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Seroprevalence and risk factors of *Chlamydia* infection in dogs in Southwestern China

Yi-Ming Tian[a,1], Jing-Feng Cao[b,1], Dong-Hui Zhou[a,*], Feng-Cai Zou[c], Qiang Miao[c], Zi-Li Liu[c], Bi-Feng Li[c], Rui-Qing Lv[c], Xiao-Peng Du[b,*], Xing-Quan Zhu[a,d]

[a] *State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province 730046, PR China*
[b] *Kunming Police Dog Base of the Ministry of Public Security, Kunming, Yunnan Province 650204, PR China*
[c] *College of Animal Science and Technology, Yunnan Agricultural University, Kunming, Yunnan Province 650201, PR China*
[d] *College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province 163319, PR China*

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ABSTRACT

Chlamydia spp. are obligate intracellular bacteria distributed globally, known to cause various forms of diseases in animals and humans. To date, there is limited information about the seroprevalence of Chlamydia and the risk factors associated with Chlamydia infection in dogs in the world. In the present study, a serological survey was undertaken to examine the seroprevalence and risk factors associated with dog chlamydiosis in Yunnan Province, southwestern China. A total of 591 dogs were sampled, antibodies to Chlamydia were determined by indirect hemagglutination assay (IHA). The overall seroprevalence was estimated at 17.6%. The risk factors associated with seroprevalence were determined by a multivariate logistic regression analysis. Gender and age of dogs were not significant in the logistic regression analysis ($P > 0.05$) and left out of the final model. Type and geographical origin of dogs were considered as main risk factors associated with Chlamydia infection, stray dogs (31.37%) were more than 16 times (OR = 16.167, 95% CI = 6.283–41.599, $P < 0.01$) at risk of acquiring the infection compared to the police dogs (7.62%), while pet dogs (14.41%) had a 3 times (OR = 2.968, 95% CI = 1.349–6.529, $P = 0.007$) higher risk. Positive dogs were found in 5 districts of Yunnan Province with prevalence ranging from 2.56% to 31.67% except Diqing (0/56). Dogs in Kunming (20.21%) had a 9 times higher risk of being seropositive compared to dogs in Lijiang (2.56%) (OR = 9.057, 95% CI = 1.211–67.714, $P = 0.032$), although no regional differences were found in other 4 administrative divisions compared to Lijiang ($P > 0.05$). Our study revealed a widespread and high prevalence of Chlamydia infection in dogs in Yunnan Province, southwestern China, with higher exposure risk in stray dogs and distinct geographical distribution. These findings suggest the potential importance of dogs in the transmission of zoonotic Chlamydia infection, and thus Chlamydia should be taken into consideration in diagnosing dog diseases.

1. Introduction

Chlamydia spp. are obligate intracellular bacteria distributed globally, known to cause various forms of diseases in animals and humans (Everett, 2000; Wheelhouse and Longbottom, 2012; Cong et al., 2012, 2013; Fernández-Benítez et al., 2013; Jiang et al., 2013). Infection of livestock, companion animals, and other animals with *Chlamydia* may result in pneumonia, abortion, rhinitis, conjunctivitis, arthritis, urethritis, atherosclerosis, or enteritis, or inapparent diseases; and some *Chlamydia* species also cause zoonotic diseases, such as *Chlamydia felis* and *Chlamydia psittaci* (Everett, 2000; Longbottom and Coulter, 2003; Livingstone and Longbottom, 2006; Reinhold et al., 2011).

Dogs as human friends were susceptible to *C. psittaci* infection (Maierhofer and Storz, 1969), and isolation of *C. psittaci* from dogs had been reported (Fraser et al., 1969; Arizmendi et al., 1992; Sprague et al., 2009), but due to some diagnostic problems such as low awareness of the disease, variable clinical presentations, chlamydiosis in dogs is often not recognized by general practitioners, naturally occurring canine *Chlamydia* infection is not often recognized (Werth, 1989). Limited epidemiological surveys of *Chlamydia* infection in dogs have been conducted in the world, and most of them were published in local journals, the serological studies have found antibodies in up to 50% of clinically healthy dogs and 61.9% in sick dogs (Liutkeviciene et al., 2009). These findings suggest the potential importance of dogs in the epidemiology

* Corresponding authors. Tel.: +86 931 8342813; fax: +86 931 8340977.
E-mail addresses: zhoudonghui@caas.cn (D.-H. Zhou), dxp_2007@163.com (X.-P. Du).
[1] These authors contributed equally to this work.

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Table 1
Prevalence of *Chlamydia* infection in dogs in the world (available data).

Country	Test[a]	No. tested	Positive (%)	Cut-off value	Article language	References
France						
Paris region	UN[b]	183	41	1:20	French	Didier (1979)
Japan	CFT	359	9.5	UN	English	Fukushi et al. (1985)
Italy						
Central-southern	CFT	781	5.5	1:16	Italian	Cerri et al. (1987)
Perugia	CFT	392	18	UN	Italian	Valente et al. (1994)
Czechoslovakia	CFT	530	5.5	1:16	Slovak	Kocianová et al. (1992)
Germany						
	CFT	UN	7.2	UN	German	Schmatz et al. (1977)
	ELISA	1127	20	UN	German	Werth et al. (1987)
Spain	ELISA	UN	8.3	UN	Spanish	Buendia et al. (1995)
Austria	UN	30	46.6	UN	German	Sixl et al. (1988)
	PCR	50	2		German	Nell et al. (2000)
Lithuania						
	CFT	1133	19.5	UN	English	Liutkeviciene et al. (2001)
	IFA	1133	38.1	UN	English	Liutkeviciene et al. (2001)
	DIF	218	61.9	UN	English	Liutkeviciene et al. (2009)
Sweden	PCR	79	0		English	Holst et al. (2010)
China						
Dongguan city	IHA	977	2.87	1:64	Chinese	Huang et al. (2010)
Lanzhou city	IHA	264	12.1	1:16	English	Wu et al. (2013)

[a] CFT, complement fixation test; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; IFA, immunoferment assay; DIF, direct immuno-fluorescence test; IHA, indirect hemagglutination test.
[b] UN, unknown.

of zoonotic *Chlamydia* infection. We have summarized the available prevalence data of *Chlamydia* infection in dogs in the world in Table 1.

The present study was aimed at determining the seroprevalence of *Chlamydia* infection in dogs in Yunnan Province, southwestern China, and identifying factors associated with the presence of *Chlamydia* antibodies in dogs in this province.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Permit No. LVRIAEC2011-006). Dogs of various categories (police dogs, pet dogs and stray dogs), from which serum samples were collected, were handled with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China. The collection of serum samples was performed as part of routine process of disease monitoring and surveillance for these dogs. The owners of pet dogs had given permission for the collection of serum samples.

2.2. Serum samples

From June 2011 to September 2012, a total of 591 dog blood samples were collected from police dogs ($n = 105$), pet dogs ($n = 333$) and stray dogs ($n = 153$) in 6 administrative districts of Yunnan Province, Southwestern China, including Kunming city, Yuxi city, Lijiang city, Diqing Tibetan Autonomous Prefecture (Diqing), Dehong Dai and Jingpo Autonomous Prefecture (Dehong) and Xishuangbanna Dai autonomous prefecture (Banna) (Table 2). 105 police dogs were all from Kunming Police Dog Base of the Ministry of Public Security, China. Whenever possible, information regarding gender, age, and geographical origin of dogs were obtained from their owners and the veterinarians, and the biometric data of stray dogs were estimated based on their body condition and dental age.

2.3. Detection of antibodies to Chlamydia by indirect hemagglutination test (IHA)

Antibodies to *Chlamydia* were determined by IHA using a commercial kit (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China) according to the manufacturer recommendations as reported previously (Cong et al., 2013; Huang et al., 2013; Wu et al., 2013; Zhang et al., 2013; Zhou et al., 2013). Briefly, 75 μl of IHA dilution solution was transferred into 96 well 110° V-bottomed polystyrene plates with 25 μl of sera added and diluted in a four-fold series from 1:4 to 1:64. The plates were shaken for 2 min and then incubated at 37 °C for 2 h without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:16 or higher, and positive and negative controls were included in each test.

Table 2
Seroprevalence of *Chlamydia* infection in dogs in Yunnan Province, southwestern China by indirect hemagglutination test (IHA).

Factor	Category	No. examined	No. Positive	Prevalence (%)
Gender	Male	322	45	13.98
	Female	269	59	21.93
Type	Police dog	105	8	7.62
	Pet dog	333	48	14.41
	Stray dog	153	48	31.37
Region	Kunming	386	79	20.47
	Banna	60	19	31.67
	Dehong	7	1	14.29
	Yuxi City	44	4	9.09
	Diqing	56	0	0
	Lijiang	38	1	2.56
Age	≤1 year	207	30	14.49
	1 < years ≤ 2	85	17	20
	2 < years ≤ 3	82	15	18.29
	3 < years ≤ 4	63	22	34.92
	4 < years ≤ 5	39	5	12.82
	>5 years	115	15	13.04
	Total	591	104	17.6