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SUSCEPTIBILITY OF ELK (*CERVUS ELAPHUS*) TO EXPERIMENTAL INFECTION WITH *ANAPLASMA MARGINALE* AND *A. OVIS*

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ABSTRACT: *Anaplasma ovis* was experimentally transmitted from domestic sheep to elk (*Cervus elaphus*) and back to splenectomized sheep. No rickettsemias were detected but serum from three of seven experimentally inoculated elk developed *Anaplasma* spp.-reactive antibody as measured by indirect immunofluorescence (IIF) or by the rapid card agglutination and complement fixation assays. Three elk were experimentally infected with *A. marginale*. The rickettsiae were detected in blood of these elk and caused disease in a splenectomized domestic bovine calf after subinoculation of blood from the elk. All three elk had positive titers with IIF. No clinical signs of illness were noted in any elk inoculated with either *Anaplasma* species.

Key words: Anaplasmosis, *Anaplasma marginale*, *Anaplasma ovis*, experimental infection, elk, *Cervus elaphus*.

INTRODUCTION

Anaplasmosis is an infectious disease of ruminants manifested by progressive anemia and sometimes death. The disease is associated with the presence of intraerythrocytic bodies of the rickettsial genus *Anaplasma* (Lincoln, 1990). The causative agents of anaplasmosis in cattle and sheep are *Anaplasma marginale* and *A. ovis*, respectively. However, these pathogens are not confined to cattle and sheep (Kuttler, 1984). Species of wild North American ruminants naturally or experimentally susceptible to both *Anaplasma* organisms include mule deer (*Odocoileus hemionus hemionus*) (Zaugg, 1988), white-tailed deer (*O. virginianus*) (Kuttler et al., 1967), bighorn sheep (*Ovis canadensis*) (Goff et al., 1993), and pronghorn antelope (*Antilocapra americana*) (Zaugg, 1987). American bison (*Bison bison*) are only susceptible to *A. marginale* (Zaugg, 1986), while American elk (*Cervus elaphus*) are susceptible to *A. marginale* (Renshaw et al., 1979) with some presumptive evidence of susceptibility to *A. ovis* as well (Post and Thomas, 1961).

The questionable susceptibility status of elk to *A. ovis* infection prompted the present investigation. Our objectives were to evaluate elk susceptibility to infection by *A. marginale*, determine if elk are susceptible to experimental infection by *A. ovis*, and determine the carrier state of both infections over a 6-mo period.

MATERIALS AND METHODS

The project was conducted in two separate studies. In Study A (10 January 1992 to 2 September 1992), eight captive born 8-mo-old male elk, obtained from the U.S. Forest Service, Starkey Research Facility, Starkey, Oregon (USA), were allotted into two groups of four animals each and maintained in adjacent paddocks at Washington State University Wildlife Research Center, Pullman, Washington (USA). The elk were provided alfalfa hay supplemented with alfalfa pellets, mineral salt block, and fresh water ad libitum. Each animal was determined to be free of *Anaplasma* spp. infections by indirect immuno-fluorescence (IIF) using both *A. marginale* and *A. ovis* antigens (Tibbitts et al., 1992), and by examination of Giemsa-stained thin blood films. All animals appeared to be healthy and had normal attitudes and appetite.

Three elk (B-32, W-76, and Y-50) (Group 1) were inoculated intravenously (IV) with 4 ml of Puck's Saline-G (PSG) (Palmer et al., 1982) containing 2.0×10^8 erythrocytes infected with

a well characterized stabilate of an Idaho isolate of *A. marginale* (P_{41}-ID_2AM) (Zaugg et al., 1986). The fourth elk (Y-750) served as an uninoculated control.

Three additional elk (B-31, B-33, and W-745) (Group 2) were inoculated IV with four ml of PSG containing 2.5×10^8 erythrocytes infected with a well characterized stabilate of an Idaho isolate of *A. ovis* (V_{52}-Id_0AO) (Zaugg, 1987). A fourth elk (W-747) served as an uninoculated control.

Each animal from both groups was monitored daily for overt signs of disease, a blood sample drawn twice a month through 50 days post-inoculation (PI) and then monthly through 172 days PI for determination of packed cell volume (PCV), examination of Giemsa stained blood films for rickettsemia, and evaluating serum with IIF serologic evaluations (Tibbitts et al., 1992). After 172 days PI, 50 ml of whole blood from each of the three inoculated animals in each group was pooled, washed free of buffy coat cells in PSG and the erythrocytes resuspended to 50 ml in PSG. The 50 ml suspensions were inoculated IV into a 5-mo-old splenectomized calf (Group 1), or into a 5-mo-old splenectomized domestic sheep (Group 2). The calf (C-560-BL) and sheep (S-138-GR) were monitored as above three times each week for periods of 24 and 37 days PI respectively.

In Study B (3 September 1993 to 26 April 1994), four 18-mo-old elk, two males (E-764, and E-767) and two females (E-18 and E-765), were wild-caught from Butte County, Idaho (USA) (43°48′N, 112°42′W) and maintained together in an outside paddock at the Idaho Department of Fish and Game Wildlife Health Laboratory, Caldwell, Idaho. Four yearling Targhee domestic ewe lambs, two splenectomized (S-314 and S-331) and two spleen-intact (S-301 and S-329) were raised from birth and maintained in an outside paddock at the University of Idaho, Caine Veterinary Teaching and Research Center, Caldwell, Idaho. All test animals were provided with free-choice alfalfa hay, mineral salt mix, and fresh water. The animals were in good flesh; their packed cell volumes and hemograms (Goff et al., 1993) and serum chemistries (Technicon SMAC III, Technicon, Inc., Tarrytown, New York, USA) were normal. Each animal was determined to be free of *Anaplasma* spp. infections by rapid card agglutination (RCA) and complement fixation (CF) assays, (Todorovic et al., 1977) and by examination of Giemsa-stained thin blood films.

The four elk and the two spleen-intact sheep (S-301 and S-329) each were inoculated IV with 35 ml of freshly collected whole sodium citrate-treated sheep blood containing 4.3×10^8 erythrocytes infected with an Idaho isolate of *A. ovis* ($V_{52}ID_0AO$).

Each animal was monitored daily for signs of disease. A blood sample was drawn weekly through 43 days PI and then monthly through 120 days PI for determination of PCV, examination of stained blood films for rickettsemias, and providing serum for CF and RCA serologic evaluations.

After 43 days PI, approximately 15 ml of blood from each of the four elk was collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant (Vacutainer®, Becton Dickinson and Co., Rutherford, New Jersey, USA). The fresh whole blood was pooled and 20 ml inoculated IV into each of the two splenectomized sheep (S-314 and S-331). The sheep recipients of elk blood were monitored as above twice per week for a period of 28 days PI at which time they were each treated with long-acting oxytetracycline (Liquamycin®, Pfizer, New York, New York, USA) at 20 mg/kg intramuscular (IM) injection.

RESULTS

All inoculated elk of Group 1 developed detectable, specific *Anaplasma* spp. antibodies between day 28 and 50 PI, and the IIF titers steadily increased to 1:1600 in each animal. *Anaplasma* spp.-like inclusion bodies occasionally were seen on monthly blood film evaluations of inoculated elk taken between day 28 and 172 PI, but never exceeded five per 3,000 erythrocytes (Table 1).

An *A. marginale* infection was detected in the splenectomized calf (C-560-BL) on day 14 PI. The highest percent parasitized erythrocytes, 45%, was noted on day 23 PI (Table 1). The PCV decreased precipitously on day 23 PI to a low of 11% and the animal was euthanized by jugular IV lethal injection of sodium pentobarbital (14 mg/kg body weight) (Anpro Pharmaceutical, Arcadia, California, USA).

Two inoculated, and the control elk in Group 2 remained seronegative throughout the 172 day evaluation period. The third animal (B-33) developed detectable antibodies between day 28 and 50 PI but maintained a weak IIF titer (1:100) until after 150 days PI when the titer increased to 1:800. No *Anaplasma* spp.-infected erythrocytes were detected throughout the

TABLE 1. Results from experimental inoculation of elk (*Cervus elaphus*) with *Anaplasma marginale* and *A. ovis*, 10 January 1992 to 2 September 1992, and 3 September 1993 to 26 April 1994.

	Animal number	Inoculum source	Maximum antibody titers			Maximum PPE[d]
			IIF[a]	CF[b]	RCA[c]	
Study A	E[e]-B32	Bovine	1,600	ND[f]	ND	≤1.0
Group 1	E-W76	Bovine	1,600	ND	ND	≤1.0
A. marginale	E-Y50	Bovine	1,600	ND	ND	≤1.0
	E-Y750	NA[g]	Negative	ND	ND	UD[h]
	C[i]-560BL[j]	Pooled elk[k]	ND	ND	ND	45
Group 2	E-B31	Ovine	Negative	ND	ND	UD
A. ovis	E-B33	Ovine	800	ND	ND	UD
	E-W745	Ovine	Negative	ND	ND	UD
	E-W747	NA	Negative	ND	ND	UD
	S[l]-138[j]	Pooled elk[m]	ND	ND	ND	25
Study B	E-18	Ovine	ND	80	Positive	UD
A. ovis	E-764	Ovine	ND	Negative	Negative	UD
	E-765	Ovine	ND	80	Positive	UD
	E-767	Ovine	ND	Negative	Negative	UD
	S-301	Ovine	ND	160	Positive	12
	S-329	Ovine	ND	160	Positive	17
	S-314[j]	Pooled elk[n]	ND	160	Positive	20
	S-331[j]	Pooled elk[n]	ND	160	Positive	22

[a] IIF = indirect immunofluorescence.
[b] CF = complement fixation.
[c] RCA = rapid card agglutination.
[d] PPE = percent parasitized erythrocytes.
[e] E = elk.
[f] ND = not done.
[g] NA = not applicable.
[h] UD = undetectable by stained blood film examination.
[i] C = bovine steer.
[j] Splenectomized.
[k] Pooled elk blood from B22, W76 and Y50.
[l] S = sheep.
[m] Pooled elk blood from B31, B33 and W745.
[n] Pooled elk blood from 18, 764, 765 and 767.

duration of the study in any elk (Table 1). There was no significant reduction in PCV, or any other evidence of clinical disease in any animal of the group.

Anaplasma spp.-infected erythrocytes first were noted in blood films from the splenectomized sheep (S-138-GR) on day 28 PI. The percent parasitized erythrocytes reached a high of 25% on day 35 PI (Table 1). Severe anemia developed and the animal was euthanized on day 37 PI. The control elk from each group remained clinically normal and serologically negative for the duration of the study.

Two *A. ovis*-inoculated elk of Study B remained *Anaplasma* spp.-seronegative for the 120 days of the study. The other two inoculated elk (E-18 and E-765) developed CF titers of 1:20 and 1:40, respectively, as early as 21 days PI. The titers remained low and never exceeded 1:80 throughout the study. A positive RCA reaction also occurred with the sera from both elk E-18 and E-765 by day 21 PI and remained positive until the project was terminated. No *Anaplasma* spp.-infected erythrocytes were detected throughout the duration of the study in any of the four inoculated elk (Table 1). There was no significant reduction in PCV values, or any other evidence of clinical disease in any of the elk.

The two similarly inoculated spleen-intact sheep, S-301 and S-329, developed

antibodies by day 21 PI based on both CF and RCA tests. The highest CF titers of 1:160 were detected 43 days PI. Rickettsemias of 1.0% and 1.2%, respectively, at 21 days PI increased to a maximum percent parasitized erythrocytes of 12% and 17% by day 26 PI (Table 1). Packed cell volume levels decreased to a low of 23% within 26 days PI, but progressively returned to normal (34%) by day 31 PI.

Anaplasma spp.-infected erythrocytes first were noted in blood films from the splenectomized sheep (S-314 and S-331) on day 17 PI. The percent parasitized erythrocytes reached a high of 20% and 22% respectively, on day 28 PI (Table 1). Pronounced PCV decreases to a low of 10% in both sheep prompted their treatment with oxytetracycline. Both sheep recovered.

DISCUSSION

In agreement with Renshaw et al. (1979) an *A. marginale* infection was induced in elk and confirmed by passage to a splenectomized domestic bovine calf. Unlike anaplasmosis in cattle or sheep, the disease in elk was subclinical. This confirmative data was useful for comparison with the experimental *A. ovis* infection.

Anaplasma ovis infections were identified serologically in elk and confirmed by passage to susceptible domestic sheep, establishing an ovine-cervine-ovine sequence. Thus, elk are capable of supporting infections of both *Anaplasma* species, and could contribute to the dynamics of each. Available serologic assays cannot easily discriminate between *A. marginale* and *A. ovis*; cross-reactivity is extensive (Jessup et al., 1993). Also, little is known about how persistent the infections with either species may be in elk. For this reason, we subinoculated blood from the recipient elk to susceptible splenectomized sheep at 43 days PI, representing a short time after seroconversion, and at 172 days PI. Each subinoculation resulted in transfer of infectious material and initiation of infection in the susceptible domestic sheep, thereby establishing the persistent nature of infection in elk. More information about the carrier state is required to establish the epizootiologic significance of elk and their role as reservoirs for tick transmission. Two ticks, *Dermacentor andersoni* and *D. albipictus* are effective vectors of *Anaplasma* spp. (Stiller et al., 1989) and both ticks naturally infest cattle, sheep, and elk in Idaho (Saunders and Baird, 1980).

Elk appeared more susceptible to infection by *A. marginale* than by *A. ovis*. Infected erythrocytes and seroconversion occurred with all three *A. marginale* inoculated elk, compared to only three of seven *A. ovis* inoculated elk which only seroconverted. In addition, in Study A, the IIF titers were greater in the *A. marginale* inoculated elk, thus perhaps more replication took place.

Most North American wild ruminants that have been studied are similar to elk in that they show little, if any, clinical anaplasmosis (Kuttler, 1984; Zaugg and Kuttler, 1985; Zaugg, 1987, 1988). One major exception are bighorn sheep (*Ovis canadensis*), which are susceptible to infection with *A. ovis* resulting in severe clinical disease (Tibbitts et al., 1992; Goff et al., 1993).

The use of the IIF assay overcame problems often associated with the CF assay (anticomplementary activity in wild ruminant sera) and RCA assay (incompatibility with bovine conglutinin used in the assay resulting in a large percentage of false negatives) (Magonigle and Eckblad, 1979). However, in the studies reported here, *Anaplasma* spp.-reactive antibody were detected by all three assays. For large scale application and better specificity, an improved assay with species-specific attributes would be useful, but until such an assay is developed, the RCA remains valuable as a general initial screening tool (Zaugg and Kuttler, 1985) which can then be confirmed with the use of IIF.

It is clear that wild ruminants play a role in the epizootiology of anaplasmosis in North America. Additional studies con-

cerning their carrier status and vector tick association will help clarify these roles. This information will benefit both wild and domestic animal managers particularly in regions where wild ruminants share habitat with livestock.

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