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Authors: Measures, Lena N., and Anderson, Roy C.

Source: Journal of Wildlife Diseases, 19(3) : 219-224

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-19.3.219>

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## CHARACTERISTICS OF NATURAL INFECTIONS OF THE STOMACH WORM, *OBELISCOIDES CUNICULI* (GRAYBILL), IN LAGOMORPHS AND WOODCHUCKS IN CANADA

Lena N. Measures and Roy C. Anderson

Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

**ABSTRACT:** Wild lagomorphs and woodchucks collected predominantly in southern Ontario, Canada were examined for subspecies of *Obeliscoides cuniculi* (Graybill). *Obeliscoides cuniculi multistriatus* was found in snowshoe hares (*Lepus americanus*). *Obeliscoides cuniculi cuniculi* was found in cottontail rabbits (*Sylvilagus floridanus*), European hares (*Lepus capensis*) and woodchucks (*Marmota monax*). Prevalence of *Obeliscoides cuniculi multistriatus* in snowshoe hares was 100% and mean intensity (and range of intensity) was 760 (9-4,198) in Lindsay, Ontario in 1980. Mean intensity in hares varied trimonthly. The highest mean intensity of worms occurred in spring when most worms were adult. Transmission occurred mainly in spring. Most worms present in fall (70%) and winter (54%) were fourth stage. Immature fifth-stage and gravid females were present in hares during fall and winter. Prevalence and mean intensity of *O. c. cuniculi* in cottontails was 15% and 29 (1-118). Prevalence and mean intensity of *O. c. cuniculi* in woodchucks was 6% and 56 (16-118). European hares were infected with *O. c. cuniculi*, prevalence was 10% and mean intensity was 60 (36-83). In Ontario woodchucks and European hares were common in areas frequented by cottontail rabbits and probably acquired sporadic infections of *O. c. cuniculi* from infected cottontails.

### INTRODUCTION

*Obeliscoides cuniculi* (Graybill) has been reported from snowshoe hares (MacLulich, 1937; Erickson, 1944; Dodds and Mackiewicz, 1961; Bookhout, 1971; Gibbs et al., 1977); blacktail jackrabbits (*Lepus californicus*) (Ward, 1934); cottontail rabbits (Alicata, 1932; Erickson, 1947; Dorney, 1963; Jacobson et al., 1978; Andrews et al., 1980); marsh rabbits (*Sylvilagus palustris*) (Tomkins, 1935; Stringer et al., 1969) and swamp rabbits (*Sylvilagus aquaticus*) (Ward, 1934; Smith, 1940). It has also been reported from woodchucks (Twichell, 1939; Rausch and Tiner, 1946; Fleming et al., 1979) and white-tailed deer (*Odocoileus virginianus*) (Maples and Jordan, 1966; Prestwood et al., 1973).

Measures and Anderson (1983a) described the two subspecies *O. c. cuniculi* (Graybill, 1923) Graybill, 1924 from cottontail rabbits and *O. c. multistriatus* Measures and Anderson, 1983 from snowshoe hares. The host specificity of these subspecies was examined experimentally (Measures and Anderson, 1983c). However, it is not known whether *O. c. multistriatus* and *O. c. cuniculi*, which will mature in experimentally infected snowshoe hares, cottontail rabbits and woodchucks, are present in these hosts in the wild. Thus, the present study was

undertaken to determine the prevalence and mean intensity of both subspecies of *O. cuniculi* in wild lagomorphs and woodchucks in Canada. In addition, the mean intensity and stages of *O. c. multistriatus* in snowshoe hares collected during 1980 were examined to determine when transmission occurred.

### MATERIALS AND METHODS

Wild lagomorphs (snowshoe hares, Arctic hares (*L. arcticus*), European hares, cottontail rabbits) and woodchucks were trapped, shot or snared during 1980 and 1981. Live animals were killed with a barbiturate (Somnotol<sup>®</sup>, MTC Pharmaceuticals, Hamilton, Ontario L4W 2S5, Canada; Nembutal<sup>®</sup>, Abbott Laboratories, Montreal, Quebec, M5W 1V7, Canada). Animals killed on the road by traffic were also examined. Carcasses were examined immediately or were frozen for examination later. Lagomorphs were categorized by sex and age. Young-of-the-year, juvenile or adult categories were based on weight, striation of the uterus and fusion of the epiphysis of the tibia (Hale, 1949; Keith et al., 1968). Woodchucks were identified as young-of-the-year or adult based on weight (Snyder et al., 1961). Young-of-the-year animals were those born in the calendar year in which they were collected.

Prevalence (the number of hosts infected ÷ the number of hosts examined; Margolis et al., 1982) and mean intensity (the mean number of parasites per infected host in a sample; Margolis et al., 1982) of *O. cuniculi* in animals examined from predominantly southern Ontario were recorded (see Table 3). Ingesta and scrapings (stomach contents) were washed with tap water on a series of three sieves, i.e., 250, 500 and 850  $\mu$ m. Stomach contents were placed in bottles containing 10% formalin and were sampled

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Received for publication 18 October 1982.

TABLE 1. Results of examining wild woodchucks and lagomorphs for *Obeliscoides cuniculi* (1980 and 1981).

Host and locality collected	Number examined	Prevalence	Mean intensity (range)
<b>Snowshoe hares</b>			
Lindsay, Ontario (44°21'N, 78°45'W)	84	100	760 (9-4,198) <sup>a</sup>
Kirkland Lake, Ontario (48°09'N, 80°02'W)	43	88	355 (165-550) <sup>b</sup>
Wellington County, Ontario (43°50'N, 80°30'W)	5	100	39 (5-70) <sup>b</sup>
Edmonton, Alberta (53°33'N, 113°28'W)	12	67	467 (20-1,045) <sup>c</sup>
<b>Cottontail rabbits</b>			
Lindsay, Ontario	8	0	—
Halton County, Ontario (43°30'N, 79°53'W)	35	20	34 (1-118)
Wellington County, Ontario	17	18	19 (11-24)
Malton Airport, Toronto, Ontario (43°39'N, 79°23'W)	5	0	—
<b>Woodchucks</b>			
Halton County, Ontario	4	0	—
Wellington County, Ontario	65	6	56 (16-118)
<b>European hares</b>			
Wellington County, Ontario	5	40	60 (36-83)
Halton County, Ontario	1	0	—
Malton Airport, Ontario	16	0	—

<sup>a</sup> Mean intensity based on 80 animals.

<sup>b</sup> Mean intensity based on three animals.

<sup>c</sup> Mean intensity based on six animals.

using a cylindrical vessel constructed of Plexiglas. Fixed stomach contents were added to this vessel and a 10 ml cylindrical vial filled with water was gently lowered into the vessel. Water was then added to bring the level of the solution to within 0.5 cm of the top of the vessel. Total volume of the vessel containing fixed stomach contents, water and vial was 330 ml. A Plexiglas lid sealed the vessel which was then gently agitated 10 times so the 10 ml vial could move freely and permit free movement of stomach contents in and out of the vial. The vessel was then placed on a table for about 10 min and contents allowed to settle. The vessel lid was taken off and the 10 ml vial carefully removed. Contents of the vial were placed in a Petri dish and were examined using a dissecting microscope. All worms were removed, counted, classified by sex and stage, returned to the vessel and the procedure repeated. The average of three samples was used to determine total number of worms in 330 ml. This method was found to be accurate and consistent.

As sample sizes of snowshoe hares collected in some months were small, data were grouped trimonthly and analyzed using single factor analysis of variance at  $P < 0.05$  (Zar, 1974). Data were transformed to logarithms (base 10) or square root to approximate a normal distribution. The Fischer significant difference test at  $P < 0.05$  (Carmer and Swanson, 1973) was applied to transformed data to locate differences between trimonthly periods. The Mann-Whitney test at  $P < 0.05$  was used to examine age and sex differences (Zar, 1974).

Worms in wild lagomorphs and woodchucks were classified by sex and stage as outlined in Measures and Anderson (1983c). Subspecies of *O. cuniculi* were

identified according to Measures and Anderson (1983a). Specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Helm. Coll. Nos. 77089, 77090, 77091) and the National Museum of Natural Sciences, Ottawa (NMCIC(P) No. 1982-0671).

## RESULTS

*Obeliscoides cuniculi multistriatus* was found only in snowshoe hares. *Obeliscoides cuniculi cuniculi* was found in cottontail rabbits, European hares and woodchucks.

*Obeliscoides cuniculi* occurred with a significantly greater prevalence and mean intensity in snowshoe hares than in cottontail rabbits, European hares and woodchucks (Table 1). The parasite was not found in 15 Arctic hares collected from Ellesmere Island, Northwest Territories (80°N, 85°W and 78°N, 83°W). *Obeliscoides cuniculi* was also found in five snowshoe hares collected from Manitoulin Island (45°50'N, 82°20'W), Sudbury (46°30'N, 81°00'W) and Barrie (44°24'N, 79°40'W), Ontario and Fairbanks, Alaska (64°50'N, 147°50'W), U.S.A.; these animals are not included in Table 1.

All snowshoe hares collected near Lindsay, Ontario were infected. Of four pregnant hares collected in April or May three had large numbers of worms (1,012, 1,001, 3,807); one had

only 231 worms. One lactating hare collected in July had 2,156 worms. There was no significant difference in mean intensity of worms in adult female hares than in adult male hares (Table 2). Significantly lower mean intensities were found in juvenile and young-of-the-year than in adult hares but only 16 of the former groups were available for examination (Table 2). As sample size was small and juveniles and young-of-the-year did not occur in all seasons, seasonal analysis was performed on pooled age and sex classes.

Mean intensity of worms in snowshoe hares was significantly higher in spring (April to June) than in winter (January to March). Mean intensity decreased significantly in summer (July to September). Mean intensity remained low in fall (October to December). Fall and winter mean intensities were not significantly different from that in summer.

Mean intensity of third-stage larvae in snowshoe hares was consistent and low during the year sampled (Table 3). Mean intensity of fourth-stage larvae in winter was significantly higher than in spring. Spring and summer mean intensities were not significantly different. Mean intensity of fourth-stage larvae was significantly higher in fall than in spring and summer. Fourth-stage larvae comprised the greatest percentage of total worms present in both fall (70%) and winter (54%). Fourth-stage larvae represented only 9% of total worms present in spring and 22% in summer.

Mean intensity of immature adults was significantly higher in winter and spring than in fall. Immature adults comprised a small percentage of total worms present throughout the year (from a high of 20% in winter to a low of

TABLE 2. Examination of 80 snowshoe hares collected in Lindsay, Ontario during 1980 for *Obeliscoides c. multistriatus* Measures and Anderson, 1983.

Age and sex	Number examined	Mean intensity (range)
Adult male	36	731 (11-3,949)
Adult female	28	1,102 (28-4,198)
Juvenile female	12	367 (44-1,056)
Juvenile male	1	11
Young-of-the-year male	3	82 (9-205)

2% in fall). Mean intensity of adult worms was lowest in winter and highest in spring compared to other times during the year. Mean intensity of adult worms decreased significantly in summer and fall. Adult worms comprised the greatest percentage of total worms present in spring (82%) and summer (76%). Adult worms represented about 26% of the total worms present in fall and winter, when temperatures were below 0 C.

Small (2 mm in diameter) ulcers and/or a large amount of yellow mucus which contained many worms were observed in six snowshoe hares. The stomachs of these hares contained over 1,000 worms.

Cottontail rabbits were collected from January to December but only those collected from January to July were infected. Prevalence and mean intensity of *Obeliscoides cuniculi* in cottontail rabbits examined was 15% and 29 (1-118). Of 65 cottontail rabbits examined, 32 were female, 31 were male and two were of unknown sex. One was young-of-the-year, three were juvenile, 60 were adult and one was of unknown age. The young-of-the-year rabbit and juvenile rabbits were not infected. Male and

TABLE 3. Results of examining 80 snowshoe hares collected from Lindsay, Ontario during 1980 for *Obeliscoides c. multistriatus* Measures and Anderson, 1983.

Month collected	Total no. examined	Mean intensity $\pm$ SE (range)				
		Total	L <sub>3</sub>	L <sub>4</sub>	Immature adult	Adult
January-March	45	573 $\pm$ 98 (11-3,069)	1 $\pm$ 0.7 (22)	312 $\pm$ 65 (11-2,145)	115 $\pm$ 25 (11-670)	145 $\pm$ 26 (10-505)
April-June	12	1,825 $\pm$ 425 (209-4,198)	2 $\pm$ 1.7 (20)	157 $\pm$ 90 (44-1,111)	170 $\pm$ 78 (165-1,022)	1,497 $\pm$ 322 (209-3,443)
July-September	10	578 $\pm$ 228 (9-2,156)	—	125 $\pm$ 84 (7-869)	15 $\pm$ 5.7 (11-43)	439 $\pm$ 206 (1-2,079)
October-December	13	645 $\pm$ 187 (99-2,596)	3 $\pm$ 1.8 (11-22)	449 $\pm$ 164 (77-2,035)	16 $\pm$ 8.6 (11-99)	177 $\pm$ 57 (11-550)

female rabbits had similar intensities of infection. Stomach lesions were not observed at gross examination of infected cottontail rabbits.

Woodchucks were collected from May to August 1981 but only those collected in June or July were infected. Prevalence in woodchucks examined was 6%. Of 69 woodchucks examined 26 were female, 41 were male and two were of unknown sex. Seventeen were young-of-the-year, 50 were adult and two were of unknown age. Young-of-the-year woodchucks were not infected. Male woodchucks had a greater mean intensity of worms than female woodchucks. Stomach lesions were not observed during gross examination of infected woodchucks.

European hares were collected in winter and summer but only hares collected in summer were infected. Prevalence was 10%. Of 21 adult hares examined seven were female, nine were male and five were of unknown sex. Both infected animals were male.

#### DISCUSSION

Prevalence and mean intensity of *Obeliscoides cuniculi* in snowshoe hares collected in Ontario was greater than previously reported (Erickson, 1944; Dodds and Mackiewicz, 1961; Bookhout, 1971; Gibbs et al., 1977). Bookhout (1971) and Gibbs et al. (1977) reported prevalences of 96% and 97% in snowshoe hares in Michigan and Maine.

Three young-of-the-year snowshoe hares collected in July and August had low intensities. Leverets (young hares) are nursed up to 4 wk and occasionally longer, but they begin to feed on grass and herbaceous plants when 10 to 12 days old (Severaid, 1942). At this time leverets could be exposed to infective larvae, especially if the lactating female is infected. In the present study, all juvenile hares collected were infected but mean intensity was considerably lower than in adults. Erickson (1944) reported greater prevalence and mean intensity of *O. cuniculi* in adult than in immature hares.

Most adult worms present in snowshoe hares collected in spring 1980 near Lindsay probably represented worms that over-wintered as fourth-stage larvae since there was a significant decrease in mean intensity of fourth-stage larvae and a significant increase in mean intensity of adults from winter to spring. The increase of adult worms in hares in spring was consistent with a return to more favorable conditions in

the external environment for development and transmission of free-living stages. Spring is probably more important for transmission of this parasite since moisture is abundant and the external environment is contaminated with large numbers of eggs from maturing adult worms that overwintered in hares. Mean intensity of worms decreased significantly during summer but the presence of fourth-stage larvae in hares in summer indicated that hares were still acquiring some infections during this period. The low mean intensity in hares in the fall suggests that hares did not acquire large infections at this time. Gibbs et al. (1977) also observed a decrease in mean intensity in snowshoe hares in Maine during summer and fall.

Gibbs et al. (1977) postulated that high temperature and low moisture levels in midsummer adversely affected free-living stages. During the present study, moisture was abundant during spring and summer. It was only during September that precipitation was lower than normal. Ambient temperatures in the Lindsay area were normal during 1980. However, local microclimatic conditions could moderate or intensify the temperature for free-living stages (Kates, 1965). The decrease in mean intensity of *O. cuniculi* in hares during summer and fall could also be accounted for by increasing levels of immunity as suggested by Gibbs et al. (1977). However, Erickson (1944) suggested that resistance was slow to develop and he found that infections tended to increase over time.

Winter in southern Ontario lasts approximately 4 mo from December to March. Measures and Anderson (1983c) have shown that infections in domestic rabbits can last a maximum of 118 days. Fernando et al. (1971) have shown that infective larvae of *O. cuniculi* exposed to 4 C arrest development at the early fourth-stage in domestic rabbits. Infective larvae of *O. cuniculi* exposed to low temperatures during fall in the external environment probably become arrested at the fourth stage in hares. Gibbs et al. (1977) observed a high percentage of fourth-stage larvae in hares collected in October, December, January and February and suggested that arrested development may be important for the parasite's survival during prolonged winter conditions. Arrested larvae would probably overwinter until more favorable conditions returned in the external environment.

In the present study, adult and immature

worms were present in fall and winter. Adult females were gravid and presumably releasing eggs at this time. Dorney (1963) suggested that some eggs in feces of cottontail rabbits infected with *O. cuniculi* in Wisconsin can survive cold temperatures in the external environment. However, it is doubtful that transmission occurs during winter. Measures and Anderson (1983b) indicated that some eggs and infective larvae of *O. c. multistriatus* can survive low or freezing temperatures. Griffiths (1937) reported that *Trichostrongylus colubriformis* could survive winter on pasture in Canada and infect worm-free sheep the following spring. Conditions in the microenvironment for free-living stages of trichostrongyles during winter are probably moderated by grass and snow cover (Andersen et al., 1966). In areas where frost penetrates deeply into the soil, survival of larvae is unlikely. However, where deep snow persists for several months, survival of larvae may be enhanced (Gibson, 1966).

In the present study, the marked difference in prevalence and mean intensity of *O. cuniculi* in snowshoe hares as compared to those in cottontail rabbits may be due to the different habitats these lagomorphs occupy. In Ontario, snowshoe hares inhabit cedar or spruce swamps which are wet most of the spring and summer. Environmental conditions in these wet areas would be favorable for the development and transmission of free-living stages of *O. cuniculi*. In contrast, cottontail rabbits inhabit deciduous woodlots or hedgerows in agricultural areas. These areas are relatively dry compared to areas inhabited by snowshoe hares and this may account for the low prevalence and mean intensity of *O. cuniculi* in cottontail rabbits examined.

Woodchucks in southern Ontario are common in habitats frequented by cottontail rabbits which occasionally utilize woodchuck burrows. Thus, woodchucks probably acquired infections of *O. cuniculi* sporadically from infected cottontail rabbits. Only woodchucks collected in June and July were infected. Woodchucks may lose infections during hibernation as suggested by Fleming et al. (1979). Infections are probably acquired only in summer after infected cottontail rabbits contaminate woodchuck burrows with their feces. *Obeliscoides c. multistriatus* developed in experimentally infected woodchucks (Measures and Anderson, 1983c). The

absence of *O. c. multistriatus* in woodchucks in Ontario is probably due to the ecological separation between woodchucks and snowshoe hares.

The occurrence of *O. cuniculi* in European hares is a new host record in North America. European hares, which were introduced to Ontario in 1912 (Dymond, 1928), share a similar habitat with cottontail rabbits. The presence of *O. c. cuniculi* in European hares indicates that they are acquiring this subspecies from infected cottontails.

*Obeliscoides c. cuniculi* developed in experimentally infected snowshoe hares and *O. c. multistriatus* developed in experimentally infected cottontail rabbits (see Measures and Anderson, 1983c). The absence of *O. c. multistriatus* in wild cottontail rabbits and the absence of *O. c. cuniculi* in wild snowshoe hares examined may be related to the different habitats these lagomorphs occupy in Ontario. The ecological separation of the two hosts may have been essential in bringing about the subspeciation of *O. cuniculi* (Measures and Anderson, 1983a).

#### ACKNOWLEDGMENTS

T. Warren and students of Sir Sandford Fleming College in Lindsay, Ontario, C. Bartlett, Dr. E. Bailey, K. Duncan, J. McCreadie, D. Schnurr, M. Whittaker, Dr. V. Thomas and others helped to collect animals. S. Van Walsum provided excellent technical help. R. J. Hines of the Department of Mathematics and Statistics, University of Guelph provided statistical expertise. J. Smith and Dr. R. Appy contributed their knowledge of statistics and computers. Dr. E. Bailey kindly reviewed the manuscript. This work was funded through a National Science and Engineering Research Council of Canada operating grant.

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