Contents lists available at ScienceDirect

IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60195-4

# Detection and characterization of *Chlamydophila psittaci* in asymptomatic feral pigeons (*Columba livia domestica*) in central Thailand

Ladawan Sariya[1*], Phirom Prompiram[1], Siriporn Tangsudjai[1], Kanaporn Poltep[1], Tatiyanuch Chamsai[1], Chalisa Mongkolphan[1], Kamolphan Rattanavibul[2], Verachai Sakdajivachareon[2]

[1]*Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Salaya, Nakhon Pathom, Thailand 73170*

[2]*Division of Veterinary Public Health, Department of Health, Bangkok Metropolitan Administration, Phra Nakhon, Bangkok, Thailand 10200*

ARTICLE INFO

*Article history:*
Received 26 October 2014
Received in revised form 10 November 2014
Accepted 22 December 2014
Available online 20 February 2015

*Keywords:*
*Chlamydophila psittaci*
Psittacosis
ompA gene
Feral pigeon

ABSTRACT

**Objective:** To detect and characterize *Chlamydophila psittaci* (*C. psittaci*) in asymptomatic feral pigeons in central Thailand. **Methods:** A total 814 swabs from the trachea and cloacae of 407 non-clinical feral pigeons in central Thailand were collected and tested for the presence of *C. psittaci*. **Results:** A 10.8% of feral pigeons in the sample group were positive as determined by nested PCR primer specific to *C. psittaci*. The *outer membrane protein A* (*ompA*) gene of positive samples exhibited amino acid identity of *C. psittaci* ranging from 71 to 100% and were grouped in genotype B. Exceptionally, BF1676-56 isolate was closely related to *Chlamydia avium* with 99% identification of the *16S ribosomal (r) RNA* gene. **Conclusions:** This is the first report on *C. psittaci* isolated from asymptomatic feral pigeons in Thailand, which provides knowledge for the disease status in pigeon populations in Thailand.

## 1. Introduction

*Chlamydophila psittaci* (*C. psittaci*), an obligate intracellular gram-negative bacterium, causes psittacosis in humans and avian species. The bacterium is classified in the family *Chlamydiaceae* and the genera *Chlamydophila*. *C. psittaci* can be classified into eight serovars (A to F, WC, and M56) by using monoclonal antibody specific to epitope on the major outer membrane protein (OMP) and into nine genotypes (A to F, E/B, WC, and M56) based on ompA gene sequencing and analysis[1,2]. Because all genotypes of *C. psittaci* can infect humans, this bacterium is thus a concern in zoonotic disease.

Feral pigeons (Columba livia domestica) present in urban and rural areas globally and often come into close contact with people. Feral pigeons have been reported as harboring a total of 60 different human pathogenic organisms including viruses, bacteria, fungi, and protozoa. However, only seven pathogens, namely *Salmonella enteric*, *C. psittaci*, *Histoplasma capsulatum*, *Aspergillus* spp., *Candida parapsilosis*, *Cryptococcus neoformans*, and toxoplasma, are routinely transmitted to humans[3]. *C. psitaci* is the most pathogenic agent found in the feral pigeons that are known vectors for the transmission of this agent to humans[4]. The presence of *C. psittaci* in pigeons was documented for the first time in 1940[5]. Thereafter, serological surveillance and the detection of *C. psittaci* antigen in feral pigeons were reported. The prevalence of *C. psittaci* in feral pigeons in Amsterdam has been reported at 7.9%[6]. Moreover, in European feral pigeons, the presence of *C. psittaci* has been reported as ranging from 3.4% to 50.0%. The seroprevalance of antibody against *C. psittaci* in the sampled European pigeon populations ranged from 19.4% to 95.6%[4]. However, the prevalence and genotype of *C. psittaci* in Thailand has not been investigated. Thus, the aims of this study were detect and characterize *C. psittaci* in asymptomatic feral pigeons in central Thailand.

*Corresponding author: Ladawan Sariya, Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Salaya, Nakhon Pathom, Thailand 73170.
Tel.: +6624415242
Fax. +6624415236
E-mail address: ladawan.sar@mahidol.ac.th

Foundation project: This work is financially supported by the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University.

## 2. Materials and methods

### 2.1. Sample collection

Tracheal and cloacal swabs were collected from 407 non-clinical feral pigeons in Bangkok, Thailand. A total 814 swabs were collected from May to September 2013. Genomic DNA was extracted from swab samples using the DNeasy blood and tissue kit (QIAGEN, Hilden, North Rhine-Westphalia, Germany). The collected DNA samples were kept at -20 ℃ until use.

### 2.2. C. psittaci detection

Primer specific to the ompA gene was used for *C. psittaci* detection in a semi-nested PCR format[7]. Primer sequences are listed in Table 1. Primers A and B produced a 260-bp fragment in the first PCR, whereas primers B and C amplified a 165-bp fragment in the second semi-nested PCR. In the first PCR, the PCR mixture contained 2 μL of template DNA, 2.5 μL of 10× $Mg^{2+}$ free buffer, 1.5 mM of $Mg^{2+}$ solution, 2.5 μL of 10 mM dNTPs, 0.5 μL of i-*Taq* DNA polymerase (iNtRON, Sungnam, Kyungki-Do, South Korea), and 0.5 μM of each A and B primer. Sterile nuclease-free water was added up to 25 μL. The PCR reaction was performed under the conditions of 2 minutes at 94 ℃ for initial denaturing, followed by 35 cycles of 30 seconds at 94 ℃, 30 seconds at 58 ℃, and 30 seconds at 72 ℃, and was terminated at 72 ℃ for 7 minutes. In the second PCR, the PCR reaction and PCR parameters were the same as those of the first PCR, with the exception of the use of 3 mM of $Mg^{2+}$ solution and primers C and D. One microliter of the PCR product produced from the first PCR was used as a template for the second PCR.

### 2.3. DNA sequencing

A nearly full-length fragment of the *ompA* gene of positive samples was amplified with primers CTU and CTL[8] or primers CTU and OMP-F, and produced a 1 070-bp fragment. Full length 16S ribosomal (r) RNA was amplified from chromosomal DNA using primers Chlamydophila_16SF and Chlamydophila_16SR and produced PCR product of approximately 1 500-bp[9]. Twenty-five microliter of PCR reaction contained 2 μL of template DNA, 2.5 μL of 10 × $Mg^{2+}$ free buffer, 1.5 mM of $Mg^{2+}$ solution, 2.5 μL of 10 mM dNTPs, 0.5 μL of i-*Taq* DNA polymerase (iNtRON, Sungnam, Kyungki-Do, South Korea), and 0.5 μM of each forward and reverse primer. For *ompA* gene amplification, the PCR reaction was performed under the conditions of 2 minutes at 94 ℃ for initial denature, followed by 35 cycles of 30 seconds at 94 ℃, 30 seconds at 58 ℃, and 30 seconds at 72 ℃, and was terminated at 72 ℃ for 7 minutes. For *16S rRNA* gene amplification, the PCR parameter was performed under the conditions of 2 minutes at 94 ℃ for initial denature, followed by 35 cycles of 45 seconds at 94 ℃, 45 seconds at 60 ℃, and 2 minutes at 72℃, and was terminated at 72 ℃ for 7 minutes. The *ompA* and *16S rRNA* PCR product was ligated into the pGEM-T easy vector (Promega, Madison, WI, USA) and transformed to competent Escherichia coli TOP10 (Invitrogen™, Carlsbad, CA, USA) using the calcium chloride method. Transformants were selected on an LB agar plate containing ampicillin and X-Gal/IPGT. Selected plasmid was extracted by MiniPrep DNA preparation kit (QIAGEN, Hilden, North Rhine-Westphalia, Germany) and subjected for DNA sequencing at the company (SolGent Co. Ltd., Yuseong-gu, Daejeon, Korea).

### 2.4. Phylogenetic tree construction

Putative amino acid sequences of the *ompA* gene were compared with the sequences that available in GenBank. Phylogenetic tree was constructed using the maximum likelihood method based on the JTT matrix-based model with a bootstrap value of 1 000 replicates. For nucleotide sequence of 16S rRNA, phylogram was constructed using the Maximum likelihood method based on the Kimura 2-parameter model with bootstrap value of 1 000 replicates. Evolutionary analyses were conducted in MEGA6 version 6.0[10]. The accession number of the sequences is shown in Table 2.

## 3. Results

A total of 814 swab samples from 407 feral pigeons were examined using nested PCR. The results showed that 44 (10.8%) pigeons were positive for primers specific to *C. psittaci*. Out of the 44 positive samples, 39 were found to have *C. psittaci* in the cloacal (59.1%) or tracheal (29.5%) swab. Five were found to have *C. psittaci* in both the cloacal and tracheal swabs (11.4%). The positive DNA samples were further characterized for *ompA* genotyping. Only 32 of 44 (72.7%) positive samples could be directly amplified to produce a nearly full length of the *ompA* gene. The

**Table 1**
Primers for *C. psittaci* diagnosis and genotyping.

| Specific gene | Primer name | Sequence | |
|---|---|---|---|
| Primer for diagnostic PCR | *ompA* | Primers A | 5′CAG GAT ATC TTG TCT GGC TTT AA 3′ |
| | | Primers B | 5′GCA AGG ATC GCA AGG ATC 3′ |
| | | Primers C | 5′TTA GAG GTG AGT ATG AAA AAA CTC 3′ |
| Primer for genotyping PCR | *ompA* | CTU | 5′ ATG AAA AAA CTC TTG AAA TCG G 3′ |
| | | CTL | 5′ CAA GAT TTT CTA GAY TTC ATY TTG TT 3′ |
| | | OMP-F | 5′TCT TTC ATT GAT TAA GCG TGC T 3′ |
| | *16S rRNA* | Chlamydophila_16SF | 5′ GCG TGG ATG AGG CAT GCA A 3′ |
| | | Chlamydophila_16SR | 5′ GGA GGT GAT CCA GCC CCA 3′ |

*ompA* phylogram in figure 1 shows that most of the positive samples were classified in genotype B with 71%–100% amino acid identity to *C. psittaci*. Unlike the other isolates, the BF1676–56 isolate was separated from the *C. psittaci* group. To classify the BF1676–56 isolate, a full length of 16S rRNA of BF1676–56 isolate was amplified from chromosomal DNA for DNA sequencing and analysis. The phylogram of the 16S rRNA nucleotide sequence shows that this isolate was closely related to Chlamydia avium (Accession no. CP006571) with 99% identity (Figure 2).

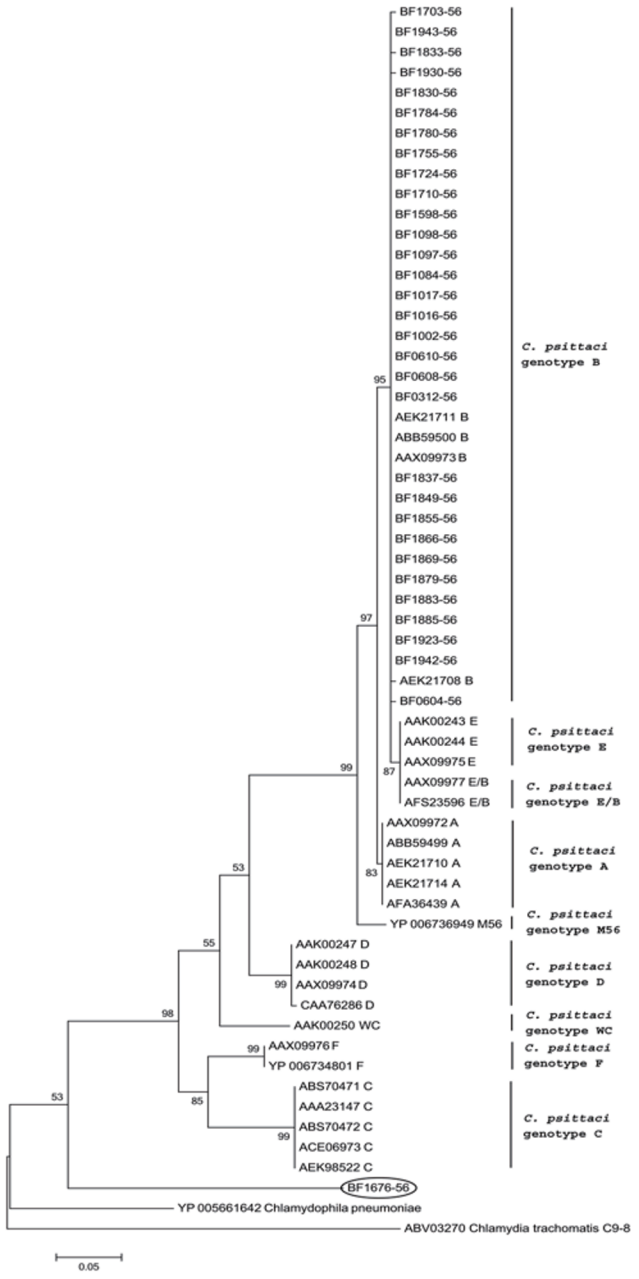


**Figure 1.** *ompA* gene phylogenetic tree.
Putative amino acid sequences of the *ompA* gene were used for phylogram construction. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1 000 bootstrap replicates). Branches without bootstrap values occurred in less than 49% of the trees. Branch lengths are measured in nucleotide substitutions.

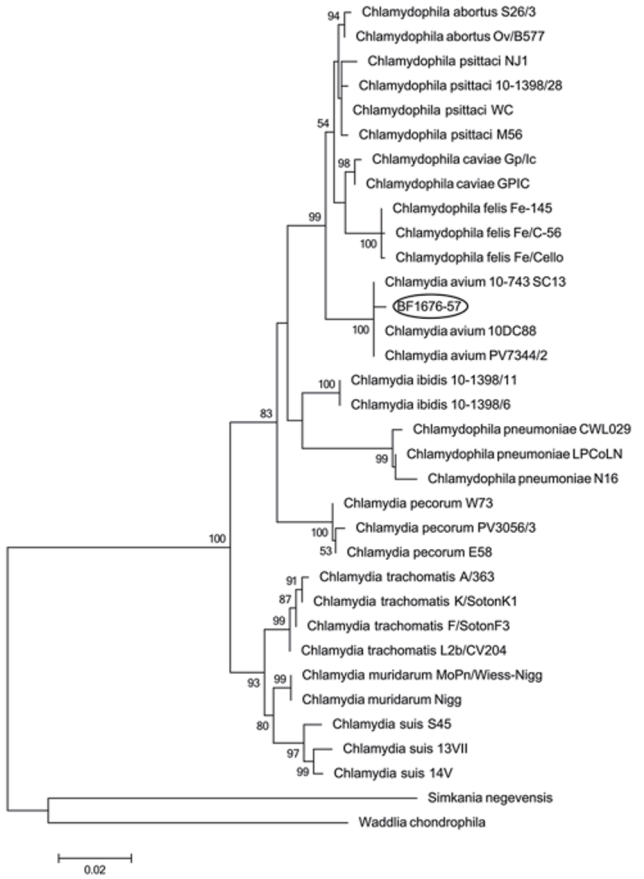


**Figure 2.** *16S rRNA* gene phylogenetic tree.
Nearly full length of *16S rRNA* gene was used for phylogenetic tree construction. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1 000 bootstrap replicates). Branches without bootstrap values occurred in less than 49% of the trees. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

## 4. Discussion

Feral or urban pigeons are an important reservoir of zoonotic diseases because they live closely with humans. *C. psittaci* is a pathogen found in these pigeons and can be transmitted to humans by direct and indirect contract with the birds. In this study, incidence of *C. psittaci* in feral pigeon populations in central Thailand was 10.8%. The percentage of positive samples in this study was similar to the percentage that has been reported in European pigeon populations[4]. *C. psittaci* was mainly found in cloacal swabs, which suggests that pigeons may shed this bacterium in the environment via their feces. The *ompA* gene in 12 of the 44 positive samples could not be directly amplified from clinical samples because the DNA contained in the samples was not large enough to amplify and generate a sufficient amount of PCR product. Moreover, the genotypic PCR was less sensitive than the diagnostically nested PCR. Thus, positive samples that were unable to be typed may require the culturing technique.

From the ompA phylogram, BF1676–56 isolate was not classified in the *C. psittaci* group. 16S rRNA sequencing

**Table 2**
Accession number of 16S rRNA sequences used in this study.

| Species | Accession No. | Species | Accession No. |
|---|---|---|---|
| *Chlamydia avium* 10–743_SC13 | KF366258 | *Chlamydia trachomatis* A/363 | HE601796 |
| *Chlamydia avium* 10DC88 | CP006571 | *Chlamydia trachomatis* F/SotonF3 | HE601806 |
| *Chlamydia avium* PV7344/2 | KF366257 | *Chlamydia trachomatis* K/SotonK1 | HE601794 |
| *Chlamydia ibidis* 10–1398/11 | HQ662954 | *Chlamydia trachomatis* L2b/CV204 | HE601960 |
| *Chlamydia ibidis* 10–1398/6 | HQ662953 | *Chlamydophila abortus* Ov/B577 | NR_036834 |
| *Chlamydia muridarum* MoPn/Wiess–Nigg | NR_036835 | *Chlamydophila abortus* S26/3 | CR848038 |
| *Chlamydia muridarum* Nigg | NR_074982 | *Chlamydophila caviae* Gp/Ic | NR_036833 |
| *Chlamydia pecorum* E58 | NR_102975 | *Chlamydophila caviae* GPIC | NR_074946 |
| *Chlamydia pecorum* PV3056/3 | CP004033 | *Chlamydophila felis* Fe/C–56 | NR_074947 |
| *Chlamydia pecorum* W73 | NC_022440 | *Chlamydophila felis* Fe/Cello | D85706 |
| *Chlamydia psittaci* 10–1398/28 | HQ662953 | *Chlamydophila felis* Fe–145 | AB001785 |
| *Chlamydia psittaci* M56 | CP003795 | *Chlamydophila pneumoniae* CWL029 | NR_074981 |
| *Chlamydia psittaci* NJ1 | CP003798 | *Chlamydophila pneumoniae* LPCoLN | CP001713 |
| *Chlamydia psittaci* WC | CP003796 | *Chlamydophila pneumoniae* N16 | U68426 |
| *Chlamydia suis* 13VII | AY661794 | *Simkania negevensis* Z | NR_029194 |
| *Chlamydia suis* 14V | AY661795 | *Waddlia chondrophila* | NR_028697 |
| *Chlamydia suis* S45 | NR_029196 | – | – |

showed that it was closely related to *C. avium*, which was reported as a new member of the family Chlamydiaceae by Sachse and colleagues in 2014 and can be found in pigeons and psittacine birds[11]. *C. avium* infected birds exhibit asymptomatic infection and the potential for zoonotic transmission of this bacterium to humans is unknown[11]. Most of the positive samples detected in this study were identical to *C. psittaci* genotype B as determined by *ompA* genotyping. *C. psittaci* genotype B was concerned for zoonosis that transmitted from pigeon to human. In the Netherlands, genotype B has been reported in three human cases with symptomatic psittacosis infection and may be an underestimated source of disease[6]. Recently, genotype B has been discovered in nine human cases in Venezuela with symptomatic and asymptomatic infection. These people reported permanent pigeon presence and activity in their homes' windows and air conditioners[12]. Besides the potential zoonotic transmission to humans, pigeon is also the risk of infection of pet birds, captive birds, and poultry that live in close contact with human beings. Thus, feral pigeon management programs and disease surveillance should be implemented to control the disease and reduce the risk of pigeon–to–human transmission by such pathogenic agent. Moreover, public education is should be done in parallel with the surveillance program. People, especially immunocompromized patients, need to know the risk of contracting diseases when feeding pigeons and handling carcasses. Additionally, people should protect themselves by wearing masks when removing pigeon feces or nests from buildings.

In conclusion, the present study reveals the first report on *C. psittaci* detection in feral pigeons in Thailand. The information about zoonotic diseases in pigeons may help to control or reduce the risk of diseases transmission to humans.

## Conflict of interest

The authors declare that they have no conflict of interests.

## References


[1] Andersen AA. Serotyping of *Chlamydia psittaci* isolates using serovarspecific monoclonal antibodies with the microimmunofluorescence test. *J Clin Microbiol* 1991; **29**: 707–711.

[2] Geens T, Desplanques A, van Loock M, Bönner BM, Kaleta EF, Magnino S, et al. Sequencing of the *Chlamydophila psittaci* ompA gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. *J Clin Microbiol* 2005; **43**: 2456–2461.

[3] Haag–Wackernagel D, Moch H. Health hazards posed by feral pigeons. *J Infect* 2004; **48**: 307–313.

[4] Magnino S, Haag–Wackernagel D, Geigenfeind I, Helmecke S, Dovc A, Prukner–Radovcić E, et al. Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Vet Microbiol* 2009; **16**: 54–67.

[5] Pinkerton H, Swank RL. Recovery of virus morphologically identical with psittacosis from thiamin–deficient pigeons. *Exp Biol Med* 1940; **45**: 704–706.

[6] Heddema ER, van Hannen EJ, Duim B, Vandenbroucke–Grauls CM, Pannekoek Y. Genotyping of *Chlamydophila psittaci* in human samples. *Emerg Infect Dis* 2006; **12**: 1989–1990.

[7] Buxton D, Rae AG, Maley SW, Thomson KM, Livingstone M, Jones GE, et al. Pathogenesis of *Chlamydia psittaci* infection in sheep: detection of the organism in a serial study of the lymph node. *J Comp Pathol* 1996; **114**: 221–230.

[8] Denamur E, Sayada C, Souriau 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**: 2525–2530.

[9] Everett KD. Chlamydia and Chlamydiales: more than meets the eye. *Vet Microbiol* 2000; **31**: 109–126.

[10] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; **30**: 2725–2729.

[11] 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**: 79–88.

[12] Arraiz N, Bermudez V, Urdaneta B, Mujica E, Sanchez MP, Mejía R, et al. Evidence of zoonotic *Chlamydophila psittaci* transmission in a population at risk in Zulia state, Venezuela. *Rev Salud Publica (Bogota)* 2012; **14**: 305–314.