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Chlamydia abortus in brown pelicans (*Pelecanus occidentalis*) from the gulf of California, Mexico

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Abstract

The brown pelican (*Pelecanus occidentalis californicus*) is a migratory subspecies that nests on the islands of the gulf of California, in the Pacific Northwest. The objective of this study was to identify *Chlamydia* species in brown pelican samples obtained in 2016, 2017, and 2021. Samples were taken from the cloacae and choanae of 86 brown pelicans in specimens ranging from three to seven weeks of age. The samples were obtained with sterile swabs and transferred to an SPG growth medium. The DNA was extracted to carry out a real-time PCR that detects the gene of the 235 rRNA subunit of the *Chlamydiaceae* family. In addition, real-time PCR directed to the ompA gene was performed to determine the *Chlamydia* species. Of the 86 sampled pelicans, four were positive for the family *Chlamydiaceae* and *Chlamydia abortus*. This is the first report of *C. abortus* in brown pelicans from the gulf of California, Mexico.

Keywords: *Chlamydia abortus*; *Pelecanus occidentalis*; Gulf of California; Mexico; Real-time PCR.

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Study contribution

Chlamydiasis is an endemic disease in our country, there are no references on the presence of *Chlamydia* spp. in brown pelicans that migrate to Mexico, this manuscript presents new information regarding the presence of *Chlamydia* and the species that precede brown pelicans in the islands of the gulf of California, and that later migrate to the USA.

Introduction

The subspecie *Pelecanus occidentalis californicus* (California brown pelican), as well as the entire species, was eliminated in 2009 from the list of endangered species in territory of North America. Contamination of the gulf of California, Mexico was the root cause that led the brown pelican to be cataloged in the list of endangered species in the early 1970. It must be noted that the pelican conservation practices in Mexico and the United States of America are not coordinated. The recent decrease of the California brown pelican subpopulation in the state of Baja California Sur, Mexico, and the subpopulations that are representative of the gulf of California have not changed significantly.(1)

The large biodiversity on the islands in the gulf of California includes endemic species from various taxonomic ranks. The reproduction cycle of more than thirty seabird, four pinniped, and two sea turtle, takes place on the islands. Moreover, the islands are home to 218 endemic animal species and subspecies, including 81 reptiles, 45 land birds, and 92 mammals.(2)

The family *Chlamydiaceae* consists of intracellular pathogens that can cause disease in humans and animals. The more we know about the genetic diversity of this type of pathogens, the greater the evidence that their bacterial infections can spread across a variety of hosts. More than 400 host species are documented around the world, mainly in wild animals.(3) Birds are the main host for multiple *Chlamydia* species. Notoriously, *Chlamydia psittaci* has been identified in various wild and domestic birds. It has been suggested that wild birds are a reservoir of *C. psittaci* and potentially other *Chlamydia* spp. because these bacteria have been found in more than 70 wild birds, identifying the largest diversity in Europe. The *Corvidae*, *Accipitridae*, and *Columbidae* are significant emergent host families.

The role of wild birds in chlamydia infection epidemiology has become of major significance ever since the characterization of new species such as *Chlamydia avium*, *Chlamydia gallinaceae*, *Chlamydia avium*, and *Chlamydia buteonis*, and the raised possibilities of new transmission pathways.(4) Some reports suggest that avian infections might be caused only by *C. psittaci*,(5) while others have confirmed infections caused by other species of *Chlamydia*, such as *Chlamydia pecorum*, and *Chlamydia trachomatis*, and *Chlamydia abortus*.(6, 7)

The aim of this study was to determine which *Chlamydia* species infect brown pelicans (*Pelecanus occidentalis californicus*) in the Gulf of California, Mexico, during an annual census of nests and general health overview.

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Materials and methods

Ethical statement

The project was evaluated and authorized by the Secretaría de Medio Ambiente y Recursos Naturales, under the authorization of the Mexican government: Nº GPA/DGVS/01798/21. The animals used in this study were free-living, no experiment was performed on them.

Study area

The sampling area comprised San Lorenzo archipelago (28° 37′ 44″ N 112° 49′ 34″ W), Encantada island in San Luis Gonzaga bay (29° 57′ 56.9″ N 114° 25′ 08.3″ W) and Ángel de la Guarda island in front of Los Ángeles bay (29° 32′ 34″ N 113° 31′ 28″ W), all located in the gulf of California.

Sample collection

In 2016, samples were taken from the cloaca and choana of brown pelicans, whilst in 2017 and 2021 only cloacae samples were collected of 86 brown pelicans ranging between three to seven weeks old were sampled. Monitoring consisted of an active annual census of nests, the capture of the offspring for bird banding, determination of measurement and weight, detection of external parasites, and general health overview. There were no previous reports of mortality during the last six weeks before to each of the samplings in such islands or nearby.

Throughout June 2016, the first sampling was restricted to the San Lorenzo archipelago. Samples were collected from the cloaca and choana of 20 healthy brown pelicans. In August 2017, samples were obtained from the cloaca of 30 healthy brown pelicans: 20 from San Lorenzo archipelago and 10 from Encantada island. The third sampling was carried out in July 2021. Thirty-six samples were collected from the cloaca of healthy brown pelicans: 19 from San Lorenzo archipelago and 17 from the Ángel de la Guarda island.

The swab samples were kept in 15 mL tubes containing 2 mL of an SPG growth medium (217 mM sucrose, 4 mM KH_2PO4, 7 mM K2HPO4, and 1 % L-glutamine), supplemented with 10 % bovine fetal serum (Gibco, USA) and antibiotics (200 mg/mL, streptomycin and 50 mg/mL gentamicin).(8)

Molecular identification of Chlamydia *spp.*

A commercial kit Qiagen DNeasy Blood and Tissue® (Qiagen, Germany) was used to extract the DNA, in accordance with the manufacturer's protocol. The family *Chlamydiaceae* was identified using real-time PCR, which amplified a 23S rRNA subunit gene.(9) Next, *Chlamydiaceae* positive samples were analyzed with real-time PCR in order to identify *C. psittaci*, *C. pecorum*, and *C. abortus* species, by amplifying the *ompA* gene.(7)

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Results

In 2016, one out of 20 cloacae samples from San Lorenzo archipelago were positive for the family *Chlamydiaceae*. This report identified the sample as *C. abortus*. None of the 20 choanae samples were positive for the family *Chlamydiaceae* or *Chlamydia* species. The 20 cloacae samples taken from San Lorenzo archipelago in 2017 were negative, like the 10 cloacae samples from Encantada island. In 2021, two out of 19 cloacae samples from San Lorenzo archipelago and one out of the 17 cloacae samples from Ángel de la Guarda island turned out positive to the family *Chlamydiaceae*. Later, the samples were identified as *C. abortus*.

Discussion

A previous study, carried out in five wildlife rehabilitation centers in the state of Baja California Sur, Mexico, focused on the topic of the *Chlamydia* genus present in birds. It discovered DNA in 3.14 % (6/191) in the species Cooper's hawk and great horned owl. Moreover, *Chlamydia buteonis* has been reported in a Swainson's hawk (*Buteo swainsoni*) and in a red-tailed hawk (*Buteo jamaicensis*).(10) With regards to brown pelicans, a report from 1947 used a complement fixation test that detected positive birds to the *Chlamydia* species.(11) In Argentina, *C. abortus* was found in birds using real-time PCR. The family *Chlamydiaceae* was detected in 30 % of the samples, of which 70.3 % were positive for *C. psittaci*, and 14.9 % for *C. abortus*.(12)

Recent studies have described whole genome sequencing of the *Chlamydia* strains classified as G1, G2, and 1V genotypes, in three *C. abortus* strains isolated from wild birds. Significantly, these strains show genetic characteristics of *C. abortus* and *C. psittaci*. However, phylogenetic analysis indicates an association closer to the prototypical *C. abortus* strains. Likewise, it has been reported that the *Chlamydia* strains classified as G1, G2, and 1V genotypes, and the strains found in water birds as well as the strains from Corvidae, a group closely together with *C. abortus* strains. Therefore, it has been proposed to broaden the *C. abortus* species, so that it includes *C. abortus* strains from mammals, as well as bird strains that were previously classified as *C. psittaci*, atypical, or intermediate.(13)

The current report found *C. abortus* in brown pelicans from the northern gulf of California, Mexico. In this northern region, where the Ángel de la Guarda island and the San Lorenzo archipelago are located, brown pelicans live together with other bird species such as yellow-footed gull, osprey, blue-footed booby, Brandt's cormorant, and wild ducks that arrive in the winter season. Because brown pelicans are also a migratory species, they can be in contact with other domestic and wild species during their migration across the USA. This could play a role in the acquisition and transmission of *Chlamydia*. Bacterial transmission among birds, or from mammals to birds, could be caused by the elimination of the bacteria through the cloaca and oropharynx. It has been suggested that infection may occur via dust or aerosols containing fecal particles, or via direct contact with fresh feces.(13, 14)

The significance of *C. psitacci* being responsible for avian chlamydiosis is well known.(15) However, recent studies have revealed other Chlamydia species, such as *C. abortus*, *C. pecorum*, *C. trachomatis*, *C. suis*, *C. muridarum*, and *C. buteonis* in birds.(10, 13, 15) The present study was focused on finding the most relevant *Chlamydia* species in Mexico using real-time PCR, which are *C. psittaci*, *C. pecorum*, and *C. abortus*.(16–19) The report found *C. abortus* in four of the samples and analyzed a novel finding in this type of seabird. Further work will be required to validate and determine the epidemiological significance of this finding.

Data availability

The original dataset used in this research and if applicable, supporting information files, are deposited and available for download at the SciELO Dataverse repository.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

Author contributions

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Writing-original draft: M Limón-González, E Díaz-Aparicio
Writing-review and editing: R Avalos-Téllez, R Hernández-Castro, EG Palomares Resendiz

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