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EXPERIMENTAL ANAPLASMOSIS IN MULE DEER: PERSISTENCE OF INFECTION OF *ANAPLASMA MARGINALE* AND SUSCEPTIBILITY TO *A. OVIS*

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ABSTRACT: An experimental *Anaplasma marginale* infection was induced in a splenectomized mule deer (*Odocoileus hemionus hemionus*) which persisted subclinically at least 376 days as detected by subinoculation into susceptible cattle. *Anaplasma ovis* was experimentally transmitted from sheep to a splenectomized and a spleen-intact mule deer, and back to sheep. The pathogenesis in deer was very similar to that seen in sheep using ovine blood inoculations.

Key words: *Anaplasma marginale*, anaplasmosis, *Anaplasma ovis*, experimental infection, mule deer, *Odocoileus hemionus hemionus*, pathogenesis, splenectomized deer.

INTRODUCTION

Anaplasmosis is an infectious disease of ruminants manifested by progressive anemia, and sometimes death, associated with the presence of intraerythrocytic bodies of the rickettsial genus *Anaplasma* (Robertson, 1976). The disease is transmitted from acutely infected and carrier animals to susceptible hosts primarily by hematophagous arthropods (Ewing, 1981), and iatrogenically (Reeves and Swift, 1977). In utero passage is also known (Zaugg and Kuttler, 1984).

While the causative agents of bovine and ovine anaplasmosis are *Anaplasma marginale* and *Anaplasma ovis*, respectively, these pathogens are not confined to cattle and sheep (Kuttler, 1984). Species of wild ruminants in North America naturally or experimentally susceptible to *A. marginale* include elk (*Cervus elaphus nelsoni*) (Magonigle and Eckblad, 1979), bighorn sheep (*Ovis canadensis canadensis*) (Howe et al., 1964), bison (*Bison bison*) (Zaugg, 1986), pronghorn antelope (*Antilocapra americana*) (Jacobson et al., 1977), white-tailed deer (*Odocoileus virginianus*) (Kuttler et al., 1967), Columbian black-tailed deer (*O. hemionus columbianus*) (Howarth et al., 1969), and mule deer (*O. hemionus hemionus*) (Howe and Hepworth, 1965). Wildlife in North America susceptible also to *A. ovis* include elk (Post and Thomas, 1961), pronghorn antelope (Zaugg, 1987) and white-tailed deer (Kreier and Ristic, 1963).

In the western United States, domestic and wild ruminants often share the same ranges. There is concern about the existence and significance of wildlife as reservoirs of anaplasmosis. A knowledge of the epizootiology of anaplasmosis would contribute significantly to the development of control programs. The most common wild ruminant in the intermountain states is the mule deer. Mule deer were proven to be susceptible to short term infections of *A. marginale* (Howe et al., 1964). The objectives of the present investigation were to determine if: (1) a long-term *A. marginale* carrier state persisted in mule deer, and (2) mule deer were susceptible to experimental infection by *A. ovis*.

MATERIALS AND METHODS

Three *Anaplasma* seronegative mule deer fawns, one male and two females (average weight of 9 kg), were obtained by special permission from the Idaho Department of Fish and Game. The fawns were maintained in an isolated, enclosed barn and hand fed fresh goat's milk for about 45 days. Then a commercial milk replacer (Lamb Milk Replacer, Land O'Lakes, Inc., Fort Dodge, Iowa 50501, USA) was fed for an additional 2 mo while the fawns were gradually weaned to solid feed. Thereafter, alfalfa hay, cracked barley, fresh water and salt were provided ad libitum. When the fawns were approximately 5 mo old, two fawns (MD-1 and MD-2) were splenectomized under halothane

(Fluothane, Aveco, Fort Dodge, Iowa 50501, USA) gas anesthesia.

Experiment one

Thirteen days after surgery the female splenectomized fawn MD-1, was inoculated intravenously (i.v.) with 3 ml of a 1:10 dilution of an *Anaplasma marginale* stabilate from a splenectomized calf which had been infected with a stabilate prepared from an infected whitetailed deer (Texas laboratory strain) in 1976 and subsequently maintained (USDA, ARS, Pullman, Washington 99164, USA) in liquid nitrogen. Splenectomized calf C-21 also received the same inoculation to verify the infectivity of the stabilate.

Blood was obtained from both MD-1 and C-21 at least weekly and examined by monitoring packed cell volume (PCV), percentage of parasitized erythrocytes (PPE) on Giesma-stained thin blood films, rapid card agglutination (RCA), and complement fixation (CF) test values and responses. Twenty-one days after inoculation C-21 was treated with oxytetracycline (Liquamycin LA-200, Pfizer, New York, New York 10017, USA) at 20 mg/kg intramuscularly (i.m.) to prevent death.

Thirty-four days postinoculation (PI) with *A. marginale*, 10 ml of whole EDTA blood was collected from deer MD-1 and inoculated i.v. into splenectomized calf C-351. Two hundred sixty-one days PI 5 ml of EDTA blood was drawn from MD-1 and inoculated i.v. into 2-yr-old, spleen-intact seronegative steer C-52K. Then, 376 days PI, EDTA MD-1 blood was obtained and 4 ml each was inoculated i.v. into splenectomized calf C-53 and splenectomized sheep S-56. Thirty-eight days after receipt of MD-1 blood, calf C-53 was treated with oxytetracycline to prevent death.

The test cattle and sheep were confined in individual outdoor pens and supplied alfalfa hay, salt and fresh water ad libitum. All animal recipients of deer MD-1 blood were monitored hematologically and serologically as described above for at least 90 days, or until therapeutic treatment. Steer C-52K was challenged with 10 ml i.v. of a 1:10 dilution of virulent *A. marginale* stabilate 93 days after MD-1 blood inoculation.

Experiment two

When deer MD-2 (splenectomized male) and MD-3 (spleen-intact female) were approximately 1 yr old they were each inoculated i.v. with 10 ml of pooled unclotted blood from three *A. ovis* infected ewes. At the time of inoculation the ewes showed an average PCV of 25%, 0.2% PPE and CF titer of 1:160. The three ewes had been infected the previous year with *A. ovis* from southwest Idaho. Splenectomized sheep S-802A also received a 10 ml pooled blood inoculum to verify the infectivity of the source. Thirty days after inoculation, sheep S-802A was treated with oxytetracycline at 20 ml/kg i.m. to prolong life.

Blood was collected from deer MD-2, deer MD-3 and sheep S-802A at least weekly, and examined as described above. Procedures followed for RCA (Amerault and Roby, 1968) and CF (Anonymous, 1974) tests utilized standard *A. marginale* antigen because no standardized *A. ovis* antigen was available.

Sixty days PI 4 ml of EDTA blood was drawn from each deer MD-2 and MD-3, and inoculated i.v. into splenectomized sheep S-377 and S-803, respectively. One hundred thirty-eight days PI a splenectomized sheep S-57 was inoculated with 4 ml of whole blood from MD-2, and splenectomized sheep S-58 was inoculated with 4 ml of blood from MD-3. On the same date an additional 3 ml of blood from each deer was pooled and inoculated i.v. into splenectomized calf C-P11. Forty-two days after exposure to MD-3 blood, sheep S-58 was treated with oxytetracycline as a life-saving measure.

The sheep and calf were confined in outdoor pens and supplied alfalfa hay, salt and fresh water ad libitum. All test recipients of deer blood were monitored hematologically and serologically at least weekly as described above for 90 days, or until therapeutic treatment or death.

RESULTS

Experiment one

Both splenectomized animals (deer MD-1 and calf C-21) exhibited characteristic parasitemias and serologic evidence of *A. marginale* infection. The prepatent period (PP), defined as the time from *Anaplasma* exposure until the time when a parasitemia of $\geq 0.5\%$ is observed (Zaugg, 1987), was 14 days for both animals (Table 1). Calf C-21 suffered acute clinical signs of anaplasmosis including depression, anorexia and weakness. The lowest PCV (19%, or a 37% decrease) was observed on day 21 PI, at the time of treatment. The highest detected PPE value was 24.4%. Clinical signs disappeared within 4 days of oxytetracycline treatment. Clinical illness was not observed in MD-1. The lowest PCV (18%) was a decrease of 56% from the pre-inoculation average of 40% 30 days PI, and the highest PPE was 20.3%. MD-

TABLE 1. Serologic and hematologic data on mule deer, calves and sheep experimentally exposed to *Anaplasma* spp.

Test animal number[a]	Disease agent[b]	Blood test[c]	Days after intravenous inoculation																
			7	14	21	23	26	27	30	32	34	39	44	47	48	53	60	63	68	74
MD-1[d]	AM	PCV	40	41	32	29		21	18		25		31		35		37		31	33
		PPE	0	0.9	17.3	20.3		2.9	4.5		0.1		0		0		0		0	0
		RCA	−	+	+	+		+	+		+		+		+		+		+	+
		CF	−	s	40	80		160	160		320		160		160		40		10	10
C-21[d]	AM	PCV	30	30	19[e]						25									
		PPE	0	2.6	24.4						0									
		RCA	−	−	+						+									
		CF	−	40	160						640									
MD-2[d]	AO	PCV	31	32	35		36			30		32		31		29	21	19	22	24
		PPE	0	0	0		0			0		0		0.6		8.6	31.5	36.0	3.3	0.9
		RCA	−	−	−		−			−		−		+		+	+	+	+	+
		CF								s						40	40	160	320	160
MD-3	AO	PCV	32	31	33		34			36		45		36		33	35	31	36	33
		PPE	0	0	0		0			0		0		0		0.1	0.4	0.5	0.6	0.1
		RCA	−	−	−		−			−		−		+		+	+	+	+	+
		CF	−	−	−		s			2 0		s		s		20	40	5	10	5
S-802A[d]	AO	PCV	34	35	37	26	22		13[e]		10				27					
		PPE	0	0	3.0	4.6	17.0		26.5		9.0				0					
		RCA	−	−	−	−	−		+		+				+					
		CF	−	−	5	10	20		20		40				80					

[a] MD, mule deer; C, calf; S, sheep.
[b] AM, *Anaplasma marginale*; AO, *A. ovis*.
[c] PCV, packed cell volume (%); PPE, percentage of parasitized erythrocytes (%); RCA, rapid card agglutination; CF, complement fixation titers; s, suspect; −, negative reaction; +, positive reaction.
[d] Splenectomized.
[e] Treated with oxytetracycline, 20 mg/kg, i.m.

1 did not exhibit a detectable parasitemia after 44 days PI.

Blood collected from MD-1 on days 34, 261 and 376 PI was infective for both splenectomized and spleen-intact bovines, with a PP average of 25 days (Table 2). The splenectomized calves (C-351 and C-53) required treatment to prevent death. The mature, spleen-intact steer (C-52K) experienced a high PPE of 3.7%, a low PCV of 30% and a high CF response of 1:320, and failed to exhibit clinical signs. Subsequent challenge of this steer with virulent heterologous *A. marginale* stabilate failed to induce clinical disease. Splenectomized sheep S-56, which received MD-1 blood 376 days PI, remained clinically, hematologically and serologically normal for at least 90 days.

Experiment two

The two deer and one sheep inoculated with *A. ovis* infected sheep blood contracted anaplasmosis. Both splenectomized animals (deer MD-2 and sheep S-802A) developed parasitemias of 36.0% and 26.5%, respectively (Table 1). The maximum parasitemia detected in blood of the spleen-intact deer MD-3 was 0.6%. The PP of 21 days in the sheep was less than one-half that of MD-2 (47 days) and one-third that of MD-3 (63 Days). Sheep S-802A exhibited acute clinical signs of illness, which regressed after treatment. Deer MD-2 experienced a 46% decrease in PCV from a pre-inoculation value of 35%. The lowest PCV noted with MD-3 (31%) only represented a 9% decrease from the normal average of 35%. Neither deer showed any clinical signs of disease and both animals spontaneously recovered from parasitemia.

Deer MD-2 and MD-3 blood inoculated into splenectomized sheep on days 60 and 138 PI was infective. All four recipient sheep experienced acute disease; three died from anaplasmosis and one recovered with treatment (Table 2). Prepatent periods ranged from 14 to 28 days with an average of 19 days. Pooled MD-2 and MD-3 blood collected and inoculated into splenectomized calf C-P11 on day 138 PI failed to induce *A. ovis* infection. All monitored parameters of calf C-P11 remained normal for at least 90 days after deer blood exposure.

DISCUSSION

In agreement with other work (Boynton and Woods, 1933; Howe et al., 1964) an *A. marginale* infection was induced in a splenectomized mule deer and confirmed by passage to susceptible cattle. After experimental infection the carrier state in MD-1 persisted at least 376 days PI. Prior to the present study, the longest reported duration of infection in mule deer was 66 days (Howe et al., 1964). Prepatent periods were identical in the splenectomized deer and calf, and parasitemias rose rapidly in both animals (Table 1), suggesting that mule deer exhibit a similar level of susceptibility as cattle to experimental *A. marginale* infections. The fact that calf C-21 required treatment while MD-1 did not suggests that the splenectomized mule deer mounts a more effective response than a splenectomized bovine calf. Although splenectomy greatly increases susceptibility to anaplasmosis, not all cases of anaplasmosis in splenectomized calves are fatal (Zaugg and Kuttler, 1984, 1985). An obvious difference between anaplasmosis infection in splenectomized deer and calves is that the disease in deer was subclinical.

Despite the substantial parasitemias exhibited by splenectomized calves after infective deer-blood inoculations (Table 2) there is an indication that *A. marginale* may have been attenuated with mule deer passage. The mature, spleen-intact Holstein steer C-52K developed a subclinical infection with minimal parasitemia (climax of 3.7% PPE) and only an 11% decrease from normal PCV values. This is in direct contrast to the usual acute susceptibility of *A. marginale* naive mature cattle to inocula of 0.5 ml of asymptomatic carrier blood (Lincoln et al., 1987). Steer C-52K did not develop clinical disease af-

TABLE 2. Hematologic, serologic and transmission data for *Anaplasma* on animal recipients of blood from experimentally infected mule deer.

Deer number	Disease agent[a]	Days post-exposure	Deer blood recipient animal number[b]	Volume of blood inoculated (ml)	Disease transmission yes/no	Prepatent period (days)	Recipient disease data (days after inoculation)[c]				
							Low PCV (%) (day)	High PPE (%) (day)	Day of first RCA positive	Day of first CF titer	High CF titer (day)
MD-1[d]	AM	34	C-351[d]	10	yes	23	17 (37)	41.2 (39)[e]	30	(29)	320(45)
MD-1	AM	261	C-52K	5	yes	25	30 (35)	3.7 (27)	25	(24)	320(34)
MD-1	AM	376	C-53[d]	4	yes	27	16 (38)[e]	33.5 (38)[e]	34	(27)	640(40)
MD-1	AM	376	S-56[d]	4	no	—	—	—	—	—	—
MD-2	AO	60	S-377[d]	4	yes	15	11 (25)[f]	60.8 (25)[c]	15	(21)	80(25)[f]
MD-2	AO	138	S-57[d]	4	yes	14	23 (18)[f]	56.8 (18)[c]	16	s (18)	s (18)[f]
MD-3	AO	60	S-803[d]	4	yes	19	6 (20)[f]	25.6 (25)	15	(17)	640(29)[f]
MD-3	AO	138	S-58[d]	4	yes	28	16 (42)[e]	18.0 (380)	30	(28)	640(38)
MD-2[g] and MD-3	AO	138	C-P11	6	no	—	—	—	—	—	—

[a] AM, *Anaplasma marginale*; AO, *A. ovis*.
[b] C, calf; S, sheep.
[c] PCV, packed cell volume; PPE, percentage of parasitized erythrocytes; RCA, rapid card agglutination; CF, complement fixation; s, suspicious <4+ at 1:5.
[d] Splenectomized.
[e] Treated with oxytetracycline, 20 mg/kg, i.m.
[f] Death occurred the following day.
[g] Pooled blood sample.

ter challenge with a known virulent heterologous *A. marginale* stabilate.

The fact that no *A. marginale* infection occurred in sheep S-56 may reflect a reduced level of infection in the deer blood inoculum 376 days PI, which simply was too low to infect a sheep which is naturally less susceptible to *A. marginale* than are cattle. Another explanation may be that deer passage did not alter the *A. marginale* organism sufficiently to infect an ovine host.

Experimental *A. ovis* infections were induced and hematologically and serologically identified for the first time in mule deer and were confirmed by passage to susceptible sheep, establishing an ovine-cervine-ovine sequence. The prepatent periods of 47 and 63 days in mule deer were two and three times longer than those observed in the sheep receiving the same inoculum (Table 1). This was similar to the extended prepatent periods of 40 days seen in pronghorn antelope inoculated with *A. ovis*-infected blood (Zaugg, 1987). Once a detectable parasitemia began, the rise in PPE of the splenectomized deer MD-2 was as rapid as that recorded from splenectomized sheep S-802A blood (Table 1). After a CF titer was first observed in MD-2 sera, its rate of increase was more rapid and the peak CF titer was higher than titers seen in S-802A sera (Table 1). The fluctuations of PPE and PCV in spleen-intact deer MD-3 closely paralleled those in spleen-intact sheep inoculated with *A. ovis*-infected blood from the same source furnishing inocula for the animals in the present study (Zaugg, 1987).

Judging from the acute disease induced in the four sheep recipients of MD-2 and MD-3 blood, very little if any attenuation of *A. ovis* occurred in a single deer passage. It is difficult to infect calves with *A. ovis* (Kuttler, 1981). Therefore, it was not surprising that pooled MD-2 and MD-3 blood did not transmit an infection to calf C-P11.

Results from the present study demonstrated mule deer are susceptible to experimental *A. ovis* infection. However, it is unlikely that mule deer would be important sources of *A. ovis* infection for domestic sheep. Still, further investigations on the epizootiology of the disease would be of value to livestock producers.

The lack of clinical signs noted in deer infected with *A. marginale* and *A. ovis* agree with previous studies of anaplasmosis in wild North American ruminants (Howe et al., 1964; Robinson et al., 1968; Renshaw et al., 1979; Howe, 1981; Kuttler, 1984; Zaugg and Kuttler, 1985; Zaugg, 1987). This subclinical susceptibility of wildlife in North America questions the accepted theory proposed by Mohler (1930) that *Anaplasma* spp. originated in European cattle and was imported to the Western Hemisphere by the Spaniards in the 16th or 17th centuries. If, in fact, the anaplasmas are relatively new to the Americas one might expect the susceptible indiginous ruminant hosts to exhibit more intense reactions to the infections.

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