

Chlamydophila psittaci assessment in threatened red-tailed Amazon (*Amazona brasiliensis*) parrots in Paraná, Brazil

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RESUMO. O papagaio-de-cara-roxa (*Amazona brasiliensis*) é uma espécie endêmica das regiões costeiras do sudeste do Brasil. Integra a lista oficial das espécies brasileiras ameaçadas de extinção e é considerado vulnerável pela União Internacional para a Conservação da Natureza e dos Recursos Naturais. O comércio ilegal de animais selvagens e a perda de habitat representam as principais ameaças para a espécie. Assim, a implementação de ações imediatas que evitem o declínio da espécie e sua futura extinção são imprescindíveis. Uma ferramenta essencial em relação à conservação de aves é a investigação do risco das doenças e o papel dos patógenos sobre as populações de espécies ameaçadas. Em relação às doenças aviárias, a *Chlamydophila psittaci* é um importante patógeno que pode causar clamidiose, doença contagiosa e sistêmica com patogenicidade variável de acordo com a cepa, hospedeiro e fatores de estresse. *C. psittaci* tem sido detectada em psitacídeos de vida livre no Pantanal. Dessa forma, neste estudo sua presença foi investigada em populações de papagaios-de-cara-roxa em vida livre no Estado do Paraná. Este trabalho faz parte das ações realizadas pelo Projeto de Conservação do Papagaio-de-cara-roxa no Brasil. Para a pesquisa de *C. psittaci* amostras de swab cloacal e traqueal foram coletadas de 117 ninhegos de vida livre e submetidas à reação em cadeia pela polimerase (PCR). O DNA de *C. psittaci* foi detectado em 1,2% (1/117) das amostras dos ninhegos analisados. Fatores relacionados à emergência de doenças podem ser complicados e exigem um trabalho contínuo baseados na vigilância epidemiológica e pesquisas laboratoriais. Em relação à população do papagaio-de-cara-roxa futuros estudos relacionados à sanidade e conservação de seu habitat ainda precisam ser realizados a fim de assegurar a sua sobrevivência em longo prazo.

PALAVRAS-CHAVE. Clamidiose, conservação, psitacídeos, saúde dos ecossistemas.

ABSTRACT. The red-tailed Amazon (*Amazona brasiliensis*) is a parrot endemic to the coastal regions of the southeast Brazil. It is part of the official list of threatened Brazilian species and is considered vulnerable by the International Union for Conservation of Nature and Natural Resources. Illegal trade and habitat loss are the main threats to this species. Thus, immediate actions in order to avoid declination and further extinction of this parrot are of utmost importance. An essential tool concerning avian conservation is to address disease risk and the role that the pathogens can play in the threatened wildlife populations. Regarding avian diseases, *Chlamydophila psittaci* is an important pathogen which can lead to chlamydiosis, a contagious and systemic disease with variable pathogenicity according to strain, host, and stress factors. *C. psittaci* has already been detected in free-living populations of Brazilian psittacines in Pantanal. In the present study *C. psittaci* investigation was conducted in free-living populations of the threatened red-tailed Amazon (*Amazona brasiliensis*) in Paraná State. This work is part of the actions carried out by red-tailed Amazon Conservation Project in Brazil. For the *C. psittaci*'s survey, tracheal and cloacal swab samples were collected from 117 red-tailed Amazon free-living nestlings and submitted to polymerase chain reaction (PCR). *C. psittaci*'s DNA was detected in 1.2% (1/117) of the samples from the parrots analyzed. Factors related to disease emergency can be complicated and would require ongoing work supported by field epidemiology and laboratory research. Concerning the red-tailed Amazon population, further health surveillance and conservation of their habitat still need to be conducted in order to assure their long-term survival.

KEY WORDS. Chlamydiosis, ecosystem health, psittacine birds, wildlife conservation.

INTRODUCTION

The red-tailed Amazon (*Amazona brasiliensis*) is a parrot species endemic to the Atlantic Forest in southeastern

Brazil. The species occurs in a narrow littoral strip, between the Serra do Mar and the coast, from Itanhaém in São Paulo through Paraná to the extreme northeast Santa Catarina, southeast Brazil and breeding areas are mostly located on small

estuarine islands (SCHERER-NETO 1989, MARTUSCELLI 1995; LALIME 1997; SCHERER-NETO & TOLEDO 2007; SIPINSKI 2003; SCHUNCK *et al.* 2011). The red-tailed Amazon is part of the official list of threatened species in Brazil and is also considered vulnerable by the International Union for Conservation of Nature and Natural Resources (MARTUSCELLI 1995, SCHUNCK *et al.* 2011, IUCN 2012). Trapping for the cagebird trade and habitat loss are the most important threats to this species (IUCN 2012). Thus, immediate actions in order to avoid declination of this endangered parrot and further extinction are of utmost importance (BENCKE *et al.* 2006).

An essential tool concerning avian conservation is to address disease risk and the role that such processes can play in the threatened wildlife populations (MILLER 2007). Currently almost all research published about the red-tailed Amazon describes issues on their nutrition and distribution (SERAFINI *et al.* 2011, GALETTI *et al.* 2006, SCHUNCK *et al.* 2011), but studies concerning their health are still lacking in the literature. Regarding avian diseases, *Chlamydophila psittaci* is the etiologic agent of chlamydiosis, a contagious and systemic disease with variable pathogenicity according to the strain, host, and stress factors (NASPH 2010). Transmission of *C. psittaci* primarily occurs from one infected bird to another susceptible bird in close proximity. The agent is excreted intermittently in faeces and exudates and the primary route of infection is through the respiratory tract, followed by the oral route. In the nest, throughout the breeding season, male psittacines feed the female by regurgitation during the incubation of the eggs and the food can be contaminated by secretions of the crop, pharynx and nasal cavity; transmission from parent to young may also occur through feeding (BRAND 1989, VANROMPAY *et al.* 1995).

The Order Psittaciformes contains by far the most *Chlamydophila*-positive bird species, 153 out of 342 (45%) (KALETA & TADAY 2003). In Brazil, the first occurrence of *C. psittaci* in psittacine birds was documented after a serosurvey conducted in captive healthy parrots from several breeders (RASO 1999). Nowadays the disease is considered endemic in the country and has been detected recurrently in different avian species including several native psittacine birds (RASO 2006). The microorganism has been identified in healthy free-living populations of Hyacinth macaw (*Anodorhynchus hyacinthinus*) and blue-fronted parrot (*Amazona aestiva*) in Mato Grosso do Sul (RASO *et al.* 2006) and in confiscated Hyacinth macaws in São Paulo State (RASO *et al.* 2013). Besides, chlamydiosis outbreaks with high mortality rates involving Psittaciformes have been reported in wildlife rehabilitation centers in São Paulo and Minas Gerais (RASO *et al.* 2004, ECCO *et al.* 2009).

Brazil is recognized as having the greatest Psittaciformes diversity in the world, from the 85 species occurring in the country, 22 are considered at risk (CBRO 2011, IUCN 2012). Since the prompt recognition and confirmation of microorganisms with pathogenic potential in vulnerable populations is of paramount importance and due to the epidemiological findings of *C. psittaci* in Brazilian psittacine birds, this study aim to describe a *C. psittaci* surveillance conducted in free-living red-tailed Amazon (*Amazona brasiliensis*) in Paraná State, Brazil.

MATERIALS AND METHODS

The survey conducted in free-living red-tailed Amazon parrots was carried out in years 2004-2007 in Paraná's coast islands where the species naturally occurs, specially at Ilha Rasa, where most nests were located. Tracheal and cloacal swab samples were collected from 117 red-tailed Amazon (*Amazona brasiliensis*) nestlings, placed into microtubes with 1 mL of ethanol 100% and stored at -4°C until analysis.

The search for these birds to be sampled started always with the quest for nests within the forest. Tree cavities suitable for parrot nests were one by one inspected for red-tailed Amazon Parrot presence and vertical climbing techniques were employed to assess the nests to confirm it. Nests were marked with numbers and were mainly located on three islands (Gamelas, Grande and Rasa). Nestlings from monitored trees were taken from the nest, carefully examined and sampled, and immediately returned to the nest. All of them were apparently healthy.

For *C. psittaci*'s DNA molecular analysis, tracheal and swab samples were pooled and vortexed for 2 minutes and centrifuged at 20,000 g for 30 minutes at 4°C. The pellet was resuspended into 40 µL of buffer (0.1 M NaCl, 10 mM TRIS, 1 mM EDTA; 5% Triton x-100) and 9 U proteinase K (Invitrogen, USA), incubated at 56°C for 90 minutes and centrifuged at 2000 g for 2 minutes. DNA extraction was performed from supernatant using GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, USA), according to manufacturer's instructions. Then, the DNA was submitted to a seminested polymerase chain reaction (snPCR) based on conserved regions of the major outer membrane protein (MOMP) gene from Chlamydiaceae according to the literature (RASO *et al.* 2006). Positive and negative control samples were included in each run. The samples were analyzed by electrophoresis on 1.5% agarose (w/v) gels (Invitrogen Brasil) stained with 0.5 µg/ml ethidium bromide. Under ultraviolet light, a 165-base pair DNA fragment was detected in the positive snPCR samples.

RESULTS AND DISCUSSION

C. psittaci's DNA was detected in 1.2% (1/117) of the cloacal and tracheal samples from the free-living red-tailed Amazon nestlings analyzed.

In the past decades, individual populations of many avian species have undergone declines and many habitats have suffered losses of the original cover. Thus, conservation actions are essential to prevent extinctions and improve population's trajectories (HOFFMAN *et al.* 2010). Several pathogens, causative agents of infectious diseases, play a complex role in conservation biology. The contribution of infectious disease to the decline and extinction of wildlife populations is increasingly documented (FRIEND *et al.* 2001, ARMSTRONG *et al.* 2008).

Regarding the red-tailed Amazon, considered vulnerable by the IUCN (2012), reports related to pathogens surveillance are still scarce. Epidemiological diseases investigation in this species is crucial, especially when taken into account the population density in certain areas of their

natural distribution. In the present study, free-living nestlings were sampled in breeding areas and it could be observed that nesting cavities were spatially very close from each other. Additionally, outside the breeding season, large groups of adult red-tailed amazon parrots remain in close proximity especially on roosting sites, making daily movements whenever they are feeding and roosting (SIPINSKI 2003). This concentration might facilitate bird-to-bird spread of microorganisms resulting in adverse consequences (BRAND 1989, FRIEND & FRANSON 1999).

In the present study *Chlamydophila psittaci*'s DNA was detected in 1.2% of the free-living nestlings samples analyzed. Similar results were described by RASO *et al.* (2006) which conducted a research in free-living blue-fronted amazon parrots (*Amazona aestiva*) nestlings, where *C. psittaci*'s DNA was detected in 6.3% of the swab samples analysed by snPCR. These results diverge from the two serological surveys in different species of wild psittacine in South America. In Bolivia, no free-ranging Blue-fronted Amazon parrots (*Amazona aestiva*) were found to have chlamydial antibodies (DEEM *et al.* 2005). Also, in Peru, no adults of wild parakeets (*Aratinga weddellii* and *Brotogeris sanctithomaei*) showed chlamydial antibodies (GILARDI *et al.* 1995).

In the study performed in Brazil *C. psittaci*-positive birds showed no clinical signs of disease. Infection of *C. psittaci* in birds might be inapparent and there are few reports of morbidity and mortality from chlamydiosis in the natural habitat (FRANSON & PEARSON 1995, FRIEND & FRANSON 1999). It is important to note that the chances of detecting low rates of mortality or morbidity in most free-living birds' populations might be remote. Sick and dead birds are rapidly removed from the environment by predators or scavengers, and the carcass may rapidly become decomposed. Only when mortality exceeds the ability of predators to remove carcasses, there will be enough numbers present in field to be noticed (BRAND 1989). The red-tailed Amazon population in Paraná State is constantly monitored and diseases outbreaks would probably be noticed by field researchers. Nonetheless, once *C. psittaci* has been detected in a free-living red-tailed Amazon nestling, further studies should be conducted in order to elucidate if chlamydiosis might be a disease that interferes somehow in this population dynamics.

Even with the fact that *C. psittaci* has been detected in only one asymptomatic nestling sampled; it should be consider that this nestling probably became infected by their parents through feeding or by infected exudates. Furthermore, this pathogen is intermittently eliminated by their host (VANROMPAY *et al.* 1995); therefore others nestlings evaluated in this study could be infected with the microorganism but not eliminating it at the time of the swab sample collection, resulting in snPCR negative results.

The occurrence of a pathogen in a free-living population must also be put into ecological perspective. Chlamydial infections in avian hosts can stay latent (thus inapparent) for years and then emerge with clinical signs following stressful episodes as molt periods, abrupt variation in weather conditions and environmental changes in surroundings (FLAMMER 1997). Impacting environmental changes include loss of habitat, which nowadays represents one of the major threats to red-tailed Amazon (SCHUNCK *et al.* 2011). Ecosystem disturbance and landscape changes can lead to disease occurrence (FRIEND & FRANSON 1999, SEHGAL 2010), which is a matter of great concern when taken into account the particular features and limited size

of the regions where the red-tailed amazon are distributed. Habitat heterogeneity is a key factor in determining the red-tailed Parrot distribution. As an example, the most striking feature of the regions where this species inhabits in Paraná State is a dynamic and complex network of channels and islands that produces a mosaic of habitats that the parrot explores, making regular movements among the different vegetation types (MARTUSCELLI 1994). Some of these areas are protected by the Brazilian Government; nonetheless deforestation still occurs in some level due to the lack of fiscalization (BENCKE *et al.* 2006). In addition, in the state of São Paulo, where the species also occurs, the majority (64%) of the red-tailed Amazon free-living population remains outside of the main protected areas, which enhances the threat to the future of this species (GALETTI *et al.* 2006).

In view on this situation, ex-situ conservation programs have been carried out with several endangered species, in order to maintain a viable population and to restore it if necessary (ARMSTRONG *et al.* 2008). An essential part of conservations programs is constancy sanitary evaluation, which may help to predict treats related to infectious diseases and to reinforce prophylactics measures to avoid potential pathogenic microorganism's dissemination. On the subject of captive birds, in some cases chlamydiosis may be devastating. In Brazil, RASO *et al.* (2004) and ECCO *et al.* (2009) reported chlamydiosis outbreaks leading to several psittacine deaths. Morbidity and mortality of wild species in captivity frequently is associated with stress of transportation, unsanitary or crowded conditions. Finally, factors related to disease emergence can be complicated and will require ongoing work supported by field epidemiology and laboratory research (STALLKNECHT 2007). Concerning the red-tailed Amazon population further health surveillance and conservation of their habitat still need to be conducted in order to assure their long term survival.

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