Epidemiological and pathogenic aspects of *Toxocara canis* and *T. cati* infection, with the evaluation of the zoonotic risk

(SUMMARY OF Ph.D. THESIS)

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ABSTRACT

Pets have become an integral part of everyday human life, the benefits of human-animal interaction on a social, physical, mental and emotional level being known and demonstrated. Along with these, the consequences of humanization and closer contact between dogs, cats and humans also require an approach from the perspective of the One Health concept, which includes multidisciplinary collaboration between veterinarians, public health specialists and the general population in terms of prevention. and control of zoonotic parasitic diseases.

Gastrointestinal disorders are one of the reasons for owners when presenting at the veterinary clinic with the pet for a medical consultation. Identifying the etiological cause can be challenging, but it is needed in order to develop an effective and complete therapeutic management. Various pathogens such as viruses, bacteria and parasites may be responsible for the occurrence of gastrointestinal symptoms in small animals (SABSHIN et al., 2012; TELLO and PEREZ-FREYTES, 2017).

Toxocara canis and *T. cati* are nematodes that inhabit the digestive tract in dogs and cats, respectively, being among the most common parasites identified in these species. Infested animals eliminate parasitic eggs along with the feces, thus contributing to environmental contamination and the infection of other definitive or paratenic hosts. In Europe, an average prevalence of 14.6% in dogs and 24.5% in cats was estimated following a systematic review of studies in 26 countries between 1994 and 2019 (OVERGAAUW and NIJSSE, 2020).

Along with their veterinary importance, *T. canis* and *T. cati* represent a public health problem, causing human toxocariasis, a severe pathology expressed by four major clinical forms of evolution: visceral toxocariasis *(larva migrans visceralis*), neurological toxocariasis (*larva migrans neuralis*), ocular toxocariasis (*larva migrans ocularis*) and covert toxocariasis (MACPHERSON, 2013). Humans and other vertebrates or non-vertebrates can accidentally become paratenic hosts by ingesting larvated eggs from the environment, through consumption of contaminated food or meat containing larval stages of the parasite (HOLLAND, 2017). The zoonotic risk is significant, as human seroprevalence can be 19% globally and an average of 10.5% in Europe (ROSTAMI et al., 2019).

The study of pathogenicity produced by the migration of *Toxocara* spp. larvae in the definitive or paratenic host, requires the development of experimental models. Rodents are the most commonly used animal models, due to their easier, less expensive maintenance, handling and reproduction. Moreover, they represent natural paratenic hosts, being involved in the life cycle of the parasite (BOES and HELWIGH, 2000).

In this frame, the present paper aims (1) to determine the epidemiological aspects regarding the prevalence of *T. canis* and *T. cati* infection in clinically healthy dogs and cats, but also with digestive pathology, (2) the pathogenesis in the definitive host and in the paratenic one by using mice as experimental models for obtaining *larva migrans neuralis* syndrome and (3) evaluation of the efficacy of disinfection measures

currently used in areas with high risk, against contamination and infection with *Toxocara* spp. eggs

The thesis is structured in two parts; the first part is entitled "Literature review" and the second part "Personal contributions".

The first part of the thesis includes information from the literature on *Toxocara canis* and *T. cati*, being structured in 6 chapters. In chapter I.1. data on history and taxonomy, morphological description and life cycle are presented. Chapter II.2 provides information on pathogenicity, clinical and morphopathological aspects in both the definitive and the paratenic host. Chapter I.3 contains epidemiological data on parasitic infection in dogs and cats, toxocariasis in humans, as well as information on environmental contamination with eggs of *Toxocara* spp. Chapter I.4 summarizes information on diagnostic methods, and chapter I.5 includes information on the treatment and control of *T. canis* and *T. cati* infection in dogs and cats. The last chapter of this part (I.6) presents data on experimental models used to obtain the "*larva migrans*" syndrome.

The second part of the thesis includes the research activity and the results obtained during the doctoral studies. Thus, it includes the enunciation of the objectives of the doctoral thesis and five chapters of original studies on epidemiological and pathogenic aspects of *Toxocara canis* and *T. cati* in dogs and cats (II.1, II.2, II.3), experimental "larva migrans" syndrome in paratenic hosts and highlighting the zoonotic risk (II.4, II.5).

Chapter II.1 aimed at retrospectively evaluating the prevalence of infection with roundworms and other gastrointestinal helminths in dogs (n = 964) and cats (n = 382) post-mortem and their correlation with morphopathological lesions and other comorbidities. The study was performed on corpses of dogs and cats necropsied at the Department of Pathological Anatomy, Necropsy Diagnosis and Forensic Medicine at the Faculty of Veterinary Medicine of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, between 2013-2018. The animals were necropsied to determine the cause of death. Information was extracted from the registers related to breed (mixed breed, purebred), age (puppy / kitten: ≤6 months, youth: ≤6 months ≤2 years and adults), sex and origin, cause of death, morphopathological lesions, comorbidities and identified endoparasites.

The extracted data were statistically processed using Epi InfoTM 7 (CDC, USA) (DEAN et al., 2011). The frequency, prevalence and 95% total confidence interval (CI) were calculated for each parasite identified and based on risk factors (breed, age, sex and origin of the animals). The risk factors, the correlation between the infection with roundworms and morphopathological lesions and comorbidities were analyzed by the Hi-square test.

Out of the total number of necropsied animals, 11.1% were infected with at least one digestive helminth, the prevalence being higher in cats (21.2%), compared to that obtained in dogs (7.1%). Roundworms were identified in 8.5% of necropsied animals and were statistically significant the most common helminths identified in both dogs (75.0%) and cats (81.5%). The following digestive helminths were also identified in necropsied dogs: *Trichuris vulpis* (14.7%), *Taenia* spp. (13.2%), *Dypilidium caninum* (7.4%) and digestive strongyls (4.4%). The following digestive helminths

were also identified in cats: *D. caninum* (23.5%) and *Taenia* spp. (14.8%). The statistical analysis identified a single risk factor in roundworm infection and only in dogs. Thus, infection with roundworms was statistically significantly more frequent in young dogs under 6 months of age (43/43). Roundworm infections in necropsied dogs and cats were frequently associated with enteritis lesions (dogs: 70.6%; cats: 56.1%) and gastritis (dogs: 41.2%; cats: 40.9%). Also, 35.3% of dogs infected with roundworms were diagnosed post-mortem with canine parvovirus and 15.2% of cats infected with roundworms were diagnosed post-mortem with feline panleukopenia.

Chapter II.2 aimed to determine the prevalence of *T. canis,* associated risk factors and other parasitic enteropathogens in dogs with and without digestive manifestations. 321 faecal samples from dogs with both digestive manifestations and clinically healthy ones, were collected and analyzed. The dogs were aged between 3 weeks and 17 years (mean 22.01 months ± 31.93). The faecal samples were subjected to the coproparasitological examination by flotation technique (Willis), within the Department of Parasitology and Parasitic Diseases (DPBP) from FMV Cluj-Napoca. The results were statistically calculated with the Epi InfoTM 7 software (CDC, USA) (DEAN et al., 2011). The frequency, prevalence and 95% CI were calculated for the total infections, each parasite identified, parasitic associations and for the variables studied. Risk factors were assessed using the Hi-square test. The variables studied were: (1) age (puppies ≤3 months: 116; youth >3 - ≤12 months: 85; and adults> 1 year: 120); (2) sex (males: 173, females: 148); (3) origin (dogs with owner: 197; dogs from kennels: 53; and dogs from associations or temporary adoption program: 71); (4) deworming in the last 3 months (yes: 163; no: 158); (5) the presence (n = 178) or absence (n = 143) of digestive manifestations.

The general endoparasitic prevalence was 64.5% in the investigated dogs; endoparasites were statistically significantly more frequently identified in dogs with digestive manifestations (77.0%) compared to clinically healthy ones (49.1%). *Toxocara canis* was the most prevalent parasitic enteropathogen, both in dogs with digestive disorders and in clinically healthy ones. The following endoparasites were also identified at the coproparasitological examination (n = 11): *Trichuris vulpis* (19.9%), *Cystoisospora* spp. (14.3%), *Ancylostoma caninum / Uncinaria stenocephala* (10.0%), *Giardia duodenalis* (5 , 6%), *Sarcocystis* spp. (0.9%), *Taenia* spp. (0.6%), *Strongyloides stercoralis* (0.3%), *Neospora caninum / Hammondia heydorni* (0.3%), *Toxascaris leonina* (0,3%) and *Dypilidium caninum* (0.3%). Although the general prevalence of monospecific infections was higher (45.2%) than that of co-infections (19.3%), pluriparasitism was positively correlated with the evolution of digestive manifestations in dogs (24%). The most common parasitic association was between *T. canis* and *Cystoisospora* spp. (5.3%) and with statistically significant differences between dogs with and without digestive manifestations.

Age was identified as a risk factor for *T. canis* infection in the investigated dogs. The highest prevalence was recorded in puppies (58.6%) regardless of clinical status (with digestive manifestations: 62.9%; without digestive manifestations: 52.1%). The infection rate decreased with age, but 10% of adult dogs were co-eliminators of *T. canis* eggs. Also, dogs that did not benefit from deworming in the last

3 months regardless of clinical status had a higher risk of being infected with *T. canis* (total: 55.1%; with digestive symptoms: 55.0%; asymptomatic: 55.2%).

Diarrhea is a symptom statistically significant associated with *T. canis* infection in dogs (42.3%).

The data obtained underlines the need to perform coproparasitological examinations for both diagnostic and routine control purposes.

Chapter II.3 aimed to determine the prevalence of *T. cati* infection and other parasitic enteropathogens and to evaluate risk factors in cats with and without digestive manifestations. The study included 137 cats presented to the Emergency and Intensive Care Unit. The age of the investigated cats was between 4 weeks and 15 years with an average of 26.03 (± 3.68) months. From these cats, faecal samples were collected and tested by coproparasitological examination at DPBP within USAMV Cluj-Napoca, FMV for differential diagnosis or routine control. Statistical results were obtained with the Epi InfoTM 7 software (CDC, USA) (DEAN et al., 2011). The frequency, prevalence and 95% CI were calculated for the total infection, each parasite identified, parasitic associations and for the variables studied. Risk factors were assessed by binary and multinomial logistic regression analysis. The variables studied were: age (≤6 months: 66; 6 months-2 years: 39; >2 years: 32); race (mixed: 128; pure: 9) sex (males: 70, females: 67), habitat (outdoor access: 96; indoor: 41), presence (n = 90) or absence (n = 47) of digestive signs and the status of internal deworming (yes: 74; no: 63).

Overal prevalence of intestinal parasites was 50.4%, with statistically significant differences between cats with digestive manifestations (66.7%) and clinically healthy ones (19.2%), either as monospecific or polyspecific infections. *Toxocara cati* (40.2%) was statistically significant the most commonly identified endoparasite, followed by *Cystoisospora* spp. (10.2%), *Ancylostoma tubaeforme* (3.7%), *Giardia duodenalis* (2.2%), *Taenia* spp. (2.2%) and *Toxoplasma gondii / Hammondia hammondi* (0.7%). The most prevalent parasitic association was that between *T. cati and Cystoisospora* spp. (8.9%) and was also the only one statistically significant in cats with digestive signs.

More than half (65.7%) of the examined cats showed one or more digestive signs such as vomiting (n = 21), diarrhea (n = 78) or loss of appetite (n = 31). Digestive signs were more common in cats with outdoor access (78.8%), in kittens (63.3%) and in cats not recently dewormed (53.3%). Of these, 53.3% were infected with *T. cati*. In clinically healthy cats, *T. cati* was observed in 14.9% of them. Regarding the age categories, there was a decreasing trend in the rate of *T. cati* infection in symptomatic cats correlated with increasing age, but without statistical significance. In kittens and youth (47/48), diarrhea was statistically significant the most common digestive sign in individuals infected with *T. cati* as the sole symptom (24/48) or in combination (23/48) with other digestive signs such as vomiting (12/48) and loss of appetite (22/48).

Lack of deworming in the last three months (OR: 15.9), access to the external environment (OR: 13.8), the presence of digestive signs (OR: 5.4) and young age (OR: 4.2) were determined as risk factors for *T. cati* infection in owned cats by logistic regression analysis.

Chapter II.4 aimed to observe the syndrome of *larva migrans* of *T. canis* and *T. cati* in the paratenic host represented by mice from the CD1 line. Aspects of pathogenicity in cerebral toxocariasis have been recorded and described. In this frame, 3 experimental groups of 14 mice were made: (1) group infected with 2000 *Toxocara canis* eggs/ mouse; (2) group infected with 2000 *Toxocara cati* eggs / mouse infesting; (3) control group to which distilled water was administered.

The electroencephalogram was performed at 30 and 60 days post infection on the same individuals, respectively 3 mice from each group.

After 30 days, 7 mice from the infected groups (*T canis, T cati*) and 3 mice from the control group were sacrificed by cervical dislocation under general anesthesia. The procedure was repeated at 60 days p.i. (post infection). Necropsy and macroscopic examination of the organs was performed, larvae of *T. canis* and *T. cati* were observed and counted in the brain, histopathological and immunohistochemical examinations were performed.

Mice from the CD1 line were a suitable experimental model for both *T. canis* and *T. cati*, especially for obtaining cerebral toxocariasis.

Electroencephalography of mice infected with *T. canis* and *T. cati* showed changes in all individuals, compared to the uninfected control group. Various epileptiform discharges and encephalic-like changes were highlighted, which were present at both 30 days p.i and 60 days p.i.

In the case of groups infected with *T. canis*, the larval recovery rate was similar between day 30 p.i (21.34%) and 60 p.i (21.77%). The larval distribution revealed a higher percentage in the right hemisphere in mice infected with *T. canis*, but without statistical significance.

The percentage of larval recovery decreased in *T. cati* infected mice, from 30 days p.i. (3.55%) at 60 days p.i. (1.98%), but statistically insignificant. The mean larval distribution revealed the highest percentage in the cerebellum at both 30 and 60 days p.i. Statistical significance was obtained at 60 days p.i. between the larvae recovered from the cerebellum and the right hemisphere.

Recovery and larval motility had higher values in the group infected with *T. canis* compared to the group infected with *T. cati*, both at 30 days p.i. and at 60 days p.i. in mice in the CD1 line.

The anatomopathological changes were correlated with the results of the histopathological examination in which the presence of larvae and inflammatory infiltrates in other organs was highlighted. In the central nervous system, the inflammatory reaction was more intense in individuals from the group infected with *T. canis*, represented by moderate congestion and interstitial edema, Wallerian degeneration, malacia, Gitter lipophage cell formation, foci of gliosis and proliferation of astrocytes in the form of gemistocytes, compared to a minimal inflammatory process characterized by vacuolations, discrete degeneration and rare foci of gliosis in the case of the group infected with *T. cati*; In both groups, granulomatous reaction in the meninges was found.

Immunohistochemistry revealed the expression of inflammatory markers at the CNS level (GFAP, iNOS and S100) in the case of both infected groups, being performed for the first time in *T. cati* infected mice;

All procedures and actions performed were in accordance with the Bioethics Comission of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (authorization nr. 125/05.12.2018).

Chapter II. 5 aimed to evaluate the efficacy of disinfectants currently used in high-risk environments on the embryogenesis of *T. canis* eggs, in order to answer the question of whether the measures currently being taken are sufficient to prevent *T. canis* infection and contamination. The disinfectants tested were: sodium dichloroisocyanurate dihydrate (0.05%) (A); sodium hypochlorite (2.4%) (B); mix of glutaraldehyde and ammoniacal salts (dimethylcoccobenziamonium chloride) (1%) (C); postassium peroxymonosulfate (1%) (D); dodecylpropane-1,3-diamine mix (0.06%) (E); 2-propanol, ethanol, benzalkonium chloride, glycoprotamine (100%) (F). These substances are indicated for their bactericidal and virucidal properties, but "off label" are used to obtain a parasiticidal action.

Disinfectants were tested at the maximum concentration recommended by the manufacturer for the biocidal effect. Each disinfectant tested was mixed with approximately 10,000 *T. canis* eggs for 5, 10, 15, 30 and 60 minutes. For each disinfectant and contact time, 3 replicates were used. After exposure to disinfectants, eggs of *T. canis* were washed and incubated in distilled water at 27 ° C for 2 weeks. During the incubation period, every two days the eggs of *T. canis* were examined microscopically (10x, 20x, 30x, 40x) assessing the morphology and motility of the larvae. The percentage of larval development was calculated using the following formula: fully embryonated eggs (L2) / total number of eggs x 100 (OH, 2016). Altered eggs were not considered. The effectiveness of disinfectants was determined as the difference between the larval development of 100% and that of each disinfectant at the contact times used. The statistical evaluation was performed using the GraphPad InStat statistical software package, using One-way Analysis of Variance (ANOVA). The comparison was performed by the Tukey-Kramer Multiple Comparison Test.

None of the disinfectants tested in the experiment were able to completely inhibit the embryonation of *T. canis* eggs or destroy them, regardless of the time of contact. The most effective disinfectant was sodium dichloroisocyanurate at a contact time of 30 minutes (31.44%).

Morphological changes in the outer shell of the eggs were observed in the samples of sodium hypochlorite, in which case the development of the eggs was faster compared to the control group.

The general conclusions were:

- roundworms were the most prevalent digestive helminths identified potsmortem in dogs and cats, along with lesions of enteritis and gastritis;
- canine parovirus and feline panleukopenia are frequently found comorbidities in dogs and cats infected with roundworms;
- prevalence of *T. canis* and *T. cati* infection were statistically significant higher in dogs and cats with digestive clinical signs (diarrhea);
- age and lack of deworming were risk factors for *T. canis* infection in dogs, regardless of the clinical status of the animals;

- age, outdoor access and lack of deworming were risk factors for *T. cati* infections in cats;
- *T. canis* and *T. cati* cause cerebral toxocarosis in mice from the CD1 line, with a higher larval recovery rate in the case of *T. canis*;
- *T. canis and T. cati* induce epileptiform and encephalitic changes on the electroencephalogram of infected mice;
- the inflammatory reaction in the central nervous system was more intense in the mice infected with *T. canis* compared to *T. cati* infected ones;
- immunohistochemistry revealed the expression of inflammatory markers in the central nervous system (GFAP, iNOS and S100) in both infected groups;
- disinfection that includes the tested chemicals is not an effective measure to prevent contamination with *T. canis*, thus the risk of infection for other animals and the zoonotic one are possible.

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