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Experimental Transmission of *Trypanosoma theileri* to Bison[1]

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ABSTRACT: Bloodstream trypomastigotes were recovered from a bison experimentally exposed to cryogenically preserved culture forms of *Trypanosoma theileri* of bovine origin. The bison trypomastigotes were compared statistically with bloodstream *Trypanosoma theileri* trypomastigotes from cattle and bloodstream *Trypanosoma cervi* trypomastigotes from North American deer. The trypanosomes from bison retained the morphological features of *T. theileri* suggesting that cattle and bison share this parasite.

Wrublewski (1908) first reported trypanosomes from bison; he found these parasites in blood cultures from the wisent, or European bison, *Bison bonasus* (L.), in Puszcza Białowieska in eastern Poland. Trypanosomes were reported in North American bison, *Bison bison* (L.), in 1981 (Kingston et al., 1981). Clotted blood samples were collected in 1979 from 39 bison that were imported into Wyoming. Samples were cultured in veal infusion medium (VIM) and eight (20.5%) were positive for trypanosomes. Culture forms were grown in cell culture through several passages using the procedures of McHolland-Raymond et al. (1978) and frozen at −70°C. Attempts to recover bloodstream trypomastigotes by the concentration methods of Kingston and Morton (1975a) and Kingston and Crum (1977) from four of the known positive animals failed. Of 79 other bison from various herds examined in Wyoming between 1979 and 1982, 2 animals were positive by VIM culture. In 1983 blood samples from 73 additional bison in Albany County, Wyoming were examined by the direct method and VIM culture. Results of these studies are reported herein. Also, in the spring of 1983, 3 yearling bison were purchased from the Durham Meat Company, Gillette County, Wyoming in order to infect them with cryopreserved material and recover bloodstream stages of bison trypanosomes. This report details the results of this experiment.

Materials and Methods

Two yearling bison and 2 bovine calves were inoculated IV per jugular with 8th passage, cryogenically preserved, culture-form bison trypanosomes obtained from the initial 1979 isolation. One yearling bison and 1 bovine calf were inoculated with cryogenically preserved, culture-form *Trypanosoma theileri* Laveran, 1902, 3rd isolate, passage 23, of bovine origin (McHolland-Raymond et al., 1978). Inoculum size was 5×10^6 trypanosomes/animal. Animals were bled on day 4 following exposure and periodically thereafter. Blood was cultured (VIM) and centrifuged blood was examined directly (DE) in microhematocrit tubes for the presence of swimming trypanosomes. Positive microhematocrit tubes were scored, broken, and thin films prepared of some of the contents (Kingston and Crum, 1977; Kingston et al., 1985). The slides were fixed, stained with Giemsa, and scanned for trypanosomes. When found these were photographed on 35-mm transparency film. Transparencies were projected and the trypanosomes traced; tracings were measured using a calibrated map-wheel reader. Measurements were analyzed and compared, using a computer programmed one-way ANOVA, with similar mensural values derived from trypanosomes from bovines (Matthews et al., 1979; McKenzie and Kingston, unpubl. data) and values derived from trypanosomes from North American Cervidae (Kingston et al., 1985).

Forty-four ml of heparinized blood that contained about 400 trypanosomes per ml from a positive experimental bison (#23) were collected (day 11 PI) and one-half inoculated IV per jugular into a bison and one-half into a bovine calf.

Hematocrit (HT) and hemoglobin (Hb) determinations, total RBC, WBC, and differential counts were carried out on the animals studied.

One positive bison and 1 positive bovine calf were

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Table 1. Results of trypanosome inoculation of bison and cattle, including subinoculation (S.I.).

Inoculum	Preinoculation culture	Recipient #	Results DE day examined							
			4	7	11	12	17	21	25	28
Cultured bison trypanosomes	–	Bis 21	–	–	–	S.I.	–	–	+	–
	–	Bov 473	–	–	–		–	ND	ND	–
	–	Bis 22	–	–	–		–	ND	ND	–
	–	Bov 198	–	–	–	S.I.	–	–	–	–
Cultured bovine trypanosomes	–	Bis 23	+	+	+		–	ND	ND	–N
(*Trypanosoma theileri*)	–	Bov 541	+	–	–		–	ND	ND	–N

N = necropsied.
ND = not done.

killed for tissue collection; necropsies were performed immediately following. Tissue imprints were made and tissues preserved and routinely handled for histopathological examination.

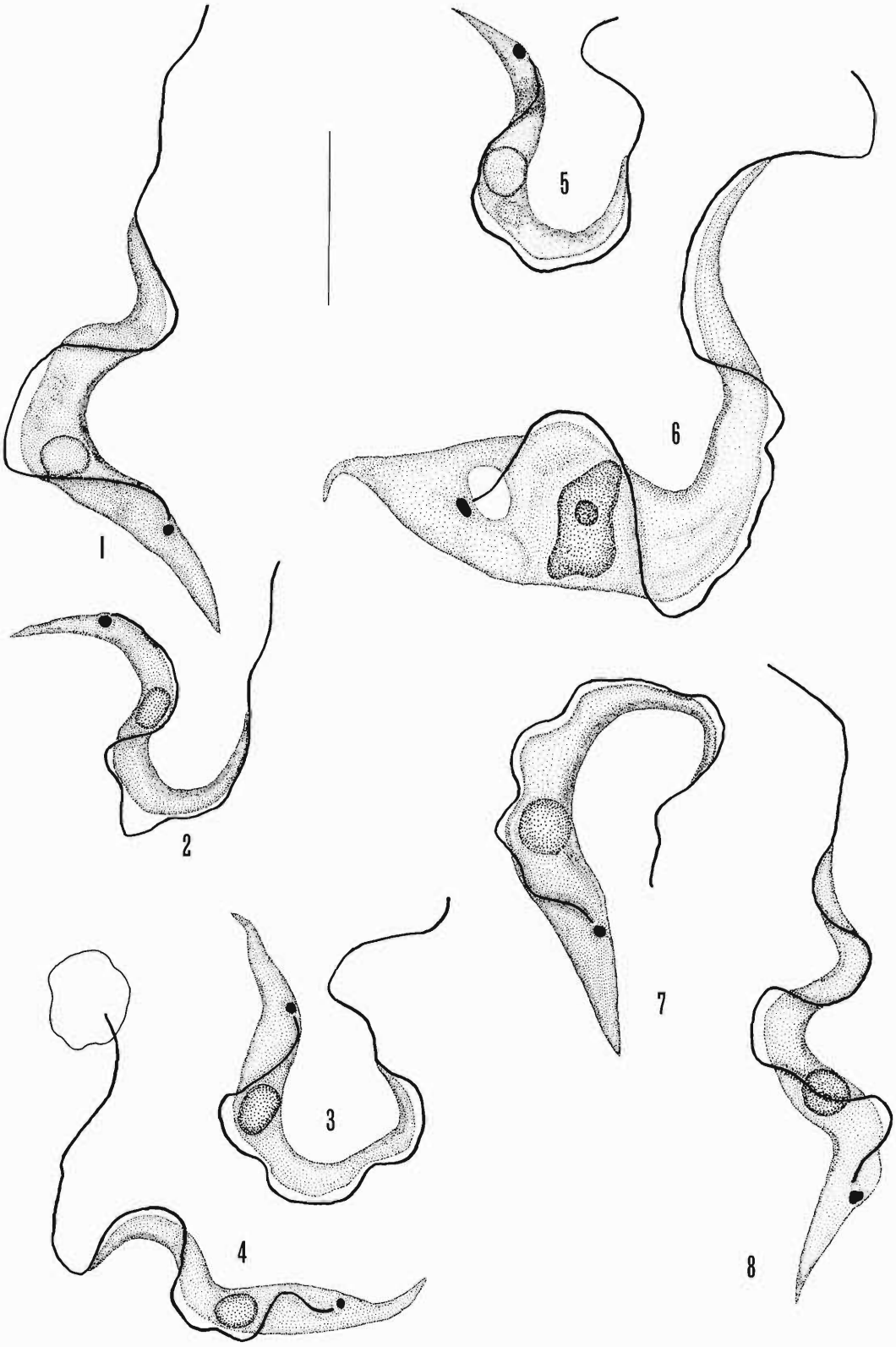
Results

TRANSFER EXPERIMENTS: The 2 yearling bison and the 2 bovine calves that received bison culture trypanosomes were uniformly negative when examined by DE and by VIM culture beginning on day 4 PI and continuing through day 17 PI (maximum of 4 examinations). The yearling bison (#23) that received bovine culture trypanosomes became patent on day 4 PI with ca. 90 trypanosomes seen per ml. On day 7 ca. 1,200 trypanosomes per ml were present and the infection declined on day 11 when there were about 400 trypanosomes per ml. No trypanosomes were observed thereafter. The bovine calf (#541) that received bovine culture trypanosomes was seen to be positive with ca. 10 trypanosomes per ml only on day 4 PI (Table 1). About 45 ml of heparinized blood from the positive bison was collected on day 11 PI and half of this amount was inoculated into 1 of the bison (#21) previously exposed to bison trypanosomes and half into a bovine calf (#198) previously exposed to bison trypanosomes. The bison (#21) became positive on day 13 PI (5 trypanosomes/ml) but infection was not detected thereafter; the bovine calf (#198) similarly exposed did not become patent (Table 1). Preinoculation cultures (VIM) were negative for all animals.

TRYPANOSOMES RECOVERED FROM BISON BLOOD: Trypanosomes were recovered from the blood of the bison (#23) (Figs. 1–8) inoculated with cultured *T. theileri* of bovine origin. Measurements of 27 trypanosomes were made and compared with bloodstream bovine *T. theileri* and bloodstream cervid *Trypanosoma cervi* (Table 2). Greater similarities were noted between trypanosomes recovered from cattle and bison than between trypanosomes recovered from the latter host and deer. Kinetoplast indices (KI) derived from trypanosomes from cattle and bison were identical (1.9) and varied widely from that value for deer trypanosomes (2.7). Comparisons of free flagellum to body length ratios (FF:BL) showed bison and cattle to be closely associated (1:2.4 and 1:2.8, respectively) and distinctly different from deer (1:6.1). Other values measured showed similar correspondence between bison and cattle trypanosomes and differences with deer trypanosomes. Measurement data for each mensural value showed an essentially normal distribution when comparing bison, cattle, and deer trypanosomes.

TISSUE IMPRINTS FROM BISON (#23): Examination of Giemsa-stained imprints of spleen, liver, brain, kidney, pituitary, and lymph nodes (hepatic, mediastinal, tracheobronchial, and mesenteric) revealed no trypanosomes.

HISTOPATHOLOGICAL EXAMINATION, BISON (#23): Sections of lung were characterized by peribronchial cuffing and submucosal infiltration of lymphocytes, plasma cells, and macrophages; bronchial and bronchiolar epithelial hyperplasia; and mild hypertrophy of bronchial smooth muscle. Scattered airways contained neutrophils and necrotic debris. Lungworms were found in bronchi on gross examination. Neutrophils were numerous in the splenic pulp and surrounding germinal centers and lymphoid follicles. Lymphoid tissue in spleen and all lymph nodes were hyperplastic and germinal centers were active. Significant lesions were not observed in liver, kidney, adrenal, ovary, uterus, and heart. Try-



panosomes were not observed in any tissue section. Acute suppurative splenitis, lymphoid hyperplasia of the spleen and lymph nodes, and mild verminous bronchitis were diagnosed.

TISSUE IMPRINTS FROM BOVINE CALF (#541): Trypanosomes were not seen in tissue imprints (made from the same organs examined as noted above) from the trypanosome-positive bovine calf.

HISTOPATHOLOGICAL EXAMINATION, BOVINE (#541): Lymphocytes lightly cuffed many airways in the lung and occasional lymphoid follicles were present. Lymph nodes had thick active cortices and many germinal centers. Other tissues were essentially normal. No trypanosomes were seen in histological sections of tissues. Lymphoid hyperplasia was diagnosed.

HEMATOLOGIC EXAMINATION—RBC, PCV AND HB: There was a decline of ~33% (day 21 PI) in the number of RBC's in the trypanosome infected bison (#23). The RBC count rose thereafter to a more normal level. Packed cell volumes (PCV) paralleled RBC counts in the infected bison (#23) and dropped 26% by day 11 PI. This was followed by a return to the day 0 value by day 28 PI. Erythrocytic parameters remained constant in the other bison and bovines.

WBC: White blood cell (WBC) counts decreased 47.5% ($4.0 \times 10^3/mm^3$ to $1.9 \times 10^3/mm^3$) in the first infected bison (#23) during the 11-day period when trypanosomes could be recovered (DE) from the blood of this animal. The leucopenia then reversed and WBC's increased sharply to $11.0 \times 10^3/mm^3$ by day 21. By day 28 PI the numbers of WBC's had declined to approximately normal values. WBC numbers in the challenge bison (#21) showed only a moderate decline (19%) on day 25 PI following inoculation and this decline was reversed at the termination of the experiment. The WBC response in the infected bovine calf (#541) was markedly different from that seen in the bison (#23) with the numbers of WBC's fluctuating sharply upwards through day 17 and returning to approximately normal by day 28.

DIFFERENTIAL WHITE BLOOD CELL COUNTS: Differential WBC counts in the trypanosome-infected animals remained relatively unchanged throughout infection except that the bovines exhibited a 21–27% increase in the number of lymphocytes between 4–11 days PI.

SURVEY OF BISON FOR TRYPANOSOMES, ALBANY COUNTY, WYOMING (1983): Examination (VIM) of blood samples from 73 bison in Albany County, Wyoming in late 1983 revealed 26 (35.6%) infected with trypanosomes; no trypanosomes were recovered by DE. Twelve bulls (46%) and 14 cows (54%) were infected. They were 1–2 years of age with the majority (85%) being yearlings.

Discussion

Previous reports (Kingston et al., 1981) refer to trypanosomes in North American bison as *Trypanosoma* sp. because culture forms only were examined and it is not possible to use such material for species identification (Trager, 1975). The trypanosomes recovered from European bison were designated *T. wrublewskii* (Vladimirov and Yakimov, 1908). Yakimov (1915) concluded later that the parasite was actually *T. theileri.*

The present report provides additional information on the identity of trypanosomes from bison. These trypanosomes were of bovine origin. Measurements of specimens (27) from the acutely infected bison when compared with the measurements of (304) trypanosomes from cattle (*Bos taurus*) and (174) trypanosomes from North American cervids indicate that the bison trypanosomes retain *T. theileri* morphology after passage through bison, especially with regard to flagellar length (FF), flagellar length to body length ratios (FF:BL), and kinetoplast index (KI) (Table 2).

Trypanosomes of the stercorarian subgenera *Megatrypanum* and *Herpetomonas* are considered to be relatively to strictly host specific (e.g., *T. melophagium* in sheep and *T. lewisi* in rat). This feature has been used in the identification of such trypanosomes (Hoare, 1972) even when bloodstream stages have not been available. The

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Figures 1–8. Representative bloodstream trypomastigotes from bison (#23) experimentally infected with *Trypanosoma theileri* of bovine origin. 1. BL 30, FF 13, FF:BL 1:2.05, W 4, KI 1:2.2. 2. BL 25, FF 9, FF:BL 1:2.8, W 2.5, KI 1:1.8. 3. BL 28, FF 15, FF:BL 1:1.9, W 3, KI 1:1.9. 4. BL 23, FF 17, FF:BL 1:1.4, W 3.5, KI 1:1.8. 5. BL 23, FF 11.5, FF:BL 1:2.0, W 2.5, KI 1:1.6. 6. BL 47, FF 11, FF:BL 1:4.3, W 9, KI 1:2.4. Largest form seen, considered predivision stage. 7. BL 30, FF 7, FF:BL 1:4.2, W 3.5, KI 1:2.2. 8. BL 30, FF 12, FF:BL 1:2.5, W 3, KI 1:2.3. Approximately average trypomastigote. See legend for Table 2 for definition of abbreviations used here.

Table 2. Comparison of mensural values of bloodstream trypomastigotes recovered from bison and *Trypanosoma theileri* from bovines and *T. cervi* from Cervidae in North America.

	PK*	KN	PN	NA	BL	FF	L	W	FF:BL	NI	KI
Bison	5.7	6.4	12.4	15.9	28.0	12.4	40.3	3.1	1:2.43	0.8	1.9
$N = 27$											
SD	2.3	0.97	2.5	3.9	6.0	3.1	6.5	1.5	0.92	0.2	0.4
Range	2–11	4–9	8–18	9–29	17–47	7–17	24–58	2–9	1:1.35–4.71	0.542–1.143	1.286–3
Bovine	7.4	8.9	16.2	20.2	36.4	14.2	50.5	3.3	1:2.8	0.8	1.9
$N = 304$											
SD	3.3	2.6	5.1	6.3	10.5	4.5	12.7	2.0	2.3	0.2	0.4
Range	0–17	2–20	5–33	7–36	13–59	1–37	16–90	1–13	1:0.89–39.0	0.4–1.7	1–4
All deer†	11.5	7.1	18.5	23.3	42.0	8.2	50.1	5.5	1:6.1	0.8	2.7
$N = 174$											
SD	5.6	2.1	6.3	7.3	12.4	3.4	13.6	2.5	3.8	0.2	0.9
Range	3–27	2–14	8–36	10–43	21–74	1–21	26–83	1–15	1:0–27	0.4–1.6	1.2–7

* PK = posterior end to kinetoplast distance, KN = kinetoplast-to-nucleus distance, PN = posterior end-to-nucleus distance, NA = nucleus-to-anterior end distance, BL = body length, FF = length of free flagellum, L = overall length, FF:BL = free flagellum to body length ratio, W = width, NI = PN/NA (nuclear index).

† All deer, see Kingston et al. (1985).

course of infection in the bison was similar to that seen in experimentally infected bovines (Matthews et al., 1979) differing mainly in the brevity of infection (trypanosomes not detected in blood of bison #23 after 11 days PI) compared with experimental infections in the bovine (where peak infections were achieved on day 12 PI and were terminated on days 20–26 PI) (Matthews et al., 1979). Differences in duration of infection of *T. theileri* in these 2 hosts might result because the bison is an unusual host for this parasite, of this strain of parasite, or to other undiscernible factors. On reflection, infection of bison with *T. theileri* of bovine origin might be expected. The 2 hosts are closely related members of the Bovidae. This is indicated by reproduction between cattle and bison, and some mammalogists place bison in the genus *Bos* along with cattle. Although it is not possible to state precisely that trypanosomes from naturally infected bison are, actually, *T. theileri,* it is highly probable that bison and cattle share this parasite.

The lack of infectivity of trypanosomes of bison origin to bison yearlings or bovine calves cannot be presently explained. The trypanosomes inoculated were alive, but had been cryogenically preserved from 1979 to the time of their use in 1983 when they were re-cultured (McHolland-Raymond et al., 1978) to provide the numbers used in this experiment. These trypanosomes appeared to have lost infectivity, which may be inferred from the fact that bison #21 became patent when challenged with trypanosomes from bison #23. A transitory infection was detected in bison #21 on day 13 PI, but the low intensity of infection and its delayed patency may be ascribed to the small numbers of trypanosomes inoculated (about 9,000 total to each of the 2 recipients) or the anamnestic response of bison #21 to *T. theileri* organisms after exposure to bison trypanosomes. This would suggest that the 2 organisms share similar antigenic determinants.

If, indeed, bison trypanosomes are *Trypanosoma theileri* Laveran, 1902 then, Wrublewski (1908) was remarkably astute in assigning the culture forms he observed from the wisent to that species.

The effect of trypanosomes on the infected bison (#23) appears to be reflected in an anemia and a transitory leucopenia, the significance of which is unknown.

The histological examination revealed immune activation in both bison #23 and bovine calf #541. No trypanosomes were found in any organs, but the neutrophil infiltration in the spleen of bison #23 suggests splenic involvement during infection perhaps similar to that seen in elk (Kingston and Morton, 1975b).

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Obituary

GERTRUDE E. MCINTOSH
November 1, 1905–April 30, 1986
Elected Member January 20, 1934

The Allen and Gertrude McIntosh Biology Student Enrichment Fund
Belmont Abbey College
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Mrs. Gertrude E. McIntosh, wife of Allen McIntosh (Member 1930–1977), established the memorial fund at the Belmont Abbey College in 1983. The fund has now been renamed.