Thesis for Ph.D. degree

PATHOGENESIS, PATHOMECHANISM, CHEMOTHERAPY AND PREVENTION OF

Larval TOXOCAROSIS

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SUMMARY

As the number of cats and dogs has increased in the past twenty years in Hungary, so has the contamination of the environment caused by the parasites originating from the faeces of these animals. In temperate climates, such as in Hungary, for humans the main risk of infection comes from *Toxocara canis* (Werner, 1782) and *Toxocara mystax* (Zeder, 1800) (*syn.* *cati* Schrank, 1788) roundworm species of dogs and cats. Humans are passively infected with the eggs of the above-mentioned species originating from the hosts’ faeces. The *Toxocara* larvae hatching in the human intestine from the infectious eggs may cause larval toxocarosis while migrating in the tissues of various organs.

In our investigations, using faeces examinations and autopsy we proved heavy contamination of local dogs and cats with various intestinal worms, as well as the high frequency of *Toxocara* species of public health interest not only in young animals. Furthermore, we studied the pathogenesis and pathomechanism of larval toxocarosis in a paratenic (mouse) host model. With histopathological examination of the existing chronic larval toxocarosis still present 13 months after artificial infection with a limited number of *T. canis* larvae and with the determination of the number of larvae recovered from the liver and brain using the digestion method, we were able to prove permanent migration in both primary and secondary infection. We studied the migration of the up to now less frequently studied *T. mystax* larvae also in a mouse model. According to our results, they may presumably cause the visceral larva migrans also in humans acting as “accidental” paratenic hosts. With a series of chemotherapeutic experiments we proved that the *Toxocara* larvae established in the paratenic hosts might be targets for antihelmintic agents. In order to reach outstanding efficiency (≥ 90 %) 384-672.8 mg (≥12.8-22.4 g/kg of bodyweight) of fenbendazole is required. As for albendazole, 1/3 of the above amount is satisfactory. In addition to dosage, other elements such as the form of the medication, the duration of the treatment, etc. can significantly modify the outcome of the treatment. Following the evaluation of questionnaires we highlighted the flaws in the cognition of both pet owners and partially veterinarians in Hungary particularly regarding *Toxocara* species and the public health hazard they represent. Finally we outlined the national measures to be taken in order to decrease the infection of humans as “accidental” hosts.
INTRODUCTION

Certain species of worms existing in animals living in closer or broader proximity of humans may cause infections in humans as well, and consequently they represent public health hazard. Among nematodes there are some that might become sexually mature in both humans and animals acting as final hosts. There are even more species which inhabit animals as final hosts when they are fully mature, but upon getting into humans – as paratenic hosts (inadequate host, carrier hosts) – their larvae may cause zoonosis manifested in larva migrans (LM) pathological condition with symptoms of varying severity.

In Hungary, the number of animals kept in the immediate vicinity of humans for various purposes, especially cats and dogs, has increased over the past twenty years. As the number of animals has increased, so has – among others – the contamination of the environment caused by the parasites originating from the faeces of these animals. In temperate climates, such as in Hungary, for humans the main risk of infection comes from *Toxocara canis* (Werner, 1782) and *Toxocara mystax* (Zeder, 1800) (*syn. cati* Schrank, 1788) roundworm species of dogs and cats. One of the causes is that these parasites are rather frequent in both dogs and cats, up to 90-100% of puppies might be infected. Humans might be passively infected with reproductory products (eggs) originating from the worm species from the faeces of the above-mentioned carnivores. The major sources of the infection might be for instance food and hands contaminated with soil, sand and earth, therefore it is particularly young children who are susceptible to the infection. *Toxocara* larvae hatching in the human intestine from the infectious eggs may cause larval toxocarosis (earlier toxocariasis*) while migrating in the tissues of various organs.

I have been dealing with worm parasites of dogs and cats for over ten years, but I also met infections and diseases caused by parasites of the abovementioned, mainly in young animals earlier while working as a veterinary practitioner after graduating. I often encountered the ignorance of people keeping animals, regarding the dangers these parasites represent to humans, and unfortunately the lack of proper awareness among some veterinaries and medical doctors could as well be witnessed.

* The spelling of the names of parasitosis(oses) – following the recommendations of the new standard nomenclature accepted by the European (EFP) and World Federation of Parasitologists (WFP) in 1990 – is generally the nominative of the parasite’s taxon name followed by the standardized -osis suffix.
Many human infections by *Toxocara* larvae are not diagnosed and this type of infection is often not even considered in the case of the majority of patients showing unspecific symptoms. We might hear certain opinions from some medical doctors that it is unwarranted to be concerned much if the infection is diagnosed and the attitude regarding the necessity of medication may vary too.

The increasing number of cats and dogs in Hungary, the lack of data concerning their level of infection and the contamination of the environment with worm eggs as well as the contradicting reports concerning the pathomechanism and pathogenesis of diseases caused by *Toxocara* larvae migrating in humans and the chemotherapy for final hosts (carnivores) and developing forms of parasites present in paratenic hosts altogether motivated me in choosing this topic.

**LITERARY BACKGROUND**

The first description of the *visceral larva migrans* (*VLM*) syndrome is attributed to Baever et al (1952), and at that time they believed that *T. canis* and *T. mystax* larvae migrating in tissues for a longer period were the exclusive pathogens. Since the first description of the *VLM* syndrome it has been proved that the larvae of other nematode species can play a role in developing almost identical syndromes in humans.

In the case of final host dogs there are several ways of becoming infected with *T. canis*. Of these, the most important and most frequent one is the infection of the foetus in the pregnant female dog through the placenta. The larvae in the small intestine of the newborn puppies reach sexual maturity within 3 weeks from birth while female worms may produce thousands of eggs starting from as soon as the 16th – 22nd day, which can reach our environment e.g. pavements, parks, sandboxes in playgrounds, gardens, etc. with the dogs’ faeces. In the case of cats transplacental infection does not occur, but the kittens become infected with the *T. mystax* larvae via the milk of the queen.

What happens in the human organism when infected as an accidental paratenic host? Almost the same as in the older dogs, that is the *Toxocara* worms migrate in the larval form, i.e. upon entering the body the larvae hatch from the eggs and reach various organs and tissues with the help of the bloodstream.
From epidemiological point of view, “normal” paratenic hosts, mainly rodents play an important role in the subsistence of *Toxocara* worms. The aim of the majority of experiments concerning *Toxocara* species on animal species acting as paratenic hosts is to apply the experience acquired in the so-called “model experiments” to investigate human toxocarosis, since these experiments may help to understand pathological and immunological processes in various disease conditions.

There have been investigations both abroad and in Hungary to estimate the level of parasitic egg contamination of the soil of urban parks and sandboxes. In most surveys 1-25% of all tested public places proved infected. According to publications in the last few years *Toxocara* worms of cats may also play a significant role in causing human toxocarosis.

According to our latest knowledge human toxocarosis may appear in three clinical forms:

1. The *visceralis larva migrans (VLM)* syndrome. This form is characterized by extremely variable, non-specific clinical symptoms and often with heavy inflammation in different organs depending on the immune reactive response of the host organism.

2. *Ocularis larva migrans (OLM)* The granulomatosus retina deficiencies caused by the migrating larvae may cause the loss of sharp vision, strabismus, uveitis, chorioretinitis, retinaablatio and the so-called “lightvision”. In some cases full blindness of one or both eyes may also occur.

3. Larva migrans without specific organic location with slight symptoms (“covert toxocarosis”), which has been described lately, i.e. with non-organ-specific clinical appearance e.g. chronic loss of weight, headache, sleeping disorders, behavioural disorders, abdominal pain, etc.

The risk factors leading to the development of human toxocarosis may differ in various countries or even regions depending on the circumstances of personal hygiene etc. of those living there. Migration caused by *Toxocara*-larvae might be without symptoms, though following the migration, the dormant larvae may re-activate years later e.g. because of immune suppression. In humans serodiagnostic procedures are the most suitable to reveal the infection.

In Hungary the first publication dealing with human VLM was published in 1977 (Kávai et al). According to the data of OEK between 1988 and 1996 following the annual examination of 1974-2876 people an average of 17.8% revealed *Toxocara*-positive tests and this ratio has been similar in the past few years. From the published data we are unable to conclude the real
number of infections (prevalence) in Hungary, since these results reflect the findings of selective examinations.

The literary references concerning the successful medication of human *VLM* are contradictory. Previously only diethylcarbamazine was applied, later thiabendazole, but the experiences regarding the success of the treatments vary greatly. In order to decrease clinical symptoms in human *Toxocara* infections corticosteroids are generally used. The majority of the experiences concerning the effectiveness of various anthelminthics were published following the treatment of experimental animals, in particular mice, shortly after infection (the so-called acute phase), with large doses and for a few days. According to the figures of the diverse model experiments, the effectiveness of the given anthelminthics against *Toxocara* larvae is not convincing.

**OBJECTIVES**

We intended to fulfill the following objectives during our work:

1. Collecting data using faeces examination and autopsy to determine the level of intestinal worm infection in dogs and cats in Hungary with special regard to *Toxocara* infection.
2. We wanted to examine the pathogenesis and pathomechanism of larval toxocarosis on a paratenic (mouse) host model. One of our objectives was to study the consequences of chronic persistant primary *T. canis* infection, the effects of repeated infection on the larval count, with special attention to pathological reactions observed in the liver, spleen and the brain. Furthermore we intended to study the migration of the previously less extensively studied *T. mystax* larvae and compare it to the behavior of the larvae of the dogs’ *Toxocara* species.
3. We wished to examine the possibilities of the chemotherapy of larval toxocarosis on a paratenic (mouse) model.
4. We wanted to examine the possible protective measures against toxocarosis with special regard to the prevention of human infection. In order to reach this objective we wished to study the knowledge of veterinarians and pet keepers on the subject of the worms of dogs and cats and their hazard to public health and their prevention. Finally we wished to examine ways of prevention in Hungary so that the risk of occasional helminthozoonosis for people getting in contact with animals could be decreased with the closer cooperation of veterinarians and medical doctors.
MATERIALS AND METHODS

1. Collection of data with faeces examination and autopsy for the parasite infections of dogs and cats, with special regard to *Toxocara* infection.

<table>
<thead>
<tr>
<th>Method</th>
<th>Site and time of collection</th>
<th>Samples</th>
<th>Number of samples /n/</th>
</tr>
</thead>
<tbody>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Budapest, 1982-86 faeces (clinic, animal shelter, on duty)</td>
<td>1674</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>faeces (11 parks &amp; playgrounds)</td>
<td>200</td>
</tr>
<tr>
<td>autopsy</td>
<td>Dog</td>
<td>Budapest, 1986 (animal health station, Phylaxia)</td>
<td>49</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Debrecen, 1994 faeces (clinic, animal shelter)</td>
<td>284</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Budapest, 1997 faeces (11 parks &amp; playgrounds)</td>
<td>200</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Mátraderecske (dogs kept in garden only)</td>
<td>206</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Monor (92,5 % kept in garden)</td>
<td>305</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Barcs (66,8 % kept in garden)</td>
<td>126</td>
</tr>
<tr>
<td>flotation</td>
<td>Dog</td>
<td>Zalalővő &amp; Csöde (96,7 % kept in garden)</td>
<td>100</td>
</tr>
<tr>
<td>flotation</td>
<td>Cat</td>
<td>Budapest, 1982-86 faeces (clinic, animal shelter)</td>
<td>150</td>
</tr>
<tr>
<td>flotation</td>
<td>Cat</td>
<td>Debrecen, 1994 faeces (clinic)</td>
<td>38</td>
</tr>
<tr>
<td>flotation</td>
<td>Cat</td>
<td>Barcs, 1998-2000 bélsár (outdoor cats)</td>
<td>27</td>
</tr>
</tbody>
</table>

- The comparison of the combination of three types of concentrating fluid, namely Breza-type/Flotol (n=514) and Breza-type/potassium carbonate (n=492) in order to improve the efficiency of the flotation method.

- The comparison of worm infection data obtained from faeces examination and simultaneous autopsy of dogs via the dissection of 49 dogs.

2. Examination of the pathogenesis and pathomechanism of larval toxocarosis using a paratenic host (mouse) model.

- We examined the effects of the primary and secondary *T. canis* [600 eggs/mouse (CD-1 conv.)] infection macroscopically and microscopically on spleen, liver and brain alterations of animals sacrificed 7, 17, 44 and 394 days post infection and also by counting recovered larvae.

We also kept record concerning the change of behavior and condition of the animals throughout the long infection period. The recovery of larvae from the organs and tissues studied was achieved using a method including digesting with HCl-pepsin, and the histopathological samples were evaluated following haematoxiline-eosin staining.
• We evaluated the route of migration of *T. mystax* larvae and the pathological reactions they caused by examining the liver, lung, brain and muscles of one fore and one hind limb of the animals sacrificed 2, 5, 8, 11, 14, 21, 36, 42 and 128 days after the 1000 eggs/mouse (CFLP) infection using the above mentioned methods.

3. Investigations on the possibilities of chemotherapy of larval toxocarosis on a paratenic host (mouse) model, with special regard to the treatment of infected people

• This chapter details the first two-phase experiment in the series of examinations on the efficiency against *T. canis* larvae as well as the development of an appropriate method of recovering larvae necessary for subsequent comparative examinations on a large number of animals. It reviews the preliminary examinations concerning the efficiency of mebendazole and albendazole against larvae in a therapeutic experiment performed on 27 female, white CFLP mice infected with a dose of 600 and 2000 eggs/animal.

• Furthermore it summarizes the series of treatments of some 600 mice against migrating and stationary *T. canis* larvae in the tissues following infection with 800-1200 eggs. The treatments were launched 2, 14, 81, 87, 104, 123 days after the infection, and were applied for different time periods and in various forms e.g. in suspension, in dry food, and in the form of various anthelmintics applied parentally (benzimidazole derivates: albendazole, mebendazole, fenbendazole, thiabendazole, flubendazole, oxfendazole; and macrocyclic lactone derivate: ivermectin).

• The chapter includes the investigations on the previously less frequently studied *T. mystax* larvae, where the treatment of 24 animals was performed 15-22 and 216-240 days post infection with 1000 eggs.

Efficiency was determined by comparing the average number of larvae recovered from various tissues, particularly from the brain and muscles employing the digestive method in experimental groups vs. untreated control groups.

4. Examination of the possible protective measures against toxocarosis with special regard to the prevention of human infections.

The examination regarding the knowledge of pet owners was carried out: 173 (Debrecen, veterinary hospital) + 126 (Barcs, veterinary consulting room) with the help of a questionnaire; the questionnaire designed to collect data on the knowledge of veterinarians: 20 (Nógrád county) + 126 (95 Budapest + 31 countryside).
RESULTS AND CONCLUSIONS

1. The prevalence of intestinal worm infection of dogs and cats with special regard to *Toxocara* infection on the basis of faeces examination and autopsy

- between 1982-86 58.7% of dogs (Budapest), in 1994 50.7% (Debrecen),
- between 1998-2000 55.0-70.0% (Monor, Barcs, Zalalövő and Csöde),
- between 1982-86 36.7% of cats (Budapest), in 1994 26.3 % (Debrecen) and
  - in 1999 51.8% (Barcs) were infected with various intestinal worms.
- In dogs the *Trichuris vulpis* and *T. canis* infections, while in cats the *T. mystax* infection proved to be the most common. In groups where the majority of dogs was younger than 1-year-old (Debrecen, Barcs) and 5-years-old (Mátraderecske) *Toxocara* infection proved to be the most frequent. In this latter examination, even in animals older than 5 years, the infection with roundworms was also remarkable (31.8 %).
- 81.6% of all dissected dogs proved to be infected with at least one worm species. *T. canis* occurred mainly in young animals. *T. vulpis* and *Dipylidium caninum* could be found among all age groups.
- Examining faeces samples collected from the very same 11 parks ten years later we could observe a 25-23% infection rate and similarly the *Trichuris*- and *Toxocara* eggs were the most frequent.
- We did not find any significant difference regarding the detectability of parasite eggs in favor of either concentrating solution comparing Breza and Flotol fluids, however when the same faecal samples were examined twice, the overall results indicated an infection of higher prevalence. In the Breza and potassium carbonate combination we could observe a higher level of infection revealed by the application of the Breza-type concentrating solution besides proving the significance of examinations performed twice.
- Comparing the worms observed and the result of the faeces examination we demonstrated that in the case of *D. caninum* worms, the flotation did not prove to be a reliable method to identify infection. On the other hand *Toxocara* and *Trichuris* eggs are relatively well detectable with the use of this method.
2. The pathogenesis and pathomechanism of larval toxocarosis in the paratenic host (mouse) model.

- **T. canis** We did not observe symptoms in the early stages of the infection with 600 larvae. At the end of our series of experiments lasting over 13 months the emerging symptoms were also in association with the pathological alterations caused by the accumulated larvae in the brain. Despite the accumulation some of the larvae does again continue visceral migration from the brain. The behavior and clinical condition of the animals is rather associated with the localization of the larvae in the brain and the actual larvae count per gram of the brain tissue than the dose used for infection. The continuous migration of some of the larvae is proved by the fact that we found larvae in the liver even at the end of our experiment, especially in those subjected to single infection. The general state and condition of these mice was significantly worse than those of the twice infected. The enormous difference observed in the earlier period (days 17-44) between primary and secondary infected animals, especially in the number of recoverable larvae from the brain almost became even in the ones killed on the 394th day. Though in the case of animals infected once, more larvae accumulated in the brain compared to the previous cases and we also found more larvae in the liver. We believe that the continuous migration of some of the larvae in primary infected animals can occur almost without obstacle since the “liver entrapment” works to a lesser extent.

In our experiment the migration of the larvae could be justified histopathologically in the weeks following the infection, since the signs of traumatic and cellular infiltration as well as some larvae could be seen in both the gray and white matter of the cerebrum, furthermore focuses could be recognized as well. The macro- and microscopic spleen and particularly liver defects of the animals sacrificed on different days showed the effect of the larvae penetrating into the given organ and the intense reaction of the organism to repeated infection.

- **T. mystax** The larvae reach the muscles even in early stages of the infection in contrast with *T. canis*. Until 11 days after the infection the histological examination results of the brain also suggests that the larvae reach the brain later. After the 21st day following the infection the larvae reappear in the liver. This could likely be explained by the fact that some of the larvae migrate constantly in the body. The proportion of larvae recovered from the muscles is 74.7% in the case of mice killed at a later stage. The results of histological examinations also confirm our observations. The signs of larvae flooding the liver, that is the traumatic tunnels, haemorrhage and later on the neutrophil granulocyte infiltrations can be well perceived in the liver tissues. These alterations are more or less severe, and are proportional to the number of
larvae. The consequences of the larvae migration can also be seen in the lungs and cerebrum. This also supports our assumption that the *Toxocara* species of cats may also play an important role in developing *VLM* in certain cases.

3. The possibilities of chemotherapy against larval toxocarosis in the paratenic host (mouse) model with special regard to the treatment of infected human

- **Experiments on the chemotherapy against *T. canis* larvae**

  - The larvae in tissues showed susceptibility to drug action. The level of effectiveness of treatments with multiple doses of fenbendazole was the same applied from the 2nd and the 14th day. However, in the treatments started on the 81st, 87th and 123rd days fenbendazole, flubendazole and albendazole at some of the applied doses proved to be effective (causing the 88-100% decrease of the number of larvae), thus proving that hypobiotic larvae remain susceptible to anthelmintics in their dormant stage, long after the 80th day following the infection.

  - The method of medication influenced effectiveness. The effectiveness of fenbendazole applied in oral suspension at early stages of the infection is similar to that of treatment as a medicated pelleted diet, but as time goes on and the larvae “get older” the effectiveness gradually decreases. The application of benzimidazoles in dry food increases effectiveness, therefore medicated dry food seem to be a simple method to make the treatment more effective versus oral suspensions. The relationship between digesta transit, delivery kinetics and drug bioavailability seems to be a feasible direction for further examinations.

  - The selection of effective drugs and doses became possible based on the findings.

- **Benzimidazoles.** According to our examinations the treatments with benzimidazoles demonstrated significant effectiveness against larvae when using the following treatment methods.
Treatments resulting in >90 % decrease in *Toxocara canis*

Total larva count in order of percentage intensity effect (IE %)

<table>
<thead>
<tr>
<th>Active ingredients</th>
<th>Dose/day</th>
<th>Treatment starting day after infection</th>
<th>Total drug uptake mg/mouse</th>
<th>IE %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>starting day</td>
<td>duration in days</td>
<td>mg/mouse</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>6,0 g/food kg *</td>
<td>123.</td>
<td>30</td>
<td>672.8**</td>
</tr>
<tr>
<td></td>
<td>6.0 g/food kg</td>
<td>2.</td>
<td>20</td>
<td>484.8</td>
</tr>
<tr>
<td></td>
<td>6.0 g/food kg</td>
<td>87.</td>
<td>30</td>
<td>642.0</td>
</tr>
<tr>
<td></td>
<td>6.0 g/food kg</td>
<td>123.</td>
<td>20</td>
<td>408.0</td>
</tr>
<tr>
<td></td>
<td>48 mg/mouse</td>
<td>2.</td>
<td>8</td>
<td>384.0</td>
</tr>
<tr>
<td></td>
<td>6.0 g/food kg</td>
<td>15.</td>
<td>20</td>
<td>506.5</td>
</tr>
<tr>
<td></td>
<td>6.0 g/food kg</td>
<td>81.</td>
<td>20</td>
<td>484.0</td>
</tr>
<tr>
<td></td>
<td>9.6 g/food kg</td>
<td>81.</td>
<td>16</td>
<td>622.0</td>
</tr>
<tr>
<td>Albendazole</td>
<td>1,6 g/food kg</td>
<td>123.</td>
<td>30</td>
<td>195.3</td>
</tr>
<tr>
<td></td>
<td>1,6 g/food kg</td>
<td>87.</td>
<td>30</td>
<td>141.5</td>
</tr>
<tr>
<td></td>
<td>1,6 g/food kg</td>
<td>81.</td>
<td>20</td>
<td>134.0</td>
</tr>
</tbody>
</table>

IE = intensity effect; *6.0 g/food kg = 30 mg/mouse/day = 1000 mg/bodyweight kg;
**672.8 mg/mouse = 22 426 mg/bodyweight kg

The 1.6 g/food kg or 6.0 g/food kg dose of flubendazole and oxibendazole had an effect of 57.8-88.2% and 81.1%, and 32.0% after a 20-days application. The 125 mg/bwkg/day dose of mebendazole, well known in human medicine as well, given on days 6-10 of infection had a weak effect on both the larvae migrating and established in tissues. In case of the also well known thiabendazole we experienced non-satisfactory effectiveness either (44.4%).

Based on the above it is conceivable that in case of human toxocarosis the condition of the patient improves because of the inflammation-reducing effect of glycocorticoids given simultaneously, and we cannot disregard the inflammation-reducing effect of thiabendazole either. Due to the weak larvicid effect most of the larvae may persist alive in the tissues.

-**Macrocyclic lactones.** We achieved only a maximum effectiveness of 47.0% with 0.6 g/bw. kg dose of ivermectine applied in various ways.

-**The blood-brain barrier proved to be permeable for the applied anthelminitics.**

According to our investigations after the treatments started on the 2nd, 14th, 81st and 87th days post infection 81.8-93.4 %, and 77.6-91.3 % of the larvae can be found in the brains of
the control and treated animals respectively. Although these differences were statistically significant the applied treatments did not affect the distribution of the larvae considerably. Based on our examinations it seems that the brains of mice do not provide a site facilitating survival of larvae. However, such aspects of the chemotherapy of larval toxocarosis require further studies.

- **Experiments on the chemotherapy against *T. mystax* larvae**

  No solid conclusion can be drawn from the results of our therapeutic experiment due to the low number of animals involved because of the limited amount of infectious material available. Despite this, it is apparent that the fenbendazole applied in suspension form starting on the 15th day after the infection proved to be completely ineffective against *T. mystax* larvae migrating in mice. In contrast the albendazole applied in a much smaller dose reached efficiency of >60%. On the other hand fenbendazole applied in food for 25 days with a total amount of active ingredient of 600 mg was just as outstandingly effective against the more than 7 months old *T. mystax* larvae as could be observed in case of the treatments against *T. canis* larvae.

4. **Studies on the prevention against toxocarosis with special regard to the prevention of human infection.**

- **Evaluation of a survey with questionnaires**

  According to the survey and based on the opinions of veterinarians, almost all pet owners (98.4%) are inadequately informed about intestinal worms. Consequently it is no surprise that almost as many asked the veterinarian whether intestinal worms of their pets could pose a threat to human health. 71.9% of veterinarians answered having had at least one owner in his practice who had been treated by a physician because of suspected infection caused by dog/cat worms. According to the survey in 1998, 88.9% of veterinarians apply anthelmintic treatment routinely and only 3.2% of them take faeces samples for parasitological examination and decide depending on their results, though 90.5% of them have the availability of faeces analysis for parasites. As indicated by the vets we asked, some of the pet owners are not even willing to foot the bill for a single faeces analysis. The veterinarians claim that due to financial reasons, more than half of the owners ask for the cheaper, potentially less effective medicines. 30-25.4% of the ones who gave an answer would and do treat puppies against helminths at the age of 4-7 weeks, which is a wrong practice. Almost 75% believe that the efforts of the veterinarians to inform pet owners is not sufficient.
The recommendation for prevention based on our findings and results

The decrease of intestinal worm infection of dogs and cats with anthelmintic treatments.

Young animals: *Puppies are to be treated first at the age of 14 days* (preimaginal dehelminthisation), *then* (because of milk-mediated infections) in *every two weeks until the age of 12 weeks* (if this frequent treatment is not feasible then at least twice, in two-weeks interval following the treatment on the 14th day). Then at the age of 4 and 5 months, after that quarterly. *Kittens are to be treated first at the age of 4 weeks* (because of milk-mediated infections), *then every two weeks until the age of 12 weeks* (if this frequent treatment is not feasible then at least twice in two weeks interval following the treatment in the 4th week), after that as in the case of dogs. It implies that the first two treatments should take place in the kennel. Nursing females: *to be treated at the same time as her offsprings.*

Grown-up animals: quarterly treatment is ideal, but if it is not available so often, then at least twice annually. With the regular treatment of adult animals the number of parasite eggs spread with faeces can drop as well. *In breeding facilities the treatment of each and every animal is to take place at the same time*, accompanied by the adequate cleaning and disinfection of the premises.

- Decrease in the number of dogs and cats. Sterilization of animals kept as a hobby or not suitable for breeding could be a satisfactory method to prevent unwanted reproduction. Veterinaries should play an important role in advising.

- Education of the public. This includes the prevention of faeces excretion of dogs and cats in public places (e.g. pavement, playgrounds). Veterinarians have an indirect role: calling the attention of the public to this and providing information. Veterinarians play the main role to enlighten people and broaden their knowledge relating to helminthozoonoses.

- The role of the media in informing people. The written and above all the electronic media could play an invaluable part in providing information and prevention. The main topic of popular programs on animals could be the importance of worm prevention, its health consequences, but attention must be paid to involve a specialist or a veterinary competent in this field, because the media can easily be misleading sometimes, of which we have quite a few examples. Even authorities might mislead the population unintentionally e.g. with inaccurate wording of certain points in the statute regulating pet keeping.

- The role of physicians. Medical doctors should be more involved in providing information, since pet owners see their physician first in certain cases. General physicians have an even bigger role, as well as health visitors and pediatricians in families with children. Professional
advice given by doctors might have a part to play. (The question can be raised: how well-informed are physicians in this topic…?)

The prevention of helminthozoonoses, such as human larval toxocarosis is a joint task for veterinarians and physicians in Hungary.

Introducing parasitology tuition as an independent subject. Primarily a chance should be given for students in postgraduate physician and nurse education, as well as during undergraduate terms to broaden their expertise on parasitology, even in facultative subjects. Currently there is no such subject, medical students receive some tuition within the field of microbiology in 2-4 hours of lecture and 2x2 hours of practical class or seminar.

NEW FINDINGS

1. With our appraising examinations we were the first to prove the high rate of various intestinal worm infections of dogs and cats in Hungary, as well as the frequent occurrence of Toxocara species relevant for public health and not only in young animals.

2. With the help of a histopathological examination of the prevailing chronic larval toxocarosis more than 13 months after the infection with few T. canis larvae as well as with the count of the larvae recovered from the liver and the brain we proved permanent migration both in the primary and secondary infection, though in the latter case fewer larvae have the chance to do so.

3. The T. mystax larvae reaching the brain and migrating or established there, several months after the infection in paratenic hosts, thus presumably in humans, acting as “accidental” paratenic hosts may develop the visceral larva migrans syndrome.

4. With a series of chemotherapeutic experiments we proved that the Toxocara larvae established in paratenic host could be subject to anthelmintic drugs, thus Toxocara larvae in both the early and later stages of their migration as well as the hypobiotic larvae of chronic infections can be targeted by chemotherapeutic drugs depending on the dosage applied.

5. In order to reach excellent efficiency (≥ 90 %) a dose of 384-672.8 mg (≥12.8-22.4 g/bwkg) is required of fenbendazole, but 1/3 of this amount is sufficient of albendazole. Other benzimidazoles like flubendazole, oxibendazole, oxfendazole and thiabendazole – applied in human medicine – and in case of the various applications of ivermectin the efficiency varies in each case, but definitely decreases greatly.
6. There is hardly any connection between the estimated amount of the active ingredient absorbed and the extent of treatment success, since besides dosage other factors such as form of medication, duration of treatment, treatment with multiple doses, etc can modify the outcome of the treatment with the applied anthelmintic.

7. As far as we know we were the first to examine the efficiency of albendazole and fenbendazole against *T. mystax* larvae migrating or resting in a paratenic host. We demonstrated that the fenbendazole applied orally and in suspension following the 15th day after the infection is completely ineffective, on the other hand the albendazole applied in a much smaller dose (1/6) has an efficiency of >60%. The fenbendazole given for 25 days in dry food was just as exceptionally effective against *T. mystax* larvae carried for 7 months as we could observe in case of treatments against *T. canis* larvae.

8. After the evaluation of the answers given in our questionnaire we pointed out the shortcomings in the awareness of pet owners and some veterinaries concerning the helminthoses of dogs and cats, especially *Toxocara* species and the public health hazard they represent.

9. After the evaluation of the possibilities of prevention against the larvae of final host animals, in particular against *Toxocara* species we outlined the overall measures to be taken in Hungary to decrease the chances of infection of humans acting as “accidental” hosts.
Bibliographics data of the author’s own publications in the subject of the dissertation


Bibliographics data of abstracts of lectures and posters presented at the international and national meetings in the subject of the dissertation.

**Refereed congress abstracts:**


**Other congress abstracts:**


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