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Serological survey of canine leptospirosis in Croatia - the changing epizootiology of the disease

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ABSTRACT

Canine leptospirosis is a well known zoonotic infection with worldwide distribution. The serovars Canicola and Icterohaemorrhagiae have traditionally been responsible for most cases of canine leptospiroses. The use of widely available bivalent vaccines containing those two serovars has greatly reduced canine leptospirosis. However, re-emergence of the disease has been detected in Europe and North America, partly due to changes in the infecting serovars. The aim of this study was to determine the prevalence of the presumed infective serovars in dogs in Croatia. During a period of four years (2006-2010), 151 canine sera were submitted to the Laboratory for Leptospires, Faculty of Veterinary Medicine, University of Zagreb. Using a microscopic agglutination test (MAT), 57 (37.7%) seropositive sera were detected. The most prevalent presumed infective serovars, in decreasing order, were: Pomona, Grippotyphosa, Icterohaemorrhagiae, Australis, Saxkoebing and Hardjo. Results showed that most infections were caused by serovars not covered by the vaccine, which raises questions concerning its efficacy in preventing leptospirosis in dogs.

Key words: leptospira, canine leptospirosis, seroprevalence, infecting serovar

Introduction

Leptospirosis is a bacterial disease occurring worldwide in many animal species and humans. It is caused by pathogenic members of the *Leptospira* genus. The diversity of the *Leptospira* genus, that comprises around 300 different serovars organized into 28 serogroups, additionally complicates the diagnosis and prevention of canine leptospirosis. Each serogroup is composed of several closely related serovars, which have similar

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antigenic determinants on their outer membrane. These outer membrane antigens are responsible for the induction of agglutinating antibodies. Animals and humans acquire the infection after contact with infected urine or a contaminated environment. Following infection, leptospires colonize the renal tubules and are excreted via the urine into the environment, where they can survive for as long as six months, in favourable conditions. An important factor in maintaining leptospires in the wild are maintenance hosts, usually various rodent species, which are typically asymptomatically infected and shed leptospires in urine for a very long time (FAINE et al., 1999). In contrast, accidental hosts, such as dogs, humans and other animals, suffer a wide range of clinical manifestations, from milder cases, with pyrexia, anorexia and vomiting, to more serious symptoms, such as hepato-renal failure and severe pulmonary haemorrhage (HARKIN and GARTRELL, 1996; FAINE et al., 1999; GEISEN et al., 2007; KOHN, 2010).

The *Leptospira interrogans* serovars Canicola and Icterrohaemorrhagiae are traditionally responsible for most cases of canine leptospirosis (FAINE et al., 1999). During the last thirty years, a bivalent vaccine containing the bacterins of these serovars has been widely used in the prevention of the disease, resulting in a decline in its prevalence. However, in the last decade, many countries have noted the re-emergence of canine leptospirosis and leptospires from other serogroups (Grippotyphosa, Pomona, Sejroe, Australis) have been confirmed as causative agents (SCANZIANI et al., 2002; WARD et al., 2004; MOORE et al., 2006; GEISEN et al., 2007; STOKES et al., 2007). The observed re-emergence of canine leptospirosis is probably associated with changes in the infecting serovars (ALTON et al., 2009). Due to the diversity of agglutinins on the outer membrane, protection provided by the current vaccines is restricted to the serogroups used in their production. Therefore, the efficacy of the vaccines currently available is questionable (SCANZIANI et al., 2002; ANDRE-FONTAINE, 2006; GEISEN et al., 2007). The purpose of this study was to determine the seroprevalence of leptospira serovars in the canine population in Croatia.

Materials and methods

Serum samples from 151 dogs were submitted to the Laboratory for Leptospires, Faculty of Veterinary Medicine, University of Zagreb, during a period of four years (January 2006 to September 2010). The majority of samples originated from dogs with some form of clinical disease, mostly hepato-renal lesions. A microscopic agglutination test (MAT) was performed following standard procedure (DIKKEN and KMETY, 1978; HARTSKEERL et al., 2006) to determine antibody titres against 12 Leptospira serovars: Grippothyphosa, Sejroe, Australis, Pomona, Canicola, Icterohaemorrhagiae, Tarassovi, Saxkoebing, Ballum, Bataviae, Poi and Hardjo. The breed, age, gender, living environment and sample submission date for each dog were recorded.

Seropositive samples were divided into two groups: (A) dogs positive for leptospirosis with titres of ≥1000, or four-fold or greater rise of a titre in the second sera and (B) seropositive, but not necessarily diseased dogs, with titres from 100 to <1000. For the purpose of investigating seropositivity, all dogs with titres ≥100 were included (groups A and B). But for the purpose of identifying the most prevalent infective serovars, we focused on dogs diagnosed with acute leptospirosis (group A).

Results

Of 151 dog sera, 57 (37.7%) were seropositive. Of the 57 seropositive dogs, 26 (45.6%) belonged to group A and 31 (54.4%) to group B. In both groups, almost all the seropositive dogs exhibited positive MAT titres to more than one serovar. In group A, the exception was one seropositive dog, which was positive only to the serovar Pomona (1600). In group B, only four dogs had single titres from 100 to 500, to serovars Grippotyphosa (2), Canicola (1), and Australis (1).

Serovars against which the sera tested from group A agglutinated in the highest titre were as follows: Pomona (8/26, 30.8%), Grippotyphosa (5/26, 19.2%), Icterohaemorrhagiae (4/26, 15.4%), Australis (4/26, 15.4%), Saxkoebing (1/26, 3.8%) and Hardjo (1/26, 3.8%). Titres ranged from 1000 to 6400. Three samples from group A agglutinated to more serovars in equally high titres: Australis/Pomona (1/26, 3.8%), Grippotyphosa/Pomona/Canicola (1/26, 3.8%) and Grippotyphosa/Australis (1/26, 3.8%). In 11/26 (42.3%) of samples from group A, the highest titre for a single serovar was two or more dilutions more than the second highest titre for other serovars. In the other 14/26 (53.8%), there were fewer than two dilutions between the highest and second highest titres.

The dogs in group B displayed titres against the all serovars tested (14 Grippotyphosa, 13 Australis, 7 Ballum, 7 Icterrohaemorrhagiae, 5 Hardjo, 4 Poi, 4 Pomona, 3 Saxkoebing, 3 Canicola, 1 Bataviae, 1 Tarassovi).

The most common breeds among the seropositive dogs (groups A and B) were: mixed breed (20/57, 35.1%), followed by German Shepherd (5/57, 8.8%), Hungarian Viszla (4/57, 7.0%), English Setter (3/57, 5.3%), Rough Collie (2/57, 3.5%) Dachshund (2/57, 3.5%) and one dog from each of the following breeds: Belgian Shepherd, Great Dane, Labrador Retriever, Doberman Pinscher, Golden Retriever, Bernese Mountain Dog, Standard Poodle, Pekingese, Standard Schnauzer, Saint Bernard, Beagle, Pitt Bull Terrier, German Pointer, Alaskan Malamute and English Pointer. Since data on the ages of the dogs were obtained for only half the samples, they were excluded from interpretation. Of 57 seropositive dogs, 37 (64.9%) were male and 20 (35.1%) female. The majority of seropositive dogs (35/57, 61.4%) were from a rural environment, while 21/57 (36.8%) dogs were from an urban environment.

Positive samples from group A were evenly distributed throughout the year (one to two positive samples during each month of the year) with a slightly higher frequency of seropositive samples detected in August, September and October (3, 5 and 4 cases respectively). Only in March were no cases detected.

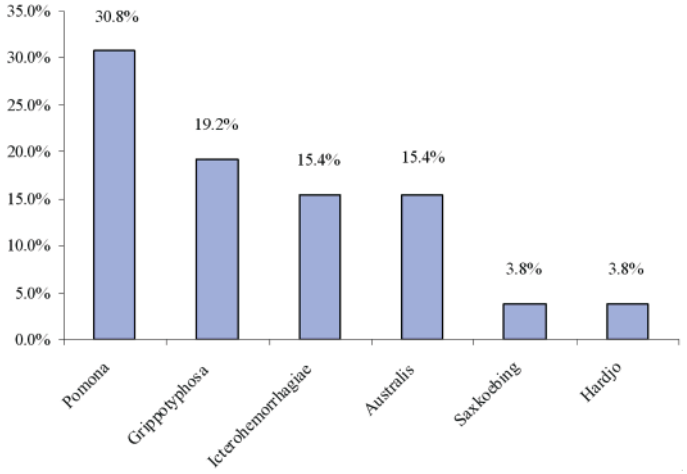

Fig. 1. Percentages of infective leptospira serovars

Discussion

Of the 151 dog sera tested in this survey, 57 (37.7%) were seropositive. This result hardly reflects the average seroprevalence of the entire dog population of Croatia, because sera were not sampled randomly, but taken exclusively from dogs showing some form of clinical disease, mostly hepato-renal symptoms. If random sampling had been performed, the seropositivity level would probably have been lower.

The most prevalent infecting serovars in group A were, in decreasing order: Pomona, Grippotyphosa, Icterohaemorrhagiae, Australis, Saxkoebing and Hardjo. Five of these six detected serovars are not included in vaccines currently used in Croatia. Three sera tested agglutinated in equally high titres to two or more serogroups (Australis/Pomona-2000, Grippotyphosa/Pomona/Canicola-2000, Grippotyphosa/Australis-1000), which was probably the result of co-agglutination, although there was also a possibility of infection by more than one serovar. The accurate identification of the infecting serovar requires the isolation of leptospires rather than serology, but this is rarely feasible. Samples are usually unsuitable for haemoculture, either because of delayed submission to the lab, or animals being treated with antibiotics prior to sampling. In 42.3% of the sera tested,

the highest titre for a single serovar was two or more dilutions more than the second highest titre of the other serovars, which provides a high level of certainty in identifying an infecting serovar. In analyzing the prevalence of the infective serovars, we focused on group A because these dogs had been diagnosed with acute leptospirosis, proving that these infecting serovars were capable of causing acute disease. This was important when estimating if these serovars should form potential components of a vaccine against canine leptospirosis. Moreover, in this way, the possibility of detecting co-agglutinations rather than infecting serovars was reduced to the minimum.

The vaccination histories of the dogs were unknown. However, antibodies to *L. interrogans* serovar Canicola were detected in only one dog, in combination with equally high titres to Pomona and Grippotyphosa. This indicates that the titres detected in this dog were not vaccinal. The possibility that vaccination induced equally high titres (2000) to serogroups Canicola, Pomona and Grippotyphosa in the only dog with antibodies against the serovar Canicola could be ruled out, because the dog had not been vaccinated against leptospirosis during the three months before sampling, and had a very low titre to another vaccinal strain, Icterrohaemorrhagiae. Very high titres of vaccinal serovars can only be induced by field infection or very recent vaccination (which was not the case in this study). Furthermore, all dogs with high titres to the serovar Icterrohaemorrhagiae had no antibodies to the serovar Canicola, not even in low titres. This led to the conclusion that all the titres detected in group A were the consequence of field infections, not vaccination.

The breed distribution identified in this study roughly represents the most prevalent breeds in Croatia. Most of the purebred seropositive dogs belonged to large and/or hunting breeds, which are often predominant in cases of canine leptospirosis, probably because they generally spend more time outside than smaller dogs, increasing the possibility of contact with urine-contaminated environments and subsequently acquiring infections. Almost two thirds of the seropositive dogs were male (65%), which can probably be explained by their tendency to roam more, which also increases the possibility of exposure to infection. Approximately two thirds (61.4%) of the seropositive dogs lived in rural areas and about one third (36.8%) in urban environments. The higher prevalence of canine leptospirosis in rural, compared to urban, areas has already been reported (GHNEIMA et al., 2007). This finding is probably the result of dogs in rural environments coming into contact with wildlife more often, especially with small rodents, as the major carriers of leptospires. In this survey, the relatively even distribution of leptospira cases (Group A) throughout the year was recorded, in contrast to the disease's typical seasonal distribution, with peak prevalence from late summer to autumn (WARD et al., 2002). The seasonal distribution of samples from group B was of little importance, because low titres usually represent residual titres, so it is impossible to tell exactly when infection occurred.

Traditionally, the serovars Icterohaemorrhagiae and Canicola were incriminated in most cases of canine leptospirosis, characterised by acute or subacute hepatic and renal failure (FAINE et al., 1999). In the 1970s, a bivalent vaccine against these two serovars was introduced in Europe and the USA. This is probably the reason for the decrease in the prevalence of canine leptospirosis caused by these serovars. However, the vaccine does not induce immunity against most serovars belonging to other serogroups. This may have led to a subsequent alteration in the infection rate caused by various Leptospira serogroups currently associated with canine leptospirosis. In the USA, mainly Grippotyphosa, Pomona, Bratislava and Autumnalis are identified today, based on seroreactivity in clinically ill dogs (BIRNBAUM et al., 1998; GOLDSTEIN et al., 2006). In Europe, data on canine leptospirosis is generally scarce. However, in the last decade, the *L. interrogans* serovars Bratislava and Grippotyphosa have been identified as the most prevalent in Italy (SCANZIANI et al., 2002), Grippotyphosa, Saxkoebing and Icterohaemorrhagiae in Germany (GEISEN et al., 2007) and Australis, Bratislava, Grippotyphosa and Pomona in Switzerland (FRANCEY, 2010). Those findings raise questions about the efficacy of the currently used vaccine in protecting against the disease (SCANZIANI et al., 2002; ANDRE-FONTAINE, 2006; GEISEN et al., 2007).

The most important factor in preserving natural sources of infection are various small rodent species, which serve as reservoir hosts for leptospires and exhibit potentially lifelong urinary shedding. In favourable environmental conditions, leptospires can remain infective for as long as six months. Dogs are often directly or indirectly exposed to such contaminated environments. Studies conducted in Croatia have demonstrated that between 7.0% and 29.9% of small rodents, depending on the area investigated, are leptospira carriers (BORČIĆ et al., 1982 and 1983; MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010). The serovars Mozdok and Tsaratsovo from serogroup Pomona, Bratislava and Lora from serogroup Australis, Saxkoebing and Istrica from serogroup Sejroe, Grippotyphosa from serogroup Grippotyphosa and Bataviae from serogroup Bataviae, have already been isolated from small rodents in Croatia (MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010). They probably all pose a threat of infection to dogs, other animals and humans.

The control of canine leptospirosis is important in canine health management, as well as in reducing the spread of the disease. Human leptospirosis is usually associated with occupational exposure and outdoor recreational activities, but also with direct contact with shedding companion animals (TREVEJO et al., 1998; JANSEN et al., 2005). Although the first goal of leptospirosis vaccination in dogs is to prevent clinical disease, the prevention of renal carriage is also essential, due to its zoonotic potential and the public health risk involved. It is well known that the clinical resolution of acute infection may lead to asymptomatic shedding. In addition, there is evidence to suggest that clinically healthy

dogs can be chronic carriers, shedding leptospires via their urine into the environment. Leptospiruria has been detected in 7.05% of domestic dogs in Ireland (ROJAS et al., 2010), 8.8% of dogs in the United States (HARKIN et al., 2003) and 22% of dogs in Iran (ZAKERI et al., 2010). However, reports on the protection of leptospiral bacterins against the renal carrier state differ (ANDRE-FONTAINE et al., 2003; SCHREIBER et al., 2005).

As the global control of canine leptospirosis is hardly feasible through controlling natural sources of infection, vaccination is still undoubtedly the best method of disease prevention. Nevertheless, due to an obvious alteration in the serovars causing the disease, vaccines containing the most prevalent infective serovars would certainly be more efficient in the prevention of canine leptospirosis than the current vaccine.

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ŠTRITOF MAJETIĆ, Z., J. HABUŠ, Z. MILAS, V. MOJČEC PERKO, V. STAREŠINA, N. TURK: Serološko istraživanje leptospiroze u pasa u Hrvatskoj - mijenjanje epizootiologije bolesti. Vet. arhiv 82, 183-191, 2012.

SAŽETAK

Leptospiroza u pasa je zoonoza proširena diljem svijeta. Najčešći uzročnici bolesti u prošlosti bili su serovarovi Canicola i Icterohemorrhagiae. Uporabom vakcine koja sadržava ta dva serovara znatno je smanjena pojavnost bolesti. Međutim, u posljednje vrijeme zabilježen je porast slučajeva bolesti u pasa u Europi i Sjevernoj Americi, dijelom i zbog promjene prevalencije vjerojatno infektivnih serovarova. Cilj ovog istraživanja bio je ustanoviti učestalost infektivnih serovarova u pasa u Hrvatskoj. Tijekom četiri godine (2006. - 2010.) Laboratorij za leptospirozu Veterinarskog fakulteta Sveučilišta u Zagrebu zaprimio je 151 uzorak pasjeg seruma. Mikroskopskim aglutinacijskim testom (MAT) ustanovljeno je 57 (37,7%) pozitivnih uzoraka. Najučestaliji zarazni serovarovi bili su Pomona, Grippotyphosa, Icterohemorrhagiae, Australis, Saxkoebing i Hardjo. Rezultati su pokazali da je većina infekcija u pasa bila uzrokovana serovarovima koji nisu sadržani u cjepivu što dovodi u pitanje njezinu učinkovitost u prevenciji bolesti.

Ključne riječi: leptospira, leptospiroza u pasa, seroprevalencija, infektivni serovar