A SEROLOGICAL STUDY OF TULAREMIA IN DOMESTIC ANIMALS AND THE POTENTIAL THREAT TO HUMANS[1]

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Abstract. Serological evidence was obtained that demonstrated previous infection with *Pasteurella tularensis* in 2 of 9 sheep raised in Monroe County, MI. Although no ectoparasites were found on the sheep in April and May when the testing was done, it was assumed that they were infected by ticks. Titers for Brucella were much less than those for *P. tularensis* and were not considered meaningful. Eight New Zealand white rabbits from nearby Risingsun, OH were serologically negative for tularemia and brucellosis. These animals had been confined in a commercial rabbitry and were free of ectoparasites. One of 8 dogs from Fulton County, OH, had a positive titer for *P. tularensis*, but not Brucella. No ectoparasites were observed on the dogs, but they had been recently dipped. The possibility that man as well as other animals might become infected with *Pasteurella tularensis* in the Toledo, OH area exists.

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This study was undertaken to determine if tularemia was a potential threat to the human population in the Toledo area. Initially, we tried to capture wild rabbits but after 2 weeks of observing 7 "live traps" in the Lucas County area without results, we decided that other methods should be used for the study. It has been reported in the American, Canadian, and Russian literature (Olsofieu and Rodneva 1960; Parker and Dade 1929; Pollitzer 1967; Simpson 1929; Watkin *et al* 1974) that sheep and dogs may be infected with *Pasteurella* (*Francinella*) *tularensis*. Sheep, dogs and domestic rabbits were made available through the Medical College of Ohio and the Toledo Zoological Gardens for study.

METHODS

Two methods are commonly used to test sera from animals for the antibodies of tularemia: the rapid slide agglutination test, for screening purposes, and the tube agglutination test, to determine the specific level of antibody or titer in the blood.

Procurement of Sera. Sterile test tubes coated with silicone (Vacutainer®, B&D) were used to collect blood. Each tube had the capacity of approximately 7 ml, which provided serum for at least 5 tests. Blood was obtained by inserting the needle into the jugular vein after elevating the animal's head and applying pressure over the thoracic inlet to tighten the skin over the neck and distend the vein.

Blood also was taken from the central or marginal ear vein of rabbits by means of silicone coated Vacutainer tubes. This procedure was difficult since the veins in the ears of rabbits readily constrict. The vacuum causes the rabbit veins to collapse, making the withdrawal of blood difficult, so an infrared lamp was directed toward the rabbits' ears to produce vasodilation. In 2 rabbits from which we could not get ear vein blood, a cardiac puncture was used to obtain blood. The dog's foreleg was clipped and the area was cleaned with alcohol prior to venipuncture. A tourniquet was placed just above the dog's 2nd joint to occlude the veins and make them easier to enter with the Vacutainer needle.

Blood from all animals was centrifuged at 100 rpm for 10 min. to remove the red blood cells. The clear serum was drawn into a capillary pipette, placed into a sterile test tube and stored in a refrigerator at 4° C until tests could be performed. Each tube was identified with the animal's number, type, and the date of withdrawal. The sera was recentrifuged just before testing to insure clear serum, as serum containing hemolyzed red blood cells is difficult to interpret.

Slide Agglutination Test. The slide agglutination test was performed as soon as possible after collection of blood. An aliquote of 0.04 ml of serum from each animal to be tested was placed in one of the squares of an agglutination testing plate. *Bacto Pasturella tularensis* antisera (Difco Laboratories), 0.04 ml, and isotonic sodium chloride solution, 0.04 ml, were added to additional squares to serve as positive and

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negative controls, respectively. One drop, 0.03 ml, of *Bacto Pasteurella tularensis* antigen was added to each drop of serum and to the positive and negative controls. Each droplet was mixed with a separate applicator stick, and the plate was rotated gently. If a slide was used, it was placed on a mechanical rotator and agitated for 4 minutes at 175 rpm. The plate or slide was then examined in a lighted viewing box. Reactions were recorded from negative (0) to positive (4+). Any specimen with 2+ or more was retested using 0.04, 0.02, 0.01, and 0.005 ml of the diluted positive serum, as well as positive and negative controls. The tests were done at dilutions of 1:40, 1:80, 1:160, and 1:320, and all samples were subsequently examined by means of the tube agglutination test.

According to the results of Schneider *et al* (1950–1951), sera bearing a slide titer of 1:80 or more were regarded as positive. Those exhibiting no agglutination at a dilution of 1:40 were considered negative, while those sera showing a weak reaction at 1:40 only were considered weakly positive.

Tube Agglutination Test. The tube agglutination test was more precise in determining the titer level. The first step was to place 9 labeled test tubes in a rack and 0.05 ml isotonic sodium chloride into each tube. A 1:15 dilution of the serum to be tested was prepared by adding 0.2 ml of serum to 0.8 ml of isotonic sodium chloride. Then 0.05 ml of the serum dilution was added to the first tube and mixed well. From the first tube 0.05 ml of fluid was transferred to the second tube and mixed. The same amount of fluid, 0.05 ml, was serially transferred to the remaining tubes. *Bacto Pasteurella tularensis* antigen (0.5 ml) was added to each tube and mixed by shaking the rack. The resultant dilutions were: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280 for tubes 1 through 7 respectively. Tubes 8 and 9 were positive and negative control tubes. The tubes were incubated in a 37° C water bath for 2 hours. After removal from the water bath, the tubes were placed in a refrigerator at 2–10° C.

Similar agglutination tests with *Brucella abortus* antigen were performed on the same serum of the sheep and dogs to determine if there was a cross-reaction or false-positive reactions with Brucella antibodies.

RESULTS

Sheep. The sheep with the highest titers (1:320) for tularemia were numbers 64 and B_2 (table 1). A Brucella test was conducted to determine the degree of cross-reaction with Brucella. Sheep #64 had a Brucella titer of 1:80, which was diagnostic, and sheep #B_2's Brucella titer was 1:40, weakly positive. If the Brucella titers, which might be concluded as nonspecific, were subtracted from the *Pasteurella* titers, 64 and B_2 still would have diagnostic titers for tularemia.

Rabbits. The 8 rabbits (New Zealand's from Risingsun, OH) used in this study were from a controlled environment where the animals were kept in separate cages which were cleaned and disinfected regularly. Rabbits are not known to be susceptible to brucellosis, which accounts for the negative results from the Brucella tests (table 1). No rabbits had titers over 1:40 by means of the tube agglutination test.

Dogs. One of 8 dogs from Fulton County had a positive titer for *P. tularensis*. The dog with the highest titer of tularemia in the tube agglutination test was #3412, whose titer was 1:160. A Brucella test also was done to eliminate the possibility of a false-positive or a cross-reaction (table 1). The Brucella titer was subtracted from the *Pasteurella* titer making it 1:80, which may be considered diagnostic for tularemia.

DISCUSSION

Tularemia is a disease which is commonly thought to be found only in rabbits. Most veterinarians do not consider tularemia as a serious disease in sheep and dogs. Philip *et al* (1935) reported an outbreak in Montana affecting 1320 ewes with the death of about 200 animals. They also isolated the causative micro-organism and incriminated ticks as the vector. In 1974, Watkin *et al* studied an outbreak of tularemia in Alberta, Canada, in a band of 850 ewes from which they succeeded in isolating *Pasteurella tularensis* from both animals and ticks. From the work of these authors and other reports of losses under similar circumstances, it is believed that tularemia can be a serious disease of sheep, causing heavy losses.

Jellison and Kohnls (1955) reported 189 human cases of tularemia in workers associated with sheep from 1934 to 1952. The source of infection was attributed to contact with sheep by skinning dead animals, shearing sheep, or by bites of infected ticks (*Dermacentor andersoni*) in 46 cases. Two sheep in our study appear to have had tularemia. This is over 20%, but the number of sheep was too small to reach a meaningful percent of infection. The sheep tested were from Monroe County, MI.

Unfortunately in our study no wild

TABLE 1

Tube tests on serum from sheep, rabbits and dogs tested for antibodies associated with tularemia and brucellosis.*

Sheep No.	Tularemia				Titer	Brucellosis				Titer
	1:40	1:80	1:160	1:320**		1:40	1:80	1:160	1:320	
62	+	+	+	−	1:160	+	+	+	−	1:160
64	+	+	+	+	1:320	+	+	−	−	1:80
66	+	−	−	−	1:40	+	−	−	−	1:40
85	+	+	+	−	1:160	+	−	−	−	1:40
89	+	−	−	−	1:40	+	−	−	−	Neg.
B_1	+	+	−	−	1:80	+	−	−	−	1:40
B_2	+	+	+	+	1:320	+	−	−	−	1:40
B_3	+	+	+	−	1:160	+	+	+	−	1:160
B_4	+	−	−	−	1:40	−	−	−	−	Neg.
+C***	+	+	+	+	Pos.	+	+	+	+	Pos.
−C***	−	−	−	−	Neg.	−	−	−	−	Neg.
Rabbit No.	Tularemia				Titer	Brucellosis				Titer
	1:40	1:80	1:160	1:320**		1:40	1:80	1:160	1:320	
1.	−	−	−	−	Neg.	−	−	−	−	Neg.
2.	+	−	−	−	1:40	−	−	−	−	Neg.
3.	+	−	−	−	1:40	−	−	−	−	Neg.
4.	−	−	−	−	Neg.	−	−	−	−	Neg.
5.	−	−	−	−	Neg.	−	−	−	−	Neg.
6.	−	−	−	−	Neg.	−	−	−	−	Neg.
7.	+	−	−	−	1:40	−	−	−	−	Neg.
8.	−	−	−	−	Neg.	−	−	−	−	Neg.
+C***	+	+	+	+	Pos.	+	+	+	+	Pos.
−C***	−	−	−	−	Neg.	−	−	−	−	Neg.
Dog No.	Tularemia				Titer	Brucellosis				Titer
	1:40	1:80	1:160	1:320**		1:40	1:80	1:160	1:320	
1415	+	−	−	−	1:40	+	−	−	−	1:40
3411	−	−	−	−	Neg.	−	−	−	−	Neg.
3412	+	+	+	−	1:160	+	−	−	−	1:40
3413	+	−	−	−	1:40	−	−	−	−	Neg.
3414	+	−	−	−	1:40	+	−	−	−	1:40
3418	−	−	−	−	Neg.	−	−	−	−	Neg.
3419	+	+	−	−	1:80	+	−	−	−	1:40
2808	+	−	−	−	1:40	+	−	−	−	Pos.
+C***	+	+	+	+	Pos.	+	+	+	+	Pos.
−C***	−	−	−	−	Neg.	−	−	−	−	Neg.

*Similar results were obtained by means of the slide test.
**Positive results were not obtained in greater dilutions.
***Control with positive antisera included with the test material = +C; saline control = −C.

rabbits were captured so domestic rabbits from a commercial rabbitry were tested instead. Medical historians have evidence that tularemia as a disease occurred at the latter end of the Miocene or early Pliocene period (Olsufieun 1963) and that rabbits contract the disease from common wood ticks (Bell 1945).

Tularemia is rarely diagnosed in dogs. Although most mammals are susceptible to tularemia, dogs are not infected unless they are exposed to infected ticks. Dogs that live outside for longer periods of time in wooded areas are apt to be infected and dogs who tend livestock may be infected by the ticks that live on

farm animals. The dogs whose blood was tested were from rural Fulton County, OH. Dogs should be examined regularly for ticks, and infected animals and their quarters should be treated with an acaricide.

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