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Isolation of *Chlamydophila psittaci* from Laying Hens in China

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Abstract: Twenty six hens which were suspected to suffer from chlamydiosis were detected with Indirect Hemagglutination test (IHA) and twelve sera samples of them were positive. The *C. psittaci* was isolated from yolk sac of embryonated eggs. The MOMP gene of *C. psittaci* was amplified with PCR and a strand of 1170 bp was got.

Key words: Isolation, *Chlamydia psittaci*, laying hen, IHA, PCR, MOMP gene

INTRODUCTION

Avian Chlamydiosis (AC), previously named psittacosis or parrot fever or ornithosis is caused by *Chlamydophila psittaci* (Friis, 1972). *Chlamydophila* is the new genus name adopted in a reclassification that separates the family Chlamydiaceae into two genera, namely: *Chlamydia* and *Chlamydophila* (Everett, 1999). At present, eight serovars of *C. psittaci* were existed and six of them (A-F) are considered endemic in birds. *C. psittaci* is known to infection most species of pet birds, poultry (including ducks, turkeys, geese and related domestic species) and wild birds (Andersen, 1997; Vanrompay *et al*., 1995). Moreover, the avian strains can infect humans and other mammals and may cause pneumonia, encephalitis and even death (Olsen and Treuting, 1944; Broholm *et al*., 1977; Meyer and Eddie, 1951).

In China in a poultry farm in Lanzhou city an infectious disease which showed eggs dropping, malformed eggs, soft shell eggs, ascites, anorexia, weight loss, diarrhea and depression took place in the laying hen. The loss of eggs can reach 50%. However, all the chickens were vaccinated with vaccines of Eggs Drop Syndrome (EDS), infectious bronchitis and mycoplasma gallisepticum. The antibody of them still kept at protective level. According to the clinical features, 24 laying hens of 1 year old from a shed of 15,000 were submitted for laboratory examination. So the study of isolation and detection were done in the laboratory.

MATERIALS AND METHODS

***C. psittaci* isolation:** Material from liver and spleen were filtered after grinding and inoculated into the yolk sac of embryonated 7 days old eggs to isolate *C. psittaci.* When 50% of the inoculated eggs died at third passage, no bacteria were observed in the Giemsa staining smears made with the dead chick embryos yolk sacs membrane but a lot of chlamydial elementary body-like particles. Subsequently, the yolk sacs of further eggs were inoculated with infected yolk sac membrane. All the eggs died at 120 h after inoculation. Duplicate yolk sac membrane smears were given.

Antibody to *C. psittaci* detection: Chicken blood was collection and Indirect Hemagglutination test (IHA) was done to detect the antibody to *C.psittaci* (Changqing *et al*., 2002). PBS was added to 96-well plates at 25 μL per well. Chick sera (25 μL) were added to the first well and successive two-fold dilutions were made from the first well to the eighth well in each row. To each well was added 25 μL *C. psittaci* antigen linked to the surface of sheep red blood cells. Positive and negative controls were set up simultaneously. The plate was shaken gently and incubated at 37°C for 2 h. The results were considered positive if the test sera showed agglutination at ≥1:16 in comparison with the controls.

***C. psittaci* DNA extraction and Polymerase Chain Reaction (PCR):** In order to detect laying hen infection caused by *C. psittaci* or not, PCR technique was used and the Major Outer Membrane Protein (MOMP) gene was detected. A pair of PCR primers was designed using

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Primer 5.0 software according to the published sequence of avian *C. psittaci* in GenBank (accession No. L25436). The forward primer was 5'-AGG AGA TCT ATG AAA AAA CTC TTG AAA TCG-3' and the reverse primer was 5'-TGG GTC GAC TTA GAA TCT GAA TTG AGC ATTC-3'. The total genomic DNA of *C. psittaci* was extracted from infected chick embryo yolk membrane (Hewinson *et al*., 1997). The amplification was performed in a 50 μL reaction mixture containing 5 μL 10× PCR buffer, 4 μL dNTP (each 2.5 mM), 50 pmol of each primer, 0.25 μL Pyrobest™ DNA polymerase (5U μL^{-1}) (Japan) and 4 μL extracted DNA. Reactions were run in a thermocycler (Techgene, UK) with the following program: denaturation at 95°C for 5 min, 35 cycles composed of denaturation at 95°C for 1.5 min, annealing at 53°C for 1 min and extension at 72°C for 2 min. Finally, extension was carried out at 72°C for 10 min.

Sequencing of PCR products: The amplified PCR products were purified by TakaRa Gel Extraction Kit (TaKaRa Biotechnology Co., Ltd, Japan), ligated with a pMD18-T Vector system (TaKaRa Biotechnology Co., Ltd, Japan), transferred into *Escherichia coli* DH 5α strain. The MOMP gene clones were sequenced by TaKaRa Biotechnology (Japan).

RESULTS AND DISCUSSION

Giemsa staining: No bacteria were observed in the Giemsa staining smears made with the dead chick embryos yolk sacs membrane but a lot of chlamydial elementary body-like particles and should be doubt that the death of eggs was caused by *C. psittaci.*

Antibody detection of *C. psittaci*: About 26 hens of 240 days age blood specimens were collected from the submitted hens that these hens had the clinical features as described previously and antibodies to *C. psittaci* were determined by IHA kit. Seven of 26 hens sera had high titer of chlamydial antibody (1:128) and 5 of them were 1:64 according to the diagnosis standard in China (if titer of sample ≥1:16, the serum sample can be seen positive sample). This means that these chicks were infected once by *C. psittaci.*

Anatomization of infected laying hens: All the hens were examined at autopsy and 11 of which showed pneumonia, pericarditis and the majorest features were hepatosplenomegaly and a part of hens showed oviduct enlargement.

PCR detected for MOMP gene and sequence comparison: A fragment of about 1170 bp was got through clone and sequence (Fig. 1). Comparisons of the MOMP amino acid sequence of isolated strain with psittacine strain VS225 (F serotype), GD (CP3 (B serotype), CT1, there were 94.4, 89.7, 82.3 and 88.7% homology with them, respectively.

The *C. psittaci* was studied from avian chlamydiosis in china in 1959. From that time, a series of reported of *C. psittaci* were given in dove, table poultry but no report in detail of laying hens was given (Cheng *et al*., 2004; Changqing *et al*., 2003; Qiong-qui and Liu, 1997).

In birds *C. psittaci* can cause a systemic infection. Typical clinical features in a susceptible host infected with a highly virulent include respiratory signs, mucopurulent nasal and conjunctival discharge, diarrhea, polyuria and dullness. Yellow-green droppings are common. Strains of low virulence will produce clinical signs that similar but less extensive. However, the typical clinical features of nasal and conjunctival discharge were not seen in laying hens the farm in China. This phenomena was different from the infection of ducks, turkeys. It may be has the same condition of reported that chickens are relatively resistant to *C. psittaci* (Andersen, 1997). The significance of the infection to the laying hens is as yet uncertain and needs further investigation.

PCR techniques were used to detecte *C. psittaci.* Different strategies have been used according to the diagnosis target such as OmpA, 16S rRNA, OmpB (Hewinson *et al*., 1997; Messmer *et al*., 1997; Moroney *et al*., 1998; Olsen and Treuting, 1944; McElnea and Cross, 1999).

These tests were reported as able to detect *C. psittac*i in samples of tissues, faeces and choanal and cloacal swabs and are sensitive, rapid and have performed better than traditional tissue staining methods and culture.

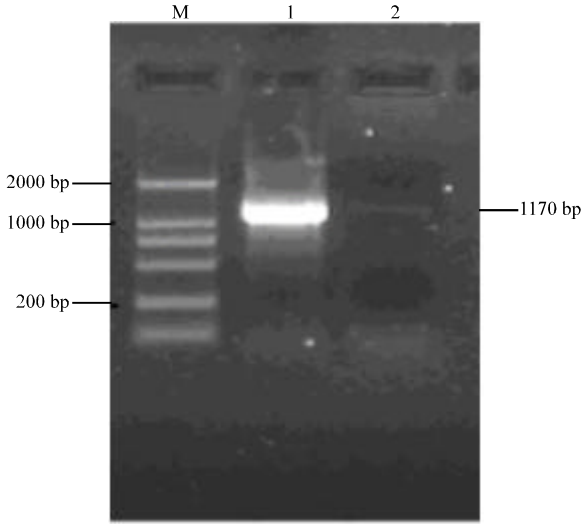

Fig. 1: PCR product of MOMP gene of *C. psittaci* (lane 1) M was DL2000 DNA Marker (Takara Biotechnology, Japan). Lane 1 was MOMP gene of *C. psittaci*. Lane 2 was negative control

In this test, the MOMP gene as target gene was detected. A brand about 1170 bp was amplified by PCR. Comparisons of the MOMP amino acid sequence of isolated strain with psittacine strain VS225, GD, CP3, CT1, there was highest homology with VS225 (reach 94.4%). It is indicated that the isolated strain from laying hens was possible have same serotype with VS225. But the host association of VS225 strain is psittacine. Where is the Chlamydia psittaci come from? How do Chlamydia psittaci transmit from parrot to laying hens? These questions made us confusion and the most possible reason is that there was a bird market near the farm (about 1 km).

The keepers of the farm always go to the birds market. May be they brought *Chlamydia psittaci* into the farm. This is the only reasonable reason to explain the phenomena. In China, there were several reports about *C. psittaci* in dove, broiler but there have no report in laying hens in detail. How many avian species were infected by *C. psittaci* and how many serotypes of them were prevailed in China this would be made us to do more researcher.

CONCLUSION

In this study comparisons of the MOMP amino acid sequence of isolated strain with psittacine bird strain VS225, there was 94.4% homology between them.

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