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Authors: MATTHEWS, MICHAEL J., KINGSTON, NEWTON, and MORTON, JAMIE K.

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Trypanosoma cervi KINGSTON AND MORTON, 1975 FROM MULE DEER, *Odocoileus hemionus*, IN WYOMING[1] [2]

MICHAEL J. MATTHEWS,[3] [4] NEWTON KINGSTON[5] and JAMIE K. MORTON[6]

Abstract: Mensural values of blood stream stages and cross-transmission studies defined the trypanosome species from mule deer, *Odocoileus hemionus*, as con-specific with *Trypanosoma cervi*, the trypanosome found in elk from the same locality. Trypanosomes were found in eight mule deer sampled in the spring and eight sampled in the winter in southeastern Wyoming.

INTRODUCTION

Identification of trypanosomes from cervids in North America generally has been limited to morphology of culture forms as trypomastigote stages in peripheral blood are not sufficiently numerous for study; thus precise species identification generally has not been possible. Most investigators studying the trypanosomes of white-tailed deer (*Odocoileus virginianus*)[8,9,10,11] mule deer (*Odocoileus hemionus*),[1,7] and elk (*Cervus canadensis*),[2,4,10,11] have considered them as *theileri*-like bcause their culture stages resemble *Trypanosoma theileri* Laveran, 1902.

Recently,[5] trypomastigotes from the blood of elk were described as *Trypanosoma cervi* Kingston and Morton, 1975, a species morphologically and biologically distinct from *Trypanosoma theileri*. Since then trypomastigotes have been recovered from the blood of mule deer in Wyoming[7] and are here identified from morphometric analysis and the results of cross-transmission studies as *T. cervi*, thus conspecific with the tryponosome of elk.

MATERIALS AND METHODS

In May, 1974, blood samples were taken by venipuncture from 12 mule deer held at the Sybille Game Research Unit, Wheatland, Wyoming. Trypomastigotes and other stages were recovered from the blood of eight. From December, 1974, through February, 1975, an additional eight mule deer were live-trapped at various locations in Albany and Carbon counties, Wyoming, and these deer were bled similarly. Trypomastigotes were recovered from the blood of all of these animals. Blood was processed as noted previously.[5,6,7] The spring and winter forms and their subsumed means were compared with *T. cervi* from elk using an analysis of variance.

Bovines, elk and mule deer recipients in cross transmission experiments were all culture (VIM) negative at the time of exposure to donor blood. They had been checked for up to 2 months (six negative cultures) prior to exposure. The mule deer donor blood was positive for trypanosomes on the day of inoculation into bovines. Organisms were directly observed in the plasma just above the buffy

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[3] Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071, USA; Some of the research reported here was in partial fulfillment of the requirements for the degree of Master of Science.

[4] Present address: New York State Department of Environmental Conservation, Wildlife Resources Center, Delmar, New York 12054, USA.

[5] Division of Microbiology and Veterinary Medicine, University of Wyoming.

[6] Wyoming Game and Fish Department, Research Laboratory, Laramie, Wyoming 82071, USA.

coat in microhematocrit tubes containing about 0.05 ml of blood.

Blood (5-10 ml) from some of these infected deer was inoculated into uninfected bovines and the bovines were checked by direct examination (DE)[7] and by culture (VIM)[4] periodically after exposure. The bovines were subsequently challenged with (50 ml) blood from bovines infected with *T. theileri.*

Blood (50 ml) from an infected mule deer was inoculated into each of two culture negative elk calves and the course of infection followed by VIM culture. Blood (40-45 ml) from bovines with a patent infection with *T. theileri* was inoculated into each of two culture negative mule deer fawns and (50 ml each) into each of two uninfected bovine calves, and the blood of these recipients was studied by VIM culture.

Observations on recipient blood after exposure was by direct examination of microhaematocrit tubes of blood for the presence or absence of trypanosomes and/or by culture

RESULTS

Trypanosomes recovered from the blood of mule deer in May, 1974, comprised both atypical and typical trypomastigotes (Figs. 1, 3) and epimastigotes and epimastigote-like dividing forms (Fig. 2). Trypomastigotes found in the spring often were longer and wider than the more typical forms, and possessed a blunt or rounded posterior end (Fig. 1) in contrast to the typical *Megatrypanum*-type with a long pointed posterior end. Spring forms averaged 33 μm long and 6.1 μm wide, some of the more typical short slender forms with pointed posterior ends also were seen in the spring material (Fig. 3). These forms ranged from 21-34 μm in length.

The winter material contained a smaller trypanosome with an average body length of 27 μm and a mean body width of 4.2 μm. They possessed a long, pointed posterior end typical of forms belonging to the subgenus *Megatrypanum* (Figs. 4-7).

The nucleus in both spring and winter forms is located posteriorly; the kinetoplast may vary from close to the posterior end to close to the nucleus in both spring and winter forms.

The means and ranges of the mensural values of Hoare[3] are given separately for the spring and winter trypomastigote forms and the combined means are subsumed to include the 55 specimens measured (Table 1). Data on *T. cervi* from elk are included for comparison.

Morphometric Analysis

Trypanosomes collected in spring and winter from mule deer differ in a number of characteristics. Spring forms have a significantly longer body length (BL, L), and the length of the free flagellum (FF) and free flagellum: body length ratios (FF:BL) are significantly different, as are nuclear distances from anterior (NA) and posterior ends (PN) and kinetoplast to posterior end (PK) and width values (W). Spring and winter forms were similar only in kinetoplast to nuclear distance (KN), width to body length ratios (W:BL) and in the nuclear position in the body (NI). Spring and winter collected trypanosomes apparently represent morphologically distinct populations. A comparison of the spring and winter forms of mule deer trypanosomes with *T. cervi* from elk show statistically significant differences at the 1% level for PK, PN, NA, BL and W:BL values.

No significant difference was found in the FF length in elk and mule deer trypanosomes and this is also true for FF:BL ratios (though absolute BL values are significantly different at the 1% level). Similarly, NI and KI indices are not significantly different ($P = < 0.01$) though again absolute measurements of PK, KN, PN, and NA values are significantly different at the 1% level. Thus the major differences in the trypanosomes from mule deer and from elk appear to be a function of body length and essentially these trypanosomes are not dissimilar except in this feature of body length. The basis for this host-associated body length difference in these two parasites is unknown.

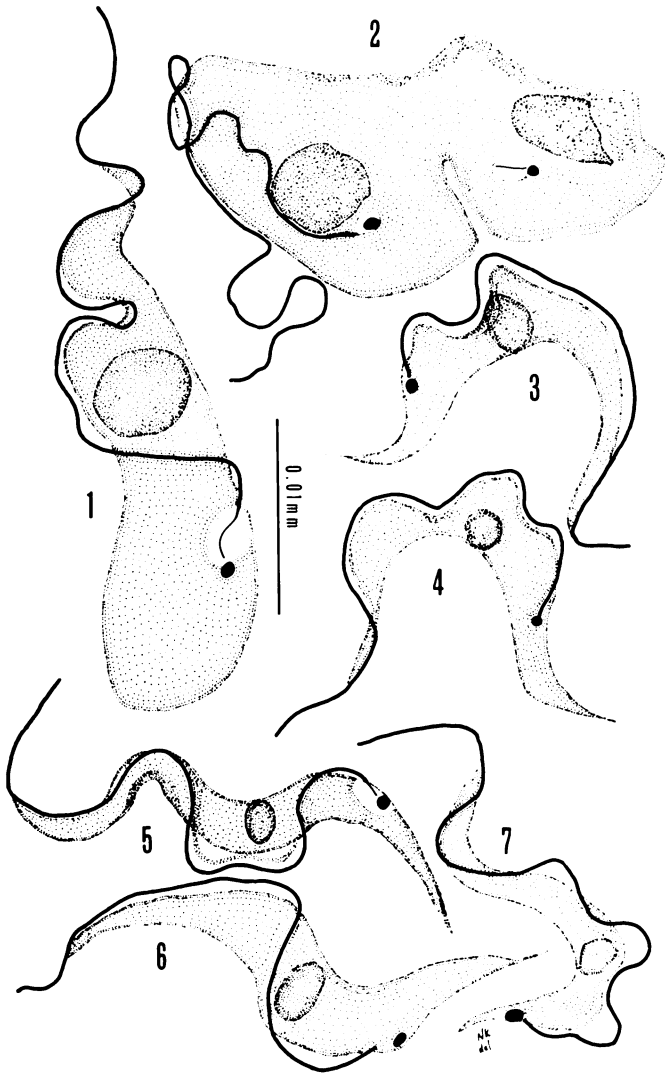

FIGURE 1. Trypomastigote from spring-sampled mule deer. Note atypical rounded posterior end. Clear space near kinetoplast is considered to be the flagellar reservoir.

FIGURE 2. Dividing form from spring-sampled mule deer. Two nuclei and two kinetoplasts present.

FIGURE 3. Typical **Megatrypanum**-type trypomastigote from spring-sampled mule deer with pointed posterior end and short free flagellum.

FIGURE 4 to 7. Typical **Megatrypanum**-type trypomastigotes from winter-sampled mule deer. The relatively short free flagellum is characteristic of **Trypanosoma cervi**.

TABLE 1. Measurements (in micrometers) of trypanosomes from mule deer and from elk.

		PK	KN	PN	NA	BL	FF	L	FF:BL	W	NI	KI	W:BL
MULE DEER:													
Spring and Winter forms	mean	7.5	5.8	13.1	17	30.3	7	37.3	1:6	5.2	0.79	2.4	1:6.6
	n=55												
Spring forms	mean	9.2	5.4	14.6	18.4	33.1	5.7	38.8	1:8.2	6.1	0.8	2.9	1:6
	range	3-15	2-13	10-19	12-26	26-42	1-14	28-51	1:2.5-27	2-9	0.4-1.3	1.2-5.5	1:3-13
	n=28												
Winter forms	mean	5.7	6	11.5	15.5	27.4	8.2	35.5	1:3.8	4.2	0.7	1.9	1:7.1
	range	3-11	4-8	8-18	10-22	21-34	0-16	26-42	1:1.5-8.3	2-8	0.5-1.2	1.2-3.2	1:3.2-12.5
	n=27												
Elk: *Trypanosoma cervi**													
	mean	12.2	7	19.4	24.8	45.4	6.6	52	1:6.9	4.6	0.78	2.8	1:9.8
	range	5-20	4-9	11-32	20-30	32-56	3-11	40-61		3-8	0.5-1.3	2.8-3.6	1:7.6-13.3
	n=14												

*From Kingston and Morton, 1975

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.

TABLE 2. Cross-Transmission Experiments.

Recipient	Donor. Size of inoculum: blood/diluent—route	Date of exposure	Days examined after exposure and results	Challenged: Days after exposure: donor (Size inoculum)	Examination: Days after exposure and results
BOVINE					
#2082 A,F	Mule deer A,M 5 ml/5 ml IV	17 II 75	7,9,11,14,18,23[c] all negative	1) 33; Bov. #3103 (15/50) 2) 93; Bov. #38 (50/50)	1) 9,11,18; all negative 2) 4,6,8,12,14,16,19; all positive
#1048 A,F	Mule deer A,F 10 ml/10 ml IV	28 11 75	3,7,12,22; all neg. 31[c] positive; 33,40 negative	49; Bov. #82 (32.5/32.5)	7,12 positive 18 negative
#9126 A,F	Mule deer A,F 50 ml/50 ml IV	8 V 75	4[c],7[c]; negative; 11 positive; 13[c],17[c],19[c] 21[c],25[c],29[c]; all C negative	36; Bov. #44 (50/50)	4,6 positive
ELK					
#CaE-1 Y, M	Mule deer A,F 50 ml/50 ml IV	8 V 75	4[c],9[c],13[c],16[c],20[c],21[c],29[c], 31[c]; all DE neg.; all C pos.	31; Elk # E-37 (50/50)	6[c]+, 10[c]−, 14[c]+, 17[c]+
#CaE-6 Y, F	Mule deer A,F 50 ml/50 ml IV	8 V 75	4[c], 9[c], 13[c], 16[c]; all DE neg. 20[c]; DE pos.; 21[c], 29[c], 31[c]; all C positive	31; Elk # E-47 (50/50)	6,10,14,17; all DE and C negative
MULE DEER					
#41 M, Fawn	Bov. #50 Calf 18 ml/18 ml IV 27 ml/27 ml SQ	4 XI 75	3, 25, 33, 40 all C neg.		
#10 F, Fawn	Bov. #50 Calf 40/40	4 XI 75	3, 25, 33, 40 all C neg.		

DE = Direct Examination (see text)
C or [c] = Culture
Y = Yearling; A = Adult; M = Male; F = Female
All donors positive on day of inoculation

Cross-Transmission Experiments

The results of the cross-transmission experiments involving inoculation of infected mule deer blood into bovines and elk calves, and inoculation of bovine blood infected with *T. theileri* into mule deer fawns, are summarized in Table 2.

The (mainly) negative results of the exposure of susceptible bovines to trypanosomes from mule deer support the contention that these trypanosomes differ in their cross-transmission characteristics from *T. theileri*. In two of the three bovines (1048 and 9126) trypanosomes were seen in the bovine blood (DE) after exposure. In the first animal two trypanosomes were seen on day 31 following exposure and not thereafter; cultures of bovine blood were negative on this day and all other days. In the second animal, a single trypanosome was seen on day 11 but not before or after and all cultures were negative. These two observations were probably of vagrant trypanosomes from mule deer which had been maintained but which were not reproducing in the blood and were observed by chance. This argument is supported by the fact that both animals were susceptible to challenge with infected bovine blood, one becoming positive on day 4 and the other on day 7 following exposure to the bovine trypanosome.

It is not understood why one bovine (2082) did not respond positively to challenge with trypanosomes from a bovine on day 33 (following exposure to bloodstream trypanosomes from mule deer); however she became positive when challenged 93 days after the primary mule deer exposure with the challenge infection running a normal course for acute bovine trypanosomiasis (D. Matthews and N. Kingston, unpublished data).

Elk calf exposures (CaE-1 and CaE-6, both yearlings-Y) to trypanosomes from mule deer resulted in a generally subpatent (non-DE+except for CaE-6 on day 20) infection which however was detectable in both animals by culture on day 4 and extending to day 31 (and possible longer in one animal, CaE-1). The apparent positive response by CaE-1 to challenge with trypanosomes from elk may be but the continuation of the mule deer infection. Inasmuch as cultures are read 1 to 3 weeks after blood collection it is not known if this elk recipient was infected on day 31 when challenged. Elk CaE-6 was negative to *T. cervi* when challenged with elk blood.

The mule deer exposures to *T. theileri* from a bovine were equivocal. These animals were not detected as being positive but, for various reasons, were not examined during the critical period between 4 to 20 days after exposure. Two challenge calves (48 and 49) given 50 ml of blood each from the same donor (Bovine 50) on the same day as the mule deer each became positive. These exposures should be repeated in other deer.

DISCUSSION

Epimastigotes and dividing stages of trypanosomes apparently are infrequent in the circulating blood of ruminants though these stages have been reported in the peripheral blood of bovines infected with trypanosomes.[3] Such stages as seen in the blood of mule deer in the spring may be associated either 1) with transmission by some putative arthropod vector at this season, or 2) a spontaneous-recrudesence due to seasonal or other stress of the deer. Further work on this phenomenon seems warranted.

The level of parasitemia seen in both spring and winter mule deer was much higher than we have noted previously in either elk or bovines. In these latter hosts we do not see trypanosomes on direct examination of centrifuged blood though they may be found following double concentration. This finding may explain the subpatent infections (detected by culture only) noted in elk calf recipients of mule deer trypanosomes.

From the close resemblance of the mule deer and elk trypanosomes and from the (limited) cross-transmission data, we believe that we are dealing with one species of trypanosome in these two host species, with the mule deer constituting a new host record for *Trypanosoma cervi*.

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