

***Lontra longicaudis* infected with canine parvovirus and parasitized by *Diocotophyma renale*¹**

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ABSTRACT.- Echenique J.V.Z., Soares M.P., Mascarenhas C.S., Bandarra P.M., Quadros P., Driemeier D. & Schild A.L. 2018. ***Lontra longicaudis* infected with canine parvovirus and parasitized by *Diocotophyma renale*.** *Pesquisa Veterinária Brasileira* 38(9):1844-1848. Laboratório Regional de Diagnóstico, Faculdade de Veterinária, Universidade Federal de Pelotas, Campus Capão do Leão, Pelotas, RS 96010-900, Brazil. E-mail: alschild@terra.com.br

This study describes a case of parvovirus infection in a river otter (*Lontra longicaudis*) assisted at the Wildlife Rehabilitation Center and Wildlife Screening Center, Federal University of Pelotas (UFPel), Rio Grande do Sul state, Brazil. Clinical signs included apathy, dark and fetid diarrhea, and crusted lesions on the palmar pads of the fore and hind limbs. The animal died after undergoing support treatment with antibiotics, anti-inflammatory, and fluid therapy. At necropsy, the intestines were reddened and edematous and the right kidney was diminished by one third of its normal size and covered with whitish, spongy material. A female *Diocotophyma renale* was found free in the abdominal cavity. Histologically, dilatation of the intestinal crypts and fusion and blunting of the intestinal villi were observed. In addition, moderate, multifocal lymphocytic enteritis with lymphoid depletion in Peyer's patches and mesenteric lymph nodes were present. Immunohistochemistry with anti-canine parvovirus monoclonal antibody (anti-CPV) was strongly positive in the bone marrow cells and enterocytes of the intestinal crypts, confirming the diagnosis of parvovirus infection. The peritoneum on the right kidney was expanded with a cuboidal cell border, forming multiple papillary projections associated with eggs of *D. renale* and severe inflammatory infiltrate (giant cells, macrophages, lymphocytes, eosinophils, and plasma cells). Areas of necrosis and mineralization were also observed. Due to fragmentation and degradation of its natural habitat, the otter approached the urban area and was contaminated with the virus, which is hosted and disseminated by domestic animals. Infection with *D. renale* can be associated with the large population of parasitized domestic animals, which eliminate the helminth eggs through urine, contaminating the environment where the parasite intermediate and paratenic hosts co-inhabit. The diseases of these animals can be a decline factor of wild populations that inhabit the region and are an alert to spillover risk.

INDEX TERMS: *Lontra longicaudis*, otter, canine parvovirus, *Diocotophyma renale*, parvovirus infection, wildlife, viroses, parasitoses.

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RESUMO.- [*Lontra longicaudis* de vida livre infectada por parvovirus canino e parasitada por *Diocotophyma renale*.] Descreve-se um caso de parvovirose em uma lontra (*Lontra longicaudis*) enviada ao Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres da Universidade Federal de Pelotas, Rio Grande do Sul, Brasil. O animal estava debilitado, apático, apresentava diarreia escura e fétida e lesões crostosas nos coxins palmares dos membros torácicos e pélvicos, morrendo após tratamento de suporte com antibiótico, anti-inflamatório e fluidoterapia. Na necropsia os intestinos estavam edematosos e avermelhados e o rim direito estava recoberto de material brancoacento e

esponjoso, com comprometimento de cerca de um terço do órgão. Foi observado, também, um exemplar de *Diocotophyma renale*, fêmea, livre na cavidade abdominal. Histologicamente havia fusão das vilosidades, dilatação das criptas intestinais com enterite linfocítica moderada multifocal e depleção linfóide nos linfonodos mesentéricos. Na técnica de imuno-histoquímica (IHQ) com anticorpo monoclonal anti-Parvovírus canino (Anti-CPV) houve marcação positiva nos enterócitos da base das vilosidades intestinais e na medula óssea, confirmando o diagnóstico de parvovirose. O peritônio sobre o rim direito estava espessado e revestido por células cubóides, formando múltiplas projeções papilares, nas quais observava-se acentuado infiltrado de células gigantes, macrófagos, linfócitos, eosinófilos e plasmócitos. Entre as projeções papilares havia ovos de *Diocotophyma renale*, áreas de necrose, calcificação e células gigantes. Conclui-se que a lontra, em função da fragmentação e degradação de seu habitat natural, aproximou-se do centro urbano e contaminou-se com o vírus, o qual é mantido e disseminado por animais domésticos. Por sua vez, a infecção por *D. renale* pode estar relacionada com a presença de animais domésticos parasitados, os quais eliminam ovos do helminto através da urina contaminando o ambiente, onde coabitam hospedeiros intermediários e paratênicos do parasito. As doenças desses animais podem ser um fator de declínio das populações de animais silvestres e alerta para o risco de *spill-over* na região.

TERMOS DE INDEXAÇÃO: *Lontra longicaudis*, lontra, parvovírus canino, *Diocotophyma renale*, parvovirose, animais silvestres, viroses, parasitoses.

INTRODUCTION

Lontra longicaudis longicaudis (Olfers, 1818) (Carnivora: Mustelidae) is a semiaquatic mustelid species, with distribution restricted to rivers, lakes, and lagoons, found from Mexico to the province of Buenos Aires and southern Uruguay. In Rio Grande do Sul state, Brazil, it occurs from coastal environments to plateau areas (Rheingantz & Trinca 2015). The species prefers white water rapids with little turbidity, but also occurs in mangroves, river estuaries and deltas, and weirs and dams (Rheingantz & Trinca 2015). The otter is listed as a threatened species due to illegal poaching and fragmentation of its natural habitat (Rheingantz & Trinca 2015). Habitat loss and degradation have provided increased contact between wild and domestic animals and humans (Seal & Armstrong 2000). This proximity fosters the sharing of diseases in a process called spillover (Schloegel & Daszak 2004). In addition to becoming exposed to trauma, wild animals become susceptible to diseases that previously only affected domestic animals (Frölich et al. 2002, Gortázar et al. 2007), e.g., parvovirus infection, which affects mainly domestic canids. Wild animals such as mustelids, procyonids, and felids are also susceptible to these diseases (Steinel et al. 2001). Transmission occurs mainly directly, through contact with feces contaminated by the virus. The disease presents with severe hemorrhagic enteritis caused by virus tropism by cells of high mitotic activity (Olson et al. 1988). In turn, diocotophymosis is caused by the nematode *Diocotophyma renale* (Goeze 1782) (Enoplidae: Diocotophymatidae), which parasitizes domestic and wild carnivores in several countries. In Canada, mustelids of the genus *Lontra* are affected by this disease mainly due to their

eating habits (Kimber & Kollias 2000). In Brazil, there are some records of this nematode in wild animals (Barros et al. 1990, Vicente et al. 1997, Ribeiro et al. 2009), but the disease is more prevalent in domestic canids and felids (Silveira et al. 2015).

The present study aimed to describe the clinical and pathological aspects of parvovirus infection and diocotophymosis in an otter (*Lontra longicaudis*) rescued in a municipality in the southern region of Rio Grande do Sul state, and discuss the probable factors that influenced the occurrence of these diseases in this wild species.

MATERIALS AND METHODS

A male, adult, otter (*Lontra longicaudis* Olfers, 1818) specimen rescued in the urban area of the municipality of Pelotas, Rio Grande do Sul state, Brazil, presenting signs of weakness and apathy was referred to the Wildlife Rehabilitation Center and Wildlife Screening Center of the Federal University of Pelotas (Nurfs-Cetas/UFPel). Due to the clinical conditions of the animal, screening examinations and antibiotic and anti-inflammatory support treatments and fluid therapy were performed; however, the animal died four days after arrival.

The otter was sent for necropsy to the Regional Diagnostic Laboratory of the College of Veterinary Medicine of the Federal University of Pelotas (LRD/UFPel). Samples from all organs were collected, fixed in 10% buffered formalin, and routinely processed for histology, stained with hematoxylin and eosin (HE). Samples of bone marrow, small intestine, and colon embedded in paraffin were sent to the Department of Veterinary Pathology of the Federal University of Rio Grande do Sul (SPV-UFRGS) for immunohistochemistry (IHC) detection of canine parvovirus (CPV) antigen. Anti-CPV monoclonal (MCA 2064-Serotec) was used at dilution of 1:1000 as the primary antibody. Antigen retrieval was performed with protease type XVI (Sigma). Subsequently, biotinylated secondary antibody conjugated to streptavidin (LSAB kit, Dako) was used. DAB (3,3'-diaminobenzidina) (Dako) was used as chromogen for color development. Slides of positive cases of CPV were used as control.

RESULTS

The otter assisted at the NURFS presented low body condition score (2/5), apathy, dark diarrhea, and crusted lesions on the palmar pads. Necropsy showed generalized pallor of the musculature and of the opening of the abdominal cavity, and opaque, thickened mesentery with multiple, dark, 1 mm-diameter spots. The intestines were edematous and reddened (Fig.1). Histologically, necrosis and fusion of the villi and crypts were observed in the small intestine, with few enterocytes adhered to the villi and crypts (Fig.2). Moderate, inflammatory infiltrate of lymphocytes was observed. The colon had lesions similar to those in the small intestine. There was lymphoid depletion in Peyer's patches and mesenteric lymph nodes. IHC with canine parvovirus monoclonal antibody (Anti-CPV) showed strongly positive staining in the epithelial cells of the base of intestinal villi (Fig.3) and in the bone marrow.

A *Diocotophyma renale* specimen was found free in the abdominal cavity (Fig.1). The right kidney was opaque, thickened and covered with whitish, spongy material. The organ was diminished, with approximately one third of the renal parenchyma left, which also presented atrophy of several *reniculi* (Fig.4). Histologically, the mesentery was thickened by edema, hemorrhage, and neovascularization. In the right kidney, serosa was thickened and covered with cuboid cells, forming multiple papillary projections



Fig.1. *Lontra longicaudis* infected with canine parvovirus. The serosa of some small intestine loops shows edematous and reddish appearance. A sample of free *Diocotophyma renale* is observed in the abdominal cavity.

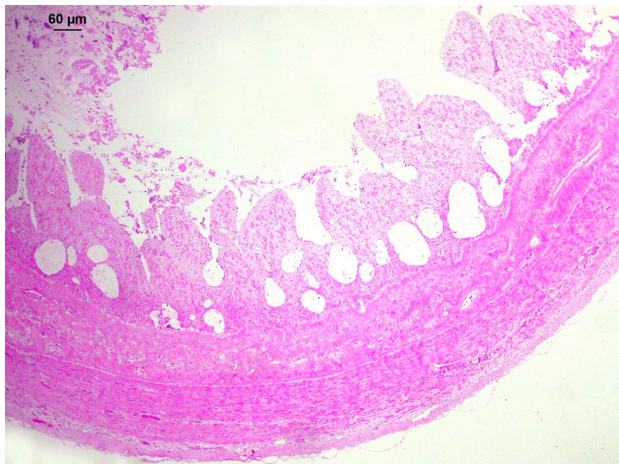


Fig.2. *Lontra longicaudis* infected with canine parvovirus. Adherence of intestinal villi is observed due to the scarce amount of coating epithelial cells, there is marked necrosis of enterocytes from the intestinal crypts. HE, obj.20x.

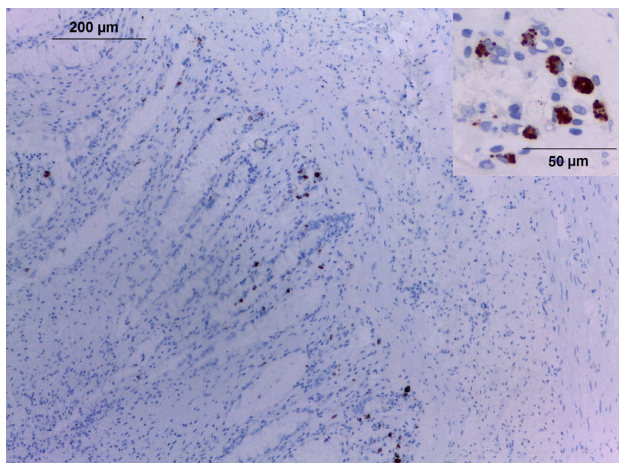


Fig.3. *Lontra longicaudis* infected with canine parvovirus. Strongly positive labeling of cryptic epithelial cells in the mucosa of the small intestine. Immunohistochemistry by the streptavidin-biotin-peroxidase method with canine anti-parvovirus antibody. IHC, obj.20x. Inset: strongly positive marking at the base in the crypts. Obj.40x.

(Fig.5) in which a marked inflammatory infiltrate composed of giant cells, macrophages, lymphocytes, eosinophils, and plasma cells was observed. In between the papillary projections there were *D. renale* eggs (Fig.5), areas of necrosis, calcification, and giant cells. Thickening of the interlobular renal septa and compression atrophy of the glomeruli and renal tubules were also observed. In addition, there were mesenteric lymph nodes and lymphoid depletion in the Peyer's patches.

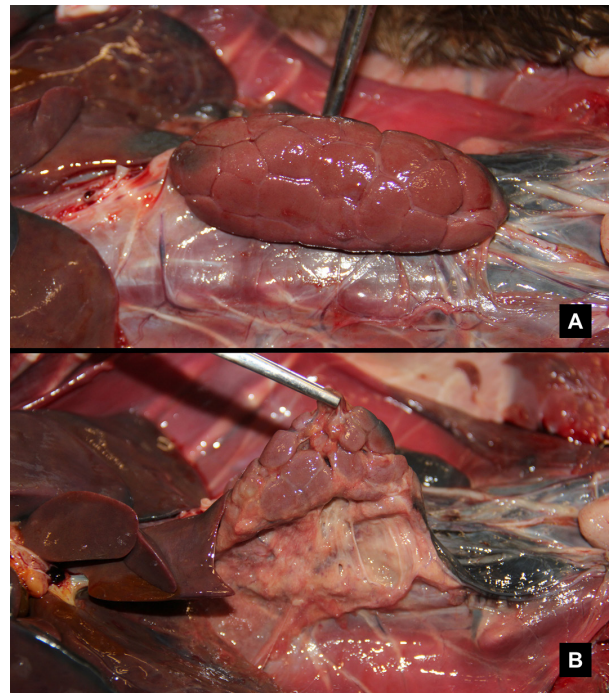


Fig.4. *Lontra longicaudis* parasitized by *Diocotophyma renale*. (A) Normal-looking left kidney. (B) Right kidney with reduced size and thickened capsule. There is a whitish and nodular tissue replacing the renal parenchyma.

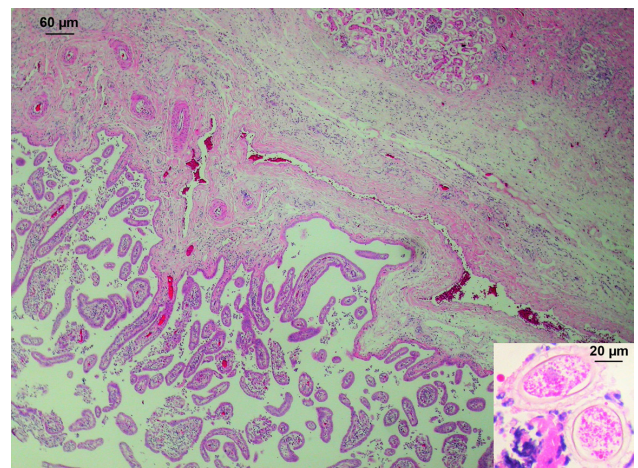


Fig.5. *Lontra longicaudis* parasitized by *Diocotophyma renale*. Right kidney. The serosa is thickened, covered by cuboid cells, forming multiple papillary projections, in which inflammatory infiltrate is observed. HE, obj.20x. Inset: *Diocotophyme renale* eggs, associated with papillary projections. HE, obj.40x.

DISCUSSION

Based on macroscopic and histological changes and on the results of immunohistochemical staining (IHC), a free-living otter was diagnosed with parvovirus infection. A *Diocotophyma renale* specimen was also found in the abdominal cavity of this animal. The clinical signs of parvovirus infection and lesions were similar to those found in domestic canids affected by this disease (Oliveira et al. 2009). The otter analyzed in this study was rescued in the urban area of the municipality of Pelotas, which has a population of approximately 66,700 canids, and approximately 80% of them are semi-domiciled or stray (Pelotas 2012). These canids spend long periods of time roaming, and are able to travel long distances (Hughes & Macdonald 2013). As a result, these individuals have been considered reservoirs of agents of various diseases, and are described by some authors as disseminators of diseases to free-living animals (Hughes & Macdonald 2013). This fact supports the hypothesis that the otter in question was infected when approaching the urban environment of Pelotas (Measures 2001, Hughes & Macdonald 2013). Parvoviruses remain for a long time in the environment, which enables maintenance of their infectious potential (Pollock & Coyne 1993, Measures 2001). In the present case, when approaching the urban area to escape from the degradation and fragmentation of its habitat, the otter was probably exposed to contact with the domestic animals. The close contact between these individuals is a facilitator of the transmission of several diseases (Seal & Armstrong 2000).

Parvovirus infection can be a decline factor for otter populations, as studies have shown the decline of wild wolf (*Canis lupus*) packs as a result of this disease (Mech et al. 2008), and the same may occur with otters in southern Brazil. The Species Survival Commission of the International Union for Conservation of Nature (IUCN) emphasized that infectious diseases are limiting for the conservation of wild mustelids (Thomas & Cole 1996), and the case herein described reinforces this assertion.

Otters inhabit rivers and lakes and feed mainly on crustaceans and fish, but may be more general depending on the environment they are in (Pardini 1998, Quintela et al. 2012). Studies conducted in the region have reported that food items, such as fish and other vertebrates, in the diet of *Lontra longicaudis* (Quintela et al. 2012) are parasitized by *D. renale* larvae (Mascarenhas & Müller 2015, Mascarenhas et al. 2016). *D. renale* life cycle requires the participation of aquatic organisms and fish, considered paratenic hosts of this parasite (Measures 2001). In the present case, it is likely that the otter became infected by ingesting paratenic or intermediate (oligochaetes) hosts infected with L3 larvae (infective form). Apparently, the urban and periurban environments of the municipality of Pelotas are contaminated, considering that there are several records of *D. renale* in wild and domestic animals in the region (Pesenti et al. 2012, Mascarenhas & Müller 2015, Mascarenhas et al. 2016, 2017, Rappeti et al. 2017). There is also a risk to public health, because cases of diocotophymosis have already been described in humans (Ignjatovic et al. 2003, Li et al. 2010). Otters have multi-lobular kidneys, composed of several *reniculi* (Baitchman & Kollias 2000). It is possible that, due to the anatomy of the organ, *D. renale* ruptured the renal parenchyma and remained free in the abdominal cavity.

The parasite is probably an incidental finding, and it did not contribute to the death of this animal.

CONCLUSIONS

The canine parvovirus infection described in the otter analyzed in this study occurred as a consequence of environmental contamination by the large number of stray or semi-domiciled canids and felids living in the municipality of Pelotas, which contaminate the environment and are a potential risk to the preservation of wild species.

Habitat fragmentation exposes wildlife living near urban areas to risk of spillover, which probably occurred in the present case.

In addition to parvovirus infection and diocotophymosis, other pathogens may soon affect other species, also contributing to the decline of their populations.

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