

# FIRST DETECTION OF LEISHMANIA OF THE SUBGENUS VIANNIA IN ALIPIOPSITTA XANTHOPS, ENDEMIC BIRD OF SOUTH AMERICA

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## Abstract

We report the first molecular detection of *Leishmania* infection (subgenus *Viannia*) in the yellow-faced parrot (*Alipiopsitta xanthops*), at a wildlife rehabilitation center located in the city of Campo Grande, Brazil, an endemic area for leishmaniasis. PCRs targeting kinetoplast DNA (kDNA) and the small subunit of ribosomal RNA of *Leishmania* spp. were performed, both positive, followed by the sequencing of the amplified region of the SSU rDNA gene, which confirmed the identity of the parasite. This is the first report of success obtained in the use of PCR targeting the IRBP (Interphotoreceptor retinoid-binding protein) gene as an internal control in the molecular diagnosis of pathogens in bird species.

**Keywords:** Birds. IRBP. *Leishmania*. PCR. rDNA. SSU.

## 1. Introduction

Leishmaniasis is a serious public health problem, especially in Brazil, considered by the World Health Organization (WHO) as an endemic country for different species of these parasites (WHO 2017), which occur throughout most of the national territory (Basano and Camargo 2004). Among wild animals, more than 60 species of mammals from seven orders have been diagnosed with *Leishmania* spp infection, with rodents being the most widely studied in relation to their role as hosts and reservoirs of these parasites (Roque and Jansen 2014).

Knowledge regarding the possible role of birds as hosts of *Leishmania* spp. is still limited due to the scarcity of studies on parasites of the genus *Leishmania* in this group of vertebrates. Chickens (*Gallus gallus domesticus*) have been reported as a food source for phlebotomines, whose presence near the housing of these animals is a risk factor for the transmission of *L. infantum* (Rodrigues et al. 1999). However, under experimental inoculation of the parasite, it was not possible to confirm the role of this bird as host of *L. infantum*, because among the three diagnostic tests performed, only molecular detection showed positive results (Otranto et al. 2010). In the same study, two birds of the species *Anser anser* and one bird of the species *Phasianus colchicus* showed anti-*L. infantum* antibodies (without experimental inoculation), being identified as possible hosts of this pathogen in nature (Otranto et al. 2010).

## 2. Material and Methods

A peripheral blood sample from the bird *Alipiopsitta xanthops*, popularly known as yellow-faced parrot, was provided by the Center for Rehabilitation of Wild Animals (*Centro de Reabilitação de Animais Silvestres* - CRAS) located in the city of Campo Grande, Mato Grosso do Sul, Brazil, to perform molecular tests for the detection of parasites. The only information provided by the CRAS was that the individual died suddenly in June 2015 and there was no report of any clinical signs that could be related to infection by *Leishmania* sp.

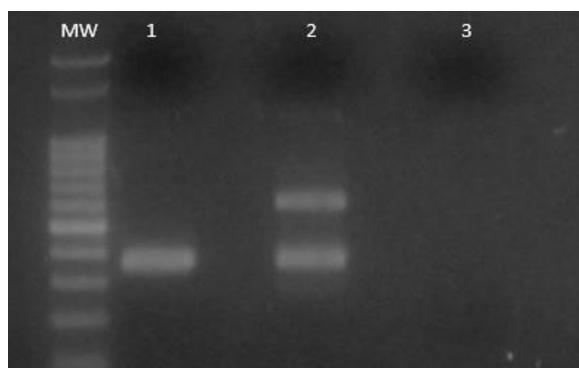
The sample was submitted to DNA extraction with the commercial Wizard® Genomic DNA Purification (Promega) kit. As an internal control to ensure the viability of the genetic material, IRBP (Interphotoreceptor Retinoid Binding Protein) PCR was performed using the primers IRBP fwd 5 'TCC AAC ACC ACC ACT GAG ATC TGG AC 3' and IRBP rev 5 'GTG AGG AAG AAA TCG GAC TGG CC 3', as described by Ferreira *et al.*, 2010.

Then, PCR targeting kDNA was performed using the primers: A: 5 '(C / G) (C / G) (G / C) CC (C / A) CTA T (T / A) T TAC ACC AAC CCC 3 'and B: 5' GGG GAG GGG CGT TCT GCG AA 3', according to Silva *et al.*, 2000. To confirm positivity, a nested PCR (Ln-PCR) targeting SSU rRNA was performed using R221: 5'GGT TCC TTT CCT GAT TTA CG 3', R332: 5'GGC CGG TAA AGG CCG AAT AG 3', R223: 5'TCC CAT CGC AAC CTC GGT T 3' and R333: 5'AAA GCG GGC GCG GTG CTG 3', as described by Cruz *et al.*, 2002, which amplifies only *Leishmania* spp., avoiding cross reactions with other trypanosomatids. The amplicon of LnPCR was purified with PureLink® Quick Gel Extraction kit (Invitrogen) and subjected to genetic sequencing for identification of the *Leishmania* species.

## 3. Results and Discussion

To certify the viability of the DNA obtained from this sample, we performed PCR targeting the IRBP gene, which is still little used as an internal control in studies in the area of parasitic infectious diseases (Ferreira *et al.* 2010; Gonçalves *et al.* 2015; Tonelli *et al.*, 2017). This gene is used mainly in phylogenetic studies to determine the relationship between mammalian species (Jansa, and Weksler, 2004). Although its effectiveness as an internal control for rodent species (Ferreira *et al.* 2010) has been shown previously, this is the first report to show that the protocol using this pair of primers can also be used for another class of vertebrates (birds) and, therefore, this gene may be an effective internal control for the molecular diagnosis of pathogens in birds.

The first PCR performed to detect *Leishmania* infection in the sample targeted *Leishmania* kDNA and gave positive results. Then, to confirm the infection and avoid cross-reactions with other trypanosomatids, LnPCR was also performed confirming the positive result and, therefore, the *Leishmania* sp. infection (Figure 1).



**Figure 1.** Ln-PCR. 2% agarose gel electrophoresis, stained with SybrSafe and observed on blue light. MW: molecular weight (100 base pairs); 1: *Alipiopsitta xanthops*; 2: *Leishmania braziliensis*; 3: negative control.

After sequencing and BLAST analysis of the LnPCR product, the parasite of the sample was identified as *Leishmania* sp., belonging to the subgenus *Viannia*. The bird had been maintained in a wild animal rehabilitation center in Campo Grande-MS, an endemic area for leishmaniasis, and according to the information sheet provided by the center, did not present clinical signs of disease.

As shown by Oliveira in 2003 and again in 2006, the city of Campo Grande has an abundant phlebotomine fauna, which includes, among species of epidemiological importance, *L. longipalpis*, a vector of visceral leishmaniasis and *Nyssomyia whitmani*, a vector of tegumentary leishmaniasis. The presence of *Leishmania* spp. in Campo Grande has been confirmed in humans (Castro et al. 2018), in wild animals (Shapiro et al. 2013; Castro et al. 2020) and in vectors (Silva et al. 2008), which suggests that the parasite transmission cycles are present and active in the capital of the state of Mato Grosso do Sul.

It should be emphasized that diagnostic studies in places such as wildlife rehabilitation centers are extremely important, since animals of several species live in close contact to each other in a forest environment prone to the presence of vectors. The presence and circulation of *Leishmania* in the Wildlife Rehabilitation Center of Campo Grande was reported by Humberg et al. in 2012, where samples of opossums (*Didelphis albiventris*) collected and treated at the center were analyzed, with a positivity index of 20.37% of specimens infected by *Leishmania chagasi*, a fact that may indicate participation of this species in the transmission cycles in the city. Furthermore, the presence of *N. whitmani*, an important vector of different *Leishmania* species of the subgenus *Viannia* (Mayrink et al., 1979; Lainson et al., 1989; Queiroz et al., 1991) was also observed at the Center (Marinho et al. 2017), indicating the possible transmission of the parasite at the site.

It is important to consider that the detection of *Leishmania* infection does not necessarily mean that this species is a reservoir of the parasite. To be considered a successful reservoir, a species must be responsible for maintaining the parasite's transmission cycle in the wild, serving as a viable source of infection for the phlebotomine vectors (Roque et al., 2014).

#### 4. Conclusions

The finding of a new wild species naturally infected by *Leishmania* sp. indicates the importance of investigating infection by these parasites in wild animals of different species, in addition to mammals, to identify new hosts and evaluate the possibility of these to act as reservoirs, contributing to a better understanding of the transmission cycles of leishmaniasis and consequently to its control.

**Authors' Contributions:** MATHEUS, L.M.D., DUARTE, P.O. and FERREIRA, E.C.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** Not applicable.

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