5974

A Study of Bovine Blood, Urine and Feces for the Presence of Bact. Abortus Bang.*

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Shaw[1] was able to demonstrate the presence of *Micrococcus melitensis* in the blood stream of patients suffering from undulant fever. Horrocks[2] demonstrated the organism in the urine but was not able to find it in the feces of these patients. Amoss and Poston[3, 4] reported a technic by which they were able to isolate, using cultural methods, Brucella organisms from the feces of undulant fever patients. There has been little reported on the presence of *Bact. abortus* Bang in the blood, urine, and feces of cattle affected with Bang's disease. Zeller[5] attempted to infect six cows by subcutaneous injections of *Bact. abortus* Bang. Two of these were inoculated with suspensions heated at 57° C. for 10 minutes. He studied the blood, urine and feces and was unable on autopsy to find the organism in any tissue of the body. Long[6] studied the urine of cattle affected with Bang's disease. Studies were made on nineteen different animals over a period of ten months. Cultural methods gave negative results from 109 samples. Friess[7] examined 131 bovine blood samples by microscopic, cultural, and animal inoculation methods with negative results. Soule[8] made blood cultures of a large number of dairy cows. Two series of tests were made; one series resulted in 299 positive blood cultures from 2,237 animals in which agglutinins were present in the blood. The second series gave 206 positive blood cultures from 2,607 agglutinin positive animals. Forty animals gave positive blood cultures without concomitant blood agglutinin.

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[1] Shaw, E. A., Reports of the Commission Appointed to Investigate Mediterranean Fever. 1905-7, Parts I-VII, 19.

[2] Horrocks, R. A., Reports of the Commission Appointed to Investigate Mediterranean Fever. 1905-07, Parts I-VII, 19, 22, and 36.

[3] Amoss, Harold L., and Poston, Mary A., *J. Am. Med. Assn.*, 1929, **93**, 170.

[4] Amoss, Harold L., and Poston, Mary A., *J. Am. Med. Assn.*, 1930, **95**, 482.

[5] Zeller, H., *Arch. f. wissensch. u. prakt. Tierh.*, 1922, **49**, 65.

[6] Long, L. E., Thesis, Michigan State College, East Lansing Mich. 1927.

[7] Friess, Ferdinand, *Jahr. Vet.-Med. Neumann-Kleinpaul u. Zietzschmann*, 1931, **50**, 1122.

[8] Soule, M. H., *Iér Congres International de Microbiologie*, Paris, 1930, **1**, 606.

We have studied the presence of *Bact. abortus* Bang in the blood, urine and feces of cattle. Samples of blood, urine and feces were collected in the following manner: Each animal was bled in a standing position. Blood was obtained by vena puncture of the jugular and collected in 100 ml. centrifuge tubes containing 1 ml. of a 20% solution of sodium citrate. Samples of urine were collected by catheterization whenever possible. In order to prevent possible contamination the external genitals and vaginal canal were washed with clean water. When this procedure caused evacuation of the bladder before the catheter could be introduced the first ounce or 2 of urine was allowed to escape and the required amount was then collected in sterile flasks. Feces were collected by dilating the anus which usually brought about evacuation of the posterior bowel. The samples were placed in small petri dishes.

Fifteen to 20 gm. of feces were suspended in 250 ml. of saline solution and allowed to stand over night. In the morning the suspension was filtered through 4 layers of hospital gauze to remove the coarser particles. The filtrate was then placed in two 100 ml. centrifuge tubes and centrifuged at low speed, 400-500 r.p.m., for 5 minutes. This was intended to throw the larger particles to the bottom of the tube. The supernatant fluid was poured into 2 other 100 ml. centrifuge tubes together with 1 ml. positive *Bact. abortus* serum with an agglutination titre of more than 1-1000 dilution. These tubes were then placed in a water bath at 37°C. for two hours in order to clump *Bact. abortus* if present. The tubes were then removed from the water bath and centrifuged at 1000 r.p.m. for 10 minutes. The supernatant liquid was discarded and the sediment washed by resuspending in 100 ml. of saline solution. This was repeated twice. Some of the sediment was streaked on gentian violet plates and incubated under 10% CO_2. The rest of the sediment was resuspended in 10-15 ml. of saline solution and 2 guinea pigs were inoculated with 2 ml. amounts.

Urine samples were treated in the same manner except that they were used immediately and were not filtered through gauze. Five ml. amounts of the final sediment after washing were injected intraperitoneally into guinea pigs.

The samples of citrated blood were collected in each of two 100 ml. centrifuge tubes. These were centrifuged at high speed (2000 r.p.m.) until the serum was free of blood cells. The serum was then pipetted off; and the cells suspended in saline solution. These were centrifuged again and the above process repeated twice. The 2 samples of blood cells were then mixed together and cultured by smear-

ing on serum agar and gentian violet plates. Guinea pigs were inoculated with the washed cells; 6-10 ml. amounts were injected intraperitoneally into each of 2 pigs.

These inoculation technics were tested by putting small numbers of *Bact. abortus* Bang into samples of blood, urine and feces and treating them as above. *Bact. abortus* Bang was recovered in every instance by guinea pig inoculation.

Cultural examinations of the blood cells, urine and feces were negative for the Bang organism. Gentian violet plates did not inhibit the colon bacilli. There was always a heavy growth of these organisms on the plates of cultures from urine and feces. Eosin methylene blue plates as recommended by Amoss and Poston (loc. cit.) were of little value as difficulty was experienced in getting *Bact. abortus* Bang to grow on them.

Fifteen cows positive to the test tube agglutination blood test for *Bact. abortus* Bang were obtained for study immediately after calving or aborting. Bacteriological examinations were made of the following materials: colostrum, milk, fetal membranes. feti, blood, urine and feces. Studies were made of blood, urine, and feces in some cases as long as 47 days after the termination of pregnancy.

Attempts were made to infect artificially 4 animals by drenching (1) with saline suspensions from cultures of *Bact. abortus,* (2) stomach contents of *Bact. abortus* infected feti and by dropping saline suspensions of *Bact. abortus* into the conjunctival sac. *Bact. abortus* Bang was isolated at the time of calving or aborting from 2 of these showing that they became positively infected. Samples of blood, urine and feces from these 4 animals were studied both before and after calving or aborting.

One hundred and twenty-three samples of citrated blood were examined, 119 were negative and 4 positive; 119 urine samples, 107 negative, 17 positive; 118 feces samples, 116 negative and 2 positive.

These results indicate that *Bact. abortus* Bang is not present in large numbers in the blood stream of infected cows. It will be noted that *Bact. abortus* Bang was isolated 4 times from the blood out of 123 samples and 2 of these positive results were from a single animal. The longest period of time after calving or aborting that *Bact. abortus* Bang was isolated from urine was 12 days. This is within the period of time that *Bact. abortus* Bang has been isolated from vaginal discharges.[9] It is evident that it would be

[9] Fitch, C. P., Delez, A. L., and Boyd, W. L., *J. Am. Vet. Med. Assn.*, 1930, **29**, 680.

impossible to be absolutely certain that the organisms did not get into the urine through its contact with the vaginal and uterine discharges. This indicates that the urine may be a means of dissemination of *Bact. abortus* Bang. Two positive feces inoculations were obtained from 2 different animals. The samples were collected one and 3 days after aborting. This small number of positive results would lead one to believe that there is not an active infection in the intestinal tract but that the presence of the organism in the feces is probably due to eating materials contaminated with *Bact. abortus* Bang.

Blood cultures were prepared from blood taken from the jugular vein. The skin and hair coat of the head, neck, and withers was thoroughly moistened. An area 15x10 cm. on either side of the neck usually midway between head and shoulders was shaved and painted with tincture of iodine. An incision was made through the skin and the incised area was lightly touched with iodine. A sterile 30 ml. all glass syringe with a 16 gauge hypodermic needle attached was then used in aspirating the blood. The blood was transferred to flasks containing 250 ml. of 10% serum beef infusion broth. These flasks were incubated at 37½°C. under 10% CO_2 for 10 days. Subcultures were made at intervals on gentian violet plates and serum agar slants. The results obtained from these blood cultures were very unsatisfactory. Sixty cultures were made in all. Four of these cultures were sterile. The other 56 cultures showed gross contaminants. From 31 of these the gentian violet plates remained sterile whereas the serum agar slants showed a variety of growths. *Bact. abortus* Bang was not found in a single instance from blood cultures. In the case of one blood sample a small number of *Bact. abortus* Bang organisms were added. *Bact. abortus* was recovered when subcultured on gentian violet plates. This broth culture was contaminated with gram positive organisms but these did not grow on gentian violet plates.

Summary. *Bact. abortus* Bang was not found often in the blood, urine and feces of cattle known to be infected with this organism. Guinea pig inoculation proved to be a much more satisfactory method of isolation than cultural procedures.