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Source: Journal of Wildlife Diseases, 25(3) : 410-412

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-25.3.410

Journal of Wildlife Diseases, 25(3), 1989, pp. 410–412

Trypanosoma kansasensis sp. n. from *Neotoma floridana* in Kansas

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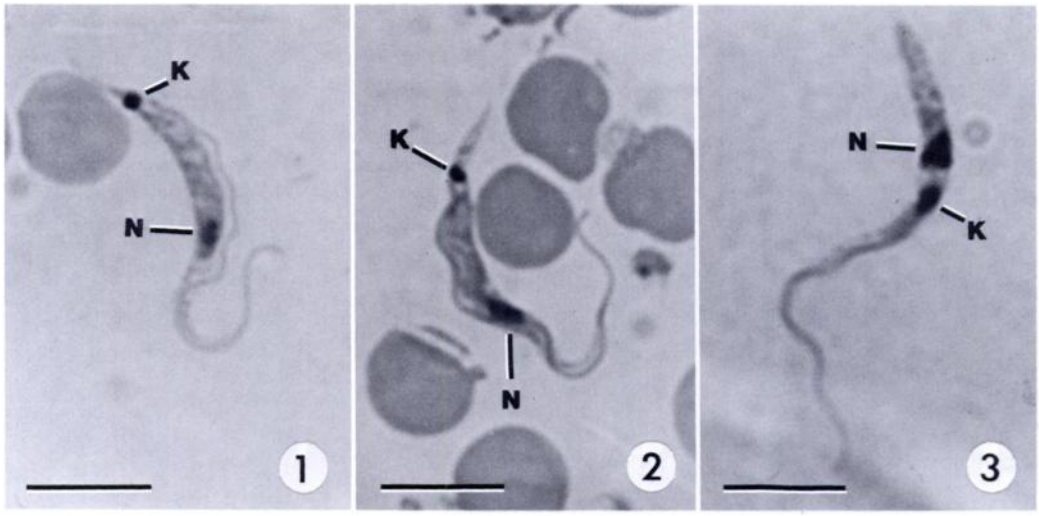
ABSTRACT: *Trypanosoma kansasensis* sp. n. (Sarcomastigophora: Kinetoplastida) is described from three of 23 (13%) eastern woodrats (*Neotoma floridana*) collected from Pottawatomie County, Kansas (USA). All flagellates found in the blood of woodrats were trypomastigotes and are larger than *T. neotomae* in overall dimensions, especially flagellar length and the distance between the posterior end of the organism and kinetoplast. Liver infusion-tryptose (LIT) cultures of infected whole blood resulted in the transformation of some parasites into epimastigotes; however, there was no apparent increase in parasite numbers.

Key words: *Trypanosoma kansasensis* sp. n., *Trypanosoma neotomae*, Sarcomastigophora, Kinetoplastida, trypanosome, eastern woodrat, *Neotoma floridana*, LIT culture, survey.

The only species of trypanosome known to infect woodrats is *Trypanosoma neotomae*, first described by Wood (1936) from *Neotoma fuscipes* in California (USA). Although similar to *T. lewisi* from *Rattus* spp., *T. neotomae* was determined to be a distinct species since it was not transmissible to susceptible Norway rats (*Rattus norvegicus*) and was capable of infecting *Neotoma fuscipes* while *T. lewisi* was not. Also, *T. neotomae* was found to have a longer incubation period and persisted in the blood longer than *T. lewisi* in respective hosts (Wood, 1936). The woodrat flea (*Orchopeas howardi* = *O. wickhami*) was found to be the intermediate host with epimastigotes ("crithidia") and trypomastigotes ("trypanosomes") within its intestines. Additional hosts reported to be infected with *T. neotomae* are *N. lepida* from California and *N. albigulae* from a locality north of Guaymas, Sonora, Republic of Mexico (Wood and Wood, 1937; Wood, 1952, 1975).

Between June and August 1988, we collected 23 eastern woodrats (*Neotoma floridana*) from Pottawatomie County, Kansas (USA; 39°13′N, 96°30′W) as part of a survey to determine whether *Trypanosoma cruzi* was present in central Kansas. All animals were trapped using Tomahawk live-traps (Tomahawk Live Trap Company, P.O. Box 323, Tomahawk, Wisconsin 54487, USA) and brought back to our laboratory at Kansas State University (Division of Biology, Ackert Hall, Manhattan, Kansas 66506, USA). Animals were killed by CO_2 asphyxiation, the thoracic cavity exposed, and blood was removed from the right ventricle by needle and a 10 cc syringe containing 10 units of heparin sulfate. Approximately 0.2 ml of whole blood from each animal was placed into each of two 25 cm^2 tissue culture flasks containing 5 ml liver infusion-tryptose (LIT) medium. The medium formulation was that described by Castellani et al. (1967), except that 0.25 mg/ml haemin was substituted for hemoglobin. All cultures were incubated at 28 C and examined at weekly intervals for a period of up to 1 mo. In addition, both thin and thick smears were made from the whole blood, air dried, fixed in 100% methanol and stained with Giemsa stain.

Although we failed to note any *T. cruzi*, a species similar to, but distinct from, *T. neotomae* was found in blood smears of 3 of 23 (13%) *N. floridana*. Only trypomastigotes were found in the blood in our study, which agrees with the information provided by Wood (1936) on *T. neotomae* except that she did note a single trypomastigote with two kinetoplasts in one blood smear. Trypomastigotes from *N. floridana* were found to be elongate, with a pointed, tapering posterior end and nucleus located anterior to the center of the body (Figs. 1, 2). Measurements (in μm), reported as the



FIGURES 1–3. Photomicrographs of Giemsa stained slides of *Trypanosoma kansasensis* sp. n. Scale bars = 10.0 μm. K, kinetoplast; N, nucleus. 1, 2. Trypomastigotes from blood smear. 3. Epimastigote from 2 wk old LIT culture.

mean ± SE (range) from 25 trypomastigotes from Giemsa stained smears of two individual woodrats (n = 15 and 10, respectively), are as follows and were made using a calibrated ocular micrometer and an Olympus BH-S photomicroscope equipped with a 100× S-Planar Apochromatic objective lens (Olympus Corporation, Tokyo, Japan).

Trypanosoma kansasensis sp. n.
(Figs. 1–3)

Description of trypomastigote: (Sarcomastigophora: Kinetoplastida) with characters of the genus *Trypanosoma*. Total body length (TL), 32.3 ± 0.22 (29.6 to 34.0); length of body excluding flagellum (BL), 23.4 ± 0.21 (21.6 to 25.6); width of body (BW), 2.2 ± 0.05 (1.6 to 2.4); free flagellum length (FL), 9.0 ± 0.23 (6.4 to 11.2); body length (BL)/flagellum length (FL), 2.7 ± 0.09 (2.0 to 3.8); nucleus length (NL), 2.7 ± 0.06 (2.2 to 3.2); nucleus width (NW), 1.7 ± 0.02 (1.6 to 1.8); nuclear index (NI) (=NL/NW) 1.6 ± 0.05 (1.2 to 2.0); posterior end to nucleus (PN), 15.1 ± 0.17 (13.6 to 17.4); posterior end to kinetoplast (PK), 5.4 ± 0.15 (4.8 to 7.6); kinetoplast length (KL), 1.2 ± 0.04 (0.8 to 1.6); kinetoplast width (KW), 1.0 ± 0.02 (0.8 to 1.2); kinetoplast index (KI) (=KL/KW), 1.3 ± 0.05 (1.0 to 2.0).

Other stages: Unknown.

Type host: Eastern woodrat, *Neotoma floridana* (Rodentia, Cricetidae).

Type locality: Pottawatomie County, Kansas, USA (34°13′N, 96°30′W).

Disposition of specimens: Syntypes, U.S. National Parasite Collection, Beltsville, Maryland 20705, USA; Accession number 80508.

Discussion: The total body length of the form we observed is slightly greater than that reported by Wood (1936) for *T. neotomae* (32.3 μm versus 29.4 μm). Although additional measurements are lacking in the original description, supplemental data are given by Davis (1952) where she examined blood smears from three *N. fuscipes*; two of which were collected by herself and the third by F. D. Wood. The means calculated from 50 individual parasites from each host include total body lengths of 26.2, 28.1, and 30.9 μm, posterior end to kinetoplast lengths of 2.7, 3.9, and 4.4 μm, and free flagellum lengths of 4.9, 4.4, and 4.8 μm. Based on these data, the greatest differences between the form we observed and *T. neotomae* is that the kinetoplast is located further from the posterior end of the body in *T. kansasensis* and the flagellum of *T. kansasensis* is considerably longer (BL/FL = 2.7 versus 5.3, 6.4, and 6.5).

Although trypanosomes inoculated along with whole blood failed to reproduce in LIT cultures, the parasites remained viable in the medium. At 2 wk postinoculation, smears were made from two of the cultures and processed as described above for blood smears. Both trypomastigotes and epimastigotes (Fig. 3) were noted, as were three specimens each with two kinetoplasts. Of 100 trypanosomes scored for specific morphologic type, 47% were trypomastigotes, 42% were

epimastigotes, 10% were a form intermediate between trypomastigote and epimastigote where the kinetoplast was immediately adjacent to the nucleus, and 1% was a form possessing two kinetoplasts but a single nucleus and flagellum. More advanced stages of division were not noted, even after cultures were held in the laboratory for 1 mo.

Based on the above measurements, we believe the form from *N. floridana* represents a previously undescribed species of the genus *Trypanosoma* and assign it the name *Trypanosoma kansasensis* sp. n. The specific epithet is derived from Kansas, the site of the type locality.

This study was supported by a grant from Bioserve Space Technologies (NASA NAGW-1197). This manuscript is Kansas Agricultural Experiment Station Contribution No. 89-226-J.

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Received for publication 8 December 1988.