

## Detection of *Lawsonia intracellularis* fecal shedding in dogs in Minas Gerais, Brazil

### Detecção de *Lawsonia intracellularis* em fezes de cães em Minas Gerais, Brasil

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#### – NOTE –

#### ABSTRACT

*Lawsonia intracellularis* is an obligate intracellular bacterium that is responsible for proliferative enteropathy, an enteric disease endemic in swine and common in foals. However, few studies have investigated this disease in dogs, and there are no reports of dogs infected with *L. intracellularis* in Latin America. The aim of this study was to evaluate the fecal shedding of *L. intracellularis* in diarrheic and non-diarrheic dogs in Minas Gerais, Brazil. A total of 58 dogs, 18 apparently healthy and 40 diarrheic, were examined in this study. DNA extracted from feces was analyzed using a nested PCR reaction to detect *L. intracellularis*. Three out of 40 (7.5%) diarrheic samples, all from 3-month-old puppies, were positive for *L. intracellularis*. These results highlight the need for additional studies to examine the role of this pathogen as a possible cause of enteric disease in dogs.

**Key words:** *Lawsonia intracellularis*, canine, proliferative enteropathy, diarrhea.

#### RESUMO

*Lawsonia intracellularis* é uma bactéria intracelular obrigatória responsável pela enteropatia proliferativa, uma doença entérica endêmica em suínos e comum em potros. Em cães, no entanto, existem poucos estudos sobre essa doença e inexistem relatos de cães infectados por *L. intracellularis* na América Latina. O objetivo deste estudo foi avaliar a presença de *L. intracellularis* em amostras de fezes de cães diarreicos e aparentemente saudáveis em Minas Gerais, Brasil. Foram incluídos 58 cães, sendo 18 aparentemente saudáveis e 40 diarreicos. Submeteu-se o DNA bacteriano fecal a uma reação de PCR nested para *L. intracellularis*. Três das 40 (7,5%) amostras de cães diarreicos foram positivas para *L. intracellularis*, estando todos esses animais com aproximadamente 3 meses de idade. Estes resultados salientam a necessidade de mais estudos para confirmar o papel deste patógeno como uma possível causa de doença entérica em cães.

**Palavras-chave:** *Lawsonia intracellularis*, canídeos, enteropatia proliferativa, diarreia.

Proliferative enteropathy (PE) is an enteric disease caused by the obligate intracellular bacterium *Lawsonia intracellularis*. PE has been described in many animal species, including sheep, hamsters, guinea pigs, foxes, ferrets and horses, and it is characterized by thickening of the intestinal epithelium due to enterocyte proliferation (McORIST & GEBHART, 2012; VANNUCCI & GEBHART, 2014). In swine, *L. intracellularis* has been recognized as an enteropathogen since 1931, and PE is now considered endemic in every country that has significant swine production (LAWSON & GEBHART, 2000). Although a small number of studies have examined *L. intracellularis* infections in dogs (COLLINS et al., 1983; LEBLANC et al, 1993; HERBST et al., 2003; KLIMES et al., 2007), the role of *L. intracellularis* as an enteropathogen in dogs is unclear, and there are no reports of dogs infected with *L. intracellularis* in Latin America. Thus, because little is known about *L. intracellularis* infections in dogs, the aim of this study was to investigate the presence of *L. intracellularis* in stool samples of diarrheic and non-diarrheic dogs in Brazil using a nested PCR method.

Stool samples were collected from 58 dogs, of which 18 were apparently healthy (10 puppies <1 year old and 8 adults, ranging from 2 months to 10 years of age), and 40 were diarrheic (19 puppies and 21 adults, ranging from 1 months to 8 years of age). The dogs investigated in this study were brought to the Veterinary Hospital of the Universidade Federal de Minas Gerais for consultations; fecal samples were

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obtained directly from the rectum of diarrheic dogs using a sterile probe at the time of consultation. These samples were only collected from dogs for which the main motivation for the veterinary appointment was the occurrence of diarrhea. Fecal samples from apparently healthy animals were collected in three public squares in Belo Horizonte, Minas Gerais, Brazil, with the prior permission of the owners, when the animal was defecating. Ethical approval for this study was granted by the Animal Experiments Committee of the Universidade Federal de Minas Gerais (CEUA, protocol number 090/10).

Fecal samples were stored at -20°C until DNA extraction. DNA was extracted using the Qiagen QIAamp Stool Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. Extracted DNA was analyzed using a nested PCR reaction, as previously described (JONES et al., 1993). Briefly, two pairs of primers were used to amplify a 217 bp-long DNA fragment. An external pair of primers, LIA (5'- TAT GGC TGT CAA ACA CTC CG - 3') and LIB (5'- TGA AGG TAT TGG TAT TCT CC - 3'), delimited a 319 bp-long fragment. An internal pair of primers, LIC (5' - TTA CAG GTG AAG TTA TTG GG - 3') and LID (5' - CTT TCT CAT GTC CCA TAA GC - 3'), was used to amplify the final DNA fragment. The resulting PCR products were analyzed by electrophoresis using a 2% agarose gel and stained with ethidium bromide. Positive and negative control DNA samples were added to each reaction.

The resulting positive PCR products were purified using spin columns containing Sephacryl 1S-400 (GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions, and each product was sequenced two times in both directions using the LIC and LID primers and the Big Dye V3.1 Terminator Kit (Applied Biosystems, EUA). Amplification was carried out under the following PCR conditions: 44 cycles of 96°C for 30s, 50°C for 15s and 68°C for 4min. The resulting PCR products were sequenced using an ABI 3130 DNA analyzer (Applied Biosystems, California, USA).

Unthawed aliquots of the positive stool samples were used to explore the differential diagnosis of *L. intracellularis* infection. Samples were analyzed for the presence of rotavirus by polyacrylamide gel electrophoresis (PAGE) followed by silver staining (HERRING, 1982), and samples were analyzed for the presence of *Giardia* sp. vegetative stages and cysts by enzyme immunoassay (EIA, Ridascreen *Giardia*, R-Biopharm, Germany). Assays to isolate and genotype *Clostridium perfringens* were performed as described previously (SILVA et al., 2013). Samples were analyzed for the presence of *C. perfringens* enterotoxin using a commercial EIA kit (Ridascreen *Clostridium perfringens* Enterotoxin,

R-Biopharm, Germany). Assays to isolate *C. difficile* were performed as previously described (SILVA et al., 2013), and samples were analyzed for the presence of *C. difficile* A/B toxin using an EIA (*Clostridium difficile* Tox A/B II, Techlab Inc., Virginia, USA). Samples were analyzed for the presence of coronavirus and parvovirus using an immunochromatographic test (Corona and Parvovirus Ag Test-Eco Diagnostica, Brazil), and assays to isolate *Escherichia coli* followed by a multiplex PCR assay for detecting pathogenicity factor genes were performed as described previously (MACÊDO et al., 2007).

Three (5.2%) of the 58 dog samples tested by nested PCR were positive for *L. intracellularis*. All of them were from puppies in the diarrheic group that were approximately 3 months old. It is important to note that a nested PCR assay is considered to be the most appropriate diagnostic test for *L. intracellularis* *in vivo* due to its high sensitivity and specificity. Specifically, the PCR protocol used in the present study is able to detect approximately 10<sup>2</sup> *L. intracellularis* cells per gram of feces (JONES et al., 1993). In addition, a BLAST search of the sequences obtained from these puppies exhibited 99-98% identity (with 100% coverage) with the *L. intracellularis* aspartate-ammonia lyase gene, confirming the diagnosis in these dogs. In addition, all positive animals were also negative for all other enteropathogens tested.

The positive rate found in the diarrheic group (7.5%) is similar to that reported by HERBST et al. (2003) (5.4%) in a study conducted in Germany. In addition, the detection of *L. intracellularis* in puppies corroborates previous descriptions of the disease in young dogs (HÚSNIK et al., 2003; TOMANOVÁ et al., 2003; KLIMES et al., 2007).

Two hypotheses could explain the detection of *L. intracellularis* in these animals. First, *L. intracellularis* could be shed sporadically by dogs, and second, *L. intracellularis* could be responsible for the diarrhea in these cases. Three factors in addition to the detection of this microorganism in dogs increase the probability that *L. intracellularis* is a pathogen in dogs: (1) in the present study, all positive dogs had no known previous contact with pigs or horses, two important sources of *L. intracellularis*; (2) all of the positive animals were diarrheic at 3 months old, while all the animals from the control group were negative, even though six out of the 18 control animals were also 3 months old; and (3) all of the positive animals were negative for all other enteropathogens tested.

In previous reports of *L. intracellularis* infection in dogs (HÚSNIK et al., 2003; LEBLAC et al., 1993; TOMANOVÁ et al., 2003), lesions were

observed and the agent was detected in the mucosa of the stomach and duodenum, which are not typically sites of infection in pigs, in which the agent has been studied most. These findings may lead to under-diagnosis of the disease due to sampling of intestinal segments that are not typically infected. Only COLLINS et al. (1983) observed characteristic lesions of the disease in the aboral segment of the small intestine.

The hypothesis that *L. intracellularis* acts as an enteropathogen in dogs was raised for the first time in 1983 after its detection by PCR in two Dalmatian puppies. These animals also had intestinal lesions similar to those described in PE in swine (COLLINS et al., 1983). *L. intracellularis* was also detected in a dog with inflammatory bowel disease in the Czech Republic using nested PCR protocols (HÚSNIK et al., 2003). In another study, KLIMES et al. (2007) found that diarrhea was the most common reported clinical sign in dogs serologically positive for *L. intracellularis*. In the same study, 40 out of 54 dogs (74.1%) had specific IgG antibodies against *L. intracellularis*; nearly all of these animals suffered from chronic diarrhea, and three dogs were confirmed to have PE (KLIMES et al., 2007).

Other infections in the differential diagnosis of PE in dogs to which dogs in the present study were not tested included infections with canine distemper virus, *Campylobacter* spp, *Cryptosporidium* and helminths. In addition, the PCR assay for *E. coli* virulence factors used in the present study was not capable of detecting the *eae* gene, which is commonly associated with diarrhea caused by this bacterium in dogs. Despite these limitations, the results of this study raise the possibility that *L. intracellularis* infection is a cause of diarrhea in dogs. Practitioners in Brazil should be alerted to the possibility that this agent is a cause of unresolved enteritis and mortality in this species. To the authors' knowledge, this is the first confirmation of *L. intracellularis* shedding by dogs in Latin America.

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