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First Record of Chlamydia Seroprevalence in Peafowls in Southwestern China

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Abstract: Chlamydiosis is a naturally occurring, contagious and zoonotic disease caused by *Chlamydia psittaci* (*C. psittaci*), affecting humans as well as domestic and wild birds globally but little is known about *C. psittaci* infection in peafowls in China. The objective of the present investigation was therefore to examine *C. psittaci* seroprevalence in peafowls in Yunnan province, Southwestern China. Serum samples from 270 peafowls including 265 blue peafowls and 5 green peafowls originated from Kunming city and Xishuangbanna Dai Autonomous Prefecture (Banna for short) were collected in 2011 to 2012 and examined independently for the presence of antibodies to *C. psittaci* by the Indirect Hemagglutination Assay (IHA). In this survey, antibodies to *C. psittaci* were found in 84 of 270 (31.11%) peafowls. Both green peafowls (*Pavo muticus*) (2/5) and blue peafowls (*Pavo cristatus*) (30.94% of 265) were positive for *C. psittaci* antibody and peafowl from zoos in Kunming had higher seroprevalence (52.70% of 74) than those from peafowl gardens in Banna (22.96% of 196 samples) but the difference was not statistically significant by logistic regression analysis ($p>0.05$). Significant difference was observed among age groups, the prevalence in adult peafowls (86.36% of 44) was significantly higher than that in adolescent birds (5.73% of 157) ($p<0.01$, OR = 104.148, 95% CI = 34.920-310.620). There were no statistical interactions between ages and geographical origins of peafowl ($p>0.05$). The results indicated a wide distribution of *C. psittaci* infections among peafowls in Yunnan, Southwestern China, posing significant public health concerns. To the knowledge, this is the first report of *C. psittaci* infection in peafowls in Yunnan province, Southwestern China.

Key words: *Chlamydia psittaci*, seroprevalence, Indirect Hemagglutination Assay (IHA), peafowl, Yunnan province, China

INTRODUCTION

Chlamydiosis is a naturally occurring, contagious and zoonotic disease that affects humans globally as well as domestic and wild birds (Rohde *et al*., 2010; Rodolakis and Yousef, 2010; Wheelhouse and Longbottom, 2012). It is caused by the obligate intracellular gram-negative bacterium *Chlamydia psittaci* (*C. psittaci*) with numerous strains vary in their pathogenicity for different avian and mammalian hosts (Everett *et al*., 1999; Vanrompay *et al*., 2007). Transmission of *C. psittaci* mainly occurs through inhalation of contaminated materials or ingestion such as aerosolized faecal dust, feather particles or dried respiratory tract secretions from infected birds and vertical transmission in birds were also reported (Wittenbrink *et al*., 1993; Lublin *et al*., 1996; Harkinezhad *et al*., 2009a). Infection typically causes influenza-like symptoms and the clinical presentation can range from mild respiratory infection to severe pneumonia and systemic illness (Vanrompay *et al*., 1995; Harkinezhad *et al*., 2009b; Rohde *et al*., 2010). Domestic and companion birds are considered as the main risk for the transmission of chlamydiosis to humans, closely and

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continued contact between humans and infected birds may also lead to outbreaks (Vanrompay *et al*., 2007; Harkinezhad *et al*., 2009a; Zweifel *et al*., 2009).

C. psittaci infection in birds has been referred as avian chlamydiosis with a wild range of hosts and infection has been documented in approximately 467 bird species in 30 different orders (Kaleta and Taday, 2003). Peafowls are large and brightly birds of the pheasant family, Phasianidae, naturally found in the tropical forests of Southeast Asia and parts of China, especially in Yunnan province, Southwestern China where has long been known as the animal kingdoms. But as an ornamental bird, peafowls are also cultured in many zoos. Earlier studies have identified a number of pathogens of potential conservation concern for this species such as avian influenza, avian pox and toxoplasmosis (Liu *et al*., 2005; Khan *et al*., 2009; Ismail *et al*., 2010; Tian *et al*., 2012). *C. psittaci* was first isolated from infected peacocks in China in 2009 (Yang *et al*., 2011) suggesting that chlamydiosis in peacocks could present a public health hazard by zoonotic transmission. However, the sample size in that study was very small (only 20 pharyngeal swabs of peafowls were sampled) and information about *C. psittaci* infection in peafowls still remains scant in the world. The objective of the present investigation was therefore to determine the seroprevalence of *C. psittaci* infection in peafowls in Yunnan province, Southwestern China which will provide base-line information on potential risk factors associated with infection and potential implications for public health.

MATERIALS AND METHODS

The investigated area: The survey was conducted in Yunnan province (97°31'-106°11'E, 21°8'-29°15'N) which is located in the far Southwest of China spanning approximately 394,000 km^2 with a population of 45.7 million. Approximately 800 bird species can be found in this province including green and blue peafowls. The serum samples of peafowls in present study were obtained from animal zoos or peafowl gardens in two administrative regions of the province, namely the Kunming city and Xishuangbanna Dai Autonomous Prefecture (Banna for short) (Tian *et al*., 2012).

Serum samples: Two hundred and seventy peafowls were sampled between November 2011 and February 2012 in Yunnan province. Blood was collected via the wing vein of the peafowl. Serum samples were obtained by centrifugation at 3000 g for 10 min and stored at -20°C until further study. Whenever possible, information the breed, geographic region and age were obtained for each bird from bird owners or veterinarians. The surveyed peafowls included 265 blue peafowls (*Pavo cristatus*) and 5 green peafowls (*Pavo muticus*). Total 196 blue peafowls from peafowl gardens in Banna were mainly bred in captivity in small groups for table delicacy as well as ornamental purposes but 74 peafowls (69 blue peafowls and 5 green peafowls) from two zoos in Kunming city (44 in the wild animal zoo, 30 in the Yuantongshan Zoo) were free-range raised for tourists to visit in peacock gardens in the zoos. Apart from 69 peafowls which lack age information, other examined peafowls were grouped into two categories based on their growth cycle: the adult birds (≥24 months, 44 birds) and adolescent birds (≥5 months and <24 months, 157 birds) according to the general practice of raising peafowls. The management data of these birds were acquired before collecting serum samples through interviews with bird owners or veterinarians.

Determination of antibodies to *C. psittaci*: Antibodies to Chlamydia infection were determined in peafowl sera using an Indirect Hemagglutination Antibody (IHA) test with a commercially available kit (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province, China) according to the manufacturer's instructions (Xu *et al*., 2010). In brief, 75 μL of IHA dilution solution was transferred into 96 well V-bottomed polystyrene plates with 25 μL of sera added and diluted in a 4 fold series starting from 1:4. After adding 25 μL Chlamydia antigens 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. Both positive and negative controls were provided by the kit and were included in each test.

Statistical analysis: Differences in seroprevalence of examined peafowls between the two age groups and among associated factors were analyzed using the binary logistic regression in SPSS (Release 18.0 Standard Version, SPSS Inc., IBM Corporation, Somers, NY) for Windows. The differences between levels within factors and interactions were considered to be statistically significant and highly significant when $p<0.05$ and $p<0.01$, respectively. Odds-Ratios (OR) with 95% confidence intervals based on likelihood ratio statistics were reported.

RESULTS AND DISCUSSION

As shown in Table 1, a total of 270 peafowls from 2 administrative regions in Yunnan province were examined, 84 of which (31.11%) were found to be positive for

Table 1: Seroprevalence of *Chlamydia psittaci* infection in peafowls in Yunnan province, Southwestern China determined by Indirect Hemagglutination Assay (IHA)

Factors	No. examined	Antibody titers 1:16	1:64	1:256	1:1024	No. positive	Prevalence (%)
Species							
Blue peafowl (*Pavo cristatus*)	265	47	29	4	2	82	30.94
Green peafowl (*Pavo muticus*)	5	0	2	0	0	2	40.00
Locations							
Banna*	196	20	19	4	2	45	22.96
Kunming city	74	27	12	0	0	39	52.70
Ages							
Adolescent (>5 <24 months)	157	5	4	0	0	9	5.73
Adult (≥24 months)	44	15	17	4	2	38	86.36
Unknown	69	27	10	0	0	37	53.62
Total	270	47	31	4	2	84	31.11

Banna*: Xishuangbanna Dai Autonomous Prefecture

Chlamydia antibodies including titers of 1:16 in 47, 1:64 in 31, 1:256 in 4 and 1:1024 in 2 peafowls. Both green peafowls (2/5) and blue peafowls (30.94% of 265) were positive for Chlamydia antibodies but the difference in prevalence between the two peafowl species was not discussed here on account of the small size of green peafowl sampled. Peafowls from zoos in Kunming had higher seroprevalence (52.70% of 74) than peafowls from peafowl gardens in Banna (22.96% of 196 samples), although the difference is not statistically significant ($p = 0.871$). Statistical analysis revealed that the prevalence in adult peafowls (86.36%) was significantly higher than that in adolescent birds (5.73%) ($p<0.01$). There were no statistical interactions between ages and geographical origins of peafowls ($p = 0.124$).

As the causative agent of psittacosis or avian chlamydiosis in psittacine birds and domestic fowls, *C. psittaci* has been known for decades and the highest prevalence rate were found in psittacine birds and pigeons (De Freitas Raso *et al*., 2002; Dovc *et al*., 2005, 2007). In addition, infections can take a subclinical and/or chronic course (Harkinezhad *et al*., 2009a). So, far, only one study on avian chlamydiosis in peafowls has been report worldwide which was conducted in China. In that study, high seropositivity for *C. psittaci* (16/20) and high mortality and severe pneumonia, airsacculitis, diarrhea symptoms in infected peacocks were observed and the infected farmers showed high fever and respiratory distress (Yang *et al*., 2011). The present investigation showed that the overall seropositivity for *C. psittaci* exposure was 31.11% in peafowls in Yunnan province and no obvious clinical signs were observed in seropositive peafowls according to the farmers and veterinarians in the peafowl gardens suggesting recessive infections of the birds.

Risk factors associated with *C. psittaci* seropositivity in peafowls was further analyzed using stepwise logistic regression. It was found that only age proved to influence the seroprevalence significantly which showed a positive correlation with the number of positive peafowls. The prevalence in adult birds (86.36%) was significantly higher than in adolescent birds (5.73%) ($p<0.01$, OR = 104.148, 95% CI = 34.920-310.620). In general, the infected bird shed *C. psittaci* intermittently and can serve as a source of infection of humans and other birds and transmission occurs mainly through inhalation or ingestion of contaminated material (Wheelhouse and Longbottom, 2012). Animals with older age have more opportunities to contact with the pathogens, suggesting higher probability of an immunological response to the pathogen. Though geographical origin is not a crucial factor for *C. psittaci* infection in the peafowl groups in this study ($p>0.05$), peafowls from zoos or peafowl gardens in Banna and Kunming both had highly seropositivity for *C. psittaci* with percentages of 22.96 and 52.70%, respectively. The scenario observed here is of important public health concern since risk of catching psittacosis is not only associated with transient exposures but also with direct contact with the birds (Telfer *et al*., 2005; Fenga *et al*., 2007) and cases of psittacosis caused by contact with infected birds including peafowls have been reported (Harkinezhad *et al*., 2009a; Rodolakis and Yousef, 2010; Yang *et al*., 2011). Peafowls in the present study were mainly kept as ornamental birds in the peafowl gardens and free-range fed on the lawn for the visitors, an increasing number of visitors in these places increase the risk of exposure of visitors to the infected peafowls which plays an important role as infection transmitters to zoo animals and humans.

CONCLUSION

The present investigation demonstrated a high seroprevalence (31.11%) of *C. psittaci* infection in peafowls in Yunnan province, Southwestern China which has implications for peafowl health and public health concern. Therefore, integrated and improved control strategies and measures should be implemented to prevent and control *C. psittaci* infection in peafowls.

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REFERENCES

De Freitas Raso, T., A.B. Junior and A.A. Pinto, 2002. Evidence of *Chlamydophila psittaci* infection in captive Amazon parrots in Brazil. J. Zoo. Wildl. Med., 33: 118-121.

Dovc, A., D. Slavec, R. Lindtner-Knific, O. Zorman-Rojs, J. Racnik, J. Golja and K. Vlahovic, 2007. The study of a *Chlamydophila psittaci* outbreak in budgerigars. Bull. Vet. Inst. Pulawy., 54: 343-346.

Dovc, A., P. Dovc, D. Kese, K. Vlahovic, M. Pavlak and O. Zorman-Rojs, 2005. Long-term study of chlamydophilosis in Slovenia. Vet. Res. Commun., 29: 23-36.

Everett, K.D., R.M. Bush and A.A. Andersen, 1999. Emended description of the order Chlamydiales, proposal of *parachlamydiaceae* fam.nov. and *Simkaniaceae* fam. Nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species and standards for the identification of organisms. Int. J. Syst. Bacteriol., 49: 415-440.

Fenga, C., A. Cacciola, C. di Nola, S. Calimeri and G.D. Lo *et al*., 2007. Serologic investigation of the prevalence of *Chlamydophila psittaci* in occupationally-exposed subjects in eastern Sicily. Ann. Agric. Environ. Med., 14: 93-96.

Harkinezhad, T., T. Geens and D. Vanrompay, 2009a. *Chlamydophila psittaci* infections in birds: A review with emphasis on zoonotic consequences. Vet. Microbiol., 135: 68-77.

Harkinezhad, T., K. Verminnen, M.D. Buyzere, E. Rietzschel, S. Bekaert and D. Vanrompay, 2009b. Prevalence of *Chlamydophila psittaci* infections in a human population in contact with domestic and companion birds. J. Med. Microbiol., 58: 1207-1212.

Ismail, M.M., O.A. Khan, G. Cattoli and H. Lu, 2010. Isolation and identification of highly pathogenic avian influenza virus subtype H5N1 in peafowl (*Pavo cristatus*). Avian Dis., 54: 357-360.

Kaleta, E.F. and E.M. Taday, 2003. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian. Pathol., 32: 435-461.

Khan, A., A. Yousaf, M.Z. Khan, M. Siddique, S.T. Gul and F. Mahmood, 2009. Cutaneous form of pox infection among captive peafowl (*Pavo cristatus*) chicks. Avian Pathol., 38: 65-70.

Liu, S., J. Chen, X. Kong, Y. Shao and Z. Han *et al*., 2005. Isolation of avian infectious bronchitis coronavirus from domestic peafowl (*Pavo cristatus*) and teal (*Anas*). J. Gen. Virol., 86: 719-725.

Lublin, A., G. Shudari, S. Mechani and Y. Weisman, 1996. Egg transmission of *Chlamydia psittaci* in turkeys. Vet. Rec., 139: 300-300.

Rodolakis, A. and M.K. Yousef, 2010. Zoonotic potential of *Chlamydophila*. Vet. Microbiol., 140: 382-391.

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

Telfer, B.L., S.A. Moberley, K.P. Hort, J.M. Branley and D.E. Dwyer *et al*., 2005. Probable psittacosis outbreak linked to wild birds. Emerg. Infect. Dis., 11: 191-197.

Tian, Y.M., F.Y. Dai, S.Y. Huang, Z.H. Deng and G. Duan *et al*., 2012. First report of *Toxoplasma gondii* seroprevalence in peafowls in Yunnan Province, Southwestern China. Parasit. Vectors., Vol. 5, 10.1186/1756-3305-5-205.

Vanrompay, D., R. Ducatelle and F. Haeserouck, 1995. *C. psittaci* infection: A review with emphasis on avian Chlamydiosis. Vet. Microbiol., 45: 93-119.

Vanrompay, D., T. Harkinezhad, M. van de Walle, D. Beeckman and C. van Droogenbroeck *et al*., 2007. *Chlamydophila psittaci* transmission from pet birds to humans. Emerg. Infect. Dis., 13: 1108-1110.

Wheelhouse, N. and D. Longbottom, 2012. Endemic and emerging chlamydial infections of animals and their zoonotic implications. Transbound. Emerg. Dis., 59: 283-291.

Wittenbrink, M.M., M. Mrozek and W. Bisping, 1993. Isolation of *Chlamydia psittaci* from a chicken egg: Evidence of egg transmission. Zentralbl Veterinarmed B., 40: 451-452.

Xu, M.J., Y. He, R. Liang, D.H. Zhou and R.Q. Lin *et al*., 2010. Seroprevalence of *Chlamydia* infection in pigs from intensive farms in Southern China. J. Anim. Vet. Adv., 9: 1143-1145.

Yang, J., Y. Ling, J. Yuan, W. Pang and C. He, 2011. Isolation and characterization of peacock *Chlamydophila psittaci* infection in China. Avian. Dis., 55: 76-81.

Zweifel, D., R. Hoop, K. Sachse, A. Pospischil and N. Bore, 2009. Prevalence of *Chlamydophila psittaci* in wild birds-potential risk for domestic poultry, pet birds and public health? Eur. J. Wildl. Res., 55: 575-581.