

SHORT REPORT

High number of asymptomatic dogs as leptospiral carriers in an endemic area indicates a serious public health concern

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SUMMARY

Asymptomatic dogs can be potential hosts of leptospirosis. However, the extension of this phenomenon in endemic areas has not yet been clearly defined. This study is aimed at evaluating the role of asymptomatic dogs as carriers of *Leptospira* in an endemic area of Brazil. A total of 131 male dogs without apparent leptospirosis symptoms were included in the study based on clinical and hematologic exams. Serum and urine samples were collected for microscopic agglutination tests (MAT) and polymerase chain reactions (PCR) targeted the *LipL32* gene, respectively. Forty-two dogs (32·1%) presented seroreactivity (titres ≥ 100). The serogroup Icterohaemorrhagiae was predominant, representing 92·7% of the seropositive samples. Overall, leptospiral DNA was detected on 26 urine samples (19·8%). PCR positivity was more common (28·6%) on seropositive dogs than on seronegative (15·7%) ones. Nevertheless, MAT was not correlated to PCR ($P > 0\cdot05$). Age was not associated with seroreactivity, but dogs older than 5 years of age had 4·07 more chances (odds ratio) of being carriers (PCR positive) than younger ones. Although the fact of knowing that asymptomatic dogs can act as leptospiral carriers is not new, the extension of this fact is impressive in an endemic region, and its role and impact on public health cannot be neglected.

Key words: Carriers, dog, *Leptospira*.

Leptospirosis is an emerging zoonotic disease caused by pathogenic species of the genus *Leptospira*. It affects domestic animals, wildlife, and humans [1]. Leptospire are maintained by carrier animals, which shed the bacterium in the urine. Humans become infected through direct or indirect contact with the urine of infected animals, by contact with water and/or contaminated soil [2, 3].

The dog's role as carriers has been increasingly studied, because it is known that they can act as a source of infection and therefore cause a problem for public health [1]. Although there is evidence that clinically normal dogs can be chronic carriers of infection (and thus maintenance hosts [3–5]), the extension of this phenomenon in endemic areas has not yet been clearly defined. Few studies were conducted in endemic regions, and a recent study conducted in Chile indicated that 19·3% of the asymptomatic dogs were reported to shed leptospire [3]. Considering this, this study aims at evaluating the role of asymptomatic dogs as carriers of *Leptospira* in an endemic area, through serological and molecular methods.

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We conducted this study after approval of the Ethics Committee of the Universidade Federal Fluminense (Fluminense Federal University) (UFF-Number 709). We studied 131 male dogs (due to the convenience of collecting samples by catheterization) without apparent symptoms of leptospirosis or any other infectious diseases in the region of São Gonçalo, located in the metropolitan region of Rio de Janeiro, Brazil. It is a low-income urban region, located at latitude: 22°49'37"S and longitude: 43°03'14"W, with a population of 1 044 058 and a population density of 4035, 90 hab/km². São Gonçalo is known to be endemic for leptospirosis and similar to other endemic regions in Brazil [6]. As an inclusion criterion, the dogs had not been vaccinated for leptospirosis in the last 12 months, in order to avoid interference on serologic tests [6].

All the animals proved healthy at the clinical examinations, which were always conducted by the same veterinarian. We performed a complete body condition examination, including variables such as temperature, hydration, renal sensitivity, mucosae color, tissue perfusion, and cardiorespiratory sounds. From each studied animal, blood, and urine were collected: blood samples, serum activity of ALT (alanine aminotransferase) and levels of urea as well as creatinine were gathered and a CBC (complete blood count) was performed in order to confirm the absence of alterations. Additionally, urine samples were collected by a urethral catheter (approximately 3 ml of urine), aliquoted (1 ml aliquots) in Eppendorf tubes with 100 µl of Phosphate buffered saline and kept on -20°C until tested as a batch.

A microscopic agglutination test (MAT) was conducted according to recommendations [7]. It was performed with a panel including eight serovars representing seven serogroups. The antigens used were *Leptospira interrogans* serovars Autumnalis (Akiyami A), Bratislava (Jez-Bratislava), Bataviae (Van Tienen), Canicola (Hond Utrecht IV), Grippotyphosa (Moska V), Icterohaemorrhagiae (RGA), Copenhageni (M 20), and Pomona (Pomona). Urine polymerase chain reactions (PCR) was conducted targeting the *lipL32* gene, referred to be specific for pathogenic leptospirosis [8]. Non-parametric variables were compared using Fisher's exact test. Odds ratio was also assessed for these analyses.

A total of 42 dogs (32.1%) presented seroreactivity (titres ≥ 100). Thirty dogs presented a maximum titer of 100, four of 200, seven of 400 and only one dog presented a titer of 800, indicative of clinical disease.

Table 1. Distribution of detection of anti-leptospiral antibodies (MAT) and leptospiral DNA (PCR) of asymptomatic dogs of different ages in the metropolitan area of Rio de Janeiro, Brazil

Age	N tested (%)	Seropositive (%)	PCRpos (%)
<1 y.o.	5 (3.9)	1 (2.4)	0
1–2 y.o.	17 (12.9)	6 (14.3)	3 (11.5)
3–5 y.o.	36 (27.5)	8 (19)	3 (11.5)
6–8 y.o.	25 (19.1)	12 (28.6)	6 (23.1)
9–11 y.o.	22 (16.8)	8 (19)	8 (30.8)
12–16 y.o.	26 (19.8)	7 (16.7)	6 (23.1)
Total	131 (100)	42 (100)	26 (100)

y.o., years old.

Reactions against strains of Icterohaemorrhagiae serogroup (Icterohaemorrhagiae RGA and Copenhageni M20) were predominant, representing 92.7% of the seropositive samples. Less frequently, reactions against Canicola were also observed (7.3%). In relation to PCR, leptospiral DNA was detected on 26 urine samples (19.8%). PCR results, which indicate the carrier status, were not associated to the serology ($P = 0.10$). From the 26 PCRpos samples, 12 (46%) were also seropositive, while among the 105 PCRneg, 75 (71%) were seronegative. Age was not associated to seropositivity ($P > 0.05$), but dogs older than 5 years of age presented 4.07 more chances (odds ratio) to be carriers (PCR positive) than the younger ones. Results are summarized in Table 1.

Although high, the seropositivity rate was not unexpected, as serosurveys conducted on endemic areas reported more than 20% of seroreactivity. Additionally, the predominance of serogroup Icterohaemorrhagiae was expected for that region, since it is the predominant serogroup not only for dogs [6, 9], but also for humans [10]. It drastically contrasts to a non-endemic area such as Belgium, where 6.5% of asymptomatic dogs tested positive (1/100) in the MAT [11]. Age has already been considered as an important associative factor for the seroprevalence among dogs, particularly after the dog reaches 4 years of age [12]. It is not surprising, as older dogs had more time to be in contact with the etiologic agent. Nevertheless, the influence of age on the possibility of being a carrier of leptospires (evidenced by PCR) had never been reported before.

An important point is that serology proved not to be a good method to identify asymptomatic carrier dogs. It has been suggested that, due to the poor positive predictive value of serologic testing to determine

whether a dog is actively shedding leptospire in the urine, it might be appropriate to consider using the PCR assay [4], which corroborates with our findings. Urinary PCR has been increasingly employed for animal leptospirosis worldwide. We hereby have demonstrated in a field study conducted in an endemic area that this tool is strongly recommended for the detection of carriers among asymptomatic dogs.

The most important outcome was the high number of leptospiral carriers among asymptomatic dogs. Despite the limitations of urine PCR, which is influenced by intermittency of shedding, barely 20% of the dogs were shedding leptospire at the moment of sampling. That outcome represents a serious public health risk. Similar studies were conducted in non-endemic regions. In those cases, rates of 8.2% were reported in the USA [4], 8% in Scotland [5] and 7% in Dublin [13], while in Germany only 1.5% of the dogs were shedding leptospire [2]. The contrast of those rates with our outcomes indicates that in tropical endemic regions the role of asymptomatic dogs as leptospiral carriers and their impact on public health cannot be neglected.

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DECLARATION OF INTEREST

None.

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