Prevalence of antibodies against *Lawsonia intracellularis* in dogs with and without gastrointestinal disease

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ABSTRACT: Blood sera from 71 dogs were examined for specific IgG antibodies against *Lawsonia intracellularis* using the indirect immunofluorescent antibody test. The dogs were divided into two groups according to the presence or absence of gastrointestinal disease, which could potentially be associated with *L. intracellularis.* In the group of dogs with gastrointestinal disease (n = 54), 40 dogs were positive (74.1%). Most positive dogs suffered from chronic or intermittent diarrhoea. In the group without signs of primary gastrointestinal disease (n = 17), antibodies were found in 13 dogs (76.5%). The overall positivity was 74.7%. These results indicate that dogs may be an important host species of *L. intracellularis.*

Keywords: canine; intracellular bacteria; diarrhoea; indirect immunofluorescence; serology

Lawsonia intracellularis is an obligatory intracellular bacterium that has been identified as the etiological agent of proliferative enteropathies, which are characterised by intestinal epithelial hyperplasia (McOrist et al., 1995). It is an important enteropathogen of domestic pigs with worldwide distribution, which typically causes diarrhoea and growth retardation resulting in economic losses (Lawson and Gebhart, 2000). Apart from pigs, proliferative enteritis associated with this bacterium has been described in a variety of mammalian and avian species (Cooper and Gebhart, 1998). For instance, *L. intracellularis* may cause infection in horses, whose clinical forms with corresponding pathological findings have so far been reported only from North America (Frank et al., 1998; Brees et al., 1999; Lavoie et al., 2000).

In dogs, proliferative lesions in the gastrointestinal tract, likely caused by *L. intracellularis* have been reported only in two cases (Collins et al., 1983; Leblanc et al., 1993). This bacterium has later been detected in a dog with inflammatory bowel disease using the nested polymerase chain reaction (PCR) and serology (Husnik et al., 2003; Tomanova et al., 2003a) and in four dogs with diarrhoea examined by PCR (Herbst et al., 2003). *L. intracellularis* has also been found in other species of the Canidae family, namely the grey wolf, red fox and probably also the blue fox (Eriksen et al., 1990; Tomanova et al., 2003b).

To our knowledge, no study of the prevalence of antibodies to *L. intracellularis* has been done so far in the canine population. Therefore the present paper describes a serological survey in dogs with

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or without gastrointestinal disease aimed at the detection of *L. intracellularis.*

MATERIAL AND METHODS

Blood sera from 71 dogs were examined for specific IgG antibodies against *L. intracellularis* using the indirect immunofluorescent antibody test (IFAT). The dogs were either patients of the Clinic of Dog and Cat Diseases, University of Veterinary and Pharmaceutical Sciences, Brno, or their sera were supplied by private veterinary practitioners from various parts of the Czech Republic (dogs with suspected exocrine pancreatic insufficiency). The dogs were divided into two groups according to the presence or absence of gastrointestinal disease, which could potentially be associated with *L. intracellularis.* The first group contained 54 dogs with various gastrointestinal conditions, namely dogs with chronic or intermittent diarrhoea of an undetermined cause (18 dogs), with signs suggestive of exocrine pancreatic insufficiency (EPI) which was later excluded (15 dogs), acute gastroenteritis (five dogs), confirmed EPI (three dogs), histologically confirmed inflammatory bowel disease (IBD; three dogs), *Campylobacter* infection (three dogs), *Giardia* infection (three dogs), intestinal lymphangiectasia (one dog), colorectal adenocarcinoma (one dog), borborygmi and flatulence (one dog) and chronic vomiting of undetermined etiology (one dog). The dogs ranged in age from four months to 13 years (median, three years). Forty-four were intact males and 10 were females. There were dogs of four crossbreeds and 22 different pure breeds. Apart from physical and basic laboratory examination, all dogs with signs suggestive of EPI (chronic diarrhoea, weight loss, polyphagia) were tested using the trypsin-like immunoreactivity assay. Bacteriological cultivation of rectal swabs was performed in most dogs with chronic diarrhoea. Gastrointestinal endoscopy with subsequent histopathological examination was performed in five dogs. Two dogs died or were euthanised due to liver failure and intestinal intussusception and underwent *post mortem* examination including histopathology.

The second group contained 17 dogs without primary signs of gastrointestinal disease that were either clinically healthy (two dogs) or were treated for various conditions including renal failure (five dogs), diabetes mellitus (four dogs), etc. The dogs ranged in age from four months to 12 years (median, eight years). Eleven were intact males and six were females. There were dogs of six crossbreeds and seven different pure breeds.

The blood samples were taken either from an antebrachial vein or from the jugular vein. Serum was separated by centrifugation within two hours and stored at –22°C until analysis proceeded. For serological examination, the indirect immunofluorescent antibody test (Eli-Lilly, USA) was performed on a slide prepared by S. McOrist with fixed *L. intracellularis* cells (NCTC 12656^T strain) and 15 wells. The sera used in the tests were inactivated at 56°C for 30 min at 1:10, 1:30 and 1:100 dilutions in phosphate-buffered saline (PBS). Serum samples were placed in individual 5 µl wells and incubated at 4°C in a wet chamber overnight. Before the next incubation, the slides were washed six times in PBS buffer followed by an incubation with anti-dog IgG conjugated with fluorescein isothiocyanate (Sigma, St. Louis, USA) at a dilution of 1:30 in the PBS at 37°C for 30 minutes. Finally, the slides were washed for 5 min in PBS buffer five times, dried and kept in a dark chamber at a room temperature. Each test on the slides with 15 wells included a positive and a negative control. The positive control serum came from a dog with a repeated positive reaction, while the negative sera were obtained from five newborn Beagle pups. Test results were read using an immunofluorescence microscope at 150 to 300 × magnification. Samples that clearly contained the fluorescent *L. intracellularis* bacterium in the viewing field were considered to be positive.

For statistical analysis, the chi-square test was used.

RESULTS

Examination of 71 serum samples revealed the presence of specific IgG antibodies against *L. intracellularis* in 53 dogs (74.7%). In the group with gastrointestinal disease (n = 54), 40 dogs were positive (74.1%). Nearly all of these animals suffered from chronic diarrhoea; only three dogs had acute diarrhoea and/or vomiting (acute gastroenteritis). One dog had colorectal adenocarcinoma and one suffered from borborygmi and flatulence. Fourteen of the positive dogs had chronic or intermittent diarrhoea of an undiagnosed cause, sometimes associated with vomiting, in 10 dogs there were signs suggestive of EPI (which was later excluded),

three dogs suffered from confirmed EPI. The likely cause if diarrhoea in the remaining dogs was IBD, namely lymphocytic-plasmacytic enteritis (three cases), the *Giardia* infection (three cases), and the *Campylobacter* infection (two cases). One dog with EPI also had chronic hepatitis and ascites, one dog with IBD was positive for *Helicobacter* spp. on cytologic examination and another dog with IBD had lymphangiectasia complicated by fatal intussusception. Among positive dogs, there were eight German Shepherds, four Poodles, four crossbreeds, three Doberman Pinschers, two Boxers, two Rottweilers and two Pitbull Terriers. The age of positive dogs was four months to 13 years (median, three years) and the age of negative dogs was five months to 10 years (median, 3.5 years). There were 32 males and eight females among the positive dogs.

In the group without gastrointestinal disease (n = 17), 13 dogs were positive (76.5%). Among the positive dogs, five were crossbreeds, three were German Shepherds and two were Dachshunds. The age of the positive dogs was 3 to 12 years (median, eight years), while the age of the negative dogs was four months to eight years (median, 2.25 years). There were eight males and five females. There was no statistically significant difference in seropositivity between both groups.

DISCUSSION

The prevalence of specific IgG antibodies to *L. intracellularis* in dogs was surprisingly high (74.7%). In the Czech Republic, Tomanova (2003) reported the presence of IgG antibodies to *L. intracellularis* in 64.9% of 801 domestic pigs examined by IFAT. In the same country, the prevalence of IgG antibodies in wild pigs was 51.4% (Tomanova et al., 2002). However, pigs are the major known host species of this bacterium, while reports on the presence of *L. intracellularis* in dogs are very scarce. On the other hand, the proportion of positive serum samples was very high in horses (60.6%), particularly in animals over one year of age (87.1%) (Tomanova, 2003). According to Williams et al. (1996), *L. intracellularis* has similar pathogenic potential in the equine species as in swine, although apparently at a lower incidence.

The IFAT was chosen for detecting antibodies to *L. intracellularis* because it is used to monitor the incidence of proliferative enteropathy in domestic pigs in both the USA and in Europe (Knittel et al., 1998). Some authors suggest that the sensitivity of the IFAT test is higher when compared with the PCR assay in pigs and foals (Knittel et al., 1998; Lavoie et al., 2000). In spite of the possibility of the occasional occurrence of false positive cases, Jacobson et al. (2004) recommend serological testing to determine the presence of *L. intracellularis* infection in swine herds. Using the PCR, the agent was found in 7% of faecal samples from dogs with diarrhoea (Herbst et al., 2003). In another study, we have found *L. intracellularis* in 43.5% of dogs with gastrointestinal disease using nested PCR (Klimes et al., unpublished results).

Considering the clinical signs in those few reported dogs with *L. intracellularis* infection and the signs in other animal species (Collins et al., 1983; Eriksen et al., 1990; Lawson and Gebhart, 2000; Herbst et al., 2003; Husnik et al., 2003), we can assume diarrhoea as the most characteristic clinical symptom. The similar frequency of positive samples in dogs with gastrointestinal disease (mainly chronic diarrhoea) and in dogs without primary gastrointestinal signs does not exclude the possibility that *L. intracellularis* may be an important intestinal pathogen of dogs. This situation can be compared to that of *Campylobacter* spp. It is a well-established pathogen of domestic carnivores which can be detected with a similar frequency in the faeces of healthy and diarrhoeic dogs or cats (Burnens et al., 1992). The seropositivity of many dogs without gastrointestinal disease can be attributed to subclinical infection. In pigs, subclinical infection is widespread and predisposing factors are important for the development of clinically and pathologically detectable disease (Moller et al., 1998). The high proportion of positive dogs in the group without gastrointestinal disease can also be partly explained by the age of animals studied. In this group, the age of all dogs and that of positive dogs was markedly higher than the age of negative dogs (medians; eight years, eight years and 2.25 years, respectively). Older animals are more likely to encounter *L. intracellularis* during their lifetime than younger dogs. Tomanova (2003) found a significantly higher proportion of positive serum samples in older domestic pigs and horses when compared with younger animals. On the other hand, the humoral immune response of pigs against the *L. intracellularis* infection is believed to be short-lived, though in some individuals it may be detectable up to 13 weeks after exposure (Knittel et al., 1998; Guedes and Gebhart, 2003). To our

knowledge, the only report concerning the persistence of IgG antibodies to *L. intracellularis* in dogs comes from a patient with IBD, where all five blood samples collected over a period of 243 days were serologically positive (Tomanova et al., 2003a).

The source of infection for dogs remains unknown. Most dogs included in this study were individually kept animals fed commercial dog food or homemade food. Contact with pigs or feeding raw pork meat to these dogs were highly improbable. However, some studies indicate that *L. intracellularis* is probably much more widespread in different animal species and thus in the environment than previously thought (Herbst et al., 2003; Tomanova et al., 2003b).

Since the antibodies merely indicate exposure to the bacterium and are not necessarily correlated to clinical disease, it is not possible to decide whether the presence of antibodies to *L. intracellularis* in our dogs reflected the cause of the observed gastrointestinal signs or was merely a sequel of a previous infection, subclinical infection or transient passage of the organism. However, the high prevalence of antibodies shows that dogs probably represent another important host species of *L. intracellularis.* Clinical and pathological aspects of the *L. intracellularis* infection in dogs certainly merit further investigation.

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