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Chlamydia psittaci exposure in pet birds

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Chlamydia psittaci is a zoonotic pathogen with a wide range of avian hosts and may be transmitted to humans and cause severe disease. To assess the risk of psittacosis posed by pet birds, the seroprevalence of *Chlamydia psittaci* antibodies in 360 Eurasian siskins (*Carduelis spinus*), 289 oriental skylarks (*Alauda arvensis*) and 36 black-tailed grosbeaks (*Coccothraustes migratorius*) in Gansu province, north-western China was detected by an indirect haemagglutination assay. Twenty-seven out of 289 (9.34 %) *Alauda arvensis*, 45 out of 360 (12.50 %) *Carduelis spinus* and 2 out of 36 (5.56 %) *Coccothraustes migratorius* were positive for *Chlamydia psittaci* infection at a cut-off dilution of 1 : 16. The prevalence of *Chlamydia psittaci* was higher in *Carduelis spinus* (12.5 %) than in *Alauda arvensis* (9.34 %) and *Coccothraustes migratorius* (5.56 %); however, the differences were not statistically significant ($P>0.05$). Statistical analysis indicated that *Chlamydia psittaci* seroprevalence in adult pet birds (12.4 %, 67/540) was significantly higher than that in juvenile pet birds (4.83 %, 7/145) ($P<0.01$). There was no statistical difference in *Chlamydia psittaci* seroprevalence between male (12.4 %) and female (8.27 %) birds. To our knowledge, this is the first report indicating the seroprevalence of *Chlamydia psittaci* exposure in pet birds in China. Our results indicate that close contact with pet birds poses the risk of zoonotic transmission of *Chlamydia psittaci*.

INTRODUCTION

Chlamydia is a genus that includes an important zoonotic obligate intracellular pathogen that causes acute diseases in birds and mammals, including humans. They may lead to a wide range of clinical manifestations including ocular, pulmonary, genital, articular and intestinal illness, but very often they induce persistent, chronic, or subclinical infections (Rodolakis & Yousef Mohamad, 2010). Psittacosis is a well-known disease caused by *Chlamydia psittaci* (Rohde *et al.*, 2010), which is prevalent in poultry, pet birds and wild birds, and causes economic losses to the poultry industry and the pet trade (Geigenfeind & Haag-Wackernagel, 2010). As far as the transmission of *Chlamydia psittaci* strains from birds to humans is concerned, veterinarians and the owners of pet birds have been reported to face a particular risk (Evans, 2011). Pet birds are considered to be close friends and companions of humans, thus playing an important role in human life. Unfortunately, pet birds pose a potential threat of transmitting *Chlamydia psittaci* to the owners and their family members.

Chlamydia psittaci infection in pet birds has been reported previously in Europe, Latin America, Africa, the USA and Brazil (Circella *et al.*, 2011; Herrera *et al.*, 2001; Mushi *et al.*, 2001; Piasecki *et al.*, 2012; Pilny *et al.*, 2012; Raso *et al.*, 2013; Sareyyupoglu *et al.*, 2007). Several studies have reported the seroprevalence of *Chlamydia* infection in domestic poultry and pigeons in China (Cong *et al.*, 2012; Yang *et al.*, 2007, 2010; Zhang *et al.*, 2003), but very limited information on the prevalence of *Chlamydia psittaci* in pet birds is available in China. Eurasian siskin (*Carduelis spinus*), oriental skylark (*Alauda arvensis*) and black-tailed grosbeaks (*Coccothraustes migratorius*) are very popular pet birds in Gansu province, north-western China. The present investigation was undertaken to study *Chlamydia psittaci* exposure and to measure its seroprevalence in these pet birds in Gansu province. This information will have implications for the improved prevention and control of *Chlamydia psittaci* infection in pet birds and humans.

Abbreviation: IHA, indirect haemagglutination assay.

METHODS

The investigated site. The present investigation was carried out in Gansu province, north-western China. Gansu has an area of 454 000 km^2, and the vast majority of its land is more than 1000 m above sea level. It lies between the Tibetan Plateau and the Loess Plateau, and the province contains the geographical centre of China, marked by the Center of the Country Monument at 35° 50′ 40.9″ N

103° 27′ 7.5″ E. Gansu generally has a semi-arid to arid continental climate, with warm to hot summers and cold to very cold winters. Most of the precipitation is delivered in the summer months.

Pet bird serum samples. The study was approved by the Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. A total of 685 blood samples were collected from pet birds in three representative administrative regions in Gansu province between August 2011 and September 2012. The samples were collected randomly from local pet markets. Blood samples were transported to the laboratory, kept at room temperature for 2 h and centrifuged at 3000 ***g*** for 10 min to separate clear serum. The serum samples were stored at −20 °C until further analysis. All birds came from the local regions in Gansu province. Details of animal husbandry practices were obtained by questioning the poultry market owners.

Serological examination. A commercial indirect haemagglutination assay (IHA) kit (Lanzhou Veterinary Institute) was used to examine serum antibodies against *Chlamydia psittaci*. The detection procedures were carried out as previously reported (Jiang *et al.*, 2013; Laroucau *et al.*, 2009). Samples that reacted at dilutions of 1 : 16 or higher were considered positive for *Chlamydia psittaci* antibodies, and those at dilutions of between 1 : 4 and 1 : 16 were retested. Positive and negative controls were included in each test.

Statistical analyses. Differences in the seroprevalence of *Chlamydia psittaci* infection in pet birds between male and female birds, between juvenile and adult groups and between locations were analysed using the generalized linear model (e.g. interactions between animal sex, age, bird species and location), using SPSS software for Windows (release 18.0 standard version; SPSS). The differences were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

In this study, specific antibodies against *Chlamydia psittaci* were detected in 74 out of 685 serum samples (10.80 %) by the IHA test at the cut-off dilution of 1 : 16. Among them, 45 out of 360 *Carduelis spinus* serum samples (12.50 %) were seropositive for *Chlamydia psittaci*. The antibody titres were diverse; the most frequent level was 1 : 32 (4.7 %), followed by 1 : 16 (2.8 %), 1 : 64 (2.5 %), 1 : 128 (1.9 %) and 1 : 256 (0.56 %). Antibodies against *Chlamydia psittaci* were found in 27 out of 289 *Alauda arvensis* (9.34 %), and the most frequent antibody titres were 1 : 32 (3.8 %), followed by 1 : 64 (2.4 %), 1 : 16 (1.4 %), 1 : 128 (1.4 %) and 1 : 256 (0.35 %). *Chlamydia psittaci* antibodies were detected in 2 out of 36 (5.56 %) *Coccothraustes migratorius* samples and the antibody titres were 1 : 16 (Table 1).

Psittacosis is also a human disease caused by *Chlamydia psittaci*. People in frequent contact with pet birds at work or in their spare time are the most predisposed to infection. Several studies have reported *Chlamydia psittaci* prevalence in pet birds in various geographical regions. One study reported that 12.39 % of scarlet macaws (*Ara macao*) in Costa Rica exhibited antibodies against *Chlamydia psittaci* by ELISA (Herrera *et al.*, 2001), and Piasecki *et al.* (2012) detected a 10.3 % prevalence from 34 different species of parrots by PCR in Poland. The overall seroprevalence of *Chlamydia psittaci* in pet birds in Gansu was 10.8 %, which was similar to that in Poland, but lower than that detected in Costa Rica and other countries. In Brazil, the hyacinth macaws (*Anodorhynchus hyacinthinus*) were evaluated for the presence of antibodies against *Chlamydia psittaci* by a complement fixation test, and for *Chlamydia psittaci* DNA by semi-nested PCR. Of the macaws, 65.4 % were positive for at least one test (Raso *et al.*, 2013).

In comparison, the overall prevalence of *Chlamydia psittaci* infection in birds in Gansu province was much lower than that in other countries. The differences could be related to variations in ecological and geographical factors such as temperature, rainfall or landscape. As mentioned before, the vast majority of the land of Gansu province is more than 1000 m above sea level. Gansu generally has a semi-arid to arid continental climate, with warm to hot summers but cold winters. Such difficult conditions may make it hard for *Chlamydia psittaci* to survive.

The varying sensitivities of the serological methods used to determine *Chlamydia psittaci* prevalence may also be a factor contributing to the observed differences in the results. Different formats and immunoreactive antigens were used for different serological tests for *Chlamydia psittaci*, and thus different assays varied in sensitivity and specificity. Although, in general, the sensitivity of IHA is not as good as that of ELISA, the commercial IHA kit used in this study has good sensitivity and specificity, and has been extensively used (Cong *et al.*, 2013; Huang *et al.*, 2013; Wu *et al.*, 2013).

As a zoonotic pathogen, *Chlamydia psittaci* was also prevalent in other animals in China. Cong *et al.* (2013) reported that 13.32 % of examined chickens, 38.92 % of ducks and

Table 1. Seroprevalence of *Chlamydia psittaci* infection in pet birds in Gansu province, north-western China, by indirect haemagglutination assay (IHA)

Host	No. of sera with IHA titres of:					No. positive	No. tested	Prevalence (%)
	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256			
Oriental skylark	4	11	7	4	1	27	289	9.34
Eurasian siskin	10	17	9	7	2	45	360	12.50
Black-tailed grosbeak	2	0	0	0	0	2	36	5.56
Total	16	28	16	11	3	74	685	10.80

Table 2. Seroprevalence of *Chlamydia psittaci* infection in pet birds in three different locations in Gansu province, north-western China

Host	Location	No. positive	No. tested	Prevalence (%)
Oriental skylark	Lanzhou	14	169	8.28
	Tianshui	2	40	5.00
	Longnan	11	80	13.75
Eurasian siskin	Lanzhou	21	120	17.50
	Tianshui	11	120	9.17
	Longnan	13	120	10.83
Black-tailed grosbeak	Tianshui	2	36	5.56

31.09 % of pigeons were seropositive for *Chlamydia psittaci* in Lanzhou, Gansu province. Jiang *et al.* (2013) reported that 58.59 % of 920 pigs examined were positive for chlamydial antibodies in Jiangxi province. Compared to other animals, the overall prevalence of *Chlamydia psittaci* infection in pet birds was much lower.

The seroprevalence of *Chlamydia psittaci* was diverse in different species of pet birds; the highest level was 12.5 % in *Carduelis spinus*, followed by 9.34 % in *Alauda arvensis* and 5.56 % in *Coccothraustes migratorius* (Table 1). However, the differences were not statistically significant. The *Chlamydia psittaci* seroprevalence in different species from different locations ranged from 5.00 % (*Alauda arvensis* in Tianshui) to 17.5 % (*Carduelis spinus* in Lanzhou; Table 2). These differences may be related to the feeding and living conditions of the pet birds. Statistical analysis indicated that the *Chlamydia psittaci* seroprevalence in adult pet birds (12.41 %, 67/540) was significantly higher than that in juvenile pet birds (4.83 %, 7/145) ($P<0.01$). This result was probably due to adult pet birds having more chance of contact with *Chlamydia psittaci* compared to juvenile pet birds. However, the difference in *Chlamydia psittaci* seroprevalence between male (12.41 %) and female (8.27 %) birds was not statistically significant ($P>0.05$), and there was no statistical difference in *Chlamydia psittaci* seroprevalence among pet birds from the three locations ($P>0.05$; Table 3).

Table 3. Prevalence of antibodies against *Chlamydia psittaci* in pet birds by gender and age in Gansu province, north-western China

Biometric data	No. positive	No. tested	Prevalence (%)
Gender			
Male	52	419	12.41
Female	22	266	8.27
Age			
Adult	67	540	12.41
Juvenile	7	145	4.83
Location			
Lanzhou	35	289	12.11
Tianshui	15	196	7.65
Longnan	24	200	12.00

Chlamydia psittaci infections occur in at least 465 bird species, spanning 30 different bird orders. In particular, Psittacidae (cockatoos, parrots, parakeets and lories) and Columbiformes (pigeons) seem to be affected (Beeckman & Vanrompay, 2009). Vanrompay *et al.* (2007) detected *Chlamydia psittaci* DNA with nested PCR/enzyme immunoassay and revealed that 6 (13 %) of 146 pet bird owners were infected by *Chlamydia psittaci*. Petrovay and Balla reported two fatal cases of psittacosis in two poultry processing plant employees presenting with pneumonia and respiratory failure; the diagnosis was confirmed by serological and PCR methods (Petrovay & Balla, 2008). Therefore, psittacine pet birds in urban and rural areas throughout the world should be regarded as the predominant reservoirs of zoonotic psittacosis (Beeckman & Vanrompay, 2009; Geigenfeind *et al.*, 2012). Since pet birds regularly live in houses, pet markets, zoos and parks, the pet bird owners, breeders, sellers, as well as veterinarians and tourists should be aware of the potential zoonotic risk.

A previous study using an immunofluorescence antibody test revealed that 13.25 % of the children examined in Lanzhou, Gansu province were infected by *Chlamydia* during 1999 to 2002 (Dong, 2003). A recent investigation indicated that 13.91 % of female patients with urogenital system diseases were seropositive for *Chlamydia* infection (Wang & Wang, 2013). The results of our present study indicated that 10.80 % (74 out of 685) pet birds were positive for antibodies against *Chlamydia psittaci*, revealing that *Chlamydia psittaci* is prevalent among pet birds in Gansu province, north-western China. *Chlamydia psittaci* in the faeces of infected birds may pollute the soil, water and air, and hence the birds could be an indicator of the environmental contamination with *Chlamydia psittaci*. The pet birds could be regarded as the potential reservoirs of zoonotic psittacosis and they may increase the risk of human infection in this region. The pet bird samples were collected between August 2011 and September 2012, which is a relatively short period of time; thus, the results obtained may not reflect the actual situation of *Chlamydia psittaci* infection during longer periods of time. However, our results provide useful information for future studies in which it would be interesting to test environmental samples (such as soil, water and air) and ventilation systems, to assess the contamination level of premises with *Chlamydia psittaci*.

CONCLUSIONS

The results of the present study revealed a high *Chlamydia psittaci* seroprevalence in pet birds in Gansu province, north-western China, which poses a potential threat to human health in this area. Therefore, it is necessary to implement integrated control and efficient management measures to prevent and control *Chlamydia psittaci* infection in pet birds. To our knowledge, this is the first report of *Chlamydia psittaci* infection in pet birds in China.

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