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Chapter IV

Important Parasitic Zoonosis in Dogs

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Abstract

The dog can be involved in the mechanical transmission of infection by helminthes and protozoa; it has an epidemiological role in these zoonoses. The high rate of dogs naturally infected by endoparasites and the great possibility of acquiring this parasite justify the importance of attempting to prevent the occurrence of this illness. The aim of this paper is to demonstrate, through literature review, some clinical, epidemic and pathological aspects of endoparasite infection in the canine species.

Introduction

Dogs play a relevant role as definitive hosts of a large number of endoparasites, shedding gastrointestinal helminth larvae and eggs and protozoan cysts and oocysts in their feces, which favors environmental contamination and possible spread of diseases.

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

Protozoal Infections

Toxoplasmosis

Infection by *Toxoplasma gondii* is particularly interesting from the point of view of public health. Although generally asymptomatic, it frequently shows severe clinical signs of reactivation or fatal outcomes in immunosuppressed patients. Behavioral changes such as attention deficit, schizophrenia, obsessive compulsive disorder, epilepsy and hyperactivity have been reported (Lafferty, 2005, Pradhan et al., 2007, Prandota, 2010).

Women in initial stages of gestation may undergo miscarriage, premature birth, neonatal death and even the classical Sabin's Triad, characterized by retinochoroiditis, cerebral calcifications, hydrocephaly or microcephaly (Jones et al., 2001, Soares et al., 2011).

Surveys of toxoplasmosis are essential for production animals and pets. Reproductive disorders were already proved in several species, including men and dogs, emphasizing the relevance of studies of congenital toxoplasmosis and its reflexes (Bresciani et al., 1999, Bresciani et al., 2001, Bresciani et al., 2009, Azevedo et al., 2010).

In its cycle, *T. gondii* shows itself as two evolutionary forms: tachyzoites, present in organic liquid in the acute phase, and tissue cysts in the chronic infection. On the other hand, oocysts are the final product of sexual reproduction and are only formed in the digestive tract of felids, definitive hosts which release oocysts together with their feces, where, through sporogony, the oocysts become infective and extremely resistant to the environmental conditions (Miller et al., 1972).

The protozoan can be transmitted especially by ingestion of tissue cysts present in raw or undercooked meat, by contamination of food with oocysts from the feces of infected felids and by transplacental route. Other less important forms are ingestion of contaminated milk, organic fluid transference and organ transplantation (Dubey, 2009).

Some dogs remain asymptomatic, a few become sick and deaths are rare (Dubey, 1985). However, pulmonary and digestive disorders (Oppermann, 1971), hyperthermia, lymphadenopathy (Bresciani et al., 2001), ocular lesions (Abreu et al., 2002) and neuropathies (Brito et al., 2002) may occur.

Detection of antibodies against *T. gondii* can be done by indirect immunofluorescence reaction, as well as by modified agglutination test, hemagglutination test and Enzyme Linked Immunosorbent Assay (ELISA).

We observed miscarriage, fetal and neonatal mortality in pregnant dogs and detected the parasite in saliva, milk and urine of infected mothers of serologically positive but apparently healthy pups (Bresciani et al., 2009). It is also important to note that we further confirmed sexual transmission (Arantes et al., 2009).

Toxoplasmosis seroprevalence is high in the canine species and increases with age, especially in stray ones (Souza et al., 2003, Cañón-Franco et al., 2004), which contribute to the mechanical spread of this disease (Frenkel and Parker, 1996; Lindsay et al.,1997, Schares et al., 2005).

Some drugs such as pyrimethamine, trimethoprim associated with sulfonamide and doxycycline, doxycycline, azithromycin, minocycline, clarithromycin and clindamycin can be employed (Greene, 1990; Lappin, 2004).

Thus, coprophagy must be prevented, while the supply of cooked meat products or commercial food is recommended. We verified higher incidence of infection by *T. gondii* among dogs kept on earth or grass ground (Bresciani et al., 2007) and isolated oocysts of this parasite from sand from parks in public schools with potential risk of contamination for children (Santos et al., 2010).

Canine Visceral Leishmaniosis or Calazar

Showing cosmopolitan distribution, enzootic infections caused by morphologically similar protozoa of the genus *Leishmania* (Monteiro et al., 2005, Lindoso and Goto, 2006) can be transmitted by sand flies (Brasil, 2006).

Leishmaniasis is considered by the World Health Organization (WHO) the second most important current protozoal infection in people. Human patients

subjected to chemotherapy are also more susceptible and, without treatment, the disease is lethal in 95% cases (Brasil, 2006). Estimates indicate that 16 to 18 millions are affected in the world (Rosypal et al., 2007).

In humans, the signs of infection by *Leishmania* spp. are generally auto-limiting and subclinical and may progress to fever, cachexia, hepatosplenomegaly, copious bleeding and even death.

The sand fly(like *Lutzomyia* spp.) becomes infected while ingesting, during blood feeding, amastigote forms of the parasite, present in the cells of the monocytic phagocyte system on the dermis of the infected host. In the digestive tube of the insect, amastigotes change into promastigotes. During a new blood feeding, the female of the infected insect inoculate these infective forms into the definitive host where they undergo phagocytosis by macrophages, returning to amastigote form and multiplying to cause cell rupture. Thus, there is hematogenic dissemination to tissues like liver, spleen, lymph node and bone marrow (Lainson and Shaw, 1987).

Some dogs manifest a few clinical signs such as rare cutaneous lesions and nodules, while others become cachectic, showing ophthalmologic changes, onychogryphosis, and dermatopathies like peeling, nodules and ulcers, especially in the edges of ears or spread all over the body. Cases of paresis of the hind limbs have been described, as well as increased volume of the spleen and lymph nodes, including diarrhea (Silva et al., 2001).

We studied cross infections by *Leishmania* spp. and *Trypanossoma* spp. using Indirect Immunoenzyme Assay (ELISA), Indirect Fluorescent Antibody Test (IFAT) and Polymerase Chain Reaction (PCR) and we observed cross infections by both protozoa in the animals analyzed in this study.

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.

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).

The fox is the main reservoir of this disease in the wild and rural environment, while the dog plays this role in the urban environment (São Paulo, 2006). The Brazilian Ministry of Health recommends euthanasia for affected animals (BRASIL, 2006, Troncarelli, 2008).

This reemerging illness has characterized an epidemiological transition process, with increasing incidence and geographic expansion in urban areas previously free from this problem (Alves and Bevilacqua, 2004).

This canine disease, has preceded the occurrence of human cases (Paranhos-Silva et al., 1996) and is associated with environments of low socio-economic level (Alves and Bevilacqua, 2004, BrasiL, 2006), showing high seroprevalence and lethality in endemic areas (Silva et al., 2001, Bepa, 2010).

Cryptosporidiosis

By means of molecular characterization, 20 species are established for the genus *Cryptosporidium* (Plutzer and Karanis, 2009, Fayer, 2010); dogs are commonly infected by *C. parvum* (Xiao et al., 1999) and *C. canis* (Thomaz et al., 2007), while humans are frequently affected by *C. parvum* and *C. hominis* (Fayer et al., 2001, Smith et al., 2009).

Cryptosporidium protozoa are included in the Neglected Diseases Initiative of the WHO due to their close relationship with deficient sanitation and low income of the population; thus, they are considered responsible for child malnutrition, organic deficit and death. For immunocompetent ones, the infection does not manifest clinically in communities from endemic areas or causes auto-limiting diarrhea with abdominal pain and vomits (Thompson et al., 2008).

In its biology, *Cryptosporidium* sporulated oocysts 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 involved by microvilli and are located in a parasitophorous vacuole, starting the asexual reproduction (merogony). The four merozoites released in the second merogony originate the sexual stages, microgametes and macrogametes; the latter unit to form the zygote, which after two divisions forms the oocyst. Sporulation occurs inside the oocyst, developing four sporozoites. Thus, thin-walled oocysts (capable of starting a new cycle inside the same host) and thick-walled oocysts are formed and released to the outer environment together with the fecal content. The infection generally remains in the gastrointestinal tract (Tzipori and Griffths, 1998, Thompson et al., 2008).

Diarrhea due to malabsorption is caused by epithelial barrier rupture and immunological and inflammatory responses of the host. Infection by *Cryptosporidium* causes atrophy, fusion and inflammation on the surface of

intestinal microvilli, which result in absorptive surface loss and unbalanced nutrient transport (Thompson et al., 2008).

Asymptomatic signs, with release of a few fecal oocysts, can be commonly detected in dogs (Thompson et al., 2008). All over the world, variable prevalence of this infection (from complete absence to rates of 44.1%) has been reported, showing higher occurrence in the young and relationship to weaning, nutritional deficiencies and agglomeration in kennels (Hammes et al., 2007, Mundim et al., 2007, Lallo and Bondan, 2006, Bresciani et al., 2008).

We observed these evolutionary forms by techniques that stain the oocysts, such as Malachite green, Ziehl-Nielsen and Kinyoun. 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 *Cryptoporidium* species and genotypes has been conclusive only by molecular characterization. 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 and Karanis, 2009).

Nitazoxanide minimized diarrhea and oocyst release and is the first drug approved for the treatment of cryptosporidiosis (Rossignol., 2010).

Considering the disease control, our research group interviewed 188 guardians of children with the aim of verifying the degree of contact between the child and respective pet.

A total of 55.9% (104/186) parents confirmed that the animals lick the face of their children, 73.6% (137/186) pets enter the bedroom, 11.2% (21/186) pets 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, 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).

Although the risk of infection for men by direct or indirect contact with small animals is not determined (Fayer et al., 2001, Smith et al., 2009), the fecal oocysts released by dogs may represent a source of human infection and are rarely resistant to the environmental conditions (Plutzer and Karanis, 2009)

Helminthiasis

Visceral Larva Migrans Syndrome (Beaver et al., 1952) can be caused by *Toxocara canis* and Cutaneous Larva Migrans Syndrome by *A. caninum* or *A. braziliense* (Lee, 1874) in humans.

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), since evolutionary forms of helminthes released in the fecal content of dogs lead to high environmental contamination (Brener et al., 2005, Pfukenyi et al., 2010).

Visceral Larva Migrans (VLM)

VLM is a cosmopolitan anthropozoonosis. Serological surveys have confirmed the dissemination of *T. canis* among the human population (Chieffi and Müller, 1976). The contact with the soil, fomites or hands contaminated by the feces of animals infected with these agents may lead to accidental transmission to people (Blazius, 2006).

Human infection by *T. canis* occurs by the ingestion of larval eggs present in the soil or hands contaminated with the feces of infected animals. The parasite does not reach maturity in these hosts, but the larvae remain alive, erratically migrating in internal organs and producing a pathology named Visceral Larva Migrans (VLM) or invading the ocular globe and causing Ocular Larva Migrans (OLM).

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

The enterohepatic pneumoenteral form is considered the most common infection form in dogs aged up to three months. Above such age range, this migration type occurred at a lower frequency. In pregnant dogs, migrating larvae mobilize at approximately three weeks before parturition and migrate to the fetal lungs, molting to third-instar larvae before birth. In the newborn pup, the cycle is completed when the larva migrates through the trachea and to the intestinal lumen, where molts to adult worms finally occur (Urquhart, 1991).

As regards pathogenic lesions caused by gastrointestinal parasites, we must consider cutaneous, pulmonary (by pulmonary migration of the larva

during its development) and intestinal changes due to the final location of the worm in the adult stage (Fortes, 2004).

In dogs, these helminthiases may lead to organic unbalance such as anemia, changes in the appetite, intestinal obstruction or perforation, limited assimilation of nutrients, diarrhea, apathy and sometimes death (Fortes, 2004).

We found high occurrence of *T. canis* (67.3%) in young dogs. Infections by this helminth are more frequent until the age of one year (Gennari et al., 2001). Although adults tend to show effective immune response against ascarids, post-parturition females can release eggs of *Toxocara* spp. in their fecal content (Urquhart et al. 1991).

The latter are known for their resistance to environmental adversities, remaining viable for many years depending on the type of climate and soil (Glickman and Schantz, 1981). In the laboratorial routine, we examine fecal samples from dogs in their first year of life containing a marked quantity of *T. canis* eggs.

Cutaneous Larva Migrans (CLM)

CLM has worldwide importance and is characterized by a parasitic dermatitis caused by the penetration and the migration of larvae of some species of Ancylostomatidae with zoonotic potential into the epithelial extract of the human skin (Broker et al, 2004, Vasconcellos et al., 2006).

Two parasites of small animals are the main etiologic agents of eosinophilic enteritis (Landmann and Prociv, 2003) and cutaneous larva migrans (Caumes, 2006): *Ancylostoma caninum*, with worldwide distribution, and *Ancylostoma braziliense*, especially evidenced in tropical and subtropical regions of the world (Hoff et al., 2008).

Reports of cutaneous larva migrans have been related to children in strict contact with sand from recreation areas, public squares, clubs and child day care centers (Santarém et al., 2004, Moro et al., 2008), as well as to adults who act in contact with the soil such as gardeners, farmers and miners (Hoff et al., 2008). It is common in poor regions of developing countries and sometimes described in places of high purchasing power like tourism areas which are endemic for this disease (Helkelbach and Jackson, 2008).

The man becomes infected by the percutaneous route and *Ancylostoma* larvae start the dermal migration, producing "inflammatory tunnels" which evidence the helminth path on the skin surface. These lesions are generally

seen in the regions of feet, legs and buttocks, which become particularly exposed in barefoot individuals or those sitting on the sand. Irritability, insomnia, intense skin pruritus and secondary bacterial infections also constitute frequent symptoms (Helkelbach et al., 2007, Hoff et al., 2008).

This hematophagous nematode of the small intestine of dogs causes acute or chronic hemorrhagic anemia followed by diarrhea which may contain blood and mucus. Its main form of transmission is by the translactational route. Young animals are most frequently affected, but this infection may occur in dogs of all ages by the percutaneous route or by the ingestion of larvae together with food and water (Urquhart et al., 1996, Boag et al, 2003; Blazius et al 2005, Táparo et al., 2006).

Ancylostomatidae can be identified by means of different parasitological techniques, reaching occurrence of 70% to 95.6% (Yacob et al., 2007, Coelho et al., 2011). We evidenced 64.2% (27/42) positivity for *Ancylostoma* in 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 (Coelho et al., 2011).

We observed that 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 (Taparo et al., 2006).

Parasitological necropsy is considered the gold standard test to elucidate clinical suspicions, either in tests of anthelmintic efficacy or in comparisons between diagnosis techniques for helminth infections (Ogassawara et al., 1986).

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.

Thus, with our scientific studies, we proved that the owners are greatly misinformed about the control of the parasitic diseases mentioned here and about responsible pet ownership, evidencing the need of continuous implantation of community awareness campaigns (Tome et al., 2005, Neto et al., 2011).

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