

### Prevalence of *Chlamydophila psittaci* in some Wild and Pet Birds

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#### Abstract

*Chlamydophila psittaci* is one of the most important, zoonotic pathogen of birds causing chlamydiosis. This study aimed to investigate the frequency of infection by *Cp. psittaci* and to determine the genotype in birds at potential risk of exposure to this pathogen. In total four species of wild birds (50 native and 40 migratory quails, 30 doves and 25 tree sparrows) and four species of pet birds, (20 Budgerigars, 10 cockatiels, 3 finches, 5 love birds) were examined for the presence of *Chlamydophila psittaci* using impression smears stained with Giemsa stain, smears from yolk sacs were stained with Gimenez stain and PCR. The results were (80%-100%), (85%-100%) and (80%-100%) in pet birds followed by wild birds (64%-85%), (76%-95%) and (80%-90%), respectively. The pathogenicity of three isolates by intratracheal route with 10<sup>6</sup> TCID<sub>50</sub>/ml in 15 days old chickens and quails was done and showed that the more pathogenic strain for chickens and quails was the pet birds strain. The observed clinical signs were respiratory signs, conjunctivitis, and diarrhea, while the pathological changes were congestion in liver, lung, spleen, and pericarditis while mild clinical and pathological changes were observed post infection by tree sparrows and migratory quails isolates. The partial *ompA* gene sequence of isolated *Cp. psittaci* strain was placed in genotype A of *Cp. psittaci* which had the highest identity (91.9-94%) with previously similar described strains of genotype A. Pet and wild birds were the major reservoir for *Cp. psittaci* which shed in their excreta and expose human and native birds to high zoonotic risk.

**Keywords:** *Cp. psittaci*, Wild Birds, Pet Birds, *ompA* Gene

#### Introduction

Chlamydiosis, known as parrot disease, parrot fever, ornithosis or psittacosis is a widespread disease caused by *Chlamydophila psittaci* which is an intracellular, Gram-negative, coccoid bacterium causing a reportable zoonotic disease in many countries [1,2].

A special feature of *Chlamydophila* is that it has a biphasic life cycle, existing as elementary, reticulate and intermediate bodies. Elementary bodies (EB) are infectious particles that exist outside the host, whereas, reticulate bodies (RB) are formed from EB inside the host cell [3]. A susceptible bird can become infected through inhalation of airborne contaminated materials or through ingestion of contaminated foods. Human infection with *Cp. psittaci* usually occurs due to inhalation of contaminated aerosol from dried feces or respiratory tract secretions of infected birds and desiccated dropping or dander. Vertical

transmission of *Chlamydophila* was described in chickens, ducks, parakeets, sea gulls, snow geese and a number of wild birds [4-6].

*Chlamydophila psittaci* infections are most prevalent in psittacine birds including parakeets, love birds, cockatiels, amazon parrots, and macaws. Among non psittacine birds, infection was seen most commonly in dove and pigeons [7,8]. Young birds are more susceptible to infection than older birds.

Usual diagnosis is based on the isolation of the organism in cell cultures and chicken embryo and staining with special stains such as Giemsa, modified Gimenez and Castaneda, Macchiavello's [6]. In addition, stamp staining to identify the organism in impression smears from exudates, lesions or the surface of organs (liver or spleen) and swabs (cloacal, tracheal or conjunctival swabs) can also be used for identification.

*Cp. psittaci* can be identified using species-specific conventional PCR which target the *ompA* or 16S–23S rRNA genes [9,10]. PCR is considered a valuable, specific, and sensitive method for the detection and differentiation of *Chlamydia* species [11]. Within *Cp. psittaci*, there are currently six serotypes (A to F) that infect birds and show host specificity. Serotypes A and F are specific for parrots, parakeets and budgerigars; B for pigeons; C for ducks and geese (water fowl); D for turkeys; and E are specific for pigeons, ducks, ostriches and rheas. Serovar A is routinely isolated from psittacine birds, in which it is associated with both acute and persistent infections [12,13].

This study was carried out for the isolation and identification of *Cp. psittaci* from sparrows, doves, native and migratory quails, cocktails, finches, lovebirds and budgerigars using different methods. The pathogenicity of three selected strains isolated from pet birds, migratory quails and tree sparrows on chickens and quails was evaluated.

## **Material and Methods**

### ***Birds***

A total of 145 birds (30 doves, 25 tree sparrows, 50 native and 40 migratory quails) and 38 pet birds (20 Budgerigars, 3 Finches, 5 Lovebirds and 10 Cocktails) were examined for the isolation of *Cp. psittaci* from the internal organs (livers, lungs, heart). In addition, 70 (15 day old) Cobb chicks and 70 (15 day old quails) were used for the experimental pathogenicity of *Cp. psittaci*.

### ***Cytological examination***

Impression smears prepared from the cut surfaces of the internal organs were air dried, fixed and stained with Giemsa stain as was previously described [14]. Stained slides were examined microscopically to detect chlamydial inclusion bodies.

### ***Chicken embryo inoculation***

Liver, heart and lungs were grinded in 10% suspension of PBS [15,16]. Aliquot of each prepared sample was inoculated into the yolk sac of 3 eggs (0.2 mL/egg). Impression smears from the yolk sac were then prepared, and

stained with Gimenez stain for the demonstration of *Cp. psittaci* inclusions [17].

### ***Molecular identification of isolated Cp. psittaci***

Genomic DNA was extracted from yolk sacs using QIA amp DNA Mini Kit (Qiagen) according to the manufacture instructions. Primers targeting 1041 bp of the major outer membrane protein (*ompA*) gene specific for *Cp. psittaci* were used. The sequences of the primers are CPsitt-F 5`-GCT ACG GGT TCC GCT CT-3` and CPsitt-R 5`-TTT GTT GAT YTGA ATCGAAGC-3` [18]. The amplification was carried out using Dream Taq™ PCR Master Mix (2X) and the primers construction was 100 pmole/μL. The reaction conditions were an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 5 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 7 min. The PCR products were visualized by Electrophoresis in 1.5 % agarose in 1X TAE and ethidium bromide was added to a concentration of 0.5 μg/ mL [19].

### ***Experimental Pathogenicity***

#### ***Titration of Cp. psittaci strains on Embryonated chicken eggs (ECE)***

Three isolates from pet birds, migratory quails, and tree sparrows were used for titration. The stock infected yolk material was diluted using 10-fold serial dilution ( $10^{-1}$  up to  $10^{-7}$ ) [20]. Only 0.2 mL of each dilution was inoculated in five ECEs and incubated at 37°C. After 72 hours, any dead embryo was collected and yolk impression smears were prepared and stained with Gimenez stain and the titer was calculated [21].

#### ***Titration of Cp. psittaci strains on tissue culture (vero cell)***

Cell monolayer was prepared according to Vanrompay [22]. Tissue culture flask was inoculated with cells in growth media containing 10% fetal bovine serum then incubated at 37°C. The flask was examined daily by inverted microscope for the cell growth. Inoculation of samples into cell culture was carried out according to McElnea and Cross [23], the strains were inoculated into

yolk sac of 6 days old ECE 20%. Tenfold serial dilution for each sample was done, then 0.2 mL of each specimen was added to each well of TC plate. Each sample was then inoculated into two wells then put in shaker for 1 hour and incubated at 37°C. *Cp. psittaci* inclusion bodies were then detected in infected vero cells stained by Giemsa stain post 48-72 hrs. The tissue culture plates were examined microscopically for the presence of intracytoplasmic bodies and to detect cytopathic effect (CPF). The titer was then estimated using the formula described previously [21].

#### Experimental design

Seventy 15 days old commercial Cobb chicks and seventy 15 days old commercial quails were used for experimental infection

with three recovered strains. Both chicks and quails were divided into 4 groups (1a, 2a and 3a) for chicks and (1b, 2b and 3b) for quails. Each group contained 20 birds except the control group contained 10 only. The birds were infected by intra-tracheal route with 0.2 mL of 3 *Cp. psittaci* culture that contained 10<sup>6</sup> TCID<sub>50</sub>. Post infection (PI), birds were observed daily for 34 days for the clinical signs and lesions. One bird from each group was sacrificed daily for the first 10 days, then in days 14, 17, 21, 24, 28, 34 days PI. Samples from liver, lungs, heart, spleen, pancreas, pericardium, intestine, air sac, trachea and kidneys were collected and subjected to both impression smears and ECE inoculation for re-isolation. Moreover, pharyngeal and cloacal swabs were collected for egg inoculation.

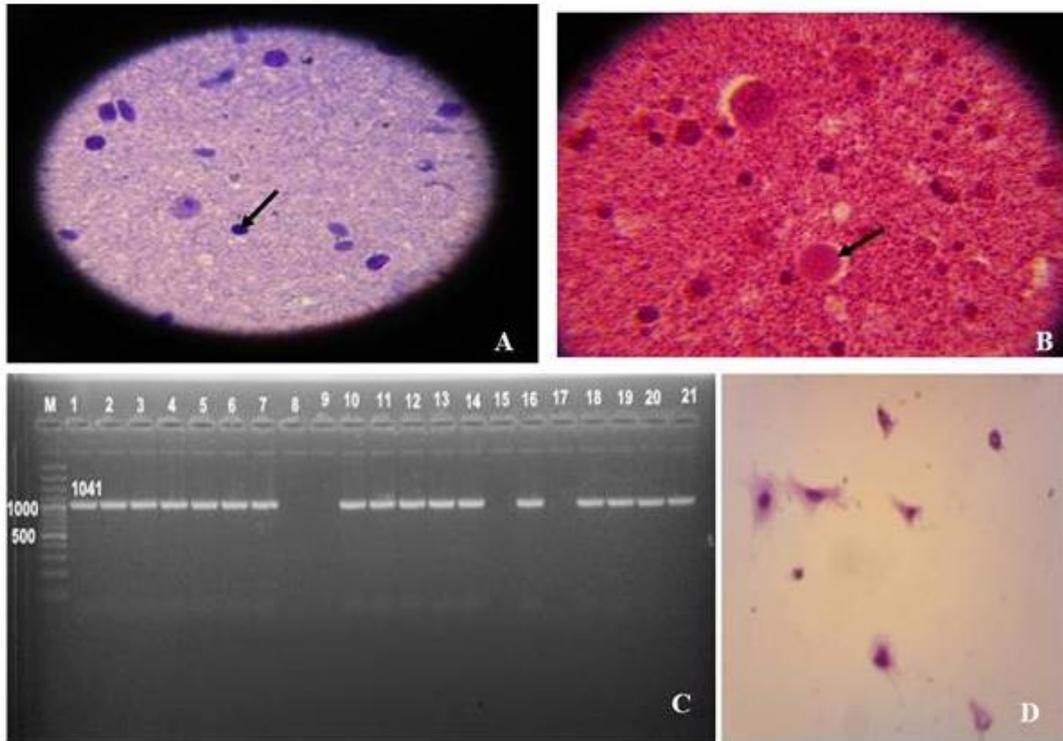
**Table 1: Isolation percentage of *Cp. psittaci* from wild and pet birds using different methods**

Bird (number)	No. (%) of positive birds by:		
	Giemsa	Gimenez	Embryonic death ratio
<b>Budgerigar (20)</b>	17(85%)	17 (85%)	14 (70%) /
<b>Lovebird (5)</b>	4(80%)	5 (100%)	3 (60%)
<b>Finch (3)</b>	3(100%)	3 (100%)	2 (66.6%)
<b>Cockatiel (10)</b>	8(80%)	9 (90%)	6 (60%)
<b>Sparrow (25)</b>	16(64%)	19 (76%)	15 (60%)
<b>Dove (30)</b>	23(76.6%)	25 (83.3%)	21 (70%)
<b>Migratory quail (40)</b>	34(85%)	38 (95%)	28 (70%)
<b>Native quail (50)</b>	36(72%)	41 (82%)	34 (68%)

#### Molecular identification

For the confirmation of *Cp. psittaci* re-isolation, one isolate was examined by PCR for the amplification of *ompA* gene. Sequencing of the amplified product was carried out using automated sequencer (ABI3730XL, DNA analyzer, Macrogen Inc., Seoul, Korea) Sequence identity was calculated using MegAlign software (DNA STAR<sup>®</sup> Laser gene<sup>®</sup> version 7.2, USA). Phylogenetic tree was constructed by the neighbor-joining method employing the

Kimura 2-parameter model in MEGA6.06 software [24] by aligning 517 bp of representative lineage specific nucleotide sequences. The tree topology was evaluated by 1000 bootstrap replicates to estimate the robustness of tree branches. The reference sequences used for comparison and phylogenetic analysis were obtained from Gen Bank with accession numbers. The analyzed sequence was submitted to NCBI Gene Bank and accession number KY296311 was provided.



**Figure 1: A: Elementary body in migratory quail liver stained by Giemsa (Black arrow), B: Inclusion bodies in yolk sac stained by Gimenez stain (Black arrow), C: Cytopathic effect, D: Exemplar of amplification of *ompA* gene (1041 bp) from *Cp. psittaci* isolates, M: 100 bp ladder, 1: positive control, (2-7, 10-14, 16, 18-21): positive samples, (8-9, 15, 17): negative samples.**

## Results

### Detection of *Cp. psittaci*

Inclusion and elementary bodies were characterized in organ smears from the examined birds. Elementary bodies are small and dense body (Figure 1A), while inclusion bodies appeared as large vacuole. In case of wild birds, chlamydial inclusions were demonstrated using Giemsa stain in migratory quails (85%), followed by doves (76.6%), native quails in (72%) and tree sparrows (64%). While in pet birds, chlamydial inclusions were observed in finches (100%) followed by Budgerigar (85%), love birds (80%) and Cockatiels (80%) (Table 1). Examination of the samples by Gimenez stain reveal that in wild birds, chlamydial inclusions were demonstrated in migratory quails (95%), followed by doves (83.3%), native quails (82%), and tree sparrows (76%). In addition, the examination showed that in pet birds, 100% of finches and love birds, followed by Cockatiels (90%) then Budgerigar (85%), were positive (Figure 1B). Embryonic death ratio

revealed that in wild birds the ratio was 60% in tree sparrows, 70% in dove and migratory quails and 68% in native quails. While, in pet birds, the ratio was 70% in budgerigars, 66.6% in finches and 60% in love birds and cockatiel (Table 1). Isolation from liver was higher in dove (73%), than other species of wild birds, on otherwise, results of impression smears from lungs and hearts were high in migratory quails (65%) and (50%), respectively. In case of pet birds, results of impression smear of liver and lungs showed high ratio in finches (100%) and (66.6%), respectively. While, heart smears showed higher ratio in cocktails (50%) (Table 2). Gimenez staining showed higher positive results followed by embryonic inoculation, and tissue smear staining with Giemsa.

### Molecular identification

Representative number of positive samples with Giemsa and Gimenez stains (n=50) were chosen for amplification of *ompA* gene. A total of 41 samples (82%) were positive by PCR (6/8 of tree sparrows, 8/10 doves, 8/10 native quails, 9/10 migratory quails, 4/5

budgerigars, 2/2 finches, 2/2 love birds, 2/3 cockatiels), Figure (1C) shows 1041 bp of *ompA* gene amplified amplicons from *Cp. psittaci* isolates.

**Table 2: Isolation percentages of *Cp psittaci* from different internal organs of examined wild and pet birds**

		Wild birds				Pet birds			
		Tree sparrow	Dove	Native quail	Migratory quail	Budgerigar	Love bird	Finch	Cockatiel
<b>Liver</b>	+ ve	15 (60%)	22 (73%)	33 (66%)	24 (60%)	15 (75%)	4 (80%)	3 (100%)	7 (70%)
<b>Lung</b>	+ ve	12 (48%)	18 (60%)	30 (60%)	26 (65%)	10 (50%)	3 (66%)	2 (66.6%)	6 (60%)
<b>Heart</b>	+ ve	10 (40%)	14 (45%)	25 (50%)	20 (50%)	8 (40%)	2 (40%)	1 (33.3%)	5 (50%)

### Experimental Pathogenicity

#### Titration of *Cp. psittaci*

Cytopathic effect of *Cp. psittaci* in tissue culture was detected by recorded changes in the shape of tissue culture cells which transfer from spindle shape to round with destruction (Figure 1D).

Titration of 3 isolates from pet birds, migratory quail, tree sparrows in tissue culture were 6.15, 6.34 and 6.4 tissue culture infective dose (TCID<sub>50</sub>) and on embryonated chicken egg the titer was 6.16, 6.33 and 6.36 (embryo infective dose) EID<sub>50</sub> respectively. Therefore, for the experimental pathogenicity, an infective dose of 10<sup>6</sup> was used.

#### Experimental infection

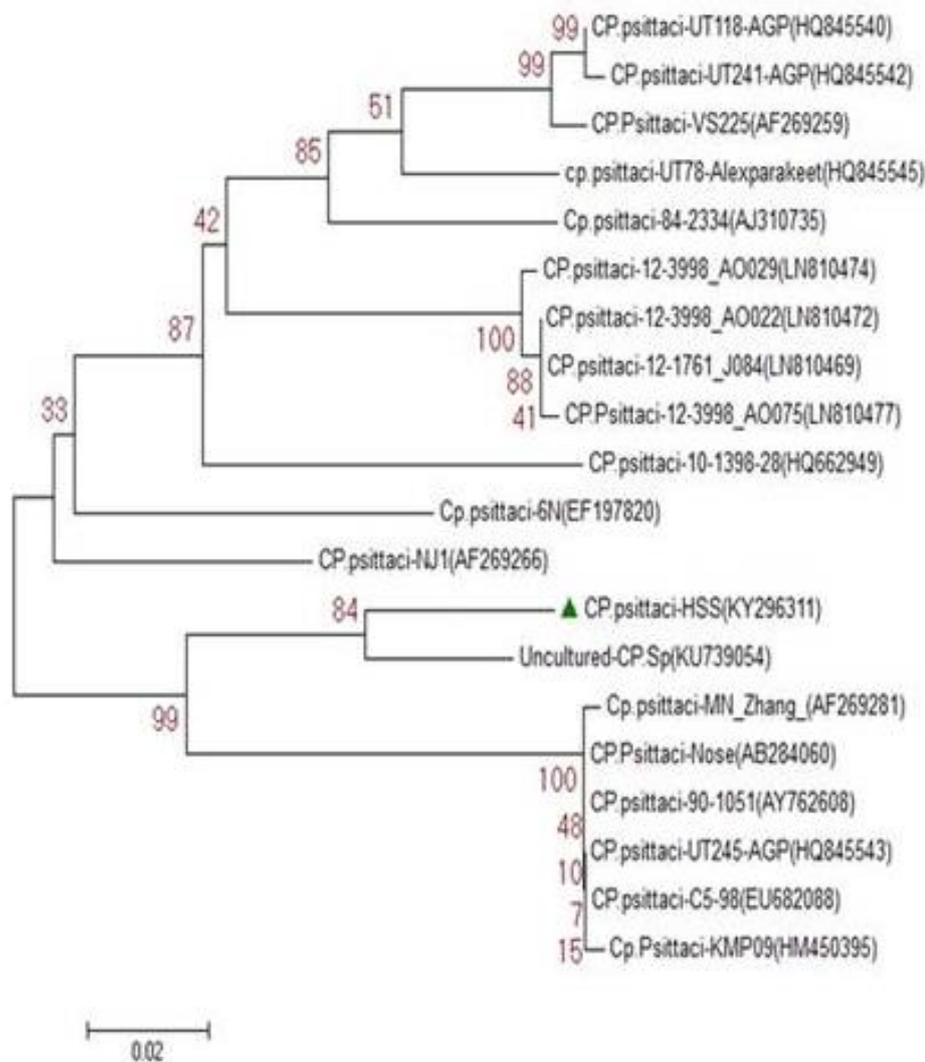
In chicks, group 1a, birds showed unilateral ocular lesion and mucoid diarrhea. Moreover, all groups showed rapid breathing and sleepiness. Three weeks PI, all groups showed rapid breathing, sleepiness, ruffled feathers, weakness and some birds showed sneezing, gasping, and conjunctivitis (groups 1a and 3a). These signs were observed along the observation period. Postmortem examination revealed mild congestion in lung, liver, heart in group 1a. While, in group 3a, mild congestion in kidney was observed from 7-10 days PI. From 2-3 weeks PI in group 1a and 3a pericarditis, pancreatitis, intestine filled with fluid and liver enlarged, lung, spleen and

muscle congested were observed. From 3 weeks all groups showed pericarditis, congested liver, congested lung heart, pericarditis, airsacculitis and intestine filled with fluid.

In quails, mucoid frothy diarrhea was observed 3-5 days PI in group 1b and 3b with unilateral ocular lesion in group 1b, while 3 weeks PI, all groups showed weakness, loss weight. In group 1b, ocular lesions were clear and gasping was observed. Greenish and mucoid frothy diarrhea in groups 1b and 2b were observed. Postmortem examination showed that groups 1b and 3b showed congested lung and liver 2-4 days PI. From 2-3 weeks PI birds in group 1b and 3b showed congested muscle, liver, lungs, spleen and hemorrhagic spots on the liver, pericarditis and enteritis. These signs were continuing along the experiment.

#### Molecular identification

For the confirmation of the re-isolation *Cp. psittaci* from experimentally infected birds, stained smears with Gimenez and Gimenez stains were examined and also isolation of the organism in ECE was carried out. The smears showed chlamydial inclusion bodies and one isolate was randomly chosen for molecular identification by amplification of *ompA* gene. The amplicon was sequenced and the sequence analysis showed identity with *Cp. psittaci* type A on the gene bank (Figures 2, 3).



**Figure 2: Phylogenetic analysis of *ompA* gene sequence of *Chlamydophil psittaci* in psittacine birds. Other sequences were obtained from gene bank (accession numbers are indicated). Bootstrap values obtained from 1000 replications are shown at branch. The scale bar represents the number of substitutions for a unit branch length.**

## Discussion

*Cp. psittaci* infection in birds (avian chlamydiosis) or humans (psittacosis) is an intracellular Gram-negative bacterium with vague clinical signs in infected birds and the organism is shed in feces and nasal/ocular discharge [2]. *Cp. psittaci* can infect a wide variety of native, wild birds and mammals including human [5,25]. One hundred and forty-five birds from four species of wild birds (30 doves, 25 tree sparrows, 50 native quails and 40 migratory quails) and thirty-eight birds from 4 species of pet birds (20 budgerigars, 3 finches, 5 love birds, and 10 cockatiels) were

examined for the presence of *Cp. psittaci* during the current study.

In this study, impression smears stained by Giemsa stain from different organs of wild birds showed high rates for *Chlamydophila* species (72, 85, 64 and 76.7%, from native quails, migratory quails, tree sparrows and doves, respectively). Similarly, high rates of *Chlamydophila* species (67.3%) from migratory birds was recorded previously in Egypt [26]. Moreover, EL -Jakee [27] recorded 81.8 and 77.4% prevalence rates for *Chlamydophila* species from cattle egret and hoopoe, respectively. In case of pet birds (budgerigars, love birds, finches, and

cockatiels), high rates of *Chlamydophila* species were also recorded (85, 80, 100 and 80%, respectively). These results agree with a previous result recorded in Egypt [28]. On the other hand, Schwartz and Fraser [29] recorded lower rates of *Chlamydophila* species in cockatiels (26.9%), love birds (21.4%) and finches (75.3%).

From the obtained results, it is clear that wild birds (Doves, tree sparrows, native quails and migratory quails) and pet birds (Budgerigar, finches, love birds, and cockatiels) are considered as natural hosts and they have a role in spreading infection by shedding the organism in their excretions [5].

*Chlamydophila* species inclusion bodies appeared as small, round, purple, red and blue dots which were the same observations reported in previous studies [25,30].

The result of microscopical examination of suspected tissues revealed that liver was the most affected organ in tree sparrows, doves,

native quails and migratory quails with isolation percentages of 60, 73, 66 and 60%, respectively. Meanwhile, the rates of *Chlamydophila* species in lung were lower than those obtained from liver in the same wild birds (48, 60, 60 and 65%, respectively). Additionally, lower ratios of Chlamydia species were recorded from the heart (40, 46, 50 and 50%, respectively) (Table 2). These results are compatible with a previous study [31], where chlamydia was highly isolated from livers (78%). On the other hand, *Chlamydophila* was highly recovered from liver of budgerigars, love birds, finches and cockatiels with percentages of 75, 80, 100 and 70%, respectively and the percentages of isolation from the lung were 50, 66, 66.6 and 60%, respectively. While lower percentage of isolation was from the heart 40%, 40%, 33.3% and 50%, respectively. The obtained results showed high incidence of infection (Table 2).

		Percent Identity																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Divergence	1	100	94.7	92.1	82.8	83.0	82.8	92.1	92.1	92.1	91.9	80.7	92.3	81.7	82.8	82.8	82.8	82.6	84.5	82.9	80.7	1	CPpsittac-HSS(KY296311)	
	2	5.5	100	85.6	85.0	85.0	84.7	85.6	85.6	85.6	85.3	83.9	85.8	85.6	86.2	86.2	86.2	85.9	88.6	86.1	83.6	2	Uncultured-CPSp(KU739054)	
	3	8.4	16.1	100	82.6	83.4	83.2	100.0	100.0	100.0	99.8	80.3	99.8	81.3	82.4	82.2	82.2	81.9	83.5	82.9	80.1	3	CPpsittac-Nose(AE284060)	
	4	19.6	16.8	20.0	100	98.0	97.6	82.6	82.6	82.6	82.4	94.3	82.4	90.7	90.1	90.3	90.3	90.1	89.0	85.1	88.2	4	CPpsittac-1S225(AF269259)	
	5	19.4	16.8	18.9	2.1	100	99.6	83.4	83.4	83.4	83.2	94.1	83.2	89.9	90.3	90.1	90.1	89.9	88.4	85.1	87.9	5	CPpsittac-UT118-ACP(HQ845540)	
	6	19.6	17.2	19.2	2.5	0.4	100	83.2	83.2	83.2	83.0	93.7	83.0	89.7	90.1	90.1	90.1	89.9	88.2	84.9	87.6	6	CPpsittac-UT241-AGP(HQ845542)	
	7	8.4	16.1	0.0	20.0	18.9	19.2	100.0	100.0	99.8	80.3	99.8	81.3	82.4	82.2	82.2	81.9	83.5	82.9	80.1	7	CPpsittac-90-1051(AV762608)		
	8	8.4	16.1	0.0	20.0	18.9	19.2	0.0	100.0	99.8	80.3	99.8	81.3	82.4	82.2	82.2	81.9	83.5	82.9	80.1	8	CPpsittac-UT245-AGP(HQ845543)		
	9	8.4	16.1	0.0	20.0	18.9	19.2	0.0	0.0	100	99.8	80.3	99.8	81.3	82.4	82.2	82.2	81.9	83.5	82.9	80.1	9	CPpsittac-CS-98(EU682088)	
	10	8.6	16.4	0.2	20.2	19.2	19.4	0.2	0.2	0.2	100	99.6	81.1	82.2	81.9	81.9	81.7	83.3	82.7	79.9	10	Cp Psittac-KMP09(HM450395)		
	11	22.3	18.2	23.0	5.9	6.2	6.6	23.0	23.0	23.0	23.2	100	91.7	88.0	88.0	88.0	87.8	87.1	85.7	88.8	11	cp psittac-UT78-Alexparakeet(HQ845545)		
	12	8.2	15.7	0.2	20.2	19.2	19.4	0.2	0.2	0.2	0.4	23.2	100	81.1	82.2	81.9	81.9	81.7	83.3	82.7	79.9	12	Cp psittac-MN Zhang(AF269281)	
	13	21.0	16.1	21.6	10.0	11.0	11.2	21.6	21.6	21.6	21.8	8.9	21.8	100	89.0	89.0	89.0	88.8	88.0	87.8	90.3	13	Cp psittac-84-2334(AJ310735)	
	14	19.7	15.3	20.3	10.7	10.5	10.7	20.3	20.3	20.3	20.5	13.1	20.5	11.9	100	99.2	99.2	99.0	87.3	86.1	88.8	14	CPpsittac-12-3998_A0029(LN810474)	
	15	19.7	15.4	20.5	10.5	10.7	10.8	20.5	20.5	20.5	20.8	13.1	20.8	11.9	0.8	100.0	99.8	87.6	85.9	88.8	15	CPpsittac-12-3998_A0022(LN810472)		
	16	19.7	15.4	20.5	10.5	10.7	10.8	20.5	20.5	20.5	20.8	13.1	20.8	11.9	0.8	0.0	100	99.8	87.6	85.9	88.8	16	CPpsittac-12-1761_J084(LN810469)	
	17	19.9	15.7	20.8	10.7	11.0	11.0	20.8	20.8	20.8	21.1	13.3	21.1	12.1	1.0	0.2	0.2	100	87.3	85.7	88.6	17	CPpsittac-12-3998_A0075(LN810477)	
	18	17.4	12.4	18.7	12.0	12.7	13.0	18.7	18.7	18.7	19.0	14.2	19.0	13.2	13.9	13.7	13.7	13.9	100	88.8	87.1	88.8	18	CPpsittac-FU1(AF269266)
	19	19.6	15.4	19.6	16.8	16.8	17.0	19.6	19.6	19.6	19.8	16.0	19.8	13.5	15.5	15.8	15.8	16.0	10.6	100	87.1	87.1	19	Cp psittac-9NIEF197820)
	20	22.4	18.6	23.3	12.9	13.3	13.6	23.3	23.3	23.3	23.5	12.1	23.6	10.5	12.1	12.1	12.1	12.4	12.2	14.2	100	20	CPpsittac-10-1398-28(HQ662949)	

Figure 3: Similarity table between *ompA* gene sequence of *Chlamydophila psittaci* in psittacine birds and other sequences were obtained from gene bank (accession numbers are indicated).

Moreover, in another study conducted in Hadia [28], the highest ratio of infection was recorded in liver samples of 93 and 100% Budgerigars and finches, respectively, while in lungs, the percentages were 40 and 0%, respectively.

Embryonated chicken eggs inoculated with *Chlamydophila* species showed congestion of embryo and yolk sac vessels. Similar observations recorded previously [32]. By examination of Gimenez stained yolk sac membrane impression smear of infected

chicken embryo by wild birds isolate, the percentage of infection was 76, 83.3, 82 and 95% in tree sparrows, doves, native quails and migratory quails, respectively. These results are in the same line with a previous study in Egypt conducted by El-Jakee [27], who analyzed *Chlamydophila* by using Gimenez stain for hoopoe and cattle egret and the positive ratios were 89.1 and 83%, respectively.

In our study, psittacine birds and migratory birds showed the highest isolation ratios, followed by dove, native quails and finally tree sparrows as also observed previously in Britain [33], where the highest isolation ratio of *Chlamydophila* species was revealed in psittacine birds, followed by doves.

PCR is used for diagnosis of *Cp. Psittaci* using species specific conventional PCR and the *ompA* gene was investigated as a target DNA sequence among family *Chlamydiaceae* [12,34]. In this study, 50 positive samples by Giemsa and Gimenez stains were randomly selected for PCR and 41 (82%) were confirmed by *ompA* amplification. These results are similar to EL-Jakee [27] who recorded that results of PCR among hoopoe and cattle egret birds were 96.4 and 90.6%, respectively. The current results showed that PCR was negative in 9 samples that showed inclusion bodies, this could be attributed to that inclusion bodies could be detected in organism other than Chlamydia. Celebi and Ak [35] recorded lower results for *Cp. Psittaci* from pet birds (34.4%) by PCR, when they used organ pools.

Titration of three selected isolates from pet birds, migratory quails, and tree sparrows for experimental infection was carried out on tissue culture (vero cell) which showed cytopathic effects and the titer was  $10^6$  TCID<sub>50</sub>. This was in accordance to previously reported studies [36,37].

Experimentally infected chicks, exhibited general signs of illness, sleepiness, ruffled feathers, weakness, eye infection as unilateral or bilateral conjunctivitis in group 1a and 3a. Furthermore, respiratory signs were observed in the form of rhinitis, sneezing, dyspnea, gasping with mucoid diarrhea at the first 10 days PI in some birds. These results are similar

to those recorded previously [37], where the clinical signs observed in experimentally infected chicken in the form of respiratory signs as gasping, dyspnea and rhinitis were also investigated.

Postmortem examinations revealed that up to 10 days PI, mild congestion in lung, liver and heart were observed in group 1a, while in group 3a, mild congestion in kidney was observed from 7-10 days PI. Post 2-3 weeks PI in groups 1a and 3a, pericarditis, pancreatitis, filled intestine with fluid and enlarged liver, lung and spleen and muscle congestion were detected. These results agree with [38], who recorded slight congested liver, spleen, lung, and pericarditis. In quails, mucoid frothy diarrhea 3-5 days PI in groups 1b and 3b with unilateral ocular lesion in group 1b was obtained herein, while 3 weeks PI, all groups showed weakness and loss weight. In group 1b, ocular lesion was clear and gasping was recorded. Greenish and mucoid frothy diarrhea in groups 1b and 2b was observed. These signs were observed along the experiment. The same observed signs were recorded in a previous research [38].

Postmortem examination of infected quails revealed that groups 1b and 3b showed congested lung and liver 2-4 days PI. From 2-3 weeks PI, birds in groups 1b and 3b showed congested muscle, liver, lungs, spleen and hemorrhagic spots on the liver, pericarditis and enteritis. These signs were continuing along the experiment. Similar post mortem changes were recorded previously [38].

From the obtained results of experimental infection, the more pathogenic isolate was from pet birds then from other two isolates from chicken and quails. This result is in agreement with Takahashi *et al.* [39], who reported that strains isolated from psittacine birds were more virulent than the pigeon strains.

Sequence analysis of *OmpA* gene fragment supported the classification of *Cp. psittaci* into genotype A as mentioned previously [12]. The genotypes of *Cp. psittaci* infection are relatively host specific and genotype A was the major genotype associated with parrot [40,41]. Our results demonstrated that the studied *Cp. Psittaci*/HSS (KY296311) strain

which was isolated from pet birds (budgerigar) belongs to genotype A and presented high nucleotide homology (94%) with the Egyptian uncultured strain isolated from song bird, 92.3% with the Germany strain (MN Zhang) that was isolated from psittacine birds as recorded in a recent study in China [41]. In addition, it showed high nucleotide homology (92.1%) with Iranian Strains Nose and UT245/AGP which was isolated from budgerigar and African grey parrot, respectively [42] in addition, homology with 90/1051 strain isolated from African grey parrot in Poland [43] was observed. Also, the nucleotide homology with the KMP09 strain isolated from psittacine birds in China was high (91.9%) [44]., While, nucleotide homology of 80.7% with UT78-Alexandria parakeet (HQ 845545) as genotype J was also reported [42].

### Conclusion

From this study, it was clear that wild and pet birds showed high incidence rates of *Cp. psittaci* in their organs and excretions exposing other native birds, workers, and human dealing with pet birds to the risk of infection as *Cp. Psittaci* has a major public health importance.

### Conflict of interest

The authors declare no conflict of interest.

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## الملخص العربي

### دراسات عن عدوى الكلاميديا في بعض الطيور البرية وطيور الزينة

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تعتبر الكلاميدوفيليا سيتاسي من أهم المسببات المرضية المشتركة في الطيور والتي تسبب مرض الكلاميديوزس. وتهدف هذه الدراسة لاستبيان نسبة العدوي بالكلاميوفيليا سيتاسي وتحديد الأنواع الجينية التي تتعرض لها الطيور حيث تم إستهداف أربعة أنواع من أجناس الطيور البريه (٥٠ من السمان المحلي، ٤٠ من السمان المهاجر، ٣٠ من البمام، ٢٥ من العصافير) وأربعة أنواع من طيور الزينة (٢٠ من البغيعاء الصغير " الدرة"، ١٠ من الكوكتيل، ٣ من طائر الحسون، ٥ من طائر الحب) وذلك عن طريق صبغة الجيمسا، صبغة الجمينز وتفاعل البلمرة المتسلسل. وكانت النتائج فى طيور الزينة تتراوح بين (٨٠٪-١٠٠٪)، (٨٥٪-١٠٠٪)، (٨٠٪-١٠٠٪) علي التوالي. وكانت النتائج فى الطيور البرية تتراوح بين (٦٤%-٨٥%)، (٧٦%-٩٥%)، (٨٠%-٩٠٪) علي التوالي. حيث كانت نسبة العزل من طيور الزينة أعلي من الطيور البرية. ومن ثم تم عمل عدوي صناعية باستخدام ثلاثة معزولات من (طيور الزينة - السمان المهاجر-العصافير) عن طريق حقن جرعة ١٠<sup>-٦</sup> في الدجاج والسمان عمر ١٥ يوم عن طريق الحنجرة وظهرت أعراض تنفسية، التهاب في ملتحمة العين وإسهال. وقد وجدت تغيرات باثولوجية مثل التهاب فى الكبد، الطحال، الرئة والقلب وقد وجد أن أكثر المعزولات تأثيرا هو معزول طيور الزينة ثم يليه العصافير ثم السمان المهاجر. وعند عمل التتابع التسلسل الجزيئي لجين *OmpA* لعترات الكلاميدوفيليا سيتاسي أظهرت تشابه بنسبة ٩١.٨-٩٤.٤% مع النوع A والذي تم تسجيله من قبل. وعليه فإن كلا من طيور الزينة والطيور البرية تمثل عائلًا هامًا لإفراز الكلاميدوفيليا سيتاسي في الإفرازات والتي يتعرض لها كلا من الإنسان والطيور مما يمثل خطرا نو أهمية مشتركة.