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Research Article

Prevalence of Chlamydia Psittaci in Domesticated and Fancy Birds in Different Regions of District Faisalabad, Pakistan

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2. Key words

Chlamydia Psittaci; Psittacosis; Prevalence; Zoonosis

1. Abstract

1.1. Introduction

The study was aimed to check the prevalence of this zoonotic bacterium which is a great risk towards human population in district Faisalabad at Pakistan.

1.2. Methodology

In present study, a total number of 259 samples including fecal swabs (187) and blood samples (72) from different aviculture of 259 birds such as chickens, ducks, pigeons, parrots, Australian parrots, and peacock were collected from different regions of Faisalabad, Pakistan. After processing the samples were inoculated in the yolk sac of embryonated chicken eggs for the cultivation ofChlamydia psittaci(C. psittaci)and later identified through Modified Gimenez staining and later CFT was performed for the determination of antibodies titer against C. psittaci.

1.3. Results

The results of egg inoculation and modified Gimenez staining showed 9.75%, 29.62%, 10%, 36%, 44.64% and 39.28% prevalence in the fecal samples of chickens, ducks, peacocks, parrots, pigeons and Australian parrots respectively. Accordingly, the results of CFT showed 15.38%, 25%, 46.42%, 36.36% and 25% in chickens, ducks, pigeons, parrots and peacock respectively.

1.4. Conclusion

The overall research results showed the prevalence of C. psittaci is significantly high in love birds and these are the carriers of this zoonotic pathogen to human population as well as to the other domesticated birds.

3. Introduction

Chlamydia psittaci is a bacterium that belongs to genus Chlamydophila, family Chlymediaceae, and order Chlamydiales [1]. *Chlamydia psittaci* that causes "Avian Chlamydiosis" also known as Psittacosis in wild, domesticated and ornamental birds having great zoonotic potential. This strict intracellular bacterium contains many serovars. The "Chlymediaceae" family has two genera Chlamydophila and Chlamydia, both genera contain nine species like *C. psittaci, C. pecorum, C. felis, C. caviae, C. abortus and C. pneumoniae* as well as *Chlamydia suis, Chlamydia trachomatis and Chlamydia muridarum* respectively [2]. *Chlamydia psittaci* is an obligate intracellular Gram-negative bacterium having zoonotic risk containing seven strains A-F, WC and M56 infecting their specific hosts for example serovar A infects psittacine birds, serovar B infects pigeons, C infects ducks and geese, D infects turkeys, E infects ducks, pigeons and other avian species, F in-

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fects parakeets, WC infects cattle and M56 infects the rodents [3].

Family "Chlymediaceae" was considered as "Viruses" due to their small size that can pass through filters of 0.45 um. These bacteria are different to other bacteria because of having an exclusive life cycle that contains elementary (EBs) and reticulate bodies (RBs). EBs are metabolically inactive but are infectious and like spores may resist to strict environmental conditions while Reticulate bodies (RBs) are non-infectious but are metabolically active. Peptidoglycan layer is absent from these bacteria and lipopolysaccharides (LPS) forms its cell wall common to all members of this family having endotoxin activity and the cytoplasmic membrane is surrounding the central core of these bacteria. These bacteria contain major outer membrane protein (MOMP) in their outer membrane which is specific to each serovar of this group important structural component that helps in serotyping of the bacteria [4].

Chlamydial members are intracellular so they depend upon host cell for energy and replication and their isolation can be done by inoculating cell monolayer cultures and yolk sac of embryonated chicken eggs for *C. psittaci* isolation and identification [5]. *C psittaci* is the major pathogen involved in psittacosis, a disease which commonly infects ornamental birds but also infects other species of birds, mammals and human beings which lead to these symptoms such as malaise conditions, chills, headache, and severe pneumonia. This disease is transmitted to a human who is involved in handling psittacine birds and is transmitted mainly by inhalation. Human psittacosis is now a notifiable zoonotic disease and this bacterium is prevalent in the whole world with zoonotic risk. 6500 cases were reported from 1996-2007 [6]. *Chlamydia psittaci* can be isolated from liver, spleen, lungs and cloacal secretions by using Buffalo Green Monkey cell and direct immunofluorescence can be performed for diagnosis of the bacterium [7].

Chlamydia psittaci can cause co-infection with other bacterial pathogens as it can evade the immune system, it involves upregulation of certain genes as expression rate of mRNA of Inca, ftsW, groEL and cpaf are increased and help to establish the *Chlamydia psittaci* infections. The complement systems are the central portion of the innate immune response. This system is activated by forty kinds of surface antigens from bacterial infection and produces an inflammatory response but *Chlamydia psittaci* causes long-lasting inflammatory responses if the bird is deficient in C3aR because these C3a peptides are protective against *Chlamydia psittaci*. This organism is proficient in evading pro-inflammatory mediators [8].

The infected birds show high mortality rates of up to 30%, as it is an obligate intracellular organism and act as a parasite it depends upon the host for energy in the form of ATP. Oral transmission of this organism is not reported yet but it can be transmitted through arthropod vectors, while the vertical transmission is limited [9].

4. Materials and Methods

4.1. Sample collection

A total of 187 fecal samples and 72 blood samples of fancy birds were collected from different regions of Faisalabad, Pakistan. These samples were transferred directly to the laboratory of Institute of Microbiology, University of Agriculture, Faisalabad.

4.2. Samples Preparation

4.2.1. Preparation of fecal samples: Fecal samples from 187 birds including 41 chickens, 27 ducks, 56 pigeons, 25 parrots, 10 peacock and 28 Australian parrots were collected. One gram of each fecal sample was taken and suspended in sterilized glass bottle containing 99.0 ml of sterile physiological saline (0.85% NaCl) with vigorous shaking. The mixture was left at room temperature for 10 to 15 minutes to the completely dissolve the droppings. The fecal material suspension was clarified, using centrifugation at 3000 rpm for 15 minutes after a vigorous shaking of the samples for 3-5 minutes. The supernatant was treated with antibiotics (Streptomycin 2.5mg/ml, neomycin 0.5mg/ml and nystatin 100 units/ml) and holded for one hour at room temperature, re-centrifuged for two times and the final supernatant was used to culture the bacteria [10].

4.2.3. Serum sample extraction from blood samples: Blood samples were centrifuged at 3000 rpm for 3 minutes in the centrifuge machine. For purification of the serum sample, again centrifugation was performed in the micro centrifuge machine at 3000 rpm for 3 minutes. Straw colored serum was stored in clear sterilized 1.5 ml Eppendorf tubes and stored at -20^{0}C until CFT performed.

4.2.4. Inoculation of the Samples in the Embryonated Eggs: SPF Embryonated eggs of 6 days were used for the inoculation of samples. The sample was inoculated in the embryonated chicken eggs via the yolk-sac route. The eggs were incubated in humidified incubator at 37^o C and the non-inoculated eggs were taken as control. The eggs were candled on daily basis for any contamination the embryos that died within 3 days' post inoculation, were discarded and considered negative while the embryos dead during3 to 10 days' post inoculation were stained using Modified Gimenez staining technique [11]. The embryos were observed for development of pathological lesions.

4.2.5. Serological Testing: The complement fixation test (CFT) was used to detect the presence of specific antibodies in the bird's serum. This test is based on the use of complement, a Biologically labile serum factor that causes the immune cytolysis i.e. lysis of antibody coated cells [12].

5. Results

In present studies, samples were collected from different domesticated and fancy bird's species from the regions of Faisalabad. Birds were sampled to check the status of *C. psittaci's* prevalence in Faisalabad regions. A number of 259 Samples including fecal samples and blood samples were collected from different birds such as Chickens (*Gallus gallusdomesticus*), Ducks (*AnasPlatyrhynchos*), Pea-coacks (*Pavocristatus*), Parrots (*Psittaciformes)*, Pigeons (*Columbidae)* and Australian parrots (*Alisterusscapularis*). *C. psittaci* were found in 31.01% (58/187) **(Table 1)** of different species of bird's fecal samples while 33.33% (24/72) of blood samples **(Table 2)**. *Chlamydia psittaci* was confirmed with cultural characteristics, morphological identification and serological approach using Compliment fixation test (CFT).

Table 1: Results of morphological identification of C. psittaci from fecal samples of fancy birds using Modified Gimenez staining Technique.

Fancy Birds	Fecal Samples			
	Total number of Samples	Positive samples	Negative samples	Prevalence (%)
Chickens	41	4	37	9.75
Ducks	27	8	19	29.62
Pigeons	56	25	31	44.64
Parrots	25	9	16	36
Peacock	10	1	9	10
Australian Parrots	28	11	17	39.28

Table 2: Results of Complement Fixation Test for detection of antibodies against C. psittaci in the serum of blood samples of fancy birds.

Fancy Birds	Blood Samples			
	Total number of samples	Positive Samples	Negative samples	Prevalence (%)
Chickens	13	2	11	15.38
Ducks	16	4	12	25
Pigeons	28	13	15	46.42
Parrots	11	4	7	36.36
Peacock	4	1	3	25

The samples were evaluated by using two parallel techniques. Firstly, all the collected fecal samples were processed and examined using Modified Gimenez staining technique for the presence of inclusion bodies of *C. psittaci* and secondly the blood samples were investigated using CFT for serological diagnosis of the presence of antibodies against *C. psittaci.*

Among the fecal samples, 58 samples were positive from 187 (31.01%) samples following chickens 9.75% (4/41), ducks 29.62% (8/27), pigeons 44.64% (25/56), parrots 36% (9/25), peacock 10% (1/10) and Australian parrots 39.28% (11/28) **(Table 3)**.

The blood samples were investigated by using CFT and 24/72 (33.33%) samples were positive for the Chlamydial antibodies in the serum of lovebirds following chickens 15.38% (2/13), ducks 25% (4/16), pigeons 46.42% (13/28), parrots 36.36% (4/11) and peacock 25% (1/4) **(Table 4)**.

Table 3: Results of cultural isolates recovered from fecal samples on the basis of cultural and morphological characteristics on region and species base.

Regions of Faisalabad	Avian Species						Total	Prevalence
	Chickens	Ducks	Pigeons	Parrots	Peacock	Australian Parrots	6 Species	%
Jhumra								
Samundari	1/8	2/5	4/9	2/7	0/2	0/3	9/34	26.47
Jaranwala	0/7	1/4	5/11	3/7	1/3	2/5	12/37	32.43
Tandlianwala	2/9	1/7	5/12	2/5	0/3	3/7	13/43	30.23
Faisalabad Sadar	0/6	1/3	5/11	1/3	0/0	2/4	9/27	33.33
Total	1/11	3/8	6/13	1/3	0/2	4/9	15/46	32.60
Prevalence (%)	4/41	8/27	25/56	9/25	1/10	11/28	58/187	31.01
	9.75	29.62	44.64	0.36	10	39.28	31.01	-

Table 4: Antibodies titer against Chlamydia psittaci detection in blood samples collected from different type of aviculture, using CFT.

	Negative titer up to 1/8		Positive titer up to 1/16		Positive titer up to 1/32		Positive titer up to ≥ 1/64		Total positive in CFT	
Chickens	11	84.61%	2	15.38%	-	-	-	-	2	15.38%
Ducks	12	75%	1	6.25%	2	12.5%	1	6.25%	4	25%
Pigeons	15	53.57%	6	21.42%	3	10.71%	4	14.28%	13	46.42%
Parrots	7	63.63%	-	-	1	9.09%	3	27.27%	4	36.36
Peacock	3	75%	1	25%	-	-	-	-	1	25%

C. psittaci were identified using eggs inoculation via yolk sac route and then smear of the yolksac membrane was stained by using Modified Gimenez Staining technique.

The fecal samples inoculated in embryonated eggs were observed for pathological lesions due to*C. psittaci* infection. Gross lesions were recorded the infected embryos were dwarf in size (Figure a) as well as developed hemorrhages on the toes of embryos (Figure b). The yolk sac membranes of infected chicken eggs were collected and subjected to stain using Modified Gimenez staining procedure. The inclusion bodies of *C. psittaci* we areas of re-appeared small pink cocci against blue to the green background. Prevalence was found 26.47%, 32.43%, 30.23%, 33.33% and 32.60% in different areas of Faisalabad such as Jhumra town, Samundari town, Jaranwala town, Tandlianwala town and Faisalabad Sadar respectively **(Table 3)**.

Figure (a) Pathological lesion, the dwarf chicken embryo developed post inoculation of processed fecal samples of fancy birds in the fertile chicken eggs to cultivate *C. psittaci*. Figure (b) Hemorrhages on the toes of embryos infected with *C. psittaci* after the inoculation of processed fecal samples of fancy birds. Figure (a)-(b) showing the growth abnormalities in the chicken embryos post inoculation of processed fecal samples, were positive to *C. psittaci*.

Figure (c)-(d) The inclusion bodies of infected yolk sac membrane stained with Modified Gimenez stain. Small pink cocci against blue-green background were the inclusion bodies of C. psittaci, appeared in the infected yolk sac membrane, inoculated with fecal samples of fancy birds.

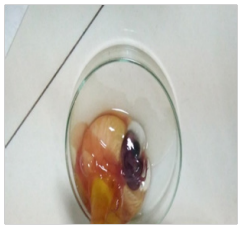

Figure (a)

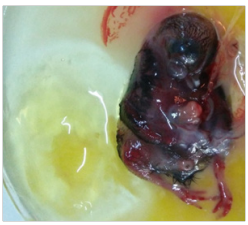

Figure (b)

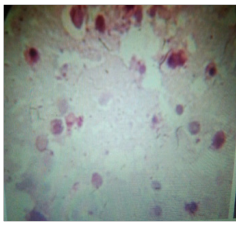

Figure (c)

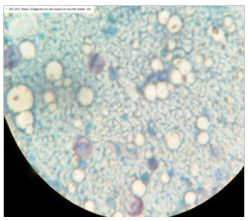

Figure (d)

Figure (a) Pathological lesion, the dwarf chicken embryo developed post inoculation of processed fecal samples of fancy birds in the fertile chicken eggs to cultivate *C. psittaci*. Figure (b) Hemorrhages on the toes of embryos infected with *C. psittaci* after the inoculation of processed fecal samples of fancy birds. Figure (a)-(b) showing the growth abnormalities in the chicken embryos post inoculation of processed fecal samples, were positive to *C. psittaci*.

6. Discussion

In the present study, the isolation and identification of *Chlamydia psittaci* were performed by using embryonated eggs. The bacterium is intracellular in nature and to culture this *C. psittaci*, samples were inoculated in the eggs by route of yolk sac membrane. After incubation of some day's identification was performed by histopathological lesions produced in the embryo as hemorrhages were developed on the head and toes of embryos, dwarfism in embryos, and congestion of yolk sac membrane was observed. These suspected positive yolk sac membranes were confirmed for the presence of *C. psittaci* by using modified Gimenez staining. In this technique, inclusion bodies of *C. psittaci* were stained as purple with the green background.

The isolation and identification of *C. psittaci* were supported by recent study in which embryonated eggs were used as a gold standard medium for *C. psittaci*. They isolated this bacterium in the samples collected from conjunctival swabs and feces. The long time incubation is only its disadvantage but it is sensitive and specific to isolate *C. psittaci*. The inclusion bodies were seen also observed in yolk sac membrane by using modified Gimenez staining technique. Isolations of *C. psittaci* can be achieved by using different cell cultures but the use of embryonated chicken eggs to isolate *C. psittaci* was conventional and gold standard [13].

The rate to *C. psittaci* from feces and conjunctival swabs was 52.94% - 72.55% and 54.17 – 79.17, respectively. This was an indication of effective isolation of *C. psittaci* using embryonated eggs and staining of *C. psittaci* by using modifies Gimenez staining. Although this method was of long time and a risk to transmit *Chlamydia psittaci* to the staff of laboratory [14].

In the present study, the prevalence of *C. psittaci* was observed in different regions of Faisalabad, Pakistan. In this Study, the prevalence was observed using cloacal swabs, feces and viscera while by serological analysis CFT was performed to assess' status of antibodies against *C. psittaci*. In the current study, prevalence was 31.01 % in feces, collected from different birds. The serological analysis showed 24 out of 72 (33.33%) blood samples were positive to *C. psittaci*. In this study, serological analysis showed prevalence in chickens (15.38%), ducks (25%), pigeons (46.42%), parrots (36.36%) and peacock (25%) respectively.

This was the first study to check the prevalence of *C. psittaci* in chickens, ducks, peacock, pigeons, parrots and Australian parrots. No other studies were found in regions of Faisalabad, Pakistan. The status of birds in Faisalabad was monitored the first time and found the significant presence of *C. psittaci* in this region. *C. psittaci* was not only found in diseased birds but also in the birds which were apparently healthy. These all birds are the risk to

transmit parrot fever to the human population. Shortly the prevalence shows, the population of Faisalabad, Pakistan is at risk.

The results were supported by previous studies, checked the status of *C. psittaci* by using different staining methods. They found 96.4%, 89.1%, 58.2%, 81.8% and 80% *C. psittaci* in the samples by using Giemsa staining, Florescent antibody test, CFT, Gimenez staining and PCR. They studied Cattle egret and Hoopoe birds for the prevalence of *C. psittaci.* They found quite high prevalence in birds and concluded that these birds were the carrier of *C. psittaci* and shed this bacterium in their droppings causing risk to the population [15].

These results were different from other studies based on the prevalence of *C. psittaci* due to the area distribution, climate of the subject area, type of birds which were studied and samples which were collected from birds for isolation and identification. Mostly scientists found prevalence in pigeons, parrots and ducks using conjunctival swabs and by using PCR and RT-PCR. My studies were based on isolation by using embryonated eggs, identification by using modified Gimenez staining and serological analysis was made by CFT.

In this study 72 blood samples were processed and CFT was performed for the detection of antibodies against *C. psittaci*. The positive samples were 33.33% for *C. psittaci* antibodies and which were supported by El-Jakee*et al.* (2014) performed CFT and found 58.2% positive in CFT. The prevalence of *C. psittaci* could be as high from 8.75% to 94.6% in any area depending upon the climate, type of birds, the serotype of *C. psittaci* and type of samples collected from birds for study [16].

7. Conclusions

Fancy birds are beautiful and people love to keep lovebirds as their pet and children are also fond of keeping such beautiful birds. The overall research results showed the prevalence of *C. psittaci* is significantly high, the fancy birds may be the carrier for disease transmission to human and other birds. Pakistani government should pay attention towards the risk of disease transmission through this zoonotic pathogen.

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References

1. Kaleta EF, Taday EM. Avian host range of Chlamydophila spp. based on isolation, antigen detection and serology. Avi Path 2003; 32: 435-62.

2. Laroucau K, Vorimore F, Aaziz R, Berndt A, Schubert E, Sachse K. Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. Infect Genet Evol. 2009; 9(6): 1240-1247.

3. Piasecki T, Chrzastek K, Wieliczko A. Detection and identification of Chlamydophila psittaci in asymptomatic parrots in Poland. BMC Vet Res 2012; 8: 233-38.

4. Harkinezhad T, Geens T, Vanrompay D. Chlamydophila psittaci infections in birds: a review with emphasis on zoonotic consequences. Vet Microbiol 2009; 135: 68-77.

5. Madani A, M Peighambari, A Barin. Isolation of Chlamydophila psittaci from pet birds in Iran. Iran J Vet Med 2011; 5(2): 95-98.

6. Feng Y, Feng YM, Zhang ZH, Wu SX, Zhong DB, Liu CJ. Prevalence and genotype of Chlamydia psittaci in faecal samples of birds from zoos and pet markets in Kunming, Yunnan, China. J Zhejiang UnivSci B 2016; 17(4): 311.

7. Yin L, Kalmar ID, Lagae S, Vandendriessche S, Vanderhaeghen W, Butaye P, Vanrompay D. Emerging Chlamydia psittaci infections in the chicken industry and pathology of Chlamydia psittaci genotype B and D strains in specific pathogen free chickens. Vet Microbiol 2013; 162(2): 740-749.

8. Sachse K, Laroucau K, Vanrompay D. La chlamydioseaviaire: nouvellesespèces et consequences. Revue Francophone des Laboratoires. 2015; 472: 59-65.

9. Cobb SP. The spread of pathogens through trade in poultry meat: overview and recent developments. Rev Sci Tech (OIE). 2011; 30: 149-64.

10. Edwin HL and Nathalie JS. Diagnostic procedure for viral, Rickettsial and Chlamydial infection. Am Public Health Assoc 1979; 32: 51-76.

11. Gimenez DF. Staining Rickettsiae in yolk sac culture. Stain technol.1964; 39:135-40.

12. OIE. Avian Chlamydiosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2012.

13. Mostafa ER, Elhariri M, Ali HA, Jakee JKE. Emergence of Chlamydia psittaci in lovebirds: A new potential risk factor of Chlamydiosis. Int J Adv Res BiolSci. 2015.

14. Spoorenberg SM, Bos WJ, van Hannen EJ, Dijkstra F, Heddema ER, van Velzen-Blad H,et al. Chlamydia psittaci: a relevant cause of community-acquired pneumonia in two Dutch hospitals. Neth J Med 2016; 74(2): 75-81.

15. El-Jakee JK, Osman KM, Ezzeldeen NA, Ali HA, Mostafa ER. Chlamydia species in free-living Cattle Egret (Bubulcus ibis) and Hoopoe (Upupaepops) in Egypt. International J Vet Sci Med 2014; 2(1): 1-6.