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Comparative studies of *Giardia* spp. in small mammals in southern Ontario. I. Prevalence and identity of the parasites with a taxonomic discussion of the genus

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Giardia microti Kofoid and Christiansen, 1915 was identified in 98.8% (322 of 326) of meadow voles (*Microtus pennsylvanicus*) and *G. peromysci* Filice, 1952 emend. in 98% (48 of 49) of deer mice (*Peromyscus maniculatus*) that were livetrapped at six locations in southern Ontario. One feral brown rat (*Rattus norvegicus*) was infected with *Giardia simoni* Lavier, 1924 and *Giardia muris* Grassi, 1881. Laboratory rats (Wistar strain) harboured only *G. simoni* and laboratory mice (C3H strain) were infected with *G. muris*. Golden hampsters (*Mesocricetus auratus*) were infected with *Giardia mesocricetus* Filice, 1952 emend.

Giardia spp. were separated into two morphologically distinct groups. Trophozoites of *G. muris* and *G. mesocricetus* were almost as wide as long and had round or oval centrally situated median bodies. Trophozoites of *G. microti, Giardia peromysci*, and *G. simoni* were elongate with long curved median bodies lying perpendicular to the long axis of the trophozoite.

Further differentiation of species was not possible by comparing trophozoite morphology but was accomplished by comparing the average lengths and widths of trophozoites.

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Giardia microti Kofoid and Christiansen, 1915 a été reconnu chez 98.8% (322 de 326) de campagnois de champs examinés (*Microtus pennsylvanicus*) et *G. peromysci* Filice, 1952 emend. a été déterminé chez 98% (48 de 49) de souris à pattes blanches (*Peromyscus maniculatus*) capturés vivants en six points du sud de l'Ontario. Un rat surmulot sauvage (*Rattus norvegicus*) était parasité par *Giardia simoni* Lavier, 1924 et *Giardia muris* Grassi, 1881. Des rats de laboratoire (souche Wistar) n'abritaient que des *G. simoni* et des souris de laboratoire (souche C3H) étaient infestées de *G. muris*. Des hamsters dorés (*Mesocricetus auratus*) abritaient des *Giardia mesocricetus* Filice, 1952 emend.

Les espèces de *Giardia* peuvent être séparées en deux groupes morphologiquement distincts. Les trophozoïtes de *G. muris* et de *G. mesocricetus* sont presque aussi larges que longs et possèdent des organites médians ronds ou ovales situés au centre. Les trophozoïtes de *G. microti*, *Giardia peromysci* et *G. simoni* sont allongés et ont des organites longs et incurbés, perpendiculaires à l'axe longitudinal du trophozoïte.

La morphologie des trophozoïtes ne permet pas la différenciation spécifique, cependant, celle-ci peut se faire par comparaison des longueurs et des largeurs moyennes des trophozoïtes.

[Traduit par le journal]

Introduction

Little is known of the intestinal protozoan *Giardia*, and their prevalence in North American rodents. Kofoid and Christiansen (1915*a*, 1915*b*) and Filice (1952) found these parasites in *Microtus californicus*, *Peromyscus californicus*, and *Peromyscus maniculatus* in California. Simon (1921*a*, 1922) reported finding *Giardia* sp. in *Microtus pennsylvanicus* in Nova Scotia and Hegner (1923) found *Giardia* sp. in wild mice (*Mus musculus*) and rats (*Rattus norvegicus*) in Maryland. Rothenbacher *et al.* (1970) discovered the parasite in a captive colony of *Peromyscus leucopus* in Pennsylvania. Laboratory colonies of rats, mice, and hamsters (*Mesocricetus auratus*) are commonly infected with *Giardia* sp. (Seamer and Chestermann 1967; Sparrow 1976).

In a preliminary survey conducted during the autumn of 1975, trophozoites of *Giardia* spp. (order Diplomonadorida) were found in the intestines of meadow voles (*M. pennsylvanicus*) and deer mice (*P. maniculatus*) livetrapped near Guelph, Ontario.

The aims of this study were to determine: (*i*) the prevalence of *Giardia* spp. infections in various small rodents, and (*ii*) the identity of the *Giardia* in these hosts by detailed morphological and morphometrical studies of the trophozoites.

Materials and Methods

Sources and Maintenance of Animals

Small mammals were livetrapped[2] during a 22-month period

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[2]Ontario Ministry of Natural Resources Scientific Collector's Permit numbers 386 and 486.

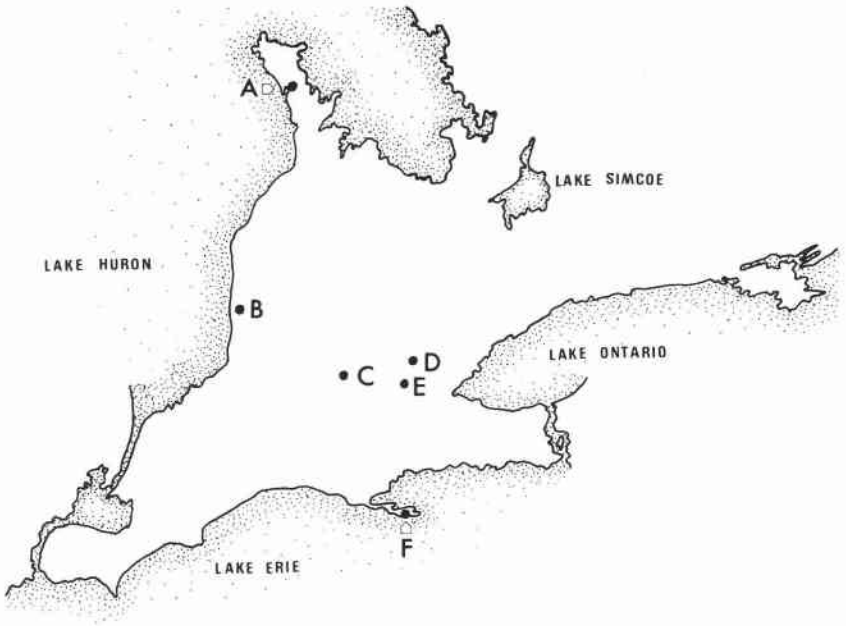

FIG. 1. Trapping locations in southern Ontario. A, Stokes Bay; B, Goderich; C, Wellesley; D, Guelph; E, Puslinch; F, Long Point.

(September 1975 to June 1977) at several locations in southwestern Ontario (Fig. 1). All species were captured in Sherman large folding aluminum traps[3] (7.6 × 8.9 × 22.9 cm) baited with raisins or a mixture of oatmeal, bird seed, peanut butter, cat food, and raisins. In midsummer traps were shaded with plywood rectangles (12 × 30 × 0.7 cm) and grass. Bedding material in the form of nonabsorbent cotton batting was placed in traps if the air temperature was expected to fall below 15°C. During winter months traps were set beneath the snow after tracing air holes from the surface to the level of runways. Traps were normally checked twice daily.

Traps were set at locations in the immediate vicinity of Guelph in all seasons. Intensive trapping was done at the Puslinch Crown Tract managed by the Ontario Ministry of Natural Resources near Cambridge during the spring and summer of 1976 and 1977. Animals were collected from Stokes Bay in the Bruce Peninsula and Point Farms near Goderich during 1-week periods in July 1976, from a farm near Wellesley during a 2-week period in June 1976, and from Long Point during 1-week periods in October 1976 and April 1977 (Table 1).

Wistar strain rats were obtained from Woodlyn Laboratories Ltd.[4] Gnotobiotic and C3H strain mice were obtained from the Department of Clinical Studies and the Department of Veterinary Microbiology and Immunology, University of Guelph. Golden hamsters (*M. auratus*) were purchased from High Oak Ranch.[5]

Animals were maintained in large opaque (28 × 47 × 14 cm) or small polypropylene (16 × 27 × 13 cm) cages in a well ventilated room. A photoperiod of 12–14 h and air temperature of 18–23°C were maintained. Absorbent cellulose bedding material[6] was used in the cages. Tap water and Purina Lab Chow[7] were provided *ad libitum*. The diets of meadow voles, deer mice, and hamsters were supplemented with carrot and apple slices.

Examination of Animals

Animals were anaesthetized using chloroform or methoxyfluorane[8] and exsanguinated by cardiac puncture. The intestinal tract was removed and rinsed briefly in phosphate buffered saline (PBS, pH 7.2) or Alsever's solution (pH 7.2). The small intestine was cut into sections 1–2 cm long. These were placed in individual wells of a titration plate with 1–2 ml of either PBS or Alsever's solution. Every other section was opened longitudinally and the contents were carefully removed. Three wet mounts of the contents and of mucosal scrapings were examined using a compound microscope (10 × objective and 10 × ocular). If trophozoites were not found, the rectal contents were suspended in PBS and three wet mounts were prepared using 22 × 22 mm glass cover slips. Each preparation was stained with Lugol's iodine solution and examined for cysts for 15 min using the 40 × objective of a compound microscope.

Morphological and Dimensional Studies of Giardia

(i) Recovery of Trophozoites

If high numbers of trophozoites were encountered in a section of intestine that was free of ingesta, the *Giardia* were washed from the mucosa into a 10-ml beaker with 2–3 ml of PBS or Alsever's solution (room temperature). Alternatively, mucosal scrapings containing trophozoites were smeared directly onto clean microscope slides.

(ii) Recovery of Cysts

Faeces from infected animals were suspended in two to three volumes of 0.85% saline and filtered through two layers of cheesecloth. If few cysts were found in wet mounts of the filtrate, they were concentrated by zinc sulfate floatation (Lennette *et al.* 1974).

(iii) Examination of Giardia using Vital Stains

Wet mounts of cysts and motile trophozoites were stained

[3]H. B. Sherman Traps, 2024 Shady Oaks Drive, Tallahassee, Florida, U.S.A. 32303.

[4]R.R. 3, Guelph, Ontario.

[5]R.R. 1, Goodwood, Ontario.

[6]Grit-o'cobs (grade 1014), The Andersons, Maumee, Ohio, U.S.A. 43437.

[7]Ralston Purina.

[8]Metofane, Pittman-Moore Inc., Washington Crossing, New Jersey, U.S.A. 08560.

with Janus green B and neutral red (Gurr 1963). Wet mounts of cysts were also stained using Lugol's iodine solution. These preparations were examined under ordinary transmitted light. Preparations were also stained with acridine orange (Strugger 1948) and examined using a halogen light source and Zeiss KP 490 exciter and LP 520 barrier filters.

(iv) Preparation of Fixed Smears

Smears of trophozoites were wet-fixed but those from faecal preparations were thoroughly air-dried prior to fixation. Smears were fixed in absolute ethanol for 3 min and air-dried before secondary fixation in 10% buffered formalin (pH 7.2). After air-drying, fixed smears were stained in Gurr's Improved Giemsa[9] (pH 7.2) for 45 min. The trophozoites from these preparations were measured and used for morphological studies.

Additional smears were fixed in Schaudinn's fluid at room temperature for 45–60 min. Residual mercuric chloride was removed with Gram's iodine solution and 5% sodium thiosulfate before staining with Heidenhain's or Ehrlich's haematoxylin using techniques described by Gurr (1963) and Humason (1972).

(v) Measurements of Giardia

Trophozoites recovered from at least three individuals of each host species from each source were measured. The first 40 parasites encountered in preparations from these hosts were measured. Outline drawings were prepared using a Zeiss drawing tube and 100 × oil-immersion lens. Measurements (Fig. 2) were taken using fine dividers set to span a distance equal to 1.0 μm on the drawings.

Results

Prevalence of Giardia spp.

Of livetrapped animals, only deer mice, voles, and the wild rat were infected with *Giardia* spp. (Table 1). Most of the voles (322 of 326 = 98.8%) and deer mice (48 of 49 = 98.0%) were infected with *Giardia microti* and *Giardia peromysci*, respectively. The deer mouse and voles that were not infected were captured at Guelph during the winter. These animals had died in the traps and frozen prior to examination.

All laboratory rats were infected with *Giardia simoni* and all hamsters with *Giardia mesocricetus* on receipt from the suppliers. Although C3H mice are maintained in isolation at the Department of Verterinary Microbiology and Immunology, University of Guelph, all animals received from this source were infected with *Giardia muris*. Gnotobiotic mice obtained from the Department of Clinical Studies, University of Guelph, were free of *Giardia*.

Morphological Studies

(i) Trophozoites

Unstained motile trophozoites were achromatic. Nuclei and median bodies were visible as areas of low density in the cytoplasm. The posterior edge of the sucking disk could be distinguished in trophozoites of *G. simoni*, *G. microti*, and *G. peromysci* as a refractile line but was less distinct in *G. muris* and *G. mesocricetus*.

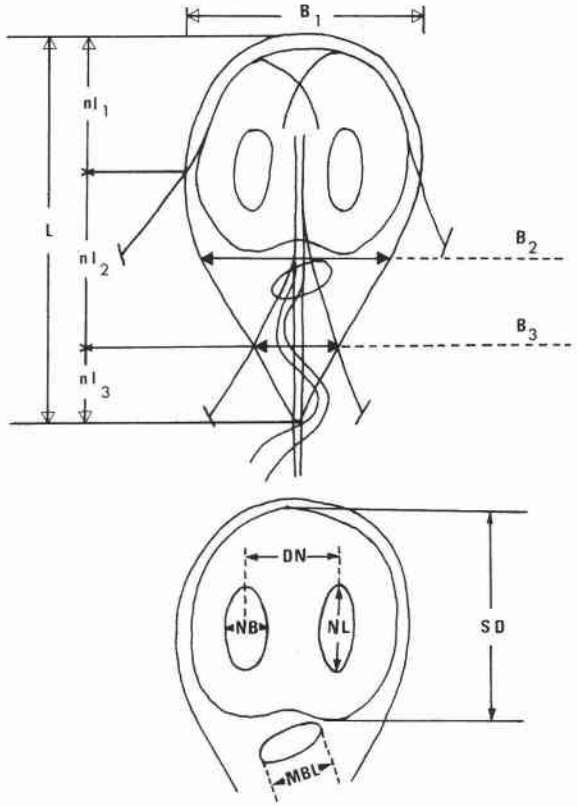

FIG. 2. Dimensions of trophozoites of *Giardia*. Top: B_1, width at level of nuclei; B_2, width midway between B_1 and B_3; B_3, width at point of emergence of lateral flagella; L, total length; nl_1, length from anterior end to middle of nuclei; nl_2, length from middle of nuclei to point of emergence of lateral flagella; nl_3, length from lateral flagella to posterior end. Bottom: *DN*, internuclear distance; *NB*, nuclear width; *NL*, nuclear length; *MBL*, length of median body; *SD*, length of sucking disk.

In Janus green B and neutral red, the nuclei of motile trophozoites were pink to light red in colour. A few small granules which stained yellow–green or red were scattered throughout the cytoplasm. These were less than 0.5 μm in diameter when measured with an ocular micrometer. In trophozoites which had attached themselves to the cover slip or slide, movement of these granules was not observed. Median bodies were not stained.

The nuclei of trophozoites in acridine orange fluoresced bright green indicating the presence of DNA (Humason 1972). A few granules in the cytoplasm also fluoresced green, but most were red indicating the presence of RNA or acid polysaccharides (Humason 1972). Median bodies and flagella did not fluoresce.

Most descriptions of *Giardia* spp. were based on haematoxylin-stained material, but during the

[9]Giemsa Improved R66, Searle Diagnostic, High Wycombe, Bucks., England.

TABLE 1. Prevalence of *Giardia* in rodents in southern Ontario

Host species	Location	No. infected per no. collected	Prevalence
Microtus pennsylvanicus	Stokes Bay	3/3	100
	Wellesley	13/13	100
	Guelph	157/161	97.5
	Puslinch	143/143	100
	Long Point	6/6	100
	Total:	322/326	98.8
Peromyscus maniculatus	Goderich	6/6	100
	Wellesley	4/4	100
	Guelph	29/30	96.6
	Puslinch	6/6	100
	Long Point	3/3	100
	Total:	48/49	97.9
Mus musculus	Goderich	0/4	
	Wellesley	0/7	
	Guelph	0/16	
	Total:	0/27	0
Rattus norvegicus	Guelph Total:	1/1	100
Blarina brevicauda	Guelph	0/9	
	Puslinch	0/6	
	Long Point	0/6	
	Total:	0/21	0
Zapus hudsonius	Guelph Total:	0/2	0
Napeozapus insignis	Guelph Total:	0/1	0

present studies the best results were obtained when Giemsa stain was used. Nuclei and median bodies were deeply stained with Heidenhain's iron haematoxylin but the posterior edge of the sucking disk stained variably. With Ehrlich's acid haematoxylin, nuclei stained deeply but median bodies and flagella were achromatic. The posterior edge of the sucking disk often could not be distinguished. The cytoplasm did not appear vacuolated or granular when stained with either haematoxylin. The edges of the sucking disk were clearly visible in most trophozoites stained with Giemsa stain. Flagella were easily traced to their origins at basal bodies between the nuclei. The median bodies and the endosomes of nuclei were deeply stained. The cytoplasm did not appear vacuolated or granular, but stained most densely in the region of the lateral shields and posterior portions of the sucking disk.

Trophozoites of the *Giardia* spp. examined could be separated into two morphologically distinct groups as follows: Type I. Motile trophozoites almost as broad as long with short flexible tail; Giemsa-stained trophozoites with large elongate nuclei in posterior region of sucking disk; sucking disk occupying large proportion of body; basal bodies anterior to nuclei; round or oval median body present in centre of body between posterior ends of nuclei (e.g. *G. muris*, *G. mesocricetus*; Fig. 3 A and B). Type II. Motile trophozoites longer than wide with long flexible tail and flexible lateral shield area; Giemsa-stained trophozoites with sucking disk in anterior half of body; pronounced indentation of medial posterior edge of sucking disk; nuclei in central region of sucking disk; single or double median body comma- or claw-shaped and situated posterior to sucking disk (e.g. *G. simoni, G. microti, G. peromysci*; Fig. 3 C–E). No consistent morphological or staining characteristics were noted which could be used to differentiate members of each type. A mixed infection was found only in the wild rat. Of 200 stained trophozoites recovered from this host, 83 (41.5%) were type I.

In all species of *Giardia*, trophozoites with median bodies appeared to be larger than those without these structures. Trophozoites of *G. mesocricetus* appeared to be larger than those of *G. muris*, but trophozoites of *G. simoni, G. microti*, and *G. peromysci* could not be differentiated by their size without taking measurements.

(ii) Cysts

Cysts of all species were morphologically similar. When examined in wet mounts, freshly excreted cysts were highly refractile. Nuclei and peripheral vacuoles were sometimes visible. Cysts were penetrated by iodine but not by the vital stains employed. Organelles were not differentially stained by iodine.

Studies of the Dimensions of Giardia[10]

The major dimensions (L, B_1, SD) of Giemsa-stained trophozoites with median bodies were compared with those of trophozoites of the same species without median bodies (Table 2). Significant differences ($P < 0.01$) were found in at least one of these dimensions in all species except *G. muris*.

Further statistical tests were conducted on the dimensions of trophozoites with median bodies (Table 3). Using Student's t test there was no significant difference ($P < 0.05$) in major dimensions within the same species of *Giardia* from different individual hosts of the same species. One-way analysis of variance (Sokal and Rohlf 1973) indicated that the variance of minor (nl_1, nl_2, nl_3, B_2, B_3, MBL) and nuclear dimensions of trophozoites was greater within species of *Giardia* than between different species. The variance of major dimensions (L, B_1, SD) was greater between than within

[10]Symbols for dimensions explained in Fig. 2.

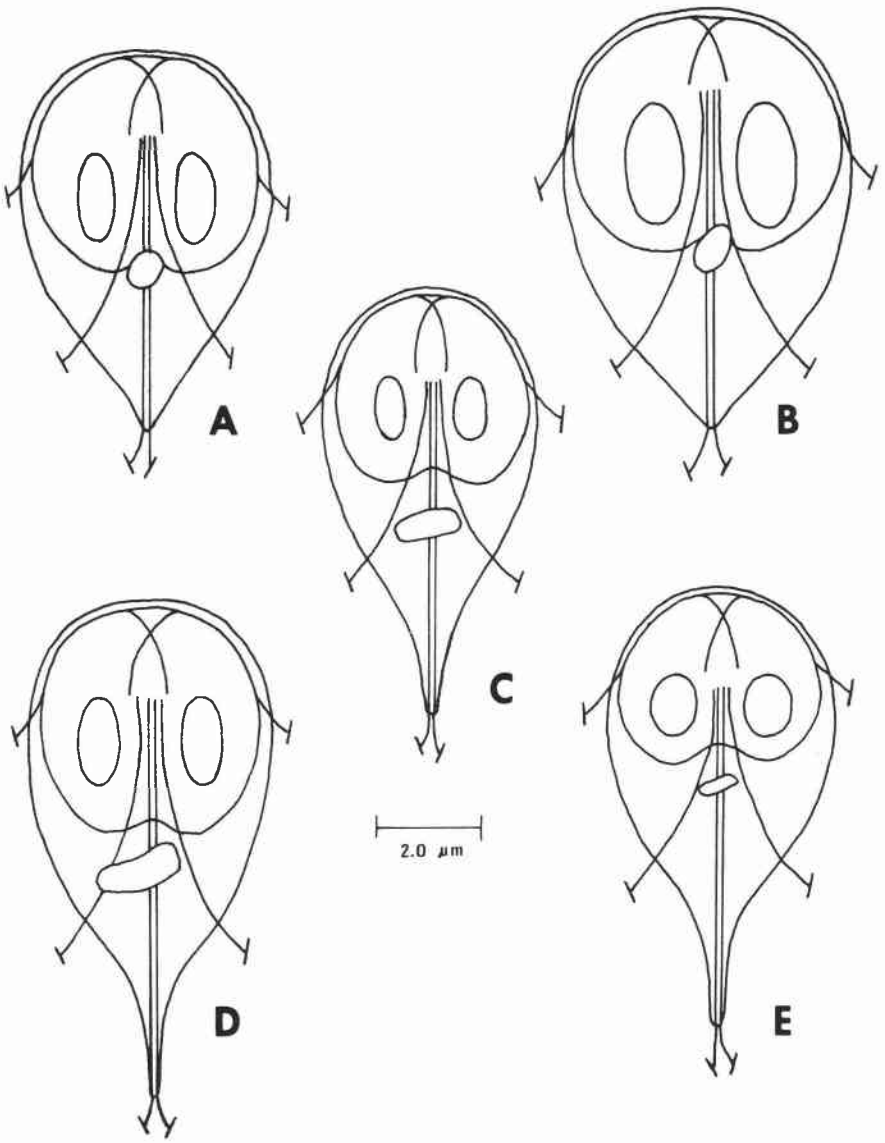

FIG. 3. Drawings of trophozoites of *Giardia* prepared from measurements presented in Table 3. Type I trophozoites: A, *G. muris*; B, *G. mesocricetus*. Type II trophozoites: C, *G. microti*; B, *G. simoni*; C, *G. peromysci*.

species. Consequently, the significance of interspecific differences was estimated by applying the Student's *t* test to the average major dimensions in all subsequent analyses.

The dimensions of species with type I trophozoites (*G. muris* and *G. mesocricetus*) were generally significantly different ($P < 0.05$) from those of species with type II trophozoites (*G. simoni, G. microti*, and *G. peromysci*). The lengths of *G. mesocricetus* and *G. simoni* were, however, not significantly different.

The lengths and widths of *G. muris* and *G. mesocricetus* were significantly different ($P < 0.05$), but the lengths of the sucking disks were not.

When species with type II trophozoites were compared, *G. simoni* was significantly different from *G. peromysci* in all major dimensions and from *G. microti* in all but trophozoite length. Only the length of trophozoites of *G. microti* and *G. peromysci* was significantly different ($P < 0.05$).

Following the method suggested by Solovjev (1975), the ratios $L{:}B_1$ of trophozoites of *Giardia* spp. were presented graphically (Fig. 4). When average lengths (L) and width (B_1) (Table 3) were plotted with their standard errors, the areas enclosed were discrete (Fig. 4). However, when L and B_1 were plotted with their respective standard deviations, there was considerable overlap be-

TABLE 2. Comparison of the major dimensions of *Giardia* spp. trophozoites with and without median bodies

Giardia species	Dimension	With median body	Without median body	Comparison* (*df*)	Significance ($P < 0.01$)
G. simoni	*L*	15.5†	14.0	1.19 (156)	–
	B_1	7.5	6.8	5.37 (156)	+
	SD	6.1	6.0	1.22 (156)	–
G. microti	*L*	13.1	12.5	3.30 (448)	+
	B_1	6.7	6.1	8.66 (448)	–
	SD	5.4	5.2	2.99 (448)	+
G. peromysci	*L*	13.8	12.9	6.26 (346)	+
	B_1	6.8	6.4	3.38 (346)	+
	SD	5.5	5.5	0.18 (346)	–
G. muris	*L*	12.1	11.6	0.03 (79)	–
	B_1	7.7	7.4	0.33 (79)	–
	SD	7.4	7.0	1.62 (79)	–
G. mesocricetus	*L*	13.2	12.4	3.25 (98)	+
	B_1	9.1	8.3	4.43 (98)	+
	SD	7.0	6.7	1.75 (98)	–

*Student's *t* test.
†Average in micrometres.

tween species. When compared using Student's *t* test, the ratios $L{:}B_1$ of trophozoites of *G. microti, G. simoni, G. muris*, and *G. peromysci* were significantly different ($P < 0.05$). This suggests that Solovjev's method is a useful tool for illustration of differences in dimensions between *Giardia* spp.

Discussion

Prevalence of *Giardia* in populations of deer mice and meadow voles was high in southern Ontario. Previously, Kofoid and Christiansen (1915*b*) found *Giardia* in 3 of 5 *M. californicus* and 4 of 59 *P. maniculatus* in California. Haiba (1953) found *G. microti* in 58% (10 of 17) of *Clethrionomys glareolus* trapped in England, while Simon (1921*a*) stated that 85% of the meadow voles (*M. pennsylvanicus*) he examined in Nova Scotia were infected. *Giardia* was not found in feral house mice (*M. musculus*) in southern Ontario during the present study. Simon (1922) did not find *Giardia* in 22 house mice collected at Baltimore and Nova Scotia, but Hegner reported infections in 2 of 20 feral house mice from Baltimore. Previous reports indicated a low prevalence of *Giardia* in feral rats (*R. norvegicus*). Simon (1922) did not find infections in 32 animals from Baltimore and Nova Scotia while Hegner (1923) found *Giardia* in three of "...more than one hundred..." feral rats in Baltimore. Prevalence of *Giardia* in wild populations appears to be variable. However, few animals were examined by some authors and there might have been differences in the sensitivity of the detection methods employed.

Characteristics of the genus *Giardia* are well defined. Alexeieff (1914) synonymized *Lamblia* Blanchard 1888 and *Giardia* Kunstler 1882. Reuling and Rodenwalt (1921) suggested *Giardia* should be used for species described from mammals and *Lamblia* for species described from tadpoles. However, most early authors (Kofoid and Christiansen 1915*a*, 1915*b*; Kofoid 1920; Hegner 1922*a*, 1922*b*) accepted Alexeiff's (1914) revision. Although *Giardia* is currently accepted as the correct generic name by many authors (Filice 1952; Noble and Noble 1971; Levine 1973), *Lamblia* was firmly entrenched in the literature and is still commonly used by East European and Russian workers (e.g. Cheissen 1964, 1965; Solovjev 1975).

The taxonomy of *Giardia* species is currently unsettled. Several authors (Meglitsch 1954; Sonneborn 1957; Simpson 1961) suggested that agamous species should be defined as populations or groups of populations which evolved as a unit. These populations or groups may be separated by spatial or other factors, but have characters in common which are retained in the species gene pool. The characters (trophozoite morphology and dimensions and host species) used to define the species listed by Ansari (1951, 1952) and those described by Abraham (1962) and Navarathnam (1969) would appear to be valid for agamous organisms. However, Filice (1952) concluded in his review that the validity of these characters had not been adequately established and he recognized only three species: *Giardia agilis*, *Giardia duodenalis*, and *G. muris*. He separated these by the shape of the median body of trophozoites, a criterion used previously by Nieschulz (1924) to separate species into three groups. Filice (1952) further proposed that all previously described

TABLE 3. Dimensions (micrometres) of trophozoites with median bodies of *Giardia* spp.

Dimension measured	*G. simoni*	*G. peromysci*	*G. microti*	*G. muris*	*G. mesocricetus*
L	15.5*	13.8	13.4	12.1	13.2
	±0.8	±1.9	±1.3	±1.8	±1.1
	(14.1–17.0)	(10.1–19.6)	(10.3–18.6)	(9.2–16.9)	(11.1–15.6)
nl_1	4.4	3.8	3.9	4.5	5.0
	±0.4	±0.5	±0.5	±0.8	±0.5
	(3.5–5.3)	(2.5–5.8)	(2.5–4.9)	(3.1–6.0)	(4.1–6.0)
nl_2	5.8	5.1	4.5	4.4	5.5
	±0.6	±0.8	±0.8	±0.7	±0.8
	(3.9–6.0)	(3.1–7.5)	(3.0–6.8)	(3.0–6.9)	(4.0–7.9)
nl_3	5.2	5.0	5.1	3.1	2.7
	±0.7	±1.1	±1.4	±0.9	±0.8
	(3.8–6.6)	(1.8–8.0)	(2.7–10.4)	(1.4–5.0)	(1.1–4.4)
B_1	7.5	6.8	6.7	7.7	9.1
	±0.7	±1.1	±0.8	±1.2	±0.9
	(6.0–9.5)	(4.1–10.9)	(4.9–9.0)	(6.6–10.8)	(7.6–11.4)
B_2	6.8	6.5	6.1	6.7	8.3
	±0.7	±1.3	±1.0	±1.6	±1.0
	(4.3–8.5)	(4.3–10.4)	(4.0–8.8)	(5.4–9.2)	(5.9–10.1)
B_3	4.3	4.1	3.9	4.2	4.9
	±0.6	±0.9	±0.7	±0.8	±0.9
	(3.3–6.0)	(1.9–7.3)	(2.1–7.4)	(2.1–5.2)	(3.4–6.9)
SD	6.1	5.5	5.4	7.4	7.0
	±0.8	±0.9	±0.7	±2.0	±0.9
	(4.0–7.4)	(3.3–7.8)	(2.3–7.6)	(4.6–9.6)	(5.2–9.0)
MBL	2.6	1.5	2.0	1.4	1.8
	±0.8	±1.2	±0.1	±0.7	±0.5
	(1.0–4.0)	(1.0–4.5)	(0.7–4.8)	(0.5–3.1)	(0.6–3.0)
NL	2.7	2.0	2.0	2.9	2.5
	±0.3	±0.5	±0.6	±0.5	±0.7
	(1.9–3.2)	(1.0–3.9)	(0.4–4.0)	(1.7–4.5)	(1.0–4.1)
NB	1.4	1.2	1.1	1.1	1.1
	±0.3	±0.4	±0.4	±0.3	±0.3
	(1.0–1.9)	(0.5–3.1)	(0.4–2.7)	(1.0–1.8)	(0.5–2.4)
DN	1.6	1.5	1.3	1.6	1.8
	±0.4	±0.8	±0.3	±0.3	±0.5
	(1.0–2.1)	(0.6–3.3)	(0.5–2.1)	(0.9–2.3)	(1.0–2.9)
L:B	2.1	1.8	2.0	1.6	1.5
	±0.2	±1.0	±0.1	±0.8	±0.2
	(1.7–2.6)	(1.4–3.1)	(1.5–2.8)	(1.3–2.0)	(1.2–1.8)
Number of trophozoites measured	78	221	215	69	50

*Average ± standard deviation (range).

TABLE 4. Statistical comparison of dimensions of trophozoites measured by Filice (1952 pp. 59, 60)

Giardia species compared	Length (*L*)	Comparison* (*df*)	Width (B_1)	Comparison* (*df*)
G. microti	11.01	12.66 (373)†	5.70	12.52 (373)†
vs.				
G. peromysci	13.88		7.16	
G. microti	11.01	4.11 (223)†	5.70	7.42 (223)†
vs.				
G. simoni	12.07		6.68	
G. peromysci	13.88	6.20 (298)†	7.16	3.02 (298)†
vs.				
G. simoni	12.07		6.68	

*Student's *t* test.
†Significantly different, $P < 0.01$.

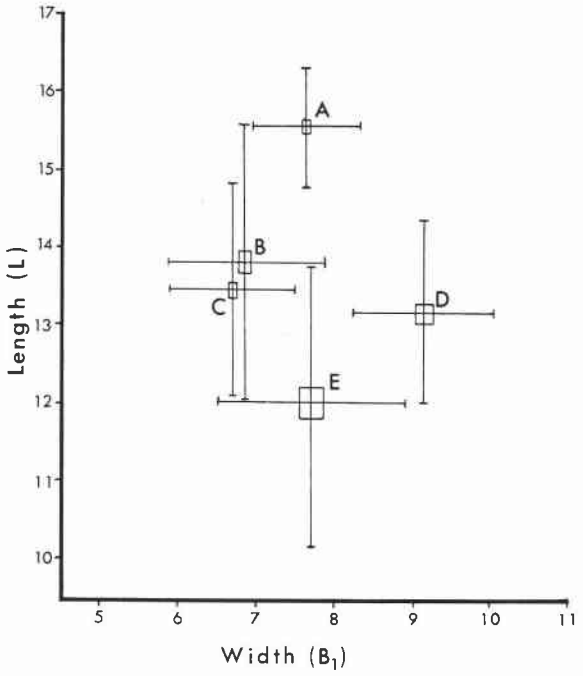

FIG. 4. Graphical representation of the ratio $L:B_1$. The values for length (L) and width (B_1) were drawn from Table 2. The bars represent one standard deviation (Table 3). The rectangles indicate the areas enclosed by plotting the standard errors of the means. A, *G. simoni*; B, *G. peromysci*; C, *G. microti*; D, *G. mesocricetus*; E, *G. muris*.

species should be given the "...nontaxonomic but pragmatic status..." of races of these three species. Although Bemrick (1962) adopted Filice's proposals, Levine (1973) suggested that, in the absence of carefully controlled cross transmission experiments, it would be more convenient to retain the original specific names for many of the *Giardia* spp. from different hosts. This approach was taken by Flynn (1973), but some workers (e.g. Meyer 1970) preferred not to name the species they studied.

During the present project, cross transmission experiments (Grant and Woo (1978) were combined with studies of the morphology and dimensions of *Giardia* spp. from several host species. The *Giardia* recovered from *M. pennsylvanicus* in southern Ontario was identified as *G. microti* Kofoid and Christiansen 1915 on the basis of similarity in the morphology and dimensions of trophozoites. Although originally described from *M. californicus*, *G. microti* was also found in *M. pennsylvanicus* in Nova Scotia (Simon 1921*a*, 1922). Others (Simon 1922; Wenyon 1926; Hegner 1930; Ansari 1952) concluded that the parasites described by Grassi (1879) from *Microtus* (=*Arvicola*) *arvalis* and by Splendore (1920) from *Microtus* (=*Pitymus*) *savii* were also *G. microti*.

Grassi (1881) indicated that *G. muris* (Syn. *Megastoma entericum*) occurs in man as well as mice. However, others (Wenyon 1907; Bensen 1908; Kofoid and Christiansen 1915*a*, 1915*b*; Simon 1922; Hegner and Taliaferro 1924; Nieschulz 1924) showed that *G. muris* is a valid species and is not found in man. The *Giardia* found in laboratory mice and one wild rat during the present study was identified as *G. muris* by comparing trophozoite dimensions and morphology with previous descriptions.

During the course of this study, hamsters were found to be infected with a *Giardia* sp. whose trophozoites were morphologically similar to, but significantly larger than, the species in laboratory mice. Filice (1952) recognized this difference in naming *G. muris* race *mesocricetus* from hamsters. It is proposed that the name *G. muris* be retained for the *Giardia* sp. from laboratory mice and that *G. mesocricetus* Filice, 1952 emend. be the name for those of hamsters. This is confirmed by experimental cross transmissions (Grant and Woo 1978).

Simon (1922) observed two morphologically distinct species of *Giardia* in a laboratory rat. One of these he identified as *G. muris*. He referred to the other, which resembled *Giardia lamblia*, as *Giardia* sp. Lavier (1924) found a similar parasite in rats (*R. norvegicus*) from the sewers of Paris, France. Since he was unable to infect rats with *G. lamblia*, he concluded Simon's *Giardia* sp. was a new species which he named *G. simoni*. The *Giardia* sp. recovered from laboratory rats during the present study was identified as *G. simoni* on the basis of trophozoite morphology and dimensions and host specificity (Grant and Woo 1978). It was also concluded that a wild rat captured at Guelph harboured both *G. muris* and *G. simoni*.

Kofoid and Christiansen (1915*a*, 1915*b*) stated that *G. muris* was found in *P. maniculatus* in California. This species was not found in deer mice in southern Ontario although most animals examined harboured another *Giardia*. This observation and the failure to transmit *G. muris* from laboratory mice and a wild rat to deer mice (Grant and Woo 1978) suggest that Kofoid and Christiansen (1915*a*, 1915*b*) may have incorrectly identified the species in *P. maniculatus*. Trophozoites recovered from *P. maniculatus* during the present studies were similar to *Giardia duodenalis* race *peromysci* Filice 1952 from *P. californicus*. It is proposed that this form be recognized as a distinct species, *G. peromysci* Filice, 1952 emend.

The reliance of many authors on minor morphological differences between trophozoites for species identification is demonstrated in Ansari's (1951, 1952) comprehensive review. Criteria used included: (*i*) the slant of the nuclei relative to the

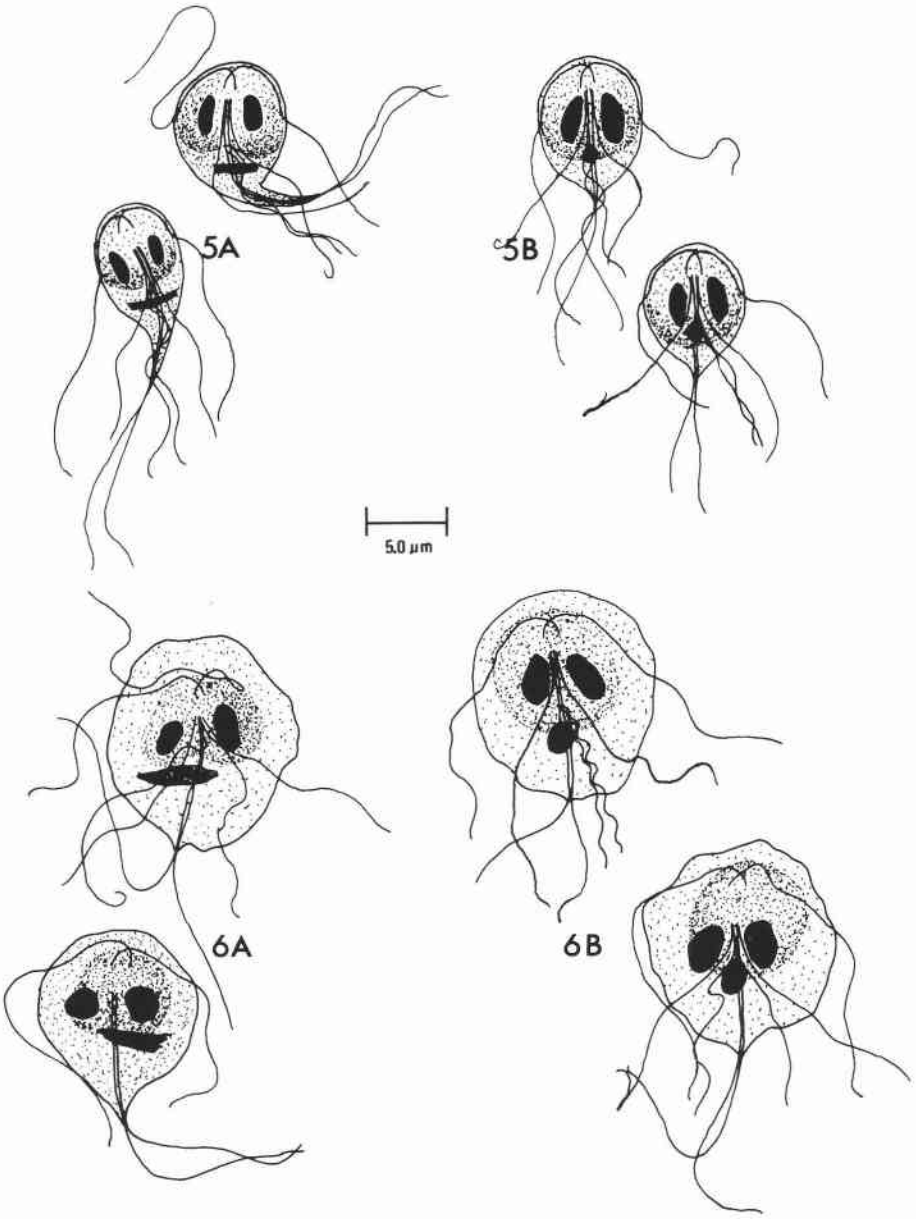

FIG. 5. Camera lucida drawings of trophozoites of (A) *G. microti* and (B) *G. mesocricetus* that were wet fixed in 100% ethanol and 10% buffered formalin prior to staining with Giemsa stain. FIG. 6. Camera lucida drawings of trophozoites of (A) *G. microti* and (B) *G. mesocricetus* recovered from the same populations as those in Fig. 5. These were air dried prior to fixation and staining.

long axis, (*ii*) the point of emergence of the anterior flagella relative to the position of the nuclei, (*iii*) the density of stained cytoplasm, (*iv*) the degree of condensation of nuclear chromatin, and (*v*) the presence of granules at the point of emergence of flagella (Nieschulz 1924; Nieschulz and Krijgsman 1925; Ansari 1951, 1952). The present investigation and earlier studies (Boeck 1917; Kofoid and Christiansen 1915*a*; Filice 1952; Haiba 1953) indicated that such features were dependent on the position and division stage of the trophozoite. Fixatives, stains, and preparation methods are also important. Trophozoites were distorted by drying smears prior to fixation and staining (Haiba 1953). During the present studies, differences in size, general morphology, density of

cytoplasmic staining, and the relative positions of organelles were produced by varying the prefixation drying time (Figs. 5 and 6). The shape of the median body was the only character relatively unaffected. These factors were seldom considered by authors in describing new species and preparation methods were rarely stated in detail. The difficulty in assessing the validity of described species is exemplified by Navarathnam's (1969) description of *Giardia qadrii* from the Indian goat (*Capra hircus*). This species was distinguished from other species by trophozoite morphology and dimensions. The extreme variation in trophozoite morphology and size reported by Navarathnam could have been produced by inconsistent preparation methods. *Giardia qadrii* may be a synonym of *Giardia caprae* Nieschulz 1924 described from domestic goats in Holland.

Noc (1909), Filice (1952), and Bemrick (1962) stated that the only consistent differences between trophozoites of different species of *Giardia* were the shape and position of the median body. The present investigation confirms that *G. muris* and *G. mesocricetus* can be differentiated from *G. microti*, *G. simoni*, and *G. peromysci* on the basis of this criterion. When consistent preparation methods were used these groups of species were distinguished by trophozoite shape and the relative positions of various structures. However, as indicated by Noc (1909) and Filice (1952), further differentiation of species was not possible by comparing trophozoite morphology.

Roberts-Thomson *et al.* (1976) used morphology of cysts to identify a *Giardia* in a hamster. They reported finding median bodies in these cysts and concluded that the species was *G. muris*. Previous authors (Kofoid and Christiansen 1915*a*, 1915*b*; Boeck 1919) reported median bodies in the cysts of several species. However, these structures were never found in cysts examined during the present investigation and no morphological differences were seen which would permit species differentiation. Recent ultrastructural studies (Sheffield and Bjorvatn 1977) failed to show the presence of median bodies in cysts of *G. lamblia* but indicated that portions of the reinforced edge of the sucking disk might be misinterpreted as median bodies when examined by light microscopy.

The dimensions of trophozoites were used to differentiate species in the past. Hegner (1922*a*) suggested that a variety of dimensions might be useful for this purpose. Although minor dimensions suggested by Hegner were used during this study to prepare composite drawings (Fig. 3), intraspecific variation was too great to allow valid statistical comparisons between species. This reflects the great variation in these dimensions within each species and the difficulty of obtaining accurate measurements of such small organisms.

The use of major dimensions of trophozoites to differentiate *Giardia* species was criticized by Filice (1952). He suggested that the disparity between the major dimensions (L and B_1) given by Simon (1922) and Lavier (1924) for different populations of *G. simoni* was greater than the disparity between either one of these and the dimensions given by Simon (1922) for *G. lamblia*. However, Filice failed to recognize that these authors had used different methods to prepare trophozoites for examination, a factor which, as indicated above, might be important in determining the measurements obtained. Filice attempted to provide further evidence from his own (1952) studies. Using Student's t test, he found significant differences in average length (L) and breadth (B_1) between some populations of trophozoites from different hosts of the same species. In addition, he found no significant differences between some populations from hosts of different species. To explain these results, he referred to Tsuchiya's reports (1930, 1931) that the size of cysts of *Giardia canis* and *G. lamblia* varied from day to day and was affected by diet and condition of the host, inferring that the dimensions of trophozoites might be similarly affected. During the cross transmission studies (Grant and Woo 1978) it was shown that the morphology and dimensions of trophozoites of *G. muris*, *G. mesocricetus*, and *G. microti* were independent of the host species. This suggests Felice's assumption might not be correct. Nevertheless, variation between and within populations of *Giardia* must occur and a large number of trophozoites from numerous hosts of the same species must be examined to obtain measurements representative of the species as a whole. If Filice (1952) had pooled measurement data for trophozoites from several hosts of the same species, he would have found significant differences (Table 4).

Some authors (Boeck 1917, 1919; Simon 1921*b*; Kofoid and Sweezy 1922; Lavier 1939) suggested that the presence of median bodies in trophozoites is related to the reproductive cycle of the parasite. The observations that trophozoites with this structure are larger than those without and that median bodies are not present in cysts seem to support this hypothesis. Presumably trophozoites with median bodies are mature forms while those without have recently divided. The function of median bodies is unknown. Recent ultrastructural studies (Cheissen 1964, 1965; Friend 1966; Holberton 1973) showed

that they are composed of microtubules and are not nutrient storage organelles as suggested by others (Boeck 1917, 1919; Simon 1921*b*; Lavier 1939). Selection of organisms without regard to the presence of these structures might result in wide variation in the dimensions obtained for different populations of the same species. Only dimensions of trophozoites with median bodies were considered in the present study when comparing differences between species of *Giardia*; this procedure was followed by Solovjev (1975).

Most *Giardia* spp. studied herein could be differentiated by comparing major dimensions of trophozoites. Significant differences were not found in some cases (e.g. the length of trophozoites of *G. mesocricetus* and *G. simoni* were not significantly different), but these species could be differentiated by comparing trophozoite morphology.

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