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Prevalence and Zoonotic Potential of Chlamydia psittaci in Domestic Pigeons from Baghdad: ELISA and Rapid Test Insights

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ABSTRACT

Because of their high prevalence and link to respiratory disorders in humans, pigeons are a common host and natural reservoir of Chlamydia psittaci, the etiological agent of avian chlamydiosis, which is regarded as a neglected zoonotic disease and public health concern. This study sought to ascertain the prevalence of C. psittaci as a possible infection source in domestic pigeon (Columba livia domestica) oropharyngeal samples. This bacterium's major outer membrane protein (MOMP), the most prevalent protein in its membrane, has been assessed for use in a number of diagnostic procedures in addition to being a potential vaccine candidate. A total of 150 swab samples from domestic pigeons were collected in Baghdad Iraq, from October 2023 to April 2024. The specific antigen (MOMP) of *C. psittaci* was detected using both the Rapid Screening Test for Chlamydia (cassette) and the enzyme-linked immunosorbent assay (ELISA). The results showed that out of the 150 samples examined, 60 (40%) tested positive for *C. psittaci* antigen using the rapid diagnostic test, while 40 (27%) were positive with the ELISA test. Our knowledge of the consequences of these infections for both human and avian health will improve if we can better understand how this bacterium affects avian host fitness and its zoonotic potential.

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1- INTRODUCTION

Recent research on the genome of Chlamydia has led to changes in its taxonomy. The genera Chlamydia and Chlamydophila are members of the Chlamydiaceae family. While Chlamydophila psittaci is a member of the recently created genus Chlamydophila, the species Chlamydia trachomatis is a member of the genus Chlamydia [18]. The Chlamydiaceae family, which includes 13 other known species, including Chlamydia pneumoniae, Chlamydia abortus, Chlamydia felis, Chlamydia suis, and Chlamydia trachomatis, is home to the Gram-negative, obligatory intracellular bacterium Chlamydia psittaci [13]. Birds are common hosts and natural reservoirs of C. psittaci, both domestic and wild [3]. Elementary bodies (Ebs), which are biologically inactive particles, are the means by which members of the Chlamydiaceae family are spread. Doves, mynah birds, pigeons (Columbi formes), and parrots (as psittacosis ornithosis) are all susceptible to avian chlamydiosis, which is most frequently caused by C. psittaci. While the infected bird may exhibit nonspecific clinical signs like weight loss, diarrhea, anorexia, polyuria, respiratory signs (dyspnea), ocular or nasal discharge, conjunctivitis, hyperthermia, abnormal excretions, decreased egg production, and sudden death, affected birds may not exhibit any symptoms at all [9]. Direct contact

with wild or captive birds or bird material, such as handling infected birds or inhaling respiratory secretions or fecal particles, can expose humans to psittacosis [22]. There are currently 15 genotypes of C. psittaci that are thought to be potentially harmful to human health and have been found in the avian reservoir [19, 25]. The genotypes of C. psittaci are classified into types A through F, E/B, M56, and WC based on differences in the outer membrane protein A (ompA) sequences [13, 22]. One of the main hosts of C. psittaci infections are pigeons and doves (order Columbiformes) [20]. C. psittaci is detected using a variety of techniques, such as fluorescent antibody tests (FATs) and enzyme-linked immunosorbent assays (ELISA). The ability to test multiple blood samples at once using colorimetric comparison is one benefit of the ELISA technique. However, in order to identify particular proteins or antigens, this method necessitates laboratory facilities. Rapid diagnostic tests, on the other hand, can be used in the field right away without the need for specialised equipment [5]. The preferred medication for treating C psittaci infections in both humans and birds is tetracycline antibiotics. For recommendations on medication dosages and treatment duration, physicians should refer to a current formulary [2]. Although the prevalence of disease and mortality rates are greatly decreased by antibiotic treatment, inappropriate or overuse of antibiotics has resulted in an increase in antibiotic resistance, which poses further difficulties [1]. The purpose of this study was to use the ELISA test to determine the prevalence of C. psittaciin in pigeon aviaries located in Baghdad Province, Iraq.

2- MATERIAL AND METHOD

Sampling. The study was conducted at AL-Nahrain University's Biotechnology Research Center. 150 domestic pigeon samples in all, representing both sexes and varying ages. A random sample was taken from 40 pigeon aviaries located throughout Baghdad. There were at least fifty pigeons in each of these privately owned and operated aviaries. Before beginning any treatment, oropharyngeal swabs were taken from each pigeon exhibiting particular clinical signs of infection. The swabs were transported aseptically in ice packs to the laboratory at AL-Nahrain University's Biotechnology Research Center in Baghdad after being placed in special transport media (VTM) (Liofilchem, Inc. USA).

Rohi *C. psittaci* Rapid Test (FlowChromatographic Immunoassay)

Is a sandwich lateral flow Chromatographic Immunoassay (cassette)(Rohi Biotechnology Co. Ltd. SH. China)for the qualitative detection of *C. psittaci* in infected secretions (nose,mouth,cloaca).

Since the result of the interaction is a visible discoloration, the test's basic idea relies on the interaction between the antigens in the sample to be tested and antibodies coated with the test plate in accordance with the manufacturer's instructions. The liquid will flow laterally across the test strip's surface when the sample is inserted into the device's sample hole. A noticeable T band will show up if the sample contains C. psittaci. To show a valid result, the C band should always show up after a sample is applied. As shown in the figure below, if it is not colored, the test is invalid. (Figure1).

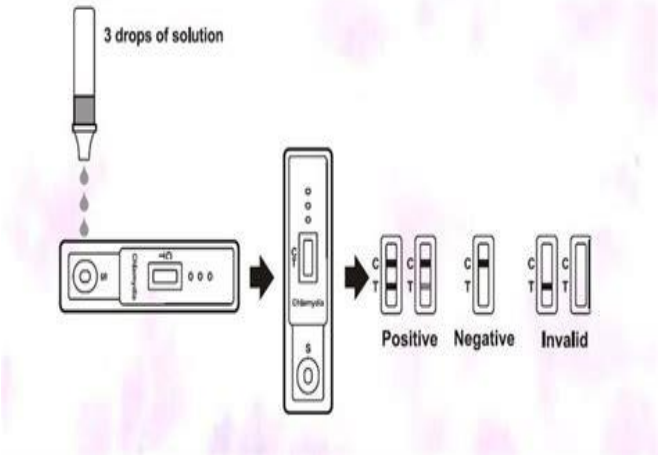

Figure 1: Cassette (Rapid test).

ELISA (enzyme-linked immunosorbent assay) kit

The antigen of *C. psittaci* was detected with ELISA kits (Pigeons *Chlamydia Psittaci* ELISA Kit, for the qualitative determination of Pigeons *C.Psittaci*, Pigeons Sandwich ELISA Detection, AFG Bioscience, USA). This test used for detection of these bacteria, it is more sensitive than other serological tests [17]. To obtain a high concentration of antigen, the samples were centrifuged at 3000 g for 20 minutes after being left at room temperature for 4 hours.

The assay kit As directed by the manufacturer, C.Psittaci was used in the sample. Purified antibody was used to coat the microtiter plate, create a solid-phase antibody, and then add the sample to wells. When combined with C.Psittaci antibody that was HRP labeled, the result was an antibody-antigen-enzyme-antibody complex. Following thorough washing, TMB substrate solution was added, which turned blue. After the HRP enzyme-catalyzed reaction is stopped by adding a 0.1N H2SO4 solution, the color change is measured spectrophotometrically (optical density OD) at a wavelength of 450 nm using an ELISA microplate reader (Expert plus microplate reader, Germany). after the non-combinative antigen and other materials have been cleaned and removed. The OD sample was considered negative when it was less than the cutoff value and positive when it was greater than or equal to the cutoff value [12].

Statistical Analysis:

The effect of different factors in study parameters was detected using the Statistical Analysis System- SAS (2018) program. In this study, a significant comparison between percentages (0.05 and 0.01 probability) was made using the chi-square test [29].

3- RESULTS AND DISCUSSION

The avian pathogen Chlamydia psittaci is frequently found in poultry, pigeons, wild birds, and pet birds. However, there are documented risks of transmission from birds, particularly pigeons, which can result in zoonotic disease in humans. Surveying and gathering baseline information on the prevalence of C. psittaci in Iraqi pigeons was the aim of this study. As far as we are aware, this study is the first to document the epidemiology of C. psittaci in Iraqi pigeons.

In this study , results of the Rohi Rapid Test showed that, out of 150 samples, 60(40%)were positive for C. psittaci, while 90 samples (60%) tested negative (Table 1). The Rapid Test/Cassette used in this study is a lateral flow chromatographic immunoassay designed for the simultaneous detection and rapid screening in pigeon's houses and laboratories aiding in the diagnosis of C. Psittaci infection.

(Table 1): The percentage of *C. Psittaci* infection according to Rapid Test/Cassette

Diagnostic test	+ve samples		-ve samples		Total	
C. psittaci Rapid Test	No.	%	No.	%	No.	%
	60	40%	90	60%	150	100%
Chi-square test $-\chi^2$ (P-value)	6.00 ** (0.010)				--	--
** (P≤0.01).						

The results from the ELIZA test, indicated that, among the 150 pigeon samples, **40 (27%)** were positive for *C. Psittaci* antigen, while **110 (73%)** were negative, , indicating an overall infection rate for chlamydiosis, as shown in (Table 2).

(Table 2): The percentage of *C. Psittaci* infection and controls according to ELISA test

Blank	+ve Control	-ve Control	+ve samples ≤ 0.38		-ve samples ≥ 0.033		Total	
			No.	%	No.	%	No.	%
0.000	0.38	0.033	40	27%	110	73%	150	100%
Chi-square test $-\chi^2$: 32.667 ** (P-value): 0.0001 .								
** (P≤0.01).								

The positive samples identified by the ELISA test (27%) were lower than those identified by the rapid test (40%) as shown in figure 2. This is attributed to the higher sensitivity and specificity of the ELISA test compared to the rapid test [28].

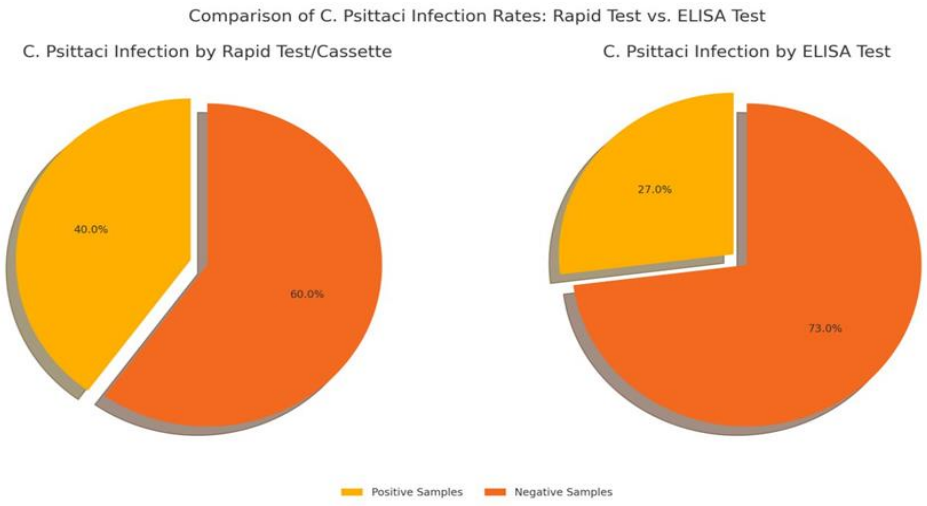

Figure 2: Comparison of C.psittaci infection rate: Rapid Test vs.ELISA Test

The seroprevalence of chlamydiosis varied across different age groups, ranging from **5%** to **32.5%** and was higher in the (1-2) months category (Table 3) and (Figure 3).

(Table3): The percentage of infected pigeon with *C. Psittaci* according to the age

NO	Age\month	No. of infected pigeon	Percentage %
1	1-2 M.	13	32.50 %
2	2-3 M.	10	25.00 %
3	3-4 M.	6	15.00%
4	4-5 M.	5	12.50 %
5	5-6 M.	4	10.00%
6	6-8 M.	2	5.00 %
Total		40	100 %
Chi-square test $-\chi^2$ (P-value)		---	76.802 ** (0.0063)
** (P≤0.01).			

(P≤0.01)= Highly Significant

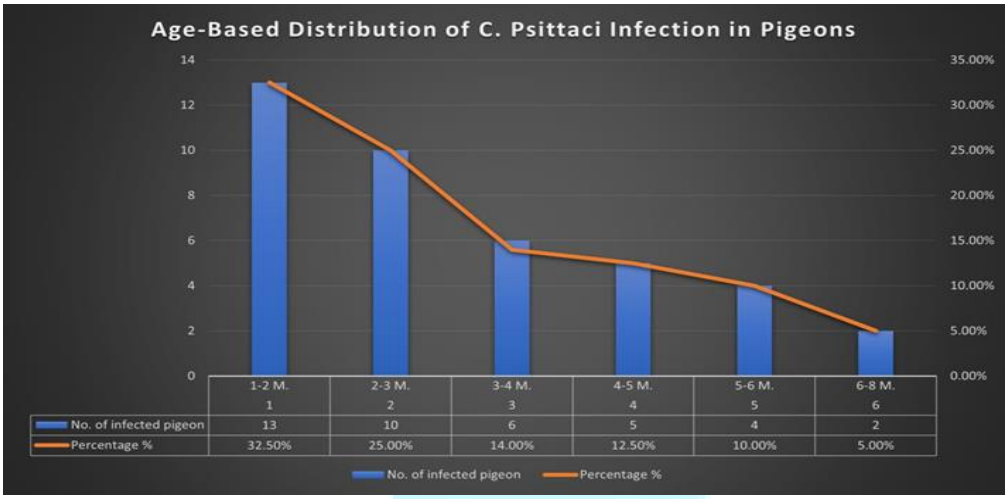

Figure 3: Distribution of chlamydial infection between the pigeon according to age

Using the C. psittaci antigen-specific ELIZA kit, a highly sensitive and specific method for detecting chlamydial antigen (Ag), 40 out of 150 oropharyngeal samples (27%) from potentially infected pigeons were found to contain C. psittaci.

Between 1966 and 2005, 38 studies examined the seroprevalence of C. psittaci in pigeons. Seropositivity rates in these studies ranged from 12.5% to 95.6% [10, 14, 16, 23]. Nevertheless, serological assays that identify antibodies against chlamydial lipopolysaccharide (LPS) antigens or chlamydial whole organisms were employed in each of these investigations.

Previous studies have reported the prevalence of *C. psittaci* in pet bird s and pigeons. Some studies found a low chlamydial infection rate, such as in the Netherlands, where the rate was (7.9%) [23, 11]. A recent study conducted in 2023on pigeon infection with *C. Psittaci*in in Sweden reported a prevalence of 14% [21], while an Iranian study found a prevalence of 14.3% in pigeons [8].

Other studies of *C. psittaci* infection in pet bird have shown a high incidence rate, such as in China reported a prevalence of 35.37% (110 out of 311) [26], and in Brazil, the prevalence of *C. psittaci* was high among pet bird (37.8%) [6]. Accordance with two previous studies, high infection rates were observed in Switzerland, where Zurich by Zweifel [27] demonstrated that 10 out of 24 (41.7 %) pigeons were positive for *C. psittaci*. and in Belgium, where 13 out of the 32 (40.6 %) pigeon in breeding facilities were found to be infected [7].

Compared with these studies, the infection rate of 27% observed in pigeon in our study was higher than some countries but close to or lowers than in others. Overall, it is challenging to compare findings across different studies due to variations in environmental conditions, diagnostic methods, feeding practices, bird husbandry practices, and bird welfare. In addition, prevalence rates can vary from one location to another due to factors such as climate, geographical area, sample size animal [15].

Our results are in line with two studies: one by Teske [24] in Germany, which reported a 29.1% infection rate of C. psittaci in young racing pigeons, and another by Cong [4], which reported a 31.09% infection rate among pigeons from bird markets in northwest China.

The climate and geographic features of Baghdad, such as the temperature, humidity, and dusty winds, may facilitate the infection and spread of C. psittaci among pigeons.

Group feeding, mixed feeding, stacked cages, and a lack of ventilation systems in pigeon houses and pet markets may all be contributing factors to the high incidence of C. psittaci infection in pigeons. Additionally, pet store owners or pigeon breeders might not promptly clean up food spills or feces.

The risk of human infection in this area may be elevated by pigeons, which may be considered potential reservoirs of zoonotic psittacosis. According to our findings, anyone who interacts with pigeons should be informed that they may be at risk of catching psittacosis from them.

4- CONCLUSION

According to the current study's findings, domestic pigeons in Baghdad, Iraq, have a high prevalence of C. psittaci infection, which could be harmful to local human health. Effective steps must therefore be taken to stop the spread of C. psittaci among domestic pigeons, as well as among staff members and clients.

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انتشار المتدثرة الببغائية واحتمال انتقالها من الحمام المنزلي الى الإنسان في بغداد: رؤى حول اختبار الأليزا والاختبار السريع

الخلاصة

يعد الحمام المنزلي مضيف نموذجي وخازن طبيعي للمتدثرة الببغائية وهو العامل المسبب لداء الببغاوات في الطيور ، والذي يعتبر من الأمراض الحيوانية المنشأ المهملة ومشكلة صحية عامة بسبب ارتفاع معدل انتشاره ولصلته باضطرابات الجهاز التنفسي لدى الإنسان.

تهدف هذه الدراسة الى تحديد مدى انتشار هذه البكتريا في عينات الفم والبلعوم للحمام المنزلي كمصدر محتمل للعدوى. بروتين الغشاء الخارجي الرئيسي لهذه البكتيريا هو البروتين الأكثر وفرة في غشائها وقد تم تقييمه ليس فقط كمرشح لتطوير اللقاح ولكن أيضا يستخدم في العديد من الاختبارات التشخيصية.

تم جمع ما مجموعه ١٥٠ عينة من مسحات الحمام المنزلي في مدينة بغداد (العراق) ، من أكتوبر ٢٠٢٣ إلى أبريل ٢٠٢٤. تم الكشف عن المستضد المحدد بواسطة الفحص السريع للمتدثرة (الكاسيت) وايضا بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم (الاليزا).

أظهرت النتائج ان الانتشار المصلي الكلي لهذه البكتريا من بين ١٥٠ عينة تم فحصها بواسطة الفحص السريع كان ٦٠ (٤٠%) ايجابية لمستضد المتدثرة الببغائية وكانت النتيجة ٤٠ (٢٧%) ايجابية مع اختبار الاليزا لنفس المستضد البكتيري. أن توضيح آثار هذه البكتريا على لياقة الطائر المضيف والامكانات الحيوانية المنشأ للمتدثرة الناشئة سيساعدنا على فهم آثار هذه العدوى على صحة الطيور والانسان.