



Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Letter to the Editor

Agglutinating antibodies against pathogenic *Leptospira* in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections

1. Introduction

Clinical leptospirosis in dogs is often associated with recent exposure, directly, or indirectly, to surface water contaminated by brown rat urine. Interestingly, Weil's disease, the most prominent clinical presentation of leptospirosis mostly occurs as an individual case, although more individuals have usually been exposed to the same presumed source. In horses, clinical leptospirosis has a low incidence and the main clinical presentation probably is abortion in a limited number of mares on a stud, typically without a likely or identified source of infection. Furthermore, Equine Recurrent Uveitis (ERU) apparently associates with intra-ocular antibodies against *Leptospira* (Wollanke et al., 1998). Interestingly, serovar Grippotyphosa is clearly predominant among *Leptospira* isolates from ERU-eyes (Hartskeerl et al., 2004), which is supported by the results from testing for intra-ocular antibodies using the Microscopic Agglutination Test (Houwens et al., unpublished).

Serosurveys among domestic animals are limited, but recent studies available show that agglutinating antibodies may occur in healthy animals and that there is considerable variation among the predominant serovars (Gautam et al., 2010; Båverud et al., 2009; Iwamoto et al., 2009; Stokes et al., 2007). It is unclear what influence the biotope in which the surveyed animals live bears on the survey results, e.g. is infection with a certain serovar associated with the way specific animal species live and where they live or is the exposure more or less ubiquitous. Since leptospires are considered to be able to survive in the environment for longer periods of time provided there is sufficient moisture and ambient temperatures are moderate, it is likely that exposure is related to the type of environment the animal lives in. Evidently, the occurrence of specific serovars is also depending on the presence and prevalence of specific host species that act as infection reservoir. Together, these factors are responsible for the considerable differences in the outcomes of serological surveys between continents and geographical areas within continents.

Pet dogs live in close contact with man and share a considerable part of his environment, but in their typical behaviour when they are being walked and particularly

when they are let loose they also penetrate the biotope of a variety of other, mainly small, mammals.

Horses typically behave differently and live in a different environment which usually combines pasture and a stable, but these biotopes are also shared with a number of small mammals. In winter, most horses are fed roughage which is almost inevitably contaminated by mouse, and often rat, urine. So, dogs as well as horses are, mainly indirectly, exposed to animals that can be reservoirs of leptospires. Some leptospiral serovars are associated with particular reservoir species, e.g. *Icterohaemorrhagiae* and *Copenhageni* with brown rats (*Rattus norvegicus*), whereas other serovars have several, or as yet unknown, reservoir species.

In order to gain insight into the level of exposure to pathogenic leptospires present in the environment, we performed a serosurvey using the Microscopic Agglutination Test (MAT) among healthy horses and dogs living in the Netherlands. Generally, dogs and horses share only a part of their environments and they have totally different feeding patterns – carnivorous versus herbivorous – hence they are exposed to different environmental sources and routes of infection. Since man shares his environment with dogs and horses, the results of this survey may also shed light on the potential exposure of humans.

2. Materials and methods

During the spring of 2003, blood samples taken by local veterinary practitioners in the framework of routine clinical procedures from 112 healthy dogs and 112 horses were made available with their owners consent. Among the dog samples, 86 were obtained through 3 veterinary clinics with a mainly urban patient population situated in various parts of the country. Sera were collected at random, provided the dogs had no complaints suggestive of an infectious disease. In addition, 26 sera from healthy dogs in a non-vaccinated hunting pack were included.

During the autumn of 2009, a total of 100 dog samples were obtained by random collection of blood samples submitted to a clinical chemistry laboratory by veterinary practitioners across the Netherlands; samples were only enrolled if they had accompanying clinical information that did rule out the likelihood of the presence of an infectious disease.

The ages of the dogs ranged from 7 months to 16 years. Apart from those in the hunting pack, the vaccination statuses of the dogs were unknown.

The horse serum samples were obtained from two different geographical areas in the Netherlands, i.e. the west of country with clay-type soils with many permanently water-filled ditches surrounding the pastures ($n = 58$), and the eastern part with sandy soils and pastures mainly surrounded by fences ($n = 54$). The maximum number of samples per owner or establishment was four. Since a vaccine for horses is not available, no horse ever received leptospiral vaccination.

Sera were stored at -20°C until tested with MAT at the WHO/FAO/OIE and National leptospirosis reference-laboratory at KIT Biomedical Research in Amsterdam (all sera were tested within 6 months after collection). The standard Dutch leptospire panel consisting of 16 strains (15 serovars) was employed. The panel included the non-pathogenic serovar Patoc, which is incorporated for its broad cross-reactivity. Since the absence of agglutination at serum dilution 1:20 was clearly detectable and a number of sera clearly showed no agglutination with any strain, titers of 1:20 or higher were considered specific and thus positive.

3. Results

3.1. Dogs

Of the total of 86 healthy family dogs, 7 agglutinated serovar Canicola; they also agglutinated serogroup Icterohaemorrhagiae (serovars Copenhageni and Icterohaemorrhagiae). Since the available leptospiral vaccines only contain serogroups Canicola and Icterohaemorrhagiae and serogroup Canicola is considered virtually extinct in the Netherlands as a consequence of the relatively high degree of vaccination (Hartman, 1984) these dogs were considered to have been vaccinated recently and their results were removed from the data. Of the remaining 79 dogs 57 (72%) had reciprocal titers ranging from 20 to 640 against at least one of the serovars in the panel; the most commonly agglutinated serovar was Copenhageni, closely followed by Patoc and to a much lesser degree Icterohaemorrhagiae.

Of the total of 100 dog samples obtained in 2009, 7 had MAT titers against serovars Canicola, Icterohaemorrhagiae and Copenhageni. These were also removed from the data for the above reason. Of the remaining total of 93 samples, 30 (32%) had MAT titers against one or more serovars with Copenhageni being predominant, also showing the highest titer (1:320).

A total of 22 of the 26 (87%) dogs in the hunting pack had titers: serovar Patoc was most commonly agglutinated, closely followed by Copenhageni and Bratislava, with Saxkoebing and Sejroe at some distance. Notably, these dogs have never been vaccinated.

3.2. Horses

A total of 89 of 112 horses (79%) had agglutinating antibodies against one or more serovars with Copenhageni and Patoc being predominant, followed by Bratislava and Grippotyphosa. Highest titers found were against Bratislava (1:320 and 1:640). The results for the two different biotopes – east/dry versus west/wet – did not clearly differ.

Note that titers against the saprophytic serovar Patoc do not signify homologous infection but indicate infection with a cross-reacting pathogenic serovar.

4. Discussion

In view of the likelihood of a certain degree of cross-reactivity between the strains in the panel, it is impossible to extract strain prevalences from these data, hence conclusions are restricted to the relative predominance of strains.

With respect to the horse samples, the results clearly show that antibodies against pathogenic serovars occur in horses that are healthy according to their owners. Even if it is argued that titers of 1:20 or 40 are less specific, this conclusion still stands because higher titers were also recorded. Since horses in the Netherlands are not vaccinated against leptospirosis, the presence of these antibodies can only be explained by the occurrence of subclinical infections, consequently that the average equine biotope in the Netherlands associates with exposure to pathogenic leptospires. Notably, the dominant serovar is Copenhageni which, together with serovar Icterohaemorrhagiae, is associated with most cases of Weil's disease in humans and dogs. Thus, horses are exposed to Copenhageni and other pathogenic serovars, but infections apparently take a subclinical course in this host species.

The results of the three groups of dog samples also show predominance of Copenhageni, although the prevalence varied between the groups. Since most dogs in this study may have been vaccinated before sampling there is a chance of a vaccination bias. However, by removing the 14 dogs that agglutinated serovars Canicola and either Icterohaemorrhagiae or Copenhageni or both (closely related serovars that both belong to serogroup Icterohaemorrhagiae) the possibility of vaccination bias was strongly reduced. Moreover, the dogs in the non-vaccinated dog pack showed clear Copenhageni agglutinating titers. And, in addition, the results of the – unvaccinated – horses demonstrate that exposure to Copenhageni is common. Together, these findings suggest that the prevalence of antibodies and the predominance of Copenhageni are not caused by vaccination but are the result of subclinical infections. There is no straightforward explanation for the difference in frequency of MAT-positivity between the samples of 2003 and 2009, but a seasonal effect has been demonstrated (Hartman, 1984; Gautam et al., 2010) and differences in exposure over the years are likely. Nevertheless, the results of the dog samples show that dogs in their average biotope are also exposed to pathogenic *Leptospira* and that they more or less regularly undergo subclinical infections. In addition, the predominance of Copenhageni agglutination is similar to that in horses which indicates that the brown rat – the main reservoir of this serovar – contaminates both biotopes leading to similar levels of exposure. Our findings are fully in concordance with a recent paper, which reported a 7% excretion rate of pathogenic leptospires by healthy dogs from sanctuaries in Ireland, which suggests a considerable seroprevalence (Rojas et al., 2010). The logically arising question is why only very few dogs (and horses) apparently develop clinical disease while others might adapt to a

commensal relationship. Basically, this could be determined by species-specific or individual host factors, or, alternatively, by differences in the infection doses or differences within the infecting serovar.

In conclusion, this study shows that dogs and horses in the Netherlands are commonly exposed to and regularly subclinically infected by pathogenic *Leptospira*. Since humans, dogs and horses in part share their biotopes, humans are also basically exposed. Note that most humans generally do not behave like dogs and horses; their hygiene will presumably result in fewer infections. These findings are in line with recent observations of others clearly demonstrating that also humans may experience sub-clinical infection with pathogenic leptospires and that only few of those exposed and infected fall ill (Speelman and Hartskeerl, 2008)

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2 August 2010