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TRYPANOSOMIASIS IN WOODLAND CARIBOU OF NORTHERN ALBERTA

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ABSTRACT: Blood was collected from 49 adult woodland caribou (*Rangifer tarandus caribou*) captured in northern Alberta, Canada, from October to February, 1991 to 1992 and 1992 to 1993. Smears of the buffy coat layer and of whole blood were examined microscopically for *Trypanosoma* sp., and blood was cultured for latent parasites. Trypanosomes were present in 41 (84%) of 49 cultures 9 days or older, but none was detected in fresh blood. Trypanosomes were pleomorphic, consisting of small oval amastigotes, 2 to 8 μm, intermediate-size epimastigotes, 20 to 30 μm in total length (including the flagellum), and large trypomastigotes, 60 to 90 μm length, with pointed ends, a well developed kinetoplast, a long free flagellum, and a prominent undulating membrane. Dividing epimastigotes appeared in pairs or rosettes of five or more organisms. Based on culture characteristics and morphologic features, the organism was identified as *Trypanosoma* (*Megatrypanum*) sp.

Key words: Caribou, *Rangifer tarandus*, *Trypanosoma* sp., survey.

INTRODUCTION

Trypanosoma spp. are universal protozoan parasites with representative species infecting nearly all vertebrate species (Hoare, 1972), but are yet to be reported and described in woodland caribou (*Rangifer tarandus caribou*). In susceptible animals, trypanosomiasis is a wasting disease which results in major losses in cattle in the subtropics (Mulligan, 1970). The disease is caused by trypanosomes which are transmitted by the bite of a blood sucking arthropod (Soulsby, 1982). The stercorarian trypanosome of the subgenus *Megatrypanum*, *Trypanosoma theileri* Leveran, 1902, is a ubiquitous but relatively harmless blood parasite of bovids (Herbert, 1964; Cross et al., 1971). Detailed ultrastructural observations have been described for developmental stages of *Trypanosoma theileri* (Moulton and Kraus, 1972).

Megatrypanum trypanosomes have been recovered by culturing blood from many North American cervids including elk (*Cervus canadensis*) (Kingston and Morton, 1973), white-tailed deer (*Odocoileus virginianus*) (Stuht, 1975), and mule deer (*Odocoileus hemionus*) (Clark, 1972). The recovered trypanosomes were considered by these respective authors to be similar to the bovine parasite *T. theileri*. However, blood stream trypomastigotes from elk blood could not be transmitted to bovids, and were sufficiently different in morphological attributes to warrant designation as a new species, *Trypanosoma cervi* Kingston and Morton, 1975 (Kingston and Morton, 1975).

Trypanosoma cervi Kingston and Morton, 1975, has been identified in North America from free ranging elk (Kingston et al., 1979), mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), moose (*Alces alces*) (Kingston et al., 1985), and reindeer (*Rangifer tarandus* L.) (Kingston et al., 1982a). Like other members of the subgenus *Megatrypanum*, *T. cervi* is usually nonpathogenic (Kingston, 1981).

Trypanosoma sp. has been recovered by culturing blood from black-tailed deer (*Odocoileus hemionus columbianus*) (Morton and Kingston, 1976), pronghorn antelope (*Antilocapra americana*) (Kingston et al., 1981), and from bison (*Bison bison* L.) (Kingston et al., 1981). Kingston et al. (1986), using comparison measurements of bison blood stream trypomastigotes and results of transfer experiments, later concluded that the bison blood stream trypanosomes were of bovine origin.

Our objective was to determine the prevalence, culture characteristics, and morphologic features of the *Trypanosoma* sp. recovered from cultures of blood from woodland caribou of northern Alberta, Canada.

MATERIALS AND METHODS

Adult free ranging woodland caribou were captured from three areas in northern Alberta, during October to February 1991 to 1992 and 1992 to 1993. Animals were obtained near Mariana Lake (55°30′N to 56°15′N, 111°15′W to 112°35′W), from an area adjacent to Pelican River (55°10′N to 55°40′N, 112°42′W to 113°30′W), and from the Grande Cache (53°40′N to 54°20′N, 119°20′W to 120°0′W) area. The animals were captured with an aerial net gun (Barrett et al., 1982), following a 1 to 3 min helicopter pursuit.

Blood was drawn from the superficial digital vein of either of the forelimbs, using 18 gauge needles. Approximately 7 ml of blood was collected into each of two Vacutainer tubes (Becton Dickson and Co., Rutherford, New Jersey, USA) containing either heparin or potassium ethylenediaminetetraacetate (EDTA-K_3). Blood smears were prepared from the EDTA blood in a field laboratory within 6 hr following collection. The smears were air-dried and fixed with CytoPrep spray (Fischer Scientific Co., Edmonton, Alberta, Canada). The blood tubes were wrapped in paper towels and transported in well insulated coolers containing ice packs to the pathology laboratory in the Alberta Research Council, Vegreville, Alberta, Canada.

To propagate parasites, blood was cultured in 25 ml Nunclon tissue culture dishes (Delta Inter Med, Roskilde, Denmark), using the method of Moorehead et al. (1960) with the following modifications. Briefly, 0.2 ml fresh heparinized blood was added to 10 ml of Media 199 with Earle's salts (Gibco BRL, Life Technologies Inc., Grand Island, New York, USA), containing 20% heat inactivated fetal bovine serum, and 0.2 ml Phytohemagglutinin-M (Gibco BRL). The cultures were incubated at 37 C for up to 21 days. Blood cultures were examined on alternate days using an inverted light microscope at 400× magnification. In addition, samples of EDTA blood were maintained in sterile condition for 42 days, at 22 to 24 C. To obtain buffy coats, samples (fresh blood and weekly from cultures) were centrifuged at 1,000 × *G* for 10 min. The layer of white blood cells (buffy coat) which formed between the serum and the pellet of red cells was collected.

Unstained wet mounts were prepared from drops of blood cultures and buffy coats and examined on a phase contrast microscope. Smears prepared in the field from fresh blood, and smears of blood cultures and of buffy coats, were stained with AJP Pack® Wright Giemsa (Ingram and Bell, London, Ontario, Canada), on an Ames Hema-Tek® slide stainer (Miles Scientific Canada Inc., Richmond, British Columbia, Canada), and examined at 1,000× magnification under oil immersion.

Samples of buffy coats and blood cultures positive for parasites on light microscopic examination were prepared for transmission electron microscopy (TEM). The samples were fixed in a mixture of 1.5% glutaraldehyde and 1.0% formaldehyde in 0.12M phosphate buffer (pH 7.4) at 4 C, for 24 hr (Karnovsky, 1965) and then centrifuged at 1,200 × *G* for 10 min. The resultant pellets washed in phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr® resin (J.B.E.M., Dorval, Quebec, Canada (Hayat, 1970). Ultrathin (90 to 120 nm) sections were cut and stained with 4% aqueous uranyl acetate and Reynold's lead citrate (Reynolds, 1963) and examined with a Hitachi H-600 transmission electron microscope (Hitachi Ltd., Tokyo, Japan). For scanning electron microscopy (SEM), a portion of the ethanol dehydrated specimens was filtered on to a 3 μm SPI® silver membrane (SPI Supplies, Division of EMicron Research Ltd., Toronto, Ontario, Canada), which was then critical-point dried, sputter-coated with gold (Bozzola and Russell, 1992) and examined on a Hitachi S-510 scanning electron microscope (Hitachi Ltd.).

RESULTS

Trypanosomes were observed in cultured blood samples from 41 (84%) of 49 animals from the three locations; 21 of 24 animals from Mariana Lakes, five of eight animals from Pelican River and 15 of 17 animals from Grande Cache.

Trypanosomes were not seen in fresh blood smears or buffy coats made from fresh blood, but many appeared after 4 days in blood cultures incubated at 37 C, and after 7 days in whole blood kept at 22 to 24 C. Developmental stages included small, round forms (amastigotes or sphaeromastigotes), intermediate-sized oval forms (epimastigotes), and large, freely motile, leaf-like trypomastigotes. An undulating membrane was present on many

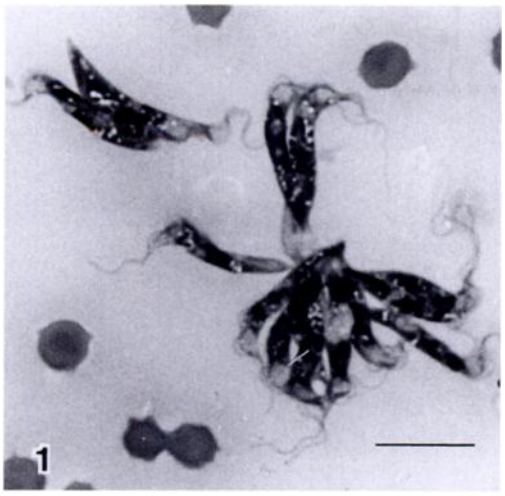


FIGURE 1. Smear of the buffy coat from cultured blood of woodland caribou with dividing epimastigotes in pairs and in a rosette. Wright Giemsa stain. Bar = 10 μm.

organisms, evident by their rapid and erratic movement in the wet mount preparations. Dividing forms were predominantly large epimastigotes. Divisions occurred by longitudinal fission and many organisms were present in pairs or in clusters (rosettes) of five or more (Fig. 1).

Using Wright Giemsa stain, cultured trypomastigotes were elongate and spindle-shaped, 60 to 90 μm in length (exclusive of the free flagellum), and pointed both at the anterior and posterior ends of the body. They had pale blue granular cytoplasm and oval, dark purple nuclei, usually located centrally or slightly posteriorly. A darkly staining kinetoplast was consistently situated in the posterior half of the organism, closer to the nucleus than to the body end. A well-developed flagellum originated at the level of the kinetoplast, followed the edge of the prominent undulating membrane to the anterior tip where it extended into a 20 to 30 μm free portion.

Ultrastructurally, amastigotes were round or oval, 2 to 4 μm in diameter with a distinct dark nucleus and less dense cytoplasm containing a small dark kinetoplast (Fig. 2a). The flagellum was usually

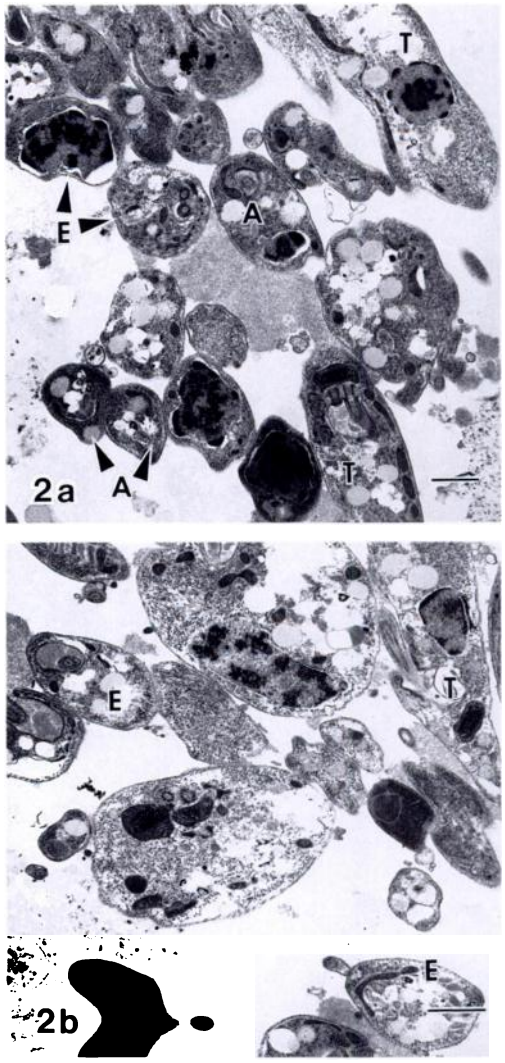
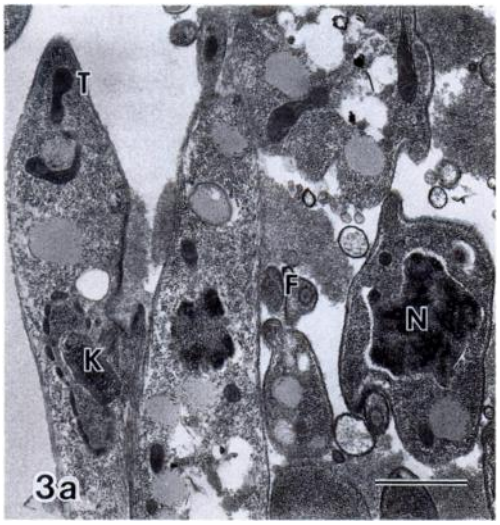


FIGURE 2a, b. Transmission electron micrograph of trypanosomes from woodland caribou blood incubated for 28 days at 22 to 24 C. Developmental stages include amastigotes (A), epimastigotes (E) and trypomastigotes (T). Bar = 1 μm.

absent or was represented by a short fibril in the cytoplasm. Epimastigotes were ovoid or elongated (Fig. 2b), 20 to 30 μm in body length. They had a granular cytoplasm, a dark round nucleus, and an anteriorly located kinetoplast near which arose the flagellum (Fig. 3b) from the flagellar pocket (Fig. 4a). The flagellum emerged from the side of the body along a shallow, undulating membrane and ended in a short free portion 5 to 10 μm long.



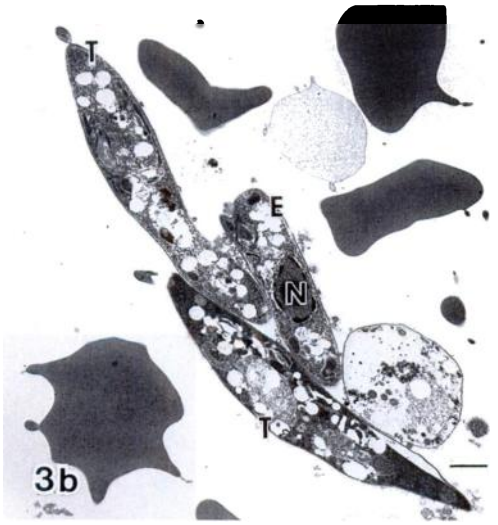
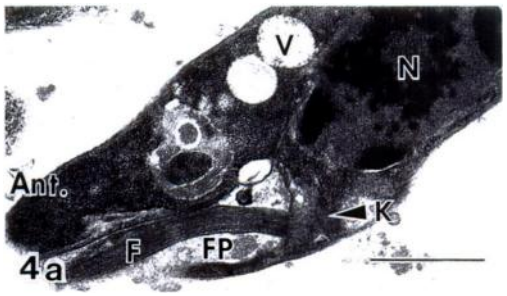


FIGURE 3a, b. Transmission electron micrograph of trypanosomes from woodland caribou blood incubated for 28 days at 22 to 24 C with details of epimastigotes (E) and trypomastigotes (T). N, nucleus; F, flagellum; K, kinetoplast. Bar = 1 μm.



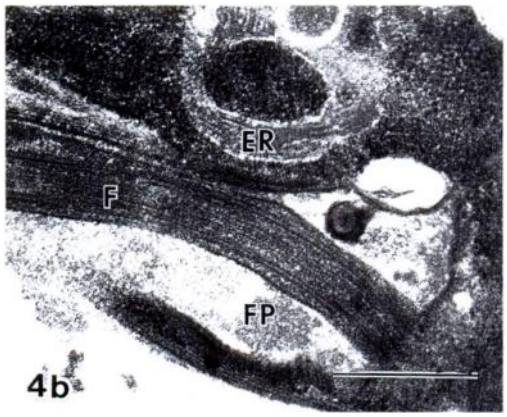


FIGURE 4. a. Transmission electron micrograph of the epimastigote stage of the woodland caribou typanosome from 5-day old cultured blood; the location of the kinetoplast (K) in relation to the nucleus (N) and to the anterior end (Ant.) is shown. F, flagellum; FP, flagellar pocket; V, vacuole. Bar = 1 μm. b. Higher magnification of Figure 4a, showing the detailed structure of the epimastigote stage including the flagellum (F), flagellar pocket (FP), and endoplasmic reticulum (ER). Bar = 0.5 μm.

The nucleus was surrounded by a distinct nuclear membrane, and usually had one, but occasionally more, round or irregular nucleoli. The endoplasmic reticulum was well developed and appeared in the form of rough and smooth-surfaced vesicles (Fig. 4b). The flagellum had an axial filament with two central and nine double peripheral microtubules (Fig. 5, top right inset).

By transmission EM, trypomastigotes were characterized by a centrally located nucleus, the kinetoplast was closer to the nucleus than to the posterior end (Fig. 2b). The polygonal or brick-shaped kinetoplast contained a central rectangular lamellar body and a less dense surrounding matrix limited by a double wavy membrane (Figs. 3a, 5). In some instances the kinetoplast appeared to be continuous with the elongated mitochondrion and a basal body extended to a double or single flagellum inside the flagellar pocket (Fig. 5). The cell membrane consisted of a characteristic trilamellar structure composed of a double osmiophilic membrane with an intermediate layer of low density. The membrane was continuously underlaid

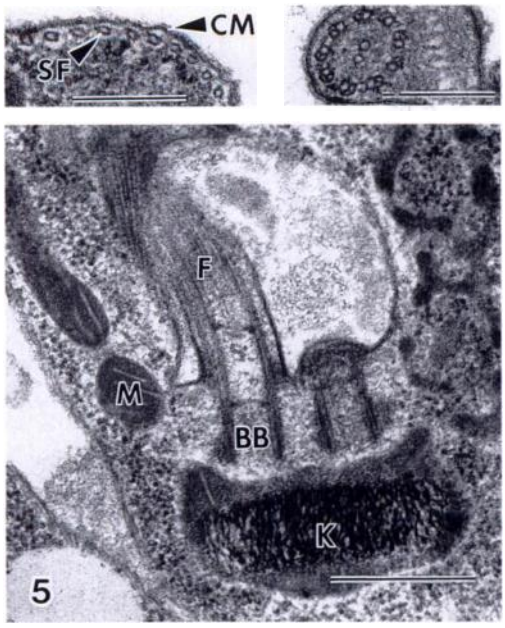


FIGURE 5. Transmission electron micrograph of woodland caribou blood incubated at 22 to 24 C for 28 days showing details of the trypomastigote stage including the flagellum (F), basal body (BB), kinetoplast (K) and mitochondrion (M). Bar = 0.5 μm. Top left inset: Cell membrane (CM) and subpellicular fibrils (SF). Bar = 0.25 μm. Top right inset: Cross section of flagellum showing axial filament with two central and nine double peripheral microtubules. Bar = 0.25 μm.

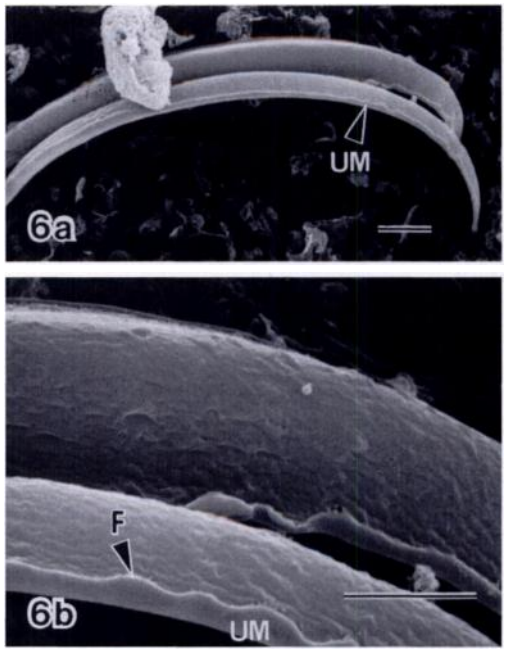


FIGURE 6a, b. Scanning electron micrograph of the trypomastigote stage in woodland caribou blood incubated for 5 days, with the flagellum (F) and distinct undulating membrane (UM). Bar 6a = 10 μm, Bar 6b = 5 μm.

with tubular subpellicular fibrils measuring, 20 to 25 nm in diameter (Fig. 5 top left inset). The cytoplasmic ground matrix was filled with ribosomes. Many parasites contained homogeneous clear cytoplasmic vacuoles which may have been glycosomes and which resembled lipid globules (Fig. 4a).

Based on a scanning electron microscopy examination of the trypomastigotes, the flagellum and flagellar sheath ran along the entire length of the parasite, contributing to a distinct undulating membrane before becoming free at the anterior end (Fig. 6a, b).

DISCUSSION

Trypanosomes appeared to be widespread among free-ranging woodland caribou of northern Alberta. The trypanosomes had culture and morphological characteristics typical of the subgenus *Megatrypanum* (Hoare and Wallace, 1966; Hoare, 1972), which have been described in several North American wild cervids (Stuht, 1975).

Trypanosomes were not seen in blood smears or buffy coat preparations made from fresh blood. The number of parasites free in the circulation may have been below the sensitivity of the concentration method used and therefore could only be detected by culture techniques.

The trypanosomes multiplied in culture both at 22 C and at 37 C. They were large, had pointed anterior and posterior body ends, and kinetoplasts located nearer to the nucleus than the posterior end (Hoare, 1972). The dividing stages consisted of very large epimastigotes similar to those reported in reindeer (Kingston et al., 1982a) and in mule deer (Matthews et al., 1977).

Splitter and Soulsby (1967) reported

that the *Megatrypanum* trypanosome, *T. theileri,* survives only 1 to 4 days in blood samples after collection, while Yakimoff (1915) found that they would survive in blood for 7 to 9 days. In our study, the storage of infected blood at 22 to 24 C did not noticeably affect the recovery and viability of trypanosomes. Furthermore, while traditional methods for culture of trypanosomes require artificial media (Splitter and Soulsby, 1967), whole blood samples which were left at room temperature (22 to 24 C) contained many trypanosomes, as did the cultures in artificial medium.

Clinical signs of disease and pathologic lesions in tissue should be looked for when *Megatrypanum* subgenus are identified (Kingston et al., 1979). Transplacental transmission occurs in mule deer (Kingston et al., 1981). There is also evidence that a related species, *T. theileri,* crosses the bovine placenta (Kingston et al., 1982b; Hussain et al., 1985) sometimes causing abortions (Woo and Limebeer, 1971).

Megatrypanum spp. trypanosomes are transmitted by hippoboscid and tabanid flies (Bose et al., 1987). In North America, trypanosomes have been collected from deer flies (Krinsky and Pechuman, 1975), ticks (*Amblyomma americanum*) from white-tailed deer (Krinsky and Burgdorfer, 1976), and from horse flies (*Hybomitra* sp.) (Davies and Clark, 1974; Morton and Kingston, 1976).

The caribou habitat harbors many blood sucking arthropods; in summer it has horseflies, blackflies and mosquitoes, and in winter it has ticks. Although the intermediate host of the caribou trypanosome is unknown, winter ticks were observed on some of the captured animals.

Our report is the first record of trypanosomes from woodland caribou in North America. Identification of the trypanosomes was based on characteristics of the culture forms as blood stream trypomastigotes were not recovered. In further studies, a more sensitive method for isolation of the caribou blood stream trypanosomes must be used, such as the double concentration method of Strout (1963). Precise morphologic speciation of the caribou blood trypomastigotes and species cross-transmission studies are required to compare the trypanosomes from woodland caribou, with *T. cervi* from other cervids, and with *T. theileri* from cattle.

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