A STUDY TO DETERMINE THE EFFICIENCY OF THE DIFFERENTIAL TEST FOR INFECTIOUS ABORTION OF CATTLE.

Major Thesis in Animal Pathology prepared

by

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June 1930

For the degree of Master of Science

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CONTENTS

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INTRODUCTION

The cattle owner has many problems with which to contend. Among the most important ones are those pertaining to the health of the herd.

Infectious abortion is one of the most widespread and dreaded diseases in the cattle industry. It is rapidly becoming more prevalent due to the development of the industry and the frequent interchange of animals.

The term "abortion" has unfortunately been used to designate any abortion that may occur. Any condition, whether infectious or otherwise, that causes the death and premature expulsion of the fetus is an abortion. This makes two classifications necessary, one of non-specific cause and the other due to specific infections. Among the latter that have caused outbreaks of abortion are: Vibrio fetus, streptococcus and Brucella abortus. That caused by Brucella abortus is the only one considered in this project.

This microorganism is responsible for great losses in the cattle industry. It kills many calves, causes many cows to become sterile, and diminishes the milk flow to a marked extent. Cattle infected with Brucella abortus show no visible lesions, and may or may nor abort due to the infection. Others known to be free of infection may abort due to traumatic or other causes.

The only practical test we have now for detecting abortion infected cattle is the agglutination test. At present there seems to be little question as to its value. The reaction to the test depends on the presence of the specific agglutinins in the blood stream. A cow may have the agglutinins in her blood, and not have Brucella abortus present. This cow would give a positive agglutination reaction the same as one that is a carrier of the organism. The cow that does not carry the live organism is in no way in danger of aborting or of spreading the infection. Therefore, if we had a way to differentiate the infected animals from the non-infected, we would be at a greater economical advantage towards controlling and eradicating the disease.

Gwatkin (1) was the first to demonstrate that a rapid macroscopic agglutination method for the diagnosis of Bang's abortion disease had possibilities. He used undiluted serum and a heavy suspension of Brucella abortus in physiologic salt solution. He mixed the two on a watch glass and observed the reactions.

Huddleson (2) reported on the observation of peculiarly shaped patterns formed when concentrated antigen was added to some serums. He noticed one type of pattern when antigen was added to the serum of cows that were known carriers of Brucella abortus, and another type of pattern when antigen was added to serum of cows that were apparently immune.

The perfection of this differential test if of such economic importance to the cattle industry that this thesis, entitled, "A Study to Determine the Efficiency of the Differential Test for Infectious Abortion of Cattle" is presented as a further study of the problem.

REVIEW OF LITERATURE

Huddleson (2) reports the observance of cows in which Brucella abortus failed to establish itself permanently. He observed that twelve cows out of a herd of thirty-two infected soon began to decline in their agglutination titre. These cows were classified as no longer carriers of Brucella abortus. The disappearance of Brucella abortus was confirmed by bacteriological examinations of suitable materials. Huddleson classified these as having an active acquired immunity. They have a fairly stable resistance to further invasion of the parasite. These animals are thought safe to be placed in herds free from Bang's abortion disease, or with infected animals, without danger of transmitting the disease or becoming reinfected. He also reports that while studying the behavior of different antigens in the rapid macroscopic agglutination test, he was surprised to find in using one of them, that a different type of reaction was manifested by serums from known infected animals than from those not infected, even though agglutinins were present in many cases in a high titre.

ANIMALS OF THE EXPERIMENT

The cattle used in this experiment were the college herds. The dairy herd consisted of approximately 150 head and the beef herd approximately 75. There has been a great deal of abortion in these herds for several years. These herds were maintained as two units, a beef and a dairy herd. Abortion control methods were adopted here in the fall of 1927. Up until this time no attempt at isolation of reactors was practiced.

The beef herd had a very limited amount of abortion disease present. It had been the practice in this herd to sell all cows for slaughter as soon as they aborted or developed breeding troubles.

In the dairy herd a high percentage of the cows had abortion disease, making it impracticable to sell the reactors. The differential test was adopted here in February, 1928, as a means of differentiating the infected animals from the non-infected. The herd was divided according to the differential test into clean, non-infected, and infected groups. They were in these groups when the experiment herein described was started.

Collection of Blood Samples.

The technic employed in bleeding the cattle is the one in general use. The cattle were placed in stanchions and their heads pulled around to their left side. The bleeding was done from the right jugular vein. With the fingers of the left hand the jugular vein was compressed about two-thirds of the way down the neck. By alternately compressing and releasing the fingers in the groove, the location of the jugular vein could be determined. After locating the vein the needle, 16 gauge, 2½ inches long, was placed directly over it and given a quick thrust so that it would penetrate the skin. The vein was kept compressed all the time. After the needle had penetrated the skin the jugular vein was re-located. The needle was withdrawn a little if necessary until it was standing directly on the vein. The needle was then given another quick thrust so that it would penetrate the vein, and blood would start flowing. Occasionally the needle would have to be withdrawn a part of the way, as it would pass completely through the vein. Occasionally also the needle would become plugged. In this case it was withdrawn completely, cleaned, and the operation started anew.

The blood was collected in sterile vials. The vials were filled about one-third full, labeled, stoppered, and laid flat until the blood was completely clotted. After each cow was bled, the needle was dropped into a pail containing a disinfectant. A wire was passed through the needle to remove any blood clots that may have been present. The needle was then rinsed in another pail of disinfectant solution. After the blood had completely clotted the samples were ready to go to the laboratory.

Preparation of Antigen

Tubes of beef liver infusion broth, pH 6.6, were inoculated with a single strain of Brucella abortus and incubated at 37° C for 72 hours. Beef liver infusion agar, pH 6.6, with gentian violet added in a final dilution of 1-10,000 was prepared (3) and twenty five cubic centimeters of this were added to each of several Blake bottles. The bottles were laid flat until agar hardened. With a sterile pipette approximately three cubic centimeters of the inoculated broth were added to each Blake bottle. The bottles were rotated back and forth until the broth had come in contact with the entire surface of the medium. These bottles, with medium side on top, were incubated at 37°C for 72 hours. At the end of incubation period the growth was washed off with phenolized 12% saline solution (Baker C.P.). Just enough of this solution was added to remove the growth. Any foreign material in suspension was removed by filtering the suspension through a thin layer of sterile absorbent cotton. The suspension was then placed in centrifuge tubes and centrifuged at approximately 2000 R. P. M. for six to eight hours. The supernatant fluid was poured off, leaving the heavy suspension of Brucella abortus. This suspension was then ready to be standardized. Sterile distilled water, containing 12% salt and .5% phenol was used to dilute the heavy suspension.

Antigen for the slow macroscopic agglutination test was standardized so that the density was equivalent to Tube One of McFarland's nephelometer.

To standardize antigen for the differential test, ten drops of phenolized 12% saline solution were added to each of the above mentioned centrifuge tubes and the contents thoroughly mixed. Three agglutination tubes were placed in a rack and one-half cubic centimeter of the heavy suspension added to each tube. Three drops of phenolized 12% saline solution were added to the first tube, four drops to the second and five drops to the third. Serum was collected from three cows, one negative to the slow agglutination test, one a reactor with a history of successive normal parturitions and negative results in attempts to isolate the abortion organism, and the other a known carrier of Brucella abortus. Each sample of the serum was then tested with the above three preparations of the antigen. The antigen giving the most satisfactory reaction with the three known serums was chosen as the basis for dilution of the suspension for routine testing.

Technic of the Agglutination Test

The technic employed here is practically the same as that of all other laboratories where infectious abortion diagnostic work is done. Two dilutions, 1-50 and 1-100, were first used on the samples. One cubic centimeter of standardized antigen was put in each agglutination tube. The serum was measured in a .1 cc pipette graduated into 0.01 cc. A rubber bulb was used on the upper end of the pipette. To the first tube, 0.02 cc of serum were added and 0.01 cc to the second, thereby making dilutions of 1-50 and 1-100, respectively. A clean pipette was used for each sample of serum. Wooden blocks with double rows of holes were used to place the tubes in. The tubes were incubated at 55° for 8 to 10 hours, and then allowed to stand at room temperature for 6 to 8 hours before reading and recording results. A positive serum caused clumping or agglutination of the organisms and these clumps settled to the bottom of the tube, leaving the supernatant fluid clear. A complete reaction was recorded as $-^{4}$, a partial reaction as $-^{1}$, $-^{2}$ and $-^{3}$, according to the degree of reaction. All samples showing complete agglutination in 1-100 dilution were carried through six dilutions. These dilutions were: 1-50, 1-100, 1-200, 1-400, 1-800 and 1-1600. Six agglutination tubes were placed in a row. Two cubic centimeters of antigen were added to the first tube and one cubic centimeter to each of the other five. Then 0.04 cc of serum were added to the first tube. With a 1 cc pipette one cubic centimeter of the solution was taken out of tube one and put in tube two, and so on through the sixth tube, leaving 2 cc in the sixth tube. These were incubated and read the same as the two dilution method.

Technic of the Differential Test

Apparatus: Glass plate and dark-field illumination box (Figure 1). This glass plate and the box somewhat resemble the one Huddleson (4) uses. The glass plate is made from double thickness window glass, ten inches square, and ruled off into inch squares with a diamond point. The box is fourteen inches long, ten inches wide and six inches deep. One end of the top is covered to a width of four inches to protect the eyes of the operator from the glow of the light which is placed in the covered end of the box. The light provides warmth and illumination. The covered part of the box behind, above, and below the bulb is painted white on the inside in order to enhance the illumination and distribute it evenly over the glass plate on top of the box. The remainder of the box, that is, the portion directly under and opposite the glass plate, is painted black. This serves to produce a black background for objects placed on the plate which receive their illumination indirectly or from an angle (4). Figure 1.

Standardized dropper pipette: This was made by drawing out thick walled glass tubing of 1/8" bore, and cutting the tubing so that its lumen corresponded to number 16 hole in a B and S wire guage. Such a pipette delivers approximately 0.02 cc of antigen in each drop.

With a clean one-tenth cubic centimeter pipette, 0.01 cc of serum was placed on the squares of the glass plate. The serum must be free of air bubbles when antigen is added as they break up the pattern. Then with the standardized dropper pipette one drop of antigen was placed on each sample of serum. Care is taken to hold the dropper pipette in a vertical position, for holding it at any other angle will make a considerable difference in the amount of antigen delivered. The glass plate is left on the dark-field illumination box, and the light kept on during the test. The serum, with antigen added, is allowed to stand, without stirring, for approximately three minutes. When the antigen remains very compact and does not spread out over the serum, the reaction is given the differential symbol "S", meaning that the cow from which this serum was taken is classified as infected with Brucella abortus (Figure 2). In cases where the reaction remains fairly compact, but the patterns formed in it appear as concentric circles, or the antigen resembles a wagon wheel, having radiating lines from the center similar to the spokes in the wheel (Figure 3). This reaction is given the differential symbol "I", meaning that the cow from which this serum was taken is classified as a non-infected reactor. In case the antigen spreads out over the entire surface of the serum and becomes cloudy or opaque so that the radiating lines disappear after about two minutes exposure, this reaction is designated as "N" (Figure 4). This cow is classified as negative.

After the above reactions are recorded, the serum is stirred with a toothpick, using a separate one for each sample tested. When the antigen and infected serum is stirred, the reaction usually shows a complete flocculation. (Figure 2).

The non-infected reactor test shows complete or partial flocculation, the flocculi being very small (Figure 3). The negative reaction shows no flocculi (Figure 4). The key to these reactions, before and after stirring, are shown in chart No. 2. The number under the heading Flocculation in chart No. 2, shows the reaction after stirring with a toothpick. In most cases negative and non-infected positive serums cannot be differentiated by patterns alone.

Collection of Samples

The milk samples were collected in sterile test tubes. Four or five streams were first stripped from each quarter. Then approximately 10 cc of the milk were collected into sterile test tubes. The cow's number and the quarter from which the sample came were written on the bottle. The sample was then ready to go to the laboratory.

Uterine and vaginal swabs were collected.

In the aborted fetus, a part of the liver and spleen was collected and kept as free from contamination as possible. The contents of the fourth stomach were removed with sterile pipettes and placed in sterile test tubes. These specimens were then ready to be taken to the laboratory.

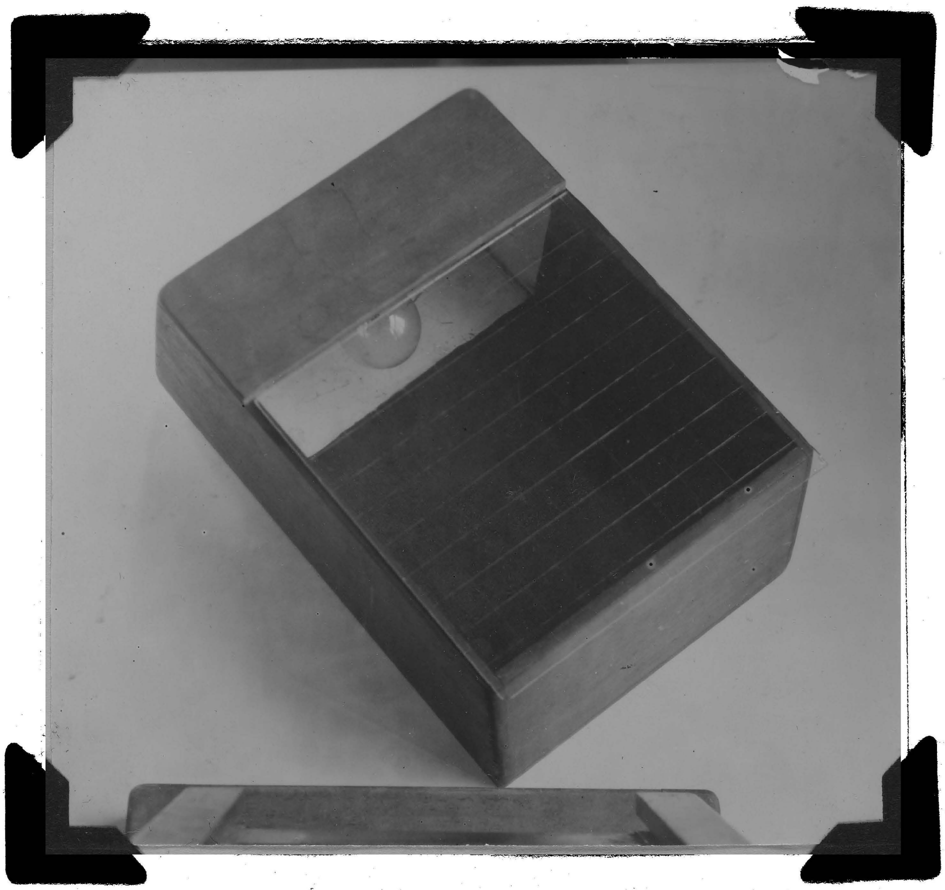

Fig. 1.- Rapid agglutination test box

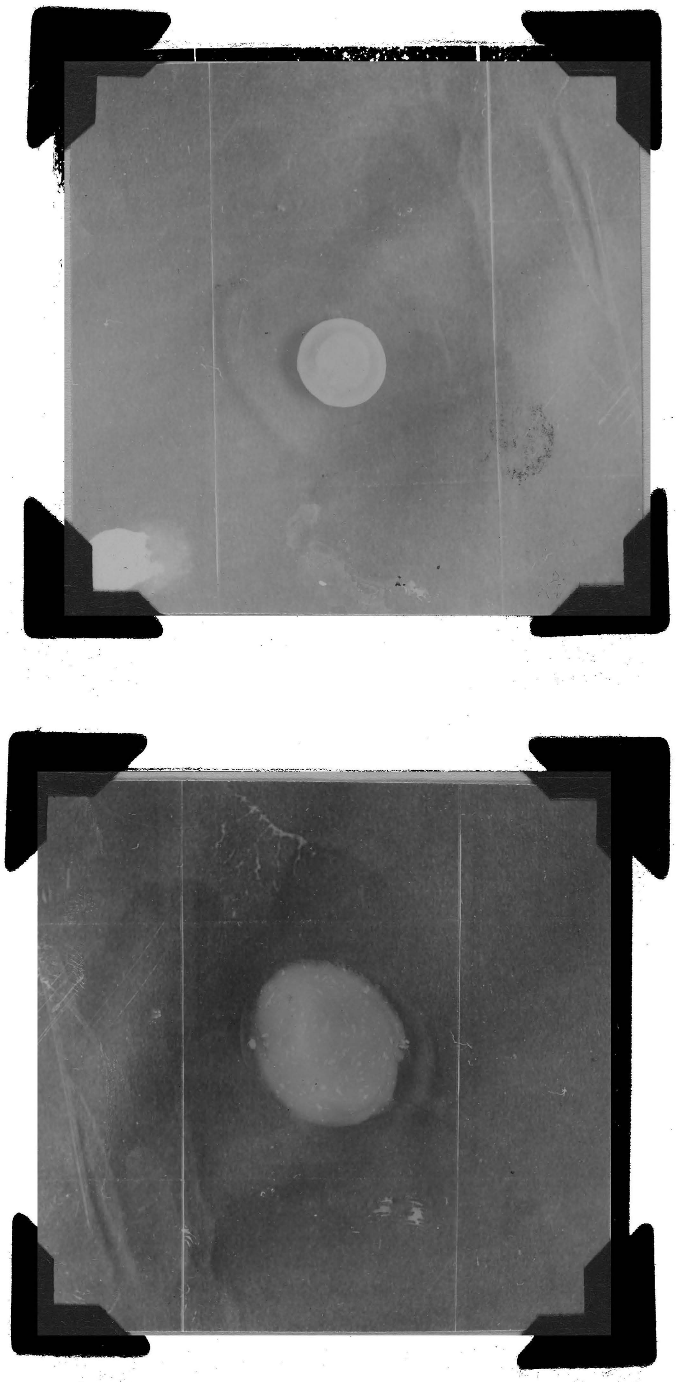

Fig. 2.- Reaction of infected serum to the differential test. Before and after stirring.

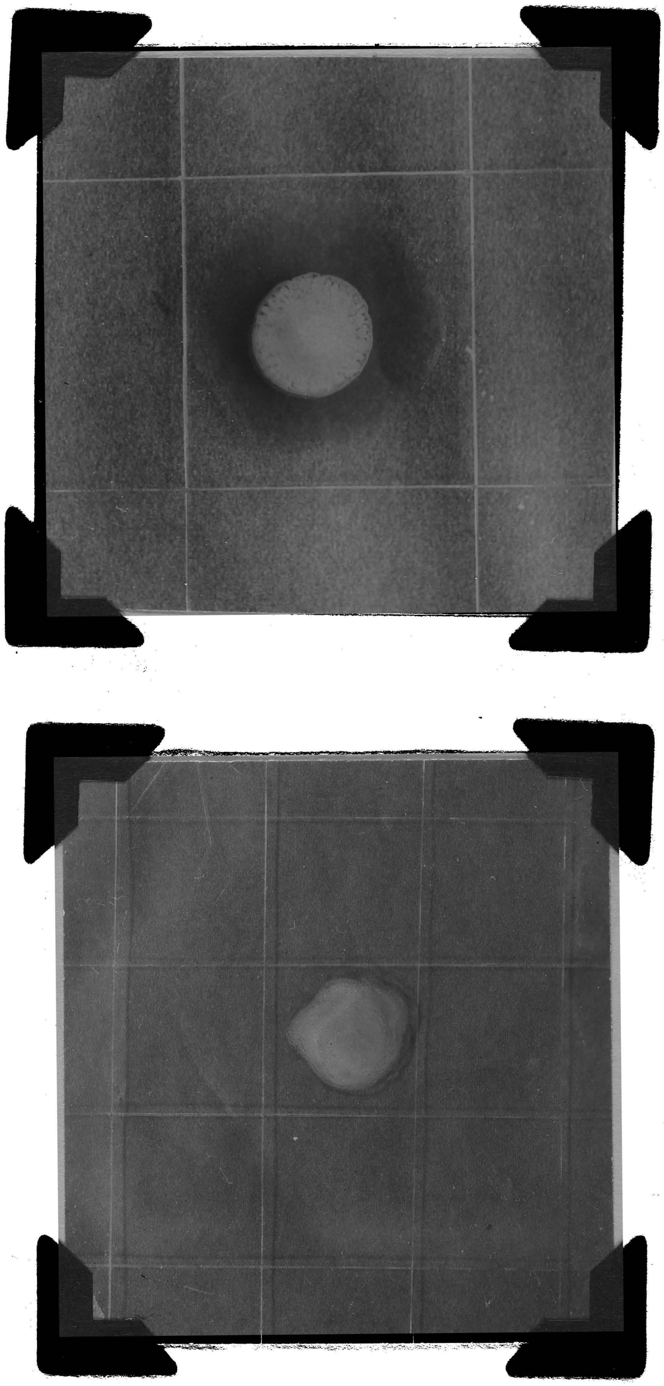

Fig. 3.- Reactions of non-infected positive serum to the differential test. Before and after stirring.

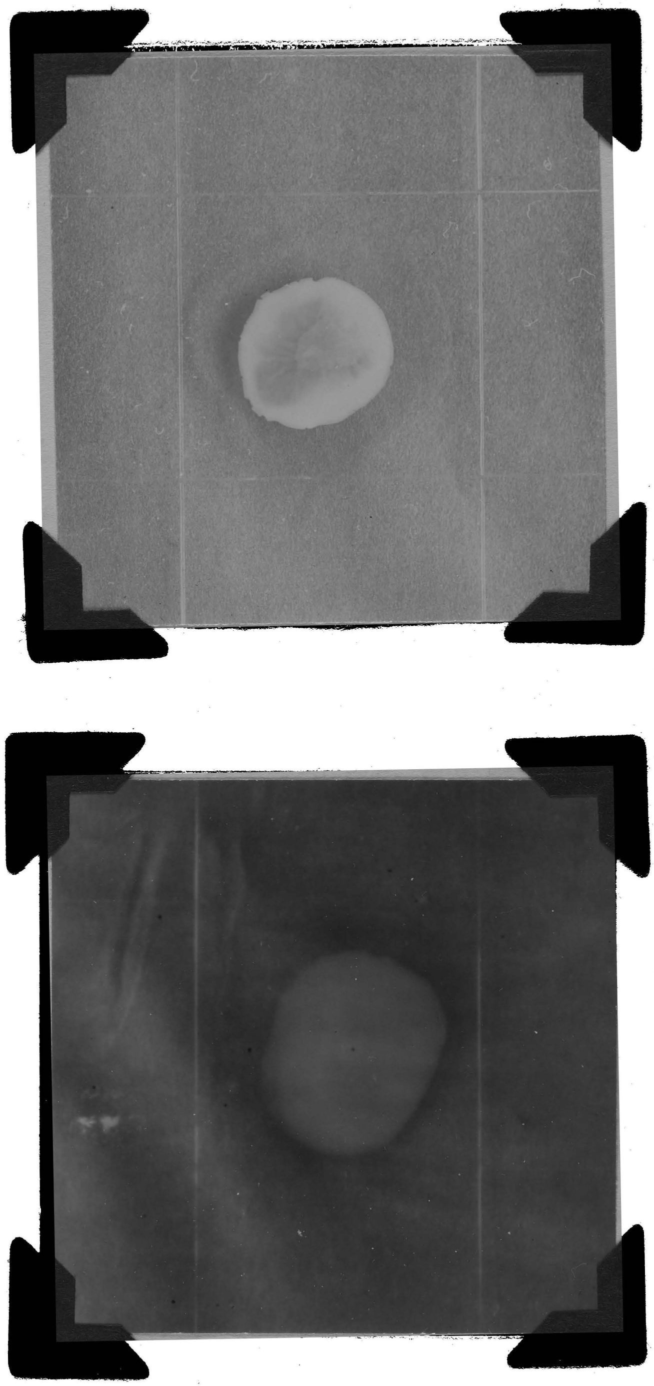

Fig. 4.- Reactions of negative serum to the differential test. Before and after stirring.

Technic of Isolation.

The initial growth of Brucella abortus is best obtained by growing it in an increased CO_2 tension of the atmosphere (5). Liver infusion agar plates, Ph 6.6 and containing gentian violet in the proportion of 1 to 10,000, was inoculated with suspected material and placed in jars to which approximately 10% CO_2 was added. The gentian violet inhibits the growth of most gram positive organisms, and gives Brucella abortus colonies a violet glistening color, but does not hinder their growth (3).

The milk samples were centrifuged at 2000 R. P. M. for two hours. About 0.2 cc of the sediment were drawn from the bottom of the tube by means of a sterile pipette, then dropped on the surface of solidified gentian violet agar in Petri plates. Brucella abortus is often found in the cream. When the pipette is passed through the cream some of it sticks to the pipette, therefore when the sediment is dropped on the medium, some of the cream gets on the Petri plate. These plates were then placed in vacuum jars, 10% CO_2 added, and incubated at 37°C for 72 hours.

The swabs from the vagina were smeared on the agar plates, and these plates were treated the same as the ones containing milk samples.

In attempting to isolate Brucella abortus from various organs of a fetus, the piece of agar was incised with a sterile knife, then smeared over the surface of gentian violet, liver agar plates (3). If the exterior of the organ was contaminated it was seared before incising. The stomach contents were removed from the test tubes with sterile pipettes and dropped on gentian violet, liver agar plates. These plates were then placed in vacuum jars, approximately 10% CO_2 added, and incubated at 37°C for 72 hours.

After the plates are inoculated, the technic was the same for all of them. The plates were taken out of the jars at the end of the incubation period. Then with a sterile platinum needle those colonies resembling Brucella abortus were fished. These are small, round, glistening, bluish colored colonies. Gentian violet, liver agar slants were inoculated with these colonies. These tubes were incubated at 37°C for 72 hours, at which time if sufficient growth was present, it was washed off with phenolized 12% sodium chloride solution. Smears were made from the slants and stained with Gram's stain to see if the culture was pure and also to see if it was Gram negative. The suspension was standardized so that the density was equivalent to Tube No. 1 of McFarland's nephelometer. This standardized solution was then used as an antigen, and the agglutination test run with it, using known negative and positive serums. If the agglutination reactions coincided with that of the known samples of serum, it was evident that the organisms isolated were Brucella abortus. Gram negative organisms having the proper cultural characteristics, that agglutinate known positive serums and do not agglutinate known negative serums, positively identifies the organism as Brucella abortus.

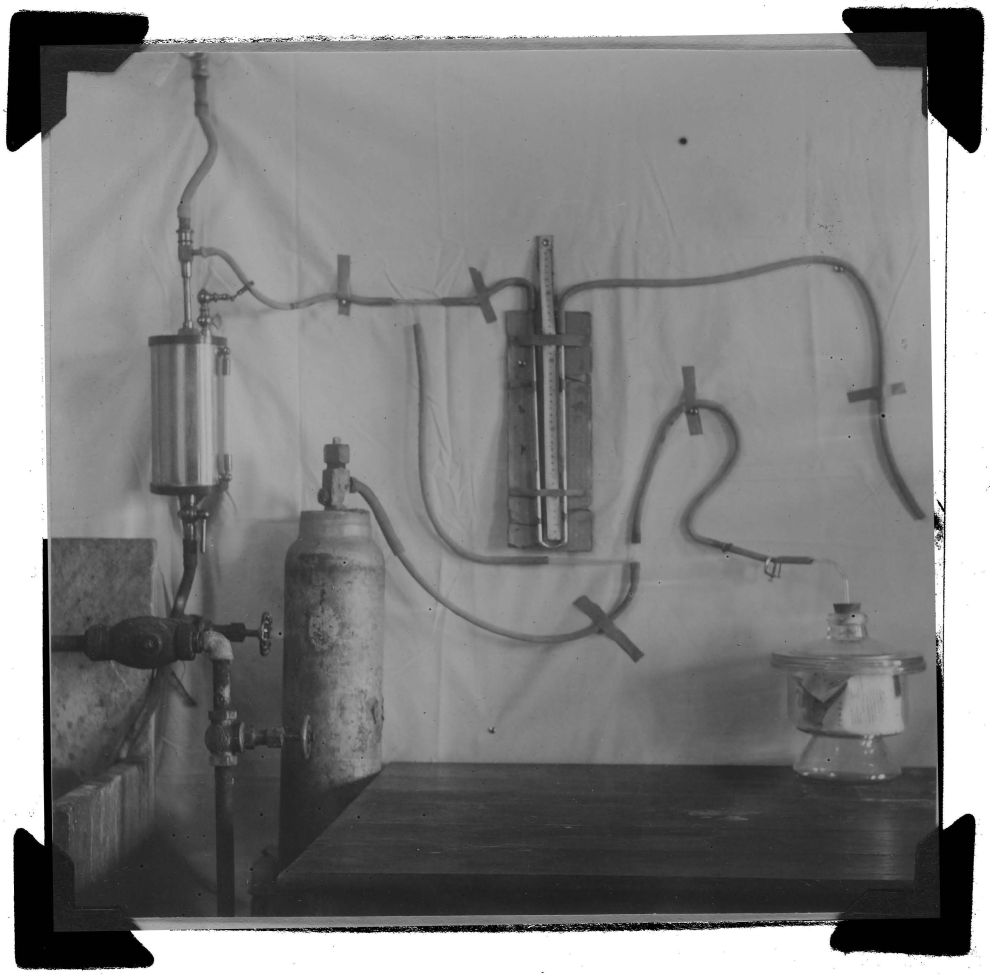

Fig. 5.- Carbon dioxide apparatus.

Water is allowed to run through the vacuum air pump shown on left hand side of the figure. This forms a vacuum and draws the air out of the vacuum desiccator in the lower right hand corner of the figure. When approximately 10% of the air was removed from the desiccator, as measured by mercury column in center of figure, the stop cock on the tubing from the vacuum air pump was closed. Then enough CO_2 from the carbon dioxide cylinder was allowed to flow into the vacuum desiccator to replace the air removed by the vacuum air pump. Then the stop cock on the tubing from vacuum desiccator was closed.

Tube Agglutination Chart No. 1

Dates tested	6-15-29						9-15-29						1-10-30						4-15-30					
Dilutions	50	100	200	400	800	1600	50	100	200	400	800	1600	50	100	200	400	800	1600	50	100	200	400	800	1600
Cow No.																								
2	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	-	Died																	
8	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+.	+	+	+	+	+
12	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-
14	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-
16	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
20	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-
22	+	+	+	+	+	-	Sold																	
26	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-
28	+	+	+	+	+	+	Sold																	
30	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	Died					
33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+
68	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-
71	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
76	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-
306	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
309	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Sold					
308	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
10	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-
13	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
3	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-
15	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
19	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-
31	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-
42	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	Died					
27	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
211	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-
218	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
220	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	Sold					
231	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	Sold					
232	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-

Differential Test Chart No. 2

Dates tested	6-15-29		9-15-29		1-10-30		4-15-30	
Cows No.	Type of pattern	Flocculation	Type of pattern	Flocculation	Type of pattern	Flocculation	Type of pattern	Flocculation
2	S	4M	S	4C	S	4C	S	4C
5	S	4C	S	4C	S	4C	S	4C
6	S	4C	S	4C	S	4C	S	4C
7	S	4C	S	4C	S	4C	S	4C
8	S	4F	S	2C	S	4C	S	4C
11	S	4F	S	4C	S	4C	S	4C
12	S	4M	S	2C	S	2C	S	4M
14	S	4F	S	2C	S	2C	S	4M
16	S	4F	S	4C	S	4C	S	4M
20	S	4C	S	4C	S	4C	S	4C
21	S	4F	S	4C	S	4C	S	4C
22	S	4C	Sold					
26	S	4C	S	4C	S	4C	S	4C
28	S	4C	Sold					
30	S	4C	S	4C	S	4C	Died	
33	S	4C	S	4C	S	4C	S	4C
64	S	2C	S	4C	S	4C	S	4C
68	S	2C	S	4C	S	4C	S	4M
71	S	4C	S	4C	S	4C	S	4M
76	S	2M	S	4M	S	2C	S	4C
306	S	4C	S	4C	S	4C	S	4C
309	S	4C	S	4C	S	4C	S	4C
308	I	2F	I	2F	I	2F	I	2F
10	I	4F	I	2C	I	2C	I	4F
13	I	4F	I	4F	I	4F	I	4F
3	I	2F	I	2F	I	2F	I	2F
15	I	2F	N	-	I	2F	I	2F
19	I	2C	I	2C	I	2C	I	2M
31	I	2C	I	2C	I	2C	I	4F
42	I	2F	N	-	Died			
27	I	2F	I	2F	I	2F	I	2F
211	I	2C	S	4C	Sold			
218	I	2F	I	2F	I	2F	I	2F
220	S	4C	S	4C	Sold			
231	S	2C	S	4C	Sold			
232	I	2F	I	2F	N	N	N	N

N - Negative

I - Non-infected

S - Infected

4C - Complete coarse flaking on warm plate when stirred with toothpick

2C - Partial coarse flaking on warm plate when stirred with toothpick

4M - Complete medium flaking on warm plate when stirred with toothpick

2M - Partial medium flaking on warm plate when stirred with toothpick

4F - Complete fine flaking on warm plate when stirred with toothpick

2F - Partial fine flaking on warm plate when stirred with toothpick

Isolation Chart No. 3

Cows No.	Date	LF	LR	RF	RR	U	F
16	8-23-29	-	-	-	+		
21	8-23-29	-	-	-	-	-	=
	11-24-29	-	-	-	-		
	1-8-30	-	-	-	-		
3	8-23-29	-	-	-	-		
	11-24-29	-	-	-	-		
68	8-23-29	-	-	-	-		
	10-1-29	-	-	-	-		
	12-1-29	-	-	-	-		
13	8-31-29	-	-	-	-		
	11-24-29	-	-	-	-		
42	8-31-29	-	-	-	-		
	11-24-29	-	-	-	-		
10	9-7-29	-	-	-	-		
	10-1-29	-	-	-	-		
	2-28-30	-	-	-	-		
15	9-7-29	-	-	-	-		
	12-1-29	-	-	-	-		
27	9-7-29	-	-	-	-		
	12-1-29	-	-	-	-		
	2-28-30	-	-	-	-		
19	12-10-29	-	-	-	-		
	1-8-30	-	-	-	-		
	2-28-30	-	-	-	-		
31	12-10-28	-	-	-	-		
	1-8-30	-	-	-	-		
8	7-1-29	-	-	+	-		
26	7-1-29	-	-	-	-		
	9-7-29	-	-	-	-		
71	7-1-29	-	-	-	+		
	4-8-30	-	-	-	+	-	
33	7-6-29	-	+	+	-	+	
	4-13-30	-	-	+	+	+	+
14	7-23-29	+	+	+	-		
28	7-13-29	-	-	+	+		
30	7-23-29	-	-	-	+		
22	7-23-29	-	-	-	-		
20	7-27-29	-	-	-	-		
	10-18-29	-	+	-	+		
11	7-27-29	-	-	-	-		
	10-18-29	+	-	-	-		
5	7-27-29	+	-	-	+		
6	7-31-29	-	-	-	-		
	10-18-29	-	-	-	-		

Cows No.	Date	LF	LR	RF	RR	U	F
2	8-6-29	-	-	-	-		
2	10-18-29	-	-	-	-		
12	8-6-29	+	-	+	-		
306	7-27-29	+	+	-	-		
309	12-8-29	-	-	-	-	-	
211	12-8-29	-	-	-	-		
220	12-8-29	-	-	+	-	+	
218	12-8-29	-	-	-	-		
315	11-24-29					-	-
76	4-8-30	+	-	-	-	+	

LF - Left front quarter of udder
LR - Left rear quarter of udder
RF - Right front quarter of udder
RR - Right rear quarter of udder
U - Uterus
F - Foetus (only three cows aborted during the period of this study)

SUMMARY

Chart No. 1 shows that during this study the agglutination titre of only two cows changed to any extent. Of these two cows the titre changed from a slight reaction in low dilution to a high reaction in high dilutions. However, both gave birth to normal, healthy calves, one in February, the other in March. Cows No. 22, 28 and 309 were sold because they were sterile. Cows No. 220 and 231 were sold because on differential test they were classified as infected with Brucella abortus, and they were from the beef herd where no isolation is practiced. Cow No. 7 died of unknown cause, No. 30 died of peritonitis, and No. 42 died of tumors.

Chart No. 2 shows that the reactions to the differential test remained practically the same in all the cows except numbers 68 and 76. Their differential agglutination titres rose correspondingly with their agglutination titres.

Isolation of Brucella abortus was tried from each cow, provided proper samples could be had. Isolation from foetuses was possible in only three cases, as only three cows aborted during this study. Cow No. 21 aborted twin calves on August 23, 1929. Isolation attempts from the foetuses were negative as were the uterus and milk examinations. This abortion was not due to Brucella abortus. Heifer No. 315 aborted a six weeks old calf on November 24, 1929. Uterus and foetus examinations for Brucella abortus were negative. This heifer had never lactated so milk examinations were impossible. The cause of abortion was undetermined. Isolation of Brucella abortus was attempted from each cow until the organism was isolated unless the cow stopped lactating, but no more than three attempts were made on any cow. By comparison of charts No. 1, 2, and 3, it can easily be seen that the isolation of Brucella abortus with one exception, cow No. 16, was from cows that gave high agglutination titre reactions, and in all cases from cows that gave infected reactions to the differential test.

Records of isolation of Brucella abortus indicate that 14 out of 23 infected reactors to the differential test are known carriers of Brucella abortus. Repeated isolation tests were run on the other nine when proper material could be obtained.

In no case was Brucella abortus isolated from the non-infected reactors to the differential test.

CONCLUSIONS

From the results the following conclusions are drawn:

1. The differential test appears to be safe to differentiate between Brucella abortus infected and non-infected reactors, but is not safe to differentiate between the non-infected reactors and the negatives.
2. The non-infected reactors and the negatives cannot be differentiated by the patterns formed by the differential test. They can be differentiated after mixing the antigen and serum. With the former the reaction consists of the formation of fine flocculi. With the latter, no flocculation occurs.
3. More experimental data should be obtained before the differential test is used in routine testing.

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