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STUDIES ON PRONGHORN ANTELOPE (*Antilocapra americana*) AS RESERVOIRS OF ANAPLASMOSIS IN MONTANA[1]

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Abstract: Twenty-six pronghorn antelope (*Antilocapra americana*) were collected in an area of eastern Montana where bovine anaplasmosis is enzootic. Their sera were examined for evidence of anaplasmosis by the complement-fixation test. Strong false positive reactions (3+ and 4+ reactors) occurred for 19 of the sera tested; 6 sera were anticomplementary. Inoculation of antelope blood into anaplasmosis-free intact calves did not produce clinical or serological evidence of anaplasmosis, and anaplasma bodies were not found in stained blood smears of antelope or recipient calves.

INTRODUCTION

Although precise data are unavailable, an estimated 50,000 to 100,000 cattle die of anaplasmosis each year.[9] Probably the greatest economic impact of the disease results from reduced milk production, weight loss and abortion of fetuses by infected cows.[9] *Anaplasma marginale* is enzootic in the eastern one-third of Montana where 1.1 million cattle reside.[10] Reports from veterinarians and ranchers in Montana indicate the disease is spreading westward into areas previously considered free of anaplasmosis.

The role of wildlife species as reservoirs of *A. marginale* in Montana has not been studied. Natural infections, or the latent carrier state of anaplasmosis have been observed in Columbian black-tailed deer (*Odocoileus hemionus columbianus*);[3,5,11] crosses of their progeny with mule deer (*O. h. hemionus*) are known reservoirs of *A. marginale* in California.[4] In a Wyoming study, blood samples from pronghorn antelope (*Antilocapra americanus*), white-tailed deer (*O. virginianus*) and elk (*Cervus canadensis*) were negative for *A. marginale* when tested in splenectomized bovine calves.[7] However, one serum pool, representing 35 mule deer was positive for anaplasmosis.[7] In a subsequent study, none of 31 mule deer in Oregon were latent carriers of the disease.[12] Similarly, 27 elk in Idaho were negative for anaplasmosis.[15] Of 132 American bison (*Bison bison*) from an area enzootic for anaplasmosis, none were positive for *A. marginale*.[13]

Although these reports suggest that natural infections occur infrequently in wild ruminant species, experimental infections of *A. marginale* have been produced in Columbian black-tailed deer,[2,6,11] white-tailed deer,[14] mule deer[2,8] elk, bighorn sheep (*Ovis canadensis canadensis*) and pronghorn antelope.[8] Clearly, the potential exists for several of these species, which co-inhabit rangeland with cattle in many areas of the western states, to serve as reservoirs of anaplasmosis. Because natural infections with

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A. marginale have not been identified in antelope, the current investigation was designed to determine if pronghorn antelope, which are numerous in eastern Montana, serve as reservoirs. A secondary objective was to extend a previous evaluation[8] of the complement-fixation test for diagnosis of anaplasmosis in antelope.

MATERIALS AND METHODS

Twenty-six antelope (16 males and 10 females) were collected from Rosebud County, Montana, in an area enzootic for bovine anaplasmosis. From 2 to 4 antelope were shot by personnel of the Montana Department of Fish and Game on each of 8 collection dates over a 13-month period (Table 1). Approximately 50 ml of blood were obtained from each antelope at the collection site, usually by cardiac puncture using a syringe containing 1% heparin solution. An additional 25 ml blood sample was collected and transported to the laboratory on ice. Following clot formation, the serum was removed and stored at -20 C until used in serological studies.

Eight Hereford calves, one for each antelope collection date, served as recipients of antelope blood. A calf was transported to the collection site in a screened enclosure to preclude exposure to natural vectors of anaplasmosis. Within an average of 15 min. after the death of each antelope, the heparinized blood was inoculated subcutaneously into the calf. Following the inoculations of blood from all the antelope on a given date, the calf was returned to the laboratory and was housed in an isolation unit.

A blood sample was obtained from each calf at weekly intervals for the first 3 weeks and thereafter twice weekly until termination of each test approximately 100 days after inoculation of antelope blood. Blood smears also were prepared on each calf bleeding date. Complement-fixation (CF) tests were conducted on antelope and calf sera,[1] and all blood films were stained with Giemsa and were examined by light microscopy for infected erythrocytes.

RESULTS

A summary of the data collected in this study is presented in Table 1. Of 25 antelope serum samples examined, 18 were 4+ reactors, 1 was a 3+ reactor, and 6 were anticomplementary in CF tests using the *A. marginale* antigen preparation supplied by the Agricultural Research Service, Beltsville, Maryland. No anaplasma bodies or any other parasites were observed in any of the antelope blood films.

All of the antelope were examined in the field for ectoparasites which might serve as vectors of *A. marginale*. The only ectoparasites recovered were two specimens of *Dermacentor* sp. (one on each of two antelope which were collected at an 11-month interval). Therefore, no attempts were made to conduct feeding tests on calves to determine if the ticks harbored *Anaplasma* sp.

As shown in Table 1, each of 8 test calves received at least one injection of blood from antelope which were 4+ reactors in the CF test. None of these calves, however, developed anaplasmosis as determined by CF tests on approximately 25 blood samples drawn serially from each calf over the subsequent 100-day period. Also, erythrocytes in blood films made at the time of each calf bleeding did not contain anaplasma bodies. Furthermore, during the test period, none of the calves developed clinical signs of anaplasmosis.

The experimental protocol provided for limited observations on seasonal prevalance of anaplasmosis in antelope. No pattern emerged either in the CF data, which showed 4+ reactivity on all collection dates, or in transmission of the disease from potentially infected antelope to calves.

DISCUSSION

In the study area of eastern Montana, outbreaks of clinical anaplasmosis characteristically occur in the spring when tick populations (primarily *Dermacentor andersoni*) are at their peak, and in the summer concurrent with the deer fly (*Chrysops* spp.) season. It has not been established experimentally that these

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TABLE 1. Experimental Design and Complement Fixation Test Results of Sera from Antelope.

Animal No.	Age (yrs.)	Sex	CF Test	Date Collected	Calf no.*
1	Adult	M	4+	18 Apr	
2	1	M	AC	18 Apr	I
3	2	M	3+	18 Apr	
4	Adult	M	4+	23 May	II
5	UD	F	4+	23 May	
6	Adult	F	4+	7 July	
7	Adult	M	AC	7 July	
8	2	M	4+	7 July	III
9	1	M	AC	7 July	
10	Adult	M	4+	15 Aug	
11	Adult	M	4+	15 Aug	IV
12	2	M	4+	15 Aug	
13	Adult	F	4+	20 Sept	
14	UD	M	4+	20 Sept	
15	Adult	F	ND	20 Sept	V
16	Adult	M	4+	20 Sept	
17	<1	F	4+	15 Nov	
18	Adult	F	4+	15 Nov	
19	<1	F	4+	15 Nov	VI
20	<1	M	4+	15 Nov	
21	UD	M	AC	3 Apr	
22	1	M	4+	3 Apr	VII
23	Adult	M	4+	3 Apr	
24	Adult	F	4+	18 May	
25	1	F	AC	18 May	VIII
26	1	F	AC	18 May	

ND = not done; AC = anti-complementary; UD = undetermined.
*Each calf was given 40-60 ml of antelope blood subcutaneously.

ticks and flies are the actual vectors of anaplasmosis in this area. Clinical disease, however, is usually observed in cattle only at times when these potential vectors are at peak population densities. In our study, 4+ reactivity in CF tests on antelope sera occurred throughout the year regardless of the month of collection. Based on the historical annual pattern of anaplasmosis in the study area, it is unlikely that such strong positive serologic responsiveness can be attributed to continuous exposure of antelope to *Anaplasma* antigens throughout the year. One might argue that active or latent infections were responsible for the CF activity. This conclusion, however, seems precluded on the basis of the consistently negative blood transfer studies.

To facilitate transmission of viable, infective organisms, every precaution was taken to insure rapid transfer of fresh whole blood from potentially infected antelope into test calves. The time interval between death of an antelope and injection of its blood into calves ranged from 5 to 45 min. with a mean of 15 min. Others[12] have observed that *A. marginale* remains viable in heparinized blood obtained from infected cattle for at least 48 h. Therefore, viable organisms should have been transmitted to test calves in our study if antelope harbored either latent or active infections.

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Collectively, the data strongly suggest that antelope in our study were not infected with *A. marginale* and that all of the antelope sera, excluding those which were anticomplementary, resulted in false positive CF reactions. Such a conclusion confirms the observation of others that the CF test as used for detection of bovine anaplasmosis is not useful for determining the status of *A. marginale* infections in antelope.[5]

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