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FURTHER STUDIES ON TRYPANOSOMES IN GAME ANIMALS IN WYOMING II [1] [2]

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Abstract: Further studies on moose revealed trypanosomes in two captive moose *(Alces alces shirasi)* and in 4 of 7 free-ranging moose in Wyoming by blood culture. Two free-ranging moose from Utah were negative. One of two additional captive moose calves was positive for trypanosomes. Trypanosomes also were detected in blood cultures of 8 of 39 American bison *(Bison bison)* being brought into Wyoming from Nebraska. Nineteen additional bison were negative for trypanosomes by blood cultures. Identification of species was not possible due to the failure to obtain bloodstream trypomastigotes from this host. Trypanosomes were recovered from 8 of 57 pronghorn antelope *(Antilocapra americana).* This is the first report of *Trypanosoma* sp. from bison and from pronghorn; the trypanosome from moose was identified as *Trypanosoma cervi* from bloodstream trypomastigotes. In 1978, natural transplacental transmission of trypanosomes was found to occur in 1 of 15 mule deer *(Odocoileus hemionus)* fetuses, examined near term by blood culture. No trypanosomes were found in 18 mule deer fetuses examined in 1979. Of 100 free-ranging elk from western Wyoming examined by blood culture in 1979, 71 were infected. These data are compared with data from 1973-74.

INTRODUCTION

Trypanosoma cervi Kingston and Morton, 1975 has been reported from free-ranging elk *(Cervus canadensis)*[8,9,16] and mule deer *(Odocoileus hemionus hemionus)*[10] in Wyoming. *Trypanosoma* sp. has been recovered by blood cultures from captive black-tailed deer *(O.h. columbianus)* and white-tailed deer *(O. virginianus)* at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit, Wheatland, Wyoming.[16] Trypanosomes from white-tailed deer examined in North Carolina, Georgia and Alabama have been identified as *T. cervi.*[7] A single captive moose *(Alces alces shirasi)* in Wyoming, examined in 1977, was positive for trypanosomes by culture.[11] Trypanosomes have been collected from other moose since that time by culture and bloodstream forms have been recovered on slides. This report adds bison *(Bison bison)* and pronghorn *(Antilocapra americana)* to the list of hosts harboring trypanosomes. Specific identification of trypanosomes from bison and pronghorn remains to be elucidated.

Additional data document transplacental transmission of trypanosomes

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from dam to fetus in mule deer. Data on prevalence of trypanosomes in 100 elk examined during the winter of 1979 from western Wyoming are presented.

MATERIALS AND METHODS

Blood was collected from putative hosts by jugular venapuncture into heparinized, or serum Vacutainer [6] tubes. Whole blood (5-10 ml) or blood clots were cultured in veal infusion medium (VIM)[8] and incubated at ambient temperatures (ca. 21 C) to identify trypanosome infected animals. Cultures were examined microscopically at weekly intervals for 3 weeks or longer after inoculation. Some blood samples were examined by direct examination (DE) of about 0.05 to 0.07 ml of heparinized blood concentrated in microhaematocrit tubes (centrifuged at 13000 g, 5 min).[1] Trypanosomes, when present, could be seen in the plasma above the buffy coat layer. Positive tubes were scored and broken above the buffy coat-plasma interface and the trypanosomes were expressed onto slides. Conventional thin blood films were prepared, air-dried, fixed in absolute methanol and stained with Giemsa. Slides were scanned under low power (16×); trypanosomes, when located, were photographed (SLR camera, color transparency film). The transparencies were projected at a standard distance, traced, and the drawings measured with a calibrated map-wheel reader. Data from moose specimens were analyzed statistically and compared with other trypanosomes previously identified as *Trypanosoma cervi* from other cervid species.

Blood to be used in cross transmission studies was collected in partial evacuated bottles containing heparin or Alsever's solution, and this mixture was inoculated intravenously into trypanosome-negative recipients.

RESULTS

Isolation of Trypanosomes From Moose. A captive 4-year old male shiras moose held since 1 week of age at the Sybille Wildlife Research Unit, was determined to be infected with trypanosomes by VIM culture in early November, 1977.[11] Blood collected in late July, 1978 from a captive female moose calf held at the same facility and examined by blood culture (VIM) contained trypanosomes.

Seven adult moose were captured in 1979 from Teton County, Wyoming for relocation, and four were positive for trypanosomes by VIM culture of blood. Blood collected in 1979 from 2 additional moose calves from Utah was negative by VIM culture.

The blood of two additional male moose calves held at the Sybille Wildlife Research Unit was examined in 1980 by culture (VIM), DE[1] and double concentration techniques.[15] One of these animals was positive for trypanosomes (Table 1).

Four trypanosomes were recovered on slides from moose blood. They were photographed and measured. The mean values and ranges of various parameters obtained from the moose trypanosomes are given in Table 2.

Cross-Transmission Studies. In December, 1978, approximately 13.5 ml of blood from a trypanosome-infected moose calf were collected and inoculated into a mule deer fawn previously determined to be negative for trypanosomes. One trypomastigote was seen in 1.5 ml of blood from the donor moose calf at that time. Subsequent examinations of the mule deer fawn by DE and VIM cultures were negative. In a second attempt at cross-transmission, approximately 33 ml of blood from the same moose calf were inoculated into an elk calf in April, 1979. No trypanosomes were seen in 28

[6] Vacutainer. Becton, Dickinson and Company, Rutherford, New Jersey 07070, USA.

TABLE 1. Big game ruminants in Wyoming infected with trypanosomes.

HOSTS SPECIES	NUMBER EXAMINED/ NUMBER POSITIVE(%)	CALVES	YEARLING	ADULTS	MALE	FEMALE	SOURCE
MOOSE	11/7 (65.4%)	2		5	2	1	Wyoming
Alces alces shirasi[1]	2/0 (0)						Utah
BISON	39/8 (20.5)*						Kimball Co., Nebraska
Bison bison[2]	6/0 (0)*						Kimball Co., Nebraska
	5/0 (0)						Laramie Co., Wyoming
	8/0 (0)						Hot Springs Co., Wyoming
	34/4 (11.8)						Goshen Co., Wyoming
PRONGHORN ANTELOPE	60/0 (0)						Albany and Laramie Counties, Wyoming
Antelocapra americana[2]	57/8 (14)						Sweetwater Co., Wyoming
MULE DEER							
Odocoileus hemionus	25/18 (72)						
	33/1 (3)	(FETUS) 1		18	1	17	Carbon Co., Wyoming
ELK							
Cervus canadensis	42/34 (80.95)	29/23	4/3	9/8	20/17	22/17	Greys River, Lincoln
		(79.3)	(75)	(88.9)	(85)	(77.3)	Co., Wyoming
	58/37 (63.7)	31/15	9/7	18/15	22/12	36/25	National Elk Refuge,
		(48.4)	(77.8)	(83.3)	(54.6)	(69.4)	Teton Co., Wyoming
TOTAL (ELK)	100/71 (71)	(48.4)					

*Now in Wyoming
[1]New host record for *Trypanosoma cervi*
[2]New host record for trypanosomes, species unknown.

TABLE 2. Mensural values of trypanosomes from moose, *Alces alces shirasi*, in Wyoming.

	PK	KN	PN	AN	BL	FF	L	W	FF:BL	NI	KI
Trypanosoma cervi from moose	7.75	7	14.5	15.75	30.25	8.25	38.5	2.75	1:3.754	1:0.927	1:2.071
Range	(6-10)	(7)	(13-17)	(14-18)	(27-35)	(7-10)	(36-44)	(2-3)	(1:2.7-4.3)	(1:077-1.1)	(1:1.86-2.4)
N=4 S.D. ±	1.7	0	1.9	2.1	3.4	1.5	3.7	0.5	0.722	0.126	0.274

PK = Posterior end to kinetoplast, KN = Nucleus to kinetoplast, PN = Posterior end to nucleus, NA = Nucleus to anterior end, BL = L-FF, Length minus length of free flagellum, FF = Free Flagellum. L = Length. W = Width. NI = PN/NA. KI = PN/KN.

haematocrit tubes (= ca. 1.4-1.9 ml) of moose blood examined by DE. No infection occurred in the elk calf which could be detected by DE and/or VIM culture. Blood from the moose calf determined to be positive for trypanosomes in 1980 was inoculated (ca 20 ml each) into the negative moose calf and into three trypanosome-negative elk calves. Donor blood was cultured. Examination of recipient blood and all cultures from donor inoculation blood cultures and recipient blood cultures did not reveal trypanosomes.

Recovery of trypanosomes from new hosts. Bison. Clotted blood samples collected in 1979 from 39 American bison *(Bison bison)* from Kimball County, Nebraska being transported to Wyoming, were cultured in VIM. Eight (20.5%) samples were infected with trypanosomes. Direct examination of haematocrit tubes of blood from an additional 6 bison from the same source did not reveal trypanosomes and none was positive by VIM culture. Blood cultures of 5 bison from the Cheyenne, Wyoming Zoo and of 8 bison from Big Horn National Park (Hot Springs Co., Wyoming also were negative for trypanosomes. Thirty-four additional bison from Goshen Co., Wyoming were examined in 1980 by culture, four (11.7%) were positive for trypanosomes. Because blood stream trypomastigotes were not available, species identification could not be made (Table 1).

Pronghorn Antelope. Recently (December, 1980) we examined blood by VIM culture from 57 pronghorn from the vicinity of Lost Creek, eastern Sweetwater county, Wyoming, and eight (14%) were infected with trypanosomes. Bloodstream trypomastigotes were not recovered on slides for identification[9] (Table 1).

Transplacental Transmission of Trypanosomes in Mule Deer. Twelve (11 females, 1 male) and 13 (12 female, 1 male) mule deer were collected in May,

1978, and May, 1979, respectively, over a 600 km² area in Carbon Co., Wyoming to determine their nutritional and reproductive status. Nineteen of 23 females were pregnant; 14 with twins and five with single fetuses. The deer were within 30-60 days of parturition.[6]

Heart blood of adult deer (1978-79) and fetuses (1978) or umbilical blood of fetuses (1979) was examined by DE and VIM culture. Eighteen of 25 (72%) adult deer were positive for trypanosomes, 11 were positive by DE and the remaining 7 infected animals were detected by blood cultures. One fetus bled in 1978, a single 1.8 kg fetus with a crown-rump length of 36 cm from a trypanosome-positive 10-year-old doe, was positive for trypanosomes by blood culture. This fetal infection demonstrated natural, transplacental transmission of trypanosomes in mule deer, a phenomenon previously not known to occur in cervids. The trypanosome presumably is *T. cervi* previously reported from mule deer[15] (Table 1).

Resurvey of Trypanosomes in Elk. Clotted blood from 100 free-ranging elk trapped on winter feedgrounds was cultured for trypanosomes in January and February, 1979. Fifty-eight of the blood samples were from elk on the National Elk Refuge feed grounds, Teton County, Wyoming; the remaining 42 blood samples were from elk on the Greys River feedgrounds, Lincoln County.

Fifteen of 31 (48%) calves, 7 of 9 (78%) yearlings, and 15 of 18 (83%) adults from the National Elk Refuge and 23 of 29 (79%) calves, 3 of 4 (75%) yearlings, and 8 of 9 (89%) adults from Greys River Feedground were positive for trypanosomes. The overall infection rates were 64% and 81%, respectively, for elk from the National Elk Refuge and Greys River Feedground.

Twelve of 22 (54%) males and 25 of 36 (69%) female elk from the National Elk Refuge were positive for trypanosomes as were 17 of 20 (85%) male and 17 of 22 (77%) female elk from the Greys River Feedground. There appear to be no significant differences in the rate of infection between males and females from either herd (Table 1).

DISCUSSION

Trypanosomes have been reported from white-tailed deer in seven southeastern states,[12] from Pennsylvania (Straneva, 1976; Pers. comm.), New York,[13] Michigan,[19] Texas[18] and Wyoming,[16] from mule deer in Colorado, New Mexico[3] and Wyoming,[10] and from elk in Michigan,[19] New Mexico[4] and Wyoming.[8] Where identification has been possible (i.e., by the recovery of bloodstream trypomastigotes for morphometric analysis and cross-transmission experiments) the trypanosomes from these hosts have been found to be conspecific.[7] They have been identified and named *Trypanosoma cervi* Kingston and Morton, 1975.

Five moose previously examined (1973-74)[16] were negative for trypanosomes. Since that time, trypanosomes have been reported from a single moose.[11] Identification of the trypanosome(s) from host species depends upon the recovery of a sufficient number of bloodstream trypomastigotes on slides to permit morphological identification and the performance of cross-transmission studies. While we do not believe the numbers of trypanosomes from moose blood on slides to be adequate for description of a new species, the four specimens seen and measured are sufficient for their analysis and comparison with *T. cervi* from other animals. The results of this analysis indicate that the values obtained from moose are not incompatible with those obtained from deer. It was concluded that moose trypanosomes are conspecific with *T. cervi* from deer.

Thus the identification of *T. cervi* in moose is consistent with the presence of this trypanosome in other species of cervids (mule deer, elk) which share the range with moose. The distribution of

moose greatly expands the geographic distribution of *T. cervi*.

The species of trypanosomes in American bison presents other problems. The only previous finding of a trypanosome in bison was by Wrublewski[21] in the wisent *(Bison bonasus)* in the forest of Bielowesch in Lithuania. The species was not identified. Hoare[5] cites this species (from Wrublewski's paper) as *T. theileri*, a common and cosmopolitan parasite of cattle *(Bos taurus)*. The forms illustrated in Wrublewski's paper are culture stages and identification from them to species is impossible. It is likely, considering the close evolutionary relationship of bovines and bison and the ubiquitous presence of *T. theileri* in bovines, that the bison were infected with this species of trypanosome. It also could be that bison harbor their own species of trypanosome, but considering the separation of the bovid and cervid families it seems unlikely that the trypanosome species in question would be *T. cervi*.

The reasons for the failure to cross-transmit *T. cervi* from moose to other cervids which were considered susceptible to this parasite are unknown. Potential hosts — mule deer fawns, elk calves and a moose calf — were considered to be free of infection prior to inoculation with moose blood but this finding may have been in error. Lack of infection in these putative hosts might also be attributed to inoculation with too few, or no, trypanosomes from the moose owing to the small size of the inoculum.

No previous records of transplacental transmission of trypanosomes are known for cervids though one elk calf, bled at 2-weeks of age (in July, 1973) was positive for trypanosome infection (Kingston, unpubl.). This latter animal could have been infected either by a horse fly vector or by transplacental transmission. Transplacental transmission of *T. theileri* has been demonstrated in bovines (Kingston, unpubl.). These and other data[5] indicate that transplacental transmission probably is not of great significance in cervids though the putative role of trypanosomes in abortion in these hosts remains to be determined.

Earlier records (1973-74) of infection of elk with trypanosomes were primarily derived by blood sampling of captive elk held at the Sybille Wildlife Research Unit. Of the total elk examined then, 79/119 (66%) were infected.[16] This is almost exactly the percentage infected among the elk of the National Elk Refuge in 1979, but lower than the prevalence of infection in the Greys River sample (81%).

Comparison of trypanosome infection rates by age showed that in the 1973-74 sample 17 to 50% of the calves were infected as contrasted with the combined prevalence of infection in elk calves in 1979 (63%) These data indicate that infection with trypanosomes is acquired early in life, probably during the first summer (<6 mo; i.e., following one summer fly season) and infections continue to be acquired as the animals age. Most elk appear to become infected by the end of their second summer (77% of yearlings). Adult elk in 1973 were between 86 and 100% infected and 85% of adults in the present sample were infected. The slight differences between prevalence of infection among 1973-74 elk and 1979 elk from western Wyoming do not reflect any significant differences in the acquisition of infection during these two periods.

Pronghorn Antelope. Previous examination by culture of blood from 60 pronghorn from Albany[16] and Laramie counties, Wyoming (unpubl.) were negative. These data strongly suggested that pronghorns were not naturally infected with trypanosomes. The finding of trypanosomes in this species of host is of particular interest because *A. americana* is the type and only species in its genus and family (Antilocapridae) and is restricted to North America.[17] The family is considered to be allied more closely to

caprine-ovine groups of bovids than to cervids.[17] The antelope trypanosome, once recovered on slides, could prove to be *T. theileri,* common in cattle in Wyoming,[14] or *T. melophagium,* known to occur in sheep in Wyoming,[20] or possibly *T. cervi,* or different from all of these.

The identity of this newly discovered antilocaprine form of trypanosome, of whatever species, may contribute to our understanding of the taxonomic position of its host. The addition of *A. americana* to the list of wild ruminants in North American harboring trypanosomes leaves only mountain sheep, *Ovis canadensis,* which have been inadequately sampled[16] and mountain goat, *Oreamnos americanus,* (Kingston, unpubl.) as the only potential hosts among ruminant game animals not known to be infected with trypanosomes. *Ovis dalli* from Alaska has been reported to be infected with trypanosomes.[2]

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