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Endoparasites with Zoonotic Potential in Domesticated Dogs

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1. Introduction

Dogs play a relevant role as definitive hosts of a large number of parasites, shedding gastrointestinal helminth eggs and protozoan cysts and oocysts in their feces, which favors environmental contamination and possible spread of diseases (Santarém et al., 2004).

Considering the close proximity between men and dogs and the zoonotic potential of these diseases, determining the occurrence of endoparasites in pets has become increasingly relevant. These diseases are frequently diagnosed in spite of the existent therapeutic and prophylactic measures.

Contact with the soil, fomites or hands contaminated with the animals' feces favors accidental human infection either through ingestion of *Toxocara canis* embryonic eggs, resulting in Visceral Larva Migrans (VLM) syndrome (Coelho et al., 2001), or through percutaneous penetration of *Ancylostoma caninum* and *Ancylostoma braziliense* infective larvae, causing Cutaneous Larva Migrans (CLM) Syndrome (Diba et al., 2004).

Studies of animal toxoplasmosis are essential in view of its transmission to men and its pathogenicity in production animals and pets (Garcia et al., 1999a; 1999b, 1999c). Although the dog is not a definitive host, it has contributed to the mechanical dissemination of this protozoal disease (Frenkel & Parker, 1996; Lindsay et al., 1997; Schares et al., 2005).

The risk of acquiring this disease is higher in the postnatal life (Escuissato et al., 2004), when severe behavioral changes such as attention deficit and schizophrenia may occur (Lafferty, 2005), reducing the life quality of individuals (McAllister, 2005). Attention should be drawn to the risk factors for acquiring this prenatal infection, considering its pathogenesis and sequelae (Bachmeyer et al., 2006; Hung et al., 2007). There is strong association of reactivation of this disease in cases of acquired immunodeficiency syndrome - AIDS (Bachmeyer et al., 2006; Hung et al., 2007), commonly leading to secondary infection of the central nervous system in immunocompromised people and severe encephalitis (PASSOS et al., 2000; Mamidi et al., 2002; Collazos, 2003; Yadav et al., 2004; Pradhan et al., 2007). Educational programs directed at reducing environmental contamination by *T. gondii* would eventually decrease the cost of treating humans with clinical toxoplasmosis (Santos et al., 2010).

The dog is considered the main domestic reservoir for *Leishmania* spp. and the Brazilian Ministry of Health has recommended euthanasia for animals affected by this disease (BRASIL, 2006). Visceral leishmaniasis (VL) is a zoonosis worldwide distributed in tropical and subtropical regions, and 90% human cases have been reported for countries like India, Sudan, Bangladesh, Nepal and Brazil (Lindoso & Goto, 2006). Immunodepressed or chemotherapy patients are most susceptible, resulting in lethality of most cases (BRASIL, 2006). The World Health Organization classified leishmaniasis as the second most important current protozoal disease (Troncarelli, 2008).

As to Chagas Disease, millions of people are infected and suffer every year due to this illness (Rosypal, et al., 2007). In São Paulo State (SP), Brazil, infection by *Trypanosoma cruzi* has been detected in dogs (Lucheis et al. 2005; Troncarelli et al. 2009).

Molecular studies have indicated that dogs can transmit the bovine genotype of *Cryptosporidium parvum*, which is known to be pathogenic to humans (Abe et al., 2002). *Cryptosporidium* oocysts are highly resistant under environmental conditions and to the action of chemical products (Plutzer & Karanis, 2009). The protozoan *Cryptosporidium* is included in the Neglected Diseases Initiative of the World Health Organization due to their close relationship with deficient sanitation and low income of the population; thus, it is considered responsible for malnutrition and death in children (Thompson et al., 2008; Bowman & Lucio-Forster, 2010). These evolutionary forms have been detected in foods such as vegetables, seafood, unpasteurized milk, citron and mineral water in several countries. A large number of cryptosporidiosis outbreaks, waterborne, are cited in the literature (Thompson et al., 2008, Smith & Nichols, 2010).

2. Parasitic agents

2.1 Infections by *Ancylostoma* spp. and *Toxocara canis*

2.1.1 Epidemiology

An epidemiological survey carried out by our research group detected *Ancylostoma* spp. in 213 dogs (53.12%), followed by *T. canis* in 83 (20.70%). The high frequency of *Ancylostoma* spp. and *T. canis* justifies the concern about this worldwide problem, emphasizing its relevance from an epidemic-sanitary point of view (Táparo et al., 2006).

Some *Ancylostoma* species present zoonotic potential (Broker et al, 2004), such as *Ancylostoma braziliense* and *A. caninum*, who are the etiologic agents of diseases known as eosinophilic enteritis (Landmann & Prociv, 2003) and cutaneous larva migrans (Caumes, 2006).

Serosurveys have proven that *T. canis* infection is disseminated among the human population (Chieffi & Müller, 1976). Contact with the soil, fomites or hands contaminated with the feces of animals infected by these agents may favor accidental transmission to people (Guimarães et al., 2005; Blazius, 2006).

Our study indicated high occurrence of *T. canis* (67.3%) among young dogs ($P < 0.0001$). Helminth and protozoal infections are more prevalent among dogs younger than one year (Gennari et al., 2001), especially when the above-mentioned helminth is involved (Fischer, 2003). There are reports in the literature about the tendency of adult dogs for presenting an

effective immune response against nematodes. However, females in the postpartum were noted to shed eggs of *Toxocara* spp. in their feces (Urquhart et al. 1991). Ancylostomatidae were identified in all age ranges of dogs in our studies (Boag et al, 2003; Blazius et al 2005, Táparo et al., 2006).

In our routine laboratory services, we commonly observe dogs in their first month showing a large quantity of *T. canis* eggs in their fecal content. These evolutionary forms of ascarids, known to have thick skin, are characterized by their resistance to environmental adversities, remaining viable for many years, depending on the soil type and the climate conditions (Glickman & Schantz, 1981, Fortes, 1994).

Contributions from Oliveira et al. (1990), Gennari et al. (1999, 2001), Oliveira-Sequeira et al. (2002), Ragozo et al., 2002 and Táparo et al. (2006), in Brazil, have designed the epidemiological aspects of these parasitic disease in the canine species.

Larvae and eggs of helminths shed in the fecal content of dogs lead to great environmental contamination, representing a public health problem (Brener et al., 2005, Pfukenyi et al., 2010) in parks (Moro et al., 2008), affecting children who are in contact with sand tanks in these places, where larvae of *Ancylostoma* spp. are present (Santarém et al., 2004).

In Poland (Borecka, 2005), Venezuela (Ramírez-Barrios et al., 2004), the United States (Bridger & Whitney, 2009) and other regions of Brazil (Sousa-Dantas et al, 2007), the percentages of *Ancylostoma* in dogs were lower than that obtained in our studies, which evidenced 64.2% (27/42) positivity for samples collected from the environment. Of this sampling, 10.86% (5/46) were from child day care centers/parks, 41.30% (19/46) from streets/sidewalks and 47.82% (22/46) from squares/gardens. The genus *Ancylostoma* was present in 65.21% (30/46) samples, *Toxocara* spp. in 15.21% (7/46), *Cryptosporidium* spp. in 4.34% (2/46) and *Giardia* spp. in 10.86% (5/46) (Coelho et al., 2011).

Using parasitological necropsy, Yacob et al. (2007) in Ethiopia and Klimpel et al. (2010) in Brazil reported the occurrence of *A. caninum* in 70% (14/20) and 95.6% (44/46) examined dogs, respectively. These values are lower and higher, respectively, when compared with that obtained in the present study for *Ancylostoma* species. Our group determined the frequency and intensity of *Ancylostoma* spp. in 33 dogs by means of coproparasitological examinations and parasitological necropsy. Willis-Mollay and Sedimentation methods indicated eggs of *Ancylostoma* spp. in 87.8% (29/33) dogs. The species *A. caninum* and *A. braziliense* were found in 63.6% (21/33) and 30.3% (10/33) dogs, respectively (Coelho et al., 2011).

2.1.2 Physiopathogenesis

In dogs, the pathogenic lesions caused by gastrointestinal parasites must be considered for cutaneous, pulmonary (due to pulmonary migration of the larva during its development) and intestinal changes due to the final location of the worm in its adult stage (Fortes, 2004).

Toxocariasis in humans leads to variable clinical signs and symptoms due to the mechanical migration of larvae and the consequent immunological responses inherent in this process (Chieffi & Müller, 1976).

2.1.3 Biology

In the biological cycle of this helminth, *T. canis* eggs are shed in the feces and become infective under ideal conditions of temperature and humidity after two to six weeks (Scjantz & Glickman, 1983, Overgaauw, 1997).

In terms of biology, the enterohepatic pneumoenteral form is considered the most common infection form in dogs aged up to three months. Above this age range, this migration type has occurred at a lower frequency.. In pregnant bitch, parental infection occurs when larvae becoming mobilized at approximately, three week prior to parturition, and migrate to the lungs of the foetus where they molt into L3 just prior to birth. In pregnant dogs, migrating larvae mobilize at approximately three weeks before parturition and migrate to the fetal lungs, molting into third-instar larvae before birth. In the newborn pup, the cycle is completed when the larva migrates through the trachea and into the intestinal lumen, where the final molts take place. In the newborn pup, the cycle completes when the larva migrates through the trachea and to the intestinal lumen, where molting into adult worm finally occurs (Urquart, 1991). Once infected, a bitch will usually harbor sufficient larvae to subsequently infect all of her litters, even if she never again encounters an infe

2.1.4 Clinical signs

Percutaneous penetration of *A. caninum* and *A. braziliense* infective larvae or ingestion of *T. canis* embryonic eggs leads to Cutaneous Larva Migrans Syndrome (Lee, 1874) or Visceral Larva Migrans Syndrome (Beaver et al., 1952), respectively.

In humans, the evidenced signs of toxocariasis are asymptomatic conditions, fever, hypereosinophilia, hepatomegaly, ocular, pulmonary or cardiac manifestations, nephrosis and cerebral lesion (Overgaauw, 1997).

In dogs, these helminth diseases can lead to organic imbalance such as anemia, changes in appetite, intestinal obstruction or perforation, limited nutrient assimilation, diarrhea, apathy and sometimes death (Fortes, 2004).

2.1.5 Diagnosis

In our laboratory, the following methods have been frequently employed for routine diagnosis of helminth infections: Willis-Mollay floating technique, using saturated sodium chloride solution of 1.182 density (Willis, 1921); Spontaneous Sedimentation in water (Lutz, 1919; Hoffmann et al., 1934); and Direct Examination. The first technique has as basic principle the fecal floating of nematode and protozoan eggs which are less dense, whereas sedimentation is more indicated to recover heavy eggs like those of trematodes and some cestodes; Direct Examination, however, is only recommended to verify barely floating structures since its sensitivity is low (Sloss et. al., 1999).

We carried out a study aimed at analyzing the efficiency of four coproparasitological techniques used in the laboratorial routine to diagnose helminth eggs and protozoan cysts or oocysts in dogs. Association of the methods Willis-Mollay and Sedimentation is considered superior, compared to the Direct method and zinc sulfate flotation centrifugation, to detect these evolutionary and parasitic forms (Táparo et al., 2006).

To elucidate clinical suspicions, either in tests of anthelmintic efficacy or in comparisons between diagnosis techniques for helminth infections, parasitological necropsy is considered the gold standard test, in which the stomach and small and large intestine content is washed in running water, sieved, fixed in 10% buffered formalin and stored in properly labeled flasks. Helminths are obtained by using a stereoscope microscope and the species are identified after clarifying the parasites with 80% acetic acid (Ogassawara et al., 1986).

As a future study, our group intends to standardize the *TF-Test*[®] kit (Three FecalTest) as the automated standard method to diagnose enteroparasites in dogs (*Canis familiaris*). A data bank will be prepared with images of eggs, cysts, oocysts and trophozoites of the main enteroparasites found and their structures. The parasitological technique *TF-Test*[®] will be restandardized and associated with a device containing optical microscope, digital camera and motorized platinum to obtain images. Computational techniques of image segmentation and classification of patterns will be employed with the aim of detecting and classifying the parasites found, determining the genus and species of helminths and protozoa, according to their signature or constant identification in the software. The results obtained with the coproparasitological techniques will be compared through statistical analysis, assessing the positivity of animals as to occurrence of enteroparasites and efficiency of techniques. This method will allow the automation of parasitological diagnosis in fecal samples from pets in a rapid and practical way with high efficacy, reducing pre-analytical, analytical and post-analytical errors.

2.1.6 Control

Important therapeutic and control measures to be adopted are addressed in the study of Heukelbach & Feldmeier. Based on the high occurrence of hookworm in dogs and cats in our studies, treatment with anthelmintics is needed, even for animals with negative stool tests, besides adopting a control of the number of animals in public places in order to decrease the likelihood of environmental contamination, since this parasite represents a potential hazard to human and animal health.

2.2 *Leishmania* spp.

2.2.1 Epidemiology

Leishmaniasis are enzootic and zoonotic diseases caused by protozoa, morphologically similar, of the genus *Leishmania* (Monteiro et al., 2005). The visceral form has in its etiology *Leishmanias* of the *donovani* complex, including *Leishmania (Leishmania) donovani* in Asia and Africa, *Leishmania (Leishmania) infantum* in Asia, Europe and Africa, and *Leishmania (Leishmania) chagasi* in the Americas (Laison & Shaw, 1987).

This disease is predominantly of places showing low socioeconomic level and promiscuity, and environmental changes have led to its urbanization (Brasil, 2006). In dogs, as well as in humans, prevalence rates are high, reaching 1 to 36%, which varies according to the region of the country (Silva et al., 2001). Among mammals, the fox is the main reservoir in wild and rural environments while the dog is the reservoir in urban sites (São Paulo, 2006).

In Brazil, insects like *Lutzomia longipalpis* and *Lutzomyia cruzi* are identified as vectors related to leishmaniasis transmission (Brasil, 2006).

Araçatuba Municipality, SP, Brazil, our study site, is in an endemic region for canine visceral leishmaniasis, and the data obtained by the Superintendence for Endemic Disease Control (SUCEN) at that locality indicated that 3227 animals were infected from 2006 to 2010. At the Secretariat of Health of that State, a total of 1512 human cases and 138 deaths were recorded from 1999 to 2009, and in 2008, 291 people were diagnosed to have this disease, of which 23 died (Bepa, 2010).

2.2.2 Biology

In studies carried out by Lainson & Shaw, 1987, the vector became infected by ingesting, during the blood meal, amastigote forms of the parasite present in the cells of the monocyte phagocytic system in the dermis of the infected host. In the digestive tube of the insect, amastigotes are transformed into promastigotes, which multiply after three to four days of the first meal. During a new meal, the female phlebotomine inoculates these infective forms into the definitive host and they are phagocytized by macrophages, returning to the amastigote form, when they multiply causing cell rupture. Thus, there is hematogenic dissemination to tissues such as liver, spleen, lymph nodes and bone marrow (Lainson & Shaw, 1987).

2.2.3 Diagnosis

The Brazilian Ministry of Health recommends ELISA as the serological screening technique and IFA as the confirmatory test for this disease, as well as euthanasia for seropositive animals, with or without symptoms (BRASIL, 2006).

ELISA (Enzyme Linked Immunosorbent Assay) represents a simple and rapid method for the survey of this canine infection (Lima et al., 2005), allowing the processing of a considerable number of samples in a short time interval (Maia & Campino, 2008).

To diagnose these diseases, serological assays of high sensitivity and specificity are used; however, infected dogs may be seronegative, and seropositive animals may not have the disease. This occurs due to the phylogenetic proximity between *Leishmania* spp. and other hematozoa, especially *Trypanosoma cruzi* (*T. cruzi*), classified into the same family Tripanosomatidae, which favors the occurrence of cross reactions in serology. In Latin countries in particular, we commonly find areas where there was the overlap of leishmaniasis and trypanosomiasis both in humans and in dogs (Troncarelli, 2008). In cases of evident cross reactions, we must consider the serological result, as well as the clinical and epidemiological factors, and carry out another diagnosis method to confirm the disease (Luciano et al., 2009).

With the development of PCR (polymerase chain reaction), a more specific and sensitive methodology, the parasite kinetoplast DNA can be identified and selectively amplified (Alves; Bevilacqua, 2004).

Considering the known phylogenetic similarity between *Leishmania* spp. and *Trypanosoma* spp. (Sundar and Rai, 2002), the consequent risk of false-positive results for these parasites (Zanette, 2006), the high occurrence of euthanized dogs due to visceral leishmaniasis in that endemic area (Nunes et al. 2008), and the absence of epidemiological surveys on canine trypanosomiasis in that region, our study was designed with the aim of detecting cross

infections by these two protozoa in Araçatuba Municipality, SP. The obtained results evidence cross reactions by both protozoa in the animals analyzed in this study (Viol, 2011).

2.2.4 Clinical signs

In men, the incubation period varies from ten days to 24 months, and the average is from two to six months. In dogs, the range is wider, between three months and several years, but generally between three and seven months (Brasil, 2006).

Some dogs show few symptoms, rare cutaneous lesions and nodules, while others manifest cachexia, cutaneous changes, peeling, nodules and ulcers, especially in the ear edges or spread all over the body. Cases of conjunctivitis, blepharitis, muzzle swelling, onychogryphosis and paresis of the hind feet have been reported, including splenomegaly, lymphadenopathy, diarrhea and intestinal hemorrhage in advanced stages of the disease (Silva et al., 2001).

2.2.5 Control

Therapeutic protocols based on antimonials, diamidines, aminoglycoside antibiotics, imidazole byproducts and purine analogues are available in the studied literature.

The treatment of canine visceral leishmaniasis, for unknown reasons, is more complicated than that of humans and no medicine is totally efficient in eliminating this parasitic infection, remaining the risk of recurrence after therapy. In endemic regions, a drug protocol consists in the association between allopurinol and pentavalent antimonial like meglumine. Cases of parasite resistance and side effects have been described (Lindsay et al, 2002).

The drugs employed in the treatment of CVL are of uncertain efficacy, and the development of a vaccine has been one of WHO's main strategic alternatives (Da Silva et al., 2001).

Several immunogens have been tested, including live or inactivated vaccines, purified fractions of *Leishmania*, recombinant antigens, antigen expression and *Leishmania* plasmid DNA through the recombinant bacterium (Lima et al., 2010).

Thus, researchers are aware of the need of conducting studies aimed at standardizing, comparing and improving diagnosis methods for both zoonoses in order to facilitate the control in reservoirs and consequently in humans. For leishmaniasis in particular, surveillance programs should recommend a highly sensitive and specific method for the early diagnosis of dogs, preventing mistakes and euthanasia of healthy dogs or the maintenance of positive animals as a source of infection for humans (Troncarelli, 2008).

2.3 Infections by *Trypanosoma* spp.

2.3.1 Epidemiology

Chagas Disease is caused by the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). Several other trypanosomes parasitize animals and men, including *T. congolensis*, *T. vivax*, *T. equiperdum*, *T. brucei*, *T. evansi* and *T. rangeli* (Herrera, et al., 2005).

This disease, both in humans and in dogs, is concentrated in rural zones and poor areas of large urban centers, where the transmitting vector proliferates at a higher frequency due to the low social condition and consequently precarious living conditions (Dias et al., 2002).

Several mammals are considered important *T. cruzi* reservoirs; in the domestic environment, however, the dog is highlighted due to the vector's feeding preference for this species (Crisante et al., 2006) and due to its close contact with men, showing great epidemiological and public health importance (Eloy & Lucheis, 2009).

2.3.2 Biology

Vectors, in any stage of their life, become infected by ingesting trypomastigote forms present in the bloodstream of the infected vertebrate host during blood feeding. These forms, which multiply in the intestine of the "barber", are eliminated in their feces during or soon after blood meal (Wanderley, 1994).

2.3.3 Clinical signs

Infected dogs develop the acute or chronic form of the disease. The acute phase, symptomatic or not, lasts about two months, evolving to a chronic phase for the rest of the patient's life. The acute phase affects young dogs aged between five and six months, where generalized infections develop, with lesions in the myocardium and central nervous system, besides anorexia, lymphadenopathy, diarrhea, myocarditis and finally death from cardiac arrhythmia. The chronic phase, after eight to 36 months of the initial infection, is characterized by ventricular arrhythmia and myocardial dilatation. Signs of cardiac failure appear first in the right side of the heart, progressing to biventricular failure (Ettinger & Feldman, 1997).

2.3.4 Diagnosis

The diagnosis of trypanosomiasis has been made by the parasite survey in blood smear, xenodiagnosis, blood culture, samples from bone marrow puncture and lymph nodes (Lucheis, et al., 2005), besides indirect assays with antibody survey. The employed serological methods are ELISA, HAI, and IFA. Cross reaction between *T. cruzi* and *Leishmania* spp. in serological tests has been frequent due to the phylogenetic proximity between these parasites (Troncarelli, 2008, Viol et al., 2011).

Thus, a more accurate diagnosis requires the use of complementary techniques like direct parasitological test and polymerase chain reaction (PCR). The latter allows amplifying DNA sequences of the parasite, consisting in a highly reliable diagnosis method with sensitivity and specificity of almost 100% (Ashford et al., 1995).

2.3.5 Control

Currently, there is no available medicine capable of eliminating *T. cruzi* infection and promoting definitive healing of all treated patients. In addition, there are regional differences due to different parasite strains. Chemotherapy of Chagas Disease remains a challenge. Only two nitroheterocyclic drugs are currently used, Nifurtimox and Benzonidazole, which are active against the blood forms of the parasite, as well as against the tissue forms once administered continuously for an ideal period of 60 days (DIAS et al., 2009).

Treatment of Chagas infection in dogs and cats is still complex since most studies aim to evaluate the canine species as experimental model for therapy in humans. Furthermore, the available drugs do not promote definitive parasitological healing and the side effects are still controversial since therapy is prolonged.

Thus, prevention of Chagas Disease is needed and prophylactic measures include covering cracks, using screens on doors and windows, avoiding the permanence of animals and birds inside the house, avoiding debris, removing the nest of birds from the house eaves, and sending suspect “barbers” to the health service (São Paulo, 2011).

2.4 Infections by *Cryptosporidium*

2.4.1 Epidemiology

The genus *Cryptosporidium* is classified into the Phylum Apicomplexa and the Family Cryptosporidiidae. Genetic analyses allowed the description, according to the host, of 20 *Cryptosporidium* species, of which 12 were in mammals; in addition, 61 *Cryptosporidium* genotypes were determined (Plutzer & Karanis, 2009; Fayer, 2010).

Although there is a report of *C. muris* in dogs (Lupo et al., 2008), the latter are generally infected by *C. canis* (Abe et al., 2002; Thomaz et al., 2007) and *C. parvum* (Xiao et al., 1999). Humans are more frequently infected by *C. parvum* and *C. hominis*. Although *C. felis*, *C. canis*, *C. meleagridis* and *C. muris* were isolated from people, the risk of human infection by direct or indirect contact with pets has not been determined yet (Fayer et al., 2001; Smith et al., 2009).

The occurrence of *Cryptosporidium* in dogs was low in our study (Bresciani et al., 2008), which corroborates other epidemiological surveys in Brazil (Mundim et al., 2007; Huber et al., 2005; Lallo & Bondan, 2006). In the world, there are reports from absence (Fayer et al., 2001) to rates of 44.1% (Hammes et al., 2007). Most authors indicate higher occurrence in pups, which is associated with weaning, nutritional deficits and overcrowding conditions in kennels and/or catteries (Thompson et al., 2008).

Oocysts shed by dogs may represent a source of human infection. According to some authors, however, this animal species does not constitute important risk in terms of public health (Thompson et al., 2008; Tzipori & Griffiths, 1998; Smith et al., 2009).

In an attempt to elucidate this issue, our current study included a total of 188 children from different villages of Andradina Municipality, SP, aged between zero and twelve years, as well as their respective pets, a total of 134 dogs and 54 cats, which were examined by using Enzyme-Linked Immunosorbent Assay (ELISA) with the *Cryptosporidium* test Kit TechLab®. To assess the degree of contact between the child and the respective pet, a questionnaire was applied to the 188 interviewees responsible for the children. Thus, 55.9% (104/186) parents confirmed that the animals lick the face of their children, 73.6% (137/186) are used to entering the bedroom, 11.2% (21/186) jump and/or sleep on the bed with the children, only 7.5% (14/186) children wash their hands after having played with the animal, 16.6% (9/54) cats jump onto the kitchen sink and 54.8% children (102/186) play with sand at home. Of the four children positive for *Cryptosporidium* spp. according to ELISA, three had their animals reactive (Coelho, 2009). The next step is to perform the molecular characterization of positive samples through ELISA for children and their respective pets.

2.4.2 Physiopathogenesis

Infection by *Cryptosporidium* causes atrophy, fusion and inflammation on the surface of intestinal microvilli, which result in absorptive surface loss and unbalanced nutrient transport. Diarrhea due to poor absorption is caused by epithelial barrier rupture and immunological and inflammatory responses by the host (Thompson et al., 2008).

2.4.3 Biology

Similarly to other parasites of vertebrates, *Cryptosporidium* spp. has a monoxenic life cycle, which is completed mainly in the gastrointestinal tract of the host. This protozoan, however, has peculiar features that differentiate it from other coccidia, including intracellular but extracytoplasmic location on the cell membrane surface of the infected host since it has a feeding organelle responsible for nutrition; in addition, it is included in a parasitophorous vacuole and has auto-infection capacity (Tzipori & Griffiths, 1998).

As to its biology, sporulated oocysts of *Cryptosporidium* are ingested by the host and, following exposure to the gastric juice and pancreatic enzymes, excystation occurs in the duodenum releasing four sporozoites. The latter are covered by microvilli located in a parasitophorous vacuole and start the asexual reproduction. They develop successive merogony generations, releasing eight and four sporozoites, respectively. The four merozoites released from the second merogony originate the sexual stages, microgametes and macrogametes, which unite to originate the zygote that, after two asexual divisions, forms the oocyst. Sporulation occurs inside the oocyst, developing four sporozoites. Thus, oocysts of thin (capable of starting a new cycle inside the same host) and thick wall (highly resistant under environmental conditions and released in the feces) develop. The infection generally remains in the gastrointestinal tract (Tzipori & Griffiths, 1998; Thompson et al., 2008).

2.4.4 Clinical manifestations

Absence of symptoms and release of few fecal oocysts can be more frequently found in dogs. In humans, infection by *Cryptosporidium* is generally subclinical in communities from endemic areas or causes auto-limiting diarrhea with abdominal pain and vomits (Thompson et al., 2008).

2.4.5 Diagnosis

The microscopic diagnosis of cryptosporidiosis requires time and experience of the observer since oocysts are hardly visualized, have small dimensions and do not contain sporocysts. In our laboratorial diagnosis routine, these evolutionary forms can be observed mainly by means of techniques that stain the oocysts, such as Malachite green, Ziehl-Nielsen and Kinyoun.

Intermittent excretion of *Cryptosporidium* oocysts is proven in small animals; thus, repeating the coproparasitological test is indicated, including new sample collection, even after a negative result (Huber et al, 2005).

In a study performed by our team, the parasitological tests of Kinyoun and Sheather were compared with ELISA and the latter was more sensitive in detecting infection by *Cryptosporidium* spp. in dogs (Bresciani et al., 2008).

The distinction between *Cryptosporidium* species and genotypes has been conclusive only by molecular characterization. Thus, continuing our study, Polymerase chain reaction (PCR) will be performed, including genotypic identification, which can be followed by genetic characterization with restriction fragment length polymorphism (RFLP), using the restriction enzyme *RsaI* of a DNA fragment amplified from the gene that codifies *Cryptosporidium* oocyst wall protein (COWP) and/or the sequencing involving the genes codifying 18S rRNA, actin, HSP-70 and GP-60. The latter has shown a high degree of polymorphisms between isolates from *Cryptosporidium* species with identification of several subgenotypes and subtypes. In spite of scientific advances, these cytogenetic analyses are costly and thus maintain the interest in searching for diagnostic methods that can be performed by veterinarians to confirm the clinical suspect and to adopt immediate therapeutic measures (Plutzer & Karanis, 2009).

2.4.6 Treatment

The indicated therapy was rehydration and electrolyte replacement during the initial stages of the infection, before the expression of the host immune response (Thompson et al., 2008). The first drug approved for the treatment of cryptosporidiosis in immunodeficient children and adults, nitazoxanide and its two metabolites, minimized diarrhea and oocyst release (Rossignol., 2010).

2.5 Infections by *Toxoplasma gondii*

2.5.1 Epidemiology

Toxoplasma gondii is a coccidian protozoan, obligate intracellular parasite, which affects almost all warm-blooded animal species (Dubey & Beattie, 1988).

Our research group verified association between dogs positive for *T. gondii* and raised on the ground or lawn ($p < 0.001$), compared to those kept in cemented environment (Bresciani et al., 2007).

As a disease of high seroprevalence, toxoplasmosis has higher incidence with age and is more common among stray dogs (Souza et al., 2003, Cánon-Franco et al., 2004).

Two important data obtained in our studies must be highlighted, with special emphasis on their zoonotic aspect: 1) after infection, we isolated the parasite by means of bioassay from saliva, milk and urine from the animals (Bresciani et al., 2001); 2) we noted that pups from those mothers were born serologically positive, infected, with antibody titers from 1:64 to 1:256, but apparently healthy (Bresciani et al., 2009).

Ingestion of tissue cysts, ingestion of oocysts from the feces of infected cats and congenital infection are the three main forms of *T. gondii* transmission. Other less important forms include ingestion of contaminated milk, transference of organic fluids and organ transplantation (Dubey, 1986; Dubey et al., 1990; Powell et al., 2001; Miró et al., 2004).

Our research group noted that *T. gondii* oocysts are widely distributed on the soil of elementary public schools in our region, likely constituting the main contamination source for these children (Santos et al., 2010).

Silva et al., 2010, showed that toxoplasmosis is related to problems of sanitary education, mainly concerning the appropriate cooking of foods, and was considered a public health problem.

2.5.2 Biology

In its cycle, the protozoan shows as two evolutionary forms in the intermediate host: tachyzoites, structures of rapid multiplication present in organic fluids in the acute phase, and bradyzoites, confined in tissue cysts, especially in the central nervous system and muscles, in the chronic infection. Oocysts, the final product of sexual reproduction, are formed only in the digestive tract of felids, definitive hosts which shed the oocysts with their feces, where by means of sporogony they become infective and extremely resistant to environmental conditions (Miller & Frenkel, 1972).

In more detail, the cycle starts with felids ingesting cysts present in the meat. The cyst wall is dissolved by proteolytic enzymes of the stomach and small intestine, the parasite is released from the cyst and penetrates into the enterocytes (intestinal mucosa cells) of the animal, asexually replicating and originating several generations of *Toxoplasma* through asexual reproduction. After five days of this infection, the sexual reproduction process starts and the merozoites formed in the asexual reproduction originate the gametes. The male (microgamete) and female (macrogamete) gametes, originated from one same or two different parasites, join to form the egg or zygote which, after segregating the cyst wall, originates the oocyst.

2.5.3 Clinical signs

In experimental inoculations, some animals keep asymptomatic, few become ill and deaths are rare (Dubey, 1985). However, there are reports of pulmonary and digestive disorders (Oppermann, 1971), hyperthermia, lymphadenopathy (Bresciani et al., 2001), ocular lesions (Abreu et al., 2002), and loss of consciousness and movement (Brito et al., 2002).

2.5.4 Pathogenesis

The multiplication of tachyzoites will lead the host cell to death, in addition to coagulation necrosis (cream to yellow, in the form of necrosis points of 1 to 2mm diameter) in organs like spleen, liver and lungs of the intermediate host.

2.5.5 Diagnosis

Detection of antibodies against *T. gondii* by indirect immunofluorescence reaction, as well as by modified agglutination test, hemagglutination test and ELISA, is recommended for the epidemiological survey of this zoonosis; however, serology is not always the best form to prove this disease (Zhang et al., 2001) since the bioassay represents an ideal tool to detect this parasite (Abreu et al., 2002). The histopathological test associated with the immunohistochemistry technique, and both in association with PCR, have been widely used in the diagnosis of this infection (Schatzberg et al., 2003, Bresciani et al., 2009).

Studies of the genetic diversity of a parasite have contributed to the evaluation of biological features such as virulence, resistance to drugs and immunological diversity, which can be

correlated to the epidemiological tracing of the agent in order to identify infection sources or transmission routes. At the same time, they can generate knowledge to improve diagnosis, treatment and control, besides valuable information to investigate genotypes from infections in animals (Tenter et al., 2000).

Molecular and isoenzymatic studies have shown that *T. gondii* has a highly clonal population structure, although it has the opportunity to genetically recombine in a highly defined sexual cycle in definitive hosts. A consequence of this clonality would be the presence of features related to pathogenicity (Sibley et al., 1995). *T. gondii* would consist thus in three predominant clonal strains, named types I, II and III, globally occurring in animals and men (Dardé et al., 1992; Howe, Sibley, 1995).

The mouse (*Mus musculus*) has been preferably used as experimental model of toxoplasmosis. Most virulent samples for mice are known to belong to type I, while almost all non-virulent isolates are included in type II or III (Howe, Sibley, 1995). Type-II genotype is associated with the reactivation of chronic infections in AIDS patients. These researchers also noted that type-I samples were more related to congenital toxoplasmosis in people and genotype III was preponderant in animals (Howe & Sibley, 1995). Nevertheless, this correspondence between virulence and the sample molecular standard may not be necessarily observed in all hosts (Grigg & Suzuki, 2003).

2.5.6 Control

Other drugs such as pyrimethamine, trimethoprim + sulfonamide and doxycycline (Quadro 2), doxycycline, azithromycin, minocycline and clarithromycin can be employed (Lappin, 2004). Clindamycin hydrochloride can be used at the dosage of 3 to 20 mg / Kg (Quadro 2) and also at 12.5 to 25 mg / Kg, orally, at every 12h for one to two weeks to shorten the oocyst elimination time. The clinical signs of toxoplasmosis resolve within two to four days with the administration of this drug (Greene, 1990; Lppin, 2004). *T. gondii* enteroepithelial stage does not occur in dogs, which become infected by ingesting sporulated oocysts or tissue cysts. Thus, coprophagy must be prevented, while the supply of cooked meat products or commercial food is recommended.

Paratenic hosts such as flies and cockroaches must be eliminated, and the contact with soil or sand possibly contaminated with cat feces must be avoided, paying special attention to the daily cleaning of the feline sandbox in order to reduce environmental contamination (Greene, 1990, Navarro, 2001; Lappin, 2004); in addition, in this context, the birth of stray animals must be controlled, which may contribute to reducing the occurrence of this important zoonosis (Jittapalapong et al., 2007).

3. Final considerations

Considering the importance of the instruction and information level of teachers from municipal early childhood education centers (EMEIS), the high occurrence of endoparasites in humans and the endemic situation of canine visceral leishmaniasis in Araçatuba Region, we investigated the knowledge degree of those teachers concerning parasitic zoonoses. Thirty EMEIS from Araçatuba were visited and 85 teachers were interviewed. Descriptive statistical analysis indicated that 96.47% (82 out of 85) teachers answered that walking barefoot may interfere in helminth infection, while 85.88% (73/85) answered that nail biting

may interfere in it. We verified that 44.71% (38/85) of them did not know the pathogenesis of helminth diseases and 63.53% (54/85) did not administer anthelmintics to small animals. The participation of cats in toxoplasmosis transmission was known by 92.94% (79/85), of which 82.35% (70/85) did not know the transmission routes. The dog was considered the disseminator of this disease by 80.00% (68/85) interviewees, and only 4.71% (4/85) cited ingestion of meat products as a route of *Toxoplasma gondii* transmission, while 67.06% (57/85) did not know about this issue. As to leishmaniasis, 91.76% (78/85) stated that dogs are the transmitters, but 58.82% (50/85) did not know how and 60.00% (51/85) recommended environmental cleaning as exclusive preventive measure. Based on the obtained data, we can infer that there is the need of implanting a community education program directed to the improvement of basic concepts of control and prevention of parasitic zoonoses (Tome et al., 2005).

Our team interviewed people in the Third Age about basic concepts related to parasitoses. The obtained results proved the need of promoting elucidation campaigns directed to the aged, addressing the control of these diseases (Lima et al. 2008).

More recently, we have carried out a study with the aim of guiding owners about concepts related to the responsible ownership of their pets in an endemic area for canine visceral leishmaniasis. Questionnaires about responsible ownership and control of this disease were applied to the owners of dogs and cats living in Araçatuba Municipality, SP, initially to assess the knowledge degree of these people. Then, based on the deficits verified in their responses, the authors of this study individually instructed the owners of these animals at their houses about the critical points to be reformulated in order to correct erroneous concepts concerning these issues. Thus, 25% (22/88) individuals had already had dogs seropositive for Canine Visceral leishmaniasis in their houses and of these, 54.55% (12/22) sent their animal to veterinarian clinics for euthanasia, 22.73% (5/22) used the services of the Municipal Zoonosis Control Center and 18.18% (4/22) paid for private treatment. However, 35.63% (31/87) dogs were never subjected to exams for the diagnosis of infection by *Leishmania* spp. (MATOS et al., 2011).

These studies indicated misinformation of owners regarding the control of canine endoparasitoses, evidencing the need of continuous implantation of campaigns for community concern.

4. References

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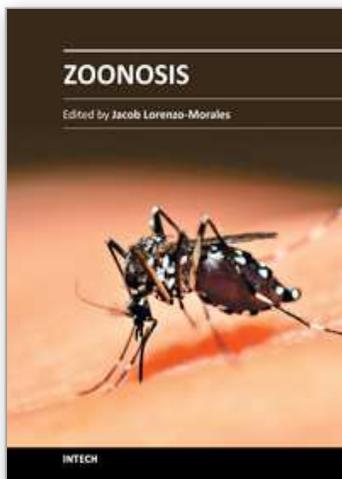
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Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

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