

MIGRATION OF SOME ROUNDWORM SPECIES IN EXPERIMENTALLY INFECTED WHITE MICE

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Abstract. The migration of four roundworm species (*Ascaris suum*, *Toxocara canis*, *T. cati* and *Toxascaris leonina*) was studied in various organs of experimentally infected white mice. The hatching of *T. leonina* and *T. cati* larvae in the paratenic host is more rapid (24—36 h) than the hatching of *A. suum* and *T. canis* larvae (1—6 days). The deposition of larvae in host organs is different in the individual species. Maximum number of larvae in the liver: *T. leonina* and *A. suum* on day 4, *T. canis* on day 2 and *T. cati* on day 1. Maximum number of larvae in the lungs: *A. suum* and *T. leonina* on day 7, *T. cati* on day 2 and *T. canis* on day 1. Maximum number of larvae in muscles: *A. suum* on day 21 only rarely, *T. canis* and *T. cati* on day 3 and *T. leonina* on day 28. In the brain occurred mostly the larvae of *T. canis* and a lower number of *T. cati* larvae, whereas the other two nematode species did not occur at all in this organ.

The roundworms parasitic in pigs (*Ascaris suum* (Goeze, 1782)) and those parasitic in dogs and cats (*Toxocara canis* (Werner, 1782), *Toxocara cati* (Schrank, 1788) and *Toxascaris leonina* (Linstow, 1909)) represent a great problem from the viewpoint of hygiene and epidemiology, because their larvae can migrate in a majority of warmblooded vertebrates including man. Many cases of larval toxocarosis (larva migrans) in man have been described by various authors (Beautyman and Woolf 1951, Beaver 1962, Beaver et al. 1952, Dent et al. 1956, Kouba et al. 1973, Redhammer et al. 1980, Šebek et al. 1975, Uhlíková and Hübner 1968, 1980), Virgala and Cesnak 1978).

Some years ago we started to study the parasite-host relationships in the paratenic hosts of various roundworm species. The present paper is a continuation of the previous ones: Figallová and Prokopič (1981), Prokopič and Klabanová (1980) and Prokopič and Figallová (1981).

MATERIAL AND METHODS

The eggs of *A. suum*, *T. canis*, *T. cati* and *T. leonina* were obtained from adult females recovered from naturally infected animals. The methods of collection, cultivation of eggs, mode of infection and examination of experimental mice were described in the paper by Prokopič and Klabanová (1980). Infective eggs of *A. suum* (2 000 eggs per host) were fed to 75 white mice at the age of 10—12 weeks, weighing 33.4 g on the average. The eggs of *T. canis* (2 100 eggs per host) were fed to 35 males of white mice at the age of 3 months, weighing 33.8 g on the average. The eggs of *T. cati* (2 500 eggs per host) were fed to 45 white mice at the age of 3 months, weighing 33.1 g on the average. The eggs of *T. leonina* (2 500 eggs per host) were fed to 50 white mice at the age of 3 months, weighing 33.9 g on the average.

RESULTS

The experiment with mice infected with *A. suum* eggs lasted 49 days. During this time the mice were gradually killed and examined (Fig. 1 A). Already 30 min after infection, the faeces of mice contained the eggs which had passed through the digestive tract. During the 1st day, as much as 277 eggs were detected in 1 g of faeces. In the 20th hour after infection, the number of eggs suddenly dropped, which indicated that most of the *A. suum* larvae hatched in the paratenic host. The last single eggs were expelled on day 6.

The macroscopical changes in the organs were very distinct. The first larvae were isolated from the mouse liver on day 4 after infection. In this organ, single larvae were found up to day 17 p.i. In the lungs, the larvae occurred from 4th to 17th day; maximum occurrence was on day 7 and after 10 days the number of larvae decreased. A high number of larvae were encountered in the larynx and trachea on days 7—14 p.i. On the 7th day, the larvae were present also in the stomach and small intestine and on days 17—21 in both the intestinal wall and contents of intestines.

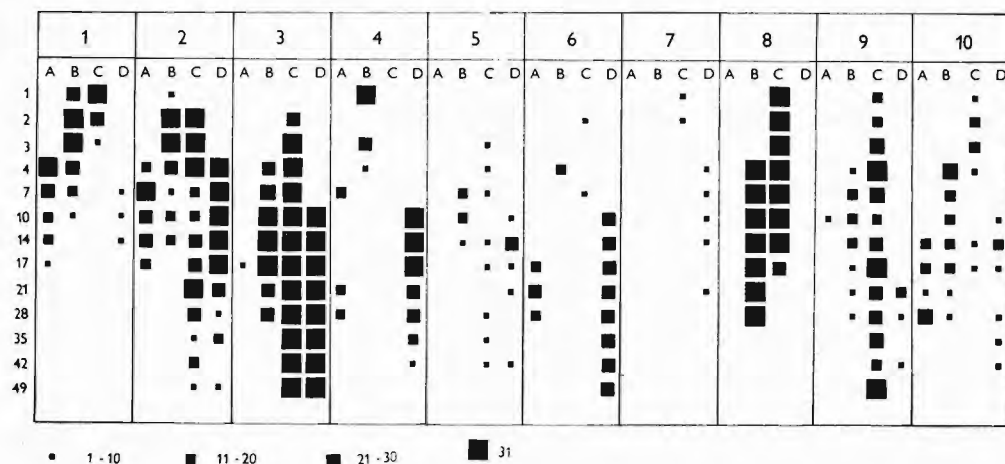


Fig. 1. Localization of larvae of different species of roundworms in the paratenic host during experimental infection. A — *Ascaris suum*, B — *Toxocara canis*, C — *Toxocara cati*, D — *Toxascaris leonina*; 1 — liver, 2 — lungs, 3 — skeletal muscles, 4 — intestinal wall, 5 — lymph nodes, 6 — genital organs, 7 — kidneys, 8 — brain, 9 — tongue and masticatory muscles, 10 — diaphragm. Vertically 1—49 No. of days in experiment. 1—10, 11—20, 21—30, 31 = mean number of larvae.

In the diaphragm, the larvae were found on days 10—21 p.i., with maximum occurrence on day 21.

The number of roundworm larvae in all organs is very low after 17 days. They survive for the longest time in the genital organs (17—21 days p.i.). No *A. suum* larvae were detected in the brain and eyes of mice.

During 28-day migration the larvae grew from 0.26×0.015 mm to 1.43×0.051 mm. After 35 days p.i., no *A. suum* larvae were found in the mice. This indicates that during this time the larvae undergo a "tracheal type" of migration and probably leave the paratenic host.

In the experiment with *T. canis* larvae (Fig. 1 B), which lasted 28 days, single eggs passed through the digestive tract of experimental animals already 30 min after infection and their number increased up to 24 h. After 48 h, they occurred only occasionally, which suggests that most of the larvae hatched in the paratenic host. No eggs were expelled after 7 days and later.

Maximum number of larvae were found in the liver after 48 h, in the lungs after 4 days, in the heart after 4 days and in the brain after 14 days. In the trachea, the larvae occurred on days 7—28, with maximum occurrence on day 14 p.i. In the muscles of legs the larvae were found from day 4, with maximum occurrence on day 28 p.i. *T. canis* larvae occurred in the liver in 82%, in the lungs in 80% and in the brain in 68%

(with maximum intensity of infection). The maximum deposition of *T. canis* larvae in the brain was observed also by Smith and Beaver (1953), Sprent (1955), Beaver et al. (1952), Burren (1971), Ho and Kobulej (1974) and Prokopič and Klabanová (1980).

In the diaphragm, the larvae occurred mostly on day 4 and then their number decreased up to day 28. In the tongue, the maximum number of larvae occurred on day 14. In the rib muscles, the larvae occurred on days 4—28 p.i. (maximum on day 21). Only 2 larvae were found in the spleen, 2 days p.i. In the eyes, the larvae were found on days 4 and 21 p.i.

The eggs of *T. cati* were expelled up to 36 h p.i. The hatching of this species in the paratenic host seems to be much more rapid than in the case of the two previous species. The maximum occurrence of *T. cati* larvae in individual organs was as follows: 159 larvae in the liver on day 1, 167 larvae in the lungs on day 2, in leg muscles on days 3—49, in the brain on days 2—28 p.i. (but in a lower number than in *T. canis*) (Fig. 1 C). The intrauterine infection of mice was also demonstrated during the experiments. The larvae of *T. cati* were found in the liver, lungs, muscles and intestinal wall of newborn animals. The mean size of larvae increased from 0.425×0.017 mm on the 1st day to 0.491×0.019 mm on the 49th day p.i.

In the experiments with *T. leonina*, some eggs were found already within 24 h after infection, which is the shortest time for hatching of larvae in the paratenic host compared to the other nematode species examined. The larvae of *T. leonina* occurred in liver on days 7—14 (Fig. 1 D), in lungs on days 4—49, and in kidneys on days 4—28 p.i. The highest number of *T. leonina* larvae were found in leg muscles, rib muscles, masticatory muscles and diaphragm from day 10 p.i. From day 10 p.i. till the end of the experiment, a small number of larvae occurred also in the genital organs. In the same period the larvae were encountered also in the small and large intestines. In the lymph nodes, the larvae were present on day 14 p.i. The intrauterine infection was also demonstrated; the larvae occurred in the intestinal wall, diaphragm and rib muscles of newborn mice. The maximum number of larvae (101 larvae per 1 g of organ) were found in the rib muscles. Their size increased from $0.61—0.73 \times 0.04$ mm on day 4 p.i. to $0.76—0.88 \times 0.042$ mm on day 49 p.i.

DISCUSSION

Already in the previous paper (Prokopič and Klabanová 1980) it was pointed out that in experimental infections, some of the nematode eggs pass through the digestive tract of their hosts before the larvae can hatch from them. Consequently, there occur differences in the intensity of infection even if the doses of infective eggs are constant. This fact was also mentioned by Buchwalder (1968).

In the present experiments, there were marked differences in hatching of larvae, expressed in the egg expulsion for a shorter or longer time after infection. For example, the expulsion of *T. leonina* eggs ceased already after 24 h and of *T. cati* eggs after 36 h, whereas in *T. canis*, the number of expelled eggs rapidly decreased after 48 h, but single eggs were found up to the 6th day after infection. A similar situation was observed also with *A. suum* eggs.

The studied roundworm species differ from one another also in the migration of larvae and their distribution in various organs of the paratenic host. The livers contain mostly *T. cati* larvae on day 1 p.i., *T. canis* on day 2 and the larvae of *A. suum* and *T. leonina* only on day 4. In the brain are deposited mainly the larvae of *T. canis* and a lower number of *T. cati* larvae, but the larvae of *A. suum* and *T. leonina* were not found in this organ. This phenomenon can be explained, in agreement with Ho and Kobulej

(1974), by the adaptation of the parasite to an easier way of penetration into the definitive host, because the paratenic host with brain infection becomes more easily a prey of the definitive host. Smith and Beaver (1953) observed mouse paralysis after one-year infection with *T. canis*. The results of Prokopič and Klabanová (1980) confirmed the statement of Sprent (1955) and Beaver et al. (1952) that the larvae of *T. canis* cumulate in the brain of the paratenic host already from days 2 and 3 and their maximum number occurs on day 14 p.i. Burren (1971) found the maximum number of *T. canis* larvae in the brain already on day 12 p.i. In our experiments, only *T. canis* larvae occurred in eyes, whereas the other species did not occur at all in this organ. Similar results were obtained also by Sprent (1955), Dubey (1968) and Burren (1971). Roneus (1963) pointed out that the larvae of *T. cati* induce similar symptoms of visceral larva migrans in pigs like *T. canis* and *T. cati* in man. This increases their significance not only in the epidemiology of larval toxocarosis in man, but also in the epizootology of domestic animals in large-scale breeding farms, as it was reported by Prokopič and Pavlásek (1977) and Prokopič and Krivanec (1978).

The larvae of *A. suum*, compared to the other studied roundworm species, are deposited in the body of the paratenic host for the shortest time (28 days). According to Sprent (1952), a majority of larvae disappear from the body of experimental host already during 3 weeks after infection. Fülleborn (1921) and Sprent (1952) observed that the migration of larvae from the lungs can be realized in two ways. The migration either of the so-called "tracheal type", when the larvae penetrate through the lung alveoli and pass through the trachea into the digestive tract, or of "somatic type", when the larvae return from the lungs to the heart and then they are carried through the arterial system to various organs.

Consequently, the migration of almost all *A. suum* larvae seems to be of the "tracheal type" in the paratenic host during 28 days and most probably the larvae leave the host through the digestive tract. On the other hand, the larvae of the remaining three nematode species, the adults of which are parasitic in the beasts of prey, are deposited in various organs of the paratenic host for a long time. In case of *T. canis* and *T. cati*, they often occur also in the brain, so that the rodents may be considered "intermediate hosts" of these species, because the larvae grow during the migration, as it was mentioned above. These nematode species can be regarded as parasites with both direct and indirect life cycle. The same conclusion was made also by Mozgovoy and Shakhmatova (1969) with *Neascaris vitulorum*. They consider the cow-mother, in which the larvae of the parasite undergo the migration, to serve as an intermediate host, as the newborn calf, in which the parasite reaches maturity, acquires the infection only inside their uterus.

МИГРАЦИЯ НЕКОТОРЫХ ВИДОВ АСКАРИД У ЭКСПЕРИМЕНТАЛЬНО ЗАРАЖЕННЫХ БЕЛЫХ МЫШЕЙ

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Резюме. Изучали миграцию четырех видов аскарид (*Ascaris suum*, *Toxocara canis*, *T. cati*, *Toxascaris leonina*) в различных органах экспериментально зараженных белых мышей. Вылупление личинок *T. leonina* и *T. cati* в паратеническом хозяине более быстро (24—36 ч), чем вылупление личинок *A. suum* и *T. canis* (1—6 дней). Количество личинок в органах хозяина различно у отдельных видов. Максимальное число личинок в печени: *T. leonina* и *A. suum* на 4-й день, *T. canis* на 2-й день, *T. cati* на 1-й день; в легких: *T. leonina* и *A. suum* на 7-й день, *T. cati* на 2-й день и *T. canis* на 1-й день; в мышцах: *A. suum* на 21-й день, *T. canis* и *T. cati* на 3-й день и *T. leonina* на 28-й день после заражения. В мозге были найдены большей частью личинки *T. canis* и небольшое количество личинок *T. cati*, тогда как другие два вида нематод в мозгах мышей не встречались.

REFERENCES

- BEAUTYMAN W., WOOLF H. L., An *Ascaris* larva in the brain in association with acute anterior poliomyelitis. J. Path. Bact. 63: 645—647, 1951.
- BEAVER P. C., Toxocarosis (visceral larva migrans) in relation to tropical eosinophilia. Bull. Soc. Pathol. Exot. 55: 555—576, 1962.
- , SNYDER C. H., CARRERA G. M., DENT J. H., LAFFERTY J. W., Chronic eosinophilia due to visceral larva migrans. Pediatrics 9: 7—19, 1952.
- BUCHWALDER R., Experimentelle Infektion mit *Ascaris suum* bei Laboratoriumstieren. Angew. Parasitol. 9: 203—211, 1968.
- BURREN C. H., The distribution of *Toxocara* larvae on the central nervous system of the mouse. Trans. Roy. Soc. Trop. Med. Hyg. 65: 450—453, 1971.
- DENT J. H., NICHOLS R. L., BEAVER P. C., CARRERA G. M., STAGGERS R. J., Visceral larva migrans — with a case report. Am. J. Pathol. 32: 777—803, 1956.
- DUBEY J., Migration of *Toxocara cati* in mice. Trop. Geogr. Med. 20: 172—176, 1968.
- FIGALLOVÁ V., PROKOPIČ J., Eosinophilia in experimental toxascariasis (visceral larva migrans) in white mice. Folia parasit. (Praha) 28: 341—342, 1981.
- FÜLLEBORN F., Askarisinfektion durch Verzehren eingekapselter Larven und über gelungene intrauterine Askarisinfektion. Arch. Schiffs. u. Trop. Hyg. 25: 367—375, 1921.
- HO T. T., KOBULEJ T., Studies on the migration of *Toxocara canis* larvae in the mouse. Parasitol. Hung. 7: 55—68, 1974.
- KOUBA K., VALNÍČKOVÁ J., LAZOVSKÁ J., Eye toxocarosis. Prakt. lékař (Praha) 53: 475—477, 1973. (In Czech.)
- MOZGOVOY A. A., SHAKHMATOVA V. J., To the study of the life cycle of *Neascaris vitulorum* (Ascaridata: Anisakidae), a nematode of ruminants. Tr. gelm. lab. AN SSSR 20: 97—101, 1969. (In Russian.)
- PROKOPIČ J., FIGALLOVÁ V., The migration of larvae of *Toxocara leonina* (Linstow, 1909) in experimentally infected white mice. Folia parasit. (Praha) 29: 309—313, 1982.
- , KLABANOVÁ V., Distribution of migrating larvae of *Toxocara canis* (Werner, 1782) in various organs of experimentally infected white mice. Čs. epidemiol. mikrobiol., imunol. 29: 171—176, 1980. (In Czech.)
- , KRIVANEC K., Comparative study of helminthological situation with pigs in large-scale breeding farms of various types. Annual Report, Institute of Parasitology, Czech. Acad. Sci., 1978.
- , PAVLÁSEK I., Endoparasites of calves in large-scale breeding farms. Vet. med. 22: 505—512, 1977. (In Czech.)
- REDHAMMER R., BOČA M., SOBOTA K., ONDREJČKA M., A serious case of *Toxocara* infection. Proceedings of the Conference "Actual problems of human parasitology", Martin 2.—3. 12. 1980. (In Czech.)
- RONEUS O., Parasitic liver lesions in swine, experimentally produced by visceral larva migrans of *Toxocara cati*. Acta vet. scand. 4: 170—196, 1963.
- ŠEBEK Z., PROKOPIČ J., SIXL W., WÜRST Z., Kann der Hundespulwurm *Toxocara canis* (Werner, 1782) dem Menschen gefährlich werden? (Nematoda, Anisakidae). Mitt. Abt. Zool. Landesmus. Joanneum 4: 61—65, 1975.
- SMITH M. H. D., BEAVER P. C., Persistence and distribution of *Toxocara* larvae in the tissues of children and mice. Pediatrics 12: 491—497, 1953.
- SPRENT J. F. A., On the migratory behaviour of the larvae of various *Ascaris* species in white mice. I. Distribution of larvae in tissues. J. Infect. Dis. 67: 165—176, 1952.
- , On the invasion of the central nervous system by nematodes. II. Invasion of the nervous system in ascariasis. Parasitology 45: 41—55, 1955.
- UHLÍKOVÁ M., HÜBNER J., Possibility of occurrence of larval toxocarosis (visceral larva migrans) in Czechoslovakia. Čs. Pediatrie 23: 715—718, 1968. (In Czech.)
- , —, Selected cases of eye and visceral form of larval toxocarosis in man. Proceedings of the Conference "Actual problems of human parasitology", Martin 2.—3. 12. 1980. (In Czech.)
- VIRGALA J., CESNAK D., Toxocariasis. Čs. Pediatrie 33: 468—470, 1978. (In Czech.)

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