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ABSENCE OF *Anaplasma marginale* INFECTION IN AMERICAN BISON RAISED IN AN ANAPLASMOSIS ENDEMIC AREA*

K. J. PETERSON and T. O. ROBY

Abstract: Blood was collected at slaughter from 132 adult American bison (*Bison bison*) raised in an anaplasmosis endemic area where the vector *Dermacentor andersoni* (= *venustus*) is indigenous. Hematologic studies revealed no indications of clinical anaplasmosis. Card agglutination and complement-fixation tests on all bison serums were negative. Eleven anaplasmosis-susceptible calves each inoculated with 204 ml of blood pooled from 12 bison did not develop anaplasmosis. Results of this study indicate American bison have resistance to natural *A. marginale* infection.

INTRODUCTION

Little is known of the susceptibility of American bison to natural *Anaplasma marginale* infection, their possible role as latent carriers, and the accuracy of either the complement-fixation test (CF) or card test (CT). An opportunity developed to investigate these problems in a large bison herd located in a bovine anaplasmosis endemic area of eastern Oregon. The herd had existed since 1962 and numbered in excess of 1,500 adults. The animals were pastured on a high desert sagebrush range where the Rocky Mountain wood tick, *D. andersoni* (= *venustus*), a known *A. marginale* vector, is indigenous.¹ Cattle ranging in surrounding areas are often infected with *A. marginale* and the percentage of latent carrier cattle is usually high. Because this herd had been in existence for 10 years, a number of bison should have become infected if this species is naturally susceptible.

The herd was infected with brucellosis, and because of eradication difficulties, most of the animals were slaughtered during the fall of 1972.

MATERIALS AND METHODS

Blood was collected during slaughter in the Coast Packing Company, Portland, Oregon, on September 29 and 30, 1972, from 132 adult male and female bison of various ages. Forty ml of blood was collected from the severed neck vessels of each bison; 20 ml was collected in a heparinized vial and 20 ml in a vial without anticoagulant. Vials were identified and immediately refrigerated at 4 C. After each day's kill, blood was transported to Oregon State University at Corvallis and immediately inoculated into unsplectomized anaplasmosis-free calves approximately 5 months old. Each of 11 calves received pooled heparinized blood from 12 bison, 24 ml intravenously (IV) and 180 ml subcutaneously (SC). All blood was inoculated within 10 h from the time of collection.

On the date of collection, packed cell volume (PCV) and hemoglobin (Hb) values (g/100 ml as measured by the Spencer Hb meter) were determined for each sample. Blood films were stained by both Wright's and Giemsa's methods

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and later examined by light microscopy for the presence of *A. marginale* or other blood parasites.

Serum collected by centrifugation of unheparinized blood samples was stored in individual vials at -60°C and later mailed in refrigerated containers to the Animal Parasitology Institute, Beltsville, Maryland, for testing by both CF and CT methods.

Body temperatures of the inoculated calves were recorded daily during the study period, each calf observed closely for signs of illness or infection at the inoculation site. During the trial, calves were maintained in isolation units with no more than four calves per unit.

Blood from each calf was collected for hematologic studies and CT and CF testing once weekly for the first 2 weeks postinoculation, twice weekly for the next 2 weeks, thrice weekly during weeks 5, 6, and 7, and then once weekly until the end of the 112-day trial. Three ml of blood was collected in vials containing EDTA (disodium salt of ethylenediaminetetraacetic acid) and 10 ml in vials without anticoagulant. Hb and PCV values were determined, and blood films stained by Wright's and Giemsa's methods were examined for each EDTA blood sample. Calf serum samples were handled in the same manner as the samples of bison serum. The criterion of infection was the presence of anaplasma bodies in at least 1% of the erythrocytes.

At the end of the trial, the susceptibility of each calf to *A. marginale* was determined by SC inoculation of 10 ml of whole blood from known *A. marginale*-infected cattle. The same procedures used to identify infection during the original trial were used after the challenge, except that serum samples for CT and CF testing were collected only once weekly.

RESULTS

The PCV of the 132 bison blood samples ranged from 33% to 68%, with a mean of 45.8%. Hb ranged from 12.8 to 20.0 g/100 ml, with a mean of 16.5

g/100 ml. Neither anaplasma bodies nor other blood parasites were observed in any of the stained bison blood smears. All CT and CF tests were negative for anaplasmosis.

No adverse clinical reactions to injection of 204 ml of pooled bison blood were observed, and all experimental calves remained healthy during the 112-day postinoculation period. Decreased PCV and Hb did not occur as they usually do in anaplasmosis-infected calves. Body temperatures remained within the normal range and no anaplasma bodies were observed in any of the stained blood smears. Serums of five of the 11 calves gave one or more non-specific serologic reactions during the first 65 days postinoculation. Twelve CF reactions (\pm to $4+$), and six CT reactions ($1+$ to $3+$) occurred during this period. Subsequent tests conducted during the next 47 days of the trial period were negative.

Upon challenge with whole blood from a known bovine *A. marginale* carrier, all calves developed anaplasmosis. Parasitemia occurred between 23 and 28 days postinoculation. A concomitant rise in body temperature and decrease in PCV and Hb also were observed.

The lowest PCV's ranged from 13% to 21% and the lowest Hb from 4 to 9.6 g/100 ml. Hemoglobin was reduced to less than 6 g/100 ml in all but one calf. The highest body temperatures ranged from 40°C to 41.4°C . All calves exhibited depression and anorexia and six developed visible icterus. None died and recovery was rapid. All developed CT and CF titers before parasitemia was observed.

DISCUSSION

Both the mean Hb (16.5 g/100 ml) and mean PCV (45.8%) values of the 132 bison sampled were greater than those reported for adult domestic ruminants.⁸ Whether these findings are normal for the species or resulted from dehydration or shipping excitement was not determined. The bison were restless, excited, and belligerent. A number were

gored and trampled to death and many other badly injured during transit and confinement in the slaughter pens.

All parameters used to identify *A. marginale* infection in bison blood samples proved negative. PCV and Hb were not decreased, no intraerythrocytic anaplasma bodies were observed, and there was no latent infection since none of the 11 anaplasmosis susceptible calves inoculated with bison blood developed either signs of anaplasmosis or seropositive CT or CF titers. Rapid refrigeration of bison blood samples and short time lapse between collection and inoculation of blood should have precluded destruction of the etiologic agent had it been present.²

No false-positive reactions were ob-

served in bison serums on either the CT or CF tests. However, the accuracy of these tests on infected blood could not be determined since none of the bison samples were infected. The CT and CF titers observed in the five calves after inoculation with bison blood were considered nonspecific because all subsequent tests were negative and all calves developed anaplasmosis upon challenge.

This trial suggests that American bison are resistant to natural *A. marginale* infection. At least some of the 132 bison should have been exposed to infection because all were raised on an anaplasmosis endemic range where *D. andersoni* is indigenous and where a high percentage of cattle in the area are latent *A. marginale* carriers.

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