

Serological Detection of Antibodies Against *Chlamydophila psittaci* Infection in Pet Parrots of Guatemala City

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ABSTRACT

Avian chlamydiosis (AC), caused by *Chlamydophila psittaci* (*C. psittaci*), is a relevant zoonotic disease transmitted to humans through psittacine or pet birds. Guatemala is a megadiverse country where parrots are commonly kept as pets. Considering such a situation and the fact that respiratory diseases are some of the main causes of morbidity in the human population, the epidemiology of AC in pet parrots has not been sufficiently investigated. The purpose of the present study was to investigate the presence and frequency of antibodies against *C. psittaci* in pet parrots in Guatemala City, Guatemala. Blood samples were collected from 100 parrots belonging to 17 species (*Amazona auropalliata*, *A. farinosa*, *A. autumnalis*, *A. albifrons*, *Agapornis roseicollis*, *Ara macao*, *A. militaris*, *Aratinga astec*, *Brotogeris jugularis*, *Cacatua alba*, *Eupsittula canicularis*, *E. nana*, *Melopsittacus undulatus*, *Ninificus hollandicus*, *Pionus senilis*, and *Psittacara strenuus*) representing 19 of the 20 zones of Guatemala. Immunoglobulins (Ig) G antibodies against *C. psittaci* were detected using Enzyme-linked Immunosorbent Assay tests. The prevalence rate of *C. psittaci* was reported at 11% (95% CI = 4.87%, 17.13%) indicating the presence of AC pet parrots in Guatemala City. Therefore, Guatemalan sanitary authorities should take some measures and the physicians must consider *C. psittaci* as a possible cause of a severe respiratory disease condition in people residing in this city.

Keywords: Avian chlamydiosis, Epidemiology, Psittacosis, Public health, Zoonosis

INTRODUCTION

Avian chlamydiosis (AC) that also known as psittacosis is a relevant zoonotic disease that affects both the health and production of animals as well as human health (Borel et al., 2018; Cheong et al., 2019; Hogerwerf et al., 2020). The causative agent, *C. psittaci*, is a global bacteria that primarily affects birds (Chahota et al., 2006; Dickx et al., 2013) and could be transmitted to mammalian hosts, including humans (Lagae et al., 2014; Sachse et al., 2015; Polkinghorne et al., 2020). Although *C. psittaci* has been found in at least 465 species of birds comprising 30 orders (Vanrompay et al., 1993; Andersen and Vanrompay, 2000), the main avian hosts belong to the orders *psittaciformes* and *columbiformes*. Clinical signs of *C. psittaci* in avian species include reproductive and enteric

disorders as well as respiratory distress (Zaręba-Marchewka et al., 2020). Non-specific signs associated with this infection commonly lead to misdiagnosis (Sylvie et al., 2009; Balsamo, et al., 2017; Weygaerde, et al., 2018). Transmission to mammals, including humans, occurs through the inhalation of sputum and secretions that *C. Psittaci*-infected animals discharge when sneezing. This agent can also be found in the feces of the birds so transmission also occurs by the inhalation exposure to pulverized feces from infected birds (Tanaka et al., 2005; Radomski et al., 2016; Kozuki et al., 2020).

Captive companion birds can be considered as reservoirs and asymptomatic shedders of *C. psittaci* (Hulin et al., 2016). In some Eastern European countries, *C. psittaci* has been detected in the serum of peoples who were in close contact with pet birds (Vanrompay et al.,

2007; Harkinezhad et al., 2009). People at risk are bird owners, aviary and pet shop employees, poultry workers, and veterinarians (Smith et al., 2011). Community-acquired chlamydiosis has also been described in Australia (Branley et al., 2014). In Guatemala, the seroprevalences of *C. psittaci* have been reported as 30-35% in captive psittacine birds (Chacón, 2001; Ordóñez, 2015).

Guatemala is a megadiverse country (Bacon et al., 2019), where native and wild parrot species are commonly kept as pets (Lepe-López and Guerra-Centeno, 2018). Respiratory diseases in Guatemala account for approximately three million cases per year in the human population, which highlights the importance of an appropriate diagnosis to cure the inflicted individuals. Moreover, it is important to investigate whether the Guatemalan population (animals and people) is close to risk factors related to the infection and transmission of *C. psittaci* (MPHSA, 2021).

With this in mind, the present study aimed to explore the presence of antibodies against *C. psittaci* infection in pet parrots in Guatemala City.

MATERIALS AND METHODS

The study area

This descriptive cross-sectional serosurvey was conducted from July to September 2019 in Guatemala City, Guatemala. Native and exotic species of parrots (order *Psittaciformes*) kept as pets in 20 zones of the city were considered for sampling. Using social networking sites and placing ads in veterinary clinics, pet parrot owners were invited to take their pet parrot to the Wildlife Unit of the Veterinary Medicine and Animal Husbandry Faculty of San Carlos of Guatemala University in Guatemala City. Only one individual parrot per owner/household was included in the study

Sampling

A consecutive sampling technique was performed until the collection of 100 individual samples (Beerendrakumar et al., 2018). Signs of respiratory disease were not considered as a sampling exclusion criterion. The collected 100 parrot's specimen samples corresponding to

17 species (*Amazona auropalliata*, *A. farinosa*, *A. autumnalis*, *A. albifrons*, *Agapornis roseicollis*, *Ara macao*, *A. militaris*, *Aratinga astec*, *Brotogeris jugularis*, *Cacatua alba*, *Eupsittula canicularis*, *E. nana*, *Melopsittacus undulatus*, *Ninicus hollandicus*, *Pionus senilis*, and *Psittacara strenuus*) were collected from 19 out of 20 zones located in Guatemala City, Guatemala (Table 1). The location of the zones is shown in Figure 1. Blood samples were collected by clipping a claw and allowing drops of blood to saturate both sides of the pre-punched filter paper disks provided in the ImmunoComb® ELISA kit (Biogal - Galed Labs, Israel). After the blood collection process, the bleeding of parrots was controlled by benzocaine powder (Kwik Stop® powder, Bimborn, LLC, United States). The samples were air-dried, identified, and transported to the Regional Reference Laboratory for Animal Health (LARRSA) at the University of San Carlos of Guatemala in Guatemala City for further processing.

Laboratory procedure

A commercial kit (ImmunoComb®) of rapid Enzyme-linked Immunosorbent Assay (ELISA) test was used to detect Immunoglobulins (Ig) G against *C. psittaci* according to the manufacturer's indications (Biogal-Galed Labs, Israel). The results were interpreted in accordance with a qualitative scale from 0 to 6. Results scored > 3 were considered as high positive while results scored ≥ 2, 1-2, and < 1 were considered as positive, suspicious, and negative, respectively.

Statistical analyses

A 95% confidence interval was calculated for the prevalence. The analysis was performed using the WinEpi calculator. By considering the 95% confidence level, unknown population size, a sample size of 100, and 11 positive samples.

Ethics committee approval

The research was approved by the Bioethics Committee of the Graduate School of the Veterinary Faculty, University of San Carlos of Guatemala. +502 24188304 MA. Ligia Rios chair of Bio-Ethics committee.

Table 1. Origin zone and number of psittacides species sampled in Guatemala City

The studied zone	Number of samples	Number of species	Parrots' species
1	3	3	1 <i>Amazona albifrons</i> , 1 <i>A. autumnalis</i> , 1 <i>Melopsittacus undulatus</i>
2	4	4	1 <i>Amazona autumnalis</i> , 1 <i>Melopsittacus undulatus</i> , 1 <i>Pionus senilis</i> , 1 <i>Psittacara strenuus</i>
3	10	7	1 <i>A. albifrons</i> , 3 <i>A. autumnalis</i> , 2 <i>A. farinosa</i> , 1 <i>Ara militaris</i> , 1 <i>Eupsittula canicularis</i> , 1 <i>Melopsittacus undulatus</i> , 1 <i>Psittacara strenuus</i>
4	2	1	2 <i>Amazona farinosa</i>
5	4	2	3 <i>Amazona albifrons</i> , 1 <i>Brotogeris jugularis</i>
6	4	3	2 <i>Amazona albifrons</i> , 1 <i>A. oratrix</i> , 1 <i>Agapornis roseicollis</i>
7	8	7	1 <i>Amazona albifrons</i> , 1 <i>A. auropalliata</i> , 2 <i>A. autumnalis</i> , 2 <i>Melopsittacus undulatus</i> , 1 <i>Pionus senilis</i> , 1 <i>Psittacara strenuus</i>
8	8	5	1 <i>Amazona albifrons</i> , 2 <i>A. autumnalis</i> , 3 <i>Agapornis roseicollis</i> , 1 <i>Eupsittula canicularis</i> , 1 <i>Pionus senilis</i>
9	2	2	1 <i>Amazona autumnalis</i> , 1 <i>Melopsittacus undulatus</i>
10	10	7	2 <i>Amazona albifrons</i> , 1 <i>A. auropalliata</i> , 2 <i>Agapornis roseicollis</i> , 1 <i>Cacatua alba</i> , 2 <i>Eupsittula canicularis</i> , 1 <i>Melopsittacus undulatus</i> , 1 <i>Psittacara strenuus</i>
11	6	5	1 <i>Amazona albifrons</i> , 2 <i>A. auropalliata</i> , 1 <i>A. autumnalis</i> , 1 <i>Eupsittula nana</i> , 1 <i>Melopsittacus undulatus</i>
12	11	8	1 <i>Amazona albifrons</i> , 3 <i>A. auropalliata</i> , 1 <i>A. autumnalis</i> , 1 <i>A. farinosa</i> , 1 <i>Agapornis roseicollis</i> , 1 <i>Melopsittacus undulatus</i> , 2 <i>Pionus senilis</i> , 1 <i>Psittacara strenuus</i>
13	4	3	2 <i>A. autumnalis</i> , 1 <i>A. albifrons</i> , 1 <i>Agapornis roseicollis</i>
14	4	2	3 <i>Amazona autumnalis</i> , 1 <i>Melopsittacus undulatus</i>
15	1	1	1 <i>Agapornis roseicollis</i>
16	11	9	1 <i>Amazona albifrons</i> , 1 <i>A. autumnalis</i> , 1 <i>A. auropalliata</i> , 3 <i>A. Farinosa</i> , 1 <i>Ara macao</i> , 1 <i>A. militaris</i> , 1 <i>Aratinga astec</i> , 1 <i>Eupsittula canicularis</i> , 1 <i>Nymphicus hollandicus</i> ,
17	3	3	1 <i>Amazona autumnalis</i> , 1 <i>Nymphicus hollandicus</i> , 1 <i>Psittacara strenuus</i>
18	2	2	1 <i>Amazona autumnalis</i> , 1 <i>Eupsittula canicularis</i>
21	3	3	1 <i>Amazona albifrons</i> , 1 <i>A. autumnalis</i> , 1 <i>A. farinosa</i>
100			

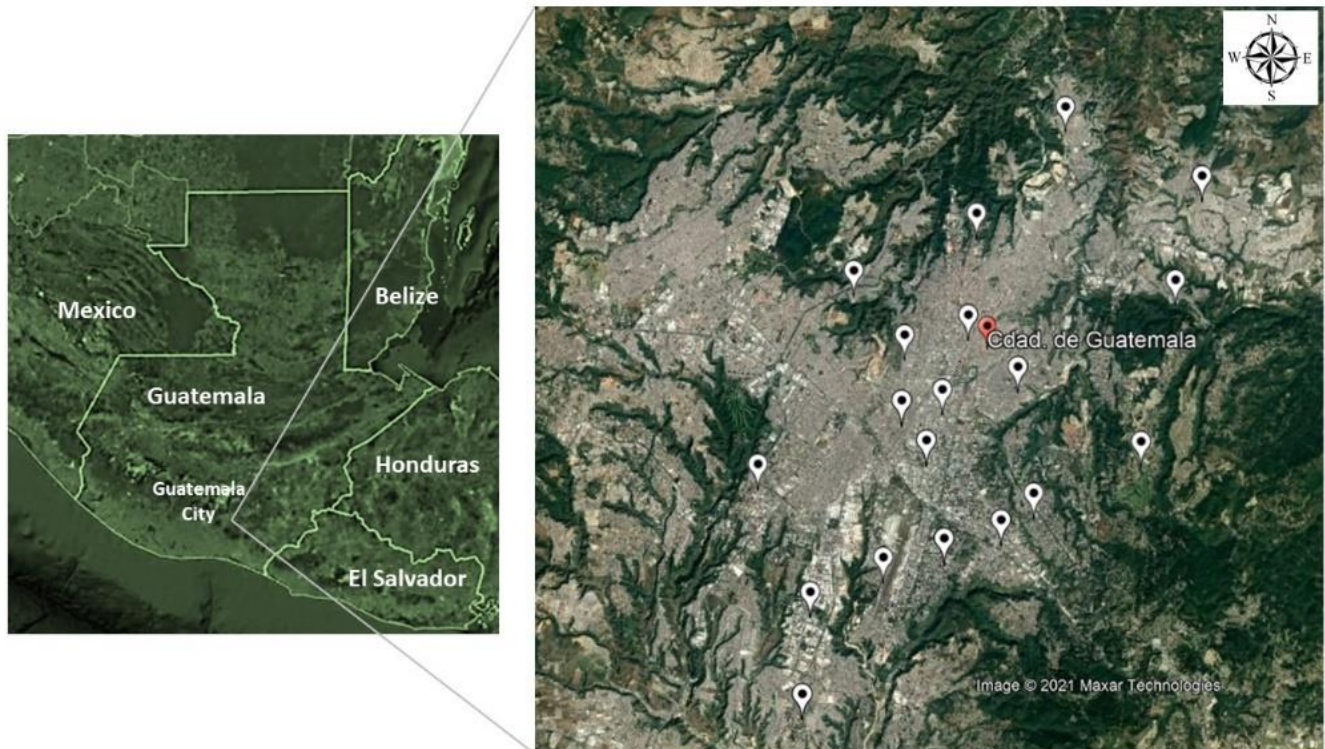


Figure 1. Location of parrots' zones in Guatemala City. Image from Google Maps, taken on May 2021

RESULTS AND DISCUSSION

Antibodies against *C. psittaci* infection were found in the current study with a prevalence rate of 11% (95% CI = 4.87%, 17.13%). Seven samples showed inconclusive (suspicious as 1-2) reactions, indicating a low reaction to *C. psittaci*, samples showed an apparent antibody response against *C. psittaci* but the antibody titers were not enough to consider the sample as positive. Positive samples were detected in the studied zones (3, 6, 10, 12, 16, 17, 18, and 21). On the other hand, suspected positive samples were from zones 2, 5, 7, 10, 13, 14, and 17. Out of 17 sampled species, 6 were native species to Guatemala and some of them were positive reactors (Table 2). Among these positive samples, 11 samples were scored as > 3 showing high antibody titers (Table 2). If the seven inconclusive samples were considered real positive, the frequency of reactors would be raised higher than 11%.

The studied population of parrots was not vaccinated against *C. psittaci*, so the detected antibodies indicated the previous contact with the field pathogen or active infection. It cannot be ruled out that the high titers of IgG indicated active cases of AC or the recovery phase of the disease, recovered birds could still harbor high titers of antibodies (Balsamo et al., 2017). It is important to mention that *A. albifrons* and *A. autumnalis* were the most affected parrot species, as 6/18 and 4/18 were serologically positive to *C. psittaci*, respectively (Table 2). Even the suspicious samples, except for *Melopsittacus undulatus*, came from the same positive species, in Guatemala. These species are the most commonly used pets suffering from stressful situations, such as poor feeding, overcrowding, and inadequate cages. Therefore, exposure to the agent and the mentioned conditions are risk factors that make successful transmission and infection with *C. psittaci*. These species may be acting as reservoirs of the pathogen in the studied area (Hulin et al., 2016; Lepe-López and Guerra-Centeno, 2018; Abd El-Ghany 2020).

The diversity of origin zones of the positive and suspicious samples in Guatemala City suggests that the pathogen could be endemic in this area and affect more susceptible species, such as *A. albifrons* and *A. autumnalis*. A high prevalence of antibodies against *C. psittaci* was found in captive parrots of the genus *Amazon* in Brazil

(Raso et al., 2002; Vilela et al., 2019). Evidence of circulation of *C. psittaci* was also found in captive *Amazon* parrots in Costa Rica (Sheleby-Elías et al., 2013).

Peridomestic wild birds (such as *Columbia livia*, *Passer domesticus*, *Quiscalus mexicanus*, *Turdus grayi*, *Zenaida Asiatica*, and *Zonotrichia capensis*), which are very common in Guatemala City, could be responsible for the transmission of *C. psittaci* in the urban landscape (Geigenfeind et al., 2012; Mahzounieh et al., 2020). This bacterium has been found also in passerine garden birds in England (Beckmann et al., 2014). It is known that *C. psittaci* can remain infectious for more than a month in organic debris (Longbottom and Coulter, 2003; Harkinezhad et al., 2009). Accordingly, there is a great possibility for the direct or indirect transmission of *C. psittaci* among wild birds, especially pigeons (Prukner-Radovicic et al., 2005) and domestic pet parrots.

Antibodies against *C. psittaci* have also been found in serum samples of captive parrots in Wildlife Rescue Centers located in the Wild-domestic Interface of Northern Guatemala and Central Mexico (Chacón, 2001; Ordóñez, 2015; Ornelas-Eusebio et al., 2016).

In the current study, the seroprevalence rate of *C. psittaci* was higher than that observed in wild populations of common parrot species in Australia (9.8%, Stokes et al., 2020) but lower than those in three Amazon parrot breeder collections in Brazil (100%, 87.5%, and 60%, Raso et al., 2002), 44% was found in captive macaws in Peru (Carlos and Luyo, 2018) or the 19% observed in pet and zoo parrots in China (Feng et al., 2016). Overcrowding and stressful conditions, which are quite common in captivity, are known to favor the occurrence of infectious diseases (Edis, 2017; Kim et al., 2021). A recent meta-analysis found a global prevalence (19.5%) of Chlamydia infections in birds without significant differences in prevalences among continents or bird's orders (Sukon et al., 2021). The obtained results of a study conducted by Lepe-López and Guerra-Centeno (2018) determined that the most frequent pet parrot species taken to the Veterinary clinics in Guatemala City were *Amazona albifrons*, *A. autopalliata*, *A. autumnalis*, *A. farinosa*, *Melopsittacus undulatus*, and *Psittacara strenuus*. These species were among the collected samples in the present investigation and *A. albifrons* and *A. autumnalis* were the most frequent species that showed antibodies against *C.*

psittaci. This finding is important from the epidemiological perspective because it means that studies and public health efforts should mainly focus on these common pet parrot species. The current study had some limitations. First, the comparisons of the results with other

prevalence studies are difficult because the used methods for the detection of *C. psittaci* antibodies are not always the same. Second, there is not enough published evidence to determine the exact levels regardless of the sensitivity and specificity of the used ELISA kits.

Table 2. Frequency, quantitative, and qualitative classification of samples

Parrot species	Classification of samples						Frequency of positive outcomes
	<i>n</i>	High positive (Score > 3)	Positive (Score ≥ 2)	Suspicious (Score 1-2)	Negative (Score < 1)	Total positive	
<i>Amazona albifrons</i>	16	2	2	2	10	4	4/16
<i>Amazona auropalliata</i>	9	1	0	1	7	1	1/9
<i>Amazona autumnalis</i>	21	0	3	1	17	3	3/21
<i>Amazona farinosa</i>	9	0	1	0	8	1	1/9
<i>Amazona oratrix</i>	1	0	0	0	1	0	0/1
<i>Agapornis roseicollis</i>	9	0	0	0	9	0	0/9
<i>Ara macao</i>	1	0	0	0	1	0	0/1
<i>Ara militaris</i>	2	0	0	0	2	0	0/2
<i>Aratinga astec</i>	1	0	0	0	1	0	0/1
<i>Brotogeris jugularis</i>	1	0	0	0	1	0	0/1
<i>Cacatua alba</i>	1	0	0	0	1	0	0/1
<i>Eupsittula canicularis</i>	6	0	1	1	4	1	1/6
<i>Eupsittula nana</i>	1	0	0	0	1	0	0/1
<i>Melopsittacus undulatus</i>	10	0	0	1	9	0	0/10
<i>Nymphicus hollandicus</i>	2	0	0	0	2	0	0/2
<i>Pionus senilis</i>	5	0	0	0	5	0	0/5
<i>Psittacara strenuus</i>	5	1	0	1	3	1	1/5
Total	100	4	7	7	82	11	11/100

CONCLUSION

The findings of the current study indicate that avian chlamydiosis (AC) is present in pet parrots in Guatemala City. The presence of antibodies in this population of birds evidences the circulation of the agent due to the absence of vaccination in Guatemala against *C. psittaci* in these species. Regarding the close interaction between the owners and their pet birds, Guatemalan sanitary authorities need to consider the necessary health care programs. Moreover, physicians and veterinarians are required to take action to reduce risk factors. Physicians must consider *C. psittaci* as a possible cause of respiratory disease in human patients and the veterinarians should scrutinize the related risk factors in their differential diagnoses to this disease as well as the diagnostic tests.

Further studies are necessary to better understand the epidemiology of AC in Guatemala City and the rest of Guatemala country. These studies could include molecular and serological investigations of *C. psittaci* infections in other avian hosts species. Moreover, the presence of other

species of *Chlamydia*, such as *C. avium* in parrots, could be considered during epidemiological investigations either in pet birds or humans.

The role of the peridomestic birds on the epidemiology of AC should also be investigated. Nevertheless, the presence of *C. psittaci* in free-ranging populations and their possible role as the reservoirs of this pathogen in Guatemala should be further studied.

DECLARATIONS

Competing interests

The authors have declared that no competing interests exist.

Consent to publish

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Authors' contribution

Estefany de León-Robles conception of the idea, administration of the project, data collection and processing and drafting of the manuscript Dennis Guerra-Centeno conception of the idea, drafting and editing the manuscript.

Ethical considerations

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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