

SEROPREVALENCE OF *Chlamydia psittaci* IN CAPTIVE MACAWS (*Ara* spp.) IN THE DEPARTMENT OF LIMA, PERU

SOROPREVALÊNCIA DA Chlamydia psittaci DE ARARAS CATIVAS (Ara spp.) NO ESTADO DE LIMA, PERU

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Abstract

The presence of *Chlamydia psittaci* in captive psittacines may have a subclinical presentation, despite its importance in immunosuppressed birds and humans because of its zoonotic potential. The aim of this study was to determine the seroprevalence of *C. psittaci* in macaws (*Ara* spp.). Birds were kept in captivity in three zoos in the department of Lima. In total, 34 adult birds (11 *Ara macao*, 15 *Ara ararauna*, 5 *Ara chloropterus*, and 7 *Ara severus*) were studied. A total of 0.5 mL of blood was obtained from the right jugular vein and placed into a coagulant-free tube. Blood serum was analyzed using an ELISA (Immunocomb Avian *Chlamidophila psittaci* kit) in order to determine IgG antibody titers. The presence of antibodies to *C. psittaci* in macaws are reported for the first time in Peru, with a seroprevalence of $44.74 \pm 15.84\%$ (17/38). No significant differences between the origin of birds and the presence of the antibodies were found.

Keywords: captive; macaw; psittacines; psittacosis.

Resumo

A infecção por *Chlamydia psittaci* pode ter uma apresentação subclínica e tem importância em aves imunossuprimidas e seres humanos por seu potencial zoonótico. O objetivo desta pesquisa foi determinar a soroprevalência de *C. psittaci* em araras (*Ara* spp.) mantidas em cativeiro em três zoológicos no departamento de Lima. Aves adultas (n=34) (11 *Ara macao*, 15 *Ara ararauna*, 05 *Ara chloropterus* e 07 *Ara severus*) foram estudadas. Sangue venoso foi obtido da veia jugular direita e colocado em tubo sem coagulante para a obtenção de soro. O soro sanguíneo foi analisado por ELISA (Kit psittaci Immunocomb *Chlamydochila psittaci*) para determinar os títulos de anticorpos IgG. A presença do anticorpos para *C. psittaci* em araras é relatadas pela primeira vez no Peru, com uma soroprevalência de $44.74 \pm 15.84\%$ (17/38). Não houve diferenças significativas entre a origem das aves e a soroprevalência.

Palavras-chave: arara; cativo; psitacídeos; psitacose.

Received on: December, 19, 2016.

Accepted on: July, 06, 2018.

Introduction

Macaws (*Ara* spp.) are charismatic parrots that belong to the order *Psittaciformes*. It is a bird group severely threatened by many anthropogenic factors⁽¹⁾. They are distributed in the tropical forests of Central and South America⁽²⁾, playing an important role in the ecosystem, and they are also considered as flagship or umbrella species. Macaws are popular in illegal wildlife trade and, after seized, they are usually hosted in zoos⁽³⁾. Health studies of these birds in Peru are still lacking; however, it is important to demonstrate the presence of agents, such as bacteria, that can severely affect this group of birds.

One of these pathogens is the *Chlamydia psittaci*, a bacterium causing Psittacosis or Chlamydiosis in wild birds. In some cases, this disease can present sub-clinical signs and after a period of stress or immune system deficiency clinical signs can also be observed^(3, 4). Therefore, it is suitable for additional tests in psittacine collections for diagnosis and treatment⁽³⁾.

In addition, these bacteria have a zoonotic potential and may produce a clinical and critical situation in humans if not detected in time. The transmission is carried out mainly via the airborne route through the dust of feathers, expectoration, and droppings of infected birds. The possibility of infection of humans is mainly due to the possession of psittacine (parrots, macaws, parrotlets, etc.) as pets or contact with these animals by occupational caregivers and veterinarians working in zoos⁽⁵⁾.

The prevalence of *C. psittaci* is variable. For captive psittacines, it was reported to be 10% (12/120) in Argentina⁽⁶⁾ and 71% (17/24) in Venezuela⁽⁷⁾. In Costa Rica, it was found in 12.39% scarlet macaws (*Ara macao*) in captivity⁽⁸⁾. In Peru, no antibodies against *C. psittaci* were found in dusky-headed parakeets (*Aratinga weddellii*) or tui parakeets (*Brotogeris sancitithomae*) studied in Manu National Park⁽⁹⁾. However, 62% (56/90) wild Humboldt penguins (*Spheniscus humboldti*) were tested positively in the department of Ica, Peru⁽¹⁰⁾.

Due to the importance of these bacteria for the health of psittacines and their zoonotic potential, the aim of this study was to determine the seroprevalence of *Chlamydia psittaci* in macaws (*Ara* spp.) kept captive in the department of Lima. Disease detection in time can allow established programs to begin timely treatment and improve biosecurity programs in order to prevent transmission to caregivers and visitors.

Materials and methods

The study was conducted in three zoos in Lima department: The Farm Villa and Magical World Zoo (GVMM), The North Farm Villa (GVN) Zoo, located in the province of Lima, and in the Warmy Zoo (WZ) located in the province of Huaral. The entire population of macaws housed in the zoos was analyzed, in total 38 individuals consisting 15 blue-and-yellow macaws (*Ara ararauna*), 11 scarlet macaws (*Ara macao*), five red-and-green macaws (*Ara chloropterus*), and seven chestnut fronted macaws (*Ara severus*). All birds were adults of unknown sex, kept in captivity for at least two years. Both zoos in the province of Lima had a macaws aviary exhibit, while in the zoo of Huaral they were kept in cages. The study was conducted under the authorization issued by the Forest and Wildlife Service in Peru (Resolution N. 075-2016- SERFOR / DGGSPFFS).

In February 2016, the birds were captured using nets. Using leather gloves, we secured the birds by placing the thumb and forefinger against the jaw and with the other hand, we wrapped the bird's wings to control their movement⁽¹¹⁾. After a brief physical examination, 0.5 mL of blood was taken from the right jugular vein of each bird using a 1-mL syringe and a 25G x 5/8" needle. The samples were placed in Vacutainer® tubes without coagulant and preserved at 4 °C for transportation. After ensuring the good condition of the animal, the birds were released into the aviary or cage.

The samples were taken to the laboratory of Ecology Diseases and Conservation Medicine Program of the Center for Ornithology and Biodiversity (CORBIDI). The serum was obtained by centrifuging the clotted blood at 2500 rpm for 10 min and analyzed by using Immunocomb *Chlamydophila psittacii* test kit (Biogal Galed Laboratories, Israel®) to determinate IgG antibodies. This test has a 95% sensitivity and 85% specificity⁽¹²⁾. The procedure and interpretation of the results were performed following the manufacturer's instructions. According to a qualitative antibody titer, the kit determines a scale of positivity in positive and high positive.

The prevalence and confidence interval of *C. psittacii* found was reported and the possible differences between species and their place of origin (CI: 95%; $\alpha = 0.005$) were assessed using Chi² test. The statistical program SPSS v21 (SPSS Inc., Chicago, Illinois, USA) was used.

Results

The seroprevalence of *Chlamydia psittacii* was 44.74% (17/38) in macaws (*Ara spp.*). The prevalence was not significantly different among the species ($P = 0.436$) or among zoos ($P = 0.785$, Table 1).

Table 1. Seroprevalence of *Chlamydia psittacii* in captive macaws (*Ara spp.*) in Lima department, Peru

Epidemiologic aspect	N	Positive	Percent	Confidence Intervals	P
Species					
<i>A. ararauna</i>	15	5	33.3	21.84	0.436
<i>A. chloropterus</i>	5	3	60	42.95	
<i>A. macao</i>	11	4	36.36	28.43	
<i>A. severus</i>	7	5	71.43	33.48	
Zoo of origin					
WZ	20	10	50	21,91	0.785
GVMM	8	3	37.5	33.5	
GVN	10	4	40	30.36	
Total	38	17	44.74	15.82	

No significant differences were found ($P > 0.05$).

GVMM: The Farm Villa and Magical World Zoo

GVN: The North Farm Villa Zoo

WZ: Warmy Zoo

Table 2 shows the presence of antibody titers among the scale of positivity (Positive and High positive) against *C. psittaci* by species and by zoos. In general, positive and high positive results were 47.06% and 52.54%, respectively. No significant differences were found according to the place of origin ($P = 0.102$)

Table 2. Presence of antibody titers among the scale of positivity (Positive and High positive) against *C. psittaci* in captive macaws (*Ara* spp.) in Lima department, Peru

Specie/ Zoo	N	Scale of positivity			
		Positive		High positive	
		N	Percent	N	Percent
Species					
<i>A. ararauna</i>	5	2	40.00	3	60.00
<i>A. chloropterus</i>	3	2	66.67	1	33.33
<i>A. macao</i>	4	1	25.00	3	75.00
<i>A. severus</i>	5	3	60.00	2	40.00
Zoo of origin					
ZW	10	3	30.00	7	70.00
GVMM	3	3	100.00	-	-
GVN	4	2	50.00	2	50.00
Total	17	8	47.06	9	52.94

GVMM: The Farm Villa and Magical World Zoo

GVN: The North Farm Villa Zoo

WZ: Warmy Zoo

Discussion

Chlamydia psittaci is a very important bacterium in avian medicine, which can affect a variety of birds, such as parrots kept in zoos. This study reports antibody titers of IgG against *C. psittaci* in parrots in Peru. Previously, only free-living dusky-headed parakeet (*Aratinga weddelli*) and tui parakeet (*Brotogeris sanctithomae*) had been studied in the National Park Manu and they were all reported as negative⁽⁹⁾.

The seroprevalence found in this study (44.74%) was lower than in other ones reported in Latin America, such as in Colombia (85%)⁽¹³⁾ and Venezuela (71%)⁽⁶⁾. However, in Costa Rica, seroprevalences from 3.4%⁽¹⁴⁾ to 12.39%⁽⁸⁾ had been reported, and in Argentina, 12 of 120 birds tested positive⁽⁶⁾; all these birds were in captivity. These differences may be due to factors such as the test used, studied species, captive management or freedom, climate, animal immune status, birds age, presence of reservoirs, genotype of the bacterium, among others⁽¹³⁾.

For the diagnosis of *C. psittaci*, the sensitivity and specificity of the test used, which can be influenced by cross-reactions, must be considered, as well as the precision and experience of the investigator. The enzyme-linked immunosorbent assay (ELISA) is the most commonly used diagnostic method

for antibody testing^(7,8,13). The Complement Fixation Test (FC), used in Guilidi's study⁽⁹⁾ have low sensitivity and presents a disadvantage as it has been observed that immunoglobulins were not always bind to complement⁽⁵⁾.

In addition, the behavior of the birds (wild or captive) and predisposition to certain species affects the presentation of bacteria and diseases. In the wild, psittacines show lower percentage of *C. psittaci*⁽⁹⁾, whereas passerines show higher rates of infection⁽¹⁵⁾. In captivity, major predisposing factors are present⁽¹⁶⁾, hence the disease and clinical signs will depend on the immune status of the birds⁽¹³⁾. As such, the highest percentage of *C. psittaci* has been observed in captive birds, according to studies from Venezuela⁽⁷⁾ and Colombia⁽¹³⁾. Different types of captive management can also largely influence the presence of the bacteria in breeding centers and zoos. The type of cage or aviary, the entry of air and light will favor the infection by avian chlamydiosis, as this is transmitted by airborne route⁽¹³⁾.

Environmental factors such as weather can affect the immune status of the birds (e.g. parrots). Environmental stress in the genus *Amazona spp.* can affect the immune system if the bird is removed from its natural habitat and is then raised in captivity⁽¹³⁾. By affecting the immune status, immune response against *C. psittaci* would favor the onset of the disease. In turn, the immune status of the bird depends on external and internal factors (mainly age and body condition)⁽¹⁷⁾.

The genotype of *C. psittaci* influences the seroprevalence that can also be influenced by certain hosts and virulence. Out of the six genotypes associated with avian hosts (A, B, C, D, E, F, and E/B), psittacines have been linked to the genotypes A, D, and F. The genotype A is the most virulent for these species and also for humans⁽⁷⁾. Due to the cosmopolitan distribution of this genotype along with its more exaggerated clinical presentation^(18, 19), it represents a serious threat to both parrots and humans.

The prevalence was not significantly different among the zoos. At Warmy Zoo, little air circulation was observed in environments, which could favor the infection since poorly ventilated places favor the presence of more concentrated fomites of *C. psittaci*^(13,17). However, at the other zoos and aviaries there is passage of passerine birds, which could also serve as a source of infection by *C. psittaci*.

According to the scale of positivity, no difference was found between the highly positive (52.94%) and the positive (47.06%) cases. In Venezuela, the disease is mostly presented in a positive scale representing medium titles (12/17) compared to birds with highly positive scale representing high titers (5/17)⁽⁷⁾. The presence of medium titles indicates birds have been in contact with *C. psittaci* or are in an acute phase, and high titles indicate active infection⁽⁷⁾. The acute phase is the generalized form that affects all organs and some clinical signs can also be observed^(13,17). However, at this stage, no birds showed clinical signs. The active phase can be fatal, with the possibility of developing clinical signs and with great possibility of transmission⁽⁷⁾; therefore, the highly positive birds (52.94%) would be actively eliminating bacteria.

Some species such as *A. ararauna* and *A. macao* had high titles and were at the active phase, while others with medium title as *A. severus* and *A. chloropterus* were mainly at the acute phase. Although individuals were sharing the same aviary and had the same type of management, immune response or condition and age of the individuals could be different⁽¹³⁾. The prevalence considering the scale of positivity was not significantly different among the zoos. Birds at Zoo Warmy had 70% high positive animals, representing birds in the active phase of infection by *C. psittaci* and showing that, in this zoo, the bacteria were circulating and there was a high, serious infection, with the great possibility of transmission to other birds, visitors, and workers⁽⁷⁾.

The seroprevalence of *C. psittaci* according to the species of macaws varies to a great extent. In blue-and-yellow macaws (*A. ararauna*), it varies from 0%⁽¹⁴⁾ to 100%⁽⁶⁾; similarly, in red-and-green macaws (*A. chloropterus*), it ranges from 0%⁽⁶⁾ to 100%⁽⁷⁾. In scarlet macaws (*A. macao*), the prevalence is relatively low in both captive and wild birds, ranging between 10%⁽¹⁴⁾ and 12%⁽⁸⁾. The chestnut fronted macaw (*A. severus*) was only studied and found negative in Poland⁽²⁰⁾, although it is known that all species of parrots are susceptible to this bacteria.

Most of the study population showed no clinical signs of the disease, except for one red-and-green macaw (*A. chloropterus*), which was positive and showed weakness and loss of feathers. Similar situations in Venezuela showed that, with high seropositivity (71%), only two of them presented clinical signs consistent with chlamydiosis⁽⁷⁾. In parrots, seropositivity can often be found without the presence of obvious clinical signs; the animals can even be asymptomatic.

Finally, we must take in consideration the zoonotic potential of this bacterium. Avian chlamydiosis is considered a disease of occupational hazard for zoo staff; therefore, it is necessary to inform staff about its risks. In addition, proper management of excreta or waste and use of biosafety material for protection is strongly recommended⁽²¹⁾. It has been reported that 78% of workers in zoos and centers were positive for *C. psittaci*⁽¹³⁾. Moreover, many of these birds were on display in the zoos, which allows more contact with visitors and could lead to an infection by *C. psittaci*.

Conclusion

These results revealed that macaws in captivity in the department of Lima, Peru, were exposed to *C. psittaci*.

Acknowledgements

To MV Eva Chomba, Warmy Zoo, MV Heedi Gonzales, Head of Wildlife Department and Ms. Cecilia Chavez, owner and manager; Farm Villa Zoos. Forest and Wildlife Service (SERFOR) in Peru for conferring the permission to conduct the study.

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