove definitely that the unidentified bacillus which the former investigators had found, through guinea pig inoculations, to be present in milk, was in reality the *Bacillus abortus*. Having thus proven the *Bacillus abortus* to be present in the milk of infected cows, they conducted numerous experiments to determine the relation of infected milk to the transmission of the disease. During this first year, they concluded that the *Bacillus abortus* was eliminated in the milk of infected cows for months and years after the active symptoms of the disease had disappeared. They also concluded, since they found the super-mammary glands to be infected, that the infection traveled through the blood stream from the udder to the uterus. The fact that a cow did not abort did not necessarily mean that she was not eliminating the *Bacillus abortus* in her milk.

References:-

1. Amer. Jour. Vet. Med. 12(1917) No. 2 p.p. 73-78.
2. Rpt. Dept. Com. Bd. Agr. and Fisheries Gt. Britain 1909 pt. 1(X)
3. Jour. Amer. Vet. Med. Assoc. 48(1915) No. 3 p.p. 304-310.

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Reinhardt and Guass were able to get positive results in both serological tests, with milk from infected cows. Cool-
²edge found infected udders in twenty seven per cent of the cows studied on seven farms. He was able to find agglutinins in the milk of infected cows and to trace the spread of infection from one infected quarter to all quarters of the udder, presumably by the hands of the milker. He concluded that these agglutinins were due to a local production in the udder rather than to generalized blood infection. He was brought to this conclusion since he noticed that the quantity of agglu-
³tinins varied in the different quarters. Evans has also found the *Bacillus abortus* to be of common occurrence in market
⁴milk. In a later publication, Schroeder and Cotton reported that, in their experiments, all cows with infected udders reacted to the blood tests. They also found that a cow could be infected through the teat and later react to the blood tests. They also call attention to the possibility of spreading infection by the hands of the milker.

Infection in Calves.

The question of infection in calves, which deals more particularly with the problem discussed in this thesis has not received much consideration until recent years. Bang found that he could cause a bull calf to give a temperature reaction last-

References:-

1. Ztsch. Infek. u. Hyg. Haust. 16 (1915) No. 4 p.p. 219-239. (X)
2. Mich. Agr. College Tech. Bul. 33 Oct. 1916.
3. Jour. Infec. Diseases v. 18 (1916) No. 5 p.p. 437-476.
4. Amer. Jour. Vet. Med. 12 (1917) No. 2 p.p. 73-78.

ing four days by giving him a subcutaneous injection of the Bacillus abortus. ¹McFaydean, Sheather and Minett states that cattle of any age and of either sex might be infected with the Bacillus abortus through natural channels. ²Following the discovery of the Bang bacillus in milk, Williams stated that he had found that calves fed on raw, infected milk reacted to the serological tests. He also stated that, after thirty to fifty days, a matting and staining of the sexual hairs occurred in calves of both sexes. Calves fed on boiled milk failed to show this matting and staining of the sexual hairs, and also failed to react to the blood tests. More recent ³researches, especially those of Giltner, Cooledge and Huddelson have failed to confirm these findings. They state that "there is no connection whatever, between the feeding of infected milk and the matting and staining of the sexual hairs of new ⁴born calves." Huddelson has done more work than others on the infection of calves through the feeding of infected milk. His work brought out the fact that some calves reacted to the blood tests at birth. He states that this fact was evidently not considered in the earlier investigations, From his feeding experiments with infected and non-infected milk, he draws the following conclusions. "Agglutinating and complement fixing bodies for Bact. abortus are very rarely demonstrated in

References:-

1. Jour. Comp. Pathol. and Thera. 26 (1913) No.2 p.p. 142-170 (X)
2. Rpt. N.Y. State Vet. College 1914-1915 p.p. 63-101.
3. Jour. Amer. Vet. Med. Assoc. 50(1916) No 2 p.p. 157-167.
4. Mich. Agr. College Tech Bul. 32 Oct. 1916.

the blood of calves as a result of ingesting naturally infected milk."

"Calves may give a positive reaction to the complement fixation test immediately after birth. The reaction probably signified a pre-natal infection." *

"There is favorable evidence that antibodies circulating in the body of the mother are not transmitted to the fetus in utero."

"The data strongly emphasize the necessity of testing the blood of calves at birth in order to differentiate between positive reactions that may be due to the ingestion of milk and positive reactions that may be present at birth."

"There appears to be no connection, whatever, between the feeding of Milk and the matting and staining of the sexual hairs of new born calves."

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Schroeder and Cotton state that it is not uncommon for calves to react at birth, but that they rarely react after three or four months. This declining power to react is not due to inability on the part of the calf to produce elements on which the reactions depend, since calves injected artificially can be made to give strong positive reactions. They conclude that these reactions are passive phenomena rather than active infection from disease germs present in the body of the calf. In a later publication ², they give the addition-

References:-

- 1.Amer.Jour. Vet. Med. 12(1917) No.2. p.p. 73-78.
 - 2.U.S. Dept. Agr.Jour.Agr. Research 9(1917) No.1 p.p. 9-16.
- *This fact was noted at the Missouri Experiment Station during the summer of 1915 and reported in the An.Rpt. of the Mo.Exp't.Sta. for the year ending June 30,1916.

al information that a calf nursing a mother with a heavily infected udder gradually lost the power of its blood serum to give a positive agglutination reaction. This led them to believe that the ingested abortion bacilli do not penetrate deeply nor abundantly into the calf's body.

TECHNIQUE OF THE COMPLEMENT FIXATION TEST.

The complement fixation test is based on two reactions namely bacteriolysis and hemolysis. If the serum of the animal under test contains specific antibodies which, in the presence of complement, destroy the bacteria or causative factor of their production(antigen), bacteriolysis takes place and these bacteria are destroyed. The second reaction depends on the hemolytic action of a sensitized rabbit's serum used in connection with the normal serum of a guinea pig. This normal serum contains the complement which is necessary to both reactions. Hence, if the serum under test contains the antibodies for which the antigen is specific, the first incubation, which is given with the hemolytic series absent, gives a chance for the antigen and antibodies to combine and fix or deviate the complement. A subsequent incubation with the other factors of the hemolytic series (hemolysin and red cells) present has no effect on the corpuscles, since the complement needed to bring about their solution has already been used. If on the other hand, the serum of the suspected animal does not contain these antibodies, the complement is left free in the solution and in a later incubation with blood cells and hemolysin, hemolysis takes place and the

corpuscles are broken down, liberating the haemoglobin which stains the solution a uniform red. In the former case, we recognize a positive reaction; in the latter, no reaction.

The five components spoken of above are named as follows; (1) Suspect's serum, (2) antigen, (3) complement, (4) washed red blood cells, (5) hemolysin.

Preparation of the Suspect's Serum.

In drawing blood from the animal to be tested, the neck is corded close to the shoulder, thus engorging the jugular vein. At the spot from which the blood is to be drawn, the skin is first washed with a disinfecting solution. A sterile 16 guage hypodermic needle is then thrust into the vein, and from the flow thus created, about 10 c.c. of blood are collected in a sterile 20 c.c. test tube. The tube is then set upright and left over night for the blood to clot. In the morning the clot is broken away from the sides of the tube with a clean knife, and the tube incubated for one half hour at blood heat. The tube is then left in the ice chest for several hours. At the end of this time, a considerable quantity of pure serum has separated from the clot and is ready to be used in the test. It has been found to be unnecessary, in most cases, to "inactivate" the serum before using it in the test. This accords with the findings of Thomsen¹ who stated that inactivation was not only unnecessary, but often harmful. However, we have found that the blood serum of some new born calves contains anti-complements which prevent hemolysis under any conditions. These anti-complements, which naturally disappear from one to four

References:-

1. Ztschr. Infek. u. Hyg. Haust. 13(1913) Nos. 3-4 p.p. 175-179. (X)

weeks, can be destroyed by heating the serum at 56°C . for one half hour.

Preparation of Complement.

The complement, or guinea pig serum, is drawn from the heart by means of an aspirating syringe and a fine needle. The animal is first etherized and placed on its back. The chest wall is then washed with a disinfectant and the sterile needle introduced into the heart. 5 c.c. can be safely drawn at intervals of five days, and if the bleedings are not too frequent, 10 c.c. can be taken from a full grown animal without killing him. This blood can be allowed to clot in a tube and the serum separated as described for preparing the serum of the suspected animal. If the tube is placed in the ice chest overnight, the quantity of serum is greatly increased and complement twelve hours old has been found to give better results than that freshly drawn. The serum is centrifugalized in a graduated tube and the pure serum is diluted with one and a half times its volume of sterile .875 per cent salt solution. It is then ready for titration, the technique of which appears in subsequent pages.

Preparation of the Antigen.

The antigen is prepared from a pure culture of the Bang bacillus. A heavy growth of this organism is obtained on slants of glycerine agar. This growth is then washed off in .875 per cent salt solution and the suspension is diluted to conform to the cloudiness of a fixed standard sample. It is then heated in the water bath to kill the bacilli. 60°C . for one hour is sufficient to bring about this result, after which, the antigen is preserved by adding one half per cent of its volume of phe-

nal solution. The suspension is then titrated in accordance with the technique hereinafter described. Storage in the ice chest in brown bottles will keep it in good condition for several months, although heating at $56^{\circ}\text{C}.$ for one half hour may be necessary, occasionally, to destroy anti-complements which tend to develop in old antigens.

Preparation of Hemolysin.

The hemolysin, or sensitized rabbit's serum, requires special care in preparation. A horse is bled from the jugular vein in the manner described for bleeding the cow. The blood is caught in a sterile Erlenmeyer flask and defibrinated by vigorous shaking. The fluid portion, containing the red and white corpuscles and serum, is then filtered through sterile gauze and placed in large centrifuge tubes. Centrifugalization throws the cells to the bottom of the tube and leaves a layer of clear serum above. The serum is then pipetted off and the layer of white cells on top of the red is also drawn off and discarded. The serum is replaced by an equal volume of sterile .875 per cent salt solution, and the red cells thoroughly churned and mixed by means of a rubber "policeman". This centrifuging and washing with fresh salt solution is repeated four times, or until the solution has lost all traces of serum. This washing is essential, as the presence of horse serum in the suspension of red cells causes the production of anti-complements in the serum of the rabbit, following injection. The washed red cells are then warmed and drawn into an aspirating syringe. At the first injection, 1 c.c. of these washed red

cells is put into the anterior ear vein of the rabbit, care being taken to avoid introducing air bubbles. Seventy two hours later, the second injection of 2 c.c. is given, followed after another seventy two hour interval by a third injection of 3 c.c. After ten days, a small quantity of blood is drawn from the heart, or by severing an ear vein of the rabbit and the serum allowed to separate. It is inactivated by heating at 56°C . for twenty minutes to destroy the complement; then diluted (1 part serum to 50 parts of .875 per cent salt solution) and titrated according to the method described in later pages. If this preliminary titration shows that the hemolytic power is sufficiently great, a larger quantity of blood is drawn and the serum prepared as described for the suspect's serum. The concentrated serum is preserved by the addition of sufficient carbolic acid to make $\frac{1}{2}$ per cent of the total volume of the liquid. The serum is then put in dark bottles and stored in the ice chest. This stock serum (hemolysin) will retain its hemolytic properties for months if properly preserved and stored. The dilute solution used in the test can be made up from the stock solution from time to time as needed. In case the preliminary titration shows the hemolytic properties to be too weak, another injection of 3 c.c. of red cells is made. After an interval of seven days following this fourth injection of blood cells, the hemolytic power of the rabbit's serum has always been found to be of the desired strength.

Preparation of Washed Red Blood Cells.

The cells for use in the test are drawn and washed in the same manner as those used for injecting the rabbit. These concentrated cells are then made up in a two per cent suspension in .875 per cent salt solution and stored in the ice chest. They will keep in good condition for several days if properly prepared.

Before accurate use can be made of the complement fixation test, the exact titre of each component must be carefully determined. By the term titre is meant the least amount of a given component that will bring about the desired reaction when used in connection with the proper amounts of the other components involved. In the case of the components of the hemolytic series, namely complement and hemolysin, the titre represents the least amount of the one that will bring about complete hemolysis of .5 c.c. of a two per cent suspension of red blood cells of the horse, when used with the proper amount of the other component. In titrating the antigen, the chief object is to determine the smallest quantity of the antigen **which** prevents hemolysis of the red cells when other factors of the complement fixation test are present in proper quantities.

Titration of the Complement.

In titrating the complement, .1 c.c of the hemolysin is assumed to be enough to hemolyze .5 c.c. of the two per cent suspension of red cells provided enough complement is

present. Graded amounts of complement are added until this proper amount is determined. The tubes are incubated for one hour at 38°C . before taking the readings. If the fresh guinea pig serum, containing the complement, is diluted one and a half times, the titre will usually fall between .04 c.c. and .06 c.c. and complement with a titre lower than this is discarded. The following table shows the method of making the titration.

TABLE No. 1

TITRATION OF THE COMPLEMENT.

Tube	1	2	3	4	5	6	7
Salt solution:	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Complement	.02	.04	.06	.08	.1	0.0	.1
Hemolysin	.1	.1	.1	.1	.1	.1	0.0
Red Cells	.5	.5	.5	.5	.5	.5	.5
Degree of Hemolysis	?	-	--	--	--	+	+

Notes:-

- + no hemolysis.
- complete hemolysis.
- + partial hemolysis.

Tube 6 is a control on the hemolysin to show that, by itself, it is unable to cause hemolysis. Tube 7 is a similar control on the complement, showing that without the addition of hemolysin it is incapable of producing hemolysis. In the actual test, one and a half times the titre is used.

Titration of the Hemolysin.

Having established the titre of the complement, the exact titre of hemolysin can be obtained by titrating graded amounts of this complement against the established quantity of the complement. The incubation period is the same. Table No. II, given below, shows the method of making the titration. The first tube in which complete hemolysis takes place is taken as the titre. Tubes 6 and 7 are controls as described under titration of the complement. In the actual test, three times the titre of the hemolysin is used.

TABLE No. II.

TITRATION OF THE HEMOLYSIN.

Tube	1	2	3	4	5	6	7
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Salt Solution	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Complement	.1	.1	.1	.1	.1	.1	0.0
Hemolysin	.02	.04	.06	.08	.1	0.0	.1
Red Cells	.5	.5	.5	.5	.5	.5	.5
Degree of Hemolysis	+	-	-	-	-	+	+

Notes:-

- + no hemolysis.
- complete hemolysis.
- ± partial hemolysis.

Titration of the Antigen.

The object of titrating the antigen is to determine the least amount of this component which must be used with a positive serum and proper quantities of the other components

to fix the complement and thus prevent hemolysis. For this work, a known positive reacting serum and a known negative reacting one, for a check, are required. Table III on the following page shows, in detail, the method of making the titration. The first tube in which there has been complete inhibition of hemolysis is taken as the titre of the antigen and in the final test five times the amount of antigen represented by the titre is used.

Tubes 9, 10, 11 and 12 are control tubes to show that large quantities of antigen in the absence of a positive reacting serum will not mechanically prevent hemolysis. Tube 8 contains known negative reacting serum and shows that the combination of serum and antigen will not prevent hemolysis unless the serum contains antibodies which are specific for the antigen used with it.

TABLE NO.III.

TITRATION OF THE ANTIGEN

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Salt Solution	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Complement	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Antigen	.02	.03	.05	.1	.15	.2	.25	.15	.2	.3	.4	.5
Positive Serum	.02	.02	.02	.02	.02	.02	.02	0.0	0.0	0.0	0.0	0.0
Negative Serum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.02	0.0	0.0	0.0	0.0

Incubate at 38°C. for one hour, add the hemolytic series shown below and incubate for another hour at 38°C.

Hemolysin	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Red Cells	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Degree of Hemolysis	+											
	-	+	+	+	+	+	+	-	-	-	-	-

Notes:-

+ no hemolysis.

- complete hemolysis.

± partial hemolysis.

In all cases, the work of making the test is facilitated by so diluting each component as to make .1 c.c. the proper quantity to use. The red cells need no titration as they always remain a constant factor to which the strength of the other components must conform.

Test of the Suspect's Serum.

Having established the titre of each component, the next step is to make the actual test of the suspect's serum. With each series of samples to be tested, two extra control tubes are included. The first of these (tube 1) controls the antigen, showing that it will not mechanically prevent hemolysis. The second is a control on the complement and hemolysin showing that these two components are working together to cause hemolysis. Each sample of serum is also controlled by using that serum without the addition of antigen (tube 3). Failure to hemolyze in this tube indicates the **presence** of anticomplements in the serum and it is necessary to inactivate it before it can be used in the test. Following the first five tubes (2 for the serum and 3 controls) each sample requires three tubes. The first of these is the serum control described above and the last two contain .02 c.c. and .03 c.c. respectively, of the serum under test and all of the other components according to titre. Table IV. on the following page shows the method of making the test.

TABLE NO.IV.

TEST OF THE SUSPECT'S SERUM.

	:control:	:control:	:control:	:	:	:
: Tube :	1 :	2 :	3 :	4 :	5 :	:
:Salt Solution:	c.c. :	c.c. :	c.c. :	c.c. :	c.c. :	:
:	1.5 :	1.5 :	1.5 :	1.5 :	1.5 :	:
:Suspect's	:	:	:	:	:	:
:Serum :	0.0 :	0.0 :	.03 :	.02 :	.03 :	:
:	:	:	:	:	:	:
:Complement :	.1 :	.1 :	.1 :	.1 :	.1 :	:
:	:	:	:	:	:	:
:Antigen :	.1 :	0.0 :	0.0 :	.1 :	.1 :	:
:	:	:	:	:	:	:
Incubate at $37\frac{1}{2}^{\circ}\text{C.}$ for 1 hour						:
:	:	:	:	:	:	:
:Hemolysin :	.1 :	.1 :	.1 :	.1 :	.1 :	:
:Red Blood :	:	:	:	:	:	:
:Cells :	.5 :	.5 :	.5 :	.5 :	.5 :	:
:Degree of hem:	:	:	:	:	:	:
:olysis after :	:	:	:	:	:	:
:second incuba:	:	:	:	:	:	:
:tion :	— :	— :	— :	? :	? :	:

Note:-

— complete hemolysis.

In reading the results of the tests, we recognize four degrees of hemolysis. The control tube is taken as the standard of complete hemolysis, and the other tubes compared with it. The — sign indicates a negative reaction and compares with the control tube in being completely hemolyzed. The + sign indicates a positive reaction, or complete inhibition of hemolysis. By w+ is meant a weak positive reaction. This indicates a slight amount of hemolysis, but still enough inhibition to be called a reaction. The - sign indicates a doubtful reaction. It is used for those samples which give a slight inhibition of hemolysis, although not enough to be called a definite reaction. The sign following the $\frac{1}{2}$ mark

indicates which way the suspicion lies. Thus $\frac{+}{-}$ + indicates that the sample is probably from an infected animal, while $\frac{+}{-}$ — indicates the opposite condition.

EXPERIMENTAL WORK.

The experimental work included in this thesis forms a portion of an experiment which is being carried out in the Experiment Station by the Veterinary Department in cooperation with the Dairy Department, for the purpose of developing more definite knowledge regarding the nature of contagious abortion in cattle. In the thesis, the following points will be considered:-

- (a) "To determine whether cows that become infected with the *Bacillus abortus* retain the infection permanently, and give a constant positive reaction to the complement fixation test, or whether the infected cows under favorable conditions may destroy and eliminate the abortion germs and become negative reactors."
- (b) "To determine to what extent the infection of contagious abortion is transmitted to the progeny in utero, or after birth through infected milk, by positive reacting dams with a history of previous abortions."
- (c) "To determine whether the positive reacting calves, dropped at full term and in apparently good health by immune, reacting dams, retain the infection in the system in a dormant condition to become active at breeding time, or whether the infection is eliminated by the calf, and the positive reac-

tions that appear later, after the heifers are bred, are due to a reinfection."

(d) "To determine the practical value of the complement fixation reaction in the detection of abortion infected bulls. And to what extent the bull is responsible for the transmission of the infection either as a permanent (systemic) or accidental infection carrier."

The original idea was to carry out the work on a definite group of cattle including all new born calves. As the experiment progressed, however, the need for more data was seen until at the present time, the entire herd of over one hundred animals is on monthly test. Each new calf has been tested as soon after birth as possible and regular monthly tests have been made thereafter. An analysis of the experimental data is given below.

PERSISTENCE OF INFECTION IN AGED COWS ON EXPERIMENT.

In these experiments, monthly tests showed that certain cows which when first tested were found to be strong positive reactors to the complement fixation test, persistently retained this reaction over a period of at least three years. This persistence of a positive reaction was probably due to the presence of a focus of infection which stimulated the constant production of the specific antibodies. If we assume that these animals have been positive reactors from the time of the last abortion, i.e. clinical evidence, this period, in some cases, is greatly lengthened. The following Tables (V. and VI.) show respectively, the breeding

records and monthly reactions of a group of persistent positive reacting cows.

TABLE NO. V.

BREEDING RECORDS OF SEVEN POSITIVE REACTING COWS.

: Cow No.:	: 61 :	: 64 :	: 89 :	: 93 :	: 96 :	: 238 :	: 313 :
: Born	: Nov.:	: Apr.:	: Nov.:	: Mar.:	: Aug.:	: Oct.:	: Apr.:
: Date	: '11 :	: '10 :	: '12 :	: '13 :	: '13 :	: '12 :	: '12 :
: 1912	: a :	: :	: :	: :	: :	: :	: :
: 1913	: a :	: :	: :	: :	: :	: :	: :
: 1914	: a :	: n :	: f :	: :	: :	: :	: a :
: 1915	: :	: :	: f :	: n :	: :	: n :	: n :
: 1916	: n :	: n :	: n :	: n :	: n :	: a :	: :
: 1917	: n :	: a :	: n :	: n :	: n :	: n :	: n :
: 1918	: p :	: p :	: k :	: s :	: p :	: s :	: n :

Notes:-

a-- abortion.

n-- normal calving.

f-- failure to breed.

p-- now pregnant.

s-- sold.

k-- slaughtered.

Blanks between calvings indicate long periods of lactation.

Cow No. 61 aborted on September 18, 1914. She showed a positive complement fixation reaction three years, six months and thirteen days after this last abortion. Cow No. 313 aborted September 24, 1914. She also continued to give a positive reaction three years, six months and seven days after this last abortion. Such of these animals as are still in the herd will be tested monthly to determine how much longer this reaction will persist.

Table VI. shows the monthly reactions of these seven animals. While this table shows that cows once strongly infected tend to constantly maintain their positive reactions over a period of at least three and a half years, certain other animals show considerable variation. Some show only an occasional reaction; or react for a period of a few months and then become negative again. Others show a rapid fluctuation, giving a strong positive reaction one month, and negative the next. Still others show a gradual loss of reaction. In such cases the reaction becomes weaker and weaker until it finally disappears entirely. One animal (307 Table VII.) is negative except at the calving period when for two or three months she shows a reaction which gradually dies out to appear again at the next calving period. This has recurred three times. If we accept the statement of Schroeder and Cotton, that the udder is the seat of infection, this fluctuation in the blood reaction may have a possible relation to the fluctuation of *Bacillus abortus* in the udders of certain cows. Concerning the latter, they have found that at certain times the abortion bacilli are numerous in the udders of cows while at other times their presence is not demonstrable. Table VII. shows the reactions of a group of these variable reactors.

TABLE VI
MONTHLY REACTIONS OF POSITIVE COWS

Date of test	61.	64.	89.	93.	96.	238.	313.
Mar. 1915	+	+	+	+	+	+	+
Apr. "	o	o	+	+	+	+	o
May "	o	o	+	+	+	+	o
June "	+	+	+	+	+	+	+
July "	o	o	+	+	+	+	o
Aug. "	o	o	o	o	o	o	o
Sep. "	o	o	+	+	+	+	o
Oct. "	o	o	+	+	+	+	o
Nov. "	o	o	+	+	+	+	o
Dec. "	o	o	+	+	+	+	o
Jan. 1916	o	o	o	o	o	o	o
Feb. "	o	o	+	+	+	+	o
Mar. "	o	o	+	+	+	+	o
Apr. "	o	o	+	+	+	+	o
May "	o	o	+	+	+	+	o
June "	o	o	+	+	+	o	o
July "	o	o	+	+	+	+	o
Aug. "	o	o	+	+	+	+	o
Sep. "	o	o	+	+	+	+	o
Oct. "	o	o	+	+	+	+	o
Nov. "	+	+	+	+	+	+	+
Dec. "	+	+	+	+	+	+	+
Jan. 1917	o	o	+	+	+	+	o
Feb. "	o	o	+	+	+	+	o
Mar. "	o	o	k	+	+	+	o
Apr. "	+	+		+	+	+	+
May "	+	+		+	+	s	+
June "	+	+		+	+		+
July "	+	+		s	+		+
Aug. "	+	+			+		+
Sep. "	+	+			+		+
Oct. "	+	+			+		+
Nov. "	+	+			+		+
Dec. "	+	+			+		+
Jan. 1918	+	+			+		+
Feb. "	+	+			+		+
Mar. "	+	+			+		k

Notes:-

k---slaughtered

s---sold

o---no test made

⊕---positive reaction (animal infected)

⊖---negative reaction (animal not infected)

TABLE VII
COWS SHOWING VARIABLE REACTIONS

Cow No.	Date	Mar. 1915	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1916	Feb. "	Mar. "	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1917	Feb. "	Mar. "	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1918	Feb. "	Mar. "
95	+	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
100	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
101	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
209	-	o	o	o	-	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
215	-	o	o	o	-	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
227	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
235	o	o	o	o	-	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
237	+	o	o	o	-	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
239	+	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
244	-	-	-	-	-	-	o	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
245	+	o	o	o	+	+	o	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
254	+	o	-	-	o	o	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
304	-	o	o	o	-	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
307	+	o	o	o	+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
316	+	o	o	o	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
415	-	o	o	-	-	-	-	-	-	-	-	+	+	-	-	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Notes:-

o---no test made
s---sold
k---slaughtered

In the foregoing table (No.VII.) cow No. 307 has shown the most interesting series of reactions. She calved August 21, 1915, December 4, 1916 and again December 23, 1917. About the time of calving each year, she gave one or more positive reactions to the monthly tests, at other times being negative.

The experimental data show that a large number of cows and heifers in an infected herd may escape infection, or at least show no clinical symptoms, calve normally and show no positive complement fixation reaction for a period of at least three years. No precautions were taken to keep infection from these animals, except to maintain sanitary barn conditions and to use sanitary maternity stalls for all cows at calving time. Table No. VIII. gives the monthly reactions of a group of cows and heifers which were evidently free from abortion infection throughout the experiment although housed in the same barn and grazing upon the same pastures with positive reacting cows for a long period.

TABLE VIII. EXPOSED COWS SHOWING PERSISTENT NEGATIVE REACTIONS.

No.	Mar. '15	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. '16	Feb. "	Mar. "	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. '17	Feb. "	Mar. "
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
241	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
242	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
246	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
248	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
251	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
314	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
317	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
318	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
412	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
413	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
414	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

k---slaughtered. s---sold. o---no test made.

REACTIONS OF CALVES AT BIRTH.

The calves under experiment, numbering more than one hundred showed at birth or near that date, almost without exception, the same reaction to the complement fixation test as that shown by their dams. Also that the positive reacting calves of both sexes gradually lose this reaction. The disappearance of the positive reaction took place in spite of the fact that the calves were fed upon skim milk, a portion of which was obtained from positive reacting cows, known to have aborted, and some of which were found, through guinea pig inoculation, to be eliminating the *Bacillus abortus* in their milk. The groups of negative reacting calves will be considered first.

Tables IX, X and XI show the reactions of calves on experiment which were negative from birth, together with the reaction of the dam nearest the calving time. Each table is divided into two sections a and b. This is necessary as it is impossible to put the entire record on a single page. However, the sections should be considered merely as one table. Section a. gives the reaction of the dam at the test nearest the date of calving together with the reaction of the calf at the test nearest the date of birth. Section b. shows the monthly reactions of this same group of calves from the first test to the present time.

TABLE IX.
(Section a.)

FIRST REACTIONS OF JERSEY CALVES FROM NEGATIVE DAMS.

Dam No.	Reaction of dam nearest calving date	Time in days between test and date of calving	Calf No.	Date of birth	Reaction at birth	Age at first test
102	-	26 after	102 b	May 17-16	-	35 da.
105	-	21 before	105 b	Dec. 2-16	-	9 "
108	-	1 after	108 b	Feb.12-17	-	1 "
108	-	5 before	108 b	Mar. 7-18	-	5 "
109	-	1 after	109 b	Jun.10-17	-	1 "
113	-	2 after	113 b	Jan.12-18	-	2 "
98	-	13 before	114	Dec.24-15	-	49 "
114	-	6 before	114 b	Mar.18-18	-	3 "
41	-	260 before	116	Mar. 1-16	-	31 "
67	-	125 after	121	Jun.30-16	-	1 "
94	-	1 before	122	July 2-16	-	6 hrs.
104	-	16 before	126	Dec.27-16	-	4 da.
59	-	92 after	127	Jan. 5-17	-	6 "
98	-	4 after	131	Apr.28-17	-	4 "
41	-	30 before	133	Jun.28-17	-	5 "
110	-	3 before	134	Jul.14-17	-	7 "
94	-	1 after	135	Aug.10-17	-	1 "
59	-	4 before	136	Jan. 7-18	-	7 "
104	-	5 after	137	Jan.29-18	-	5 "

TABLE IX.
(Section b.)

MONTHLY REACTIONS OF NEGATIVE JERSEY CALVES.

Calf No.	Date											
	Feb. 16	Mar."	Apr."	May"	Jun."	July"	Aug."	Sep."	Oct."	Nov."	Dec."	Jan. 17
102 b												
105 b												
108 b												
108 b												
109 b												
113 b												
114	-	-	-	o	-	-	-	-	-	-	-	-
114 b												
116			-	o	-	-	-	-	-	-	-	-
121												
122						-	k					
126												
127												
131												
133												
134												
135												
136												
137												

Notes:-

o---no test made.

s---sold.

k---slaughtered.

TABLE X.
(Section a)

FIRST REACTIONS OF HOLSTEIN CALVES FROM NEGATIVE DAMS.

Dam No.	Reaction of dam nearest calving date	Time in days between test and date of calving	Calf No.	Date of birth	Reaction at birth	Age at first test
209	-	6 after	209 b	Nov.27-17	-	6 da.
211	-	100 "	211 b	Jul.25-16	-	17 "
235	-	5 before	235 b	Nov. 7-16	-	4 "
235	-	6 after	235 b	Dec.28-17	-	11 "
236	-	197 bef.	236 b	Dec. 9-15	-	2 "
237	-	168 "	237 b	Nov.25-15	-	8 "
241	-	5 after	241 b	Apr.16-16	-	15 "
246	-	8 after	246 b	Aug.23-17	-	19 "
248	-	11 "	248 b	Dec.31-16	-	1 "
250	-	5 before	250 b	Apr.26-17	-	6 "
251	-	11 "	251 b	Mar.23-17	-	11 "
253	-	2 after	253 b	May 20-17	-	2 "
239	-	18 bef.	259	Aug. 8-15	-	4 "
259	-	13 "	259 b	Nov.15-17	-	6 "
235	-	121"	260	Oct.13-15	-	30 "
209	-	142"	261	Oct.30-15	-	34 "
210	-	184"	263	Dec.11-15	-	52 "
215	-	215"	265	Jan.15-16	-	16 "
243	-	9"	266	Feb.20-16	-	21 "
242	-	14"	267	Feb.25-16	-	15 "
244	-	7 after	269	Apr.24-16	-	7 "
246	-	6 before	270	Apr.27-16	-	4 "
239	-	3 after	271	Jul.29-16	-	13 "
227	-	1 after	272	Aug.31-16	-	32 "
209	-	6 before	273	Nov. 8-16	-	3 "
237	-	25 "	274	Jan.12-17	-	10 "
236	-	38 "	275	Jan.25-17	-	12 "
242	-	9 after	276	Jan.28-17	-	9 "
243	-	6 "	277	Jan.31-17	-	13 "
249	-	25 before	278	Feb. 5-17	-	8 "
210	-	25 "	279	Feb.12-17	-	1 "
211	-	17 "	281	Nov.19-17	-	2 "
257	-	8 "	282	Jan.22-18	-	2 "
242	-	11 after	283	Jan.23-18	-	1 "

The monthly reactions of these calves are found in table X
Section b.

TABLE X. Section b.
MONTHLY REACTIONS OF NEGATIVE HOLSTEIN CALVES.

Calf No.	Aug. '15	Sep. "	Oct. "	Nov. "	Dec. "	Jan. '16	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. '17	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. '18	Feb. "	Mar. "
209 b																																
211 b																																
235 b																																
235 b																																
236 b																																
237 b																																
241 b																																
246 b																																
248 b																																
250 b																																
251 b																																
253 b																																
259	-	o	o	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
259 b																																
260																																
261																																
263																																
265																																
266																																
267																																
269																																
270																																
271																																
272																																
273																																
274																																
275																																
276																																
277																																
278																																
279																																
281																																
282																																
283																																

Notes:-

s---sold.

k---Slaughtered.

o---no test.

TABLE XI.
(Section a.)

FIRST REACTIONS OF
AYRSHIRE AND SHORTHORN CALVES FROM NEGATIVE DAMS.

Dam No.	Reaction of dam nearest calving date	Time in days between test and date of calving	Calf No.	Date of birth	Reaction at birth	Age at first test
301	-	34 after	301 b	Sep.29-16	-	3 da.
301	-	11 before	301 b	Nov.13-17	-	8 "
304	-	84 after	304 b	Aug.10-16	-	1 "
315	-	11 after	315 b	Nov.22-17	-	11 "
315	-	1 before	319	Oct. 4-15	-	39 "
314	-	1 before	322	Jan.12-16	-	10 "
305	-	102 after	323	Jul. 3-16	-	29 "
315	-	14 before	325	Oct.16-16	-	36 "
317	-	2 before	326	Nov.13-16	-	8 "
413	-	10 before	413 cb	Feb.21-16	-	30 "
413	-	10 after	413 b	Mar.24-17	-	10 "
414	-	24 after	414 cb	Jan. 7-16	-	15 "
414	-	same day	414 cb	Feb. 6-18	-	6 "
414	-	10 after	414 b	Jan.27-17	-	17 "
415	-	1 before	415 b	Mar. 3-18	-	9 "
416	-	19 before	416 b	Jun.30-17	-	3 "

Notes:-

k-----slaughtered.

s-----sold.

o-----no test.

300-400-----Ayrshires.

400 and over-Shorthorns.

TABLE XI.
(Section b)

MONTHLY REACTIONS OF
Negative Ayrshire and Shorthorn Calves.

Calf No.	Nov.1915	Dec. "	Jan.1916	Feb. "	Mar. "	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan.1917	Feb. "	Mar. "	Apr. "	May "	June "	July "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan.1918	Feb. "	Mar. "
301 b																													
301 b																													
304 b																													
315 b																													
319	-	-	o	-	-	-	s																						
322			-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
323																													
325																													
326																													
413 cb				-	-	-	o	-	-	s																			
413 b																													
414 cb			-	-	-	-	o	-	-	s																			
414 cb																													
414 b																													
415 b																													
416 b																													

Notes:-

k-----slaughtered.

s-----sold.

o-----no test made.

300-400-----Ayrshires

400 and above---Shorthorns.

The foregoing tables represent a total of sixty nine calves all from negative reacting dams and all showing negative reactions at birth. This negative reaction has in all cases been maintained from the time of birth to the present date, although, as already explained, the calves were fed on raw, skim milk from a composite of many animals, some of which were known to be eliminating the bacillus abortus in their milk. Whether the calves would have remained negative if fed exclusively on the milk of infected cows is a question. As stated in the introduction, however, Schroeder and Cotton noted the loss of agglutinating power of serum from a calf nursing a mother with a strongly infected udder.

Records of Calves Showing a Positive Reaction at Birth.

The experimental data also showed that apparently normal calves dropped by immune positive reacting mothers, gave a positive reaction at birth, but that this reaction gradually disappeared. The time required for this change to occur varied from forty two to two hundred days. The temporary nature of the positive reaction in this group of calves suggests the probability that the reaction in most of these cases was due to the passage of preformed antibodies from the dam to the fetus, rather than to the passage of active infection and the subsequent production of antibodies in the fetus, although the possibility of the latter must be conceded. The recur-

rence of a positive reaction in Jersey heifer No. 111, noted in Table XII, is considered, or at least suspected, to be due to a reinfection. The fact that the calves were fed on raw, skim milk, some of which was known to be infected with the *Bacillus abortus*, did not prevent the disappearance of the positive reaction, nor seem to have any effect upon it. This fact bears out the statement of Schroeder and Cotton referred to in the introduction, that the bacilli ingested in infected milk do not penetrate deeply nor abundantly into the body of the calf. It is interesting to note that in the case of the twin calves Nos. 106 and 107, the reaction persisted for the same length of time in both animals. Unfortunately No. 106 died before being old enough to breed. The records of these animals are found in Table XII on the following page. This table and the following one, XIII, show the histories and reactions of two groups of calves from immune, positive reacting dams. As in the case of Tables IX, X and XI, these tables are divided into two sections a and b.

TABLE XII
(Section a)

FIRST REACTIONS OF JERSEY CALVES FROM POSITIVE DAMS

Dam No.	Reaction of dam nearest calving date	Time in days between test and date of calving	Calf No.	Date of birth	Age at first test	First Reaction
10	+	184 after	10 cb	May 1-16	6 hrs.	+
10	+	43 before	10 b	Apr.27-15	9 da.	+
23	+	50 after	23 b	Aug. 5-15	7 "	+
61	+	44 before	61 b	Jan.31-16	11 "	+
87	+	105 before	87 b	Sep.30-15	1 "	+
87	+	6 before	87 b	Nov. 8-16	3 "	+
96	+	11 after	96 b	Mar.20-16	32 "	+
96	+	11 after	96 b	Jun.22-17	11 "	+
101	+	same day	101 b	Aug. 1-17	6 hrs.	+
11	+	55 after	106	Jan.17-15	47 da.	+
11	+	55 after	107	Jan.17-15	47 "	+
3	+	8 before	109	Mar.23-15	4 "	+
93	+	6 after	110	Jun.25-15	5 "	+
83	+	17 before	111	Jul. 4-15	8 "	+
111	+	1 after	111 b	Nov. 1-17	1 "	+
17	+	35 before	112	Jul.19-15	2 "	+
95	+	17 after	115	Feb. 5-16	34 "	+
101	+	9 before	117	Apr.10-16	11 "	+
93	+	10 after	120	Jun.20-16	2 "	+
89	+	7 after	128	Jan.31-17	6 "	+
95	+	3 after	130	Apr.22-17	3 "	+
61	+	20 after	132	May 8-17	3 "	+

The monthly reactions of these calves are found in table XII Section b.

TABLE XII. Section b.

MONTHLY REACTIONS OF JERSEY CALVES FROM IMMUNE POSITIVE REACTING DAMS.

Calf No	Date	Mar. 1915	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1916	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1917	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1918	Feb. "	Mar. "	Time for reaction to change.	
10 cb																																						82 da.		
10 b				+	+	k																																		
23 b							+	+	-	-	-	-	-	-	-	o	s																					67 "		
61 b													+	+	+	o	-	-	-	-	-	-	-	s														143 "		
87 b									+	+	+	-	-	-	-	o	s																						114 "	
87 b																							+	+	+	-	-	-	-	-	-	-	-	k				91 "		
96 b															+	o	+	-	-	-	s																		123 "	
96 b																															+	+	+	+	-	-	-	-	s	152 "
101 b																																+	+	+	-	-	-	-	k	113 "
106		+	+	-	-	-	k																																	109 "
107		+	+	-	-	-	o	-	-	-	-	o	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		109 "	
109		+	o	o	+	-	-	-	-	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	k			111 "	
110						+	+	+	+	+	+	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200 "	
111						+	+	+	+	-	-	-	-	-	-	-	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	109 "	
111 b																																			+	+	-	-	k	63 "
112						+	+	k																																
115														+	+	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		117 "	
117														+	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s				52 "	
120																	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		62 "	
128																										+	+	+	+	-	-	-	-	-	-	-	o	-	-	131 "
130																											+	o	+	+	-	-	-	-	-	-	-	-	-	121 "
132																												+	+	+	+	-	-	-	-	-	-	-	k	136 "

K---slaughtered. S---sold. o---no test made.

TABLE XIII
(Section a.)

FIRST REACTIONS OF SIXTEEN
HOLSTEIN AYRSHIRE AND SHORTHORN CALVES FROM POSITIVE DAMS.

Dam No.	Reaction of dam nearest calving date	Time in days between test and the date of calving	Calf No.	Date of birth	Reaction at test nearest birth	Age at first test
213	+	11 before	213 b	Dec.29-16	+	2 da.
238	+	24 before	238 b	Jul.25-15	+	18 "
245	+	7 after	245 b	Mar.25-16	+	27 "
231	+	15 after	255	Mar. 2-15	+	8 "
223	+	146 before	262	Nov. 7-15	+	5 "
213	+	200 before	264	Dec.31-15	+	30 "
238	+	12 before	280	Mar.14-17	+	7 "
307	+	65 before	307 b	Aug.21-15	+	11 "
307	+	32 before	307 b	Dec. 4-16	+	7 "
311	+	56 before	311 b	Aug.12-15	+	6 hrs.
313	+	24 before	313 b	Jan.11-17	+	6 hrs.
313	+	130 before	321	Oct.25-15	+	39 da.
307	+	51 before	327	Dec.23-17	+	16 "
313	+	14 after	328	Jan.20-18	+	4 "
404	+	84 after	404 b	Aug.12-16	+	9 "
404	+	3 before	416	Jun.19-15	+	11 "

Notes:-

The monthly reactions of these calves are found in
Table XIII Section b.

200-300-----Holsteins.

300-400-----Ayrshires.

400 and above-----Shorthorns.

TABLE XIII
(Section b.)
MONTHLY REACTIONS OF HOLSTEIN AYRSHIRE AND SHORTHORN CALVES
FROM IMMUNE POSITIVE REACTING DAMS.

Calf No.	Mar. 1915	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1916	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1917	Feb. "	Mar. "	Apr. "	May "	Jun. "	Jul. "	Aug. "	Sep. "	Oct. "	Nov. "	Dec. "	Jan. 1918	Feb. "	Mar. "	Time for reaction to change
213 b																							+	-	-	-	-	-	s							54da		
238 b						+	+	+	+	-	-	s																									120"	
245 b														+	o	-	-	-	-	-	-	-	s													89"		
255	+	o	-	-	-	o	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s							65		
262								+	+	o	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	114"		
264											+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91"		
280																									+	+	+	+	-	-	-	-	-	-	-	-	129"	
307 b						+	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	s													44"		
307 b																							+	+	-	-	-	-	-	-	-	-	-	s		71"		
311 b																							+	+	-	-	-	-	-	-	-	-	-	s		60"		
313 b																							+	+	+	+	+	+	-	-	-	-	-	-	-	-	k 181"	
321									+	o	+	+	+	o	-	o	s																			200"		
327																																			+	-	- 42"	
328																																			+	+	+	
404 b																		+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	s			178"	
416					+	o	+	+	+	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	175"	

k---slaughtered.
s---sold.
o---no test.

Numbers 200-300-----Holsteins.
300-400-----Ayrshires.
400 and above---Shorthorns.

The foregoing Tables, XII and XIII, representing four breeds of dairy cattle, contain the records of thirty eight calves, all from immune, positive reacting mothers and all showing a positive reaction at birth. Of this number, two have died while still showing a reaction, and one is positive at the present time. The remaining thirty five have gradually lost the positive reaction. The time required for this change to occur ranges from forty two to two hundred days. With the exception of No. 111, these animals have maintained the negative reaction to the present time as shown by monthly tests. No. 111, as stated, is thought to have become reinfected although we must concede the possibility of the infection remaining in a dormant condition at some focus within her body, during the time she was showing the negative reactions. The breeding records of the heifers of this group, including No. 111, are given in later pages.

During the course of the work, a few calves have failed to show the same reaction as that shown by their dams at the test nearest the date of calving. Table XIV (Sections a. and b.) gives a list of such calves with their histories and monthly reactions.

TABLE XIV.
(Section a)

CALVES NOT REACTING THE SAME AS THEIR DAMS.

Dam No.	Reaction of dam nearest date of calving	Time in days between test and date of calving	Calv No.	Date of birth	Reaction nearest birth	Age at first test
100	± -	9 bef.	100b	Jul.12-17	-	9 da.
67	+	35 aft.	108	Feb. 8-15	-	25 "
228	+	35 bef.	256	Apr.17-15	-	19 "
227	+	79 bef.	257	May 31-15	-	30 "
211	-	52 bef.	258	Aug. 5-15	+	7 "
215	-	17 bef.	215b	Aug.18-17	+	3 "
312	+	125 bef.	320	Oct.19-15	-	45 "
415	-	3 aft.	418	Dec. 8-16	+	3 "

Notes:-

± -----doubtful reaction.

+ -----positive reaction.

-----negative reaction.

Numbers.100-200-----Jerseys.

200-300-----Holsteins.

300-400-----Ayrshires.

400 and above--Shorthorns.

The monthly reactions of these calves are found in
Table XIV Section b.

TABLE XIV.
(Section b.)

MONTHLY REACTIONS OF CALVES NOT REACTING THE SAME AS THEIR DAMS.

Calf No.	Date
100b	Mar. 1915
108	Apr. "
256	May "
257	Jun. "
258	Jul. "
215b	Aug. "
320	Sep. "
418	Oct. "
	Nov. "
	Dec. "
	Jan. 1916
	Feb. "
	Mar. "
	Apr. "
	May "
	Jun. "
	Jul. "
	Aug. "
	Sep. "
	Oct. "
	Nov. "
	Dec. "
	Jan. 1917
	Feb. "
	Mar. "
	Apr. "
	May "
	Jun. "
	Jul. "
	Aug. "
	Sep. "
	Oct. "
	Nov. "
	Dec. "
	Jan. 1918
	Feb. "
	Mar. "

Notes: -

k-----slaughtered.

s-----sold.

0-----no test made.

In the data given in Table XIV, dam No. 312 gave a positive reaction one hundred and twenty five days before calving but was not tested at or near the calving time. As the infection is presumably more active at the calving period, the reaction of the dam was probably positive. On the other hand, the calf, No. 320, was negative when first tested, but this was forty five days after its birth, a time sufficient for a change from a positive to a negative reaction, since in one instance observed in this experimental work, a calf which gave a positive reaction at birth changed to a negative reaction in forty two days. And it is possible that calves No. 108, 256 and 257 may have been positive at birth and changed to negative within the shorter period of nineteen to thirty days which elapsed between the date of birth and the first test. It is probable that the dams of these calves were all positive reactors at the time of the birth of their calves, since variable reactors are more likely to be positive at this period.

The serum of calf No. 215b. was found to contain anti-complimentary substances. At the first test, through error, no control on the serum was made. Later tests showed the presence of these bodies, which disappeared after heating at 56°C. for twenty minutes. If a control had been run when the first test was made, the presence of these bodies would no doubt have been detected and the serum found to be a negative reacting one. It is possible that the positive reactions shown by calf No. 258 and No. 418 soon after

birth may also have been due to anticomplements which, through error, were not detected at the first test.

BREEDING RECORDS OF HEIFER CALVES SHOWING A POSITIVE
REACTION AT BIRTH.

It has been shown that heifer calves from positive reacting dams although positive at birth, gradually lose this reaction and thereafter remain negative reactors. The experiment has shown that, unless reinfected, these heifers will in turn produce normal, non-reacting calves at the first breeding period.

Below are given the breeding records of a group of eleven heifers, all of which were positive reactors at birth and all of which have since lost the reaction and have remained negative as shown by monthly tests, with the exception of one heifer. Some of these have brought strong, healthy, non-reacting calves. Others are safely with calf at the present time and have never shown signs of aborting.

Heifer No. 107.

A complete record of her reactions will be found in Table XII. This heifer was born Jan. 17, 1915. Her dam, No. 11, gave a strong positive reaction fifty five days after calving. Calf No. 107 was tested when forty seven days old and gave a strong positive reaction. This reaction persisted for one hundred and nine days after which she became negative and has remained so. She was bred May 22, 1916 and calved normally Feb. 27, 1917. The calf, No. 129,

gave a negative reaction March 21, 1917, or twenty two days after birth. She was bred again Aug. 15, 1917, conceived at one service, and is now eight months pregnant and will apparently carry this second calf safely to term.

Heifer No. 109.

The complete record of her reactions is found in Table XII. She was born Mar. 23, 1915. Her dam, No. 3, gave a positive reaction eight days before calving. Calf No. 109 was tested when four days old and gave a strong positive reaction. This reaction persisted for one hundred and eleven days. It then disappeared and she has since been negative to the monthly tests. She was bred Sep. 3, 1916 and calved normally June 10, 1917. The calf, No. 109b, was tested when one day old and gave a negative reaction. No. 109 died in December 1917 without having been rebred.

Heifer No. 110.

The complete record of her reactions is found in Table XII. She was born June 25, 1915. Her dam, No. 93, gave a positive reaction six days after calving. No. 110 was tested when five days old and gave a positive reaction. This reaction persisted two hundred days. It then disappeared and she has since been negative to the monthly tests. She was bred Aug. 21, 1916 and again Oct. 11, She calved normally July 14, 1917. The calf No. 134 gave a negative reaction July 21, 1917 or seven days after birth. No 110 was bred again Dec. 25, 1917, conceived at one service and is again four months pregnant and apparently carrying her calf safely.

Heifer NO.111.

The complete record of her reactions is found in Table XII. She was born July 5, 1915. Her dam, No. 83 gave a positive reaction seventeen days before calving. The calf, No. 111, also gave a positive reaction the day following birth. This persisted for at least one hundred and nine days, after which she became a non-reactor and remained negative to the test for at least one hundred and eighty days or until Apr. 11, 1916. At the next test, fifty two days later, she gave a positive reaction which has continued until the present time. This heifer was first bred on Jan. 20, 1917 or seven months after the return of the positive reaction. And although the monthly tests showed a positive reaction, this heifer calved normally Nov. 1, 1917. Her calf, No. 11b, gave a positive reaction the following day. She was bred again Mar. 14, 1918 and has apparently settled to this one service. The fact that the original positive reaction disappeared, and the heifer was a negative reactor for more than six months, indicates very strongly that the reappearance of the positive reaction was probably due to a re-infection, rather than to infection retained in the body from birth.

Heifer No. 115.

The complete record of her reactions is found in Table XII. She was born Feb. 5, 1916. Her dam, No. 95, gave a plus minus-minus reaction seventeen days after calving. This dam is a variable reactor. No. 115 was tested when

thirty four days old and gave a strong positive reaction. This reaction persisted for one hundred and seventeen days after birth, when the reaction became negative and has since remained so. She was bred Aug. 16, 1917 and again Sep. 4, 1917 when she was seventeen months old. She conceived to this last service, and is now seven and one half months pregnant and shows no signs of impending abortion.

Heifer No. 117.

/ The reactions of this heifer are found in Table XII. She was born Apr. 10, 1916. Her dam, No. 101, gave a positive reaction nine days before calving. The calf, no. 117, was tested when eleven days old and gave a strong positive reaction. This reaction persisted for fifty two days, after which it disappeared and the subsequent monthly tests have shown a negative reaction. She has never come in heat. This is probably due to lack of development of the ovaries resulting from an injury to the pelvis which occurred while the heifer was quite young.

Heifer No. 120.

The reactions of this heifer are found in Table XII. She was born June 20, 1916. Her dam, No. 93, gave a positive reaction ten days after calving. This heifer was tested when two days old and gave a strong positive reaction. This reaction persisted for sixty two days, after which it disappeared and she has since remained negative. She was bred Nov. 5, 1917, and again Nov. 23, 1917. She settled

to this second service, and is now five months pregnant and has shown no signs of impending abortion.

Heifer No. 255.

The reactions of this heifer are found in Table XIII. She was born Mar. 2, 1915. Her dam, No. 231, gave a positive reaction fifteen days after calving. The Calf No. 255, was tested when eight days old and gave a strong positive reaction which later disappeared. The first negative reaction recorded, was on the sixty fifth day following birth. She was first bred Sep. 25, 1916 and at each succeeding period until Jan. 22, when she conceived. Four and a half months later this heifer was sold. She was still a negative reactor and our information is that she calved normally.

Heifer No. 262.

The record of her reactions is found in Table XIII. She was born Nov. 7, 1915. Her dam, No. 223, gave a positive reaction one hundred and forty six days before calving. No. 262 tested when five days old, also gave a positive reaction. This reaction persisted for one hundred and fourteen days and then disappeared. She has since remained a non-reactor. She was first bred Sep. 14, 1917 and again on Oct. 31, Dec. 7 and again on Oct. 31, Dec. 7 and Dec. 26. She conceived at this last service and is now apparently safe with calf, being four months pregnant.

Heifer No. 264

The record of her reactions appears in Table XIII. She was born Dec. 31, 1915. Her dam, No. 213, gave a positive

reaction two hundred days before calving. No. 264 was tested when thirty days old and also gave a positive reaction. This reaction persisted for at least fifty nine days and disappeared sometime between the fifty ninth and the ninety first day following birth. She has been negative to the subsequent monthly tests. She was first bred June 26, 1917 and again July 26, Sep. 2, Sep. 30, and Nov. 7. She settled to this last service and is now apparently safe with calf, being five and a half months pregnant.

Heifer No. 416.

The record of her reactions is found in Table XIII. She was born June 19, 1915. Her dam, No. 404, gave a strong positive reaction three days before calving. No. 416 was tested when eleven days old and also gave a strong positive reaction. This reaction persisted for one hundred and seventy five days and then disappeared. She has given negative reactions to all subsequent tests. She was bred Oct. 2, 1916, conceived at this one service and calved normally June 30, 1917. The calf No. 416b, gave a negative reaction when three days old. She was bred again Dec. 25, 1917 and is at present apparently safe with calf, being four months pregnant.

It is thus shown that of the eleven heifers which at birth gave a positive reaction to the complement fixation test for abortion, and whose dams were also positive reactors, all became negative reactors in periods varying from 52 days to 200 days following birth. And all except one

have since remained continuously negative to the test; the youngest for more than two years, and the oldest for more than three years. Four of the group have produced normal non-reacting calves and three of these are again with calf. The fifth heifer was bred and sold and our information is that she calved normally. Four others were bred and are pregnant; the present stage of gestation varying respectively from four to seven and a half months. No signs of abortion have appeared in these heifers. One heifer of this group, a Jersey, now two years and a month old, has never come in heat. It is probable that an injury to the pelvis, received when this heifer was quite young, has prevented the proper development of the ovaries.

In only one heifer of the group was there a return of the positive reaction after its disappearance several weeks following birth. This heifer, No. 111, evidently became re-infected (or on the other hand must have harbored prenatal abortion infection in a dormant condition for a period of Six months.) The positive reaction returned when she was about nine months old, and has since remained positive. She was bred when seventeen months old and carried the calf to full term. The calf was apparently healthy but showed a positive reaction. Excluding the heifer with the injured pelvis which will probably prove a non-breeder, nine out of ten, or 90 per cent of the heifers whose dams were positive reactors, have become negative reactors and have remained so.

REACTION OF HERD BULLS TO THE COMPLEMENT

FIXATION TEST FOR ABORTION.

In Table XV. is recorded the monthly complement fixation tests for abortion infection which were carried out on the eight breeding bulls that were used in the herd on experiment within the three years period just closed.

Bull No.1, Missouri Chief Josephine's Lad, (Holstein), had been in service in the herd for some time before he was put on the regular monthly tests for abortion disease. The monthly tests were made from July, 1915 to April, 1916 inclusive when he was taken out of service. During these ten months this bull was continuously negative to the abortion test, although he had served positive reacting cows before and during the experimental period. During this latter period he served six positive reacting cows and four negative reactors; all of which calved normally, the positive reacting cows dropping positive reacting calves and the negative cows dropping negative reacting calves. After removal from breeding service this bull was retained ten months longer. And to test his susceptibility to abortion infection he was injected subcutaneously with cultures of *B. abortus*. Eleven days later he gave a weak positive reaction. A second subcutaneous injection of *B. abortus* was made, which produced a strongly positive reaction to the abortion test. This positive reaction persisted until the bull was slaughtered six months after the second injection of a *B. abortus* culture.

Bull No. 2, Sir Korndyke Hengerveld De Kol (Holstein), had likewise been in service in this herd before he was put on the monthly test list. He has been kept in service in the herd during the entire three years experimental period. At no time during this period has this bull shown a positive reaction to the abortion test; although, among the cows he served were eleven that gave a positive reaction to the abortion test. This bull evidently did not harbor during this period any "systemic" abortion infection capable of producing a reaction to the complement fixation test.

The same report can be made with respect to the Ayrshire herd bull No. 3, Sir Melrose canary, which had served five cows which were positive reactors to the abortion test.

Bull No. 4, Duke Darlington, (Shorthorn milking strain), has been on the monthly test list for abortion disease for two years and seven months, during which time he served three cows which were positive reactors to the abortion test. He has, however, never shown any evidence of a positive reaction.

Four Jersey bulls were on the monthly test list for varying periods. Bull No. 5, Bessie Bates' Lad, which had been in service in the herd before the monthly abortion tests were begun, was sold at the close of the first year's experimental work, but during this time the monthly tests gave no evidence of systemic abortion infection. He had served during this experimental period six positive reacting cows.

Bull No. 6, Sultana's Virginia Lad, was in service throughout the full three year period. His monthly tests for abortion infection were all negative. These two Jersey bulls (Nos. 5 and 6) had served twenty two cows which gave positive reactions to the abortion test.

Bull No. 7 was about six months old when received. He was put in clean quarters apart from other cattle to avoid any danger from abortion infection that might come from the positive reacting cows of the herd. This bull when first tested gave a negative reaction to the abortion test and remained negative through his period of service in the herd, which was unfortunately brief as he had served only two heifers when he died. Both of these heifers dropped non-reacting calves at full term.

Bull No. 8 was bought to replace the dead bull No. 7 and was also carefully isolated from the other cattle. He was about nine months old when received and probably had not served any cows nor heifers in the herd from which he was purchased. The first two tests for abortion infection made in January and February, 1916 were negative; the next monthly test gave a suspicious reaction. The next test showed a definitely positive reaction and the bull remained positive over the next two monthly test periods; thus showing a continuous positive period of approximately four months. A negative phase of two months succeeded this when another single suspicious reaction occurred. This was followed by a series of negative reactions during the breeding period covering the next six months.

As it was not feasible to obtain another young bull to serve the group of non reacting heifers it was decided to use this bull; in spite of the suspicion which the variable abortion reactions gave that he was carrying systemic infection, at least in a mild form. Eight non reacting heifers were bred to this bull. All remained negative to the monthly test for abortion and all dropped at full term negative reacting calves.

The foregoing history of the breeding bulls shows that a systemic abortion infection was not transmitted to any of these bulls by temporary contact with any of the thirty seven, more or less, persistent positive reacting cows of the herd. And in the case of the young bull, Sophie's Demonstrator, the mild systemic infection which this bull carried (whether of pre-natal origin or from ingestion of infection after its birth) seems not to have affected the generative organs. At least no transmission of abortion infection from this bull to the abortion-free heifers occurred.

Note:- The young bulls, Nos. 7, and 8 were purchased by the Department of Dairy Husbandry for a special experiment; that of demonstrating the possibility of building up an "abortion-free" herd by breeding non reacting heifers to non reacting bulls which had never served abortion reacting cows. And when Bull No. 8 developed a positive reaction indicating a systemic infection, this was regarded as an unfortunate circumstance; as their original plan could not be carried out. It seems however that what was regarded as an unfortunate incident in the experiment may, in another way, prove advantageous in advancing our knowledge of the nature of abortion disease; since the indications thus far, from the use of this bull, are that a positive reacting bull, that is, one that is presumably carrying systemic abortion infection, does not necessarily eliminate infection through the generative organs; and may be safe for breeding. The data however from this work, and from the literature, is too meager to justify a positive statement that the "systemic" abortion infection of the male is never transmitted to the female by copulation.

TABLE XV.
MONTHLY REACTIONS OF HERD BULLS.

Date.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Mar. '15	o	-	-	o	-	-	o	
Apr. "	o	o	o	o	o	o	o	
May "	o	o	o	o	o	o	o	
Jun. "	o	-	-	o	-	-	o	
Jul. "	-	-	-	o	-	-	-	
Aug. "	-	-	-	-	-	-	-	
Sep. "	-	-	-	-	-	-	-	
Oct. "	-	-	-	-	-	-	-	
Nov. "	-	-	-	-	-	-	d	
Dec. "	-	o	-	-	-	-		
Jan. '16	-	-	-	-	-	-		-
Feb. "	-	-	-	-	-	-		-
Mar. "	-	-	-	-	-	-		±
Apr. "	-	-	-	-	s	-		+
May "	*	o	o	o		o		o
Jun. "		-	-	-		-		+
Jul. "		o	-	-		-		-
Aug. "		-	-	-		-		-
Sep. "		-	-	-		-		±
Oct. "		-	-	-		-		-
Nov. "		-	-	-		-		-
Dec. "		-	-	-		-		-
Jan. '17		-	-	-		-		-
Feb. "		-	-	-		-		-
Mar. "		-	-	-		-		-
Apr. "		-	-	-		-		-
May "		-	-	-		-		w+
Jun. "		-	-	-		-		-
Jul. "		-	-	-		-		-
Aug. "		-	-	-		-		-
Sep. "		-	-	-		-		-
Oct. "		-	-	-		-		-
Nov. "		-	-	-		-		-
Dec. "		-	-	-		-		k
Jan. '18		-	-	-		-		
Feb. "		-	-	-		-		
Mar. "		-	-	-		-		

Notes:-

- No.1-----Missouri Chief Josephine's Lad. (Holstein)
 " 2-----Sir Korndyke Hengerveld De Kol. (Holstein)
 " 3-----Sir Melrose Canary. (Ayrshire)
 " 4-----Duke Darlington. (Shorthorn-milking strain)
 " 5-----Bessie Bate's Lad. (Jersey)
 " 6-----Sultana's Virginia Lad. (Jersey)
 " 7-----Gold Medal Torone. (Jersey)
 " 8-----Sophie's Demonstrator (Jersey)

*-----Taken from experiment.

s-----sold.

d-----died.

k-----Slaughtered.

SUMMARY AND CONCLUSIONS.

The data given in the preceding pages, particularly the records of monthly complement fixation tests for abortion carried out on a herd of dairy cattle over a period of three years show:

1. (a) That some mature breeding cows which have once become infected with the contagious abortion disease may retain the infection within the body continuously (i.e. without a probable reinfection) for a period of at least three years as shown by the persistent monthly reactions of a group of experimental cows; and by other confirmatory facts in these experiments. Evidently a somewhat permanent focus of active infection is localized in some organ of all persistent positive reactors. In three cows of this experimental group the bacilli of abortion, which were infectious to guinea pigs, were in the milk. And the presumption is, that in these cases, an active focus of infection was located in the udder.

(b) That some mature breeding cows after infection with contagious abortion disease give a variable reaction to the complement fixation test. That is, some positive reactors become negative and remain so for a considerable period; and in some instances the data indicate that the negative reaction may be permanent, so far as any effect of the original infection is concerned. It thus appears that the abortion infection may die out and disappear in some breeding cows. In such cases the infection is probably localized in tissues or glands which

are not so favorable to the continued vitality of the B.abortus as the udder appears to be. Another variable reaction which has been observed is the occurrence of an occasional positive reaction in breeding cows whose monthly reactions are in the main negative. It appears that in such animals the positive reaction is more liable to occur at or near the calving period and the most rational interpretation of these cases is that a focus of dormant infection is localized in some portion of the body and that this infection becomes active only when pregnancy is well advanced.

2. Other mature breeding cows, although exposed to the same conditions in barns and on pastures, and served by the same bulls, as the two groups of cows mentioned above, escaped infection, or at least showed no clinical nor serological indications of it during a three year period in which monthly tests for abortion were made.

3. A permanent systemic infection is apparently not often transmitted from the mother to the viable fetus; nor by the ingestion of infected milk or other foods, after the birth of the calf and prior to the breeding period. But the possibility of the transmission of a durable systemic infection by one or the other of these methods, must be conceded, since in this experiment one heifer which was a positive reactor at birth and from a positive reacting dam, had a recurrence of the positive reaction after a six months negative phase, and seven months before breeding.

4. Calves from immune, positive reacting dams, show a positive reaction at or near birth but this positive reaction gradually disappears and they become non reactors. In this experiment, the time for this change to take place varied from forty two, to two hundred days, the average being one hundred and ten days. The fact that this positive reaction disappears in three to four months, indicates very strongly that the original positive reaction is due to the presence of preformed antibodies transmitted from the dam to the fetus, rather than due to infection transmitted from the dam to the calf with the consequent production of antibodies in the latter. Subsequent positive reactions in these calves are probably due to reinfection.

5. "The probability of the abortion infection passing from an immune positive reacting dam to the calf in utero is not great; or, if such apparently normal calf is infected at birth, the abortion infection is probably not conserved in its tissues until the first breeding period. Moreover, the danger of the permanent transmission of abortion infection to the calf thru raw milk is small."¹

6. Heifer calves from immune positive reacting dams lose the positive reaction present at birth and can produce normal, non-reacting calves at their first breeding period.

7. Bulls cohabiting with positive reacting, immune cows rarely become systemically infected to a degree in which they show a reaction to the complement fixation test.

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8. The bull is apparently not a major source of the spread of infection of the abortion disease from foci of systemic infection.

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