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MORPHOLOGY OF TRYPANOSOMES FROM WHITE-TAILED DEER AND WAPITI IN MICHIGAN

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Abstract: Trypanosomes were isolated from a wapiti (*Cervus canadensis*) and 72 white-tailed deer (*Odocoileus virginianus*) from several locations in Michigan. Although significantly fewer fawns were infected, there were no significant differences in rate of infection between sexes or among geographic areas. From appearance of the trypomastigote, the trypanosome from white-tailed deer belongs in the genus *Trypanosoma* and the subgenus *Megatrypanum*. It was morphometrically similar to the common trypanosome of cattle, *Trypanosoma theileri*.

INTRODUCTION

There are at least three species of trypanosomes which infect cervids, *T. vivax*, *T. evansi* and *T. mazamarum* (Table 1). Of the three, *T. evansi* is known to be pathogenic to cervids.^{1,7,10,14} There is apparently nothing known of the effect of these parasites on wild populations.¹⁰

In 1969, unidentified trypanosomes were reported from white-tailed deer in Alabama, North and South Carolina, Virginia, Georgia, and Louisiana.¹³ Recently, they have been isolated from mule deer² and wapiti.⁶ The purpose of this study was to determine if white-tailed deer in Michigan were infected.

METHODS

Blood samples from 106 white-tailed deer from several locations in Michigan were cultured for trypanosomes: Rose Lake Wildlife Research Center¹ (RLWRC) (42° 48'N, 84° 25'W), Houghton Lake Wildlife Research Station¹ (HLWRS) (44° 19'N, 84° 52' W), Cusino Wildlife Research Station¹

(CWRS) (46° 21'N, 86° 29' W), Edwin S. George Reserve² (ESGR) (42° 27'N, 84° 00'W). In addition, blood from a wapiti from the lower peninsula (45° 12'N, 84° 36'W) was also cultured.

January through March 1973, captive deer at the HLWRS, RLWRC, and CWRS were immobilized with CI-744,³ Sucostrin⁴ or leg shackles and bled from the jugular. The deer at the ESGR were shot in December 1973, and bled immediately from the jugular or from the ascending aorta at the dressing station. The wapiti was immobilized with Sucostrin in the field 12 June 1973, and bled from the jugular.

Following the method of Kistner and Hanson,¹³ 5 ml of blood from each animal was inoculated into screw-cap culture tubes containing 10 ml of veal infusion medium.⁵ The blood was cultured as soon after collection as possible (usually immediately). Each ml of medium contained 500 U potassium penicillin G and 500 U dihydrostreptomycin sulfate. Cultures were held at room temperature (22-25C) and examined for trypanosomes at 7, 14, and 21 days post-inoculation.

[1] Michigan Department of Natural Resources facility.

[2] University of Michigan, Ann Arbor facility.

[3] Experimental drug, Parke Davis and Co., Detroit, Michigan.

[4] Succinylcholine chloride, E. R. Squibb and Sons Inc., New York, New York.

[5] Difco Laboratories, Detroit, Michigan.

TABLE 1. Trypanosomes from the family Cervidae.

Trypanosome*	Common Name	Scientific Name*	Location	Reference
<i>Trypanosoma vivax</i>	White-tailed deer	<i>Odocoileus gymnotis</i>	Venezuela	9
<i>Trypanosoma hippicum**</i>	White-tailed deer	<i>Odocoileus chiriquensis</i>	Panama	3
	Brocket deer	<i>Mazama sartarii reperticia</i>		
<i>Trypanosoma evansi</i>	Sambar deer	<i>Cervus unicolor</i>	Mauritius	1
	Barking deer	<i>Muntiacus muntjak</i>	Indonesia	14
	Axis deer	<i>Axis axis</i>		
	Rusa deer	<i>Cervus timorensis</i>		
	Roe deer	<i>Capreolus capreolus</i>	Kazakstan	10
<i>Trypanosoma mazamarum</i>	Brocket deer	<i>Mazama simplicornus</i>	Brazil	7
		<i>Mazama rufa toba</i>	Argentina	16
		<i>Mazama nemorivaga</i>		
<i>Trypanosome spp.</i>	White-tailed deer	<i>Odocoileus virginianus</i>	United States	13
	Mule deer	<i>Odocoileus hemionus</i>		2
	Elk (Wapiti)	<i>Cervus canadensis</i>		6
	White-tailed deer	<i>Odocoileus virginianus gymnotis</i>	Colombia	5
	Brocket deer	<i>Mazama guasubira medemi</i>		

* Scientific names as cited in references.

**Synonymous with *T. evansi*.

A small drop of medium (approx. 0.01 ml) was removed from near the surface of each culture with an inoculating loop, placed on a microscope slide, covered with a coverglass (22 mm²) and examined at 125X until trypanosomes were seen or until the entire slide had been examined.

Using a method similar to that used by Woo *et al.*,²⁰ allantoic cavities of 10-day-old chicken embryos were inoculated with 0.2 ml of veal infusion medium containing active epimastigote trypanosomes. Inoculum was from cultures 7 to 10 days old that were free from contamination with filamentous fungi, yeasts, or bacteria. The embryos were incubated at 37C and a drop of allantoic fluid from each examined for trypanosomes as described above, at 3 and 5 days post-inoculation.

Thin films of culture medium and allantoic fluid containing trypanosomes were air dried, fixed in methanol, stained with Giemsa, and examined under oil immersion (1250X). Trypanosomes were measured with the aid of a camera lucida.

Infection data was tested for statistical significance using the Chi-square test for two independent samples.¹⁸

RESULTS

The wapiti and 72 of 91 deer were infected with trypanosomes.⁶ There were significantly fewer fawns infected (39/58) than older deer (33/33) ($P < 0.001$) and about as many male fawns infected (11/16) as females (28/42). There was no significant difference in rate of infection between fawns from different geographic areas (CWRS 11/14, HLWRS 27/43, ESGR 1/1).

Trypanosomes seen in veal infusion medium were usually typical epimastigotes (Fig. 1). Those of both the deer and wapiti trypanosomes were similar in size and morphology. They were slender and tapered to a point at the posterior

end. The kinetoplast was usually marginal and located in the anterior end near the nucleus. The nucleus was acentric and usually in the anterior end. A free flagellum was present. A random sample of 10 epimastigotes of the deer trypanosome were measured. Excluding the free flagellum, the body was 18.51 to 23.70 (20.92) μm long and 2.22 to 3.33 (2.62) μm wide. The flagellum was 5.92 to 12.59 (9.22) μm long.

Allantoic fluid from six of eight embryos inoculated with epimastigotes of the trypanosome from deer contained trypomastigotes on days 3 and 5 post-inoculation (Fig. 1). They were slender and tapered to a point at the posterior end. The kinetoplast was large and oval, usually marginal, and located in the posterior end near the nucleus. A random sample of 50 trypomastigotes were measured (Table 2). The kinetoplast was near the nucleus and far from the posterior end ($KI = 4.31$). The nucleus was near the center of the body and in the anterior end ($NI = 1.23$). Excluding the free flagellum, the body was 29.98 to 53.13 (41.53) μm long and 2.58 to 6.27 (4.32) μm wide. The flagellum was 5.53 to 20.66 (13.04) μm long. Trypomastigotes were not seen in either of two embryos inoculated with epimastigotes of the wapiti trypanosome.

DISCUSSION

Trypanosomes are very common parasites of white-tailed deer in Michigan, literally every animal examined over 1 year old was infected. Some fawns were either not infected or they had such low parasitemia that their infections were not detected.

The high infection rate in older animals suggests deer do not develop an immunity capable of ridding them of infection or protecting them from acquiring new ones. However, they must develop some form of protective immunity or have innate resistance which prevents lethal infections. There is evidence that

⁶ Cultures from 15 deer not included became contaminated with yeasts and/or filamentous fungi.

the immune system may be important in preventing severe *T. theileri* infection in cattle.¹²

The epimastigotes of the trypanosomes from both deer and wapiti were similar in size and morphology to those previously described from white-tailed deer,¹³ but were somewhat larger than those previously described from mule deer² and wapiti.⁶

The trypomastigote seen in this study was morphometrically similar to *T. theileri* (Table 2) and morphologically similar to *T. mazamarum*. *Trypanosoma theileri* is thought to infect only bovids. However, trypanosomes that looked like *T. theileri* have been reported from white-tailed deer and brocket deer in South America.⁵ *Trypanosoma mazamarum* has been reported from brocket deer



FIGURE 1. Trypanosomes from white-tailed deer and wapiti in Michigan.

Camera lucida drawings: Trypomastigotes of deer trypanosome (Strain CWRS 48806-07) (1-2), epimastigotes of deer trypanosome (Strain HLWRS 730) (3-4), epimastigotes of wapiti trypanosome (Strain WPL 73-334) (5-6).

TABLE 2. Measurements of Trypanosomes from white-tailed deer in Michigan and cattle in Ontario.

Trypanosome	PK	KN	PN	NA	F	AP	NI	KI	PK/AP	NA/AP
<i>Trypanosoma</i> sp. (Deer—Michigan)	16.68	5.32	21.94	19.39	13.04	41.53	1.23	4.31	.40	.45
<i>Trypanosoma theileri</i> (Cattle—Ontario)	12.80	5.53	18.33	15.37	13.87	33.70	1.19	3.31	.38	.46

Measurements are means in microns.

Abbreviations: PK, the distance from the posterior end to the kinetoplast; KN, the distance from the kinetoplast to the center of the nucleus; PN, the distance from the posterior end to the center of the nucleus; NA, the distance from the center of the nucleus to the anterior end; AP, the length of the body excluding the free flagellum; F, the length of the free flagellum; NI, (PN/NA), nucleus index; KI (PN/KN), kinetoplast index.

Data on *T. theileri* from Woo *et al.*²⁰

in Argentina¹⁶ and Brazil.⁷ It has not been reported as infecting other cervids.

Undoubtedly, the trypanosome from white-tailed deer in Michigan belongs in the group Stercoraria.¹¹ Members of this group are characterized by trypanosomes with a free flagellum, a large kinetoplast that is not terminal and a pointed posterior end. There is little doubt that it also belongs in the genus *Trypanosoma* and the subgenus *Megatrypanum*.¹¹ Members of this subgenus are rather large trypanosomes of mammals whose kinetoplast is typically located near the nucleus and far from the posterior end. The type species is *T. theileri*.

Even though *T. theileri* has been known for about 70 years, there is still

controversy regarding its effect upon cattle. The prevailing view is that the trypanosome is harmless. However, many still feel that it is potentially pathogenic, its dormant virulence being activated in the presence of some intercurrent infection, such as piroplasmosis or rinderpest.¹² In cattle, *T. theileri* has been associated with abortion,^{8,15} decreased milk production¹⁷ and signs of illness such as lymphocytosis, lymphadenopathy and eosinophilia.^{4,17}

Unfortunately, no conclusions can be made concerning the pathogenicity of trypanosomes from deer or wapiti until they are properly identified or until animals are infected experimentally under controlled conditions.

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