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Distribution of *Leptospira* Serogroups in Dogs from Berlin, Germany

Anne Mayer-Scholl,[1] Enno Luge,[1] Angelika Draeger,[1] Karsten Nöckler,[1] and Barbara Kohn[2]

Abstract

Leptospirosis is a bacterial zoonosis in which dogs can act as a reservoir for human infection. The annual vaccination of dogs can prevent leptospirosis caused by serovars included in the vaccine. To date, all available vaccines in Germany include only the serovars Icterohaemorrhagiae and Canicola, the most commonly found serovars prior to the introduction of the leptospirosis vaccines. Yet, the involvement of additional serovars in the clinical presentation of leptospirosis in dogs has been described. The objective of this sero-epidemiological study was to examine the different *Leptospira* serovars currently circulating in a population of dogs suspicious for leptospirosis from Berlin. In 329 dogs presenting at the Small Animal Clinic in Berlin, the predominant serogroup was Australis (24%), followed by Grippotyphosa (20%) and Pomona (9%). A total of 18% of the dogs were diagnosed with clinical leptospirosis; here the most prevalent serogroups were also Australis (28%), Grippotyphosa (18%), and Pomona (14%). The serovar prevalence data presented here confirm that a change of pattern of infecting *Leptospira* serovars in dogs has taken place in Berlin. This data corresponds to further sero-epidemiological studies from other regions in Germany. To ensure human and canine health, available vaccines should be adapted to include the most important circulating serovars.

Key Words: Leptospirosis—Dogs—Vaccine—Germany—Serology—Microagglutination test.

Introduction

Leptospirosis is a bacterial zoonosis caused by spirochetes of the genus *Leptospira. Leptospira* have been isolated from many animal species, including rodents, pigs, cattle, and dogs. The spectrum of human and canine disease is variable and can range from subclinical infection to severe signs of multiorgan dysfunction (Sykes et al. 2011). Dogs can act as a reservoir for human infection and may be an important source of human outbreaks (Levett 2001).

Historically, *L. interrogans* serovars Icterohaemorrhagiae and Canicola were responsible for most cases of canine leptospirosis worldwide. The global incidence of illness attributed to these serovars has decreased in the past years. The common serovars seen today in Europe and the United States include Grippotyphosa, Bratislava, and Pomona (Sykes et al. 2011).

To date, all available vaccines in Germany include only the serovars Icterohaemorrhagiae and Canicola. Only 1 vaccine combining the 3 serovars Grippotyphosa, Icterohaemorrhagiae, and Canicola has obtained a license at the Paul Ehrlich Institute and will be available from 2012. Because there is little or no cross-immunity between *Leptospira* serovars (André-Fontaine 2006), inclusion of circulating serovars in a vaccine is needed for canine, and thus human, protection. The objective of this sero-epidemiological study was to examine the different *Leptospira* serovars currently circulating in a population of dogs from Berlin and Brandenburg suspicious for leptospirosis.

Materials and Methods

At the Small Animal Clinic, Freie Universität Berlin, dogs with acute renal failure or hepatopathy are regarded as suspicious for leptospirosis if other causes such as toxin ingestion or acute pancreatitis are ruled out or seem unlikely. For this study, patients from the Berlin and Brandenburg region presenting at the clinic from October, 2006, to January, 2011, were included. Serological diagnosis of leptospirosis was performed by microagglutination test (MAT) according to the standard protocol (Office International des Epizootics 2008), with a log2 dilution series between 1:25 and 1:25,600. The

[1]Federal Institute for Risk Assessment, Berlin, Germany.
[2]Clinic of Small Animals, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany.

following 17 serovars comprising 13 serogroups (in parentheses) were used: *L. interrogans* serovar Australis (Australis), *L. interrogans* serovar Bratislava (Australis), *L. interrogans* serovar Autumnalis (Autumnalis), *L. interrogans* serovar Bataviae (Bataviae), *L. interrogans* serovar Canicola (Canicola), *L. interrogans* serovar Copenhageni (Icterohaemorrhagiae), *L. interrogans* serovar Icterohaemorrhagiae (Icterohaemorrhagiae), *L. interrogans* serovar Hardjo (Sejroe), *L. interrogans* serovar Hebdomadis (Hebdomadis), *L. interrogans* serovar Pomona (Pomona), *L. interrogans* serovar Pyrogenes (Pyrogenes), *L. interrogans* serovar Saxkoebing (Sejroe), *L. borgpetersenii* serovar Tarassovi (Tarassovi), *L. borgpetersenii* serovar Ballum (Ballum), *L. borgpetersenii* serovar Javanica (Javanica), *L. borgpetersenii* serovar Sejroe (Sejroe), and *L. kirschneri* serovar Grippotyphosa (Grippotyphosa).

Because more than 80% of all dogs presenting at the clinic are vaccinated against leptospirosis (B. Kohn, unpublished data), antibody titers against Canicola, Icterohaemorrhagiae, and Copenhageni (due to its cross-reactivity with Icterohaemorrhagiae) were considered vaccination titers. To assess exposure to the locally circulating *Leptospira* serovars, antibody titers of ≥1:100 in all nonvaccine serovars were considered positive. To minimize assay random variation, animals with vaccination titers were only defined as having had contact with other *Leptospira* serovars when the nonvaccine serovar titer was at least 2 dilutions higher than the vaccination titer.

Criteria for clinical leptospirosis were acute renal failure or acute hepatopathy associated with 1 or more of the following laboratory findings:

1. MAT titers of ≥1:800 against the nonvaccine serovars with a negative or low vaccination titer.
2. A greater than 2-fold rise of nonvaccine serovar titers within 2–3 weeks.
3. A positive PCR result in urine or whole blood.

A duplex PCR and a lipl32 PCR were performed, as described by Mayer-Scholl et al. (2011).

Results and Discussion

A total of 329 dogs presenting with signs suspicious for leptospirosis were included in the study. The mean age of the study population was 7.3 years, with 4% of the dogs aged less than a year; 30% were between 1–5 years, 44% 5–10 years, and 20% older than 10 years. Age was unknown for 2% of the dogs. In the sample, 39% of the dogs were male, 14% male-neutered, 30% were female, and 17% female-neutered. The sex was not recorded in 0.6% of the dogs.

Of the study population, 32% (106) were MAT positive for 1 of the vaccination serovars (titers between 1:100 and 1:3200, median 1:100); 81 dogs (25%) had had contact with locally circulating *Leptospira* serovars, excluding the vaccine serovars. These titers ranged between 1:100 and 1:25,600, with a median of 1:800.

The distribution of the serogroups in the dogs that had contact with *Leptospira* other than the vaccination serogroups is shown in Figure 1. The predominant serogroup was Australis (24%), followed by Grippotyphosa (20%) and Pomona (9%). Furthermore, 21% of the dogs showed mixed reactivity that included the serogroup Australis (Australis/Grippotyphosa [13%], Australis/Pomona [4%], Australis/Grippotyphosa/Pomona [4%]). In all dogs where Australis was the most common infecting serogroup, the serovar Bratislava induced the highest MAT titer.

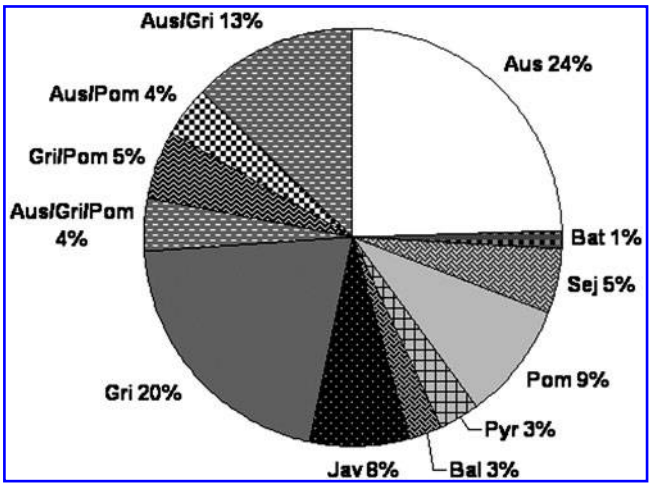

FIG. 1. The distribution of *Leptospira* serogroups in dogs presenting with signs suspicious for leptospirosis. MAT results for dogs that had contact with *Leptospira* other than the vaccination serogroups. Aus, Australis; Bat, Bataviae; Bal, Ballum; Gri, Grippotyphosa; Jav, Javanica; Pom, Pomona; Pyr, Pyrogenes; Sej, Sejroe.

In all, 58 of the 329 tested dogs (18%) met the criteria for the diagnosis of clinical leptospirosis. Amongst these dogs, 55% (32) had a positive vaccination titer (median, 1:200; range, 1:100–1:3200) and 84% (49) were seropositive for 1 or more nonvaccination serovars (median, 1:1600; range, 1:200–1:25,600). These data underline that the vaccines are not cross-protective against other serogroups. Dogs aged 6–10 years were mostly affected (50%), followed by the age groups 1–5 years (29%) and <1 year (12%). The most prevalent serogroup was Australis (28%), followed by Grippotyphosa (18%) and Pomona (14%). Of the dogs with clinical leptospirosis, 24% showed mixed MAT titers, including Australis and Grippotyphosa (Australis/Grippotyphosa [18%], Australis/Grippotyphosa/Pomona [6%]).

In comparison to the MAT, PCR enables acute-phase diagnostics. Forty-seven dogs diagnosed with leptospirosis were examined by PCR; 17 of these dogs had a positive PCR result in either urine or blood samples. Among the PCR-positive dogs, 7 had not developed an antibody response yet.

MAT data can only give a broad idea of the common serogroups present in a population, but cannot be interpreted reliably in individual patients (Levett 2003). Typing of dog isolates could provide better information of the leptospirosis situation, but isolation is difficult because antibiotic therapy had been performed in more than 50% of the dogs presenting with signs suspicious for leptospirosis (B. Kohn, unpublished data). In studies from the 1950s in Germany, the most prevalent serogroups found in dogs suspicious for leptospirosis were Canicola and Icterohaemorrhagiae (Mochmann 1957). Ten years later, a shift of prevalence of infecting *Leptospira* serovars was described, with Grippotyphosa constituting the most common infecting serovar in dogs from Berlin, followed by Canicola and Icterohaemorraghiae (Horsch and Horsch 1957). More recent studies in dogs with clinical leptospirosis

described Grippotyphosa and Sejroe (Geisen et al. 2007) as the most prevalent serovars in southern Germany. In northern Germany, the most common serogroups in dogs suspected of leptospirosis were Australis and Copenhageni (Gerlach and Stephan 2007).

The serogroup prevalence data presented here confirm that a change of pattern of infecting *Leptospira* in dogs has also taken place in the eastern region of Germany. Australis, Grippotyphosa, and Pomona have emerged as the predominant infecting *Leptospira* serogroups. It is generally considered that years of intensive vaccination with Canicola and Icterohaemorrhagiae have favored a shift in the causative serogroups. The change in the prevalence of infecting serogroups is also exacerbated by natural infection pressure from *Leptospira* strains shed into the environment by wild reservoir animals, such as rodents (*e.g.*, Bratislava, Grippotyphosa) and wild boar (Pomona) (André-Fontaine 2006, Jansen et al. 2007).

To protect dog owners against leptospirosis, veterinarians should be aware of the zoonotic potential of these leptospiral serogroups. Vaccines inducing protection against all major circulating strains should be licensed in the near future.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Address correspondence to:
Anne Mayer-Scholl
Federal Institute for Risk Assessment
Diedersdorfer Weg 1
12277 Berlin
Germany

E-mail: anne.mayer-scholl@bfr.bund.de