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STUDIES ON THE LIFE CYCLE OF *TRICHURIS VULPIS*

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Summary

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The present studies followed out the dynamics of the entire life cycle of *T. vulpis*.

The development of *Trichuris vulpis* eggs to the infective stage was studied in laboratory conditions under various temperatures. It was found out that infective larvae were formed for 14 days at 37 ^{o}C, for 17 days at 30 ^{o}C, for 45 days at 20 ^{o}C and that no development occurred at 4 ^{o}C.

The development of *T. vulpis* in host's organism until the stage of sexual maturity was followed out in 11 experimentally infected dogs, euthanized 1, 3, 6, 10, 15, 20, 30, 42, 50, 67 and 100 days after the infection. It was observed that following the ingestion of parasite's eggs, the larvae hatched in hosts's small intestines and migrated into the mucosa where they developed for 10-15 days. Afterwards thay fell into the intestinal lumen and penetrated caecal and colonic mucosa, where the stage of sexual maturity was reached. The duration of the prepatent period was 112 days while that of the patent one – more than an year.

Key words: dogs, life cycle, *Trichuris vulpis*

INTRODUCTION

Trichurosis is a nematodosis on carnivores, caused by *Trichuris vulpis* Froelich (1789) belonging to the *Trichuridae* family.

T. vulpis is a geohelminth with a direct cycle of development. The data about the duration of the separate stages of its life cycle are quite contradictory. The eggs released by female parasites pass in the environment with faeces. According to Rubin (1954), Euzeby (1961) and Leib *et al.* (1991), *T. vulpis* eggs become infective after 9–10 days whereas according to Akbaev (1998) – after 25–26 days.

The carnivores become infected after ingestion of eggs containing infective larvae. Miller (1947), having studied the early stages of *T. vulpis* development (from infection to day 10) evidenced that larvae were hatched in canine small intestine 30 min after the infection, and between post infection hours 48 and 96 were found in the base of the crypts of Lieberkühn in the first half of small intestine, coiled and sluggish. At post infection day 10 larvae returned to the lumen and were passively transferred to large intestine.

Rubin (1954), Kikuchi and Okuyama (1964) and Leib *et al.* (1991) have studied the entire life cycle of *T. vulpis*. The larvae, freed in small intestine, penetrated the crypts of Lieberkühn for 2-10 days and afterwards passed in the caecum and the colon, where they underwent 3 moultings in the superficial layer of the mucosa. Later, the anterior end of larvae became deeply embedded in mucosa and later reached sexual maturity in 10-13 weeks.

The opinion of Akbaev (1998) is just the opposite: according to the author, the development of larvae occurs only in large intestine. Initially, larvae penetrate

into the mucosa where they undergo moultings and then they fall into the lumen, become attached with their thinner end to the mucosa and reach sexual maturity in 30–107 days.

The studies upon trichurosis among carnovires in Bulgaria refer primarily to the incidence and the clinical course of the disease (Matov, 1958; Prelesov and Grosev, 1994; Georgieva *et al.*, 1999) but they do not elucidate the life cycle of the parasite. Considering the incomplete and contradictory results for *T. vulpis* life cycle, the present study aimed to examine the complete life cycle of the parasite.

MATERIALS AND METHODS

The experiments were performed on 14 mongrel dogs aged 6–12 months and weighing 5–15 kg. Following a double dehelminthization at a 14 days period with the combination praziquantel, pyrantel and febantel (Drontal plus, Bayer, Germany) at a dose of 1 tablet per 10 kg body weight and desinsection with permethrin and carbaryl (Tapilan B, Dorvet, Israel), the dogs were housed in cages with a grid floor in order to avoid accidental superinvasions.

The duration of the prepatent and the patent periods was established in 3 dogs, used as donors during the patent period, experimentally infected with 10000 *T. vulpis* eggs.

Faeces obtained from donor dogs were processed by the method of Darling. Whipworm eggs were dialysed against overboiled water. The obtained eggs were put in 14-cm Petri dishes without conservant and were incubated in a thermostat at 37 ^{o}C for 15 days.

The optimal period of development of eggs to the infective stage was found out by incubating eggs at various temperatures: 4, 20, 30 and 37 ^{o}C. Periodically, the embryonation and the development of infective larvae were followed out. Prior to the use of eggs for experimental infections, they were tested for being infective by the method of Miller (1947) and Beer (1973b).

The life cycle of *T. vulpis* in host's organism was followed up in 11 dogs, orally infected with 10000 *T. vulpis* eggs per 1 kg body weight.

Experimental dogs were euthanized at post infection days 1, 3, 6, 10, 15, 20, 30, 42, 50, 67 and 100 by intravenous injection of 0.3 mg/kg thiopental sodium after a 24-hour fasting. Immediately after the euthanasia, the organs of the alimentary system were removed. The mucosa of small and large intestines was scraped and the material – collected in Petri dishes. The collected larvae were observed live and after killing by heating. The number of larvae in 1 mL stomach content, intestinal content and scraped material was counted.

The morphological description of larvae was performed on 50 larvae isolated from each dog and included the dynamics of the following parameters: body length and width, metric data for the oesophagus, the cell body, intestine, rectum and the organs of the male, resp. female reproductive system.

RESULTS AND DISCUSSION

Development of T. vulpis eggs

It was observed that eggs in fresh faeces of experimentally infected donors were non-segmented (Fig. 1). Their cytoplasm contained two nuclei-like areas that fused in one during the embryogenesis. The cellular division was initiated in the thinner end of the egg with the formation of two unequal blastomers. Afterwards, the bigger blastomer was divided into two,

again in the thinner end of the egg. The three formed blastomers then divided each into two, thus forming a little blastule of 6 cells. The division continued until the blastule was formed by multiple small blastomers. After an unilateral getting concave along the long axis from the side of one of polar plugs, followed by an unilateral longitudinal getting concave along the short axis, a cylindrical larva was formed.

Initially, the larvae were motile but then the motility rate gradually decreased and they finally remained permanently immobile (Fig. 2). Infective larvae were released without destruction after compression of eggs with a covering glass. The larva left the egg after opening of one of the polar plugs (Fig. 3). According to the criteria of Miller (1947) and Beer (1973b) for determination of the viability and the infectiveness of *Trichuris* larvae, the larvae isolated and described by us were infective. They were morphologically characterized by a body, slightly thinner at both ends, with a length of 313.25 μm (237.5–375 μm) and width 12.58 μm (10–15 μm). In the anterior end, a lancet-like stylet was present, that was able to prolapse or invaginate in a small cavity. Its posterior end was connected to a small, thin and dark structure. The oesophagus was not clearly differentiated. The entire body of the larva was filled with a non-differentiated mass of cells forming the alimentary tract. In the oesophageal region, the cells were less densely arranged while in the intestinal region they were more compactly situated.

The opinions of the different authors by respect to the fact whether the *Trichuris* larva, formed inside the egg undergoes moulting and which is its infective stage, are quite contradictory. According to Opitz (1963) and Dalchow (1964), in

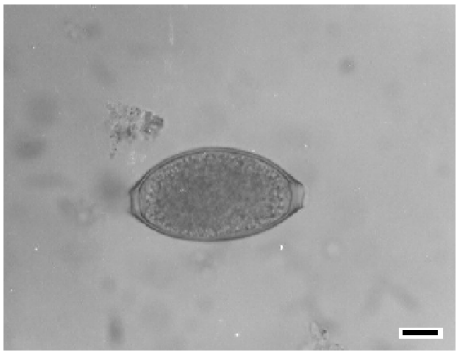

Fig. 1. *T. vulpis* egg in the moment of its discharge with faeces of infected dog. Bar = 20 μm.

Fig. 2. *T. vulpis* eggs containing infective larvae. Bar = 20 μm.

Fig. 3. Hatching of infective *T. vulpis* larva from the egg. Bar = 20 μm.

infective eggs of *T. vulpis* and *T. ovis* there are two larval stages (L_1 and L_2). This belief was based on some morpho-

logical characteristics, especially on the presence of a stylet in the anterior end, but none of authors described a moulting of the larva inside the egg. On the contrary, there was no moulting of larvae in the eggs and L_1 was the infective larva, characterized by the well-formed stylet as stated by Thapar and Singh (1954), Deo (1960), Powers (1961) for *T. ovis*, Wakelin (1969) and Shlikas and Mezjavichus (1969) for *T. muris* and Beer (1973a) for *T. suis*.

Our observations upon the dynamics of the development of *T. vulpis* eggs suggested that there was no moulting of larvae inside the egg and that L_1, with a formed stylet, was the infective larva.

The comparison of the effect of various temperatures (4°C, 20°C, 30 °C and 37 °C) on *T. vulpis* eggs development revealed that the optimal one was 37 °C. At the 14th day of the incubation, eggs contained infective larvae. The viability test showed that all isolated larvae possessed a well-formed stylet and became motile after a gentle heating. It is known that the relationship between temperature values and the period of development of infective larvae has an important epizootological significance. Our results confirmed data that the development of infective *T. vulpis* larvae was temperature-dependent. At 30 °C, infective larvae were present by day 17 while at 20 °C – by day 45. No development of larvae occurred at 4 °C for an year.

The data about the period of development of *T. vulpis* eggs to the infective stage are also contradictory. Under optimal temperatures (28–32 °C) the period lasted 9 days (Rubin, 1954) but according to Akbaev (1998) – 3 weeks. Our results were similar to those of Opitz (1963).

Development of T. vulpis in host's organism

The necropsy findings 24 hours after the infection showed a large amount of eggs containing larvae in stomach content and the duodenum. The jejunal and ileal content contained single eggs and the result for both caecal and colonic content was negative. A small number of hatched larvae were present in duodenal mucosa scrapings.

At post infection hour 72, 8% of hatched larvae were located in small intestine content while 92% were in small intestine mucosa. No larvae were present in both caecum and colon. By hour 72, the dimensions of larvae were 313.25 μm (237.5–375 μm) in length and 12.58 μm (10-15 μm) in width, with a well-expressed motility. The alimentary tract consisted of a slender anterior part that was nearly 1/5 of the body length and a gradually enlarging and fine granulated posterior part. No oesophagus, cell body or intestines were differentiated. A well formed stylet was visualized in the anterior end.

Six days after the infection, 11% of larvae were detected in small intestine content and 89% – in small intestine mucosa. Again, they were larvae neither in the caecum nor in the colon. Larvae isolated from the mucosa were coiled and sluggish, with a length of 381.25 μm (350–400 μm) and a width of 15 μm (Fig. 4).

Ten days after the infection, 94% of larvae were embedded in small intestine mucosa, 2% – in small intestine content and 4% – in caecal mucosa. Isolated larvae were 455.75 μm (375-500 μm) long and 18.5 μm (15–20 μm) wide. They were coiled and sluggish. There were no clearly formed cells composing the cell body. In the anterior end of some larvae, an externally prolapsed stylet was present.

At post infection day 15, 8% of larvae were located in small intestine lumen, 18% – in small intestine mucosa, 4% – in large intestine lumen and 70% – in large intestine mucosa (Fig. 5). The total length of larvae was 488.25 μm (375-590 μm) and the width – 19.73 μm (17.5–21 μm). The larvae were straight shaped or coiled. Those, located in the caecum were with the highest motility. An oesophagus and an intestine could be differentiated. Only the anterior end of the cell body had well formed cells.

By the 20th day of the infection, no larvae were present in small intestine but only in large intestine scrapings. The dimensions were 716.5 μm (475–1170 μm) in length and 24.08 μm (17.5–27.5 μm) in width. The oesophagus, the cell body, intestine and rectum of 20-days-old larvae were clearly visible (Fig. 6). The cell body was composed of two rows of cells. Genital primordium cells existed.

Thirty days after the infection, there were no larve in the material obtained from small intestine with the exception of single individuals in the end part of the ileum. In the caecal and colonic mucosa there were multiple larvae. The internal structure was similar to that of 20-days old larvae, only the dimensions were different – 1194 μm length (590–2220 μm) and 26.8 μm width (20-35 μm).

Forty-two days after the infection, larvae were present only in caecal and colonic lumen and mucosa. The length of parasites was 2836.79 μm. For the first time, the width of the different parts of the body in 42-days old larvae was different: at the cell body-intestine junction it was 47.5 μm, in the anterior part, at the oesophagus–cell body junction – 37.7 μm and in the posterior part – 44.2 μm. The intestine was with a formed lumen. In some individuals, there was a clearly

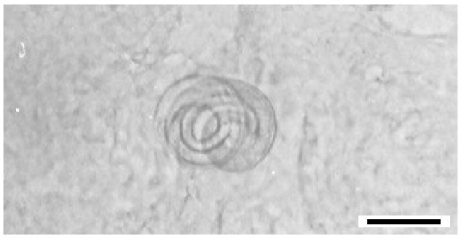

Fig. 4. Four-days old *T.vulpis* larva, isolated from small intestine mucosa. Bar = 30 μm

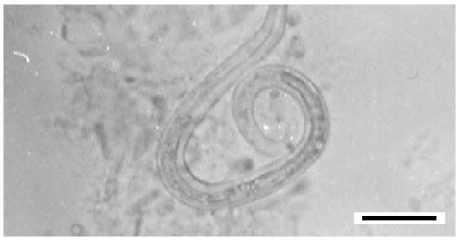

Fig. 5. Fifteen-days old *T. vulpis* larva isolated from large intestine mucosa. Bar = 30 μm.

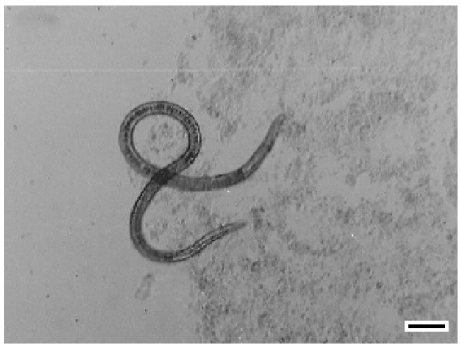

Fig. 6. Twenty-days old *T. vulpis* larva isolated from large intestine mucosa. Bar = 80 μm.

shaped cloacal tube, in others - a beginning of vagina at the cell body – intestine junction.

By the 50th day after the experimental infection, whipworms were isolated only from the caecum and colon, embedded in the intestinal mucosa. Compared to day

42, body dimensions were sharply enlarged and the slender anterior part could be clearly differentiated from the larger posterior part. The development of reproductive system was completed, so male and female whipworms were apparently distinguished. The total body length of males was 25.8 mm, the slender part width – 48.3 μm and the posterior (larger) part width - 218.7 μm. A well-formed spicule and spicule sheath with a slight bend of the posterior end. The total body length in females reached 22.4 mm and the width of anterior and posterior end were 45 μm and 167.8 μm respectively. Both the vagina and vulva were well differentiated.

All whipworms isolated from the caecum and the colon by day 67 after the infection were sexually differentiated. The increase in body dimensions compared to day 50 was not significant. The total body length of male whipworms was 27.9 mm, anterior part width – 53.7 μm and posterior part width – 228.3 μm. The respective values in female parasites were 26.8 mm, 59.5 μm and 181.8 μm.

One-hundred days after the infection, the whipworms, isolated from the colon and the caecum were characterized not only by a complete development of all organs, but also by a considerable increase in body dimensions. The total body length in males reached 43.5 mm with width of the slender anterior and the large posterior end of 96.2 and 410 μm, respectively. The spicule length was 6.8 mm and its width – 25 μm. The dimensions of the spicule sheath were 0.95 mm in length and 40 μm in width. It was covered with spines. Isolated female whipworms presented a total body length of 39 mm, with anterior end width of 82.5 μm and posterior end width – 430 μm. Within the uterus there were well formed eggs with dimensions of 80×37.5 μm.

The first release of eggs with faeces in experimentally infected donor dogs was observed between days 108-114 (day 112 on the average). The prepatent period observed by us do not correspond to that, described by Miller (1941) and Rubin (1954). According to them, sexually mature female whipworms begin to release eggs 70–100 days after the infection. By day 100 after the infection were observed female parasites with intrauterinely formed eggs, but their discharge with faeces in donor dogs occurred between days 108 and 114.

In experimentally infected donor dogs, the discharge of eggs was followed up for an year. According to Miller (1941) and Rubin (1954), the duration of the patent period is from several months to years.

On the basis of results, obtained during the present study, it could be summarized that during the development of *T. vulpis* in host's organism, larvae hatched between hours 24–72 and penetrated in small intestine mucosa. Larvae were characterized with a poorly differentiated internal content, but a well-formed stylet in their anterior end, that helped them to penetrate the intestinal wall. During the period between hour 72 and post infection day 10, larvae remained within the intestinal mucosa. Between days 10–15 they left the small intestine mucosa, fell into the lumen and migrated towards the caecum and the colon. By post infection day 15, larvae were predominantly located in caecal and colonic mucosa and in rare instances in small intestine. Between days 15 and 30 – they were found only in caecal and colonic mucosa. From day 42 until the end of the experiment (day 100), they appeared in intestinal lumen and only the anterior end was embedded in the mucosa.

Comparing our data for *T. vulpis* localization during the different stages of its

development with those of Miller (1947), we found out significant discrepancies. According to the author, larvae hatched from eggs as early as 30 min after the ingestion, by hour 24 they were already embedded in small intestine mucosa and by day 6 left small intestine mucosa and migrated towards large intestine. Our observation showed however that this migration occurred between days 10 and 15, because the biggest number of larvae embedded in large intestinal mucosa was observed 15 days after the infection. Opitz (1963) rejected the statements for migration in small intestine and considered that only larvae, having directly reached large intestine, were developing.

Following infection with *T. suis* eggs, Beer (1973a) observed hatching of larvae in small intestine after 9 hours followed by localization and development in caecal and colonic mucosa. Development of larvae only in large intestine was also described by Dalchow (1964) for *T. ovis* and Shlikas and Medjavichus (1969) for *T. muris*.

Taking into account that the only study upon the life cycle of *T. vulpis*, made by Miller (1947), included the period up to the 10^{th} day after the infection of the host, we compared the dynamics of development of morphological features with those of *T. suis*, provided by Beer (1973b). The sequence in the formation and development of alimentary and reproductive organs was identical, but the periods were different because of the shorter life cycle of *T. suis*. The author described sexually mature *T. suis* 41 days after the infection, while we observed the appearance of eggs within the uterus of female whipworms by day 100 after the infection.

CONCLUSIONS

Animals infected with *T. vulpis*, discharged eggs that contained one or two cells and that were not infective with faeces. The optimal temperature for formation of infective larvae in the egg was 37 ^{o}C for 14 days, and no development was present under 4 ^{o}C. Hatched infective larvae are motile, with a well formed stylet. After ingestion of eggs, the activation and the hatching of larvae in host's organism occurred in small intestines. They penetrated the mucosa all along the small intestine, where they developed for 10-15 days. Afterwards they fell into the lumen of small intestine, migrated towards caecal and colonic mucosa where they developed until sexual maturity.

The prepatent period in experimentally infected dogs was 112 days, and the patent period - longer than one year.

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