Letter to the Editor

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First report of *Chlamydia psittaci* seroprevalence in black-headed gulls (*Larus ridibundus*) at Dianchi Lake, China

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Abstract: *Chlamydiosis* is an important zoonosis which can transmit from birds to humans, and investigation first reported the seroprevalence of *Chlamydia psittac*i in *black-headed gulls* (*Larus ridibundus*) at the Dianchi Lake, China. A total of 1029 serum samples collected from *black-headed gulls* between 2012-2015 were analyzed. The gulls were randomly caught and blood collected at Dianchi Lake, China. All the samples were analyzed for the presence of antibodies to *C. psittaci* by indirect hemagglutination assay (IHA). In this survey, the total infection rate was 11.86% (122/1029). The results of the present survey documented the existence of relatively high *C. psittaci* seroprevalence in black-headed gulls, which have a potential risk to the wild bird health and human health. Comprehensive practical control approaches and measures should be executed.

Keywords: *Chlamydia psittaci*, Black-headed gulls, Seroprevalence

1 Introduction

Chlamydiosis is a naturally occurring zoonotic disease that causes serious psittacosis, myocarditis, and pneumonia in humans [1]. *Chlamydia psittaci* is an obligate intracellular bacterium that is evident to be responsible for the infection of different species (such as parrots, pigeons, doves, waterfowl, songbirds and the like) of wild birds and humans [2]. The oldest report of *chlamydiosis* can be dated as far back to 1880, when humans were infected by *C. psittaci* in a case report within Switzerland [3]. Upon further studies, researchers isolated the same pathogen in certain patients and amazon parrots, which led to the awareness of posing public health concerns of *Chlamydia* in humans and animals (such as infected avian and birds) [4-7]. *C. psittaci* can be detected in the droppings of infected birds, and the bacterium can remain infectious in the environment over months [8]. In addition, wild birds usually act as carrier during long-distance migration; therefore, a portion of birds could contribute to the potential interspecies and intraspecies transmission of *Chlamydiosis* [9]. Human infection usually occurs when a person inhales the bacterium shed in faeces and secretions of infected birds [10]. One important avian carrier is the black-headed gulls, a species of migratory birds distributed in Eurasia and along the east coast of North America. During the winter season, they migrate to North Africa, the Philippines, Japan, or China from Siberia. Black-headed gulls could potentially be hosts of *C. psittaci* and act as notable carriers in spreading *Chlamydiosis* via seagulls to humans. However, no information is available regarding the seroprevalence of *Chlamydia* infection in the migratory Black-headed gulls located at the Dianchi Lake of China [11]. Black-headed gulls migrate from Siberia to the Dianchi Lake between December and March every year. During these periods, favorable temperatures allow the spread of *C. psittaci* from black-headed gulls to humans through manual feeding, petting or other direct physical contact [12]. However, the symptoms of

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Chlamydia in human and animals are mild and difficult to diagnose, thus the *C. psittaci* infections within the wild birds and general public may be underestimated [13]. *C. psittaci* is a major concern for public health. If black-headed gulls carry these pathogens, infectious diseases maybe transmitted through water, resulting in infection in humans during the migration process. Thus, the objective of the present study is to determine the seroprevalence of *Chlamydial* infection in black-headed gulls at the Dianchi Lake, near Kunming of Yunnan Province, southwest of China.

2 Materials and Methods

2.1 Sample Collection

1029 black-headed gulls were caught at Wildlife Bird Observatory near Dianchi Lake between the months of December and February, from 2012 to 2015. The black-headed gulls were taken randomly and blood samples of black-headed gulls were collected from the wing veins of each bird. Blood samples were centrifuged (3,000×g) for 5 min at room temperature (15°C), and serum samples were stored at -20°C for the detection of *Chlamydia psittaci* antibodies.

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. The sampling protocols were approved by an independent Wildlife Animal Care and Ethics Committee of Yunnan Agricultural University (YNPAA2015001).

2.2 Determination of antibodies against *C. psittaci*

Antibodies to *Chlamydia psittaci* were determined by indirect hemagglutination assay (IHA) using commercial kit as reported previously [14-19]. The kit was purchased by Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences. The detection procedures were carried out as previously reported [17-18]. In summary, 75 μL of dilution IHA solution was transferred into 96-well V-bottomed polystyrene plates supplemented with 25 μL of serum and diluted in a four-fold series from 1:4 to 1:64 with physiological saline. The plates were shaken for 2 min and incubated at 37ºC for 2 h without shaking. The result was considered positive when a layer of agglutinated erythrocytes was formed at dilutions of 1:16 or higher, and both positive and negative controls (blank controls) were included in each test.

3 Results

We tested for anti-*C. psittaci* antibodies from 1029 serum samples of *black-headed gulls* using IHA. Out results demonstrated that 112 of 1029 (11.9%) serum samples were positive for chlamydial infection by IHA (Table 1). Furthermore, the prevalence was 6.3% (17/270) in 2012, 10.1% (57/565) in 2013, 20.4% (23/113) in 2014, and 30.9% (25/81) in 2015, respectively, suggesting that the annual prevalence of chlamydial infection among black-headed gulls had increased progressively throughout the years at the Dianchi lake.

4 Discussion

This data indicates that chlamydial infection was present in migratory black-headed gulls at the Dianchi Lake from Siberia. In this study, the overall *Chlamydia* seroprevalence in black-headed gulls was 11.9%, comparatively higher to then previous reports of wild birds (5.7% of feral pigeons (*Streptopelia chinensis*)) in Slovakia [13], 6.0% of wild birds in Poland [14], yet the chlamydia seroprevalence in black-headed gulls is a lower value than that of North Atlantic Seabirds (Morus bassanus, Larus argentatus, Uria aalge), at 18.5% [20]. The difference is likely due to variations in ecologic factors, geography, and hygienic conditions. More importantly, the new findings of our study further

Table 1. Prevalence of chlamydial infection among *black-headed gulls* in the Dianchi Lake, China, examined by indirect hemagglutination test (IHA).

Location	No. Tested	No. positive(%)	Prevalence
Dianchi Lake, China	270	17	6.3
	565	57	10.1
	113	23	20.4
	81	25	30.9
Total	1029	122	11.9

indicate that the seroprevalence of *C. psittaci* in the black-headed gulls of China is increasing. This result urges the implementation of comprehensive control measures to reduce *Chlamydiosis* prevalence in migratory black-headed gulls.

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