Occurrence of *Chlamydia* spp. in wild birds in Thailand

Suksai Parut\(^1\), Onket Rattanaporn\(^2\), Wiriyarat Withthawat\(^1,3\), Sangkachai Nareerat\(^1\), Lekcharoen Paisin\(^1\), Sariya Ladawan\(^1\)

\(^1\)The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Salaya, Nakhon Pathom, Thailand

\(^2\)Faculty of Veterinary Technology, Kasetsart University, Nong Wichit, Nong Bua, Nakhon Ratchasima, Thailand

\(^3\)Department of Preclinical Sciences and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand

**Objective:** To determine the occurrence of *Chlamydia* spp. in wild birds in Thailand.

**Methods:** Cloacal and tracheal swabs of 313 wild birds from 11 orders, 27 families, and 51 species were tested to determine the occurrence of *Chlamydia* infection. The outer membrane protein A (ompA) gene was amplified from positive samples to construct a phylogenetic tree.

**Results:** At the time of sample collection, none of the birds showed clinical signs of any disease. Of 313 wild birds, two Asian openbill stork (*Anastomus oscitans*) were positive for *Chlamydia* spp., representing 0.64% (2/313) and 4.9% (2/41) occurrence for birds overall and for the Asian openbill stork, respectively. Phylogram analysis based on deduced amino acid sequence of the ompA gene showed that *Chlamydia* spp. in Asian openbill storks was closely related to that in wildfowl (*Pica pica* and *Cygnus olor*) from Poland in a different branch with a 95% bootstrap value and had a shorter evolutionary distance to *Chlamydia abortus*.

**Conclusions:** Asymptomatic Asian openbill storks could be a potential source of *Chlamydia* infection in domestic animals, poultry, and humans who share their habitat.

1. **Introduction**

Chlamydomysis is an infectious disease of several animal species, including wild birds and humans. The disease is caused by an obligate intracellular gram-negative bacteria in the family Chlamydiaceae. To date, Chlamydiaceae comprises 11 species, namely *Chlamydia psittaci* (*C. psittaci*), *Chlamydia felis* (*C. felis*), *Chlamydia abortus* (*C. abortus*), *Chlamydia avium*, *Chlamydia caviae*, *Chlamydia gallinacea*, *Chlamydia muridarum*, *Chlamydia pecorum*, *Chlamydia suis*, *Chlamydia pneumoniae*, and *Chlamydia trachomatis*, and three candidate chlamydial species, namely *Chlamydia ibidis*, *Chlamydia sanziniae*, and *Chlamydia corallus*. Within the chlamydial species, *C. psittaci*, *C. felis*, and *C. abortus* have zoonotic potential\(^7\). Chlamydomysis in birds can range from asymptomatic infection to severe disease with life-threatening illness, depending on the host species affected and the chlamydial species involved. Wild birds are important to public health because they can be infected with *Chlamydia* species that are transmissible to humans and domestic animals\(^8\). Several reports have shown the prevalence of *Chlamydia* in wild birds. In 2015, the positive rate for chlamydial DNA in wild birds in Poland was 7.3% (27/369)\(^9\). Two years later, a large number of wild birds in Poland were tested, and the results revealed Chlamydiaceae prevalence of 14.8% (132/894)\(^10\). Moreover, 10.3% (125/1214) of wild birds in Austria and the Czech Republic have been found to be *Chlamydia* spp. positive\(^11\).
Dna polymerase (iNtRON, South Korea), 0.5 µM of each primer, 1 mM MgCl₂, 2.5 units of iTaq DNA polymerase (iNtRON, South Korea), and 0.5 µM each of forward and reverse primer. The PCR reaction was worked under the conditions of 2 min at 94 °C for initial denaturing, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C, and was terminated at 72 °C for 7 min. After that, DNA fragment of each sample was ligated to the pGEM-T easy vector (Promega, USA) and transformed to competent Escherichia coli TOP10 (Invitrogen™, USA) using the calcium chloride method. Transformants were selected by blue-white screening method. Plasmid was extracted by the QIAprep spin miniprep kit (QIAGEN, Germany) and submitted to Macrogen Inc. (South Korea) for DNA sequencing. A phylogram of deduced amino acid sequences of the *ompA* gene was generated by the maximum likelihood method and the JTT matrix-based model with a bootstrap value based on 1 000 replicates[18]. Evolutionary analyses were conducted with MEGA7 version 7.0 software[19].

3. Results

For all bird samples, the housekeeping gene (*12S rDNA*) was detected to examine the quality of the DNA. All samples were found to be positive for the *12S rDNA* gene, indicating the good quality of the DNA. For Chlamydiaceae detection, of 313 wild birds, two (0.64%) were positive for Chlamydiaceae with asymptomatic infection. These birds were Asian openbill storks (*Anastomus oscitans*), which belong to the order Ciconiiformes. The positive rate for Asian openbill storks was 4.9% (2/41). The *ompA* gene of the positive samples was amplified and sequenced. Nucleotide sequencing of the *ompA* gene (Accession No. MK007613 and MK007614) in our study showed only 94.1% genetic similarity to *Chlamydia* spp. of Eurasian magpies (*Pica pica*) and mute swans (*Cygnus olor*) in Poland (Accession No. KX870484.1, KX424658.1, KX062052.1, KX062055.1). The *ompA* phylogenetic tree analysis
showed that the *Chlamydia* spp. detected in Asian openbill storks can be grouped together with 99% bootstrap support and was closely related to *Chlamydia* spp. detected in Eurasian magpies and mute swans in Poland but had a different cluster creation with a 95% bootstrap value (Figure 1). Additionally, the *Chlamydia* spp. found in this study had a closer relationship to *C. abortus* than any other known *Chlamydia*.

### Table 1

| Order                | Family         | Species                                    | Common name                      | No. of tested bird |
|----------------------|----------------|--------------------------------------------|----------------------------------|--------------------|
| Anseriformes         | Anatidae       | *Anas platyrhynchos domesticus*            | Domestic duck                     | 4                  |
| Galliformes          | Phasianidae    | *Porot cristatus*                          | Indian peafowl                    | 4                  |
| Ciconiiformes        | Ciconiidae     | *Anastomus oscitans*                       | Asian openbill                    | 41                 |
| Pelecaniformes       | Ardeidae       | *Egretta garzetta*                         | Little egret                      | 1                  |
|                      |                | *Isobythys sinusis*                        | Yellow bittern                    | 3                  |
|                      |                | *Phalacrocorax fuscicollis*                | Indian cormorant                  | 2                  |
|                      |                | *Microcarbo niger*                         | Little cormorant                  | 2                  |
| Gruiformes           | Rallidae       | *Anasornis phoenicurus*                    | White-breasted waterhen           | 1                  |
|                      |                | *Porphyrio poliocephalus*                  | Grey-headed swamphen              | 3                  |
| Charadriiformes      | Laridae        | *Chroicocephalus genei*                    | Slender-billed gull               | 2                  |
|                      |                | *Sternula spp.*                            | Unidentified tern                 | 5                  |
|                      |                | *Chroicocephalus brunnicephalus*           | Brown-headed gull                 | 113                |
| Columbiformes        | Columbidae     | *Spilopelia chinensis*                     | Spotted dove                      | 1                  |
|                      |                | *Geopelia striata*                         | Zebra dove                        | 8                  |
| Strigiformes         | Tytonidae      | *Tyto alia*                                | Eastern barn owl                  | 3                  |
|                      |                | *Alope athis*                              | Common kingfisher                 | 4                  |
| Coraciiformes        | Alcedinidae    | *Halcyn smyrnensis*                        | White-throated kingfisher          | 2                  |
|                      |                | *Alectro athis*                            | Common kingfisher                 | 4                  |
| Piciformes           | Picidae        | *Jynx torquilla*                           | Eurasian wryneck                  | 1                  |
|                      |                | *Alcedo athis*                             | Common kingfisher                 | 4                  |
| Passeriformes        | Laniidae       | *Lanius cristatus*                         | Brown shrike                      | 1                  |
|                      |                | *Dicrurus macrocercus*                     | Black drongo                      | 1                  |
|                      |                | *Rhipidura javanica*                       | Malaysian pied fantail             | 3                  |
|                      |                | *Hypophymis azurea*                        | Black-naped monarch               | 2                  |
| Pycnonotidae         | Pycnonotidae   | *Pycnonotus sinica*                        | Black-headed bulbul               | 1                  |
|                      |                | *Pycnonotus aurigaster*                    | Sooey-headed bulbul               | 3                  |
|                      |                | *Pycnonotus corvus*                        | Streak-eared bulbul               | 15                 |
|                      |                | *Pycnonotus sinica*                        | Prinia inornata                   | 3                  |
|                      |                | *Prinia inornata*                          | Plain prinia                      | 3                  |
| Hirundinidae         | Hirundidae     | *Hirundo rustica*                          | Barn swallow                      | 2                  |
|                      |                | *Phylloscopus fuscatus*                    | Dusky warbler                     | 19                 |
| Phylloscopidae       | Laniidae       | *Phylloscopus fuscatus*                    | Dusky warbler                     | 19                 |
| Acrocephalidae       | Acrocephalidae | *Acrocephalus bistrigiceps*                | Black-browed reed warbler         | 12                 |
|                      |                | *Acrocephalus orientalis*                  | Oriental reed warbler             | 13                 |
| Locustellidae        | Locustellae    | *Helioptilus certicola*                    | Pallas's grasshopper warbler      | 2                  |
| Cisticolidae         | Cisticolidae   | *Prinia flaveolens*                        | Yellow-bellied prinia             | 1                  |
|                      |                | *Prinia inornata*                          | Prinia inornata                   | 3                  |
| Pellorneidae         | Pellorneiidae  | *Pellornea rubicola*                       | Puff-throated babbler             | 1                  |
|                      |                | *Acridotheres grandis*                     | Great myna                        | 1                  |
|                      |                | *Sturnia malabarica*                       | Chestnut-tailed starling          | 1                  |
|                      |                | *Gracupica nigricollis*                    | Black-collared starling           | 1                  |
|                      |                | *Acridotheres tristis*                     | Common myna                       | 2                  |
| Muscicapidae         | Muscicapa spp. | *Motacilla cinerea*                        | Unidentified flycatcher           | 1                  |
|                      |                | *Ficedula albicilla*                       | Taiga flycatcher                  | 3                  |
|                      |                | *Calliope callope*                         | Siberian rubythroat               | 1                  |
|                      |                | *Copsychus saularis*                       | Oriental magpie-robin             | 2                  |
|                      |                | *Saxicola stejnegeri*                      | Stejneger's stonechat             | 2                  |
| Passeridae           | Passeridae     | *Passer domesticus*                        | House sparrow                     | 2                  |
|                      |                | *Passer montanus*                          | Eurasian tree sparrow             | 1                  |
|                      |                | *Passer flavipes*                          | Plain backed sparrow              | 2                  |
| Ploceidae            | Ploceidae      | *Ploceus hypoxanthus*                      | Asian golden weaver               | 1                  |
|                      |                | *Ploceus philippinus*                      | Baya weaver                       | 3                  |
|                      |                | *Ploceus minor*                            | Streaked weaver                   | 8                  |
| Estrildidae          | Estrildidae    | *Lonchura punctulata*                      | Scaly-breasted munia              | 1                  |
|                      |                | *Lonchura striata*                         | White-rumped munia                | 1                  |
| Motacillidae         | Motacillidae   | *Anthus rubinulus*                         | Paddyfield pipit                  | 1                  |

### 4. Discussion

Wild birds may play a role as a potential source of Chlamydiaceae that can be transmitted to humans, domestic animals, and poultry[8,20,21]. In the present study, we demonstrated the overall occurrence of *Chlamydia* spp. in several species of wild birds was 0.64%, suggesting low occurrence in wild birds in Thailand. The primers used in this study can detect Chlamydiaceae DNA as low
as 1 fg, indicating high sensitivity of the test\[16\]. The occurrence found in the study was slightly lower than the rate in other countries, which ranges from 2.7% to 14.8%, depending on the bird species and detection method\[9,10,12\]. Phylogram-based ompA gene analysis of the positive samples found that the Chlamydia detected is closely related to Chlamydia detected/isolated in wildfowl in Poland and to C. abortus, which causes abortion and fetal death in ewes and goats, and abortion in women in close contact with aborting animals[7]. The wildfowl Chlamydia strains can presumably be classified as avian C. abortus based on MLST analysis. However, the pathogenicity of the avian C. abortus strains from wildfowl remains unknown[10]. The positive samples detected in our study were from Asian openbill storks in the order Ciconiiformes. A 4.9% prevalence level was found for these birds. Other species in Ciconiiformes were previously reported as having a Chlamydiaceae positive rate of 5.3% (2/38) for white storks[10] and 11.5% (13/113) for herons and allies[20], respectively. The variation of prevalence in Ciconiiformes may depend on the sample size of birds. However, to the best of the authors’ knowledge, Chlamydiaceae has not been previously reported in Asian openbill storks. The Asian openbill stork is a migratory bird, and the migration of Asian openbill stork populations along various migration pathways may be a potential means of spreading of Chlamydiaceae. Asymptomatic birds can transmit the bacterium to domestic birds and humans that share their environment or habitat by handling via fecal shedding and direct contact. In conclusion, this study demonstrates the occurrence of Chlamydia spp. in wild birds in Thailand is 0.64%. Chlamydia spp. in Asian openbill stork could be a potential source of infection in domestic animals, poultry, and humans who share their habitat.

**Conflict of interest statement**

The author declared that they have no conflict of interest.
Foundation project

This work was financially supported by the Faculty of Veterinary Science, Mahidol University. The sample used in this study were collected by the project of Establishment of zoonotic viral networking system: developmental phase; subproject of Influenza A virus surveys in migratory and residence birds of Thailand granting from Cluster and Program Management Office (P-15-50535), the National Science and Technology Development Agency, Thailand.

References

[1] Stephens RS, Myers G, Eppingter M, Bavoil PM. Divergence without difference: Phylogenetics and taxonomy of Chlamydia resolved. FEMS Immunol Med Microbiol 2009; 55(2): 115-119.

[2] Everett KD. Chlamydia and Chlamydiiales: More than meets the eye. Vet Microbiol 2000; 75(2): 109-126.

[3] Vorimore F, Hsia RC, Huot-Creasy H, Bastian S, Deruyter L, Passet A, et al. Isolation of a new Chlamydia species from the feral sacred ibis (Threskiornis aethiopicus): Chlamydia ibidis. PLoS One 2013; 8(9): e74823.

[4] Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, et al. Evidence for the existence of two new members of the family Chlamydiaceae and proposal of Chlamydia avium sp. nov. and Chlamydia gallinacea sp. nov. Syst Appl Microbiol 2014; 37(2): 79-88.

[5] Taylor-Brown A, Bachmann NL, Borel N, Polkinghorne A. Culture-independent genomic characterization of Candidatus Chlamydia sanzinia, a novel uncultivated bacterium infecting snakes. BMC Genomics 2016; 17: 710.

[6] Taylor-Brown A, Spang L, Borel N, Polkinghorne A. Culture-independent metagenomics supports discovery of uncultivable bacteria within the genus Chlamydia. Sci Rep 2017; 7(1): 10661.

[7] Essig A, Longbottom D. Chlamydia abortus: New aspects of infectious abortion in sheep and potential risk for pregnant women. Curr Clin Microbiol Rep 2015; 2(1): 22-34.

[8] Reed KD, Meece JK, Henkel JS, Shukla SK. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clin Med Res 2003; 1(1): 5-12.

[9] Krawiec M, Piuszeki T, Wieliczko A. Prevalence of Chlamydia psittaci and other Chlamydia species in wild birds in Poland. Vector Borne Zoonotic Dis 2015; 15(11): 652-655.

[10] Szynska-Czerwinska M, Mitura A, Niemczuk K, Zareba K, Jodzko A, Pluta A, et al. Dissemination and genetic diversity of chlamydial agents in Polish wildfowl: Isolation and molecular characterisation of avian Chlamydia abortus strains. PLoS One 2017; 12(3): e0174599.

[11] Konicek C, Vodrazka P, Bartak P, Knotek Z, Hess C, Racka K, et al. Detection of zoonotic pathogens in wild birds in the cross-border region Austria-Czech Republic. J Wildl Dis 2016; 52(4): 850-861.

[12] Jeong J, An I, Oem JK, Wang SJ, Kim Y, Shin JH, et al. Molecular prevalence and genotyping of Chlamydia spp. in wild birds from South Korea. J Vet Med Sci 2017; 79(7): 1204-1209.

[13] Sariya L, Prompiram P, Tangsudjai S, Poltep K, Chamsai T, Mongkolphan C, et al. Detection and characterization of Chlamyphphila psittaci in asymptomatic feral pigeons (Columba livia domestica) in central Thailand. Asian Pac J Trop Med 2015; 8(2): 94-97.

[14] Suksa P, Lorsunyaluck B, Dittawong P, Sanyathitiseree P, Lertwatcharasarakul P. Genetic detection and identification of Chlamydia psittaci in captive psittacine birds in Thailand. Thai J Vet Med 2016; 46(1): 67-75.

[15] Miyaki C, Matioli S, Burke T, Wajntal A. Parrot evolution and paleogeographical events: Mitochondrial DNA evidence. Mol Biol Evol 1998; 15(5): 544-551.

[16] Condon K, Oakley J. Detection of Chlamydiaceae DNA in veterinary specimens using a family-specific PCR. Lett Appl Microbiol 2007; 45(2): 121-127.

[17] Denamur E, Sayada C, Souriou A, Orfila J, Rodolakis A, Elion J. Restriction pattern of the major outer-membrane protein gene provides evidence for a homogeneous invasive group among ruminant isolates of Chlamydia psittaci. J Gen Microbiol 1991; 137(11): 2525-2530.

[18] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980; 16(2): 111-120.

[19] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016; 33(7): 1870-1874.

[20] Kaleta EF, Taday EM. Avian host range of Chlamydiaceae DNA in veterinary specimens based on isolation, antigen detection and serology. Avian Pathol 2003; 32(5): 435-461.

[21] Szymanska-Czerwinska M, Niemczuk K. Avian chlamydiosis zoonotic disease. Vector Borne Zoonotic Dis 2016; 16(1): 1-3.