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SHORT COMMUNICATION

Serological Survey of Canine Leptospirosis in Southern China

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ABSTRACT

The present study conducts a serological survey on the presence of canine leptospirosis in domestic dogs using ELISA kit. A total of 314 household dogs (168 females and 146 males) in Chongqing, Kunming, Nanchang, Fuzhou, Guangzhou, Shenzheng, and Nanning in Southern China were examined. Of the 314 dogs, 23 (7.3%) were seropositive for leptospirosis. No statistically significant difference was observed in terms of sex and age in the seroprevalence of leptospirosis, but statistically significant differences were observed among different regional groups in the seroprevalence of leptospirosis ($P<0.05$). The results of this survey indicate that the control and treatment of leptospirosis have been effective in some cities of Southern China. However, further implementation of integrated strategies is necessary to prevent and control leptospirosis in dogs.

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INTRODUCTION

Leptospirosis, caused by *Leptospira*, is a widespread zoonotic disease that affects humans and many species of domesticated and wild animals, such as cattle, sheep, horse, swine, canine, raccoon, skunk, and wild boar (Cerqueira *et al*., 2011). The disease may manifest as acute, subacute, and chronic forms. The clinical signs that can be seen in affected animals include fever, mastitis-like changes in milk, acute hemolytic anemia, stillbirths, abortion, neonatal death, infertility, weak neonates, fever, jaundice and milk drop syndrome. Canine leptospirosis is characterized by acute renal and/or hepatic failure with or without coagulopathies (Azevedo *et al*., 2005).

The epidemiology of leptospirosis is characterized by a primary host species that acts as the reservoir for each serovar (Sykes *et al*., 2011). Dogs are the natural reservoir for the serogroup Canicola, whereas rodents, especially rats, for icterohaemorrhagiae. More recently, other serovars such as *Leptospira interrogans* serovar bratislava, *L. kirschneri* serovar grippotyphosa, and *L. interrogans* serovar pomona have been described. These serovars have a range of reservoir species, including voles, raccoons, skunks, opossums, mice, pigs, and cattle (Cerqueira *et al*., 2011).

Leptospirosis can be diagnosed by bacterial isolation or by the presence of specific antibodies (Sykes *et al*., 2011). The microscopic agglutination test (MAT) is regarded as the "gold standard" for the detection of leptospiral antibodies. However, MAT has some limitations because during acute phases, elevated antibody titers may be non-specific. Moreover, it also requires highly trained personnel and living organisms as antigens. In addition, the cost of strain maintenance is high, making it difficult to use as a routine method in diagnostic laboratories (Roach *et al*., 2010). In contrast, the ELISA has been used for the diagnosis of several diseases, including leptospirosis in dogs (Sykes *et al*., 2011) because it is easier to implement, highly sensitive; therefore, it yields reliable results. The current study aims to determine the seroprevalence of leptospirosis in household dogs in Chongqing, Kunming, Nanchang, Fuzhou, Guangzhou, Shenzheng, and Nanning in Southern China using ELISA test.

MATERIALS AND METHODS

This study was performed in Chongqing, Kunming, Nanchang, Fuzhou, Guangzhou, Shenzheng, and Nanning

[§]Equally contributed to this study.

in Southern China (Fig. 1). Kunming, called the "Spring City" in China, is the special city. It has a temperate subhumid climate with a mean annual temperature of 15°C (range of 8-19°C during winter and summer) and an altitude of approximately 1891 meters. In the other six cities , the climate is characterized as subtropical humid climate with mean annual temperatures of 23–29°C (range of 10–42°C during winter and summer) and relative humidity of approximately 70–80%. Annual rainfall is approximately 1500–2500 mm.

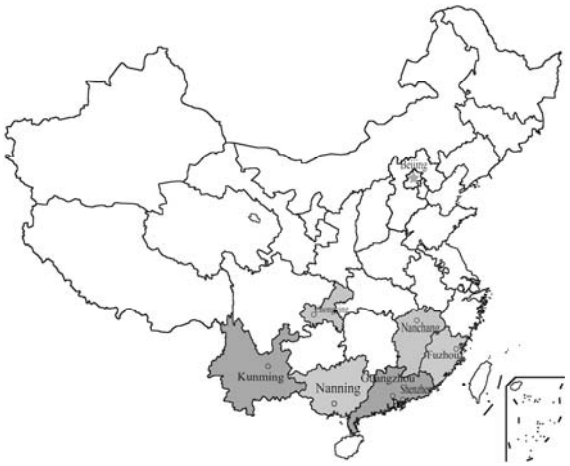

Fig. 1: The map of China. o is the sampling location in the present study.

Blood samples (simple random sampling) were taken by local veterinary clinics between November 2010 and April 2011 in the framework of routine clinical procedures from 314 healthy dogs (146 males and 168 females). The samples were collected from the saphenous veins of the dogs into sterile plain test tubes and were left to clot at room temperature for 20–30 min and centrifuged at 1000 ×g for 15 min. The separated sera were stored at -20°C until used for analysis by ELISA. Biometric data from the dogs, including age, gender, and region, were recorded. The data were acquired from the pet owners or medical records.

Leptospira antibodies were detected using a commercial ELISA kit (Multiscience Biotech Co Ltd, China) according to the manufacturer's recommend-dations. Positive and negative control sera were provided in the ELISA kit. The tests were performed at the Institute of Veterinary Medicine, Guangdong Academy of Agricultural Sciences, Guangzhou (Guangzhou, Guangdong Province). Briefly, a 96-well ELISA plate was coated with *Leptospira*-specific antigens. After incubating the diluted serum sample (1:100) in the test well and subsequent washing, a conjugate was added. The plate was washed again and a chromogenic enzyme substrate was then added. The optical density (OD) at 450 nm was read using a photometer (Bio-Rad, Hercules, California).

Test validity: $\bar{x}$(positive control) > 1.00, $\bar{x}$(negative control) < 0.10. Cut off = $\bar{x}$(negative control) + 0.15. The sera were considered negative for *Leptospira* if the OD of the sample was less than the cut off value and positive if the OD of the sample was greater than the cut off value, according to the manufacturer's recommendations.

The data were analyzed using the statistical software package SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Identification of a risk factor required at a 95% confidence level ($P<0.05$), as well as a biologically plausible association between the factor and seroreactivity to leptospirosis.

RESULTS

The results of the ELISA examinations on the canine leptospirosis positive samples are shown in Table 1. In the 314 canine samples, 23 (7.3%) were positive by the ELISA kit examination. The prevalence of *Leptospira* infection was 6.3% in dogs <1 years old age group, 9.2% in the 1–4-year-old age group, and 5.8% in the >4 years old age group. 8.9% (13 of 146) in male and 6% (10 of 168) in females were found in the current study. Statistical analysis revealed no significant difference among age groups and gender. No risk factor for dogs was found in age groups and gender groups. Among the 314 sera samples, the seroprevalence in Chongqing City (16.7%) and Nanning City (23.3%) were significantly higher than that in the other five cities (Chongqing City, $P = 0.015$, OR = 0.167, 95% CI = 0.030–0.917; Nanning City, $P = 0.006$, OR = 0.110, 95% CI = 0.021–0.567).

Table 1: Seroprevalence of *Leptospira* infection in dogs in different cities of Southern China determined by ELISA

Variable	Number of dogs (%)	Positive dogs (%)	Odds ratio (95%CI)	P
Total	314(100)	23(7.3)		
Gender				
Females	168(53.5)	10(6.0)	1	
Males	146(46.5)	13(8.9)	0.648(0.275-1.524)	0.737
Age(years)				
0-1	79(25.2)	5(6.3)	1	
1-4	131(41.7)	12(9.2)	0.670(0.227-1.979)	0.857
> 4	104(33.1)	6(5.8)	1.104(0.324-3.755)	0.959
Region				
Guangzhou	62(19.7)	2(3.2)	1	
Kunming	30(9.6)	3(10.0)	0.300(0.047 -.900)	0.508
Nanchang	30(9.6)	1(3.3)	0.967(0.084-1.102)	0.964
Fuzhou	50(15.9)	3(6.0)	0.522(0.084-3.254)	0.995
Chongqing	30(9.6)	5(16.7)	0.167(0.030-0.917)	0.015
Shenzhen	82(26.1)	2(2.4)	1.333(0.183-9.738)	0.864
Nanning	30(9.6)	7(23.3)	0.110(0.021-0.567)	0.006

DISCUSSION

Leptospirosis exists widely in both temperate and tropical climates. It has become a serious public health threat in both developed and developing countries. Leptospires colonize the renal tubules of maintenance host species such as dogs, rats, and cattle. They are excreted via the urine into the environment, where they can survive in suitable moist conditions (Erdogan *et al.*, 2008; Sykes *et al.*, 2011). Human infection results from exposure to the urine of infected animals, either directly or via contaminated soil or water (Davis *et al.*, 2008). Therefore, the dogs are a potential risk for public health in major cities.

The overall seroprevalence in dogs in the seven cities was 7.3% (23/314), which was higher than that observed in South Africa (Roach *et al.*, 2010 at 4.7% by ELISA),

lower than that observed in Iran (Zakeri *et al*., 2010 at 22% by PCR), and similar to that observed in Brazil (Rojas *et al*., 2010 at 7.05% by PCR) and the United States (Harkin *et al*., 2003 at 8.8% by PCR). These differences may have resulted from the use of different tests, survey periods, sample sizes, types of dog populations, and cut-off values for the test. Climatic factors may have also affected the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and may have influenced the overall prevalence of *Leptospira* (Azevedo *et al*., 2005).

In the present study, *Leptospira* antibodies (146 males and 168 females) were investigated in 314 dogs using ELISA. Among the 314 sera samples, the seroprevalence in Chongqing City (16.7%,) and Nanning City (23.3%) were significantly higher than that in the other five cities(Chongqing City, P = 0.015, OR = 0.167, 95% CI = 0.030–0.917; Nanning City, P = 0.006, OR = 0.110, 95% CI = 0.021–0.567) . This may be related to suitable humid in the cities of Nanning and Chongqing than the other cities. The seroprevalence in males was 8.9% (13 of 146), whereas the seroprevalence was 6.0% (10 of 168) in females. Gender was not significantly associated with the presence of *Leptospira* antibodies in the current study. No risk factor for dogs was found in gender groups, a similar finding was reported by other authors (Aslantaş *et al*., 2005).

Age is not an important risk factor for dogs in present study. The prevalence of *Leptospira* infection was 6.3% in dogs <1 years old age group, 9.2% in the 1–4-year-old age group, and 5.8% in the >4 years old age group. Statistical analysis revealed no significant difference among age groups. In contrast, Aslantaş *et al.* (2005) reported that adult dogs were more commonly infected than juvenile dogs (P<0.05).

The control strategies for leptospirosis include improving hygiene, control of rodents, and vaccination. Commercial Leptospira vaccines for dogs are available in many countries. The vaccines available in China contain different combinations of serovars Canicola, Icterohae-morrhagiae, Pomona, and Grippotyphosa. Currently, the vaccine for dogs contains the Icterohaemorrhagiae, Canicola, and Grippotyphosa serovars. It is widely used to protect dogs from Leptospira infection.

In conclusion, leptospirosis is very common among household dogs in Southern China. These dogs are an important source of infection for other animals and humans. The seroprevalence of leptospirosis is relatively low. However, asymptomatic dogs should be considered as a significant reservoir with regard to the spread of the disease.

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