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ARTICLE

Prevalence of *Chlamydia* in free-living birds in Distrito Federal, Brazil

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ABSTRACT: *Chlamydia* is a genus of obligate intracellular bacteria that may cause lethal endemic avian chlamydiosis, epizootic outbreaks in mammals, and respiratory psittacosis in humans. At present few studies have been carried out on the prevalence of this microorganism in neotropical free-living avian species. The aim of this study was to investigate the prevalence of *Chlamydia* in the free-living avian species in Distrito Federal (DF), Brazil. We anayzed blood samples and cloacal swabs of 53 avian species from two conservation units in DF. The detection of *Chlamydia* was performed by amplification of a major outer membrane gene (*omp*A) fragment by a nested polymerase chain reaction. The prevalence of *Chlamydia* was observed in 83% of the avian species studied. In most of them, we found evidence that *Chlamydia*-positive individuals were shedding the bacterium in their feces, representing a significant source of infection for other wild and domestic avian species, and particularly for humans.

KEY-WORDS: Avian chlamydiosis; *Chlamydia psittaci*; Ornithosis; PCR assay; Psittacosis.

INTRODUCTION

Chlamydia is a genus of gram-negative and obligate intracellular bacteria that have been found in many vertebrate species such as mammals (Takáčová *et al*. 2010) and birds (Kaleta & Taday 2003). Most avian strains belong to *Chlamydia* (formerly *Chlamydophila*) *psittaci* but occasional detection of *C. abortus* (Herrmann *et al*. 2000), *C. pecorum* and *C. trachomatis* has been reported (Sachse *et al*. 2012). Infections caused by *Chlamydia psittaci* range from asymptomatic infections to serious outbreaks of the disease according to the species and the host. This bacterium is excreted in the feces and nasal discharges of infected birds. Some infected birds can appear healthy and shed the organism intermittently over long periods, contributing to the dissemination of the agent and representing a significant source of infection for other birds (Fudge 1996).

Chlamydia psittaci can also infect humans. Transmission from birds to humans can occur mainly via contaminated aerosol and airborne dust, causing symptoms associated with atypical pneumonia (Andersen & Vanrompay 2003). This disease is of public health significance because of the popularity of birds as pets and placement of birds in childcare facilities and rest homes (Harkinezhad *et al*. 2009).

In more recent times, advances in the field of molecular biology have allowed for the development of extremely sensitive and specific *Chlamydia* detection methods based on amplification of the MOMP genes, *omp*A or *omp*B genes, by the polymerase chain reaction (PCR, for review see Kaleta & Taday, 2003). The PCR increases the sensitivity and specificity of detection of pathogenic microorganisms by amplifying target DNA molecules by a factor of up to 10^6 (Saiki *et al*. 1988).

Recently, Kaleta and Taday (2003) presented a review of the avian host range of *Chlamydia psittaci* that contains 469 different domestic, pet and free-living bird species comprising 30 orders. It became clear by this review that some groups of birds (e.g., psittacines and domestic pigeons) are frequently investigated. However, other orders (e.g. passerines) received less attention. Thus, although free-living-birds are recognized as important reservoirs of this bacterium in nature (Brand 1989), there are only few studies reporting the occurrence of this microorganism in free-living neotropical avian species (Raso *et al.* 2006, Uhart *et al.* 2006).

The objective of this study was to investigate the prevalence of *Chlamydia* in the free-living avian species in Distrito Federal (DF), Brazil, based on PCR assay. Distrito Federal is embedded in the Cerrado biome, the Brazilian savanna. This biome is considered one of the

25 world's hotspots for biodiversity conservation and has relatively high avian diversity, with more than 830 bird species (Silva 1997), 456 (54%) of which occurs in DF (Faria 2008).

MATERIAL AND METHODS

The survey was conducted in two conservation units in DF: Águas Emendadas Ecological Station (ESECAE) (15°34'27"S; 47°36'28"W) and Gama/Cabeça-de-Veado Environmental Protection Area (APAGV) (15°55'63"S; 47°52'24"W). These units have 10,547 and 25,000 ha, respectively, and are ~ 60 km from each other.

During 2009 and 2010, birds were captured using five to eight mist nets (12 m x 2.5 m x 36 mm). The nets were opened from 6am to 6pm for two to four days, giving a total of 48 and 288 net-hours in ESECAE and APAGV, respectively. The birds were banded with numerical plastic (rings to avoid collecting samples in duplicate, and were released after samples were obtained at the capture sites (license IBAMA/SISBIO number 14341-1). Bird identification was made based on Sigrist (2007) and classified as resident or migrating based on Sick (1997).

From each captured bird, we collected three drops of blood from brachial vein using disposable needles and capillary tubes, and cloacal swabs using sterile cotton tips. For some individuals, it was only possible to collect one type of sample (cloacal swab or blood). Blood and swab samples were placed in microtubes containing absolute ethanol and ethanol 70%, respectively. Total genomic DNA was extracted by digestion with proteinase K/SDS followed by purification using the standard phenol-chloroform-isoamyl alcohol method (Bruford *et al*. 1998) for blood samples, and purification with saturated NaCl (6M) (Abrão *et al*. 2005) for cloacal samples.

The detection of *Chlamydia* in our samples was performed by amplification of a gene MOMP (Major Outer-Membrane Protein) fragment by nested PCR, using two primers pairs described by Buxton *et al*. (1996): primers A and B for first reaction, and primers B and C for the second reaction. As this methodology can detect different *Chlamydia* species (see Buxton *et al*. 1996 for details), our study allows the determination of the chlamydial prevalence in birds. Each reaction consisted of an initial step of 95°C for 7 min., 20 cycles of 95°C (1 mdnain.), 60°C (40 s), 72°C (40 s), and a final extension of 72°C for 10 min. The semi-nested PCR reaction was similar, except that 1.5 µL of the amplified product was added and 35 cycles with annealing temperature at 52^{oC} was performed. Positive (*Clamydia* DNA) and negative control (water instead of DNA) samples were included in each run. Semi-nested PCR products were analyzed by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under ultraviolet light.

When at least two individuals per species were sampled in both areas, we compare the prevalence level between the two areas using Fischer exact test. The efficiency between the two sampling methods (blood and swab) to detect *Chlamydia* in our samples in each area was evaluated using the Wilcoxon Signed Rank test. We used a nonparametric test procedure because the Shapiro-Wilk normality test shows that our data cannot be considered as normally distributed. All statistic analysis were performed using the R package version 3 (R Core Team 2012).

RESULTS

We sampled 229 birds (41 in ESECAE and 188 in APAGV) of 53 species (44 passerine and nine non-passerine species) belong to 19 families (Table 1). We collected 156 cloacal swab and 174 blood samples in ESECAE, and 38 swab and 38 blood samples in APAGV (Figure 1). All birds captured did not show evidence of clinical disease during field work. However, the prevalence of *Chlamydia* was detected in 44 of 53 (83%) bird species studied (Table 1). *Chlamydia* was detected in all bird families studied, except in Conopophagidae (Table 1), but only one individual of that family was analyzed. In all migratory and in three out of four endemic species studied were detected *chlamydia*-positive individuals (Table 1).

For all birds investigated, 70.7% and 80.5% were *Chlamydia*-positive in APAGV and ESECAE, respectively (Table 1, Figure 1). However, considering only the seven species sampled in both areas, the *Chlamydia* prevalence was higher exclusively in *Elaenia cristata* sampled in ESECAE than those sampled in APAGV ($p = 0.007$). In both areas, the frequency of *Chlamydia*-positive individuals using cloacal swabs as DNA source was higher ($p < 0.029$) than that using blood samples (Figure 1).

DISCUSSION

This is the first extensive study of *Chlamydia* prevalence in free-living avian species of the Brazilian Cerrado and the second one in free-living birds in Central Brazil. Raso *et al*. (2006) previously detected this parasite in free-living nestlings of two parrot species in the Pantanal of Mato Grosso do Sul, Brazil. Most of the species studied here were analyzed for the first time. Our results showed that *Chlamydia* had a wide host range in free-living species in DF avifauna, including three migratory species (Table 1). It is well established that migratory species have the potential to disperse certain pathogenic microorganisms. Migratory species can either facilitate rapid spread of infections to other birds across regions, especially those species that congregate before, during or after migration, and can introduce it

to new localities. Some chlamydial strains not normally pathogenic to wild avian hosts can be highly virulent for domestic birds and humans (Hubalek 2004).

Chlamydia prevalence appears to be higher in birds sampled in ESECAE than those sampled in APAGV. The high prevalence found in ESECAE may be due to biased sample in that area. Alternatively, may be influenced by anthropic habitat perturbation. ESECAE has smaller area than APAGV and the site where birds studied were sampled in ESECAE is a regeneration pasture area that has been suffering intense pressure from surrounding human communities. These conditions may be contributed to reduction of available natural area and consequently increase of host population density. High host density has been considered one of those factors that may increase transmission efficiency of the parasite (Dobson 1988). However, more data are necessary to confirm our results.

Cloacal swab samples seem to be more sensitive to *Chlamydia* diagnosis than blood samples. This result is in accordance with previous studies (Raso *et al*. 2006) and evidence that, although none of the birds studied showed clinical signs suggestive of chlamydiosis, they may be shedding the microorganism actively in feces.

Our results showed that *Chlamydia* had wide host range in the DF avifauna. We find evidence that *Chlamydia*-positive birds were shedding the microorganism through the cloaca, representing a significant source of infection for other birds and humans. However, more studies are necessary to better understand the contribution of these species in spread the pathogen for other wild and domestic bird species, and particularly for humans. It is particularly important in the case of *T. melancholicus* since this species also occur in urban areas, and it may spread the bacterium in the urban environments.

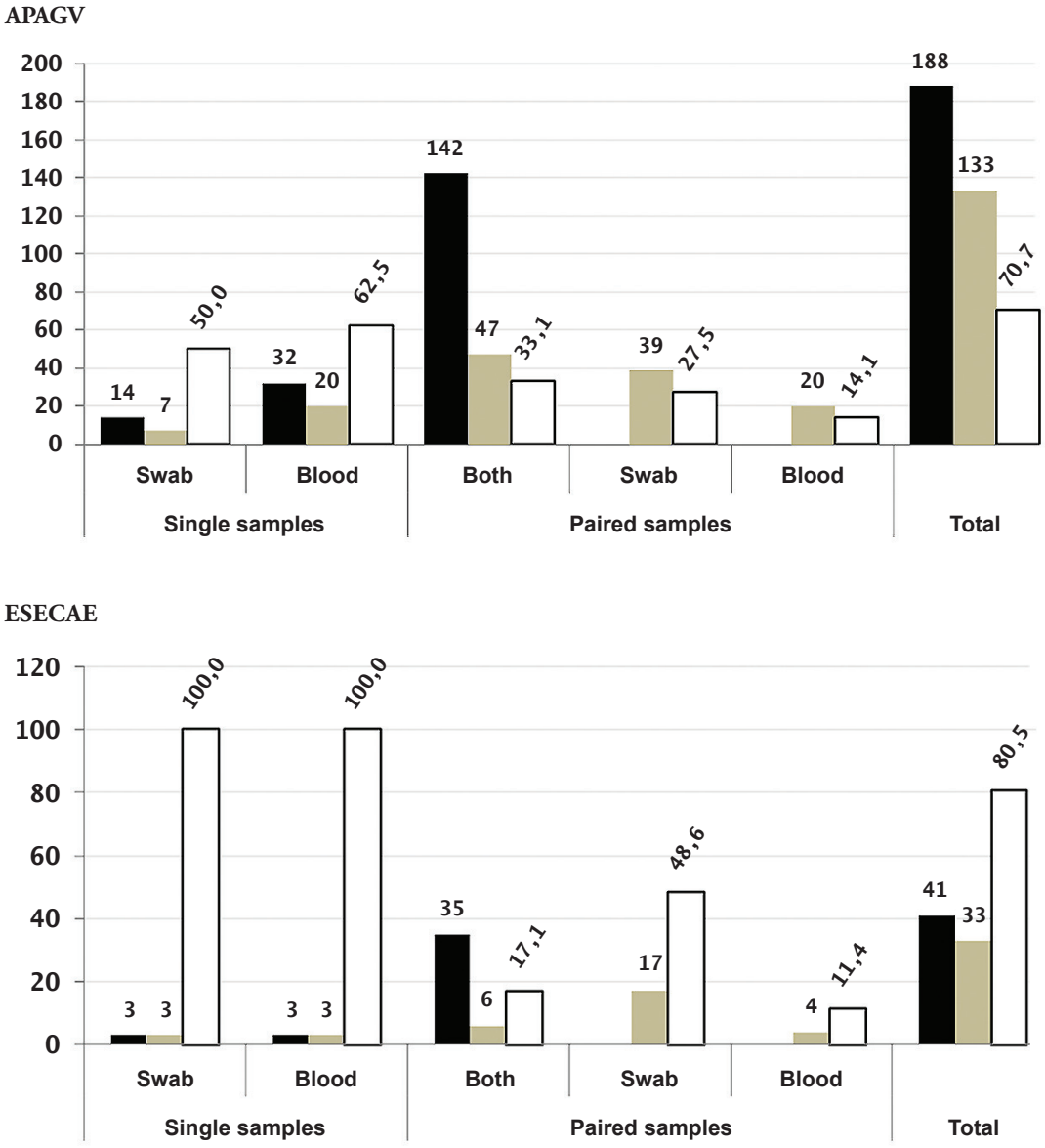

FIGURE 1. Number of birds sampled (black), number (gray) and percentage (white) of *Chlamydia*-positive individuals in two conservation units in Brazilian Cerrado: APAGV and ESECAE (see material for abbreviation). Single samples represent birds sampled with only one procedure, while paired samples represent birds sampled with both procedures.

TABLE 1. *Chlamydia* prevalence in several avian species sampled in two conservation units (APAGV and ESECAE) in Distrito Federal, Brazil.

Order - Family	**APAGV**			**ESECAE**		
Species	**N_I**	**N_{I+}**	**%**	**N_I**	**N_{I+}**	**%**
Tinamiformes - Tinamidae						
Crypturellus parvirostris				1	1	100
Columbiformes - Columbidae						
Columbina minuta				1	1	100
Columbina passerina				1	0	0
Columbina talpacoti				1	0	0
Apodiformes - Trochilidae						
Eupetomena macroura				1	0	0
Amazilia fimbriata	1	1	100			
Amazilia lactea	1	0	0			
Coraciiformes - Alcedinidae						
Chloroceryle americana				1	1	100
Piciformes - Picidae						
Picumnus albosquammatus	2	1	50			
Passeriformes						
Conopophagidae						
Conopophaga melanops	1	0	0			
Furnaridae						
Furnarius rufus				5	4	80
Hylocryptus rectirostris[E]	1	1				
Dendrocolaptidae						
Lepidocolaptes angustirostris				2	2	100
Sittasomus griseicapillus	1	0				
Tyrannidae						
Tyrannus melancholicus[M]				1	1	100
Arundinicola leucocephala				1	1	100
Myoipagis viridicata	1	1	100			
Pitangus sulphuratus				4	3	75
Elaenia mesoleuca[M]	7	7	100			
Elaenia cristata	16	3	19	4	4	100
Elaenia chiriquensis[M]	25	17	68	3	3	100
Elaenia obscura	5	2	40			
Elaeinia spectabilis	3	0	0			
Hemitriccus margaritaceiventer	1	1	100			
Pseudocolopteryx flaviventris	1	1	100			
Myiophobus fasciatus	2	2	100			
Myiarchus tyrannulus	2	2	100			
Pipridae						
Antilophia galeata[E]	12	6	50			
Polioptilidae						
Polioptila dumicola				2	2	100
Troglodytidae						
Cantorchilus leucotis	3	2	67			

Order - Family	APAGV			ESECAE		
Species	**N_I**	**N_{I+}**	**%**	**N_I**	**N_{I+}**	**%**
Turdidae						
Turdus leucomelas	10	7	70	3	3	100
Turdus rufiventris	3	3	100			
Vireonidae						
Cyclarhis gujanensis	2	2	100	1	1	100
Coerebidae						
Coereba flaveola	6	2	33	1	1	100
Thraupidae						
Neothraupis fasciata[E]	2	0	0			
Thlypopsis sordida	3	3	100			
Schistochlamys melanopis	3	2	67			
Tangara cayana	4	4	100	2	2	100
Tangara palmarum	3	3	100			
Tachyphonus rufus	2	2	100			
Ramphocelus carbo	2	2	100			
Saltator similis	16	14	88	3	2	67
Saltator maximus	3	3	100			
Emberezidae						
Geothlypis aequinoctialis	9	7	78			
Basileuterus hypoleucus	3	3	100			
Basileuterus leucophrys[E]	2	2	100			
Basileuterus flaveolus	2	2	100			
Zonotrichia capensis	9	8	89			
Arremon flavirostris	3	3	100			
Volatinia jacarina	13	12	92			
Lanio cucullatus	1	0	0			
Icteridae						
Gnorimopsar chopi				3	1	33
Fringillidae						
Cyanoloxia brissonii	2	2	100			
Total	**188**	**133**	**70.7**	**41**	**33**	**80.5**

E = endemic species; M = migratory species in the Distrito Federal; N_I = number of individual analyzed; N_I+ = number of *Chlamydia*-positive individuals; % - prevalence rates.

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