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EXPERIMENTAL INFECTIONS OF *ANAPLASMA OVIS* IN PRONGHORN ANTELOPE

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ABSTRACT: *Anaplasma ovis* was experimentally transmitted from sheep to pronghorn antelope (*Antilocapra americana*) and back to sheep. *Anaplasma ovis* was recovered in splenectomized sheep, from two of three spleen-intact pronghorns following their inoculation with blood from known *A. ovis* carrier sheep. These two pronghorns exhibited a 0.5% or higher *A. ovis* parasitemia within 48 days after exposure, and an anaplasmosis-positive serological response 91 days after exposure. Clinical signs of illness were not observed. Blood from the infected pronghorns produced disease in four splenectomized sheep.

Key words: Pronghorn antelope, *Antilocapra americana*, anaplasmosis, *Anaplasma ovis*, experimental infection.

INTRODUCTION

Anaplasmosis, a disease of ruminants characterized in severe cases by anemia and death, is caused by intraerythrocytic organisms of the rickettsial genus *Anaplasma* (Blood et al., 1979). While the causative agent of bovine anaplasmosis (*A. marginale*) has received the greatest attention, it is not the only pathogen of the genus (Ristic, 1960). Another important, but less pathogenic, species is *A. ovis*, which primarily infects sheep (Splitter et al., 1956). Sheep from Colorado, Montana and Wyoming yielded the first isolates in the western hemisphere (Splitter et al., 1955). Naturally occurring *A. ovis* infections were reported in Idaho (Magonigle et al., 1981) and suspected in Utah (Jensen, 1955). The discovery of anaplasmosis in range sheep stimulated speculations concerning wildlife reservoirs of the organism in the western United States (Magonigle et al., 1981).

Natural *A. ovis* infections in North American wild ruminants have not been reported (Kuttler, 1984). However, there is some presumptive evidence that elk (*Cervus elaphus*) may harbor *A. ovis* (Post and Thomas, 1961). Also, white-tailed deer (*Odocoileus virginianus*) are readily infected experimentally (Kreier and Ristic, 1963).

Pronghorn antelope (*Antilocapra americana*) have been experimentally infected with *A. marginale* (Howe et al., 1964). Jacobson et al. (1977) reported obtaining strong positive complement fixation test reactions in 19 of 26 pronghorn sera collected in Montana. They felt that these reactions were false positives for *A. marginale* because subinoculations into bovine splenectomized calves failed to produce disease. Commonly used serological diagnostic tests are not *Anaplasma* species-specific. Therefore, the 1977 Montana study by Jacobson et al., may have dealt wtih *A. ovis*. The objective of the present investigation was to determine if pronghorn antelope are susceptible to experimental infection by *A. ovis*.

MATERIALS AND METHODS

Three pronghorn fawns, one female and two males (average weight of 8 kg) were obtained by special permission from the Wyoming Game and Fish Department, Sybille Wildlife Research Unit, Wheatland, Wyoming. Five ml of whole jugular blood was collected in citrated tubes from each fawn subsequent to their arrival in Caldwell, Idaho. Blood samples were pooled and inoculated intravenously (i.v.) into an *Anaplasma*-susceptible, splenectomized sheep. The fawns were maintained in an isolated pen within an enclosed barn, and fed fresh goat's milk for about 45 days. Thereafter, a commercial milk replacer (Lamb Milk Replacer, Land O Lakes, Inc., Fort Dodge, Iowa 50501, USA) was used,

and cracked barley, alfalfa hay, salt and fresh water were provided ad libitum.

Fifty-seven days after arrival, when fawns were approximately 10 weeks old, each fawn was inoculated i.v. with 10 ml of fresh whole (EDTA) blood pooled from three known *A. ovis* carrier ewes. Four anaplasmosis-naive, mixed breed sheep (two splenectomized and two spleen-intact) were similarly inoculated. The carrier ewes were originally inoculated 12 to 14 months previously with whole blood from a ewe naturally infected with *A. ovis* from southwest Idaho.

Blood was obtained from all test animals at least once each week and examined by monitoring packed cell volume (PCV), percentage of parasitized erythrocytes (PPE) on Giemsa-stained thin blood films, rapid card agglutination (RCA), and complement fixation (CF) test values and responses. Procedures followed for RCA (Amerault and Roby, 1968) and CF (Anonymous, 1974) tests utilized standard *A. marginale* antigen as no standardized *A. ovis* antigen was available. Blood was collected from surviving animals for at least 90 days (sheep) and 150 days (pronghorns) after inoculation.

Thirty-eight days after *A. ovis* exposure, 60 ml of whole citrated blood was collected from pronghorn PH-2 and inoculated i.v. into splenectomized ewe S-121A. Seventy-one days after *A. ovis* exposure, 10 ml of EDTA blood was obtained from pronghorn PH-1 and inoculated i.v. into splenectomized ewe S-121. One hundred twenty-six days after exposure, 20 ml of heparinized blood was transferred from pronghorn PH-3 into splenectomized sheep S-59. Forty-nine days later (175 days postexposure) 5 ml of heparinized blood from PH-3 was inoculated i.v. into each of the splenectomized ewes S-128 and S-137. The test sheep were penned in an isolated outdoor pen. They were maintained on alfalfa hay, fresh water and salt ad libitum. All blood recipient sheep were monitored hematologically and serologically as described above for at least 90 days, or until death.

RESULTS

The recipient splenectomized sheep of the pooled pronghorn blood collected at the beginning of the study remained seronegative by RCA and CF tests for *A. ovis* 90 days. Parasitemia was not detected, and the sheep was later proven susceptible by challenge inoculation with *A. ovis*-infected blood.

Two pronghorns (PH-1 and PH-2) accidently injured themselves and required euthanasia at 71 days and 38 days, respectively, after *A. ovis* exposure. Two of the three pronghorns (PH-1 and PH-3) exhibited characteristic parasitemias and other signs of *A. ovis* infection. The third pronghorn (PH-2), which died on postexposure day 38, showed what appeared to be a 0.1% parasitemia on the day of death. This, however, was not confirmed by animal inoculation. The prepatent period, defined here as the time from *A. ovis* exposure until the time when a parasitemia of 0.5% or greater is observed, was 38 days for PH-1 and 48 days for PH-3. Pronghorn PH-2 did not exhibit a PPE of 0.5% before being euthanized. Animal PH-3 was the only pronghorn that exhibited a parasitemia and became seropositive as detected by both the RCA and CF tests. Clinical illness was not observed in the pronghorns. All four sheep inoculated with carrier blood developed a parasitemia, with an average prepatent period of 22 ± 2 days, and the positive CF response an average of 19 ± 3 days after inoculation. Sheep S-801 died of acute anaplasmosis 27 days postinoculation.

The average time sequence and magnitude of parasitemias exhibited by the pronghorns closely resembled those of the spleen-intact sheep except that the pronghorn parasitemias peaked about 16 days after those of the sheep (Fig. 1). Parasitemias in the splenectomized sheep were much higher than in the intact animals. A high PPE value of 34.1% was recorded from splenectomized sheep S-801 three days before its death (Table 1). The lowest PCV values were also observed in the splenectomized animals. Sheep S-802 survived even after its PCV dropped to 10%. The intact sheep experienced only a slight decrease in PCV values associated with the *A. ovis* parasitemia. Lowest PCV levels for sheep S-805 and S-806 were 26% and 28%, respectively, an approximate 25% decrease from pre-exposure averages. There were no detected PCV decreases associ-

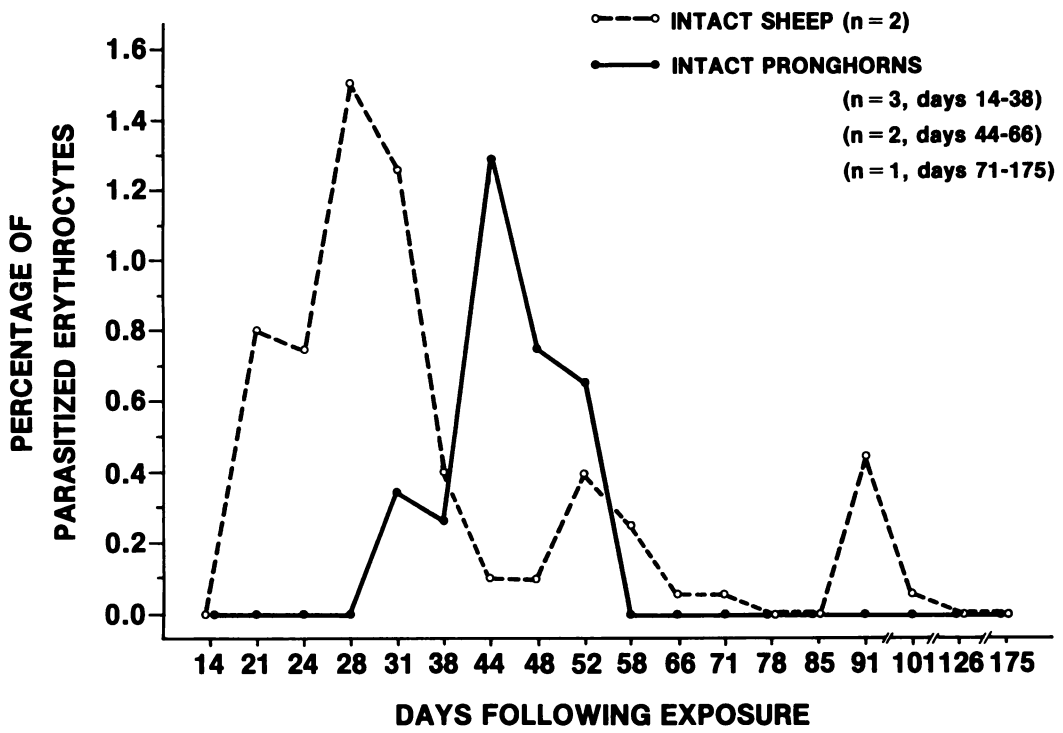

FIGURE 1. Comparison of average parasitemias in blood of spleen-intact pronghorn antelope and sheep experimentally exposed to *Anaplasma ovis*.

ated with observed parasitemias in any of the pronghorns (Table 1).

CF test-positive reactions in all inoculated sheep began in concert with detected ascending parasitemias. PH-3 was the only pronghorn which exhibited a CF *Anaplasma* titer. Titers were first observed 91 days after inoculation and 39 days after the last detected parasitemia. All 4 sheep became RCA test-seropositive within 48 days after inoculation. Pronghorn sera did not become RCA positive before 101 days after *Anaplasma* exposure.

Four of the five *Anaplasma*-susceptible, splenectomized sheep inoculated with pronghorn whole blood developed clinical anaplasmosis within 43 days of inoculation (Table 2). One sheep (S-137) died of acute anaplasmosis 55 days after exposure. Sheep S-121A, a recipient of 60 ml of PH-2 blood, remained clinically, hematologically and serologically normal at least 90 days after inoculation.

DISCUSSION

Anaplasma ovis infections were identified serologically and hematologically and were confirmed by passage to susceptible sheep, establishing an ovine-pronghorn-ovine sequence. The average prepatent periods of 40 days seen in pronghorns in the present study were longer than the average of 22 days observed in sheep receiving the same inoculum. However, the levels of parasitemia detected in the pronghorns were essentially the same as those of the intact sheep (Table 1, Fig. 1).

It was unfortunate that injuries prevented full evaluation of *A. ovis* infections in two of the pronghorns. However, observations on PH-3 and PH-1 indicated that pronghorns did not exhibit any outward signs of illness associated with anaplasmosis. The lack of clinical signs agrees with previous studies of wild ruminants and anaplasmosis (Howe et al., 1964; Robinson et al., 1968; Renshaw et al., 1979;

TABLE 1. Serologic and hematologic data on pronghorn fawns and sheep experimentally exposed to *Anaplasma ovis*.

Test animal number[a]	Blood test[b]	Days after inoculation (IV)																		
		7	14	21	24	28	31	38	44	48	52	58	66	71	78	85	91	101	126	175
PH-1	PCV	38	37	36	40	39	37	46	44	44	49	44	44							
	PPE	–	–	–	–	–	0.1	0.6	2.4	1.0	–	–	–							
	RCA	–	–	–	–	–	–	–	–	–	–	–	–							
	CF	–	–	–	s	–	–	s	–	–	–	–	–							
PH-2	PCV	45	44	46	49	49	52	53												
	PPE	–	–	–	–	–	–	0.1												
	RCA	–	–	–	–	–	–	–												
	CF	–	–	–	–	–	–	–												
PH-3	PCV	46	48	46	47	46	48	50	52	48	43	47	49	46	44	51	60	56	55	58
	PPE	–	–	–	–	–	–	0.1	0.2	0.5	1.3	–	–	–	–	–	–	–	–	–
	RCA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
	CF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	s	10	40	80	160
S-801[c]	PCV	28	26	27	20															
	PPE	–	0.4	9.1	34.1															
	RCA	–	–	+	+															
	CF	–	s	80	160															
S-802[c]	PCV	32	30	37	26	22	13	10	18	20	22	24	27	15	18	23	36	22	27	32
	PPE	–	0.1	3.0	4.6	17.0	26.5	9.2	3.0	0.1	0.1	0.1	11.8	22.3	0.6	0.5	0.6	0.2	–	0.1
	RCA	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+
	CF	–	s	5	10	20	20	80	160	40	40	40	40	40	20	20	20	20	20	10
S-805	PCV	35	36	33	26	27	29	29	32	32	30	32	33	27	31	36	36	38	37	35
	PPE	–	–	1.2	1.0	2.5	2.2	0.1	0.1	0.1	0.4	0.2	–	–	–	–	0.8	0.1	–	–
	RCA	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–
	CF	–	5	40	80	80	80	40	40	80	20	10	10	10	10	10	10	10	s	–
S-806	PCV	37	38	28	28	32	33	33	35	38	36	35	37	36	40	41	40	41	40	40
	PPE	–	–	0.4	0.5	0.5	0.3	0.7	0.1	0.1	0.4	0.3	0.1	0.1	–	–	0.1	–	–	–
	RCA	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	–
	CF	–	s	40	40	40	80	160	80	40	40	20	10	20	10	10	20	10	s	5

[a] PH, pronghorn; S, sheep.
[b] PCV, packed cell volume; PPE, percentage of parasitized erythrocytes; RCA, rapid card agglutination; CF, complement fixation titers; s, suspect; –, negative; +, positive.
[c] Splenectomized.

TABLE 2. Hematologic, serologic and transmission data for *Anaplasma ovis* on splenectomized sheep recipients of pronghorn blood.

Pronghorn no.	Days postexposure	Pronghorn blood recipient sheep no.	Volume of blood inoculated (ml)	Disease transmission yes/no	Prepatent period (days)	Recipient disease climax data (days after inoculation)[a]		
						Low PCV (%)	High PPE (%)	High CF titer
PH-1	71	S-121	10	Yes	43	8.0 (57)	21.8 (52)	320 (57)
PH-2	38	S-121A	60	No	–	–	–	–
PH-3	126	S-59	20	Yes	36	9.0 (45)	20.3 (45)	320 (44)
PH-3	175	S-128	5	Yes	28	31.0 (35)	1.6 (34)	320 (31)
PH-3	175	S-137	5	Yes	41	7.0 (54)[b]	38.7 (51)	320 (51)

[a] PCV, packed cell volume; PPE, percentage of parasitized erythrocytes; CF, complement fixation; –, negative.
[b] Animal died the next day, 55 days after inoculation.

Howe, 1981; Kuttler, 1984; Zaugg and Kuttler, 1985). This either suggests a degree of resistance or adaptability to a familiar infection.

The relatively slow rise in pronghorn CF titers is unexplained (Table 1). Several investigators considered the bovine anaplasmosis CF test misleading and of no diagnostic/survey value in studies conducted with elk (Post and Thomas, 1961; Howe et al., 1964; Vaughn et al., 1976), deer (Osebold et al., 1959; Christensen et al., 1960), pronghorns (Howe et al., 1964; Jacobson et al., 1977) and bighorn sheep (Howe et al., 1964). However, when pronghorn anti-anaplasmosis titers were finally detected, 70 days after the appearance of titers in inoculated sheep, the rise was constant. Maximum detected titers in pronghorn sera were as high (1:160), as were those observed in the similarly inoculated sheep (Table 1). Much the same pattern of occurrence was seen with regard to the other serologic test (RCA) used in this study. When a positive response was first detected in pronghorn sera, 53 to 80 days after the first positive response in sheep, the successive samples remained positive. Others considered the RCA test as a reliable and valuable tool in wild ruminant anaplasmosis studies (Howarth et al., 1976; Magonigle and Eckblad, 1979; Renshaw et al., 1979; Zaugg and Kuttler, 1985). From the present study it appeared that the bovine anaplasmosis CF and RCA tests were reliable for detecting the presence of anti-anaplasmosis antibodies after 90 days post-experimental-exposure in pronghorns. Hopefully, more sensitive diagnostic tests using monoclonal technology will be forthcoming. These should allow earlier detection and identification of carrier-states in wild ruminants.

Further work is warranted to determine if infected pronghorns, remain as long term carriers of anaplasmosis. The reservoir status of pronghorns and the implications of cross transmission between the host and sheep by natural vectors of anaplasmosis needs further investigation.

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