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Transmission of Brucella Suís From Swine to Cattle Under Pasture Conditions

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SUMMARY

Eight cows were exposed to swine artificially infected with *Brucella suis* and kept in close contact with the hogs in a small pasture.

There was no evidence of spread of infection from the artificially infected swine to any of the cattle on experiment. All attempts to isolate Brucella organisms from the cattle were negative.

Evidence of elimination of infectious material from the artificially infected swine was proved by the fact that control negative gilts acquired the infection when running in the same pasture.

Culturs of *Brucella suis* were isolated from both infected hogs and previously negative gilts which served as controls.

Three abortions in the cattle occurred while the experiment was in progress. One cow aborted her calf in 252 days. Another aborted after 258 days of the gestation period had elapsed. In the third, abortion occurred at the end of 157 days. In no case was it possible to isolate Brucella organisms from the aborted fetuses or the cows.

Several of the cows on experiment developed low blood agglutination titres and varied from negative to complete agglutination in dilutions of 1-160. There was no proof that these low titres were actually due to *Brucella suis*, but this possibility must be recognized even though we were unable to isolate Brucella organisms from any of the animals.

The data in this bulletin reports on 36 gestation periods with an average length of 278 days.

There was no evidence of any sterility or breeding difficulty and cases of retained placenta were not observed.

Previously negative swine when artificially inoculated gave evidence of becoming infected, as indicated by a blood agglutination titre of 1 to 100 or over, in an average of seven days. Our records showed this occurred in from 4 to 14 days in different individuals.

Swine recover much faster from *Brucella suis* infection than cattle do from *Brucella abortus*. If infection in swine is assumed when the blood titre is 1 to 50 or above, nineteen artificially infected swine on this experiment maintained such a titre for from 39 to 923 days. The average was 285 days, but it should be kept in mind that all but five of the hogs were sold while they still maintained a blood titre of 1 to 50 or higher.

Because *Brucella suis* has been isolated from cows' milk by other workers the possibility of contamination of the milk with this organism and the invasion of the cow's udder must be recognized. There was no evidence that this occurred in this experiment when the cattle were exposed to artificially infected swine under pasture conditions.

Transmission of Brucella Suís From Swine to Cattle Under Pasture Conditions

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There is still considerable question regarding the possibility of infected swine transmitting brucella infection to cattle under farm or pasture conditions. This question is most important when one is dealing with cows producing milk for human consumption. When the cattle herds have been rid of Bang's disease infection, frequent inquiry is made as to the safety of running infected swine along with those cattle herds. To determine just how much danger there was from practices of this kind, an experiment was set up so that the contact would be under pasture conditions.

REVIEW OF LITERATURE

Huddleson (1) states there is insufficient evidence to indicate exactly what role the infected hog plays in transmitting the disease to cattle. In work Huddleson did at the Michigan Brucellosis Laboratory, he concluded on the basis of cultures studied that *Brucella suis* was involved in infections of cattle in many of the southern states.

Cotton (2) raised the question of the possibility of the presence of porcine organisms in the udders of cows being subjected to the use of vaccine containing this type of micro-organism. This was not certain. He thinks the indications are that swine abortion and cattle abortion are not inter-communicable to any great extent by natural means. He stated the relation of swine abortion to that of the bovine species should be more definitely determined.

Cotton and Buck (3) reported that the porcine strain failed to infect cattle by natural means, but that cattle could be infected by intravenous injections. According to them, later experiments indicated that cattle were highly resistant to the swine type microorganism. Their work showed without question that the swine type of *Brucella abortus* is less pathogenic for cattle than is the bovine type. They thought it possible, and probably likely, that under unusual conditions the udders of cows may become infected with the swine type. The same authors (4) reported further work by them tended to confirm the results obtained in their previous experiment.

Cotton, Buck, and Smith (5) concluded from their experimental

work that pregnant cattle can sometimes be artificially infected and caused to abort by subjecting them to severe *Brucella abortus* (porcine) exposure through the conjunctiva, or conjunctiva and digestive tract. They found that cattle negative to the agglutination test developed temporary agglutinin reactions when kept for considerable time in close contact with swine infected with *Brucella abortus*. They were unable to isolate *Brucella abortus* from the cattle. They concluded cattle had considerable resistance to infection with *Brucella abortus* (porcine) and seldom contract infectious abortion from the swine as the result of natural exposure to them.

Cotton (6), reporting on experiments of the Federal Bureau of Animal Industry Station, stated that attempts were made to infect pregnant heifers with the porcine type of *Brucella abortus* through intense natural exposure. It was found that some of the heifers reacted in low titre for a brief period only, but no other evidence of infection was found. In these cases he thought it was probable that the infection was soon overcome by the resistant host after which the agglutinins disappeared. He thinks that infection transferred by natural exposure from swine to cattle would not be any more than a transient infection. Some of the cows on the B.A.I. experiment reacted to the agglutination test for Bang's disease for a brief period, but it was impossible to isolate *Brucella abortus* from these animals.

Thomsen (7) in his textbook says that *Brucella suis* seems to be demonstrated frequently in cases of cattle abortion in some of the states, but he does not think swine are dangerous to cattle. Later in the same publication he states there is no true relation between swine brucellosis and infectious abortion in cattle. In his summary the statement is made that there is a lack of evidence as to any demonstrable spreading of swine brucellosis to cattle.

Delez (8) attempted to infect bulls with the porcine strain by exposure through different routes, but was unable to isolate the porcine strain from the experiment animals. Moore (9) reports that Schroeder and Cotton produced abortion in pregnant cows by intravenous injection of cultures obtained from swine. Harms (10) reported experimental infection of a heifer by intravenous injection with porcine strains. This was followed by abortion. This author thinks cattle sometimes may become naturally infected with the porcine strain of Brucella abortus. Giltner (11) states that Brucella suis is transmissible to cattle. Hayes (12) states there is much evidence that Brucella suis can readily establish itself in the body of the bovine. Starr (13) applied live cultures of Brucella suis to the scarified skin of one calf and to the eye of another. This was followed by the ap-

pearance of low-titre agglutinins in the blood stream, which persisted for a short time and then disappeared. Howarth and Hayes (14) showed a previously negative cow which reacted positively to the agglutination test after 91 days close contact with positively reacting sows. Giltner (15) thinks the suis strain of brucella can invade cattle regularly. Graham, Boughton, and Tunnicliff (16) reported difficulty in infecting heifers when confining them with reacting sows, but do suggest the danger of *Brucella suis* in breeding cattle. They were able to produce abortion by intravenous injection of a heifer with porcine strains. Kitselman (17) thinks that although the evidence is not conclusive one should avoid allowing infected swine to come in contact with cattle.

Hayes (18) is of the opinion that there is danger from undulant fever through the use of raw milk from udders harboring the suis variety of Brucella and that the frequent isolation of *Brucella suis* from udders of cows emphasizes the potential danger of the swine infection to cattle. Graham and Michael (19) say every possible safeguard should be employed to prevent brucellosis from being passed from swine to cattle. Hutchings (20) suggests that swine brucellosis may be even more important in public health than an economic problem and states *Brucella suis* is not infrequently transmitted from hogs to cattle. Hasseltine (21) reported *Brucella suis* has been isolated from cows' milk.

Beattie and Rice (22) report that they found, in undulant fever due to brucella of the porcine type, one milk-producing cow that was shedding brucella in her milk. The organism was of the Brucella suis type. They felt justified in assuming that they were dealing with a milk-borne epidemic of undulant fever due to Brucella suis. They do state in their publication that under natural conditions there is some doubt if cattle will become infected with Brucella suis. Fitch and Bishop (23) stated that it had been demonstrated that Brucella suis may infect a cow. These authors have had several brucella strains isolated from cattle and found some to be suis strains. Several authors have reported that Brucella suis has been isolated from cows' milk. Among these reports is one of a milk-borne epidemic of brucellosis in Iowa reported by Borts, Harris, Joynt, Jennings, and Jordan (24) due to Brucella suis in a milk supply. As a result of their investigations they concluded hogs should not be permitted to run on the same lot with dairy cows.

Several authors are of the opinion that brucellosis is not transmitted from cattle to swine. Work done by the Missouri experiment station would tend to verify these opinions.

METHODS AND PROCEDURE

The cows on the experiment were known to be disease-free animals: that is, they were negative to successive blood tests. were placed in a pasture of approximately three acres in which there was one rather small shelter shed. Along with the cattle were placed artificially infected swine which also were negative to the blood tests before they were artificially infected by drenching them with live cultures of Brucella suis. Being comparatively small, the pasture afforded ample opportunity for the infected swine to be in close contact with the cows. Frequent blood tests were made on the cattle and swine, the interval between tests depending upon circumstances. This interval varied in some cases from three days up to thirty days. most cases the tests were made every week or at least every two Negative gilts which were not dosed were placed in the pasture along with the artificially infected swine and the cattle to serve as controls. The controls being in the pasture in close contact would prove, if they became infected, that the artificially infected swine were eliminating live virulent Brucella suis organisms. In addition to the blood tests, samples of milk, fetal membranes, vaginal discharges and stomach contents from any dead fetuses were cultured on liver infusion agar, and on liver infusion agar to which had been added gentian violet to make a 1-10,000 dilution. The same material was also injected intraperitoneally into male guinea pigs which were held for ten weeks at the beginning of the experiment but later on were held only six weeks. In the guinea pigs which were held ten weeks occasional blood tests were made to determine whether or not the guinea pigs showed any evidence of infection. The blood tests were not deemed necessary in the animals that were held only six weeks. In both cases the guinea pigs were killed at the end of the respective times mentioned before. When they were killed their blood was tested and material from them was used to inoculate liver infusion agar plates and liver infusion agar plates to which had been added gentian violet. Part of these plates were incubated under a reduced oxygen content, approximately 8% CO₂. The balance were incubated under ordinary atmospheric conditions. In all cases the plates were held for ten days before they were discarded. During this time they were examined two or more times and any colonies resembling brucella organisms were subcultured onto liver infusion agar slants. subcultures were later stained and examined microscopically and if typical both morphologically and by staining they were later checked with known positive and negative serum. When the cows and the hogs were taken off this experiment they were slaughtered locally, at which time other material was collected for culturing and guinea pig inoculation. In cattle the following tissues were selected for this work: uterine wall and cotyledons, udder and supra-mammary lymph glands. In hogs the tissues collected were uterine wall and fetal membranes if present, pieces of udder and supra-mammary lymph glands. The tissues collected were ground up in sterile mortars, using sterile sand and sterile physiological salt solution. This material was found quite suitable for both bacteriological examination and guinea pig injection. The plate cultures thus obtained and the guinea pigs were handled in exactly the same manner as above described. While the guinea pigs were on test and under observation bi-weekly weights were taken as infected guinea pigs usually show loss in weight.

EXPERIMENTAL WORK

In the beginning seven negative sows were placed in a pasture with six pregnant cows. Four of the sows were dosed with *Brucella suis* and three were left untreated as controls. The virulence of the organisms for swine was proved in both the treated animals and the control animals and the organism was re-isolated from the infected hogs in pure culture. Isolations were made from placental membranes, colostral milk, and aborted pigs.

The next year more gilts and young sows were added to the experiment, making a total of thirteen gilts or young sows during the first sixteen months. At different times during this period four of the gilts aborted. Included in this number were both dosed and control animals. The latter proved that there was sufficient infectious material spread by the infected animals to produce abortion in the controls, indicating the cows had been subjected to considerable exposure. During this period pure cultures of brucella organisms were isolated from seven of the hogs. Several of the cows had titres varying from 1 to 20 to 1 to 160, but attempts to isolate brucella organisms from them gave negative results.

During the second year one cow aborted her calf after a gestation period of 252 days, but the calf lived and was later sold to the butcher. This cow varied in blood titre from negative to 1 to 50. Attempts to isolate brucella organisms from this cow were also negative. No evidence of any great variation in the blood titre was obtained at or near the time of this abortion, which may further indicate that it was not due to the brucella organisms to which the cattle had been exposed. The other cows during the second year varied from negative to an incomplete 1 to 80. Additional gilts freshly dosed were added to the pasture to continue the exposure.

In the latter part of the third year of the experiment, all positive sows were sold and no hogs were in contact with the cattle for the succeeding six months, except some which had a negative blood titre. The reason for this rest period was to determine, if possible, if the cow titres would fade out and then to see if fresh infective material would have any marked effect following the rest period. It was found that the rest period had very little, if any, effect upon the blood titres of the experiment cattle. Following the rest period of six months, two sows were added to the pasture; one was dosed and one was left as a control. Later more hogs were added and dosed with pure cultures of *Brucella suis* in order to keep the cattle exposed to the swine infection.

During the experiment one cow dropped a living calf at 258 days. This cow was killed 38 days after calving. Autopsy revealed severe sarcoma of the abdominal organs. Just what part this sarcomatous condition played in relation to the shortened gestation period is not known. This cow had the highest blood and colostral milk titre of any animal on experiment. Her blood titre varied from negative to an incomplete 1 to 80 during this particular year.

Another cow aborted after being bred 157 days. Her blood titre remained no higher than 1 to 25 and most of the time after abortion was negative to the test. Colostral milk samples from two of the cows varied from incomplete 1 to 100 to an incomplete 1 to 200 when quarter samples were tested.

While on experiment the cows varied in titre from negative to as high as complete agglutination in dilutions of 1 to 160. At no time were brucella cultures isolated from any of the experiment cattle.

At the close of the investigation the cattle were sold and slaughtered locally but material from them was available for culture and guinea pig inoculation. Cultures were made from the uterus, udder, and supra-mammary lymph glands. All cultures and guinea pig inoculations were negative for brucella.

The cows were kept on the experiment for several different gestation periods. The results of this experiment might be criticized in that the cows should have been killed and cultures made at the end of one gestation period after they had been in contact with the artificially infected swine. It was felt that the examination of colostral milk, fetal membranes and dead fetuses when available would give sufficient evidence if infection had taken place and that it might be better to maintain the animals for several gestation periods.

The data in this bulletin reports on a total of thirty-six gestation periods in eight cows. The average length of the gestation period was

found to be 278 days. This figure is in agreement with normal gestation periods found in known Bang's disease-free accredited herds and is considered a normal average. That exposure to *Brucella suis* did not interfere with breeding efficiency of the cattle is shown by the fact that most of the cows settled with calf with one service. Sterility or trouble from retained placentae was not observed and from our experience gave further evidence that we were not dealing with brucella infection. In no instance were we able to isolate *Brucella suis* from any of the cows on the experiment, either from material collected while they were still living or from the material collected at the time of slaughter.

Controls Give Evidence of Exposure

Several of the control animals became reactors to the blood test and some of them lost their pigs prematurely with typical symptoms of infectious abortion. This proved that the artificially infected swine had eliminated sufficient virulent material in the pasture to infect the control animals. It was felt that if sufficient material was eliminated

Identification Number	Blood Reaction Previous to Exposure	Number days elapsed before blood titre reached 1-100 or over	Maximum Titre Reached	Number of days blood titre stayed 1-50 or over	Remarks
PC7		10	5120	055 *	
PC11		7	20480	255 * 258 *	6 6
D9		4	20480		
H65		10	2560	247 *	
PC10		14	2560 ^I	45	aborted
PC6		5	51 2 0	127 *	
PC14		. 7	2560	64 *	
PC15	Name of the last o	6	5120 ^I	665 *	aborted
D30		. 0		43 *	aborted
H31		í e	5120I	91 *	
H41	202	6	1280 ^I	64 *	aborted
H9		6	5120I	341 *	0.0
H111			10240 ^I	923 *	
H30	222	9	2560 ^I	780 *	
H40		7	2560 ^I	453	
D31		7	2560 ^I	332	
D33		5	2560I	309 *	
		9	640 ^I	39	6.9
CW42		8	640	193 *	
CW43		8	2560	196	

TABLE 1. - HOGS ARTIFICIALLY DOSED WITH CULTURES OF BRUCELLA SUIS

to cause abortion in the swine that the cows in the same enclosure had ample opportunity to pick up brucella organisms. Cultures of typical *Brucella suis* were isolated from the aborting control animals, proving that the abortion was actually due to *Brucella suis*.

^{*} Indicates hogs which were sold before titre dropped below 1-50.

Artificial Infection of Swine

The method used to artificially infect the swine was to administer live, actively growing cultures of *Brucella suis* by means of a dose syringe. All hogs that were used in the experiment were known to be negative to the blood test before being used. In all, 19 hogs were artificially infected by this means. A total of twelve were left untreated as controls.

Identification Number	Blood Reaction Previous to Exposure	Number days elapsed before blood titre reached 1-100 or over	Maximum Titre Reached	Number of days blood titre stayed 1-50 or over	Remarks
					
PC8		27	320	40	
H34		20	200 ^I	. 63	
H11		273	. 200 ^I	379 *	
H10		14	200 ^I	14	
H20		14	320I	18	
PC180		17	100	20	
D85			50 <u>I</u>	(1)	
CW41			50 <u>I</u>	(1)	
CW44			50 ^I	(1)	
PC1		27	1280	238 *	
PC9		27	10,240_	238 *	aborted
PC40		91	640 ^I	279 *	

TABLE 2. - CONTROL HOGS PLACED IN PASTURE WITH ARTIFICIALLY INFECTED HOGS

The time it takes for hogs to become infected artificially is comparatively short if one measures infection by the appearance of an agglutination titre of 1-100 or above. Where the gilts have been dosed artificially by drenching with a live culture of Brucella suis the average period of incubation has been under two weeks; actually in all the animals studied the average was seven days. We have measured infection by the appearance of a blood titre of 1-100 or above rather than by the act of abortion, because in our experience abortion does not always take place even though the swine are infected. The maximum titres were determined in the infected hogs and in two cases were as high at 1 to 20,480. The highest maximum titre in the control animals was 1 to 10,240. The very high titre in most of our dosed animals was probably the result of the large numbers of organisms used.

Findings in the artificially infected animals and the controls left in contact with them are shown in Tables 1 and 2.

^{*} Indicates hogs which were sold before titre dropped below 1-50.

⁽¹⁾ Blood titre never went higher than an incomplete 1-50 dilution.

DISCUSSION

How Soon Will Infected Hogs Recover?

It is the general opinion among many investigators that swine will recover from brucellosis much more quickly than will cattle. Some investigators, particularly Huddleson, think swine will apparently recover from the infection, at least they will lose their blood titre to the agglutination test in about six months. Johnson and Huddleson (25) reported most infected swine will recover within five months from the time agglutinins first appear in the blood.

In the artificially infected hogs which we have had on our experiment we have found some of the hogs to retain their blood titre as long as 12 or 18 months, when we consider a titre of 1-100 as indicating infection. If a titre as low as complete agglutination in 1-50 is looked upon as indicating an infected animal then some of the experimental animals have maintained titre for many months.

In four of the sows which were used in this investigation the blood titre persisted for well over a year; in one case being as long as 30 months. The maximum time a blood titre might have been maintained could not be determined in all of the hogs, as the animals had apparently served their purpose on the experiment and were sold. Other artificially infected hogs were found to lose their blood titre much sooner. (See Table 1.) When this occurred it might be assumed that the hogs had recovered from the infection.

One control sow on the experiment, No. PC-40, had gone back to negative or was of low titre only several different times when blood This animal was sold for slaughter and taken off the experiment, but arrangements were made to collect material for culture and guinea pig inoculation. Brucella suis was isolated from this animal. Previously she had carried a blood titre for over nine months. Since it was possible to isolate Brucella suis from her, it appears that she should be looked upon as a potential spreader during the entire time that she showed a blood titre even though this later dropped to negative or very low. The history of this experimental animal might be of interest. Blood tests taken at weekly intervals showed completely negative on two tests during the last three months the animal was alive. She showed a titre of incomplete 1 to 20 three times and complete agglutination of 1-20 once. The interpretation in many laboratories would have classified such reactions as negative. At the time of her slaughter her blood titre had gone back to an incomplete 1 to 80. This shows that hogs may have a negative reaction to the blood agglutination test and still carry live Brucella suis organisms

in their system. Other authorities have reported isolating *Brucella* suis from negative reacting animals.

In addition to the animals that have been on this experiment we have also had access to the blood test records on the main University breeding herd. Here, we have found animals which were carrying a high blood titre that lost their titre in the course of several months. An appreciable number of hogs under our observation have remained infected for a period longer than six months. Since blood titre in swine drops down in a comparatively short time, and eventually the animals become negative, it has been the policy of some investigators to make somewhat different recommendations in the control of brucellosis in swine than are usually followed in the control of Bang's disease of cattle. If swine do recover completely from brucellosis it appears more feasible in well-bred animals to hold such individuals in isolation and not sacrifice them. In the average so-called commercial herd of hogs the safer and quicker procedure would be the sale for slaughter of all infected animals even though one might expect them to recover in from six months to one year.

Low Reactions May Be Evidence of Brucella Suis Invasion

At several times during the experiment low titres in the cattle were found by the blood agglutination test. With few exceptions did these go high enough to classify the animal as a reactor (our interpretation of a reactor is complete agglutination in 1-100 dilution or higher). There was some evidence that the low agglutination titre may have been due to the *Brucella suis* organism but our results were negative in attempts to isolate and culture this organism. The question is raised as to whether or not low blood titres are indicative of *Brucella suis*. A few investigators have suggested that this might be the case. No definite proof was established on this point in this experiment.

Field Infection of Dairy Cattle With Brucella Suis

Although our experimental work has shown that the danger of cows becoming infected when running with infected swine is not very great, it must be assumed that infection of dairy cattle does in some instances occur. There are outbreaks of milk-borne brucellosis which have been definitely proved due to *Brucella suis*. In some instances the *Brucella suis* organism has been isolated from the cows. This was reported by Beattie and Rice (22) in 1934. In several instances *Brucella suis* has been isolated from the milk supply. Evidence is not at hand to prove that this always came from the cow's udder. It is conceivable to think that in some cases the porcine

organism gained entrance to the milk supply accidentally. If this is true the udder tissue of the cow was not necessarily involved. It also seems reasonable to believe that *Brucella suis* would be found more frequently in the milk if the danger of spread of this organism from swine to cattle was very great. Under most conditions hogs frequently run with cattle and have rather close association with them. Since *Brucella suis* has been isolated from the milk supply and from the cows themselves even though the number of cases recorded is not great, this constitutes a public health problem and must be looked upon as a possible public health hazard. Proper precautions should be taken in the handling of milk and dairy products to insure that they are not accidentally contaminated with the *Brucella suis* strain.

Swine Are Not Susceptible to Brucella Abortus

There seems to be little, if any, record in the literature of swine becoming infected with *Brucella abortus* as a result of pasture or close contact with infected cattle. In our experience many pregnant sows and gilts which were looked upon as being susceptible have failed to contract the infection from cattle. Although the number of such swine being exposed to infected cattle was great, we have not in a single instance observed any hogs becoming infected. Many authors in literature have recorded the inability of swine to contract the disease from cattle.

CONCLUSION

If spread of infection from swine to cattle does take place it would have a very important bearing on our present control and eradication measures because of the public health problem involved. Several abortions have occurred in the artificially infected hogs. The abortions that occurred in the cattle could not be proved to be due to *Brucella suis*. Low agglutination titres developed in the cattle, but in no instance could we prove these were due to *Brucella suis* infection. Low titres in cattle may be indicative of *Brucella suis* infection.

All attempts to isolate *Brucella suis* from the cattle were negative. *Brucella suis* was not isolated in any instance. Although it has been reported that strains of *Brucella suis* have been isolated from cattle, the results of our experiments indicate that there is not a great deal of danger of spread of infection from swine to cattle when these animals are kept together in the same pasture. Owners of cattle herds free from Bang's disease need not have much fear of spread of infection from swine which may be running with these cattle even though the swine have not been blood tested. This has a very important significance from the standpoint of human health, since

Mrucella suis is more pathogenic for man than is Brucella abortus. It minimizes the danger of man contracting undulant fever from milk produced by cows which have untested hogs running with them. It may be in regard to very virulent outbreaks of swine abortions that some transmission could take place, but the results of our experiment indicate that the danger is not great under average pasture conditions.

No evidence is available from work done at this experiment station to show that *Brucella abortus* infection is spread from cattle to swine.

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