AN INTERPRETATION OF THE AGGLUTINATION REACTION TO BACILLUS ABORTUS IN 75 CASES OF BOVINE ABORTION BACTERIOLOGICALLY CONTROLLED.

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In the following pages are given the results of a study of the agglutination test in 75 cases of abortion of which bacteriological data were at hand. Concerning these data and the methods employed in obtaining the results here used to interpret our findings, the reader is referred to Dr. Smith's paper.[1] Although bacteriological data on a much larger number of cases were available, yet in many instances the cows were removed from the herd before samples of blood could be taken. While this work was in progress certain discrepancies came to the surface and the search for a focus of infection with *Bacillus abortus* in the udder was deemed essential by Dr. Smith. Accordingly the milk from certain selected cases was brought into the investigation.

The existing literature on the agglutination test is voluminous and the work in most cases of high quality, yet there is lacking the bacteriological basis of a series of spontaneous cases for a more accurate interpretation of the test. The fact that Smith[1] found in 109 cases of abortion or premature expulsion of a living calf 46 not associated with *B. abortus* shows that our information in regard to the significance of the agglutination test has been built on somewhat insecure foundations.

Most writers have confined their attention to a comparison between the agglutination and the complement fixation test. This was done by Mohler and

[1] Smith, T., *J. Exp. Med.*, 1919, xxx, 325.

Traum,[2] Moore and Fitch,[3] Larson,[4] Rettger and White,[5] Wall,[6] and Zwick and Zeller.[7] Others have contented themselves with associating the serological reactions with abortion pure and simple. Wall,[6] Grinsted,[8] and Moore and Fitch[3] studied among other things the rise and decline of the agglutination curve. Since none of these researches bears directly upon the substance of this communication any detailed analysis would be out of place.

Methods.

Agglutination Technique.—The obtaining of blood samples from cattle is frequently difficult, since it depends on the way the animals are stabled and their degree of wildness or domestication. Those from which samples were collected for the present investigation were in the main milking cows. They were secured in stanchions, facing on a wide alleyway. A rope halter was placed on the head of the animal and the neck and head were extended by fastening the rope, so attached, to an iron rail supporting stanchions on the other side of the alley, and tightening the rope gradually until the jugular furrows were fully exposed. Strong bull nose forceps were then put on the anterior part of the nasal septum to hold the attention of the animal, and also to steady the head. One assistant can handle both the rope and bull nose forceps.

When the animal was well under control the field of operation was thoroughly wiped off with 95 per cent alcohol. Pressure was applied over the jugular vein at the posterior end of the furrow to make the vein prominent and a $2\frac{1}{2}$ inch needle No. 14 was inserted. 25 to 30 cc. of blood were collected in a sterile 50 cc. centrifuge tube. A separate, sterile needle was used for each sample. The samples were stored at room temperature until the clot had formed, after which it was separated from the sides of the tube with a heavy sterile wire.

[2] Mohler, J. R., and Traum, J., *U. S. Dept. Agric., Bureau of Animal Industry, Bull. 216*, 1913, 147.

[3] Moore, V. A., and Fitch, C. P., *Rep. N. Y. State Vet. College*, 1912–13, 82.

[4] Larson, W. P., *J. Infect. Dis.*, 1912, x, 178.

[5] Rettger, L. F., and White, G. C., *Storrs Agric. Exp. Station, Bull. 93*, 1918.

[6] Wall, S., *Z. Infektionskrankh. Haustiere*, 1911, x, 23, 132.

[7] Zwick and Zeller, *Arb. k. Gsndhtsamte.*, 1912, xliii, 1.

[8] Grinsted, P., Maanedsskrift f. Dyrlaeger, 1909, xxi, Review in *Berl. tierärztl. Woch.*, 1909, xxv, 831.

The blood samples were then placed in the refrigerator for 12 hours to allow the clot to contract completely. Each tube was centrifuged for 10 minutes, to throw the clot and free corpuscles to the bottom. With sterile bulb pipettes the clear serum was transferred from the centrifuge tubes to sterile, corked test-tubes for storage. No disinfectant was added to the serum as it was found unnecessary if each step of the procedure was carried out carefully.

The antigen used was always prepared fresh as needed. A stock culture of *Bacillus abortus* recovered early in this work by Dr. Smith was used. A 48 hour slant agar culture was washed off with 2 cc. of normal saline solution and the suspension transferred to an agar surface within a rectangular 16 ounce bottle. The bottle was sealed and incubated for 72 hours at 37°C., then washed off with 20 cc. of normal saline solution, and the suspension transferred to a sterile bottle. This very dense suspension was standardized by reducing the density with normal saline solution to a point at which the translucency equals that of a 24 hour bouillon culture of the typhoid bacillus. It was then ready for use.

In order to simplify the technique as much as possible, reduce the amount of glassware used, and safeguard the operations by using the same amounts of fluids in the series of tubes in which clumping was to be observed the following method was used throughout. Eight stock dilutions of serum were used as a starting-point. To the first tube 4 cc. of salt solution and 1 cc. of undiluted serum were added to make a stock dilution of 1:5. Into each of the other tubes $2\frac{1}{2}$ cc. of salt solution were placed. Then $2\frac{1}{2}$ cc. of the 1:5 serum dilution were added to the second tube, the mixture was drawn up several times, and $2\frac{1}{2}$ cc. of this were transferred to a third tube, and so on successively to the last. Each tube, except the final dilution, then contained $2\frac{1}{2}$ cc. of salt solution and $2\frac{1}{2}$ cc. of serum dilution, making 5 cc. in all and furnishing dilutions of serum beginning with 1:5 and ending in 1:640. To eight fresh tubes $\frac{1}{2}$ cc. of each serum dilution was now added, beginning with the highest dilution and using the same pipette up to the most concentrated solution. Finally $\frac{1}{2}$ cc. of the suspension of bacteria was added, making a series of serum dilutions beginning with 1:10 as shown in Table I. Particles in the culture suspension, such as bits of agar and clumps of bacteria, were

removed by withdrawing the suspension with a pipette through a piece of sterile cotton dropped into the stock suspension.

The distance or interval between the last tube of the series which was completely cleared by the clumping and sedimentation and the tube which showed the agglutination limit varied somewhat from case to case, as shown in Table II, which contains three illustrative examples taken from the protocols.

TABLE I.

Serum Dilution. (First Series of Tubes.)

Tube No.	1	2	3	4	5	6	7	8
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Salt solution added	4	2½	2½	2½	2½	2½	2½	2½
Undiluted serum to first tube	1							
Diluted serum to other tubes in succession		→2½	→2½	→2½	→2½	→2½	→2½	→2½
Dilutions	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640.

Final Dilution. (Second Series of Tubes.)

Serum dilutions	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Amount of serum dilution added to each tube	½	½	½	½	½	½	½	½
Culture suspension	½	½	½	½	½	½	½	½
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280

This method of using at least eight final dilutions served to reveal any possible errors and control any so called paradoxical reactions. Unless the series showed a definite regular decline in agglutinins, it was done over again. By this means most paradoxical reactions were traced to some irregularity or accidental error in manipulation. Table II illustrates the protocols. Readings were made after 3 to 4 hours in the incubator, followed by refrigeration over night.

The final agglutination titer adopted was shown by that tube in which there was some definite clumping as indicated in the suspension

or amount of deposit when compared with the next lower and negative tube and the culture control. In Table II the limit is shown as 1:640 in Case 31. Nos. 19 and 101 require further dilutions to obtain the precise limit. When clumping occurred in the control the series was rejected and a fresh one prepared. The figures given in Tables III to V represent, therefore, the agglutination limit under the conditions of the test as described above.

Test for Bacillus abortus in the Udder.—The milk was collected directly into sterile glass jars during the latter part of milking. The samples averaged about 650 cc. At the laboratory the milk was transferred to sterile separatory funnels and kept over night at

TABLE II.

Illustration of the Range between Complete Sedimentation and Slight or No Clumping.

Case No.	Dilutions.								Control.
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	
31	C.*	C.	C.	C.	++ ++	++ +	++	−	−
101	"	"	"	"	C.	++ ++	++ ++	++	−
19	"	"	"	"	"	C.	C.	++ +	−

* C. indicates complete clearing of the fluid, ++++ slight clouding plus heavy deposit, and so on to −, which means no trace of clumping.

about 4–5°C. The next morning the milk was withdrawn from the funnel, leaving the cream, distributed into large centrifuge bottles, and centrifuged for 20 minutes at a speed of about 1,500 revolutions per minute. Any cream remaining was removed with a section lifter, some of the top milk withdrawn with a pipette or syphon, and the remaining milk and sediment were well mixed and distributed into smaller centrifuge tubes and centrifuged a second time for 15 minutes at a speed of about 1,400 revolutions per minute. The top milk was again withdrawn, the remaining milk and sediment were well mixed, and each sample was injected intraperitoneally into three guinea pigs. Each animal received 5 to 7 cc. Films of the milk

fresh and fixed in absolute alcohol and ether and stained with alkaline methylene blue or Giemsa's stain were examined to determine in general the character of the sediment injected. After a period of 6 to 8 weeks the guinea pigs were chloroformed and three cultures made from each animal by tearing out bits of spleen tissue and transferring to agar slants whether lesions were present or not. All cultures were sealed.

Agglutination Titer towards Bacillus abortus when the Fetus Contains Only Spirilla (Vibrio fetus).

For more detailed information concerning the bacteriological data used in the tables to follow, the reader is referred to earlier papers by Smith.[1,9,10] Certain unpublished data were also furnished by him in individual cases to be discussed later.

In Table III are given data pertaining to nineteen cases in which spirilla were found and agglutination tests made. The remaining seven cases of the same group are omitted because blood samples were not obtained.

Taking an agglutination titer up to and including 1:40 as negative as to the presence of *Bacillus abortus*, we find that in eleven cases the agglutination titer agrees with the bacteriological findings. Of these eleven cases, the milk of three was examined for *Bacillus abortus* and found free. In two cases (Nos. 256 and 290) a maximum titer of 1:1,280 for *Bacillus abortus* was associated with the presence of this bacillus in the milk. This high titer is thus accounted for. The first four cases in the table have titers which indicate that the animals were infected with *Bacillus abortus* at some earlier date, since in three of these the milk tests were negative. The fourth was not examined. Of these No. 246 is of interest. This cow aborted May 28, 1918, with spirilla in the fetus. The fetus was small, only 7½ inches long. On September 5 and 12, blood samples registered a titer of 1:160. On February 4, 1919, a sample of milk proved free from *Bacillus abortus*. On April 30, the cow aborted again (now Case 350). *Bacillus abortus* was found in the fetus. The titer of the

[9] Smith, T., *J. Exp. Med.*, 1918, xxviii, 701.
[10] Smith, T., *J. Exp. Med.*, 1919, xxx, 313.

blood on May 16 was 1:640. Evidently a fresh infection with *Bacillus abortus* had taken place since the previous abortion. In No. 159 there was a decline in the agglutination titer following the birth of a normal calf, asphyxiated at birth. Case 251 is the only one in the table whose titer cannot be satisfactorily explained with the data on hand. This cow aborted June 10, 1918, with positive spirilla cultures. The results were negative as to *Bacillus abortus*. On September 5, the blood titer for *Bacillus abortus* was 1:640. Two milk tests with samples, collected November 20, 1918, and March 4, 1919, were both negative. The only explanation that suggests itself is that there was associated *Bacillus abortus* with the spirilla in the fetal membranes but not in the fetus in the June abortion. The absence of any tests with uterine exudate immediately after abortion leaves this interpretation open. It is also possible that *Bacillus abortus* was in the milk in very small numbers. This case is thus the only one in the table in which the serological test does not harmonize with the bacteriological data.

One of the most instructive cases in Table III is Cow 290. The peculiarity of the agglutination titer makes a more detailed history of this case of interest. This cow gave birth to twins March 13, 1917. Both were unable to drink and too weak to stand. One was brought to the laboratory dead March 15. From Dr. Smith's notes on this case we find that there was marked fatty degeneration of the liver and extreme congestion of the medullary zone of the kidneys. The mucosa of the fourth stomach was covered with a thick viscid layer of mucus. In the lungs there were many lobules and groups of lobules collapsed. The postmortem changes made examination of the small intestines useless but the calf probably died of what is usually known as scours. Histological examination of fixed and hardened tissues showed bronchopneumonic collections of cells resembling polynuclear leucocytes, general vacuolation of liver cells, and intense congestion of the medulla of the kidneys approaching hemorrhage. Pieces of the collapsed lung tissue ground and inoculated into two guinea pigs produced the abortion disease in both cases. From these *Bacillus abortus* was isolated.

A dead fetus was discharged November 19, 1918, 24 inches long (No. 290). Spirilla were isolated in pure culture from the fourth

TABLE III.

Agglutination Test for B. abortus in Cases of Abortion Associated with Spirilla.

Case No.	Length of fetus.	Native or purchased cow.	Date of abortion.	No. of pregnancy.	Date of blood sample.	Agglutination titer (limit).	Date of milk sample.	Test for *B. abortus* in milk.	Remarks.
	in.								
159	16¾	Purchased.	Dec. 17, 1917	3	Aug. 29, 1918	1:160			Preceding pregnancy also terminated in abortion. Etiology in doubt. Asphyxiated at birth.
(324)	Normal calf.		Feb. 12, 1919	4	Mar. 24, 1919	1:20			
192	36	Purchased.	Jan. 23, 1918	3	Aug. 29, 1918	1:80	Dec. 17, 1918	—	
213	21	"	Mar. 18, 1918	4	" 29, 1918	1:160	" 17, 1918	—	
246	7½	"	May 28, 1918	2	Sept. 5, 1918	1:160	Feb. 4, 1919	—	
					" 12, 1918	1:160			
(350)	Calf alive.		Apr. 30, 1919	3	May 16, 1919	1:640			*B. abortus* found in calf.
251	17½	Purchased.	June 10, 1918	2	Sept. 5, 1918	1:640	Nov. 20, 1918	—	
							Mar. 4, 1919	—	
256	19	"	" 18, 1918	4	Oct. 3, 1918	1:1,280	Nov. 27, 1918	+	
258	27	"	" 29, 1918	2	" 3, 1918	1:20	Feb. 11, 1919	—	
267	23	"	Aug. 12, 1918	3	" 3, 1918	1:40			
269	20	"	Nov. 18, 1918	2	Nov. 21, 1918	1:20	Jan. 7, 1919	—	
					" 28, 1918	1:20			

290	24	Native.	Nov. 19, 1918	3	Nov. 14, 1918 " 21, 1918 " 28, 1918 Dec. 11, 1918	1:10 1:1,280 1:1,280 1:1,280	Nov. 27, 1918	+	Preceding pregnancy (twins) associated with *B. abortus* (Mar. 13, 1917). Agglutination titer still 1:1,280 Apr. 4, 1919.
308	31½	Purchased.	Dec. 30, 1918	1	Jan. 2, 1919	1:40			
317	25	"	Jan. 27, 1919	2	Feb. 7, 1919	1:10	Mar. 21, 1919	−	
318	26	"	" 27, 1919	2	" 7, 1919	1:10			
331	8	Native.	Mar. 15, 1919	6	Mar. 24, 1919	1:10			
333	26	Purchased.	" 27, 1919	1	" 29, 1919	1:10			
339	35½	"	Apr. 9, 1919	3	" 29, 1919	1:40			
351	28	"	May 5, 1919	1	May 15, 1919 June 2, 1919	1:20 1:160			Fetus contained *B. abortus* also.
356	26½	"	" 23, 1919	1	" 2, 1919	1:80			
357	21¾	Native.	" 24, 1919	8	" 2, 1919	1:10			

stomach, large intestine, lungs, kidney, spleen, and liver. Guinea pigs inoculated with lung tissue, meconium, fourth stomach contents, and suspensions of exudate from the vagina obtained with a swab November 18 were normal when killed after 7 weeks.

On November 14, 1918, 5 days before abortion, the agglutination titer was only 1:10. 7 days later and 2 days after discharge of the fetus it was 1:1,280. The same high titer was found on November 28 and December 11. Milk drawn November 27 was found infected with *Bacillus abortus*. On April 4, 1919, the titer was still 1:1,280.

The main facts in the history of this cow are: Twins March 13, 1917, one of which carried *Bacillus abortus* in lungs. Discharge of fetus November 19, 1918, containing spirilla but not *Bacillus abortus*. Agglutination titer towards *Bacillus abortus* jumped from 1:10, 5 days before, to 1:1,280, 2 days after abortion. We have thus far been unable to find a second case with conditions paralleling this and hence are unable to present a confirmation of this somewhat unique case. Possible errors in collecting samples and in assigning the fetus to the right dam can be eliminated. The sudden rise in agglutinins may be tentatively ascribed to a rapid absorption of *Bacillus abortus* or some antigen from the udder into the circulation during discharge of the fetus. The mechanism for producing agglutinins towards *Bacillus abortus* having been established since the preceding calving, it acted promptly on stimulation by discharging large amounts of agglutinin into the blood.

No. 356 has a suspicious titer probably due to earlier *Bacillus abortus* infection. No. 351, a double infection, is discussed farther on.

In Table IV are brought together such cases of abortion as were characterized by sterile fetuses or else by miscellaneous infection of the digestive and respiratory tracts. Bacteriological details will be found elsewhere.[1]

Of the eleven cases, eight show a titer of 1:20 or lower. One (No. 211) with a high titer of 1:640 is explained by an earlier *Bacillus abortus* infection and by a demonstration of *Bacillus abortus* in the milk. The first sample of milk was negative, the second positive. In another (No. 259) with a titer of 1:160 this may be regarded as a remnant of the preceding pregnancy which terminated in abortion associated with *Bacillus abortus*. The same may be true of a third

TABLE IV.

Agglutination Titer in Cases of Abortion with Sterile Fetus or Miscellaneous Infection.

Case No.	Length of fetus.	Native or purchased cow.	Date of abortion.	No. of pregnancy.	Date of blood sample.	Agglutination titer (limit).	Date of milk sample.	Test for *B. abortus* in milk.	Remarks.
	in.		*1917*		*1918*		*1919*		
103	4	Native.	June 24	3	Oct. 30	1:10			Normal births Aug. 8, 1918, and Mar. 26, 1919.
122	24	Purchased.	July 31	1	Nov. 14	0			Normal calf Nov. 16, 1918.
126	(75 lbs.)	"	Aug. 15	2	" 14	1:20			" " " 19, 1918.
143	(35 ")	"	Oct. 11	1	Oct. 30	1:20	Jan. 10	—	" " Oct. 17, 1918.
			1918						
201	34	"	Feb. 12	2	" 9	1:10	" 10	—	
211	23	Native.	Mar. 13	4	Sept. 27	1:640	" 10	—	Preceding pregnancy terminated Apr. 4, 1917, by *B. abortus* infection.
					1919				
					Apr. 26	1:1,280	Apr. 11	+	Normal calf Apr. 4, 1919.
					June 3	1:2,560			
					1918				
259	20	"	July 18	2	Oct. 3	1:160	Jan. 2	—	Preceding pregnancy infected with *B. abortus*; present one with *B. pyogenes*.
262	29	Purchased.	" 28	1	" 3	1:20			
279	8	Native.	Sept. 27	2	" 3	1:320			Aborted Jan. 20, 1918. No bacteriological data.
291	32	"	Nov. 19	4	Nov. 28	1:10	Jan. 2	—	Infection with *B. pyogenes*.
300	35	Purchased.	Dec. 7	6	Dec. 11	1:20			

TABLE V.

Agglutination Reaction of Cows, Native and Purchased, from Which B. abortus Was Isolated.

Case No.	Length of fetus.	Native or purchased cow.	Date of abortion.	No. of pregnancy.	Date of blood sample.	Agglutination titer (limit).	Date of milk sample.	Test for *B. abortus* in milk.	Remarks.
	in.		*1917*		*1918*		*1918*		
17	27	Native.	Mar. 9	1	Nov. 7	1:40			Normal calf Apr. 14, 1918.
26		"	" 27	1	" 21	1:20			" calves Jan. 17, 1918, and Mar. 22, 1919.
31	27	Purchased.	Apr. 5	1	" 28	1:640	Dec. 17	+	Normal calf Oct. 3, 1918.
							1919		
33	26	Native.	" 6	1	" 21	1:640	Feb. 18	+	" " June 8, 1918.
34*	Living calf.	"	Feb. 23		" 21	1:320	" 20	+	" calves Feb. 23, 1917, and Mar. 3, 1918. Cow aborted Nov. 10, 1918. No bacteriological examination.
41	Nearly mature.	"	Apr. 22	1	" 14	1:40	" 20	+	Normal calf Oct. 17, 1918.
43	(8 mos.)	"	" 23	1	" 21	1:640	" 20	+	" " Apr. 23, 1918.
86	21½	"	May 16	1	" 14	0			" " Oct. 25, 1918.
89	Alive.	"	" 16	1	" 21	1:40			" " Aug. 6, 1918.
91	30	Purchased.	" 5	1	" 21	1:80			" " " 1, 1918.
101*	Normal calf.	Native.	June 28	1	Oct. 30	1:1,280	Feb. 28	+	Placenta appears normal. Normal calf June 9, 1918.
128	Calf dies (scours).	Purchased.	Aug. 17	1	" 20	1:10			*B. abortus* isolated from calf's intestine. Normal calf Sept. 26, 1918.
134	Calf alive.	Native.	Sept. 4	1	Nov. 14	1:320	Feb. 18	+	*B. abortus* obtained from placenta. Normal calf Nov. 8, 1918.

144*	Normal calf.	Purchased.	Oct. 8	1	Oct. 30	1:640	Mar. 4	+	Normal calf Mar. 21, 1919.
164	Alive.	Native.	Nov. 20	1	" 9	1:320	" 4	+	7 wks. premature.
					1919				
188*	Calf alive.	Purchased.	Dec. 31	1	Jan. 2	1:1,280	Feb. 26	−	
			1918		*1918*				
200	21	Native.	Feb. 12	1	Oct. 17	1:640	" 26	+	Calf very sick with scours when killed.
203	Calf alive.	"	" 15	1	Sept. 27	1:640			
206	28	"	" 24	1	" 27	1:160			
210	Calf alive.	"	Mar. 6	1	" 27	1:40			
214	" "	Purchased.	" 15	1	" 27	1:320			
215	" "	Native.	" 11	1	" 18	1:1,280			
							1918		
220*	" "	"	" 30	1	" 18	1:640	Nov. 12	+	Multiple staphylococcus abscesses in lung.
222	" "	Purchased.	Apr. 11	1	" 18	1:1,280			
224	" "	Native.	" 12	1	" 18	1:640			
226	" "	"	" 12	2	" 12	1:1,280			
227	" "	"	" 15	1	" 12	1:640			
253	25	Purchased.	June 13	2	" 27	1:160			
					Oct. 3	1:320			
266	31	Native.	Aug. 5	3	Sept. 5	1:640	Nov. 20	+	
							1919		
270	29½	Purchased.	" 22	2	" 5	1:1,280	Jan. 11	−	
273	16	"	" 27	2	" 5	1:320			
278	14½	"	Sept. 19	1	Oct. 4	1:40			
281	30½	"	Oct. 4	1	" 9	1:160			
					1919				
285	28½	Native.	" 23	1	Jan. 2	1:640			
288*	Calf alive.	Purchased.	Nov. 17	1	" 2	1:1,280	Feb. 4	+	Calf has scours.

* Included because the milk was examined. *B. abortus* was not isolated from the calf.

TABLE V—*Concluded.*

Case No.	Length of fetus.	Native or purchased cow.	Date of abortion.	No. of pregnancy.	Date of blood sample.	Agglutination titer (limit).	Date of milk sample.	Test for *B. abortus* in milk.	Remarks.
	in.		*1918*		*1918*		*1918*		
298	34	Native.	Dec. 2	1	Dec. 11	1:80			
301	Calf alive.	"	" 11	2	Nov. 28	1:1,280	Dec. 10	+	*B. abortus* isolated from uterine exudate. *B. abortus* found in fetus expelled Mar. 20, 1917.
			1919		*1919*				
309	(58 lbs.)	Purchased.	Jan. 2	1	Jan. 8	1:320			
311	(38 ")	"	" 7	1	" 16	1:640			
313	29	Native.	" 12	4	" 23	1:640			No record of former abortions.
316	22¼	"	" 18	2	" 18	1:1,280			
328	31	"	Mar. 9	2	Mar. 24	1:640			
329	30	Purchased.	" 11	1	" 24	1:640			
350	Calf alive.	"	Apr. 30	3	May 16	1:640			Aborted in preceding pregnancy.
351	28	"	May 5	1	" 15	1:20			Fetus contains both spirilla and *B. abortus*.
					June 2	1:160			

case (No. 279). Unfortunately a milk sample from this cow was not obtainable.

In Table V all cases of abortion associated with *Bacillus abortus* in the fetus or membranes or in the udder are brought together. The agglutination limits as given easily bring the cases into several categories. Taking those cases in which the serum clumps *Bacillus abortus* in dilutions of 1:20 and 1:40, but not up to 1:80, we find, out of 44, 8 in this class. The low titer in certain cases (Nos. 17, 26, 41, 86, 89, and 128) may be referred to the long period between abortion and the blood test, ranging from 14 to 20 months. In No. 210, the low titer of 1:40 may be due to a mild, possibly quite recent infection of the fetal membranes. The calf was vigorous and apparently in normal condition. No. 278 with a titer of 1:40 discharged a macerated fetus about 3½ months old. Here also the infection may have been too recent to stimulate antibodies.

No. 351 is of interest since both *Vibrio fetus* and *Bacillus abortus* were isolated from the fetus. The dam was well along (6½ to 7 months) in pregnancy. *Bacillus abortus* infection may have been superinduced late on the spirillum infection, since the agglutination titer for *Bacillus abortus* rose after abortion.

There are but two cases with a titer of 1:80. In one (No. 91) this was determined 18 months after abortion and may have fallen from a higher level. The second (No. 298) cannot be explained in the same way since the blood test was made 9 days after abortion. In this case, however, the fetus was large and the duration of pregnancy over 7 months. Here also the immune reaction of the dam may not have reached its maximum owing to late infection.

The two cases with a titer of 1:160 are probably explainable in much the same way as the two preceding ones. In one the blood test was made 5 days, in the other 7 months after abortion. Both fetuses were large.

Taking the next higher titer, 1:320, we find six on this level. Nos. 134 and 164 carried *Bacillus abortus* in the milk according to recent tests. How far back this milk infection dates cannot be determined. Most likely it started with the abortion indicated in the table. In the case of Nos. 214, 253, 273, and 309 the longest period between the discharge of fetus or calf and the agglutination test was less than 7 months.

The next highest titer, 1:640, was held by sixteen cases. In the two earliest cases recorded (Nos. 31 and 33) the blood test followed abortion by 19 months. The titer should have been low after such a long period but the udder was infected. In the next case (No. 144) the blood test followed abortion by nearly 13 months. In this case also the high titer is explainable by the udder infection. In the remaining thirteen cases the high titer is amply accounted for by the recent abortion. The milk, tested in only three of these, was found infected in all.

The highest recorded titer in this group, 1:1,280, was held by nine cases. The agglutination test in No. 101 is referable to the milk infection. No. 188 is an unexplained case. The test for *Bacillus abortus* in the milk was negative. A second sample is now being examined.[11] The other cases may be explained by the presence of *Bacillus abortus* in the fetus. No. 270 is of interest in that a high titer was present 14 days after abortion, although the sample of milk tested subsequently was free from infection.

SUMMARY.

The agglutination test when carried out so as to give the entire range of serum dilutions to the limit of clumping is a delicate test which reflects a variety of conditions involved in infection with *Bacillus abortus*. Among these conditions are its time relation to the act of abortion and the length of time the abortion bacilli live and multiply in the pregnant uterus. It is obvious that if a uterus be infected in the 8th month of pregnancy, the opportunity for agglutinins to accumulate are poorer than if the uterine infection lasts 3 or 4 months. The presence of *Bacillus abortus* in the udder determines in many cases the intensity of the reaction. No definite rules can therefore be formulated for the interpretation of the agglutination reaction quantitatively, since it is bound up with a complicated process varying from case to case. In the individual cow in general a titer of 1:40 or less may be regarded as indicating that the cow is not infected with *Bacillus abortus* at the time of the blood examination. It does not exclude former infections in the case of older cows, nor does it absolutely exclude very recent infection (Nos. 278 and 351).

[11] The second test of the milk of No. 188 was negative.

The highest titers, 1:640 and above, generally indicate recent infection and in the absence of recent premature births infection of the udder. Even when abortion has just occurred, it may be due to other agencies and the high titer maintained by a chronic infection of the udder dating from an earlier uterine infection with *Bacillus abortus*. Intermediate titers may indicate a gradual rise or decline of agglutinins preceding or following abortion without infection of the udder. They may also stand for a relatively high resistance or partial immunity of the cow.

In any herd a uniformly low titer (1:40 or less) in all animals may be regarded as indicating the entire absence of *Bacillus abortus*. A high titer in any one cow serves to indicate quite definitely the presence of infection in the herd. To determine more accurately the character of the infection in any individual cow there is needed in addition to the quantitative agglutination test a bacteriological study of the milk and of any prematurely discharged calf or fetus.