

## Some comments on the clinical neurological signs in a mongrel puppy during the suckling period - Case report\*

Larissa Licurci de Oliveira Barbosa<sup>1</sup>, Maria Isabel Menezes Ramos<sup>1</sup>, Paulo Daniel Sant'Anna Leal<sup>2\*</sup> and Carlos Wilson Gomes Lopes<sup>3</sup>

**ABSTRACT.** Barbosa L.L. deO., Ramos M.I.M., Leal P.D.S. & Lopes C.W.G. **Some comments on the clinical neurological signs in a mongrel puppy during the suckling period - Case report.** [Alguns comentários sobre os sinais clínicos neurológicos num cão lactante sem raça definida - Relato de caso]. *Revista Brasileira de Medicina Veterinária*, 38(Supl 3):103-108, 2016. Programa de Pós-Graduação de Ciências Veterinárias, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro. BR 465 km 7. *Campus Seropédica*, RJ 23.890-000, Brasil. E-mail: pauloleal@ctiveterinario.com.br

In the clinical routine of dogs, to perform an effective treatment of the morbid condition the diagnosis is fundamental and this objective is reached with observation of the evolution and in laboratory findings where the intercurrent infections have been observed frequently. We report a case of clinical laboratory diagnosis in *Toxocara canis* infection associated with the serological response to *Neospora caninum* in a lactating canine, with neurological and gastrointestinal clinical signs. In this way, the value of the clinical examination of feces in the serological diagnosis, demonstrating the need for greater attention to the diagnosis of multiple infections by different parasitic agents, allowing recovery of the patient after adequate treatment.

**KEY WORDS.** Neosporosis, toxocariasis, coccidia, concomitant infections, helminthes, zoonosis.

**RESUMO.** Na rotina clínica de cães, para se efetuar um tratamento eficaz da condição mórbida o diagnóstico é fundamental e este objetivo é alcançado com a observação da evolução e em achados laboratoriais onde as infecções intercorrentes têm sido observadas com frequência. Relata-se um caso de diagnóstico clínico laboratorial na infecção por *Toxocara canis* associado à resposta sorológica para *Neospora caninum* em canino filhote lactente, com sinais clínicos neurológicos e gastrintestinais. Comprovando assim, o valor do exame clínico, de fezes, no diagnóstico sorológico, demonstrando desta maneira, a necessidade de maior atenção para o diagnóstico de múltiplas infecções por agentes parasitários distintos, permitindo recuperação do paciente após tratamento adequado.

**PALAVRAS-CHAVE.** Neosporose, toxocaríase, coccídios, infecções concomitantes, helmintos, zoonose.

### INTRODUCTION

Several pathologies affect dogs, especially young dogs, care should be taken in the history, epidemiology, affections that are possible to affect the age group, as well as the races, being the diagnosis based on the clinical manifestations of the diseases, their interpretations Associated with the clinical examination and the difficulty of the various etiological agents that can cause disease in dogs, even concomitantly, which requires multiple diagnoses (Goldston & Hoskins 1999, Gough 2007, Leal et al. 2012a, Leal & Coelho 2014). The main causes of de-

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<sup>1</sup> Médica-veterinária. Programa de Pós-Graduação em Ciências Veterinárias. Anexo 1, Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ). BR 465 km 7. *Campus Seropédica*, 23.890-000, RJ. E-mails: mariaisabelmrm@gmail.com; larissalicurci@gmail.com - CNPq Scholarship.

<sup>2</sup> Médico-veterinário, DSc. PPGCV. Anexo 1, IV, UFRRJ. BR 465 km 7. *Campus Seropédica*, 23.890-000, RJ. \*Autor for correspondence. E-mail: pauloleal@ctiveterinario.com.br - Post doctoral program.

<sup>3</sup> Médico-veterinário, PhD, LD. Departamento em Parasitologia Animal, Anexo 1, IV, UFRRJ. BR 465 km 7. *Campus Seropédica*, 23.890-000, RJ. E-mail: lopesw@ufrrj.br - CNPq Scholarship.

ath in puppies are infect-parasitic diseases, mainly parvovirus, distemper and gastrointestinal parasites (Norris et al. 2006, Dezengrini et al. 2007, Silva et al. 2007, Figuera et al. 2008, Trapp et al. 2010, Fleming et al. 2011, Leal et al. 2011, Leal et al. 2012a, Leal et al. 2012b, Leal et al. 2015). Several species have already been reported parasitizing dogs, with diarrhea being the most frequent clinical manifestation, the most common being: *Ancylostoma caninum*, *Cystoisospora canis*, *Cystoisospora ohioensis*, *Giardia* spp., *Toxocara canis*, *Trichuris vulpis*, *Dipylidium caninum*, *Taenia* spp., *Sarcocystis* (dos Santos et al. 2007, Leal et al. 2011, De La Rue et al. 2012, Flausino et al. 2012, Vital et al. 2012, Leal et al. 2015) and the frequency decreases with increasing age (Fahrion et al. 2008, Nijse et al. 2016). Clinical signs are typically associated with gastrointestinal tract, diarrhea, vomiting, delayed growth, abdominal discomfort in severe cases, obstruction (Bowman 2009).

The genus *Toxocara* is classified in the family Toxocaridae (Despommier 2003, Bowman et al. 2009, Otero et al. 2015). Of the several known species, the most important in Veterinary Medicine and Public Health are *T. canis* Werner, 1782 and *Toxocara cati* Schrank, 1788, not only for the cosmopolitan distribution, but mainly for their zoonotic potential. *Toxocara canis* has as definitive hosts the dog, fox and wolf, among other wild canids, while *T. cati* has the cat, lynx and genet, among other felids and wild viverrids; however, it is important to note that both species can utilize almost any mammal as a paratenic host, including man (Bowman et al. 2009). The adults of *T. canis* present a mouth at the anterior end, surrounded by three lips, one dorsal and two sub ventral (Bowman et al. 2009). The zoonotic importance, especially in children, immunosuppressed and adults, makes toxocariasis, due to *T. canis* an important and widespread zoonosis throughout the world (Shields 1984, Janecek et al. 2015, Kuenzli et al. 2016). It is the infection in the human host by the larvae of this nematode that parasitize dogs. It is known as a visceral larva migrans syndrome, an important public health problem, with the possibility of potentially fatal cardiac lesions with tissue manifestation, mainly in the liver (abscesses), pulmonary and cardiac lesions, as well as in cases of organic manifestations With or without allergic skin rashes (Ha et al. 2016, Kuenzli et al. 2016) and the migration of larvae in the central nervous system, including humans, may cause neurological lesions resulting in a variety of Neurological symptoms (Janecek et al. 2015).

Confirmation by the frequent demonstration of seropositivity for *T. canis* antigens varies with factors such as geographic location, socioeconomic status and eating habits, with risk factors for infection including geophagy and low level education (Lee et al. 2010, Otero et al. (41.62%), imposing the awareness of the tutors and owners of dogs for a management, in order to avoid environmental contamination, since the gastrointestinal parasitism in domiciled dogs is very high (41.62%), Diagnosis, and especially collection and disposal of appropriate feces, avoiding contamination of the environment, promoting protection, since it is a zoonoses responsible for potentially serious polymorphic symptoms and morbidity (Leal et al. 2015). The maintenance of quality continuing education for veterinarians and information properly presented to pet owners is of paramount importance and closer collaboration between veterinary professionals and public health professionals is also required (Beaver et al. 1952, Leal et al. 2000, Kuenzli et al. 2016). It is important to emphasize that the association of different techniques and direct examination are important tools in the detection of multiple infections (Lloyd et al. 1983, Santos et al. 2008, Leal et al. 2011, Ramsey 2011, Flausino et al. 2012, Vital et al. 2012, Leal et al. 2015). Thus, parasitic diseases have become more prominent in their study, due to their high zoonotic potential, results show that lactating bitch can be an important source of contamination of the environment with *T. canis* eggs and especially for the respective puppies, Where almost 100% of the pups are infected in the uterus by somatic larvae reactivated from the 42nd day of gestation (Lloyd et al. 1983), and can transmit in future pregnancies, even without new infections (Webster 1958). The control of gastrointestinal parasites in animals is of fundamental importance, they may be predisposing to other etiologies, such as neosporosis, infection by *N. caninum*, protozoa of the phylum Apicomplexa, family Sarcocystidae, obligatory intracellular, cyst-forming. It infects domestic and wild canids, ruminants and equines (McAllister et al. 1998). Being a canine definitive host (McAllister et al. 1998, Dubey 2003), a parasitic disease of economic importance, with worldwide distribution (Dubey et al. 2007). It is observed mainly in dogs and cattle, occasionally can be found in coyotes, sheep, goats, horses, cats, deer and buffaloes, where abortion and encephalomyelitis are more frequent, but can be found in several organs (Dubey & Lindsay 1996, Dubey 2003, Leal et al. 2012, Munhoz et al. 2013). In dogs, neuromuscu-

lar disorders, such as: encephalomyelitis and myositis, as well as hepatopathies (Dubey & Lindsay 1996), or not present clinical manifestations (Leal et al. 2012a), but with significant lesions in several organs (McAllister et al. 1998, Buxton et al. 2002, Munhoz et al. 2013). The dog represents the only significant source of infection in the process of dissemination of the sexual form of *N. caninum*, thus assuming an important role in the natural chain of the agent (McAllister et al. 1998). The diagnosis of this parasitosis is based on the history, clinical signs and findings of oocysts in dogs' feces (McAllister et al. 1998), indirect immunofluorescence (IFR) techniques, enzyme-linked immunosorbent assay (ELISA), direct agglutination test, DNA from *N. caninum* by Real-time PCR and immunohistochemistry, contribute to the diagnosis (Leal et al. 2012, Balthazar et al. 2013, Munhoz et al. 2013). Although seropositive animals do not develop clinical manifestations, they may have lesions in the lungs, spleen, liver and lymph nodes (Munhoz et al. 2013), and diseases that produce immunosuppression may allow parasite manifestation (Greca et al. 2010, Leal et al. 2012a, Leal et al. 2012b). Congenital transmission is one of the most important forms of infection. Infected bitches can transmit this etiologic agent to their successive fetuses and litters, with newborns being infected (Munhoz et al. 2013).

In the clinical routine the diagnosis is based, with attention to the evolution and in laboratory findings where the intercurrent infections have been observed frequently. The present study points out the importance of clinical and laboratory diagnosis in *Neospora caninum* infections associated with *Toxocara canis* infection, thus confirming the value of the visualization of these etiological agents in feces and serology in the diagnosis of *N. caninum* (Moraes et al. 2008, Greca et al. 2010, Leal et al. 2012a, Balthazar et al. 2013, Munhoz et al. 2013, Leal et al. 2015).

## HISTORICAL FINDINGS

Male, undefined, 34-day-old male, adopted 14 days ago, from a wandering mother. It presented motor incoordination, ataxia, ventro-flexion of the neck, bilateral greenish ocular secretion, abdomen bulging, tense and with moderate palpation pain, normocorated mucosa, normal capillary filling time, normal lymph nodes, tenesmus, soft yellowish stools. A 3 mL syringe with a 25 x 7 mm needle was collected, and 1 mL was filled into a pediatric assay tube with anticoagulant ethylenediaminetetraacetic acid (EDTA), and a blood sample was collected for blood counts, biochemical profiles and serology of the right jugular vein by using a 1 mL in pe-

diatric test tube without anticoagulant. With the material of the syringe itself was made two blood strains in glass slides. Samples were processed at the site, using an automated device (Ms4-Vet-Melet Schloesing Laboratoires coulter), Portable Clinical Refractometer and Microcentrifuga (E3500108 Microspin CDR), for complete blood count (leukogram, erythrogram, platelet, total protein) and Leukocyte and leucocyte concentration in two stretches of glass slides. The samples without anticoagulant were centrifuged in a centrifuge (Mod. 208N, Excelsa Baby, Marca Fanem Ltda.) at 350 x G for 10 minutes, for serum separation and using a 32µl automatic pipette. Feces were collected after defecation for parasitological examination. (Roche Diagnostics GmbH, Mannheim-Baden-Württemberg), abdominal ultrasound (ureia, creatinine, ALT, AF, lactate, total proteins and fractions, potassium and glucose), in vitro Reflectance photometry (GAL), as well as in the presence of a large amount of gastric mucosal retention. Urine sample was collected by cystocentesis using a 5 mL syringe and a 25x7 needle. Urinalysis was performed using 3 mL of freshly collected urine, which was centrifuged in a conical tube for 5 minutes with a centrifugal force of 500 x G in centrifuge Mod. 208N, Excelsa Baby, brand Fanem Ltda. After centrifugation of the sample, the urine supernatant was used to perform the physical and chemical urine exams through its own reactive strips (Roche Combur10 Test® UX), the density determined by a manual clinical refractometer model Q667 (Quimis Scientific Apparatus). To carry out the urinary sediment test, the remaining material (0.5 mL) of the conical tube was used, with the resuspension of this material with a plastic pipette. A drop of this suspended material was removed, placed on a microscope slide and covered with a cover slip, and read on a 40-fold magnification Nikon E 200 microscope, a result compatible with urinary tract infection, showing hematuria, Bacteriuria, proteinuria (Seguin et al. 2003, Harada et al. 2015, Weese et al. 2015). For the parasitological diagnosis a fraction of the faeces were diluted in five drops of 0.9% saline solution stained with a drop of Lugol and observed under a Nikon E 200 microscope. Another fraction was submitted to the technique of centrifugation in saturated solution of sucrose (Leal et al. 2015). The parasites found in adult form were classified according to the morphological characteristics of the adult form, as well as the specified eggs (Figure 1) (Leal et al. 2011, Otero et al. 2015, Leal et al. 2015). ELISA test (Anigen Rapid CDV Ag Test Kit) was used for the presence of distemper virus antigen, noting the absence of distemper virus antigen (Leal et al. 2012b). Hematocrit (GV = 30%), hemoglobin (9.7 g / dL), erythrocytes (4.6 million / mm<sup>3</sup>), values within normal limits for the age group, White leukocytosis was observed from 22,800 (9,000 to 15,000,000 / mm<sup>3</sup>), with eosinophilia, monocytosis, lymphopenia, left nuclear deviation of regenerative neutrophils, neutrophilia, plasma protein close to the maximum limit (5.6g / dL), with albumin 2.5 G / dL and globulins 2.5 g / dL and biochemical parameters.

Enzyme Linked Immunosorbent Assay (ELISA) was



Figura 1. *Toxocara canis* in a mongrel puppy. A non embryonated egg. Sheather's saturated sugar solution. Obj. 40X.

used with the positive result (1/100) for neosporosis and negative for toxoplasmosis, using the Indirect Immunofluorescence Test (IFAT) method. In the 1/80 dilution (Leal et al. 2012a).

The specific treatment for the parasites was based on the use of antibiotic, for neosporosis, clindamycin at a dose of 15mg/kg every 12 hours, oral administration for 15 days (Ramsey 2011, Leal & Coelho 2014, Reis et al. 2016), Used strategically to also treat urinary tract infection (Harada et al. 2015) and for the treatment of toxocariasis, a combination of febantel and pyrantel pamoate at the dose of 15mg of febantel and 14.4mg of pyrantel pamoate per kg of body weight, single dose, with recommendation to repeat after weighing the patient, every 14 days, total of 4 doses (Ramsey 2011, Leal et al. 2015). After the first administration of the combination of febantel and pamoate of pirantel, there was a great elimination of adult worms in the feces of the same species observed in the first sample. Said patient recovered with the treatment used and was discharged after three days.

## DISCUSSION

Neosporosis has several clinical manifestations, with multisystemic presentation, including cardiac lesions, through myocarditis (Munhoz et al. 2013, Agudelo et al. 2016), including gastrointestinal signs as in the present study and in agreement with other reports (Reis et al. 2016). It is uncommon in dogs from urban areas in Brazil and in some parts of the world, but frequent in areas of cattle breeding (Guimarães et al. 2009, Leal et al. 2012a, Balthazar et al. 2013, Nogueira et al. 2013, Igarashi et al. 2006), but it is cosmopolitan (Dubey et al. 2007) and its clinical manifestation is typically neurological, according to the present study, but may pro-

mote adenopathies, splenomegaly and hepatomegaly, or alkaline phosphatase enzyme elevation, as in this report (Munhoz et al. 2013), but it should not be forgotten that neurological signs may also be related to *Toxocara canis* parasitism, a frequent parasitism in young dogs and adults (Fahrion et al. 2008, Leij et al. 2016) because larvae have affinity for the central nervous system (Janecek et al. 2015). The elevation of the alkaline phosphatase activity (AFL) is common among the findings in parasitized animals, this increase in the value of the AFL enzyme probably occurred as a result of neosporosis (Munhoz et al. 2013) or the passage of *T. canis* larvae through the liver (Lloyd et al. 1983, Overgaauw & Nederland 1997, Foreyt 2005), due to hepatic lesions (Foreyt 2005, Munhoz et al. 2013), by pathogenic action of the agents involved in this report (Munhoz et al. 2013). The AFL activity may also be increased in value due to systemic stress caused by other diseases, such as the concomitant urinary tract infection present (Almosny 1998). Therefore, the laboratory findings, as well as the history and clinical examination only suggest the diagnosis, but the unoccupied oocysts in the faeces of *N. caninum*, in the feces samples of the patient (Munhoz et al. 2013) or the demonstration of their antibodies, confirm the diagnosis (Leal et al. 2012a). Eosinophilia observed in the present study agrees with the observed parasite infection, since such cells have a consistent function in the killing of helminth parasites (Meeusen & Balic 2000) and are present whenever there is parasitic infection by *T. canis* (Lloyd et al. 1983, Fahrion et al. 2008). *Neospora caninum* infection through the transplacental route is the most common in pups and when the diagnosis occurs in a timely manner, there is a resolution of the disease according to the current study (Munhoz et al. 2013, Reis et al. 2016, Agudelo et al. 2016), also occurs with toxocariasis, where tracheal migration predominates in dogs less than three months of age, as in the present study, and the presence of adult worms in feces indicates that the infection occurred transplacentally, Or from the early days of ingestion of colostrum larvae (Webster 1958, Overgaauw & Nederland 1997, Nijse et al. 2016), since the pre-patent period is 40 and 56 days (Overgaauw & Nederland 1997, Fahrion et al. 2008). Parasite infections are opportunistic and concomitant with other infections in pups (Leal et al. 2012a, Leal et al. 2012b), predisposing to urinary infections, where impaired immunity is a risk factor for urinary tract infections (Seguin et al. 2003) And cause pain, leukocytosis, proteinuria, bacteriuria, as observed in

the present study (Finco et al. 1979, Harada et al. 2015), indicating the need for adequate antibiotic therapy, justifying the use of clindamycin (Harada et al. 2015, Weese et al. 2015). Toxocariasis and its diagnosis in dogs, as in the present study, become important, due to the zoonotic condition, environmental contamination (Lloyd et al. 1983) and frequent aggravation, especially in children (Beaver et al. 1952, Shields 1984, Overgaauw & Nederland, 1997, Despommier 2003, Lee et al. 2010, Gandolfi et al. 2003, Overgaauw et al. 2013, Janecek et al. 2015, Kuenzli et al. 2016, Ha et al. 2016).

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