Incidence of Chlamydia psittace in wild and pet birds and its severity for chickens and quails

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A B S T R A C T

Chlamydia infections are occurring in wild bird's. Efforts to detect and identify Chlamydia are important because it is often accompanied with concurrent infection and variable outbreaks. Human being in contact with wild and pet bird's shops are exposed to hazards of infection. In the present study 145 wild birds(doves, tree sparrows, and domestic and migratory quails) and 65 Pet birds(Budgerigars, Finches, Love birds and Cockatiels)were examined to detect Chlamydia inclusions, smears from livers, lung, heart and spleen from these birds were stained by Giemsa stain to demonstrate the presence of Chlamydia inclusions .pooling of internal organs were inoculated in ECE via yolk sac route for isolation of Chlamydia psittace and smears from yolk sacs were subjected to Gimenez stain .Chlamydia psittace detection was high from liver of pet birds 70-100% while from wild birds was 60-73 %. In a Comparison between PCR, Giemsa and Gimenez stains revealed that the PCR was more sensitive in identification of Chlamydia psittace from wild and pet birds and the incidence was higher in pet birds (80-100%) than in wild birds (64-85%). After experimental infection with Chlamydia psittace the more pathogenic isolates were from pet birds for chickens and quails than other isolates. By PCR sequencing for ompA gene of isolated strain was found to belongs to genotype A of Chlamydia psittace.

Keywords: Chlamydia psittace, chickens, quails, PCR

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1. INTRODUCTION

Chlamydia are Gram-negative, obligate and non-motile organisms, it is a zoonotic disease caused by intracellular bacteria, which represent a problem in veterinary and human medicine all over the world. Numerous wild and domesticated bird species fall ill, wild birds potentially play role in the transmission of the disease in humans (Eugster,1980 and Krizek and Prukner, 2009). Chlamydophila psittace infection in birds (Avian chlamydiasis) and humans(psittacosis) chlamydophila psittace is shed in the faeces and nasal /ocular discharge (west,2011. Marta et al .2015 and Hopkins et al .2016) recorded that avian chlamydiosis is a zoonotic disease occurring in human, poultry and exotic birds. They suggested that some wild bird species play an important role as reservoirs for Chlamydia, especially chlamydophila psittace whereas C. psittace is the predominant chlamydial agents in birds. Hala, 2015 succeeded in isolation of chlamydophila psittace in high incidence from (turkeys, chickens, ducks and pigeons) with high incidence from liver, lung, spleen and heart. Vertical transmission of Chlamydia was described and transmission is primarily from one infected birds to another (witten brink et al. 1993, Andersen et al .1997 and Dovc et al., 2007). The usual duration between exposure to c. psittace and onset of illness ranges from 3days to several weeks (2-8 weeks) (Fudge 1996 and Dhama et al 2008). Jenkins, 1989; Harrison 1989; Vanrompay et al., (1995); Andersen et al., (1997) and Woldehiwet, (2001) reported that clinical signs in birds infected with Chlamydophila psittaci include sinusitis, and respiratory problems, yellow green dropping, anorexia, loss of body weight, pneumoenteritis, polyuria and dullness, unilateral or bilateral conjunctivitis and keratoconjunctivitis. Chlamydophila psittaci can be identified using

species-specific conventional PCR (Messmer et al., 1997; Sachse and Hotzel, 2003; Van Loock et al., 2005), current PCR tests for detection of Cp. psittaci target the ompA gene or the 16S–23S rRNA gene (Everett et al., 1999b; Geens et al., 2005 and Messmer et al., 1997).

Aim of this study was the detection and identification of Chlamydia from wild and pet birds

2. MATERIAL AND METHODS

2.1. Birds

145 Wild birds (30 doves, 25 tree sparrows,50 domestic and 40 migratory quails and 65 Pet birds (20 Budgerigars, 30 Finches, 5 Love birds and 10 Cockatiels) were examined.
Seventy broiler chicken 15 days old and seventy 15 days old quails were use in this study for experimental infection with the isolated and identified c. psittace.

2.2. Samples

Specimens were collected from internal organs (livers, hearts and lungs) of wild and pet birds for detection of chlamydia.

2.3. Chicken embryo inoculation

were applied according to (Busby *et al.,* 1964; Gimenez, 1964)

2.4. PCR according to (Doosti, 2011) Titration of Chlamydophila psittaci strains on ECE and tissue culture

were applied according to Alethea *et al.* (2014)

2.5. Experimental design:

To study the pathogenicity of the isolated Chlamydophila Psittaci: Seventy 15 days old commercial Cobb chicks and seventy 15 days old commercial quails were divided into 4 groups. The birds were infected intra tracheally with 0.2 ml of 3 different types of *Chlamydophila psittaci* strains from (Pet birds, migratory quails and tree sparrows). Post infection, birds were observed daily up to 34 days for observation of the clinical signs and lesions. One bird from each group was sacrificed daily for the first 10 days' post infection then each 3days 1,2,3,4,5,6,7,8,9,10, 14,17,21, 24,28,34 days P.I. The sacrificed birds were necropsied and lesions were recorded. Samples from liver, lungs, heart, spleen, pancreas, pericardium, intestine, air sac, trachea, and kidneys were collected and subjected to both impression smear and ECE reisolation.

2.6. Sequencing of isolated strain: the analysis of ompA gene of Chlamydophila psittaci applied according to (tamura et al .2013).

Table (1): Experimental infection with *Chlamydophila psittaci* strains* in chickens and quails

Group	Chlamydia isolate	Age/day	Birds no. in each group	Route of infection	Dose
1a	Pet birds	15	20 C	I/T	10^6 TC ID/ml
2a	Migratory quails		20 C		
3a	Tree sparrows		20 C		
1b	Pet birds		20 Q		
2b	Migratory quails		20Q		
3b	Tree sparrows		20Q		
Non infected control	-		10 chickens 10 quails	-	-

**Chlamydophila psittaci* from: 1- Pet birds, 2- Migratory quails, 3- Tree sparrows, I/T: Intratracheal, C: chickens, Q: quails

3. RESULTS:

3.1. Clinical signs and p.m. lesions in chickens and quails infected experimentally:

Clinical signs in chickens appeared in the form of mucoid diarrhea, unilateral ocular lesions, sleepiness, rapid breathing, sneezing, conjunctivitis, weakness and ruffled feathers. The clinical signs appeared in quails in form of mucoid greenish diarrhea, gasping, loss body weight and ocular lesions.
PM lesions in chickens: congestion of internal organs (liver, heart, lung, kidneys and spleen), pericarditis, and intestine filled with fluids, airsacculitis and congested muscle. Similar lesions occur in quails.

3.2. Results of Sequence analysis for one strain of pet birds

The partial ompA sequence of isolated strain was placed in genotype A of Chlamydophila psittaci which had the highest identity with previously described strains of genotype A. Phylogenetic analysis of ompA gene sequence of Chlamydophila 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 (Fig. 1).

Table (2): result of different methods used for identification of *Chlamydophila psittaci.* Comparison between different methods of identification using tissue smears stained with Giemsa, and yolk sac impretion smear stained by Gimenez, and ECE inoculation: -

Bird	Tissue smear* (Giemsa)			Gimenez		Embryonic death	
	Liver	Lung	Heart	positive	Negative	Positive	Negative
Budgerigars	15	10	8	17	3	14	6
(20)	(75%)	(50%)	(40%)	(85%)	(15%)	(70%)	(30%)
Lovebirds (5)	4	3	2	5	0.0	3	2
	(80%)	(66%)	(40%)	(100%)	(0.00%)	(60%)	(40%)
Finches	3	2	1	3	0.0	2	1
(3)	(100%)	(66.60	(33.3%)	(100%)	(0.0%)	(66.6%)	(33.3%)
Cockatiels	7	6	5	9	1	6	4
(10)	(70%)	(60%)	(50%)	(90%)	(10%)	(60%)	(40%)
Tree Sparrows	15	12	10	19	6	15	10
(25)	(60%)	(48%)	(40%)	(76%)	(24%)	(60%)	(40%)
Doves (30)	22	18	14	25	5	21	9
	(73%)	(60%)	(46%)	(83.3%)	(16.7%)	(70%)	(30%)
Migratory	24	26	20	38	2	28	12
quails (40)	(60%)	(65%)	(50%)	(95%)	(5%)	(70%)	(30%)
Domestic	33	30	25	41	9	34	16
quails	(66%)	(60%)	(50%)	(82%)	(18%)	(68%)	(32%)

*stained with Giemsa stain

Table (3): Comparison between result of PCR and other conventional methods for detection of *Chlamydophila psittaci*

Species of birds	Total number of birds	PCR	Giemsa	Gimenez
Tree sparrows	8	6 (75%)	7 (87.5%)	8 (100%)
Migratory quails	10	9 (90%)	9 (90%)	10 100%)
Domestic quails	10	8 (80%)	10(100%)	9 (90%)
Doves	10	8 (80%)	9 (90%)	10 100%)
Budgerigars	5	4 (80%)	5 (100%)	4 (80%)
Finches	2	2(100%)	2 (100%)	2 (100%)
Cockatiels	3	2(66.6%)	2 (66.6%)	3 (100%)
Lovebirds	2	2 (100%)	2 (100%)	2 (100%)

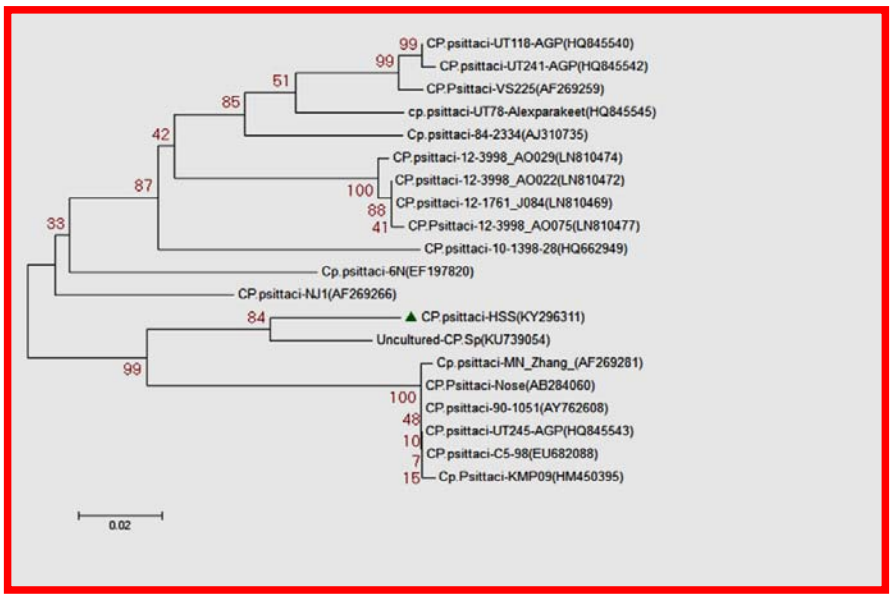

Fig. (1): Phylogenetic tree of sequence analysis

4. DISCUSSION

In this study impression smears stained by Giemsa stain from different organs of wild birds (domestic quails, migratory quails, tree sparrows, and doves) showed high percent of positive Chlamydophila psittace (72%), (85%) (64%) and (76.6%), respectively. High incidence of Chlamydophila psittaci recorded by Hadia (1987) 67.32% from migratory birds, also El Jakee et al. (2014) who recorded 81.8% and 77.4% from cattle egret and hoopoe, respectively. In case of pet birds (budgerigars, love birds, finches, and cockatiels) also high incidence of Chlamydophila psittaci was recorded (85%), (80%), (100%) and (80%), respectively.

These results agree with Hadia (1984) which recorded the highest ratio of infection in liver (93%-100%) in Budgerigars and finches respectively, while in lung was (40%-0%), respectively. While Schwartz and Fraser (1981) recorded lower incidence of Chlamydophila psittaci cockatiel (26.9%), love birds (21.4%) finches (75.3%) were positive. From the obtained results it is clear that wild birds (Doves, Tree sparrows, Domestic quails and migratory quails) and pet birds (Budgerigars, finches, love birds, and cockatiels) considered as natural host and shed the organism in their excretions, similar results were also reported by Brand (1989) and Andersen et al., (1997). The internal organs (liver, heart, and lungs) were examined by impression smears stained by Giemsa, Chlamydophila psittaci inclusion bodies appeared as small, round, purple, red and blue dots in liver, lung, heart which agree with that mentioned by Hadia (1987), Andersen (1996) and Wittenbrink et al. (1993).

The result of microscopical examination of suspected tissues reveals that liver was the most affected organ in tree sparrows, doves, domestic quails and migratory quails and results were (60%), (73%), (66%) and (60%) respectively. While the incidence of Chlamydophila psittaci in lung was high but lower than liver in the same wild birds (48%), (60%), (60%) and (65%), respectively. The lesser ratio was recorded from the heart and the percentage was (40%), (46%), (50%) and (50%), respectively. These results agree with Moore and Petrok (1985) who recorded that chlamydia in liver was (78%).

On the other hand, Chlamydophila psittaci incidence of pet birds was high in liver of (budgerigars, love birds finches, and cockatiels), the percentage was (75%), (80%), (100%) and (70%), respectively and the percentage in the lung was (50%), (66%), (66.6%) and (60%), respectively. While lower percentage of detection was from the heart (40%), (40%), (33.3%) and (50%), respectively. The obtained results showed high incidence of infection. Pathogencity of Chlamydophila psittaci for embryonated chicken eggs showed congestion of embryo and yolk sac vessels, similar observations recorded by Bougioukils et al. (2000).

By examination of yolk sac membrane impression smear stained by Gimenez from infected chicken embryo by wild birds isolate the percentage of infection was (76%), (83.3%), (82%) and (95%), respectively in (tree sparrows, doves, domestic quails, and migratory quails). These results agree with El-Jakee et al. (2014) which

analyzed Chlamydia by using Gimenez stain for Hoopoe and Cattle egret and the Chlamydophila psittaci positive ratio was 89.1% and 83.0%, respectively. On other hand, in our study Chlamydophila psittaci from infected yolk sac by pet birds isolates revealed (85%), (100%), (90%) and (100%) from (budgerigars, finches, cockatiels, and love birds), respectively.

In our study, psittacine birds and migratory birds showed the highest isolation ratio followed by Doves, Domestic quails and finally tree sparrows which agree with the results recorded by Beven and Bracewell (1986) who found the highest isolation ratio of Chlamydophila psittaci in psittacine birds then followed by doves. Polymerase chain reaction (PCR) used for diagnosis of Chlamydophila psittaci using species specific conventional PCR and the ompA gene was investigated as target DNA sequence among family chlamydiacae as mentioned by Takashima et al., (1996), Geens et al., (2005), Geigenfeind et al., (2012) and use 16S rRNA as target sequence among family chlamydiacae (Messmer et al., 1997; Everett et al., 1999b; Clarridge, 2004 and Maira et al., 2012).

In this study, ten positive samples from domestic quails, eight from tree sparrows, ten from migratory quails, ten from doves were selected according to severity of infection. The amplified product specific for Chlamydia ompA gene was demonstrated in domestic quails (80%), tree sparrows (78%), doves (80%) and migratory quails (90%) (Fig. 1). These results are similar to El Jakee et al. (2014) who recorded that result of PCR among Hoope, and cattle egret birds were 96.4% and 90.6%, respectively. Twelve positive samples from pet birds 5 budgerigars, 2 finches, 2 love birds, and 3 cockatiels were selected according to severity of infection. The amplified product specific for Chlamydophila psittaci at 1041 bp found the ompA gene was demonstrated in (90%) in budgerigars, (100%) in finches, (66.6%) in cockatiels, and 100% love birds. While Celebi and Seyyel (2006) recorded lower results from pet birds (34.4%) by PCR but they used organ pools.

Sequence analysis of ompA gene fragments supported the classification of Chlamydophila psittaci into genotype A as mentioned by (Geens et al., 2005). The genotypes of Chlamydophila psittaci infection are relatively host specific (Andersen and Vanrompay, 2000). Chlamydophila psittaci genotype A was the major genotype associated with parrot (Zhang et al., 2015). Our result demonstrated that the studied Cp. Psittaci/HSS (KY296311) strain which isolated from pet birds (budgerigar) belongs to genotype A and showed high nucleotide homology (94%) with the Egyptian uncultured strain isolated from song bird, 92.3% with the Germany strain (MN Zhang) that isolated from psittacine birds as recorded by (Zhang et al., 2015). In addition, it was showed high nucleotide homology (92.1%) with Iranian strains Nose and UT245/AGP which isolated from budgerigar and African grey parrot, respectively (Madani and Peighambari, 2013) and 90/1051 strain isolated from African grey parrot in Poland (Tomosz et al., 2015). Also, the nucleotide homology with the KMP09 strain isolated from psittacine birds (in China) was high (91.9%) (Feng et al., 2016).

In experimentally infected chickens all groups exhibit general signs of illness, sleepiness, ruffled feathers, weakness, eye infection as unilateral or bilateral conjunctivitis in group 1a and 3a also, respiratory signs were observed in form of rhinitis, sneezing, dyspnea, gasping with mucoid diarrhea at first 10 days PI in some birds. These results similar to that recorded by Yin et al. (2013) who recorded clinical signs in experimentally infected chicken in form of respiratory signs as gasping, dyspnea, and rhinitis.

Postmortem examination revealed that up to 10 days PI mild congestion in lungs, liver, heart in group 1a. While, in (group 3a) mild congestion in kidney was observed from 7-10 days PI. Post 2-3 Weeks PI in group 1a, 3a pericarditis, pancreatitis, intestine filled with fluid and liver enlarged, lungs, spleen and muscle congestion was observed. These results agree the observation of Yin et al. (2013) and Andersen (1996) who demonstrated the importance of obtaining a pharyngeal specimen for isolation of Chlamydia from cocktails and turkeys, pharyngeal swabs were more perfect than cloacal swabs, Birds sampled early in the infection were most likely to have Chlamydia in the pharyngeal samples only. By reisolation of Chlamydophila psittaci by impression smears from organs of infected chickens and stained by Giemsa stain, were positive from 2nd day PI, while in quails from 2-4 days PI. These results agree with that recorded by Batta et al. (1999). By reisolation of Chlamydophila psittaci from infected chickens and quails on ECE and staining with Gimenez stain reisolation was positive from 2-3 days in chickens PI, while in quails from 4-5 days PI.

5. CONCLUSION:

From this study, it is clear that wild and pet birds showed high incidence of Chlamydophila psittaci in their organs and excretions which expose other domestic birds, workers and human dealing with pet birds to the risk of infection as Chlamydophila psittaci has major public health importance. By

sequence of isolated Chlamydophila Psittaci for determination of the serovar (subtype), Chlamydophila psittaci was subtype A, hence the identification can indicate the source of the isolate for epidemiological studies.

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