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# Survey of pathogens in threatened wild red-tailed Amazon parrot (*Amazona brasiliensis*) nestlings in Rasa Island, Brazil

Q1 Frederico Fontanelli Vaz<sup>a,\*</sup>, Patrícia Pereira Serafini<sup>b</sup>, Rosangela Locatelli-Dittrich<sup>a</sup>,  
Rafael Meurer<sup>b</sup>, Edison Luiz Durigon<sup>c</sup>, Jansen de Araújo<sup>c</sup>,  
Luciano Matsumiya Thomazelli<sup>c</sup>, Tatiana Ometto<sup>c</sup>, Elenise Angelotti Bastos Sipinski<sup>d</sup>,  
Rafael Meirelles Sezerban<sup>d</sup>, Maria Cecília Abbud<sup>d</sup>, Tânia Freitas Raso<sup>e</sup>

<sup>a</sup> Department of Veterinary Medicine, Federal University of Paraná, Rua dos Funcionários, 1540, 80035-050 Curitiba, Paraná, Brazil

<sup>b</sup> National Center for Bird Conservation and Research, Chico Mendes Institute for Biodiversity Conservation, Estação Ecológica de Carijós, Rodovia Maurício Sirotski Sobrinho, SC 402, km 2, 88053-700 Florianópolis, Santa Catarina, Brazil

<sup>c</sup> Department of Microbiology, Biomedical Sciences Institute, University of São Paulo, Av. Prof. Lineu Prestes, 1374, 05508-900 São Paulo, Brazil

<sup>d</sup> Society for Wildlife Research and Environmental Education, Rua Victório Viezzer, 651R, 80810-340 Curitiba, Paraná, Brazil

<sup>e</sup> Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, 05508-270 São Paulo, Brazil

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### ABSTRACT

The red-tailed Amazon parrot (*Amazona brasiliensis*) is a threatened species of psittacine bird that inhabit coastal regions of Brazil. In view of the threat of this species, the aim of this study was to perform a health evaluation in wild nestlings in Rasa Island, determining the prevalence of enterobacteria and infectious agents according to type of nest. Blood samples were collected from 64 birds and evaluated for antibodies of *Chlamydia psittaci* by commercial dot-blot ELISA. Cloacal and oropharyngeal swabs samples were collected from 23 birds from artificial wooden nests, 15 birds from PVC nests and 2 birds from natural nests for microbiological analysis. Swab samples were collected from 58 parrots for *C. psittaci* detection by PCR and from 50 nestlings for Avian Influenza, Newcastle Disease and West Nile viruses' detection analysis by real-time RT-PCR. Ten bacterial genera and 17 species were identified, and the most prevalent were *Escherichia coli* and *Klebsiella oxytoca*. There was no influence of the type of nest in the nestlings' microbiota. All samples tested by ELISA and PCR were negative. There is currently insufficient information available about the health of *A. brasiliensis* and data of this study provide a reference point for future evaluations and aid in conservation plans.

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\* Corresponding author.

E-mail: [fredfontanelli@yahoo.com.br](mailto:fredfontanelli@yahoo.com.br) (F.F. Vaz).

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## Introduction

There is a wider need for greater effort in research on disease in wild bird populations, especially in threatened species.<sup>1</sup> Health monitoring of these species should be encouraged being that, at local scales, infectious disease is a common driver of population declines.<sup>2</sup> Moreover, wild birds may carry or be infected with microorganisms that can affect their health and the health of livestock, pet animals and human beings.<sup>3</sup>

The red-tailed Amazon parrot (*Amazona brasiliensis*) is listed as vulnerable according to the International Union for the Conservation of Nature Red List of Threatened Species.<sup>4</sup> This species is endemic to the Atlantic Forest, inhabiting southern coastal regions of São Paulo, Paraná and northern Santa Catarina in a narrow coastal strip.<sup>5</sup> Breeding areas are mostly located in small estuarine islands, and these facts make the parrot especially vulnerable to environmental disturbance.<sup>6</sup>

Since 1997, the Society for Wildlife Research and Environmental Education (SPVS) has developed the red-tailed Amazon Conservation Project in an attempt to minimize population decline.<sup>7</sup> Environmental modification by human activities in Rasa Island destroyed many natural nests and the lack of tree cavities can limit the population growth of parrots. One of the efforts of SPVS is to build artificial nests made from wood (Fig. 1) and polyvinyl chloride (PVC) (Fig. 1B), and install them next to the lost natural nests in large native trees that have the potential to become future natural shelters along the coast of Paraná. Since 2003, the artificial nests have been widely used by the parrots for breeding.<sup>8</sup>

In the case of the threatened red-tailed Amazon population, a health study is essential to address disease risk and to establish normal parameters previously unavailable, assisting the conservation of this species.<sup>9</sup> It would be also important to differentiate the parrots' microbiota in the different types of nests, since the avian gastrointestinal microbiota can be affected by environment.<sup>10</sup> No microbiological studies comparing wild birds from different nests under same conditions are available in the literature for parrot species.

A few studies reporting health status of wild psittacine species in southern Brazil have been described in the literature.<sup>9,11</sup> Chlamydiosis is one of the main infectious diseases for the order Psittaciformes because this group has the highest positivity rate (45%) among birds.<sup>12</sup> Avian influenza virus (AIV), Newcastle disease virus (NDV), and West Nile virus (WNV) are three of the most important viral diseases in industrial aviculture and have a significant burden to public health.<sup>13-15</sup> Besides holding several bird migratory routes, Brazil is considered free from high pathogenic viruses of these viral diseases, and monitoring wild birds is essential to analyze virus circulation, because the risk of introduction is always present. Until now, wild red-tailed Amazon parrot populations in Brazil had never been evaluated for such viruses.

In view of the limited information available on the species, the aim of this study was to perform a health evaluation in wild *A. brasiliensis* nestlings in Rasa Island, Paraná, Brazil, by assessing the cloacal and oropharyngeal microbiota according to type of nest; and by evaluating the presence of *Chlamydia psittaci*, AIV, NDV-1 and -2 and WNV.

## Material and methods

### Study area

This study was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Federal University of the State of Paraná, Southern Brazil (protocol number 050/2013) and by the SISBIO (number 41035-1). Sample collection was performed in Rasa Island, located in the Environmental Protection Area of Guaraqueçaba, Paraná, Brazil (Fig. 2). It is a protected area with an extensive area of Atlantic forest, consisting of estuaries, islands, mangrove forests, plains, mountains and plateaus where the parrot breeds.

### Sampling

Sample collection of *A. brasiliensis* was carried out concomitantly with the monitoring of nestlings during the 2013/2014 breeding season in five field expeditions (December to February). The nests on the trees were accessed using climbing equipment and ladders. The birds were taken from the nest and carefully sampled. The age was estimated, the nestlings were weighed, and immediately put back into their nests.

Blood samples were collected from nestlings from the superficial ulnar vein using 1 mL sterile syringes pretreated with 1000 IU sodium heparin for antibody test. The samples were placed in tubes and refrigerated for up to 24 h. The tubes were centrifuged for 5 min to obtain plasma, which was frozen at  $-40^{\circ}\text{C}$  until analysis for *C. psittaci*.

Cloacal and oropharyngeal swab samples were collected from nestlings for microbiological analysis and kept refrigerated in Stuart transport medium up to 48 h until analysis in the laboratory. Cloacal and oropharyngeal swab samples were collected for *C. psittaci* detection by PCR in microtubes containing 1.0 mL of PBS pH 7.4, and for AIV, NDV and WNV detection by real time RT-PCR (rRT-PCR) in 2 mL microtubes containing antifungals, antibiotics and glycerol. All samples were kept frozen at  $-40^{\circ}\text{C}$  until analysis in the laboratory.

### Microbiological analyses

Samples were plated on brain heart infusion and incubated for 24 h at  $37^{\circ}\text{C}$ . Subsequently, they were stored in 30% glycerol solution at  $-20^{\circ}\text{C}$ . When thawed, the samples were inoculated by streaking method on blood and MacConkey's agar media and incubated at  $37^{\circ}\text{C}$  for 24 h for the bacteria grow. Later, the cultured bacteria were picked up for the preparation of smear, stained with Gram's stain and examined under microscope for staining and morphological characterization of the isolates. Catalase test were also performed in the cultured bacteria. Each pure colony was inoculated in triple sugar iron agar by stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant and incubated at  $37^{\circ}\text{C}$  for 24 h, for detecting carbohydrate fermentation and production of  $\text{H}_2\text{S}$  and gas. Finally, the fermenting colonies were inoculated in an enterobacteria kit (Newprov - Pinhais, Paraná, Brazil) for biochemical tests at  $37^{\circ}\text{C}$ , examined at 24 and 48 h of incubation.

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