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PREVALENCE OF AVIAN CHLAMYDOPHILA PSITTACI IN CHINA

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Abstract

Examinations were carried out in 46 intensive farms in northern China to investigate avian *Chlamydophila psittaci.* Five hundred and twenty-five avian sera were selected for examining antibodies to *C. psittaci* by ELISA. One hundred and fifty-five clinical samples from throat swabs and oviduct tissues were tested for the presence of chlamydial antigen using IDEIA^{TM} PCE chlamydia dual amplification immunoassay, and 60 samples were tested by ompA gene-based PCR. *C. psittaci* antibodies were detected in 387 (77.8%) out of 525 serum samples, with seroprevalences ranging from 50% to 100%. Among the tested samples, 98/150 (65.3%) in broilers, 173/210 (82.3%) in ducks, and 116/165 (70.3%) in laying hens were detected to be positive, respectively. Using PCE-ELISA test kits, in 91 out of 155 clinical samples the presence of antigen was confirmed, while 64 samples were negative. Forty-three PCR's were tested as positive out of 60 samples, while 17 samples were confirmed to be negative. Both higher positive antibodies and the presence of antigens were found in avian flocks associated with typical clinical signs suggestive of chlamydiosis. This study showed a severe prevalence of *C. psittaci* among different species of domestic birds in China.

Key words: poultry, *Chlamydophila psittaci,* chlamydiosis, antibodies, immunodiagnosis, China.

Avian chlamydiosis (AC) is caused by the bacterium *Chlamydophila psittaci*（*C. psittaci).* It has been isolated from a wide range of avian species (1, 3). Chlamydiae are responsible for a variety of infections in birds and are important aetiological agents in respiratory diseases. Moreover, this pathogen is known to be transmissible to humans causing significant zoonotic infection (7, 8). *C. psittaci* infection in turkey has been present in the US already for more than 50 years, and has become prevalent in turkey farms in Europe today, resulting in economical problems (3, 12). There are no commercial vaccines available for chlamydiosis control in poultry. Antibiotics are the only current means of control; however, *C. psittaci* is becoming resistant to a number of antibiotics due to their administrative abuse (7). Hence, it is important for scientists to control avian chlamydioses and the cross-species transmission.

Since 1980s, outbreaks of respiratory diseases in poultry were recorded in ducks, pigeons, imported flamingo, and black swan in China. Later on, avian *C. psittaci* strains were isolated and identified in laying hens and broilers (4, 9). Chlamydial infection was ignored in recent years due to the outbreak of avian influenza in China and there are no recent data on the prevalence of chlamydial infection in Chinese poultry. Hence, the study was carried out in order to determine the prevalence of chlamydial infection in poultry flocks from different regions of China.

Material and Methods

Serum and clinical samples. The examinations were performed on 525 blood samples from 46 farms, representing 7 main intensive poultry areas in China, 1 farm of specific pathogen free (SPF) hens, 5 farms of commercial ducks, 6 farms of breeder ducks, 10 farms of parent laying hens, and 24 farms of laying hens. The blood samples were collected from birds between 20 and 40 weeks of age. Serum samples were kept frozen at -20°C until testing.

One hundred and fifty-five clinical samples were collected from suspected birds, including 10 throat swabs from SPF hens. Ten oviduct membranes from laying hens with cystic oviduct, 30 throat swabs from boilers, 50 throat swabs from laying hens, and 55 throat swabs from laying ducks. Approximately 100 mg of minced tissues from cystic oviduct were suspended in Eagle's minimal essential medium (MEM). The samples were vortexed with 3-mm-diameter glass beads, centrifuged at 500 x *g* for 5 min at 4°C, and the supernatants were collected for the detection of chlamydial antigens. Throats samples for the antigen test and PCR were placed in chlamydial transport medium and stored at -20C° until use (1).

Detection of chlamydial antibodies and antigens. Sera were tested for the presence of antibodies

using RIDASCREEN *Chlamydia psittaci* ELISA (R-Biopharm, Darmstat, Germany) according to manufacturer's recommendation. The collected tissues samples and throats were screened using IDEIA^{TM} PCE chlamydia dual amplification immunoassay (Dako Cytomation Ltd., UK) following the manufacturer's procedure. IDEIA^{TM}PCE-ELISA is based on the identical monoclonal antibody for binding and capturing C*hlamydia* from a sample matrix. The value of optical density (OD) below 0.119 were considered as negative, while values above 0.147 as positive. Positive values were subdivided into weakly (OD=0.147-0.222), moderately (OD=0.230-0.310), and strongly positive (OD=0.320). This allowed discrimination between farms with more recent and severe chlamydial infection.

PCR analysis. Genomic DNA of autopsy samples was prepared from purified *Chlamydia* using the DNeasy Tissue Kit (Qiagen) following the manufacturer's instructions. The PCR procedure was performed using a pair of oligonucleotide primers CTU/CTL (CTU, 5'-ATG AAA AAA CTC TTG AAA TCG G-3', CTL, 5'-CAA GAT TTT CTA GAC TTC ATT TTG TT-3') chosen in the highly conserved regions of the published *omp1* sequences (5,6,11). One reference strain of the genomic DNA was *Chlamyidophila psittaci* ISN 1528 from Dr David Longbottom (Moredun Research Institute, UK). A non-infected McCoy cells preparation was included as a negative control. PCR products were separated by 2.5% or 1.0% agarose gel (Sigma) electrophoresis and visualised by ethidium bromide staining. The DNA molecular weight marker (Boehringer Mannheim) was used as a size marker.

Results

Antibodies against *Chlamydia* in poultry flocks. Out of 525 blood samples, 387 (73.7%) revealed a significant level of *C. psittaci* antibodies, while 138 (26.3%) were negative. With respect to regions, there was 74.5%, 84%, 70%, 78%, 61.3%, 53.3%, and 73.3% positive, respectively, in different provinces. The highest percentage of seropositive birds was found in both breeder hens and SPF laying hens from Beijing (Table 1). Sera from laying hens in the Shanxi province had the lowest incidence of positive results (53.3%). It is obviously evident that parent flocks have a higher positive rate than commercial flocks. Regarding species, positive rates were 65.3%, 70.3%, and 82.3% in broilers, laying hens, and ducks, respectively (Table 2).

Antigen detection in throat swabs and oviduct tissues in birds with clinical signs. Using PCE-ELISA kits, 91 out of 155 clinical samples were detected to be positive (58.7%), while 64 samples were negative (Table 3). It was interesting to find that laying hens with oviduct cysts revealed more positive results (70%). The highest positive rate was observed in duck flocks (93.3%) with a lower egg production, while 73.3% positive results were detected in laying hens with similar clinical signs. As antigen presence is concerned, broilers with a severe pneumonia revealed 60% positive results, as compared to 30.0% in the control group. In this epidemiological survey, in clinically healthy SPF hens a lower incidence of antigen presence was detected.

Analysis of avian clinical samples by PCR test. As expected, PCR reactions using genomics DNA purified from clinical samples as templates with CTU/CTL primers generated amplication products of an average size of 1200-bp as well as a slightly smaller band. Forty-three out of 60 (71.6%) tissues samples were PCR positive, while 17 (28.4%) were negative (Table 4). Particularly, 9 out of 10 samples taken from laying hens with severe cystic oviduct form were PCR positive, while 8 of 10 samples were ELISA positive in laying hens with a lower egg production. Higher positive rate was also found in duck samples (90%) as well as in broilers with clinical signs (70%). PCR analysis revealed 1 positive result in 10 throat swabs in SPF hens.

Discussion

In the present study, ELISA, PCE-ELISA, and PCR were used to examine the prevalence of avian chlamydiosis in China. In average, all of the 46 poultry farms examined were 77.8% positive for *Chlamydia* antibodies by ELISA, 57.4% positive for antigen by PCE-ELISA, and 71.6% positive for antigen detected by PCR. Between 1990 and 2000, positive antibodies to *C. psittaci* were found in 21.0% of laying hens and 20.6% of broilers by indirect haemagglutination inhibition (IHI) test (9). Recently, a report showed that an average of 24.9% positivity was confirmed using IHI test kits (2). In the current study, 77.8% positivity was detected by ELISA kits. In contrast to the serological results, 57.4% of 155 clinical samples were antigen positive. This is the first report on the presence of chlamydial antigen using PCE-ELISA and PCR. The evidence shows that avian chlamydiosis is prevalent in laying hens, ducks, and broilers.

In European countries, avian chlamydiosis has also been increasing in recent years. In Belgium in 2002, 188 out of 200 (94%) turkey sera reacted positively, compared to 175 of 200 (87.5%) in 1992 (13). In Bosnia and Herzegovina, and in Macedonia, 45.5% of intensively bred chickens, 12%-32.8% of free living pigeons, and 8% of parrots investigated with PCE-ELISA were positive to *C. .psittaci* antigen (10). Laying hens with poor egg production revealed the presence of antigens in 22 out of 28 (78.5%) triple swabs (conjunctiva, pharynx, and cloaca) (8). Therefore, a higher presence of antigens and seropositivity in avian species demonstrates again that a potential relationship between chlamydial infection and avian health should be considered.

Table 1
Seroprevalence of *C. psittaci* in different regions

Regions	Species	Numbers	Positive	Negative	Positive (%)
Beijing	Breeder hens[a]	30	27	3	90.0
	Ducks[b]	30	25	5	83.3
	Broilers[c]	40	20	20	50.0
	SPF hens[d]	10	10	0	100.0
Hebei	Laying hens[b]	45	35	10	77.7
	Breeder ducks[b]	80	70	10	87.5
Tianjin	Broilers[c]	50	35	15	58.3
Inner Mongolia	Ducks[b]	100	78	22	78.0
Shandong	Laying hens[b]	50	28	22	56.0
	Broilers[c]	30	21	9	70.0
Shanxi	Laying hens[b]	30	16	14	53.3
Henan	Broilers[c]	30	22	8	73.3
Total		525	387	138	73.7

[a] Birds with a oviduct cyst; [b] Birds with an unknown egg drop; [c] Broilers with a sever pneumonia; [d] SPF laying hens with a clinical health

Table 2
Seroprevalence of avian *C. psittaci* in different poultry flocks

Species	Number of birds	Positive	Negative	Positive (%)
Broilers	150	98	52	65.3
Laying hens	165	116	49	70.3
Ducks	210	173	37	82.3

Table 3
Detection of *Chlamydia* antigen in avian clinical samples by PCE-ELISA

Species	Numbers of birds	Positive	Negative	Positive (%)
Breeder hens[a]	10	7	3	70.0
Laying hens[b]	30	22	8	73.3
Control hens[c]	20	3	20	15.0
Ducks[b]	30	28	4	93.3
Control ducks[c]	25	15	10	60.0
Broilers[d]	20	12	8	60.0
Control broilers[e]	10	3	7	30.0
SPF hens[e]	10	1	9	10.0
Total	155	91	64	58.7

[a] Breeder hens with oviduct cyst; [b] Birds with a lower egg production (≤50%); [c] Birds with a higher egg production (≥85%); [d] Broilers with a severe pneumonia; [e] Clinically healthy birds

Table 4
Detection of *C. psittaci* in avian clinical samples by PCR

Species	Numbers of birds	Positives	Negatives	Positive (%)
Breeder hens[a]	10	9	1	90.0
Laying hens[b]	10	8	2	80.0
Ducks[b]	20	18	2	90.0
Broilers[c]	10	7	3	70.0
SPF chickens[d]	10	1	9	10.0
Total	60	43	17	71.6

[a] Birds with cystic oviducts; [b] Birds with lower egg production (≤50%); [c] Broilers with a severe pneumonia; [d] Clinically healthy birds

Interestingly, swab samples revealed 10% antigen positivity, while their corresponding serum showed 100% seropositivity. SPF eggs are widely used for growing attenuated vaccine and purifying bio-drugs for humans. Once SPF chickens are infected, chlamydia will be transmitted directly to each vaccinated chicken and in consequence to human beings. Whether it is true or not, further studies will be done to assess the morbidity of SPF chickens and potential risk for human health in the future. It is high time that attention will be paid to *Chlamydophila psittaci* infection and its implications.

A complement fixation test (CFT) and cell cultures are considered as a golden standard (1, 3). However, CFT is unsuitable for other poultry species (turkey, duck, and chickens) except for pigeon, due to poor cross-species sensitivity and reactivity. Moreover, it is time-consuming and needs standard test reagents. In contrast to CFT, PCE-ELISA and PCR, based on *ompA* gene, are reliable methods to identify the typical inclusion bodies in suspected samples. Current PCR tests for the detection of *C. psittaci* target the *omp1* gene. As far as avian chlamydial diagnosis is concerned, tracheal swabs extracts used in this study provided a suitable material for analysis. PCR, with the use of CTU/CTL primers, performed on extracted DNA was more sensitive than PCE-ELISA. The test was also useful when isolation of chlamydia was a less favourable method, and when the bacterium cannot be cultured from pathological samples due to the need for rapid diagnosis (5, 6). In the present study and earlier studies, the question arises of the diagnostic and economic significance of *C. psittaci* as a causative agent of pneumonia, oviduct cysts, and poor laying performance. Additional investigations will focus on the transmission route and efficient measure for control of avian chlamydial infection.

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