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OBSERVATIONS ON *TURGIDA TURGIDA* (RUDOLPHI, 1819) (NEMATODA: PHYSALOPTEROIDEA) IN THE AMERICAN OPOSSUM *(DIDELPHIS VIRGINIANA)*

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Abstract: Turgida turgida is a common parasite of the stomach of the American opossum in Florida. Worms were usually found attached to the greater curvature of the corpus of the stomach. Ulcers always occurred at the site of the attachment. Large ulcers were associated with groups of adult parasites and were found in the oldest experimental infections. *Turgida turgida* fed on food within the stomach of the opossum and attached to the stomach wall when not feeding. Third- and fourth-stage larvae were found in opossums in Florida mainly from May to August suggesting that this is a major period of transmission.

INTRODUCTION

Turgida turgida (Rudolphi, 1819) Travassos, 1920 has been reported in the American opossum in Florida (Pournelle, 1950), Louisiana (Dikmans, 1931; Green, 1980), Texas (Chandler, 1932), Georgia (Blumenthal and Kirkland, 1976; Nettles et al., 1975; Stewart and Dean, 1971), Tennessee (Reiber and Byrd, 1942), North Carolina (Feldman et al., 1972; Miller and Harkema, 1970), Virginia (Holloway, 1966; Holloway and Dowler, 1963; Sherwood et al., 1969), Illinois (Babero, 1957; 1960; Leigh, 1940), Pennsylvania (Blumenthal and Kirkland, 1976), New York (Hamilton, 1958; Stoner, 1945) and Minnesota (Odlaug, 1956). The parasite causes marked lesions in the stomach wall but the pathogenicity has not been investigated in any detail (Krupp, 1966; Nettles et al., 1975; Sherwood et al., 1969).

The present study was undertaken because of the paucity of information about this common parasite. Wild American opossums from Florida were examined to determine prevalence and intensity and to observe lesions. Laboratory reared opossums were infected to determine if lesions were related to size of inoculum and/or length of the patent period. Also, preliminary studies of the feeding behavior of *T. turgida* were undertaken.

MATERIALS AND METHODS

Examination of wild opossums from Florida

Ninety-six stomachs of freshly-killed opossums were obtained for examination. The opossums had been captured in 1979 and 1980 near Riverview, Florida and kept in captivity for a maximum of two wk prior to necropsy.

Using a dissecting microscope the stomachs were examined and the numbers, stage and location of nematodes recorded. Worms were removed and fixed in a mixture of glycerine and 70% alcohol. Tissue with lesions was fixed in 10% buffered formalin, embedded in paraffin, sectioned at approximately 5 μm and stained with haematoxylin and eosin. Most stomachs were placed also in a digest (10 ml HCL, 1000 ml distilled water and 6 g pepsin) in a Baermann apparatus. The digest was examined hourly for 5 to 10 hr for the presence of larvae.

Experimental studies

Eggs and adults of the common field cricket *(Acheta pennsylvanicus)* were

maintained at 20-21 C or 30 C, 35-55% RH and 16 hr photoperiod following methods of Harris and Svec (1964) and Cawthorn and Anderson (1976). Crickets were infected with *T. turgida* by the methods of Cawthorn and Anderson (1976).

Six adult opossums were obtained from a commercial supplier in Riverview, Florida, and four opossums with young in their pouch were obtained from Georgia. Young were weaned at 5 wk of age. All opossums were housed individually in cages (94×53×30 cm) and maintained on a 16 hr photoperiod at 20-24 C. Cloth was provided as nesting material. Animals were fed commercial food supplemented with Nutriderm, [1] mink food, fruit, chicks and mice.

Nine laboratory-reared opossums were infected with third-stage larvae of *T. turgida* obtained from experimentally infected field crickets. Opossums were examined 20, 40, 60, 80, 100, 120, 138, 160 and 360 days after oral inoculation. The number of worms recovered was recorded and parts of the stomach where worms were attached were preserved for future study.

Ten opossums infected with *T. turgida* (naturally or experimentally) were given food containing the marking powder chromium sesquioxide. [2] Food was withheld from opossums 1 to 3 days prior to feeding marked food. The opossums were killed 3 to 5 hr after the animal had eaten the marked food. Worms removed from the stomachs of the opossums were examined for marking powder.

RESULTS

Examination of wild opossums from Florida

Prevalence of *T. turgida* in the 93 opossums examined was 72%. The sex of some opossums had not been recorded. However, prevalence in 28 females and 29 males was 80% and 59% respectively. Intensity in the 67 infected opossums was 15 (range 1-140). Mean intensity in 28 females and 29 males was 29 and 7 respectively.

Thirty-seven animals were examined from May to August. A total of 778 nematodes was found. Of these, 47% were third stage, 18% were fourth stage and the remainder (35%) were adult. Thirty animals were examined from September to April. A total of 231 nematodes was collected. Of these, 4% were third stage, 8% were fourth stage and the remainder (88%) were adult.

Lesions associated with T. turgida

Most worms were found attached to the greater curvature of the corpus of the stomach, immediately posterior to the fundus. Larvae were scattered throughout the corpus but adults tended to occur in one to three groups in the anterior part of the corpus.

Pinpoint ulcers, less than 1 mm in diameter, were associated with attachment of larvae. Small ulcers, 2 to 3 mm in diameter, were associated with the attachment of one or a few adults. These lesions were observed in all infected opossums. Pinpoint ulcers extended superficially into the submucosa and the larger ulcers extended deeply into the submucosa. Necrotic debris and eosinophilic material were present at the surface of the ulcer and appeared to be associated with attachment areas.

Large ulcers, 6 to 15 mm in diameter, were found in three of the 91 opossums examined. Specimens associated with large ulcers were exceptionally large adults and the number varied from 17 to 23. The mucosa and submucosa of one opossum was destroyed but some of the muscularis externa remained intact in the region of the ulcer. Only a thin layer

[1] Nordon Co., 1815 Myerside Drive, Unit 11, Mississauga, Ontario L5T 1G3, Canada.

[2] Cr_2O_3, Canlab, Canadian Laboratory Supplies, J.T. Baker, Chemicals, 80 Jutland Rd., Toronto, Ontario M87 2H4, Canada.

of granulation tissue separated nematodes from the peritoneal cavity in two of the opossums. Fibrinous exudate was present on the serosal surface of one stomach. A few large arterioles and many small ones in the area of the submucosa contained thrombi. Granulation tissue surrounded the arterioles. Most of the muscularis externa was replaced by granulation tissue. There were numerous eosinophils, neutrophils, fibroblasts and collagen fibers under the layer of necrotic debris and eosinophilic material at the surface of the ulcer. A few plasma cells were visible in the granulation tissue.

Examination of experimentally infected opossums

Larvae were scattered in the corpus and adults were generally concentrated in one to three large groups in the anterior part of the corpus near the fundus. The percentage of larvae recovered decreased with the length of time the opossum had been infected (Table 1).

Lesions were related to the stage of development of worms. In early infections (20, 40, 60, 80 days) in which only third and fourth-stage larvae were recovered, pinpoint ulcers (less than 1 mm in diameter) were present at sites of attachment of larval stages. In older experimental infections (100, 122, 138, 160 and 360 days) ulcers in the stomach were 2 to 10 mm in diameter and the older the infection the larger the ulcer. These ulcers were associated with sites of attachment of groups of adults (usually more than 12 worms).

One hundred days after infection the mucosa was destroyed and the submucosa was infiltrated with granulation tissue. In older infections (122, 138 days) large ulcers penetrated deeply into the stomach wall and the submucosa was usually severely damaged and the muscularis externa infiltrated with granulation tissue. Large ulcers were composed of numerous small depressions where individuals or a small group of worms had attached. At the surface of most of the small depressions was an amorphous eosinophilic material and just below this there were numerous eosinophils. Below the layer of eosinophils there was extensive granulation. The granulation tissue consisted of numerous fibroblasts and collagen fibers which were infiltrated by eosinophils, neutrophils and a few plasma cells.

Three adult worms were associated with an ulcer from an opossum infected

TABLE 1. Developmental stage and total number of *Turgida turgida* recovered from American opossums at various times after inoculation.

No. larvae given	Time necropsy (days after inoculation)	Worms recovered		No. worms recorded (%)[b]					
		no.	(%)[a]	Third stage		Fourth stage		Adults	
126	20	90	(70)	5	(6)	85	(94)	0	(0)
90	35	45	(50)	1	(2)	30	(67)	14	(31)
126	40	73	(58)	2	(3)	15	(20)	56	(77)
126	60	49	(39)	8	(16)	1	(2)	40	(82)
126	80	70	(55)	0	(0)	0	(0)	70	(100)
126	100	40	(32)	0	(0)	3	(8)	37	(92)
126	122	11	(15)	0	(0)	2	(18)	9	(82)
142	138	25	(16)	1	(4)	4	(16)	20	(80)
126	160	33	(26)	0	(0)	1	(3)	32	(97)
142	360	14	(10)	0	(0)	0	(0)	14	(100)

[a]percent of inoculum in parentheses.
[b]percent of total worms found in the opossum.

for 160 days. The lesion was approximately 3.5 mm wide at the base and extended into the muscularis externa which was replaced in some areas by granulation tissue. The muscularis externa at the base of the ulcer was damaged.

Feeding behaviour of T. turgida in opossums

Chromium sesquioxide, mixed with food of the opossums, was found in many of the *T. turgida* collected when the animals were killed a few hours later (Table 2). The compound was found in both larvae and adults and was most prevalent in those collected from opossums which had been denied food for two and a half to three days prior to the feeding of the marked food.

DISCUSSION

Prevalence and intensity in American opossums from Florida were lower than those previously reported in the United States. The opossums studied herein had been held in captivity for up to two weeks prior to necropsy and under these conditions, some worms might have been lost. Prevalences and intensities were higher in females than in males. However, more females were collected in summer than males, and since this was the period of greatest prevalence and intensity, the results could be biased in favor of females. Blumenthal and Kirkland (1976) reported that there was no significant difference in the mean intensities in male and female opossums from Pennsylvania.

Larval *T. turgida* were most common in animals collected from May to August indicating that this was an important period for transmission. Perhaps during this period opossums consume large numbers of insects. Studies have shown that opossums eat food which is seasonally abundant (Blumenthal and Kirkland, 1976; Fitch and Sandidge, 1953; Hamilton, 1958). Hamilton (1958) stated that insects, when available, are eaten more frequently than any other food. Wiseman and Hendrickson (1950) found that insects were the main component of the diet of opossums in late spring and early summer in southwest Iowa. Reynolds (1947) reported that insects were the most commonly found food items in opossums from Missouri.

Most *T. turgida* were attached to the greater curvature of the corpus of the stomach. *Physaloptera* spp. also appear to be site selective. Most *P. hispida* attach to the pyloric region in the stomach of cotton rats *(Sigmodon hispidus)* (Schell, 1952). *Physaloptera maxillaris* is generally found attached to the greater curvature of the fundic portion of the stomach of striped skunks *(Mephitis*

TABLE 2. The presence of chromium sesquioxide powder in the alimentary tract of *Turgida turgida* from ten American opossums given marked food at various times prior to necropsy.

Time food withheld (days)	Total worms found	Total no. with chromium sesquioxide (%)		No. found (% with chromium sesquioxide) larvae		adults	
1	25	0	(0)	5	(0)	20	(0)
1	10	2	(20)	3	(0)	7	(29)
2	11	3	(28)	2	(0)	9	(33)
2	20	8	(40)	7	(56)	13	(36)
2	18	11	(60)	2	(50)	16	(63)
2	7	5	(71)	0	(0)	7	(71)
2.5	33	33	(100)	1	(100)	32	(100)
3	9	7	(78)	0	(0)	9	(78)
3	5	4	(80)	0	(0)	5	(80)
3	6	6	(100)	1	(100)	5	(100)

mephitis) (Lincoln and Anderson, 1973). *Physaloptera praeputialis* attaches to the lesser curvature of the cardiac portion of the stomach of domestic cats (Zago Filho, 1959b).

Larvae of *T. turgida* were widely scattered throughout the corpus but adults tended to occur in one to three large groups in the anterior part of the corpus near the fundus. Schell (1952) reported larvae of *P. hispida* were well separated during the first 30-40 days post infection and following this period they congregated in a compact group. According to Zago Filho (1959b), *P. praeputialis* do not aggregate until they have matured. The aggregation of adult but not larval *T. turgida* and *Physaloptera* spp. suggests that pheromones may be involved. Pheromones have been reported to play an important role in the attraction of the sexes in several species of nematodes (Green, 1980).

Small lesions were observed in the stomach walls of all opossums infected with *T. turgida*. These lesions were presumably the result of trauma associated with the attachment of worms. However, eosinophilic material at the base and the sides of lesions may be secretions of the parasites. An eosinophilic matrix has been observed around some cestodes (Mackiewicz and McCrae, 1962). Hayunga (1979) believed that amorphous eosinophilic material separating caryophyllid tapeworms from host tissue was secreted by the parasite.

Small lesions have been associated with *T. turgida* in opossums (Nettles et al., 1975; Sherwood et al., 1969; Zago Filho, 1959a). Lincoln and Anderson (1973) noted similar lesions in skunks infected with *P. maxillaris*. Lesions have been reported in the mucosa and submucosa at the point of attachment of *P. praeputialis* (Zago Filho, 1959b).

Major stomach ulcers were observed in only three of the 91 wild opossums examined in the present study. Little of the stomach wall remained beneath these ulcers and had the opossum survived, perforation would probably have resulted. Experimental studies suggest that the size of the ulcer may be related to the number of adult worms present and the length of time the animals have been infected. *Turgida turgida* has been reported to perforate the stomach wall of opossums (Feldman et al., 1972; Krupp, 1966). Schell (1952) reported that ulcers, 7 to 10 mm in diameter, were associated with adult *P. hispida*.

Larvae and adults of *T. turgida* consume stomach contents. On two occasions blood was found in worms but, since blood from ulcers was observed in the stomach contents, it may have been accidentally ingested when the worms were feeding on the food bolus. Schell (1952) and Gier and Ameel (1959) suggested that species of the genus *Physaloptera* feed on the stomach wall but these conclusions require confirmation. Lincoln and Anderson (1973) showed that *P. maxillaris* feeds on food consumed by the host.

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