Prevalence of antibodies to Chlamydiaceae in pet dogs in Shenzhen, Guangdong Province, China

Tian Cheng[1], Shang-shu Zou[1], Xin-Qiu Wang[1], Yuan He[1], Yi Liang[1,2], Rui-Qing Lin[1]

[1]South China Agricultural University, College of Veterinary Medicine, Guangzhou, People's Republic of China
[2]Shenzhen Ruipeng Animal Hospital, Shenzhen, People's Republic of China

Received April 7, 2014
Accepted October 22, 2014

Abstract

The prevalence of chlamydiosis in pet dogs was surveyed in Shenzhen, Guangdong Province, China. A total of 442 serum samples were collected from three districts of Shenzhen, namely the Futian, Nanshan, and Luohu Districts, and assayed for Chlamydiaceae antibodies by indirect haemagglutination assay. The results showed that the mean positive rate was 6.11%, and the positive rate for the Futian District was the highest (9.52%), followed by the Nanshan District (7.08%), and the Luohu District (4.02%). The positive rate for male dogs was 6.08%, and for female dogs 6.16%. Out of all the 38 breeds of dogs examined, 14 breeds were positive. The positive rate for the Cocker Spaniel dog was the highest (14.2%), followed by the Pekinese dog (10.71%), and the Border Collie dog (10.34%). This is the first time that the seroprevalence of Chlamydiaceae infection in pet dogs in Shenzhen, Guangdong province, China was reported, and the results indicated that pet dogs may be an important source for human infection by Chlamydiaceae in Shenzhen and other areas of China.

Chlamydiosis, seroprevalence, indirect haemagglutination assay, survey

Being a kind of zoonosis, chlamydiosis occurs throughout the world (Bodetti et al. 2002; Jee et al. 2004; Greco et al. 2005; Magnino et al. 2009; Dickx et al. 2010; Lin et al. 2011) and is responsible for various diseases in humans and animals such as enteritis, perinatal mortality, pneumonia, conjunctivitis, polyarthritis, and reproductive disorders (Longbottom and Coulter 2003; Kauffold et al. 2006; Huang et al. 2010). This disease can cause considerable damage to animals, and can also be transmitted from animals to humans, and cause a rare but potentially severe and life-threatening disease (Gaede et al. 2008; Yang et al. 2010).

In China, Chlamydiaceae agents have been isolated from many animal and bird species, such as the pigeon, duck, chicken, pig, cow, and sheep (Liu and Luo 2004). It can also infect dogs and mainly cause conjunctivitis, keratitis, encephalitis or pneumonia, and the signs are similar to canine distemper (Huang et al. 2010). Raising pet dogs as companion animals in Shenzhen city is more popular than in many other cities in China. Chlamydiaceae is an infectious agent in dogs and is capable of being transmitted to humans. The chlamydial antibodies have been found very high in some serological studies worldwide, and dogs may be reservoirs of Chlamydiaceae agents (Sprague et al. 2009).

The objective of the present survey was to determine the seroprevalence of Chlamydiaceae in pet dogs from Shenzhen city, China, which will help understand the developing trends of chlamydiosis in Shenzhen city and even in China, and will be helpful in the prevention and control of this disease.

Materials and Methods

Study site

Shenzhen is located in the very south of the Guangdong Province, overlooking Hong Kong to the south and bordering Kowloon. It has an area of 1,984.69 km^2 and consists of 6 districts: Luohu, Futian, Nanshan,

Address for correspondence:
Rui-Qing Lin, BVSc, MVSc, PhD
College of Veterinary Medicine
South China Agricultural University
Guangzhou, Guangdong Province 510642, People's Republic of China

Phone: +86 20 85285954
Fax: +86 20 85280234
E-mail: rqlin@scau.edu.cn
http://actavet.vfu.cz/

Yantian, Bao'an, and Longgang. It has a mild subtropical oceanic climate with an annual mean temperature of 22.3 °C.

Sampling of pet dogs

A total of 442 serum samples of pet dogs were randomly collected from the Futian (105 samples), Nanshan (113 samples), and Luohu Districts (224 samples) of Shenzhen, Guangdong Province, China. These dogs were admitted to several veterinary clinics for various situations, including health examination, vaccination, internal medicine, castration, and surgery (sick or injured animals), but no pregnant animals were included. Of the examined pet dogs, 146 were females and 296 were males, of different ages, belonging to 38 breeds. The samples examined were divided into three age groups, namely a group of 1–12 months of age (156 dogs), a group of 12–36 months of age (147 dogs), and a group of over 36 months but under 13 years of age (139 dogs). Serum samples were obtained by centrifugation at 3.000 × *g* for 10 min, and stored at -20 °C.

Serological examination

Antibodies to Chlamydiaceae in dogs were determined using an indirect haemagglutination assay, which can detect all the antibodies of Chlamydiaceae, but not specific species and is used as routine test in China. The commercially marketed IHA kit was purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The detection procedure followed the manufacturer's instructions and previous descriptions (Zhang et al. 2013; Zhou et al. 2013). In brief, 75 µl of IHA dilution solution were transferred into a 96-well V bottomed reaction plate with 25 µl of serum sample added and mixed gently with a pipette. The mixture liquid was 4-fold gradually diluted into other 2 holes and 25 µl mixture in the third hole was discarded at last to maintain a 75 µl system. The dilution in the wells 1, 2, and 3 was 1:4, 1:16, and 1:64, respectively. Positive, negative, and blank controls were included in the same plate. After 25 µl of Chlamydia antigen were added to each well, the plate was shaken slightly with a vibrator for 2 min followed by incubation at 37 °C for 2 h. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:16 or higher. The mean prevalence rate was calculated by dividing the number of positive samples with the total number of samples examined, and was expressed as percentage of all pet dogs sampled.

Ethical statement

We declare that the experiments comply with the current laws and regulations of the People's Republic of China in which the experiments were performed.

Results

A total of 442 serum samples of pet dogs collected from 3 districts of Shenzhen City were examined for the antibodies of Chlamydiaceae using IHA. The results showed that the mean positive rate for all the samples was 6.11%; the positive rate for the Futian District

Table 1. Positive rate of Chlamydiaceae infection in different breeds of pet dogs in Shenzhen, Guangdong Province, China

Breed	Total number	Positive (%)
Poodle Caniche	81	1 (1.23%)
Golden Retriever	39	3 (7.69%)
Pomeranian	34	5 (1.47%)
Papillon	32	3 (9.38%)
Border Collie	29	3 (10.34%)
Pekinese	28	3 (10.71%)
Shih Tzu	22	2 (9.09%)
Bichon Frise	19	1 (5.26%)
Schnauzer	18	1 (5.56%)
Interbreed	18	1 (5.56%)
Siberian Husky	13	1 (7.69%)
Samoyed	11	1 (9.09%)
Pug	11	1 (9.09%)
Cocker Spaniel	7	1 (14.29%)

was the highest (9.52%), followed by the Nanshan District (7.08%), and the Luohu District was the lowest (4.02%). The positive rate for male dogs was 6.08%, and for female dogs 6.16%, with no obvious difference in sex.

Serum samples in the present survey came from 38 breeds of pet dogs. Of all the samples examined, 14 breeds were positive. The positive rate for the Cocker Spaniel dog was the highest (14.2%), followed by the Pekinese dog (10.71%), and the Border Collie dog (10.34%) (Table 1). The positive rate for the age group of 12–36 months was the highest (7.48%), followed by the group of under 12 months (6.41%), and the positive rate for the age group over 36 months was the lowest (4.32%) (Table 2).

Table 2. Positive rate of Chlamydiaceae infection of pet dogs of different ages in Shenzhen, Guangdong Province, China

Age	Total number	Positive %
Group 1		
1–12 months	156	10 (6.41%)
Group 2		
12–36 months	147	11 (7.48%)
Group 3		
36 months–13 years	139	6 (4.32%)

Discussion

The role and importance of animals as a reservoir of zoonotic pathogens is being increasingly recognized (Sprague et al. 2009; Rohde et al. 2010). As companion animals, pet dogs have great emotional value because of the loving friendship between them and their owners. But a downside for humans may be the high risk of exposure to some infectious diseases from pet dogs. As a Special Economic Zone of China, Shenzhen is more developed than many other cities in China. Raising pet dogs has become a kind of fashion accompanied with the high speed development of city economy and the raised living standard in Shenzhen, but a diverse range of infectious diseases threaten the health of pet animals and their human owners. Chlamydiosis is one of the important zoonotic diseases which infects many animal species and can be transmitted between humans and pet dogs.

There have been few reported surveys of chlamydiosis in dogs in China. These reports were published locally in Chinese journals (Huang et al. 2010; Ma and Wang 2010) and therefore are not easily accessible to international readers. In the present survey, the mean positive rate of serum samples was 6.11%, which was markedly lower than reported in a previous survey of Tibetan mastiff in Xining, Qinghai Province (27.97%), but higher than in a survey in Dongguan, Guangdong Province (2.87%), possibly due to different ecological and geographical conditions, different management level and different breeds of dog examined. There may be different susceptibilities to Chlamydiaceae in different breeds of dog, more surveys and further researches are needed to confirm that finding. Although the results of the present survey showed the positive rate for the age group 12–36 months was higher than for the other two age groups, no obvious difference was found for sex and age. On the other hand, information about the prevalence of Chlamydiaceae infections in dogs in other countries is limited. A research study using direct immuno-fluorescence (DIF) in Lithuania revealed high prevalence (61.9%) in diseased dogs with urogenital and/or conjunctival lesions (Liutkeviciene et al. 2009). Nevertheless, an investigation

in Sweden using real-time PCR analysis showed that no Chlamydiaceae were detected from any dog (Holst et al. 2010). Serological studies also revealed various positive rates and up to 50% in clinically healthy dogs (Kocianova et al. 1992; Liutkeviciene et al. 2001; Sprague et al. 2009). The varying data indicate that more investigations need to be conducted to master the epidemiological characteristics and risk factors, and to predict the trend of the Chlamydiaceae disease.

This is the first survey of Chlamydiosis in pet dogs in Shenzhen, Guangdong Province, China. All the examined pet dogs were household dogs and were less outgoing but still had chances of contact with Chlamydiaceae origins, such as birds. It is thought that dogs and humans have become infected with Chlamydiaceae through contact with each other (Sako et al. 2002), therefore the public awareness should be raised regarding chlamydiosis in dogs. This survey provides baseline data of pet dogs infected with Chlamydiaceae in Shenzhen, which will help understand the developing trends of this disease in Shenzhen city and even in China, and will be helpful in the prevention and control of this disease.

Acknowledgements

Project support was provided, in part, by the Scientific and Technological Programs of Guangdong Province (Grant No. 2012A020100001), and the Open Funds of the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Grant Nos. SKLVEB2012KFKT003).

References

Bodetti TJ, Jacobson E, Wan C, Hafner L, Pospischil A, Rose K, Timms P 2002: Molecular evidence to support the expansion of the host range of *Chlamydophila pneunoniae* to include reptiles as well as humans, horses, koalas and amphibians. Syst Appl Microbiol **25**: 146-152

Dickx V, Geens T, Deschuyffeleer T, Tyberghien L, Harkinezhad T, Beeckman DS, Breackman L, Vanronmpay D 2010: *Chlamydophila psittaci* zoonotic risk assessment in a chicken and turkey slaughterhouse. J Clin Microbiol **48**: 3244-3250

Gaede W, Reckling KF, Dresenkamp B, Kenklies S, Schubert E, Noack U, Irmscher HM, Ludwig C, Hotzel H, Sachse K 2008: *Chlamydophila psittaci* infection in humans during an outbreak of psittacosis from poultry in Germany. Zoonoses Public Health **55**: 184-188

Greco G, Corrente M, Martella V 2005: Detection of *Chlamydophila psittaci* in asymptomatic animals. J Clin Microbiol **43**: 5410-5411

Holst BS, Hanas S, Bolske G, Forsberg CL 2010: An investigation on the presence of Chlamydiaceae in Swedish dogs. Acta Vet Scand **16**: 52-63

Huang BC, Hu JC, Wu ZP, Xie HY, Zhang XP 2010: Investigation on serology of dogs and cats Chlamydia in Dongguan Guangdong (in Chinese). J Vet **44**: 9-10

Jee J, Degraves FJ, Kim T, Kaltenboeck B 2004: High prevalence of natural Chlamydophila species infection in calves. J Clin Microbiol **42**: 5664-5672

Kocianova E, Lisak V, Kopcok M 1992: *Coxiella burnetii* and *Chlamydia psittaci* infection in dogs (in Slovak). Vet Med (Praha) **37**: 177-183

Kauffold J, Melzer F, Berndt A, Hoffman G, Hotzel H, Sachse K 2006: Chlamydiae in oviducts and uteri of repeat breeder pigs. Theriogenology **66**: 1816-1823

Lin RQ, Wang XQ, Yan C, He XH, Cheng T, Wang YN, Xu MJ, Yuan ZG, Zhang YB, Zhu XQ 2011: Seroprevalence of *Chlamydophila* infection in chickens, ducks, geese and pigeons in southern China. Afr J Microbiol Res **5**: 4240-4242

Liu ZJ, Luo QH 2004: The research situation of animal chlamydiosis (in Chinese). Prog Vet Med China poultry **25**: 29-31

Liutkeviciene V, Bagdonas J, Stankevicien M, Baliukoniene M, Ganusauskaite L, Gerulis G, Masilionis K, Stankevicius A, Akunyte O 2001: Serological investigations of chlamydiosis in Lithuanian dog's population (in Lithuanian). Vet Zootechn **13**: 20-25

Liutkeviciene V, Mockeliunine V, Sengaut J, Salomskas A, Stankeviciene M, Mockeliunas R, Alwksejuninen I 2009: Chlamydia prevalence in sick dogs with uro-genital and/or conjunctival lesions. Rev Med Vet – Toulouse **160**: 547-551

Longbottom D, Coulter LJ 2003: Animal Chlamydioses and zoontic implications. J Com Pathol **128**: 217-244

Ma WL, Wang GP 2010: Chlamydia serological survey of Tibetan mastiff in Xining, Qinghai province (in Chinese). Anim Husbandry Vet **43**: 111

Magnino S, Haag-Wackernagel D, Ceigenfeind I, Helmecke S, Dovc A, Prukner-Radovcic E, Residbeqovic

E, Iliwski V, Laroucau K, Donati M, Martinov S, Kaleta EF 2009: Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. Vet Microbiol **135**: 54-67

Rohde G, Straube E, Essig A, Reinhold P, Sachse K 2010: Chlamydial zoonoses. Dtsch Arztebl Int **107**: 174-180

Sako T, Takahashi T, Takehana K, Uchida E, Nakade T, Umemura T, Taniyama H 2002: Chlamydial infection in canine atherosclerotic lesions. Atherosclerosis **162**: 253-259

Sprague LD, Schubert E, Hotzel H, Scharf S, Sachse K 2009: The detection of *Chlamydophila psittaci* genotype C infection in dogs. Vet J **181**: 274-279

Yang XH, Bai RN, Li ZY, Zhang GS, Wei HT, Yu CM, Wang HQ, Zhang FC, Liu TZ, He C, Chen H, Yuan JL, Zhang HY, Liu J 2010: Investigation on serology of pigeon *Chlamydophila psittaci* in Beijing (in Chinese). China poultry **32**: 66-67

Zhang NZ, Zhou DH, Shi XC, Nisbet AJ, Huang SY, Ciren D, Wu SM, Zhu XQ 2013: First report of Chlamydiaceae infection in Tibetan pigs in Tibet, China. Vector Borne Zoonot Dis **13**: 196-199

Zhou DH, Zhao FR, Xia HY, Xu MJ, Huang SY, Song HQ, Zhu XQ 2013: Seroprevalence of chlamydial infection in dairy cattle in Guangzhou, Southern China. Ir Vet J **66**: 2