A Study on the Transmission of *Bacterium abortus* (Bang) thru Milk.

THESIS.

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By

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Introduction.

Numerous investigations have shown the infectiousness for animals of milk containing pathogenic organisms. The milk may become infected indirectly thru unsanitary conditions and thru human carriers of disease producing microorganisms. The milk may become infected directly thru infections of the udder, tuberculosis and mammitis being two common infections of the udder in which the organisms may be excreted continually or occasionally in the milk thruout the infection.

The investigations relative to the transmission of Bacterium abortus thru milk are as yet in an experimental stage. Very little study has been completed, but numerous suggestions have been made as to the possibility of infectious abortion being acquired in this manner. The researches regarding the transmission of Bacterium abortus thru milk have been confined largely to animal inoculation, to observations and to a large collection of clinical evidence. The possibility of infectious abortion being acquired by animals thru the ingestion of raw milk was first suggested by Williams (1). His researches are, however, very incomplete. Considering the economic importance of the control of infectious

abortion, an experimental study of the numerous observations and of the clinical evidence now on record covering the infectiousness of raw milk would aid materially in solving one of the modes of transmitting Bacterium abortus.

Historic Review.

Researches concerning the presence of pathogenic organisms in milk and the possibility of a disease resulting therefrom have been extensive.

Fisher(2), many years ago, reported a paratyphoid epidemic due to infected milk, two cows of a herd supplying the milk were suffering from enteritis. Anderson(3) found the tubercle bacilli in 11 percent of the dairies supplying milk to Washington, D. C. Hess(4) likewise isolated the human type of tubercle bacilli from the milk supplying the city of New York. Griffith(5) making a study of the milk of cows and goats, vaccinated subcutaneously and intravenously with live tubercle bacilli, showed by means of guinea pig inoculations that the bacilli, bovine or human, not only made its appearance in the milk 24 hours after inoculation, but continued to be excreted therein for long periods subsequently. Capp and Davis(6) reported a septic sore throat epidemic due to milk from a herd of 15 cows, 3 of which were affected with mastitis.

That Bacterium abortus is present in the milk from apparently normal cows and is excreted therein for long periods, was first demonstrated by Schroeder and Cotton(7) in 1911.

After an extensive examination of a large number of samples of milk, the abortion bacterium was isolated by cultural methods and identified by means of the complement-fixation test. The most noteworthy feature of their work, was the demonstration of lesions in guinea pigs inoculated intra-abdominally with samples of the milk identical to those produced by a culture of Bacterium abortus inoculated in the same manner.

Zwick and Krage(8) successfully demonstrated the presence of Bacterium abortus in the milk of cows fourteen days, six months and thirteen months after abortion. Fabyan (9) likewise demonstrated the presence of Bacterium abortus in the cream and milk sediment of the milk from a herd of twelve cows, two of which had previously aborted, by means of guinea pig inoculation. The animals inoculated with material from the cows which had records of abortion revealed characteristic lesions, the other guinea pigs showed no histological changes. Larson and Sedgwick(10) examining the blood serum of four hundred and twenty-five children demonstrated the presence of Bacterium abortus antibodies in seventy-three, or seventeen percent of the total number examined. They were not able to demonstrate antibodies in milk, so concluded that probably the antibodies present in the blood serum of the children were due to an active immunity. Schroeder(11) feeding raw, pasteurized and heated milk to young guinea pigs, found that there was a greater mortality and tendancy to unthriftiness among the animals fed raw milk, than among those fed pasteurized or heated milk. At the end of the experiment an autopsy was performed

on the entire number fed, many animals receiving raw milk showed lesions characteristic of abortion disease. The first investigators to demonstrate the presence of Bacterium abortus antibodies in milk, were Reinhard and Gauss(12). They were able to show the relation between the antibody index of the blood serum and that of the milk serum by goats inoculated subcutaneously and intravenously and testing same by means of the agglutination and complement-fixation test for long periods subsequently. Their results showed that there was a direct relation between the antibody indices of the two serums, but antibodies could be demonstrated to be present in the milk long after they had ceased to appear in the blood.

The studies of Evans(13) on the bacterial flora of the udder revealed Bacterium abortus in 30 percent of the milk samples examined from four dairies in numbers varying from one hundred to fifty thousand per cubic centimeter. In a recent investigation(14) she examined one hundred and ninety-two samples of milk from five dairies. A Bacterium abortus-like organism was isolated from twenty-three percent of the samples. Three different types of organisms were distinguished, a variety designated as Bacterium abortus var. lipolyticus on account of its tendancy to decompose butter fat. Two other varieties resembled cultures isolated from pathogenic sources.

The observations and researches of Williams(15) have developed a new phase of the subject which is of economic importance from the standpoint of controlling the disease, that is, the possibility of an infection being acquired thru the ingestion of raw milk. He firmly contends from his researches that Bacterium abortus is transmitted to calves

fed upon raw milk, also that the matting and staining of the sexual hairs and granular vaginitis are in direct relation to the infection.

Cooledge(16) has successfully shown the difference existing between infected milk and non-infected milk by means of the agglutination test. He further showed that by inoculating one quarter of the udder of a cow through the teat orifice with a culture of Bacterium abortus, agglutinins appeared in the quarter inoculated and gradually spread to the other quarters of the udder.

Method of Investigation.

The literature reviewed has not revealed any definite results as to the possibility of transmitting Bact. abortus to animals thru the ingestion of milk. With this object in view, experiments were outlined in such a manner with susceptible animals so as to procure more complete data and to show the relationship between the feeding of infected milk and non-infected milk.

The experiments were outlined in the following manner: (1) The result of feeding infected milk to guinea pigs; (2) the result of feeding infected milk to rabbits; (3) the result of feeding infected milk to calves; (4) the significance of the matting of the sexual hairs around the sheath of the bull and that below the vulva of heifer calves.

The term infected milk upon which the study is based implies milk reacting positively to the agglutination and

complement fixation tests using Bact. abortus as antigen. The milk was fed to the guinea pigs and rabbits daily, and to the calves twice daily thruout the experiment. Every precaution was taken to guard against an infection from an outside source. The milking utensils were thoroughly sterilized, and the udder and teats cleansed with a damp cloth before each milking.

The effect of the infected milk fed was studied by means of agglutination and complement-fixation tests on the blood serum of the animals, using Bact. abortus as antigen. The guinea pigs were fed for a period of several weeks and then autopsied. The blood serum was tested and any anatomical changes noted. The rabbits were bled from the marginal vein of the ear or from the heart at intervals of about two weeks and the blood serum tested. They were autopsied at the conclusion of the experiment and any anatomical changes noted. The calves were bled from the jugular vein at intervals of about one week and the blood serum tested. Observation and microscopic examination of the sexual hairs of the calves were made during the course of the feeding.

Technique Employed.

The Complement Fixation Test.

The application of the complement fixation test to the diagnosis of infectious abortion has been fully discussed by Hadley and Beach(17), Surface(18) and many others.

The technique used thruout this investigation is a slight modification of the previously described methods for the preparation of the different components and the quantities used.

The writer has found that by using a 2.5 percent solution of sheep erythrocytes, more satisfactory results could be obtained than by using either a 1 percent or a 5 percent solution as is generally used by most workers. A much better differentiation between partial and complete fixation of complement is obtained over that of a 1 percent solution, while using a 5 percent solution of cells would require the use of a larger quantity of the other necessary components.

The following components are necessary in making the complement fixation test.

(1) Antigen.

(2) Serum to be tested.

(3) Complement.

(4) Hemolysin.

(5) Erythrocytes.

(6) Sterile physiological salt solution (.9%).

Preparation of Antigen.

The antigen was prepared by growing a culture of Bact. abortus for 48 hours upon plain agar. The growth was then washed off with a solution containing phenol (.5 percent) and sodium chloride (.9 percent) and filtered thru cotton. The suspension was then standardized so that the turbidity corresponded to tube 5 of McFarland's Nephelometer (19). The Antigen if kept at ice box temperature will retain the same titer for many months.

Preparation of Serum to be Tested.

The manner in which the serum to be tested was prepared differed according to the different animals tested and the nature of the serum, as blood serum and milk serum both were examined during the experiment.

Guinea pigs: The blood serum from the guinea pigs was obtained by slightly anesthetising the animal, clipping the hair directly over the sternum, making a diagonal incision about one-half inch long in the skin covering the sternum, severing the sternum with a pair of small dissecting scissors, at the same time extending them forward far enough to sever the chambers of the heart completely. The blood is then collected in a sterile 25 cubic centimeter centrifuge tube, and the serum allowed to separate from the clot. The clot is then centrifuged and the serum pipetted off and inactivated at 56°C. for thirty minutes.

This last operation is very important on account of the complementary properties of the serum. Many workers do

not regard inactivation of importance if the serum stands any length of time before testing. A serum will lose a certain amount of its complementary properties on standing for any length of time, but sufficient complement often remains in the serum to produce aberrant results. After inactivation, the serum is preserved by adding .05 of a cubic centimeter of a 20 percent solution of phenol per 5 cubic centimeters of serum.

Rabbits: The rabbits were bled from the marginal vein of the ear or from the heart by means of a small hypodermic needle. When bleeding from the ear, xylol should be used to dilate the blood vessels. This may be done by saturating a small piece of cotton with xylol and massaging the ear gently until the vessels present a dilated appearance. The vessels will remain dilated for a considerable length of time. The xylol will not injure the ear in any way if removed with a damp cloth when the operation is finished.

Obtaining blood from the heart is not a complicated operation. The animal should first be firmly fastened on its back to an animal board, the hair should be clipped closely from over the left side of the thorax ribs, the spot disinfected with 5 percent phenol and a small hypodermic needle inserted into the left ventricle. Anesthesia is not necessary as the animal suffers no pain whatever, and only a few seconds are required to obtain sufficient blood. The blood is collected in a sterile test tube and allowed to clot. The clot is centrifuged and the serum pipetted off, inactivated and preserved in the same manner as described for the guinea pigs serum.

Calves:- The blood of the calves to be tested was obtained by bleeding from the jugular vein by means of a large hypodermic needle. The bleeding may be facilitated by dilating the vein. The thumb pressed firmly below the point of puncture will cause a dilation of the vein. The blood is collected in a sterile centrifuge tube, allowed to separate from the clot, centrifuged, pipetted off, inactivated and preserved in the same manner as described for the guinea pigs serum.

The milk serum used in the test was prepared by coagulating milk by adding .01 cubic centimeters of rennet per 10 cubic centimeters of whole milk. The milk was then placed in a water bath at a temperature of 40°C. About forty-five minutes is sufficient to completely coagulate the milk and for the separation of the serum. The milk should be about two degrees Fuller's Scale acid, otherwise a considerable length of time is required for the separation of the serum. As soon as the serum is sufficiently separated from the clot, it is filtered thru filter paper to remove any material which would have a tendancy to produce a cloudy serum. It is not necessary to inactivate the milk serum as Lane-Claypon(20) has shown that there is very little complement in milk. Two cubic centimeters is the smallest quantity in which complement can be demonstrated. If the milk serum is to be retained for any length of time it is advisable to add a few drops of 20 percent phenol to prevent microbial growth.

Preparation of Complement.

The guinea pig produces the most potent complement, therefore the blood serum of this animal was used as complement. The animal was bled as in the previously described method for bleeding guinea pigs, or by bleeding from the heart without killing or anesthetising the animal. This operation was performed in the following manner; The animal was firmly fastened on its back to an animal board, a small space over the left thorax clipped free from hair, the space disinfected with 5 percent phenol and a small hypodermic needle inserted between the fourth and fifth rib into the heart. If the needle enters the left side of the heart the blood will flow from the needle very rapidly. If the right side of the heart is entered instead, the blood flow will not be rapid, but by drops. Six cubic centimeters of blood may be taken from a large guinea pig weekly without the slightest discomfort or injury to the animal. In order to obtain sufficient complement from a large number of tests, the writer used the pooled blood of three guinea pigs. The blood was collected in a sterile centrifuge tube and the serum allowed to separate. The writer has obtained a more potent complement by bleeding the animal and allowing the blood to stand in the ice box from twenty to twenty-four hours before the serum is separated from the clot for use. Complement serum should always be kept at a very low temperature. Its strength diminishes very fast when allowed to remain for a short time at room temperature.

Preparation of Sheep Erythrocytes.

The blood is drawn from the jugular vein of a sheep by means of a large hypodermic needle. The blood is collected in a sterile Erlenmeyer flask containing small pieces of glass. The flask is shaken vigorously for several minutes in order to defibrinate the blood. The blood is then strained thru sterile gauze into a large centrifuge tube to remove the glass and fibrin and centrifugalized for several minutes in order to separate the cells from the serum. The supernatant liquid is syphoned off, and physiological salt solution (.9 percent) added and again centrifugalized. This washing is repeated three times in order to completely wash out all remaining serum from the erythrocytes.

A 2.5 percent solution of erythrocytes is used in all the titrations.

Preparation of Hemolysin.

The hemolysin was prepared by either the slow or the quick method. The slow method consists in injecting intraperitoneally into a rabbit 8, 12 and 15 cubic centimeters of concentrated corpuscles respectively at intervals of five days. Five days after the last injection the blood is tested for its hemolytic property. The quick method is most desirable as it involves less time and a smaller quantity of corpuscles. The quick method consists in three intravenous injections into the marginal vein of a rabbit of two cubic centimeters of concentrated corpuscles at intervals of three

days. Three days after the last injection the rabbit is bled from the marginal vein of the ear, and the blood serum obtained tested for its hemolytic properties according to the method described in Table II. If .001 of a cubic centimeter will completely hemolyze .5 cubic centimeters of a 2.5 percent suspension of sheep erythrocytes in 30 minutes, the serum is considered satisfactory for use. The rabbit may now be slightly anesthetised and bled from the heart. The blood is collected in a deep culture dish and the serum allowed to separate from the clot. The serum is collected in small vials, inactivated at 56°C. for 30 minutes, preserved by adding .05 cubic centimeters of phenol per 5 cubic centimeters of serum and sealed with paraffin. A hemolytic serum prepared in this manner and kept in a dark cool place will retain the same titre for many months.

Titration of Components.

The serum obtained from the guinea pig by the previously described method is diluted 1:3 with sterile physiological salt solution (.9 percent). The mixture is then titrated to determine the smallest quantity necessary to completely hemolyze .5 cubic centimeters of a 2.5 percent solution of sheep erythrocytes mixed with a constant quantity of hemolysin maintained in a water bath at 37°C. for 30 minutes. The amount of complement required to cause complete hemolysis is not constant with all guinea pigs, but usually .05 cubic centimeters of the dilute serum is sufficient. In the final test, two times the original titer was used. This

is necessary because the antigen absorbs a small amount of complement, which leads to aberrant results.

The titration of the complement is shown in Table I.

Table I.

Tube No.	Complement Dil. 1:3	Hemolysin 1%	NaCl .9%	Sheep Erythrocytes 2.5%	Incubate in H_2O bath 30" @ 37°C.	Results
1	.1 cc.	3 x t.(a)	1.5 cc.	.5 cc.		Hemolysis
2	.06 cc.	3 x t.	1.5 cc.	.5 cc.		"
3	.05 cc.	3 x t.	1.5 cc.	.5 cc.		"
4	.04 cc.	3 x t.	1.5 cc.	.5 cc.		No Hemolysis
5	.1 cc.		1.5 cc.	.5 cc.		" "
6		.5cc.	1.5 cc.	.5 cc.		" "
7			1.5 cc.	.5 cc.		" "

(a) t. = the titer found on titration.

From the table it may be seen that .05 cubic centimeters is the smallest quantity causing complete hemolysis. Two times this amount is used in the final test. Tube 5 is a control to show that the complement serum will not cause hemolysis. Tube 6 is a control to show that the hemolytic serum will not cause hemolysis. Tube 7 is a control to show that the salt solution will not cause hemolysis. These controls are very essential so as to avoid any possible error.

Titration of Hemolysin.

It is necessary to know the smallest quantity of hemolysin that will completely hemolyze .5 cubic centimeters of a 2.5 percent solution of sheep erythrocytes mixed with a constant quantity of complement.

The hemolysin prepared in the previously described

manner is prepared in a 1 percent physiological salt solution for titration.

The titration of the hemolysin is shown in Table II.

Table II.

Tube No.	Hemolysin 1%	Complement Dil. 1:3	NaCl Sol. .9%	Sheep Erythrocytes		Results.
1	.1 cc.	2 x t.[a]	1.5 cc.	.5 cc.	Incubate in H_2O bath 30' @ 37°C.	Hemolysis
2	.05 cc.	2 x t.	1.5 cc.	.5 cc.		"
3	.04 cc.	2 x t.	1.5 cc.	.5 cc.		"
4	.03 cc.	2 x t.	1.5 cc.	.5 cc.		No Hemolysis
5	.2 cc.		1.5 cc.	.5 cc.		" "

(a) t. = the titer found on titration.

In the above table .04 is the smallest quantity which will give complete hemolysis. Tube 5 is a control to show that a large amount of hemolysin will not itself cause hemolysis. In the final test it was necessary to employ three times the original titer to insure a sufficient quantity for the solution of the corpuscles.

Titration of Antigen.

Before performing the final test it is necessary to know the smallest quantity of antigen required to fix complement in the presence of a specific antibody. Since the antigen, when used in large quantities has the property of absorbing complement, it is necessary to know the smallest quantity that will absorb complement in the absence of a specific antibody.

In titrating the antigen, serum containing specific antibodies must be employed. This specific serum may be obtained

by injecting a rabbit intraperitoneally with 5 cubic centimeters of a 48 hour old bouillon culture of Bact. abortus. Two injections are given at intervals of five days. Five days after the last injection the rabbit is anesthetised and bled from the heart, the blood collected in a deep culture dish and the serum allowed to separate from the clot. The serum is inactivated at 56°C. for 30 minutes, preserved with phenol (.05 cc. of a 20 percent solution per 5 cc. of serum) placed in small vials and sealed with paraffin. An immune serum prepared in this manner and kept in a dark cool place will retain the same titer for many months.

The titration of antigen is shown in Table III.

Table III.

Tube No.	Antigen	Immune serum 10%	Complement Dil.(1:3)	NaCl .9%	Incubate in H_2O bath 30" @ 37°C.	Hemolysin 1%	Sheep Erythrocytes 2.5%	Incubate in H_2O bath 30' @ 37°C.	Results
1	.01 cc.	.1 cc.	2 x t.(a)	1.5cc.		3 x t.(a)	.5 cc.		Inc. Hemolysis
2	.02 cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		" "
3	.05 cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		No "
4	.1 cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		" "
5		.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		Hemolysis
6	.01 cc.		2 x t.	1.5cc.		3 x t.	.5 cc.		"
7	.02 cc.		2 x t.	1.5cc.		3 x t.	.5 cc.		"
8	.05 cc.		2 x t.	1.5cc.		3 x t.	.5 cc.		"
9	.1 cc.		2 x t.	1.5cc.		3 x t.	.5 cc.		"
10	.5 cc.		2 x t.	1.5cc.		3 x t.	.5 cc.		Inc. "

(a) t. = the titer found on titration.

In the above table tube 3 shows the smallest quantity of antigen that will fix complement in the presence of a specific antibody. Tube 5 is a control toshow that the immune serum will not prevent hemolysis in the absence of the antigen. Tubes from 6 to 10 inclusive show the amount of antigen that may be

employed without causing absorption of complement. Tube 10 shows the smallest amount of antigen that will absorb complement in the absence of the specific antibody.

The writer has obtained satisfactory results in the final test by using one-fourth the quantity of antigen necessary to absorb complement.

The Complement Fixation Test.

The object of the complement fixation test is to determine the presence of a specific antibody in a given serum and to what concentration it may be present.

The outline followed in the complement fixation test is shown in Table IV.

Table IV.

Tube No.	Serum tested (2)	Antigen (1)	Complement Dil.(1:3) (3)	NaCl .9%	Incubate in H2O bath 30' @ 37°C.	Hemolysin 1% (4)	Sheep Erythrocytes 2.5% (5)	Incubate in H2O bath 30' @ 37°C.	Results
1	.1 cc.	.1 cc.	2 x t.(c)	1.5cc.		3 x t.(c)	.5 cc.		No Hemolysis
2	.04 cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		" "
3	.02 cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		Inc. "
4	.005cc.	.1 cc.	2 x t.	1.5cc.		3 x t.	.5 cc.		Hemolysis
5	.1 cc.	.	2 x t.	1.5cc.		3 x t.	.5 cc.		"
6(b)	.1 cc.		2 x t.	1.5cc.			.5 cc.		No "

(a) Add 1/4 complement Absorption Titer.
(b) Add sheep cells on first incubation.
(c) t. = the titer found on titration.

In the above table, tube 2 shows the smallest quantity of serum which will completely fix complement.

A number of serums, especially rabbit serum have been found to inhibit hemolysis in the absence of the antigen.

Tube 5 is a control to show that the serum itself will not inhibit hemolysis. Tube 6 is a control to show that the serum being tested will not cause hemolysis in the absence of the antigen and the hemolysin.

The Agglutination Test.

In order to determine if there was any possible relation between the lytic antibodies and the agglutinating antibodies in a serum, the agglutination test was used in conjunction with the complement fixation test.

Preparation of Antigen.

The antigen was prepared in the same manner as in the complement fixation test. The suspension was prepared so that the turbidity compared with tube 1.5 of McFarland's Nephelometer. Four cubic centimeters of the bacterial suspension is placed in each of the small test tubes used and the following quantities of blood serum or milk added; .1, .05, .025, .01, and .005. From these approximate dilutions of 1:50, 1:100, 1:200, 1:500 and 1:1000 were obtained. The turbidity of the milk does not interfere with the readings of the final results.

Experimental work.

(1) The result of feeding infected milk to guinea pigs.

The susceptibility of guinea pigs to infectious abortion has been demonstrated by numerous investigators,

chiefly by the inoculation route.

Schroeder(19) states in a discussion of a paper read before the American Association of Medical Milk Commission that he and Cotton have not only produced the abortion disease in guinea pigs by injecting them with milk, but have successfully caused the disease in guinea pigs by feeding them seemingly normal milk from apparently normal cows that had become chronic carriers of the abortion bacillus.

Table 5 shows the result of feeding infected milk to eighteen normal female guinea pigs, ~~including~~ and to six controls, two of which were fed upon non-infected milk, two upon non-infected milk plus five cubic centimeters of a 48 hour bouillon culture of Bact. abortus and two received no milk.

A milk having a high antibody index was fed daily to the eighteen guinea pigs. The milk was tested every fourteen days to determine its antibody content.

The lesions which were found present in the liver of the guinea pigs when autopsied cannot be said to be characteristic lesions caused by Bact. abortus. Many of the guinea pigs which were killed for complement serum during the past year showed the same kind of pathological changes in the liver. The pathological changes in the liver consisted of irregular brownish yellow areas from 1 to 3 mm. in area scattered over the surface. There seemed to be no uniformity in size of the lesions. The size of the liver did not seem to be affected. The histological changes consisted of small focal necrotic areas scattered diffusely thruout the tissue. The areas were filled with polynuclear and endothelial cells. There was almost a complete atrophy of the surrounding liver cells.

Table 5.

Showing the result of feeding infected milk to guinea pigs.

No. of G. Pig	No. Days of feeding infected milk	Result of Autopsy	Reaction of Blood Serum								
			Agglutination					Comp. Fix.			
			.1	.25	.05	.01	.005	.1	.04	.02	.005
1	38	Spleen slightly enlarged. Many lesions in liver.	-	-	-	-	-	-	-	-	-
2(a)	287	Few scattered lesions in liver.	-	-	-	-	-	-	-	-	-
3(b)	287	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
4	38	Died of pneumonia.	-	-	-	-	-	-	-	-	-
5	38	Died of pneumonia.	-	-	-	-	-	-	-	-	-
6	48	Died of pneumonia.	-	-	-	-	-	-	-	-	-
7	80	No anatomical changes.	+	P	-	-	-	+	+	-	-
8	124	No anatomical changes.	-	-	-	-	-	-	-	-	-
9	96	No anatomical changes.	-	-	-	-	-	-	-	-	-
10(c)	60	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
11	33	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
12	33	No anatomical changes.	-	-	-	-	-	-	-	-	-
13	73	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
14	73	Few scattered lesions in liver.	-	-	-	-	-	-	-	-	-
15	95	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
16	95	Few scattered lesions in liver.	-	-	-	-	-	-	-	-	-
17	95	Many scattered lesions in liver.	-	-	-	-	-	-	-	-	-
18	95	No anatomical changes.	-	-	-	-	-	-	-	-	-
Controls Fed Non-Infected Milk.											
1	98	No anatomical changes.	-	-	-	-	-	-	-	-	-
2	98	No anatomical changes.	-	-	-	-	-	-	-	-	-
3	Fed no milk	No anatomical changes.	-	-	-	-	-	-	-	-	-
4	" " "	No anatomical changes.	-	-	-	-	-	-	-	-	-
5	60										
Fed non-infected milk plus cult Bact. abortus.		No anatomical changes.	+	+	-	-	-	+	+	-	-
Do	60	No anatomical changes.	+	+	+	-	-	+	+	+	-

(a) Gave birth to one live and two dead fetus on the 109th day of feeding.

(b) Gave birth to three live and one dead fetus on the 266th day of feeding.

(c) Aborted two fetus on 59th day of feeding.

One abortion and two abnormal births occured during the time of feeding of infected milk. Guinea pig No. 10 aborted two fetus on the 59th day of feeding. Pig No. 2 gave birth to one live and two still births on the 109th day of feeding. Pig No. 3 gave birth to three live and one still birth on the 266th day of feeding.

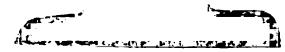
Two or eleven percent of the guinea pigs which were fed upon infected milk developed Bact. abortus antibodies in the blood during the time of feeding. The blood serum of guinea pig No. 7 gave a positive reaction at the time of autopsy. The blood serum of No. 3 gave a positive reaction eight days before autopsy just after parturition. The blood was negative ~~when~~ at time of autopsied.

(2) The result of feeding infected milk to rabbits.

That rabbits are susceptible to the abortion infection has been demonstrated by Bang(21), Nowak(22) and Mohler and Traum(23). This fact has been demonstrated only by inoculation experiments. No data are available pertaining to infedtions being acquired orally by rabbits thru the ingestion of infected milk or culture of Bact. abortus itself.

Fourteen normal females pregnant and non-pregnant were selected for the experiment. The effect of infected milk upon rabbits after continued ingestion was studied by means of agglutination and complement fixation tests on the blood serum. The frequency of abortions during pregnancy was also observed.

Table 6 shows the result of feeding infected milk to fourteen normal rabbits ~~including~~ and four controls, two of which were fed upon non-infected milk, the remaining two



received no milk for a period of one hundred and twenty-four days. A milk having a high antibody index was fed daily to the fourteen rabbits. The milk was tested every fourteen days to determine its antibody content. The reactions and partial reactions shown in the table were that to be due to a passive immunity. In order to clear up this point, four of the same rabbits as shown in Table 6 were fed no milk for a period of seventy four days, tests being made on the blood serum every two weeks. From the data, Table 7, one may easily see that there is very little difference in the blood reaction after feeding no milk and that during the feeding of infected milk. Rabbit No. 7 developed a pasitive reaction after being fed upon infected milk for 124 days. The reaction seemed to remain fairly constant after feeding of milk was discontinued.

The milk controls, (rabbits fed upon non-infected milk) and controls that were fed no milk show the same reactions and partial reactions as thos rabbits fed infected milk.

The reactions and partial reactions seem to be non-specific. It is a well known fact that old serum or serum containing an excess of hemoglobin often contains antilytic bodies, this could not have been the cause of the non-specific action of the above serums as they were separated from the clot in a clear state, inactivated, and titrated twenty-four hours after being drawn. Anticomplementary bodies cannot be considered, since there was no inhibition of hemolysis in control tubes containing serum and antigen controls. A conclusion may be crawn in two different ways, viz, either specific antibodies were present, or an absorption of complement occured due to the union of a substance in the serum with the antigen and complement.

In order to determine if the non-specific reaction was due to the presence of specific antibodies, three normal serums which were titrated and found to fix complement. The complement and antigen were incubated for two hours. The mixture was then centrifugalized and the clear serum drawn off and tested. The serums possessed the complement fixing properties in the same dilutions as before.

Non-specific reactions of serum have been studied with particular care by Noguchi(24) and to them he has given the name of proteotropic or false complement fixing bodies.

Kolmer and Trist(25) found non-specific substances present in the blood serum of rabbits and dogs against bacterial antigens such as staphlococci, colon and typhoid bacilli.

Table 8 shows the result of feeding non-infected milk plus a culture of Bact. abortus to six normal rabbits. The rabbits developed antibodies as a result of feeding the mixture. The antibodies are not persistent. They gradually develop to a maximum, remain for a short time and gradually disappear while the mixture is continually being fed.

No anatomical changes occured in any of the rabbits autopsied during, or ~~at the time~~ after the feeding of infected milk or non-infected milk plus a culture of Bact. abortus.was discontinued.

Each of the rabbits used were bred one or more times during the feeding of infected milk or non-infected milk plus a culture of Bact. abortus. No abortions occured in any of the rabbits.

(3) The result of feeding infected milk to new-born calves.

That calves may acquire an infection thru the ingestion

of milk has neither been definitely proven to occur, nor has it bee proven not to occur.

The possibility of a calf acquiring an infection in utero has been given very little consideration in previous researches upon the subject. The literature reveals no important experimental data concerning infection in utero.

In order to obtain unquestionable experimental data, calves (excepting those fed non-infected milk) were separated from their respective dams shortly after birth in order to prevent a possible infection from infectious material which might be deposited on the surface of the udder or teat. The infectious material being ingested at the time of sucking. The calves were bled from the jugular vein and the serum test for the presence of Bact. abortus antibodies was made before they received any milk whatever. Six calves were fed upon naturally infected milk and six calves were fed upon non-infected milk. The calves fed upon non-infected milk were not separated from their respective dams after birth due to unavoidable circumstances. They were allowed to remain and suck their dams for three days and then separated. A control was fed pasteurized naturally infected milk in order to compare the results with those fed infected milk. A second control was fed non-infected milk plus 5 cubic centimeters of a 48 hour ~~old~~ bouillon culture of Bact. abortus with each feeding in order to compare the effect of a naturally infected milk with that of an artificially infected milk. One gallon of the milk was fed twice daily thruout the experiment.

As accurately as possible, the history of each dam was obtained, showing the number of normal parturitions, the

number of abortions and the reaction of the blood serum to the agglutination and complement fixation tests at the time of the past parturition or abortion.

Table 9 shows the history of the dams of the calves used in the experiment.

The blood of cow 995 was negative up to the time of calving. Two days after calving, the blood and milk both became positive and have remained positive up to the time of this writing. Guinea pigs inoculated intra-abdominally with 5 cubic centimeters of the milk from each quarter showed characteristic Bact. abortus lesions when autopsied twelve weeks later. The blood serum of the guinea pigs gave a positive reaction to the agglutination and complement fixation tests. Bact. abortus was isolated from the diseased spleens of the guinea pigs. This cow has had one calf and no abortions.

The blood and milk of cows 997, 998, 999, 1000, 1002, 1005 and 1006 were negative before and after the last parturition.

The blood and milk of cow 1007 were not tested before the last parturition. Immediately after parturition both the milk and blood gave a negative reaction. This cow has had six calves. The calf of the second pregnancy was aborted.

No data was obtained on the blood reaction of cow 1001 before the last parturition, but immediately after the blood gave a partial reaction and the milk gave a positive reaction. The milk continued to react for four weeks at which time she died from an inflammation of the udder. This cow

Table Showing History of Dams of Calves Used in Feeding Experiments.

Dam No.	Age	Number of Normal Births	Number of Abortions	Reaction of Blood Serum before Last Parturition or Abortion	Reaction of Blood Serum after Parturition or Abortion	Reaction of Milk after Becoming Fresh
995	2	one	None	Negative	Positive	Positive
996	2	one	None	Negative	Positive	Positive
997	2	one	None	Negative	Negative	Negative
999	2	one	None	Negative	Negative	Negative
1000	2	one	None	Negative	Negative	Negative
1001	7	five	None	No Test Made	Partial (Susp.)	Positive
1002	5	Three	None	No Test Made	Negative	Negative
1003	4	Two	One 2nd Calf	Negative	(b)	
1004	8	Six	One 2nd Calf	No Test Made	Negative	Positive
1005	2	One	None	No Test Made	Negative	Negative
1006	7	Four	None	No Test Made	Negative	Negative
1007	9	Six	One 2nd Calf	No Test Made	Negative	Negative
1008	5	Three	None	No Test Made	Positive	Positive
1009	3	Two	None	Positive	Positive	Positive
86	3	None	Two	Positive	Positive	Positive
87	3	Two	None	No Test Made	Positive	Positive
88	3	One	One 2nd calf	No Test Made	Positive	Positive

(b) died of milk fever

has had five normal calves. No abortions have occurred.

The blood and milk of cow 1003 was negative before parturition. Two days after parturition she died of milk fever. This cow has had two normal calves. The calf of the second pregnancy was aborted.

No data was obtained on the blood and milk reaction of cow 1004 before parturition. After parturition the blood was negative, but the milk was positive for one week. This cow has had six normal calves. The calf of the second pregnancy was aborted.

The milk of cow 1008 has given a positive reaction for more than two years. The blood was not tested before the last parturition, but immediately afterward gave a positive reaction. This cow has had three normal calves. No abortions have occurred.

The milk and blood of cow 1009 was positive before the last parturition. Both milk and blood were positive when tested after the last parturition. This cow has had two normal calves. No abortions have occurred.

The milk of cows 86, 87 and 88 has shown a high antibody index for more than a year and a half, and was suspected of containing Bact. Abortus, which was later demonstrated to be true. The milk of these cows, including cow 995, was the "infected milk" which was fed to the guinea pigs, rabbits and calves.

Repeated trials to isolate Bact. Abortus directly from the milk fed to the animals failed, but by inoculating guinea pigs with the milk and allowing twelve weeks for incubation, lesions were produced in the spleen of guinea pigs

from which B ct. abortus was isolated.

Bull calf 995 A was fed upon naturally infected milk for a period of thirteen weeks. The blood of the calf was tested before feeding, and was found to be negative. A slight reaction developed the first week after feeding. A high antibody index developed at the end of the second week, and remained constant until the end of the third week. When tested at the end of the fourth week, the blood was negative. The blood remained negative during the remainder of the time feeding was continued.

At no time during the feeding did the sexual hairs around the sheath show any tendency to become matted. Approximately three weeks after feeding of milk was discontinued, the sexual hairs showed a brownish red staining and matting. A small portion of the matted hairs were clipped off and washed with saline solution. The mixture was then centrifugalized and the sediment examined microscopically. The microscopical findings consisted of a few epithelial cells, uric acid crystals and many bacteria. No pus cells were found in the sediment.

Heifer calf 996A was fed upon naturally infected milk for a period of ten weeks. The blood of the calf was tested before feeding, and was found to be negative. The blood remained negative throughout the time of feeding.

At no time during the feeding did the sexual hairs below the vulva show a tendency to become matted, nor was there any discharge of muco-pus from the vulva.

Table 10.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Bull Calf 995 A (a)		Reaction of Blood Serum									Reaction of Milk Being Fed.								
		Agglutination					Comp.Fix.				Agglutination					Comp.Fix.			
	Date	.1	.25	.05	.01	.005	.1	.04	.02	.005	.1	.25	.05	.01	.005	.1	.04	.02	.005
Before Feeding	1-15-16	-	-	-	-	-	-	-	-	-	+	+	+	p	p	+	+	+	+
1 week after feeding,	1-22-16	p	-	-	-	-	+	+	p	-									
2nd " " "	1-29-16	p	-	-	-	-	+	+	p	-	+	+	+	p	-	+	+	p	-
3rd " " "	2- 5-16	p	-	-	-	-	+	+	p	-									
4th " " "	2-12-16	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-
5th " " "	2-19-16	-	-	-	-	-	-	-	-	-									
6th " " "	2-26-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
7th " " "	3- 4-16	-	-	-	-	-	-	-	-	-									
8th " " "	3-11-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	-	-	-
9th " " "	3-18-16	-	-	-	-	-	-	-	-	-									
10th " " "	3-25-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	-	-
11th " " "	4- 1-16	-	-	-	-	-	-	-	-	-									
12th " " "	4- 8-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
13th " " "	4-15-16	-	-	-	-	-	-	-	-	-									
14th "(b) " "	4-22-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
17th " " "	5-11-16	-	-	-	-	-	-	-	-	-									
20th " " "	6- 3-16	-	-	-	-	-	+	p	-	-									
22nd " " "	6-15-16	-	-	-	-	-	-	-	-	-									
25th " " "	7-10-16	-	-	-	-	-	-	-	-	-									

(A) Born 1-15-16.
(b) On this date feed of milk was discontinued.

Bull calf 997A was fed upon naturally infected milk for a period of two weeks. The calf died of acute intoxication during the third week of feeding. The blood reaction was negative before feeding milk. A slight reaction developed at the end of the first week. At the end of the second week the blood was again negative. The sexual hairs around the sheath showed no signs of matting.

Heifer calf 1000A was fed upon naturally infected milk for a period of fourteen weeks. The blood was negative before the feeding of milk, and continued to remain negative throughout the period of feeding. At no time during the feeding did the sexual hairs below the vulva show a tendency to become matted, nor was there any discharge of muco-pus from the vulva.

Bull calf 1001A was fed upon naturally infected milk for a period of five weeks. The calf was slaughtered for veal at the end of the fifth week. The blood of the calf gave a positive reaction before feeding milk and continued to give a positive reaction up to the end of the third week. The blood gave a negative reaction on the fourth week and continued to remain negative.

The sexual hairs around the sheath became slightly matted and stained black the second week of feeding. A microscopic examination was made of the sediment washed from the hairs. Only epithelial cells, uric acid crystals, and bacteria were found.

Table 11.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Heifer Calf 996A (a)		Reaction of Blood Serum									Reaction of Milk Being Fed								
		Agglutination					Com.Fix.				Agglutination					Com.Fix.			
	Date	.1	.25	.05	.01	.005	.1	.04	.02	.005	.1	.25	.05	.01	.005	.1	.04	.02	.005
Before Feeding	5-28-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
1 week after feeding,	6- 5-16	-	-	-	-	-	-	-	-	-									
2nd " " "	6-12-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	-	-
3rd " " "	6-19-16	-	-	-	-	-	-	-	-	-									
4th " " "	6-26-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	p	p	-	-
5th " " "	7- 3-16	-	-	-	-	-	-	-	-	-									
6th " " "	7-10-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	p	p	-	-
7th " " "	7-17-16	-	-	-	-	-	-	-	-	-									
8th " " "	7-24-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	p	p	-	-
9th " " "	7-31-16	-	-	-	-	-	-	-	-	-									
10th " " "	8- 7-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	p	p	-	-

Born 5-28-16

Table 12.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Bull Calf 997A (a)		Reaction of Blood Serum									Reaction of Milk Being Fed								
		Agglutination					Com.Fix.				Agglutination					Com.Fix.			
	Date	.1	.25	.05	.01	.005	.1	.04	.02	.005	.1	.25	.05	.01	.005	.1	.04	.02	.005
Before Feeding	2- 5-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
1 week after feeding.	2-12-16	-	-	-	-	-	p	-	-	-									
2nd " " "	2-19-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	-	-
3rd " " " (b)																			

(a) Born 2-5-16
(b) Died of acute intoxication 2-21-16.

Table 13.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Heifer Calf 1000A (a)	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp.Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp.Fix. .1	.04	.02	.005
Before Feeding	2-25-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	+	+
1 week after feeding,	3- 3-16	-	-	-	-	-	-	-	-	-									
2nd " " "	3-10-16	-	-	-	-	-	-	-	-	-									
3rd " " "	3-17-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
4th " " "	3-24-16	-	-	-	-	-	-	-	-	-									
5th " " "	3-31-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
6th " " "	4- 7-16	-	-	-	-	-	-	-	-	-									
7th " " "	4-14-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
8th " " "	4-21-16	-	-	-	-	-	-	-	-	-									
9th " " "	4-28-16	-	-	-	-	-	-	-	-	-	+	+	+	p	p	+	+	+	-
10th " " "	5- 5-16	-	-	-	-	-	-	-	-	-									
11th " " "	5-12-16	-	-	-	-	-	-	-	-	-	+	+	+	+	p	+	+	+	-
12th " " "	5-26-16	-	-	-	-	-	-	-	-	-									
13th "(b) " "	6- 2-16	-	-	-	-	-	-	-	-	-	+	+	+	+	p	+	+	p	-
15th " " "	6-15-16	-	-	-	-	-	-	-	-	-									
20th " " "	7-10-16	-	-	-	-	-	-	-	-	-									

(a) Born 2-25-16.
(b) On this date the feeding of milk was discontinued.

Table 14.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Bull Calf 1001A (a)	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp.Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005
Before Feeding	4-28-16	+	p	-	-	-	+	+	p	-	+	+	-	-	-	-	-	-	-
1 week after feeding	5-6-16	+	-	-	-	-	+	+'	+	-									
2nd " " "	5-13-16	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-
3rd " " "	5-22-16	-	-	-	-	-	+	-	-	-									
4th " " "	5-29-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5th "(b)" "	6- 5-16	-	-	-	-	-	-	-	-	-									

(a) Born 4-27-16
(b) Killed 6-7-16

Bull calf 1002A was fed upon a non-infected milk for the first week after birth and then fed upon naturally infected milk for thirteen weeks.

The blood was negative before feeding milk and continued to remain negative thruout the period of feeding.

The sexual hairs about the sheath developed a slight matting and brownish black staining when the calf was four weeks old. The matting was not persistent. The hairs would matt together after urination, but would separate again on drying. Several microscopic examinations were made of the washings from the sexual hairs. No pus cells were ever found present.

In order to compare a possible blood reaction due to a naturally infected milk with that due to an artificially infected milk, a calf was fed upon non-infected milk plus a culture of Bact. abortus.

Bull calf 1003A was fed upon non-infected milk plus five cubic centimeters of a forty eight hour boillion culture of Bact. abortus (the culture being added to the milk at each feeding) for a period of twelve weeks. The blood of the calf was negative before feeding the mixture. A high antibody index developed after the first week of feeding, which remained constant until the end of the fourth week. At this time the antibody index began to decrease. On the ninth week the blood was negative and remained negative up to the time of this writing.

A distinct matting and staining of the sexual hairs around the sheath appeared after twelve weeks of feeding. A microscopic examination of the hairs showed only a few epithelial cells, uric acid crystals and many bacteria. No pus cells were found.

Table 15.

Showing the Result of Feeding Naturally Infected Milk to a New Born Calf.

Bull Calf 1002A (a)	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005
Before Feeding	4-27-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 week after feeding	5- 3-16	-	-	-	-	-	-	-	-	-									
2nd " " "	5-10-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
3rd " " "	5-17-16	-	-	-	-	-	-	-	-	-									
4th " " "	5-24-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
5th " " "	5-29-16	-	-	-	-	-	-	-	-	-									
6th " " "	6-5-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-
7th " " "	6-12-16	-	-	-	-	-	-	-	-	-									
8th " " "	6-19-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	+	-
9th " " "	6-26-16	-	-	-	-	-	-	-	-	-									
10th " " "	7- 3-16	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
11th " " "	7-10-16	-	-	-	-	-	-	-	-	-									
12th " " "	7-17-16	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	p
13th " " "	7-24-16	-	-	-	-	-	-	-	-	-									
14th " " "	7-31-16	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	p

(a) Born 4-26-16

Table 16.

Showing the Result of Feeding Non-Infected Milk plus a Culture of Bact. Abortus to a New Born Calf.

Bull Calf 1003A (a)	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp.Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp.Fix. .1	.04	.02	.005
Before Feeding	2-21-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
" "	3- 6-16	-	-	-	-	-	-	-	-	-									
1 week after feeding	3-13-16	-	-	-	-	-	±	±	±	p	-	-	-	-	-	-	-	-	-
2nd " " "	3-20-16	-	-	-	-	-	±	±	±	p									
3rd " " "	3-27-16	-	-	-	-	-	±	±	±	p	-	-	-	-	-	-	-	-	-
4th " " "	4- 3-16	-	-	-	-	-	±	±	±	-									
5th " " "	4-10-16	-	-	-	-	-	±	±	p	-	-	-	-	-	-	-	-	-	-
6th " " "	4-17-16	-	-	-	-	-	±	±	-	-									
7th " " "	4-24-16	-	-	-	-	-	±	±	-	-	-	-	-	-	-	-	-	-	-
8th " " "	5- 1-16	-	-	-	-	-	±	±	-	-									
9th " " "	5-8-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10th " " "	5-15-16	-	-	-	-	-	±	-	-	-									
11th " " "	5-22-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12th "(b)" "	5-29-16	-	-	-	-	-	-	-	-	-									
15th " " "	6-15-16	-	-	-	-	-	-	-	-	-									
19th " " "	7-10-16	-	-	-	-	-	-	-	-	-									

(a) Born 2-21-16
(b) On this date the feeding of milk was discontinued.

In order to maintain a control on the naturally infected milk fed to the calves, the infected milk was pasteurized at a temperature of 63 deg. C. for twenty minutes and fed to a calf. A temperature of 63 deg. C. for twenty minutes has no effect upon the Bact. abortus antibodies, but is sufficient to destroy all Bact. abortus organisms.

Heifer calf 999A was fed upon pasteurized infected milk for a period of thirteen weeks. The blood reaction was negative before feeding and remained negative thruout the time of feeding.

At no time during the feeding did the sexual hairs below the vulva become matted, nor was there any discharge of muco-pus from the vulva.

Table 17:

Showing the Result of Feeding Pasteurized Naturally Infected Milk to a New Born Calf.

Heifer calf 999 A	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005
Before Feeding	2- 3-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
1 week after feeding	2-10-16	-	-	-	-	-	-	-	-	-									
2nd " " "	2-17-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	p	-
3rd " " "	2-24-16	-	-	-	-	-	-	-	-	-									
4th " " "	3- 2-16	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
5th " " "	3- 9-16	-	-	-	-	-	-	-	-	-									
6th " " "	3-16-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	-	-	-
7th " " "	3-23-16	-	-	-	-	-	-	-	-	-									
8th " " "	3-30-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	-	-
9th " " "	4- 6-16	-	-	-	-	-	-	-	-	-									
10th " " "	4-13-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	-	-
11th " " "	4-20-16	-	-	-	-	-	-	-	-	-									
12th " " "	4-27-16	-	-	-	-	-	-	-	-	-	+	+	+	p	-	+	+	+	-
13th "(b) " "	5-11-16	-	-	-	-	-	-	-	-	-									
17th " " "	6- 3-16	-	-	-	-	-	-	-	-	-									
19th " " "	6-15-16	-	-	-	-	-	-	-	-	-									
23rd " " "	7-10-16	-	-	-	-	-	-	-	-	-									

(a) Born 2-3-16
(b) On this date the feeding of milk was discontinued.

Tables 18, 19, 20, 21, 22, and 23 show the result of feeding non-infected milk to calves. Contrary to the other feeding experiments, these calves were not separated from their dams immediately after birth. They were allowed to suck their dams three days and then separated and fed upon non-infected milk.

Bull calf 1004A was fed upon non-infected milk for a period of twelve weeks. The blood of this calf gave a postive reaction before it was fed upon milk. The blood continued to give a postive reaction up to the fourth week of feeding. The blood tests were discontinued until the ninth week. The blood at this time was negative. It was negative when tested again at the end of the twelfth week.

No matting of the hairs around the sheath occurred during the time of feeding.

Heifer calves 1005A and 1006A were fed upon non-infected milk for a period of twelve weeks. The blood of each calf was negative before feeding, and continued to remain negative thruout the time of feeding.

No matting of the sexual hairs below the vulva occurred during the time of feeding.

Table 18.

Showing the Result of Feeding Non-Infected Milk to a New Born Calf.

Bull Calf 1004A (a)	Date	Reaction of Blood Serum Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005	Reaction of Milk Being Fed Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005
Before Feeding	4-28-16	+	+	p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 week after feeding	5- 7-16	+	p	-	-	-	+	+	p	-									
2nd " " "	5-14-16	+	-	-	-	-	p	-	-	-	-	-	-	-	-	-	-	-	-
3rd " " "	5-22-16	+	p	-	-	-	-	-	-	-									
9th " " "	6-29-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12th " " "	7-24-16																		

(a) Born 4-27-16

Table 19.

Showing the Result of Feeding Non-Infected Milk to a New Born Calf.

Heifer Calf 1005A (a)	Date	Reaction of Blood Serum Agglutination .1 .25 .05 .01 .005	Comp.Fix. .1 .04 .02 .005	Reaction of Milk Being Fed. Agglutination .1 .25 .05 .01 .005	Comp. Fix. .1 .04 .02 .005
Before Feeding	4-20-16	- - - - -	- - - -	- - - - -	- - - -
1 week after feeding	5- 3-16	- - - - -	- - - -		
2nd week " "	5-10-16	- - - - -	- - - -	- - - - -	- - - -
3rd " " "	5-17-16	- - - - -	- - - -		
9th " " "	6-29-16	- - - - -	- - - -	- - - - -	- - - -
12th " " "	7-24-16				

(a) Born 4-19-16

Table 20.

Showing the Result of Feeding Non-Infected Milk to a New Born Calf.

Bull Calf 1006 A (a)	Date	Reaction of Blood Serum Agglutination .1 .25 .05 .01 .005	Comp.Fix. .1 .04 .02 .005	Reaction of Milk Being Fed Agglutination .1 .25 .05 .01 .005	Comp. Fix. .1 .04 .02 .005
Before Feeding	4-19-16	- - - - -	- - - -	- - - - -	- - - -
1 week after feeding	4-26-16	- - - - -	- - - -		
2nd " " "	5- 3-16	- - - - -	- - - -	- - - - -	- - - -
3rd " " "	5-10-16	- - - - -	- - - -		
10th " " "	6-29-16	- - - - -	- - - -	- - - - -	- - - -
13th " " "	7-10-16				

(a) Born 4-19-16

Table 21.

Showing the Result of Feeding Non-Infected Milk to a New Born Calf.

Bull Calf 1007A (a)		Reaction of Blood Serum									Reaction of Milk Being Fed								
		Agglutination					Comp.Fix.				Agglutination					Comp. Fix.			
	Date	.1	.25	.05	.01	.005	.1	.04	.02	.005	.1	.25	.05	.01	.005	.1	.04	.02	.005
2nd week after feeding	4-21-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3rd " " "	4-29-16	-	-	-	-	-	-	-	-	-									
4th " " "	5- 6-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5th " " "	5-15-16	-	-	-	-	-	-	-	-	-									
6th " " "	5-22-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14th " " "	7-24-16	-	-																

(a) Born 4-2-16

Bullc lf 1007A was fed upon non-infected milk for a period of twelve weeks. The blood of this c calf was not tested until two weeks after birth. The blood was negative and remained negative thruout the feeding. This calf was selected more especially on account of the ifficulty of the dam had in expelling the calf. Eight hours were required for the complete expulsion of the calf. A muco purelent discharge came from the vulva of the dam continually for two weeks before parturition and continued for several weeks afterward.

No matting of the sexual hairs occurred around the sheath during the time of feeding.

Heifer calves 1008A and 1009A were allowed to suck their dams for four days after birth and then fed upon non-infected milk. The milk of each dam has continually reacted to seriological tests for more than eighteen months. The blood of the two calves gave a positive reaction when first tested. The reaction seemed to decrease with each succeeding test. No tests were made on the blood at the time of birth, therefore, the mode of infection is a question.

There was no matting of the sexual hairs of either calf.

Table 22.

Table Showing the Presence of Antibodies in the Blood of a Calf Fifty Nine Days Old Before the Blood Was Tested.

Heifer Calf 1008A	Date	Reaction of Blood Serum Agglutination .1 .2 .0 .01 .00	Reaction of Blood Serum Comp. Fix. .1 .04 .02 .00	Reaction of Milk Being Fed Agglutination .1 .2 .0 .01 .00	Reaction of Milk Being Fed Comp. Fix. .1 .04 .02 .00
8th week of feeding	4-19-16	p - - - -	+ + + p	- - - - -	- - - -
9th " " "	4-26-16	- - - - -	+ + - -		
10th " " "	5- 3-16	+ + p - -	+ + +, -	- - - - -	- - - -
11th " " "	5-10-16	- - - - -	+ + p -		
12th " " "	5-17-16	- - - - -	+ p - -	- - - - -	- - - -

Form 2-20-16

Table 23.

Table Showing the Presence of Antibodies in the Blood of Calf Seventeen Days Old Before the Blood Was Tested.

Heifer calf 1009A	Date	Reaction of Blood Serum: Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005	Reaction of Milk Being Fed: Agglutination .1	.25	.05	.01	.005	Comp. Fix. .1	.04	.02	.005
3rd week of feeding	4-19-16	p	-	-	-	-	±	±	±	p	-	-	-	-	-	-	-	-	-
4th " " "	4-26-16	-	-	-	-	-	±	±	p	-	-	-							
5th " " "	5-2-16	p	-	-	-	-	±	±	±	-	-	-	-	-	-	-	-	-	-
6th " " "	5-9-16	-	-	-	-	-	±	-	-	-									
7th " " "	5-16-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Born 4-2-16

General Discussion.

The experimental work has been conducted in such a manner as to determine more definitely than have the former researches the infectiousness of milk fed to animals.

Repeated trials were made to isolate Bact. abortus directly from the milk upon which the animals were fed without success. However, by employing an indirect method, that is, by inoculating guinea pigs with the milk and allowing a period of twelve weeks to elapse for incubation, Bact. abortus was isolated from the diseased spleen of the animals when autopsied.

The gross pathological and histopathological changes that take place in the liver and spleen of guinea pigs due to Bact. abortus are described by Fabyan (26) in the following manner: In the liver the characteristic pathological changes are, adhesion to the surrounding tissues; the size of the organ does not seem to be affected by the disease; a tense, congested appearance is often present. Scattered over the surface are a few or many grayish, translucent, pin-point foci, just visible or two or three m. m. in diameter extending into the substance of the organ. When newly formed, these foci have a glistening, pearly appearance and later assume a yellowish, opaque color. The gall bladder appears normal. The hepatic lymph nodes are sometimes enlarged. The histopathological changes are characteristic foci scattered diffusely thru the tissue, especially at the border of the lobule in close

posed of a few cells or they may occupy an area the size of two or more lobules. The liver cells are pushed aside or become occluded and degenerate, the protoplasm staining a diffuse eosin-red. Focal necroses are common in some cases. Polynuclear leucocytes are present, and rarely a few isolated giant cells. Dilatation and proliferation of the bile ducts is noted.

The gross pathological changes in the spleen are enlargement, distention of the capsule, the organ fairly bursting. The surface may be normal or present numerous fine, grayish, pin-point elevations. Occasionally a few or numerous grayish, opaque, pinhead foci are distinguished just beneath the capsule. The color is darker than normal. On section the surface is very moist; the pulp usually swollen, soft, obliterating in part the normal markings. When the disease becomes chronic, the spleen appears only moderately enlarged. The histopathological changes are dilation of the blood sinuses, cellular proliferation of the tissue, the epitheloid cells appearing in groups. These may be present in the Malphigian bodies or lie just beneath the capsule. Polynuclear lucocytes are occasionally quite numerous. Giant cells are usually are but may be present in large numbers. We seldom found cells containing typical bacilli. The connective tissue changes are not well marked.

The lesions which were found present in the liver and spleen of guinea pigs resulting from the injection of naturally infected milk fed to the animals, are

That Bact. abortus antibodies develop in the bodies of guinea pigs when fed naturally infected milk is considered without doubt. The danger resulting therefrom appears to be a negligible factor. Eleven per cent of the guinea pigs gave reactions to serological tests either when autopsied or before autopsy. The two guinea pigs fed upon a mixture of non-infected milk plus a culture of Bact. abortus developed antibodies, but no lesions were found in the organs when autopsied.

The antibodies which developed in the guinea pigs while being fed upon naturally infected milk were due either to a passive transmission of the antibodies present in the milk or to the presence of the antigen in the bodies of the animals. Ehrlich (27) has shown that a high degree of immunity is conferred to the suckling thru milk when the mother is highly immune to bodies such as ricin, robin, abrin and tetanus toxin. One cannot overlook the fact that the results obtained by Ehrlich concern antitoxic bodies and not bactericidal bodies.

The failure to demonstrate antibodies in guinea pig No. 3 can be explained only by a negative reaction which often occurs directly after an infection. The still birth that occurred in pigs No. 2 and No. 3 cannot, as yet, be attributed to Bact. abortus. There is no literature available on this phase of the question.

Out of fourteen rabbits fed upon infected milk, one, or seven percent, developed Bact. abortus antibodies. The present of these antibodies in the body

of the rabbit (no 7, Table 6) cannot be attributed to a passive immunity. As the blood serum of the rabbit continued to show a high antibody index after the feeding of infected milk was discontinued. The remaining reactions and partial reactions that are shown in the table can be attributed only to the non-specific complement fixing property possessed by rabbit serum. For this reason the rabbit is not a suitable animal for experimental work on infectious abortion.

That rabbits will develop antibodies in the body when fed continually upon a culture of Bact. abortus is shown in table 8. The antibodies do not persist thruout the feeding of the mixture. They begin to gradually disappear after the fifty-sixth day of feeding.

Out of six calves fed upon infected milk, one calf, 995A, or sixteen per cent, developed Bact. abortus antibodies. The antibody index remained constant for four weeks. At the beginning of the fifth week and during the remainder of the time the calf was fed upon infected milk, antibodies could not be demonstrated. The antibodies which were present in the blood serum cannot be looked upon as being due to a passive transmission of antibodies which were present in the milk, as the development of antibodies in calf 1003', which was fed upon a mixture of non-infected milk plus a culture of Bact. abortus, compare very closely with the development of antibodies in calf 995A. The development of antibodies in the calves as a result of ingesting naturally infected milk seems to be a demonstrated fact, but the cause and significance

of the appearance of the antibodies cannot be explained until further development of the studies on the calves.

That Bact. abortus antibodies occur in the fetus at the time of birth has never before been demonstrated. The blood serum of calves 1001A and 1004A, as the serological tests show, possessed antibodies at the time of birth. The antibodies maintained a fairly high index for three weeks, after which they gradually disappeared. Were the antibodies due to the present of the antigen in the body of the fetus, or to a possible transmission of antibodies from the mother?

If one studies the history of the mothers of the calves, it can be readily seen that there is no relation between the antibodies present in the blood of the two fetus and that of the mothers. Cow 1001 showedonly a partial fixation of complement in one cubic centimeter of blood serum at the time of calving, while the blood serum of the calf showed a fairly high antibody index. Cow 1004 showed no antibodies in the blood serum at the time of calving, while the blood serum of the calf showed a fairly high antibody index. Pfandler (28) says that antibodies circulating in the body of the mother have not been proved to pass thru the placenta into the body of the fetus, even if the mother becomes immune to the disease. Altho, he says, this may occur from pathological changes in the placenta and from the presence of the antigen in the placenta which serves to stimulate the production of antibodies that pass into the circulation of the fetus.

The matting of the sexual hairs of bull and heifer calves is a subject to which Williams (29) has attached a great deal of importance in his researches. He concludes from his observations that calves fed upon raw milk in almost every instance acquire a matting of the sexual hairs from thirty to sixty days after birth and that Bact. abortus may be the cause of this phenomenon.

In only two cases of those recorded in the foregoing data was there any matting and staining of the sexual hairs observed. The sexual hairs about the sheaths of calves 1001A and 1004A became slightly matted and stained brownish black about two weeks after birth. The sexual hairs seemed to be matted only during the time and shortly after urination. The hairs were clipped from the sheaths of both calves on several occasions, washed with saline solution, smears made of the sediment from the centrifugalized washings and examined microscopically. The examination always revealed epithelial cells, uric acid crystals, and may bacteria. Pus cells were never found present.

Most of the bull calves developed a matting of the hairs after the feeding of milk was discontinued which was usually from ninety to one hundred days after birth. The vulvar hairs of heifers never showed any tendency to become matted after feeding of milk was discontinued.

Only one conclusion can be drawn from the date, and that is, there has been no connection shown to exist between the matting of sexual hairs of new born calves and the feeding of either infected or non-infected milk.

That the experimental work is more conclusive than is Williams' in showing that antibodies develop in animals from the ingestion of milk in that, (1) the blood serum of guinea pigs, rabbits, and calves develops antibodies when fed upon infected milk, but does not develop when fed non-infected milk; (2) the blood of the calves was tested before feeding milk, (3) the calves were separated from their dams directly after birth and not allowed to suckle, thus eliminating a possible chance for an infection to occur from a source other than the milk. Williams did not take any one of these factors into consideration in similar researches.

That the blood of calves should be tested before feeding for Bact. abortus antibodies is clearly emphasized in tables 22 and 23. It would be unjustifiable to say that the two calves became infected from the ingestion of milk, since no serological tests were made of the blood before the calves were fed upon milk.

It occurred to the writer that perhaps the antibodies present in the naturally infected milk might be highly bactericidal for Bact. abortus, thus being capable of destroying Bact. abortus after sufficient time has elapsed for bactericidal action to take place.

In order to demonstrate this point an experiment was outlined according to table 24. A milk which contained Bact. abortus antibodies was heated intermittently for one hour at 60 deg. C on three successive days in order to destroy all microorganisms present. Two cubic centimeters of boillon were placed in small sterile

test tubes and to these were added in order .2, .1 and .05 cubic centimeters of milk, 24,000 Bact. abortus organisms, and .1 cubic centimeter of complement serum. Two controls were used, one containing boillon, .2 cubic centimeters of milk and 24,000 bacteria, the other containing only boillon and 24,000 bacteria. The tubes were incubated for two hours at 37 deg. C. One tenth cubic centimeter of the mixture from each tube was plated out before and .1 cubic centimeter after incubation.

Table 24.

Showing the Cytolytic Effect of Milk Containing Bact. Abortus Antibodies.

Tube No.	Milk	No. Bacteria Added	Complement Dil.(1;3)	Bullion	No. Bacteria Present After 2 hrs Incubation @ 37
1	.2 cc.	24,000	.1 cc.	2 cc.	1200
2	.1 cc.	24,000	.1 cc.	2 cc.	None
3	.005 cc.	24,000	.1 cc.	2 cc.	None
4	.2 cc.	24,000		2 cc.	Many thousand
5		24,000		2 cc.	43,800

The results show that there is a decided reduction in the number of bacteria treated with milk containing Bact. abortus antibodies. Tube 1 contained 1200 bacteria after inculbation. Tubes 2 and 3 were sterile. The control tubes 4 and 5 contained approximately 48,000 bacteria. The presence of bacteria in tube 1 can be explained only as being due to the inhibiting effect of such a large amount of serum on the complement. Since a milk containing Bact. abortus antibodies is highly bactericidal for Bact. abortus, it would be logical to assume that such a milk when heated at a sufficient temperature to kill the organisms and not the antibodies, would be beneficial to new born calves. The milk containing antibodies should give the calf a possible immunity during its early development.

- SUMMARY -

Guinea pigs, rabbits, and new born calves develop Bact. abortus antibodies as a result of ingesting naturally infected milk.

No serious danger seems to result from the appearance of the antibodies in the blood of the animals.

The possibility of infections being acquired in utero seems to be a phase of the abortion question that should be given more attention, since 16 per cent of the twelve calves used in this experiment possessed Bact. abortus antibodies at the time of birth.

There appears to be no connection between the matting of the sexual hairs of bull and heifer calves

and the ingestion of naturally infected milk or of non-infected milk.

A milk containing Bact. abortus antibodies appears to be highly bactericidal for Bact. abortus in vitro.

In concluding I wish to acknowledge my indebtedness to Mr. L. H. Cooledge and to Dr. Ward Giltner for suggestions and assistance received in this investigation.

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Fig. 1. Showing the unmatted sexual hairs of calf 995 A after feeding naturally infected milk for twelve weeks.

Fig. 2. Showing the unmatted sexual hairs of calf 996 A after feeding naturally infected milk for four weeks.

Fig. 3. Showing the matting of the sexual hairs of calf 995 A three weeks after the feeding of naturally infected milk was discontinued.

Fig. 4. Showing the matting of the sexual hairs of calf 1003 A one week after the feeding of milk was discontinued.

Fig. 5. Showing the apparent matting of the sexual hairs of calf 1002 A. This matting is due to urination.

Fig. 6. Showing a distinct muco pus discharge from the vagina of cow 996.

Fig. 1

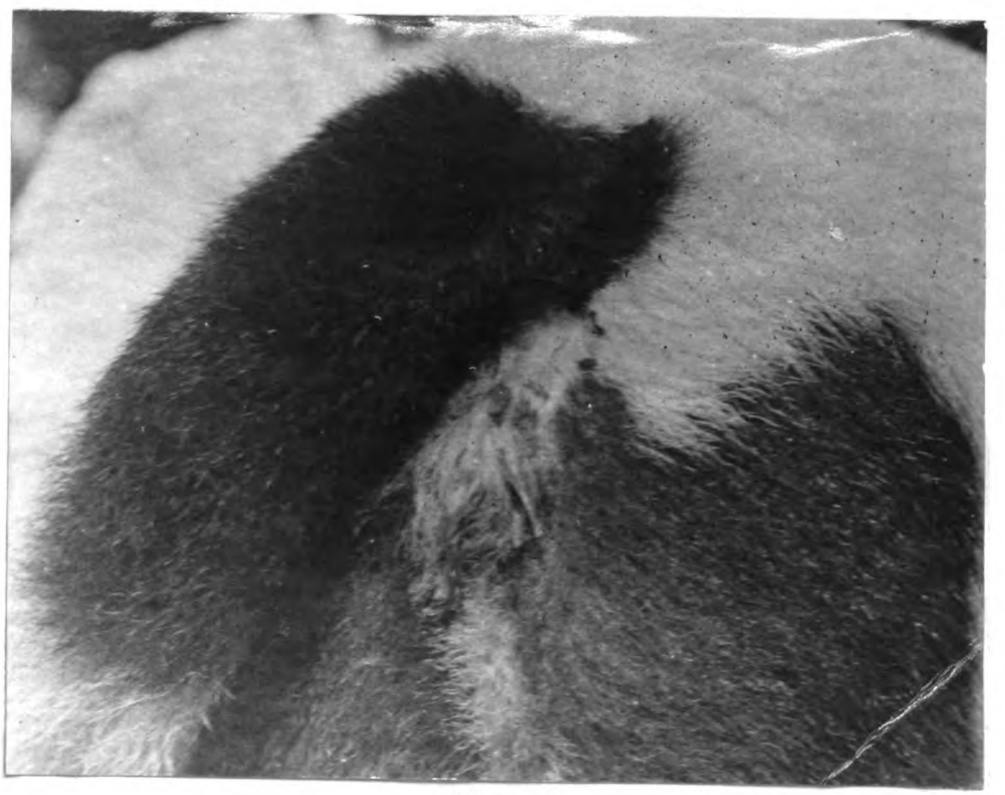

Fig. 2

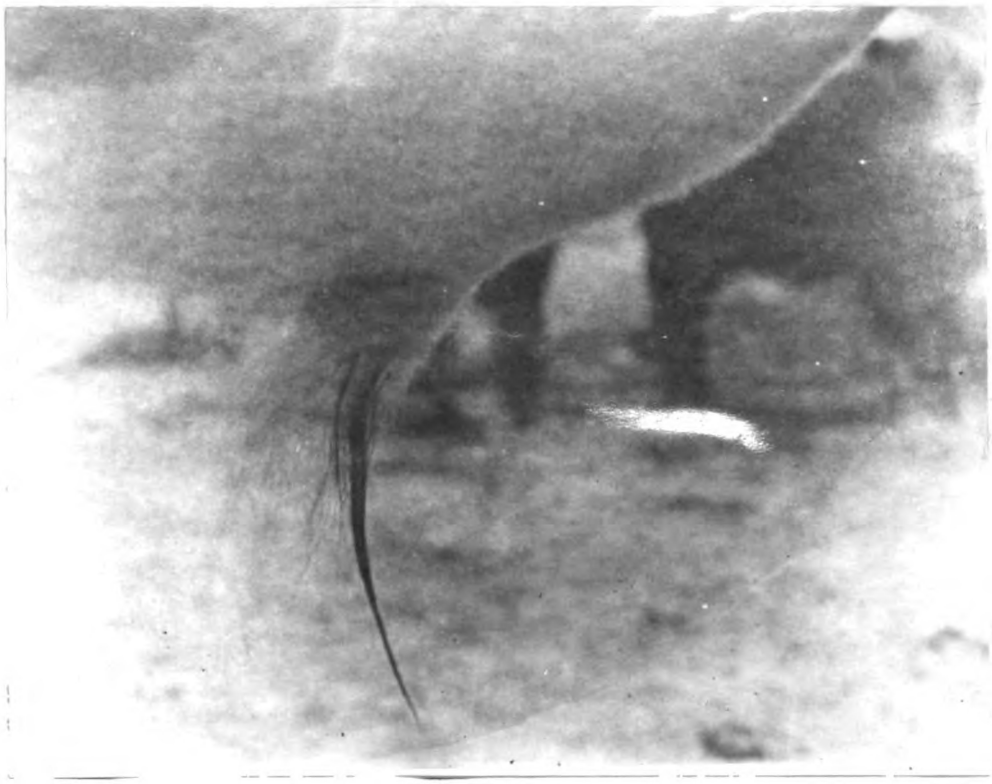

Fig. 3

Fig. 4

Fig. 5

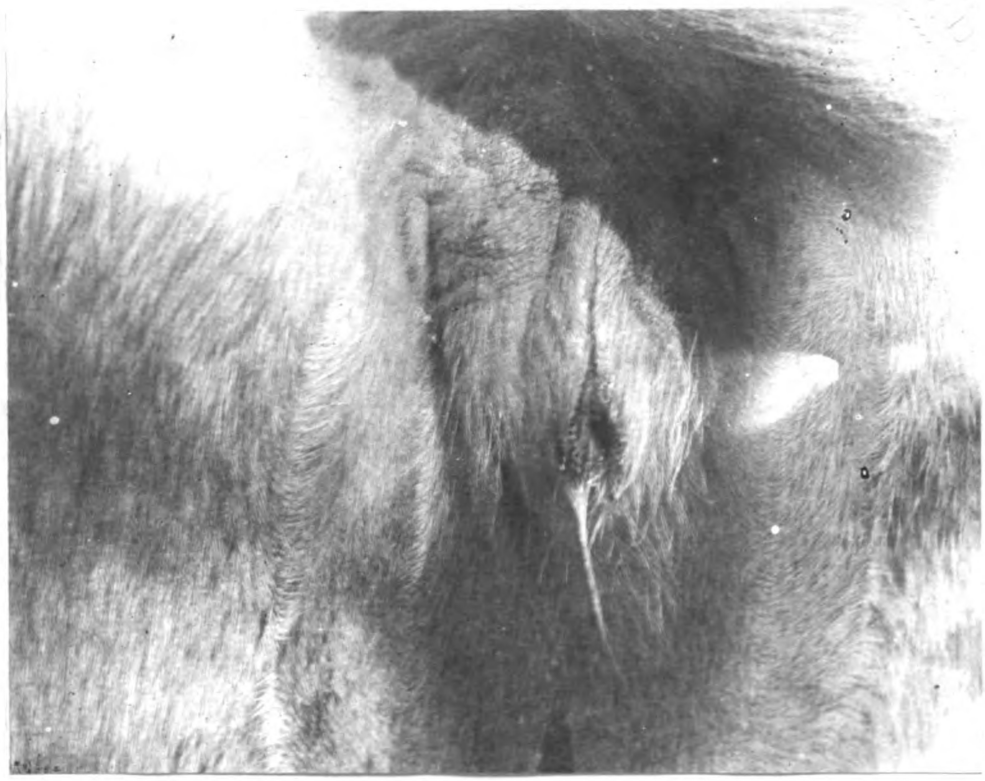

Fig. 6