Studies Relating to Brucella Abortus Infection

I— On the Occurrence of the Organismin the Blood Stream

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ALTHOUGH a positive serological reaction against *Brucella abortus* may be considered prima facie evidence of Brucella infection, it is often difficult to obtain proof by isolation of the organism, particularly from the blood stream. Soule, (1930) examined a large number of dairy cows and reported approximately 13.3 per cent positive cultures from the blood in one series of tests, and 7.9 per cent in a second series. By guinea pig inoculations, Fitch, Bishop and Boyd, (1932) found *Br. abortus* to be present in four of one hundred and twenty-three samples examined (two of these four samples being from a single animal). Haring and Traum, in 1937 reported positive results, obtained by a special technique (described later), from eight (approximately 11 per cent) of 72 specimens of blood collected from cows infected with previously cultured strains of *Br. abortus.*

More recently (since completing the work herein reported) Fitch, Bishop, Boyd and Kelly (1939) have reported further studies in this subject. They made daily cultures from the blood of seven artificially infected heifers, using the technique recommended by Haring and Traum and succeeded in making 22 isolations over a period of 114 days from one animal and a lesser number from each of the other animals. From one animal only one isolation was obtained. They decided that a definite bacteremic period occurs at the time agglutinins begin to appear in the blood.

With naturally infected animals, as disclosed by serological test, there are few references to the successful isolation of the organism from the blood stream.

A greater degree of success occurs with blood from infected human beings and laboratory animals, but even here the finding of the organism is uncertain and, with most workers, appears to be the exception rather than the rule. Carpenter and Boak, 1930, reported obtaining cultures from 60 per cent of the blood samples submitted to them from cases of undulant fever, but Giordano and Sensenich, 1930, obtained cultures from only two or ten per cent of twenty cases and state "blood cultures have been extremely difficult to obtain in this series of cases." Henry, Traum and

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Haring, 1932, obtained cultures from 47.6 per cent of 126 guinea pigs known to be infected with *Br. abortus.*

A higher percentage of positive findings in the blood of human subjects and Laboratory animals is to be expected, since the technique of culturing can be more carefully carried out. Culturing from large animals, often some miles from the laboratory, is difficult and contaminations are always a problem.

In this study, an attempt has been made to gain further information on:

1 the occurrence of *Br. abortus* in the circulating blood.
2 the type of medium and technique most suitable for the isolation of the organism from the blood.
3 the atmospheric requirements of the organism on initial culture.

METHODS

Laboratory Animals

Group 1.—Guinea pigs inoculated for the examination of suspected material—hygromatous fluid, milk, and in one instance pure culture—were bled from the heart at intervals of approximately four weeks following inoculation until three bleedings had been made. Animals showing agglutinins in their blood on first bleeding were noted. On second bleeding, the blood of the positive animals was cultured. In this way, culturing of negative animals was avoided, and the planting were made at a time when the infection could be expected to be well advanced (7 to 8 weeks after inoculation). Plantings were made in duplicate in both glucose bouillon and on liver agar containing gentian violet 1:100,000—one set for incubation under increased carbon dioxide tension, the other for incubation in ordinary atmosphere. For at least a month, at intervals of about ten days, transfers were made from the broth to liver agar slants, and incubation was again carried out in both carbon dioxide and ordinary atmosphere. If no growths occurred in a month, the tubes were discarded.

Blood from 84 positive-reacting guinea pigs was cultured. The guinea pigs were later destroyed and if on necropsy lesions were visible the lesions too were cultured, a high percentage of them yielding *Br. abortus.*

In addition to blood cultures, subinoculations also were made from thirty-five of the positive guinea pigs. Five cubic centimetres of freshly drawn heart blood was inoculated intraperitoneally into another guinea pig. Bleedings of the subinoculated pigs were carried out monthly as with the original pigs. Following the third bleeding, they were destroyed for necropsy. Details are shown in tables 1 and 2.

Group 2.—At a later date, blood from a second series of 48 positive guinea pigs, (infected by inoculations of milk), was cultured in both ten per cent carbon dioxide and ordinary atmosphere as in the previous series.

Group 3.—Using the technique recommended by Haring and Traum, blood was cultured from a third series of 25 infected guinea pigs. Several cubic centimetres of heart blood were placed in a sterile vial containing

Table 1

GUINEA PIG BLOOD LESION CULTUTE RESULTS

Inoculum Obtained From Herd	Inoculum	Guinea Pig No.	Heart Blood Culture Results In		Medium on Which Growth was Obtained	Necropsy	Lesion Culture Results			
							Spleen		Testicle	
			CO_2	Atmos.			CO_2	Atmos.	CO_2	Atmos.
A	Milk	28	+	—	Glucose Bouillon	Spleen+++ Test.++++	+	—	+	—
A	Milk	40	—	+	Liver Agar	Spleen++	—	—	—	—
A	Milk	66	+	—	Liver Agar	Spleen++++	—	—	—	—
B	Hygro-matous Fluid	163	—	+	Liver Agar	Spleen+ Test.+++	+	—	—	—
C	Culture	198	—	+	Glucose Bouillon	Spleen++++ Test. ++++	+	—	—	—
A	Milk	201*	+	—	Glucose Bouillon	Spleen+++	—	—	—	—
A	Milk	210*	+	—	Glucose Bouillon	Spleen++++ Test.+	—	+	—	—
A	Hygro-matous Fluid	225*	+	—	Glucose Bouillon	Spleen++++	+	—	—	—
C	Milk	269*	+	+	Glucose Bouillon	Spleen++ Test. +	+	—	+	+
C	Milk	275	+	+	Glucose Bouillon	Spleen++++ Test. +	—	—	—	—
C	Milk	277	+	+	Glucose Bouillon	Spleen++++ Test. ++	+	+	+	+
C	Milk	278*	+	+	Glucose Bouillon	Spleen++ Test. ++	+	+	—	+

Legend: *—indicates guinea pigs from which subinoculations (blood) were made.
Culture Colums—+indicates Br. abortus obtained. —indicates negative results.
Necropsy Column—+, ++, +++, ++++, indicate degrees of pathological change.
Test.— testicle

five or six drops of a 1.6 per cent solution of sodium citrate, agitated and then heated in a water bath at 56°C. for 15 minutes. The blood was then poured over two large cooked blood agar slants and incubated, one slant in ten per cent carbon dioxide, the other in ordinary atmosphere. At intervals of three to four days after the first week the slants were examined and gently tipped to re-spread the blood over the surface. Tubes showing no growth after three weeks were discarded.

Cattle

Group 1.—Blood from ten positive-reacting cows was collected by vena-puncture of the jugular after cleansing the area and incising the skin. Owing to the inconvenience of transporting tubes of bouillon and making inoculations at the farm, single plantings only, for incubation in carbon dioxide, were made. Six of the animals were bled directly into tubes of glucose bouillon. Within a few hours the tubes were placed in the carbon dioxide chamber and incubated. Later, it was thought better

Table 2

POSITIVE GUINEA PIGS IN WHICH BR. ABORTUS IN THE BLOOD STREAM WAS INDICATED, BY EITHER CULTURAL OR GUINEA PIG INOCULATION METHODS.

FIRST ANIMAL PASSAGE				SECOND ANIMAL PASSAGE				
G. Pig No.	Agglut. Titre	Blood Culture Results	Necropsy	G. Pig No.	Agglut. Titres Days after Inoc. 34	59	91	Necropsy
192	1:12000	—	Spleen++	324	—	—	1:200	Spleen—
197	1:10000	—	Spleen++++	329	—	—	1:2000	N.V.L.
201	1:12000	+	Spleen+++	333	—	—	—	N.V.L.
207	1:8000	—	N.V.L.	339	—	—	1:1000	N.V.L.
210	1:8000	+	Spleen++++ Testicle+	342	—	—	1:200	N.V.L.
220	1:8000	—	Spleen++++ Testicle+	352	—	—	1:1000	N.V.L.
225	1:8000	+	Spleen++++	357	—	—	—	N.V.L.
263	1:1000	+	Spleen++ Testicle+	418	—	—	—	N.V.L.
270	1:2000	+	Spleen++ Testicle++	436	—	—	—	N.V.L.

Legend: Culture Column—+indicates Br. Abortus obtained
Necropsy Columns—+,++,+++,++++ indicate degrees of pathological change.
N.V.L.—indicates no visible lesions.

results might be obtained if the blood serum, which might be bactericidal, were removed and only the clots were cultured. From four of the animals, blood was collected in large centrifuge tubes (200 c.c.), allowed to clot, and then centrifuged. The serum was drawn off and replaced by glucose bouillon. Incubation was carried out in ten per cent carbon dioxide. From three of the samples, which became contaminated, only one sub-culture to liver agar was made. From the other, three subcultures were made at ten-day intervals.

In addition to subcultures from the four blood clot cultures, a further check was made for the presence of Brucella by means of animal inoculations. After ten days incubation the cultures were thoroughly agitated, and from each tube a few cubic centimetres of blood bouillon were withdrawn and injected into each of two guinea pigs.

Group 2.—Since the technique of citrating and heating recommended by Haring and Traum appeared to be more conducive to results than previously tried methods, it was decided to pursue the study further by means of this method. As opportunity permitted, blood specimens were collected from time to time from known, long standing, positive reactors and from newly infected animals. One set of specimens (24) was collected from calves one day to fourteen days after vaccination with fresh live culture, Strain 19 (currently used in calfhood vaccination experiments). Most of the calf-blood specimens were collected while the calves were showing quite marked systemic reactions; e.g., swelling at point of inocualtion, high temperature (104° to 106° F.), noticeable lassitude and slight

anorexia. From two vaccinated calves blood was collected one, two and four hours after injection and daily thereafter for several days, and then every few days for about two weeks.

Another set of specimens were taken from a mature cow infected for the purpose of preparing a high titred antiserum. This animal was exposed three times, twice conjunctivally and once by drenching, over a period of six weeks, to a virulent strain of *Br. abortus*. One to several days following each exposure, blood specimens were collected and cultured. Some months later the animal aborted and following the act of abortion several more specimens were cultured. The blood serum showed a high agglutination titre at this time, and the uterine discharges and milk contained *Br. abortus* as evidenced by guinea pig inoculations. The blood was collected in each instance from the jugular vein by means of a small needle on a 10 c.c. syringe. This method greatly reduced the problem of contamination,—only a few specimens being lost. The blood was immediately forced into a vial containing sodium citrate solution. As soon as possible, this was heated (56° C. for 15 minutes) and poured onto large slants, either cooked blood agar or liver agar, pH 6.8, containing gentian violet 1:100,000. Most, but not all, of the specimens were seeded in duplicate so that incubation could be carried out in both ordinary atmosphere and ten per cent carbon dioxide. Each tube was periodically examined and tipped to spread the blood over the surface of the medium.

RESULTS AND DISCUSSION

Laboratory Animals

Group 1—Br. abortus was obtained, *by cultural methods, from the* blood of twelve of 84 guinea pigs (14 per cent). The blood and lesion culture findings from these twelve guinea pigs are shown in table 1.

By *subinoculations, Br. abortus* was indicated in five of 35 guinea pigs (14 per cent). Positive findings, *by both methods,* were indicated in nine or 26 per cent of the animals examined. Table 2 indicates the cultural and inoculation results from these nine animals.

From some animals, positive results were obtained by one method, from others, by the other. From only one guinea pig were positive findings obtained by both methods.

A comparison of the results of blood cultures and lesion cultures indicated that *Br. abortus* was sometimes isolated from the blood stream, while attempts to culture the organism from the lesions in the spleen and testicles were unsuccessful.

In considering the atmospheric requirements, if the growth from guinea pig 198 is omitted—since the infecting organism in this instance had already been grown in atmosphere—it will be observed that five strains grew only in carbon dioxide, two grew only in ordinary atmosphere and four grew in both. If table 1 is examined further, and guinea pig 198 is again omitted, it is seen that, from both blood and lesions, eighteen cultures were obtained in carbon dioxide and twelve were obtained in ordinary atmosphere.

Cattle

In this series of experiments 116 specimens of venous blood from forty naturally and artificially infected animals were cultured with negative results in every instance. The results with four specimens were verified after ten days incubation in bouillon, by guinea pig inoculations. No evidence of infection was induced in the inoculated guinea pigs.

These results, at variance to some extent with those of other investigators, indicate either that *Br. abortus* is rarely present in the circulating blood of infected cattle or that some factor not yet determined prevents its detection in this medium. A possibility that the culture medium was unsuitable for growth of the organism can be discarded since on the same series of media isolations were readily obtained from other sources; e.g., hygromata and guinea pig spleens. The negative findings in the one pregnant animal artificially infected for the purpose of preparing antiserum are of interest. This animal at first appeared resistant and showed only a weak agglutinin response until repeated exposures had been made. Immediately following the third exposure the agglutination titre rose rapidly, but on none of the several bleedings made near this time was a bacteremia demonstrated. As previously mentioned, according to Fitch, it is at about this time that a bacteremia occurs. The culture used to infect this animal was one that had been easily grown. When originally isolated it grew in both ten per cent carbon dioxide and ordinary atmosphere and on several occasion since, it has been recovered easily from the spleens of guinea pigs. One would expect, therefore, to experience little difficulty in recovering the organisms if these were present in the blood.

A surprising feature of the study was the negative finding immediately following the act of abortion. A similar finding was noted on two other occasions, with naturally infected animals. Bleedings were made at this time in the expectation that a bacteremia would be found since following an abortion the animal appears to be over-loaded with organisms and the agglutination titre often rises. Fitch and his associates (1939) found that a bacteremia might be present until the time of parturition or abortion, but in only two animals were they able to demonstrate it after parturition.

Summary

1. Heart blood specimens from three groups of infected guinea pigs comprising 157 animals were cultured. Blood from 35 of these was passaged to other guinea pigs.

2. *Br. abortus* was isolated in pure culture from 21 guinea pigs (13 per cent) and its presence was indicated in four others by animal inoculation, a total of 25 or 16 per cent.

3. Twelve cultures grew only under increased carbon dioxide tension, five grew only in ordinary atmosphere and four grew in both.

4. 116 specimens of bovine blood collected from 40 different animals, both

naturally and artificially infected, were cultured, but not a single specimen yielded a culture of *Br. abortus.*

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II. On the Occurrence of the Organism in Serous Swellings or Hygromata on Cattle.

IN CONTRAST with the difficulty of isolating *Brucella abortus* from the blood stream of infected cattle, as indicated in the preceding paper,[1] is the relative ease with which the organism can be isolated from a large percentage of serous swellings or hygromata of cattle. Boyd, Delez and Fitch,[2] first reported finding *Br. abortus* in hygromata in 1930, and Delez[3] in 1934 reported finding the organism in the serous effusion of the hip joint of an infected cow.

For a number of years, the writer had several dairy herds under close observation for the purpose of studying Bang's disease. Some of the herds were extensively infected, and in two in particular, the disease was long standing. Positive and negative units were maintained, but little or no actual effort was being made to rid the premises of reactors. Thus an opportunity was afforded, over a considerable period, to observe the occurrence of hygromata on infected animals. The positive unit of each herd was maintained in a separate quarter, and in each of these the relatively frequent appearance of swellings on knees and hocks was rather striking. On one occasion two cows were observed to have recently developed hygromata. Previous blood tests (the most recent of which had been applied about two months earlier) had always been negative. On again testing these animals, however, it was found that both had become positive.

Later, as time permitted, the examination of the contents of a number of the swellings was undertaken with a view to obtaining further information on their relation to Bang's disease.

Observations of Hygromata

The cause of hygromata, has not been determined. From their usual locations, they appear to be the result of injury, such as the continual bruising that occurs on cement floors. Exceptions to this, however, are seen

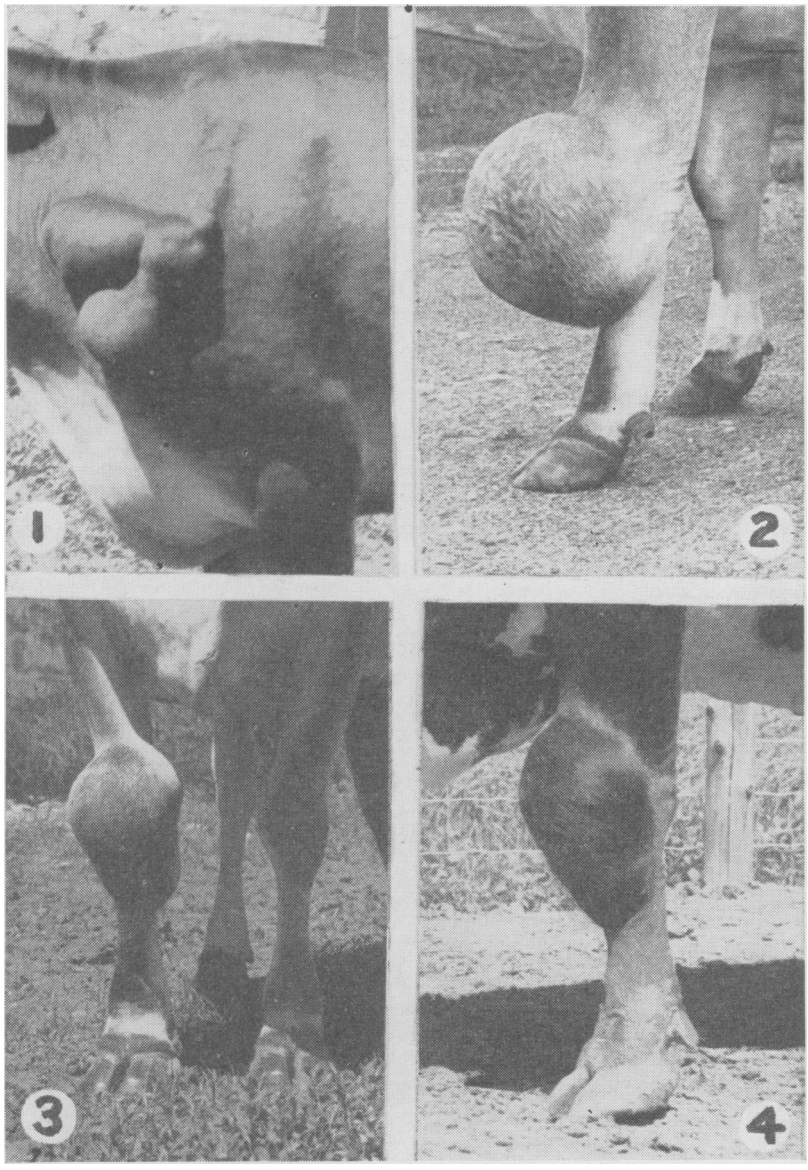

Fig. 1.—Animal No. 39 (A. & B). Tri-saccule hygroma on face of shoulder and side of neck. *Brucella abortus* was obtained from the fluid on two different occasions.
Fig. 2.—Animal No. 22. Hygroma on knee Fluid yielded pure cultures of *Brucella abortus* Suppuration occurred following aspiration.
Fig. 3—Animal No. 32 (Table II). Blood asd serous fluid positive to agglutiation tests but cultural and guinea pig inoculation results negative.
Fig. 4.—Animal No. 42 (Table II). Blood and serous fluid negative to agglutination tests but cultural and guinea pig inoculation results positive.

in those instances where the swellings occured on the side of the neck; e.g., fig. 1. Here, the initial injury, if present at all, would seem to have been very slight.

Hygromata have been observed frequently in the herds infected with *Br. abortus*. In this study, thirty-six of the affected cows were positive reactors and three others, although negative, were subsequently found to be carrying infection. In other words, approximately 93 per cent of the animals which developed hygromata were found to be infected with *Br. abortus*. On the other hand, hygromata have been observed only rarely in other herds that have been free of infection for a number of years. The method of stabling each herd was similar. The hygromata seen on negative animals have been small and have shown little tendency to increase in size, while those on the infected animals have, with few exceptions, tended to become larger and in some cases, have attained enormous proportions; e.g., fig. 2. On neither the negative nor the positive animals have they appeared highly sensitive or painful. When reduced by simple aspiration, they soon regained their former size. Only one of them, No. 22 (fig. 2.), suppurated, and this not until after fluid had been aspirated several times for experimental study. Pyogenic infection was evidently introduced by the aspirating needle.

Method of Examination

Twenty-five to thirty cubic centimetres of fluid were aspirated from the swelling with aseptic precautions by means of a hypodermic syringe and placed in a sterile bottle. A blood sample was drawn from the jugular vein at the same time for a comparative agglutination test. With a few of the earlier specimens collected, no effort was made to increase the bacterial concentration for inoculation. Five cubic centimetres of fluid were removed and injected directly into each of two guinea pigs; the remainder of the specimen was used for agglutination tests against *Br. abortus*. In each case, the test was carried to the end point, and the titre recorded for comparison with the reaction of the blood serum and the inoculation results. Later, with a view to concentrating the organisms, the samples were centrifuged for thirty minutes at 2000 revolutions per minute, the supernatant fluids pipetted off and the substrata only used for inoculation. Smears were made from a number of substrata for staining and microscopical examination. The supernatant fluids were held and their agglutination titres determined as before.

Subsequently, as a further check on the presence of organisms, it was decided also to culture the specimens. When the study was started, media with a reaction of pH 7.4 to 7.6 was being used for stock cultures and although some authorities (Duncan & Whitby—1930)[5] give the optimum reaction for the growth of *Br. abortus* as pH 7.4, it appeared from our own experiences that a medium of a lower pH might be more suitable for isolations.

To verify this point, duplicate sets of media, one pH 6.8, the other pH 7.6 were used for a number of specimens. Culturing from the substrata was

carried out in both glucose bouillon and liver agar containing gentian violet 1: 100,000 plantings being made in duplicate sets—one for incubation in 10 per cent carbon dioxide, the other for incubation in ordinary atmosphere. The remaining portion of each substratum was re-suspended in normal sodium chloride solution and injected into two guinea pigs. If growth appeared from the direct seeding on the solid medium, the broth cultures were discarded. Otherwise, transfers from the broth to agar slants were made at eight to ten day intervals, until at least a month had elapsed.

Each guinea pig used was tested prior to inoculation, to insure that only negative animals were being employed. Following inoculation, when possible, each pair of guinea pigs was placed in separate cages. When a number of guinea pigs were inoculated at the same time and separate cages were not available, several non-injected control pigs were placed with them as a check on the possibility of infection spreading from animal to animal. A positive reactor has never been found in the guinea pigs from the breeding stock, and none of those placed with the inoculated pigs in this series of experiments has developed infection. The guinea pigs were injected intraperitoneally and at intervals of three to four weeks following inoculation each guinea pig was bled from the heart, for serological test. After three bleedings, the pigs were destroyed and necropsies made.

Results

Forty-five serous swellings were examined, forty-one of which were located on the knee, two on the lateral surface of the hock and two on the side of the neck and shoulder. Results of the examination of twenty-one of these serous swellings, by serological and guinea pig inoculations, are given in table I. In table II are indicated the results obtained from the remainder, which also were studied by cultural methods.

The presence of *Br. abortus* was indicated in nine of the former group and in eighteen of the latter. Thirty-six of the cows on which hygromata occurred showed positive blood reactions, while six were negative. The hygromatous fluid from one cow, whose blood contained no agglutinins (No. 1., table I), showed a titre of 1:50 and one guinea pig inoculated with the fluid became positive. The hygromatous fluids from two negative (blood) cows (No. 30 & 42, table II) were also negative when tested for agglutinins, but each yielded a culture of *Br. abortus* and each proved infectious to one of two guinea pigs. One specimen of hygromatous fluid from a positive cow (No. 33) was negative on agglutination test, but yielded *Br. abortus* on culture and proved infectious to one guinea pig.

Ten guinea pigs gave definitely positive serological reactions without macroscopic lesions and one pig indicated a questionable (1:50) reaction without lesions, but no guinea pig with a negative reaction developed lesions. In several instances, only one of the two guinea pigs inoculated became positive.

Increased carbon dioxide tension appeared to be essential for the growth of *Br. abortus* in the direct seeding of hygromatous fluid. No growths were obtained in ordinary atmosphere. The medium with a

Table I—Hygromatous Fluid—Serological and Biological Test Results

Cow No.	Type of Swelling	Agglutination Titre		Guinea Pig No.	Days Following Inoculation	Serological Reaction Shown by Guinea Pigs	Necropsy
		Blood	Serous Fluid				
1	Hygroma on Knee	Negative	1:50	87	108K	Positive	Emaciated, Spleen++++
				88	108K	Negative	No visible lesions
2	"	1:100 ±	1:4000	89	58D	Positive	Spleen++
				90	87K	Positive	Spleen++++, Testicles++++
3	"	1:100 ±	1:100	91	98K	Negative	No visible lesions
				92	92K	Negative	No visible lesions
4	"	1:100 ±	1:300	93	47D	Positive	Spleen++++
				94	98K	Positive	No visible lesions
5	"	1:100 ±	1:300	99	98K	Negative	" " "
				100	98K	"	" " "
6	"	1:100 ±	1:1500	101	98K	"	" " "
				102	98K	"	" " "
7	"	1:100 ±	1:400	103	98K	"	" " "
				104	98K	"	" " "
8	"	1:700	1:700	108	98K	"	" " "
				109	98K	"	" " "
9	"	[redacted] 1:2000	1:1500	110	98K	Positive	Spleen++++
				111	30D	Questionable	Died from bleeding. No visible lesions
9	Serous Swelling on side of neck	[redacted] 1:2000	1:1000	112	98K	Positive	Emaciated, Spleen++++
				113	98K	Positive	Spleen+++
10	Hygroma on Knee	1:100 ±	1:800	191	93K	Negative	No visible lesions
				192	94K	"	" " "

Legend: 100 ± : indicates serum dilutions carried only to 1:100
Plus signs (+): Indicate various degrees of pathological change.
D : Died K: Killed.
* : indicate animals from which blood was cultured.

Table 1 (Continued) — Hygromatous Fluid—Serological and Biological Test Results

Cow No.	Type of Swelling	Agglutination Titre		Guinea Pig No.	Days Following Inoculation	Serological Reaction Shown by Guinea Pigs	Necropsy
		Blood	Serous Fluid				
11	Hygroma on Knee	1:100 ±	1:400	193	93K	Positive	No visible lesions
				194	93K	"	Spleen +++
12	Serous Swelling on lateral surface of hock	1:100 ±	1:200	195	93K	"	No visible lesions
				196	93K	Negative	" " "
13*	Hygroma on Knee	1:200	1:100	153	82K	"	" " "
				154	82K	"	" " "
14*	"	1:1000	1:8000	155	13D	Not tested	Pneumonia
				156	82K	Positive	No visible lesions
15*	Serous Swelling on lateral surface of hock	1:2000	1:800	157	82K	Positive	Spleen ++, Testicles ++++
				158	14D	Not tested	Pneumonia
16*	Hygroma on Knee	1:1000	1:1000	159	82K	Negative	No visible lesions
				160	82K	"	" " "
17*	"	1:100	1:400	171	72K		" " "
				172	72K	"	" " "
18*	"	1:1000	1:1000	169	19D	Not tested	Pneumonia
				170	72K	Negative	No visible lesions
19	"	1:500	1:4000	173	72K	"	" " "
				174	72K	"	" " "
20	"	1:400	1:400	163	13D	Not tested	Pneumonia
				164	13D	"	"

Legend: 100 ± : indicates serum dilutions carried only to 1:100
Plus signs (+): Indicate various degrees of pathological change.
D : Died K: Killed.
* : indicates animals from which blood was cultured.

Table II Hygromatous fluid — Serological, Cultural and Biological Test Results.

Cow No.	Type of Swelling	Agglutination Titre		Culture Results		Guinea Pig No.	Days Following Inoculation	Agglutination Titre of Guinea Pigs	Necropsy
		Blood	Serous Fluid	in Atmosphere	in 10%CO_2				
21* R. Knee	Hygroma on Knee	1:2000	1:1000	—	+	220	82K	1:8000	Spleen++++ Testicle++
						221	82K	Negative	No visible lesions
21 L. Knee	"	1:2000	1:1000	—	+	222	82K	Negative	" " "
						223	82K	Negative	" " "
22*	"	1:2000	1:2000	—	+	224	68D	1:200	Spleen++++ Testicle+++
						225	82K	1:8000	Spleen++++
23*	"	1:2000	1:800	—	—	226	82K	Negative	No visible lesions
						227	70D	Negative	" " "
24	"	Negative	Negative	—	—	263	68D	Negative	" " "
						264	95K	Negative	" " "
25	"	Negative	Negative	—	—	285	95K	Negative	" " "
						286	95K	Negative	" " "
26 R. Knee	"	1:100	1:50	—	+	287	127K	1:1000	Spleen++++ Testicle++++
						288	127K	Negative	No visible lesions
26 L. Knee	"	1:100	1:50	—	+	289	90K	1:200	" " "
						290	121K	1:200	Spleen++ Testicle++
27	"	1:400	1:200	—	+	291	121K	1:3000	Spleen++ Testicle+++
						292	97D	1:200	No visible lesions
28	"	1:100	1:25	—	—	293	27D	Not tested	" " "
						294	121K	Negative	" " "
29	"	1:200	1:400	—	+	295	121K	1:1000	Spleen++ Testicle+++
						296	121K	1:1000	No visible lesions
30 R. Knee	"	Negative	Negative	—	+	548	97K	1:3200	Spleen++
						549	97K	Negative	N.V.L.

Legend: *— indicates animals from which blood was cultured.

Table II —(Continued) Hygromatous fluid — Serological, Cultural and Biological Test Results.

Cow No.	Type of Swelling	Agglutination Titre		Culture Results		Guinea Pig No.	Days Following Inoculation	Agglutination Titre of Guinea Pigs	Necropsy
		Blood	Serous Fluid	in Atmosphere	in 10%CO₂				
31 R. Knee	Hygroma on Knee	1:200	1:25	—	—	551	97K	Negative	N.V.L.
						552	97K	Negative	N.V.L.
32 R. Knee		1:400	1:200	—	—	582	92K	Negative	N.V.L.
						584	18D	not tested	N.V.L.
33 L. Knee	"	1:400	Negative	—	+	583	92K	1:400	Spleen+: R. testicle+
						585	50D	Negative	N.V.L.
34 L. Knee	"	1:200	1:50	—	—	586	42D	Negative	Liver abscess
						587	91K	1:1600	N.V.L.
35 R. Knee	"	Negative	Negative	—	—	588	86D	Negative	N.V.L.
						589	83D	Negative	N.V.L.
36 L. Knee	"	1:800	1:3200	—	+	590	88K	1:800	N.V.L.
						591	56D	1:3200	N.V.L.
37 L. Knee	"	1:400	1:200	—	+	592	69D	1:200	R. testicle abscessed
						593	24D	Negative	N.V.L.
38 R. Knee	"	1:400	1:3200	—	—	594	88K	1:800	Spleen++, Testicles+++
						595	88K	Negative	N.V.L.
39A*	Hygroma on shoulder	1:6000	1:7000	—	+	604	77K	1:1600	Spleen++
						605	77K	1:800	Spleen++
39B	Hygroma on Side of neck	1:1600	1:6400	—	+	786	61K	1:3200	Spleen+++
						787	61K	1:3200	Spleen+++
40	" on Knee	1:600 Positive	1:800	—	—	682A	54K	1:50	N.V.L.
						683	54K	Negative	N.V.L.
41 R. Knee	"	1:100	1:200	—	+	784	61K	1:800	Spleen+++
						785	61K	1:100	Spleen+++, L. testicle++
42 L. Knee	"	Negative	Negative	—	+	788	30D	Negative	N.V.L.
						789	61K	1:1600	Spleen++++ R. testicle++

Legend: *— indicates animals from which blood was cultured.

reaction of pH 6.8 was found to be more satisfactory than that with the higher pH value. In most instances growth did not occur on the more alkaline medium. There appeared to be no advantage, in this series, in culturing in bouillon and subculturing on solid media, since in each instance when growths were obtained by this method, they also were obtained by direct seeding on the agar.

The agglutination titres of the blood serum and hygromatous fluid in the same cow did not always correspond. In some cases, the titre of the blood serum was higher, in others that of the hygromatous fluid was the higher, and no constant relationship existed between the titre of each and the presence of *Br. abortus*.

Discussion

The primary aetiological factor in the occurrence of hygromata appears to be injury, but since they are so frequently seen in positive or infected herds and only rarely seen in negative herds, it is difficult to dissociate them from Bang's disease. In this study hygromata were examined as they appeared, without any attempt at selection as to positive or negative animals, but only three of the 42 animals studied failed to show evidence of Brucella infection. Unfortunately, these three animals were removed from the herd before further studies could be made.

In the development of the swellings on the side of the neck where injury must have been slight, *Br. abortus* probably played an important role. The animals bearing the swellings appeared to be heavily infected. Both aborted and their sera showed high agglutination titres. The swelling on animal No. 39, when first seen was a soft, flabby, single saccule covering only a small area near the face of the shoulder. Subsequently it developed into three relatively large distinct saccules and when last seen, nearly a year later, covered a relatively large area on the side of the neck (fig. 1). *Br. abortus* was readily isolated from it when first observed and again on a second examination nine months later (table II). Indicated also in this table (*) are some of the animals whose blood was cultured with negative results. Of particular interest in the study was the finding of *Br. abortus* in the hygromatous fluid of three animals whose blood serum was negative.

It has long been recognized that *Br. abortus* possesses a peculiar predilection for colonizing in the gravid uterus and the udder, and it is possible that a similar selection of tissues has taken place when the organisms occur in hygromata. The association of *Br. abortus* with poll evil and fistulous withers of horses, as reported by Rinjard and Hilger [7] (1928); Fitch, Delez and Boyd [6] (1930); and Duff [4] (1933) may be more or less analogous to its occurrence in hygromata of cattle. A difference of interest, however, is noted in that hygromata, unlike poll evil and fistulae, seldom suppurate.

As a rule, guinea pig inoculation is considered more reliable than direct culturing as a method for the isolation of *Br. abortus*, but it will be

noted (table II) that in one instance, organisms were obtained by direct culture, while guinea pig inoculation gave negative results. The importance of injecting two or more guinea pigs with each specimen is obvious, since in four instances one pig remained negative, while its mate developed definite evidence of infection. The number of organisms inoculated and the individual resistance of the host in these cases are no doubt important factors in the development of infection.

While increased carbon dioxide appeared to be necessary for the growth of the organism in direct culture from serous fluid, it will be seen in table 1 of the preceding paper,[1] that from the heart blood of one guinea pig (No. 163), infected by means of hygromatous fluid, growth was obtained in ordinary atmosphere.

Summary

1. The contents of forty-five serous swellings (hygromata) on thirty-six positive and six negative animals were examined.
2. The presence of *Br. abortus* was indicated.
   (a) By successful isolation in pure culture in 15 of the 25 specimens cultured.
   (b) By guinea pig inoculation, and the resulting serological reactions, in 25 of the swellings.
   (c) By both methods combined, in 27 or 60 per cent of the swellings.
3. In one animal whose blood serum was negative and whose hygromatous fluid did not agglutinate *Br. abortus* above 1:50, the presence of *Br. abortus* was indicated by guinea pig inoculation.
4. In two animals in which *both* the blood serum and hygromatous fluid were negative, the presence of *Br. abortus* in the hygromatous fluid was proven by direct isolation of the organism.
5. Evidence of *Br. abortus* infection was educed in approximately 93 per cent of the animals that developed hygromata.

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