Intraspecific differences between cottontail and woodchuck isolates of the trichostrongylid nematode Obeliscoides cuniculi (Graybill)

by Nelson Samuel

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
Montana State University

Abstract:
The investigations reported in this paper were Undertaken to determine whether isolates of Obeliscoides cuniculi from the southern woodchuck (Marmota monax monax) and the eastern cottontail rabbit (Sylvilagus floridanus mearnsi) are identical or two distinct strains. Infections were induced in laboratory rabbits by oral inoculation of 3,000 infective larvae of woodchuck and cottontail origin. In the first experiment, the course of the infection was studied in two 5-animal groups, by following fecal egg counts twice weekly during a 5 month period. The second experiment comprised two groups, each containing 6 animals, all of which were necropsied 28 days postinoculation.

The prepatent period averaged about 17 days in the woodchuck strain and 15 days in the cottontail strain. Worm egg output by the cottontail strain was much higher than in the woodchuck strain. The cottontail strain exhibited 2 peaks before egg production decreased from the maximum to a low level. In two rabbits the woodchuck strain also showed a second peak. At 161 days postinoculation, the average number of cottontail strain eggs passed in a 24-hour period was 17,931, compared with 2,998 in the woodchuck strain.

More worms were recovered at necropsy from rabbits infected with the cottontail strain than from rabbits infected with the woodchuck strain. The female-to-male ratio in the cottontail strain was 1:1.3 at 28 days and 1:2.03 on day 168 postinoculation. A similar sex ratio was obtained in the woodchuck strain.

The associated pathological changes were primarily those of gastritis, the mucosa being studded with petechial haemorrhages in small localized areas, edematous fundic rugae, and a few areas of ulceration. Histological studies revealed that the woodchuck strain in some instances produced the most severe lesions. In older infections a few crateriform ulcers were more common in rabbits infected with the woodchuck strain than in the cottontail strain.

Measurements of adult Obeliscoides cuniculi of both strains showed significant differences ($P<0.01$) in length of body, esophagus, spicules, and in the distance from anterior end to vulvar region.

Retardation of the parasite at the fourth stage was the most consistent phenomenon observed in rabbits infected with the cottontail strain. The inhibited larvae remained in the mucosa as long as 225 days.

In accordance with the above findings, it is concluded that 0. cuniculi isolates of woodchuck and the cottontail origin which were used in this study represent distinct strains having a number of unique physiological and morphological characteristics.

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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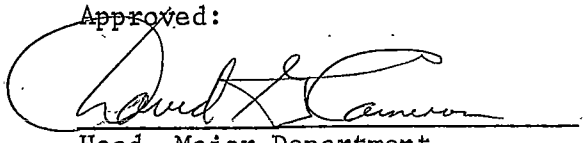
Approved:



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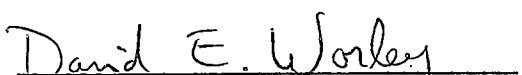

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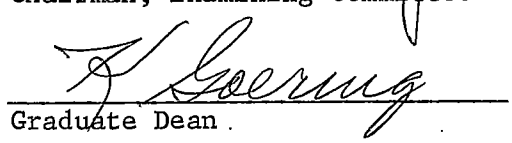

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ABSTRACT

The investigations reported in this paper were undertaken to determine whether isolates of *Obeliscoides cuniculi* from the southern woodchuck *(Marmota monax monax)* and the eastern cottontail rabbit *(Sylvilagus floridanus mearnsi)* are identical or two distinct strains. Infections were induced in laboratory rabbits by oral inoculation of 3,000 infective larvae of woodchuck and cottontail origin. In the first experiment, the course of the infection was studied in two 5-animal groups, by following fecal egg counts twice weekly during a 5 month period. The second experiment comprised two groups, each containing 6 animals, all of which were necropsied 28 days postinoculation.

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Retardation of the parasite at the fourth stage was the most consistent phenomenon observed in rabbits infected with the cottontail strain. The inhibited larvae remained in the mucosa as long as 225 days.

In accordance with the above findings, it is concluded that *O. cuniculi* isolates of woodchuck and the cottontail origin which were used in this study represent distinct strains having a number of unique physiological and morphological characteristics.

INTRODUCTION

Graybill (1923) based his original description of *Obeliscoides cuniculi*, a trichostrongylid nematode parasite of rabbits, hares, and rodents, on specimens obtained from the stomach of domestic rabbits *(Oryctolagus cuniculus)* in New Jersey. Originally he assigned the generic name *Obeliscus*, but the following year he proposed *Obeliscoides* because the name *Obeliscus* was preoccupied in the phylum Mollusca (Graybill, 1924). The morphological features of the adult *Obeliscoides cuniculi* have been described by Graybill (1923) and Chandler (1924). Alicata (1932) described the life history and developmental stages in the host. He also described preparasitic development and the responses of third stage larvae to various physical stimuli. Neither he nor Dixon (1965) were able to establish percutaneous *O. cuniculi* infections in rabbits. Alicata (1932) followed the course of infection in the guinea pig, which is an abnormal host for this nematode. He also used *O. cuniculi* larvae for the immunization of rabbits against this parasite (Alicata, 1965).

Worley (1963) investigated whether *O. cuniculi* infections in rabbits resembled gastric nematodiasis of ruminants, and concluded that a number of similarities existed. He also suggested this rabbit nematode infection as a laboratory model of ruminant helminthiasis. Later Worley and Thompson (1963) investigated the efficacy of using rabbits infected with *O. cuniculi* as a system for anthelmintic screening.

Tiner (1965) found that the effect of thiaxanthene delayed embryonal development and destroyed growing larvae *in vitro*. Russell *et al.*, (1966) concluded that many similarities exist between the infection produced by this gastric nematode of rabbits and *Trichostrongylus* or *Ostertagia* infections of cattle and sheep. They suggested that rabbits infected with this stomach worm could be used as a model in the laboratory for the study of ruminant trichostrongyloidosis. Schwartz and Shook (1931) reported that rabbits infected with stomach worms suffer from diarrhea, emaciation, ulceration of the stomach wall, anemia and may even die from the infection.

Fernando (1968) studied the distribution of hematin compounds within various tissues of *O. cuniculi*. Hemoglobin was detected within the intestinal cells of fourth stage larvae approximately 6 days after host infection, and continued to increase throughout development. It was also present in perienteric fluid and in the body wall of both female and male adult worms. *O. cuniculi* has been used as a source of test tissue for certain histochemical techniques in the study of histochemistry of the trichostrongylidoses by Frandsen (1965), who also reported that carbohydrates and collagen were present in different tissues of this parasite (Frandsen, 1966).

Natural *Obeliscoides cuniculi* infections in rabbits and hares have been reported in many areas of the United States. Dorney (1963) reported that 98% of the fecal samples collected during two consecutive

winters from cottontail rabbits *(Sylvilagus floridanus)* in Green Bay, Wisconsin contained *Obeliscoides* ova. Morgan and Waller (1940) found that the incidence *O. cuniculi* was 11.9% in the Iowa cottontail *(Sylvilagus floridanus mearnsi)*. Clancy *et al.* (1940) reported that this parasite occurred in the eastern cottontail *(Sylvilagus floridanus mallurus)* and in the New England cottontail *(Sylvilagus transitionalis)* throughout Connecticut. Worley (1963) referred to Albrecht's unpublished report that 68% of the cottontail rabbits examined in Ohio were infected with this stomach worm. The incidence of infection with *O. cuniculi* in Kansas rabbits *(Sylvilagus floridanus)* was 16.6% (Franklin *et al.* 1966) and in Cleveland County, Oklahoma, was 81.7% in swamp rabbits *(Sylvilagus americanus aquaticus)* (Smith, 1940). Ward (1934) found this parasite in rabbits of central Oklahoma. Aldous (1936) examined 54 stomachs of snowshoe hares *(Lepus americanus phaeonotus)* from a study area near Cushing, Minnesota and reported that half of them were infected with an average of 99 *Obeliscoides cuniculi* per hare. Dunagan (1957) and Lubinsky (1957) reported *O. cuniculi* in the snowshoe hare of Alaska and Canada, respectively, and Park (1932) found the parasite in a Kansas jack rabbit *(Lepus californicus melanotis)* in the New York Zoological Park. Erickson (1944) noted that 1116 out of 1428 snowshoe hares from Minnesota were infected with this species. He considered that this stomach worm infection was significant in producing mortality in the hare population.

Obeliscoides cuniculi has previously been reported from woodchucks *(Marmota monax monax)* in Missouri (Twichell, 1939); Itasca Park, Minnesota (Wallace, 1942); central Ohio and East Lansing, Michigan (Rausch and Tiner, 1946). The latter authors suggested that the woodchuck may be a common host for this parasite. This was substantiated in one locality in eastern Ohio by Worley (personal communication) who found all 7 woodchucks examined were infected with moderate or heavy worm burdens.

Because of a number of discrepancies in the descriptions and measurements of *Obeliscoides cuniculi* specimens from domestic rabbits in Texas and New Jersey, Chandler (1924) felt that there could be more than one North American species of *Obeliscoides*. However, there has been no report of comparative studies on the biology of *O. cuniculi* from different hosts.

Alicata (1932) found immature stages of worms in infected rabbits necropsied at day 31 postinoculation. It was not evident whether the term "immature stage" was synonymous with fourth stage larvae which he described. Russell *et al.* (1966) recovered numbers of immature parasites in direct proportion to the size of the inoculum given to rabbits necropsied 51 days postinoculation. Sollod *et al.* (1968) contributed further to the description of the parasitic stages of *O. cuniculi*. They classified as histotropic forms fourth stage larvae obtained by digestion of gastric mucosae scraped from rabbits necropsied during

prepatency.

The term histotropic phase has been used to denote the period during which nematode larvae are present in the tissues. Some of the larvae remain a longer time than the normal 1 to 2 week period in the mucosa and fail to grow after molting to the fourth stage. Sommerville (1954) observed this process in *Ostertagia circumcincta* in sheep and found the larvae remained in the mucosa as long as 3 months. However, there has been no report of a prolonged histotropic phase of development for *O. cuniculi* which resembles the larval tissue stage of *O. circumcincta* described by Sommerville (1954).

This project was initiated to compare the biological characteristics of woodchuck and cottontail isolates of *O. cuniculi* and to study its histotropic phase.

MATERIALS AND METHODS

The nematode strains, isolated from an eastern cottontail rabbit *(Sylvilagus floridanus mearnsi)* and a southern woodchuck *(Marmota monax monax)* in Ohio in 1959 and 1965 respectively, were maintained in laboratory rabbits *(Oryctolagus cuniculus)*. The maintenance of *Obeliscoides cuniculi* has been described previously (Worley, 1963). The rabbits were individually housed in wire bottomed (half-inch mesh) cages. They were fed nonmedicated Peavey rabbit pellets (Peavey Company, Minneapolis, Minnesota). Infective (third stage) larvae were cultured by incubating a mixture of sterilized peat moss and freshly passed feces from infected rabbits for 8 to 10 days at 20° to 24°C in covered glass dishes. Infective larvae were harvested by the Baermann technique. Larvae were washed, concentrated by sedimentation and stored in water at 3° to 4°C for 3-5 days before innoculation.

The method used for culturing feces to obtain infective larvae was kept as uniform as possible in both the strains. A few larvae were heat killed for measurements and preserved in a mixture of 95 parts 70% ethanol and 5 parts glycerol. All measurements were made on larvae from cultures 8 days old. Adult worms recovered from a naturally infected woodchuck in Ohio in 1963 were used for morphological studies. These specimens were compared with adult worms of the woodchuck strain after 7 passages in laboratory rabbits and adults of the cottontail strains after more than 10 passages. The total length of adult worms

was measured with the aid of a B & L microprojector. The larval and other measurements of the adults were made with a Leitz Ortholux microscope equipped with an ocular micrometer. Morphological differences between larvae and adults of the two strains were compared statistically using standard deviations, analysis of variance, and Duncan's multiple range test (Duncan, 1955). The data were processed by the computer Sigma 7 BPM/BTM-D_1B at the computing center, Montana State University.

Ten Californian cross-bred rabbits of two litters, born and reared in the colony of the Veterinary Research Laboratory, were allotted to two groups, W_1 and C_1, each containing 5 animals. The age of the animals ranged from 128 to 153 days and they weighed 6-8 pounds at the start of the experiment. Infections in each animal were induced by oral inoculation of 3,000 infective larvae given with a "Bard" plastic catheter. Groups W_1 and C_1 received equivalent numbers of woodchuck and cottontail strain larvae. Fecal egg counts (Lane, 1928) were carried out twice weekly in each group from day 25 postinoculation to week 23. All rabbits were weighed weekly and were kept under daily observation. Preinoculation fecal examinations were performed with saturated sodium chloride flotation to ensure that all animals were free of gastrointestinal parasites. Fecal examinations were performed routinely from the 13th day postinoculation until eggs first appeared in the feces. Two rabbits from group W_1 and one from C_1 were removed during the test and the rest were necropsied on the 168 day postinoculation.

In the second experiment, twelve Californian cross-bred rabbits ranging in age from 196 to 199 days were allotted to 2 six-animal groups (W_2 and C_2). They were inoculated with 3,000 infective larvae of the woodchuck strain (group W_1) and an equivalent dose of cottontail larvae (group C_2). Two rabbits from group C_2 were eliminated from the experiment. One of these died and the other gave birth to young early in the course of the project. The remaining 10 rabbits were killed on the 28th day postinoculation. In both experiments littermates were separated into pairs by sex and one of each pair was allotted to each experimental group. All the rabbits were killed by injecting about 5 ml. of air in the outer marginal vein of the ear or by overexposure to chloroform. The stomach of each animal was ligated at the esophagus and the duodenum to retain its contents. It was removed from the abdomen and transferred to a petri dish containing 0.86% saline solution. Approximately one sq. cm. of stomach tissue along the lateroventral side of the greater curvature and opposite the esophageal opening was taken for histological studies. The stomach tissue was preserved in 10% buffered formalin. The tissues were sectioned at 6 microns and stained in azure eosinate using Hadlow's method (Lillie, 1954). The stomach was opened along the lesser curvature and the mucosal lining was scraped. The ingesta and the mucosal scrapings were washed through a 100-mesh screen to remove finer debris. The washings were examined and the number and sex of the adult worms were recorded. After thorough washing, the stomach was cut

into anterior and posterior halves. The two portions of the stomach were incubated overnight on a mechanical shaker at 37°C in widemouthed bottles containing 0.86% saline solution to free histotropic forms from the mucosa. The stomach tissue and contents of the bottles then were washed on a 200-mesh screen, centrifuged and immature forms recovered with the aid of a dissecting microscope.

RESULTS

Worm egg output in feces: The prepatent period averaged about 17 days (range 17-19) in the woodchuck strain and 15 days (range 15-17) in the cottontail strain. Egg output in the feces of rabbits (groups W_1 and C_1) given 3,000 infective larvae is illustrated in Figure 1. Egg production during the first weeks of patency increased more rapidly in the cottontail strain (group C_1) than in the woodchuck strain (group W_1). The egg output fluctuated at a high level for 6-11 weeks postinoculation, after which it decreased abruptly. In the later stage of infections egg output was diminished gradually in both strains. In all 4 rabbits infected with the cottontail strain, two peaks of egg output were observed. The first peak averaged about 889 eggs/g of feces and was between the 6th and 8th week of infection. The second peak occurred between 8 to 11 weeks postinoculation with an average of 1000 eggs/g of feces. Group W_1 shwed a peak averaging 499 eggs/g of feces during the 8th or 9th week of infection. Two of four rabbits in this group showed a second peak of 559 eggs/g of feces between the 11th and 16th week postinoculation, approximately 25 and 21 days after the first peak. At 161 days postinoculation, the number of eggs passed in a 24-hour period by rabbits in groups W_1 and C_1 was 2,998 and 17,931 respectively. The mean ratio of eggs per gram of feces to worms in the woodchuck strain was 1 egg/g/worm or 2.95 eggs/g/female. In the cottontail

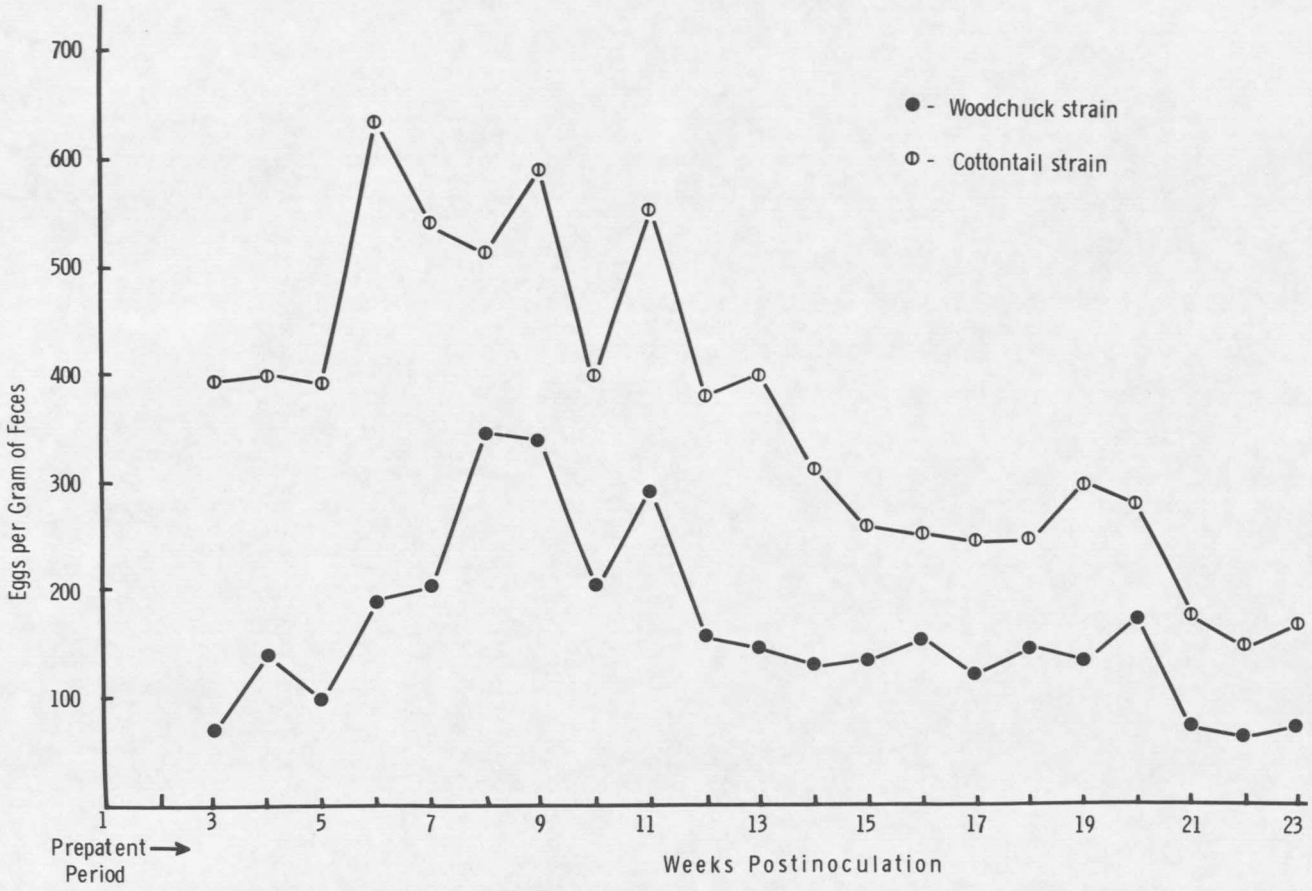

Figure 1. Average fecal egg counts of laboratory rabbits exposed to 3,000 larvae of O. cuniculi. Points shown are mean values for 4-animal groups.

strain, the ratio was 0.93 egg/g/worm or 2.56 eggs/g/female.

Worm sex ratios: The female-to-male worm ratio in a 28 day-old infection was about 1:1.19 in woodchuck strain and 1:1.30 in cottontail strain infections. In 168-day infections, the female-to-male ratio was 1:1.75 in the woodchuck and 1:2.03 in cottontail strains.

Distribution of worms in the stomach: The average recovery of inhibited fourth stage larvae from the anterior half of the stomach was 75 worms in the woodchuck strain and 201 in the cottontail strain. In the posterior half, very few larval forms of either strain were recovered. The total worms and the fourth stage larvae recovered are shown in Tables I and II. At 168 days postinoculation, 41.49% of the group W_1 were fourth stage forms, versus 55.04% of the group C_1. In a 28 day-old infection, the fourth stage larvae comprised 28.3% and 31.6% of the worm burden in woodchuck and cottontail strains respectively.

The adult worms of both strains were generally confined to the cardiac and anterior portion of the fundic region in early patency. In the later patent period, the gastric mucosa of the body and the fundic regions had an even distribution of worms. No evidence was found in the present study of a pyloric infection with *O. cuniculi*, although the pyloric wall was thickened in one rabbit (group W_1).

Pathogenicity: Gross pathological observations in groups W_2 and C_2 show that petechial lesions were concentrated in the cardiac and fundic regions. The associated pathological changes were primarily