

DETECTION OF ZOONOTICAL MICROSPORIDIA *ENCEPHALITOOZON INTESTINALIS* IN GIANT ANTEATER *MYRMECOPHAGA TRIDACTYLA* (LINNAEUS, 1758) FROM BRAZIL

Beatriz Regina Rodrigues Carvalho¹, Ronalda Silva de Araujo², Danilo Kluyber³, Bruno de Lima Araújo¹, Maria Anete Lallo¹

¹Programa de Patologia Ambiental e Experimental, Universidade Paulista (UNIP), São Paulo, Brazil

²CETESB-Companhia Ambiental do Estado de São Paulo, São Paulo, Brazil

³ ICAS - Instituto de Conservação de Animais Silvestres, Campo Grande, Mato Grosso do Sul, Brazil

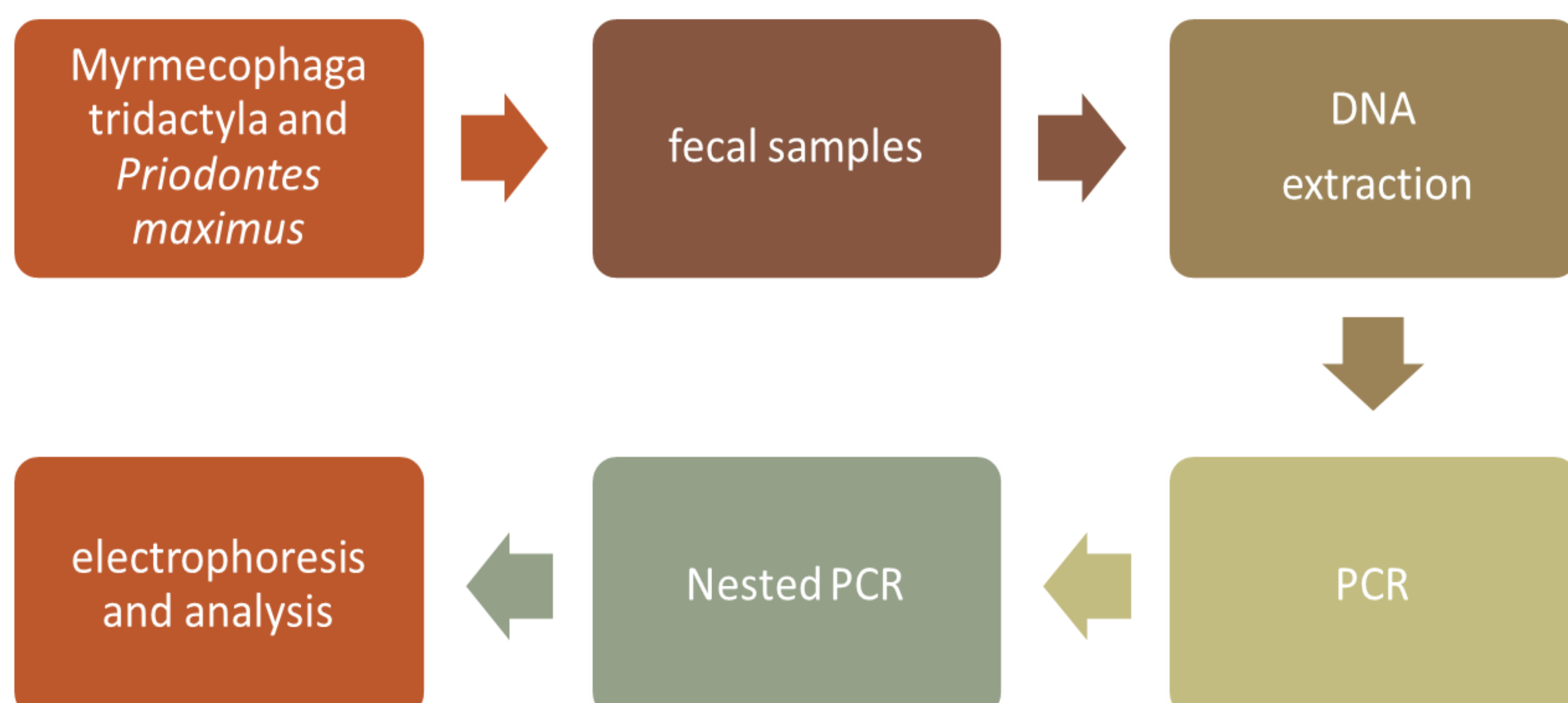
Introduction:

Microsporidia are opportunistic, emergent fungi that infect an exceptionally diverse group of hosts, including vertebrates and invertebrates. Among the more than 1,700 species identified, 17 were described as potential zoonoses in immunosuppressed and/or immunocompetent vertebrates [1,2]. The aim of this study was to analyze the occurrence of zoonotic microsporidia *Encephalitozoon cuniculi*, *E. intestinalis*, and *Enterocytozoon bieneusi* in the feces of giant armadillos (*Priodontes maximus*) and giant anteaters (*Myrmecophaga tridactyla*) monitored on highways.

Methods:

In this research, fecal samples from *Myrmecophaga tridactyla* and *Priodontes maximus* were used, donated by the Institute of Wildlife Conservation (ICAS), located in Campo Grande, Mato Grosso do Sul, Brazil. These samples belong to free-living animals from the Cerrado and Pantanal biomes.

Fortyfour fecal samples (220mg) were subjected to thermal shock (cold and heat) and then subjected to DNA extraction with the QIAamp fast DNA stool mini kit (Qiagen), according to the manufacturer's recommendations. The DNA was amplified by polymerase chain reaction (PCR) with generic primers and the product generated from this reaction was submitted to Nested PCR with specific primers: EBIEF1/EBIER1 for *E. bieneusi*; ECUNF/ECUNR for *E. cuniculi* and SINTF/SINTR for *E. intestinalis*.



The samples amplified in the second reactions were stained with 0.5 µL of SYBR™ Safe (Invitrogen™) and subjected to electrophoresis on a 1.5% agarose gel (Amersham Bioscience), under a current intensity of 6v/cm. Fragment sizes were estimated by comparison with the 100bp Plus DNA Ladder molecular weight marker (Invitrogen™). The gel was visualized under ultraviolet light (Life Technologies™) and photographed.

References:

- [1] MAGALHÃES, T. R.; et al., A multidisciplinary review about Encephalitozoon cuniculi in a One Health perspective. Parasitology Research, p. 1-17, 2022.
- [2] RUAN, Y. et al. "The largest meta-analysis on the global prevalence of microsporidia in mammals, avian and water provides insights into the epidemic features of these ubiquitous pathogens." Parasites & vectors v. 14, p.1-186. 2021.
- [3] DA SILVA FIUZA, V. R., Lopes, C. W. G., de Oliveira, F. C. R., Fayer, R., & Santin, M. (2016). New findings of Enterocytozoon bieneusi in beef and dairy cattle in Brazil. Veterinary Parasitology, 216, 46-51.
- [4] DESBIEZ, A. L. J. et al. O que sabemos sobre os tatus do Pantanal? Revisão do conhecimento sobre ecologia, biologia, morfologia, saúde, conservação, distribuição e métodos de estudo. Boletim do Museu Paraense Emílio Goeldi-Ciências Naturais, v. 17, n. 1, p. 11-69, 2022.
- [5] STENTIFORD, G. D. et al. Microsporidia—emergent pathogens in the global food chain. Trends in parasitology, v. 32, n. 4, p. 336-348, 2016.

Results:

From the 5 samples collected from *Priodontes maximus* – giant armadillo, none tested positive for microsporidia. However, out of the 38 samples from *Myrmecophaga tridactyla* - giant anteater analyzed, two tested positive for *E. intestinalis* (Fig.01) (5.2%), one of the most described zoonotic species of microsporidiosis. These samples belonged to a female anteater and her male offspring (Fig.02), with their samples (4 and 11) collected at the respective geolocations 21°38'33.47"S 53°36'10.15"W and 21°38'21.08"S 53°36'16.07"W.

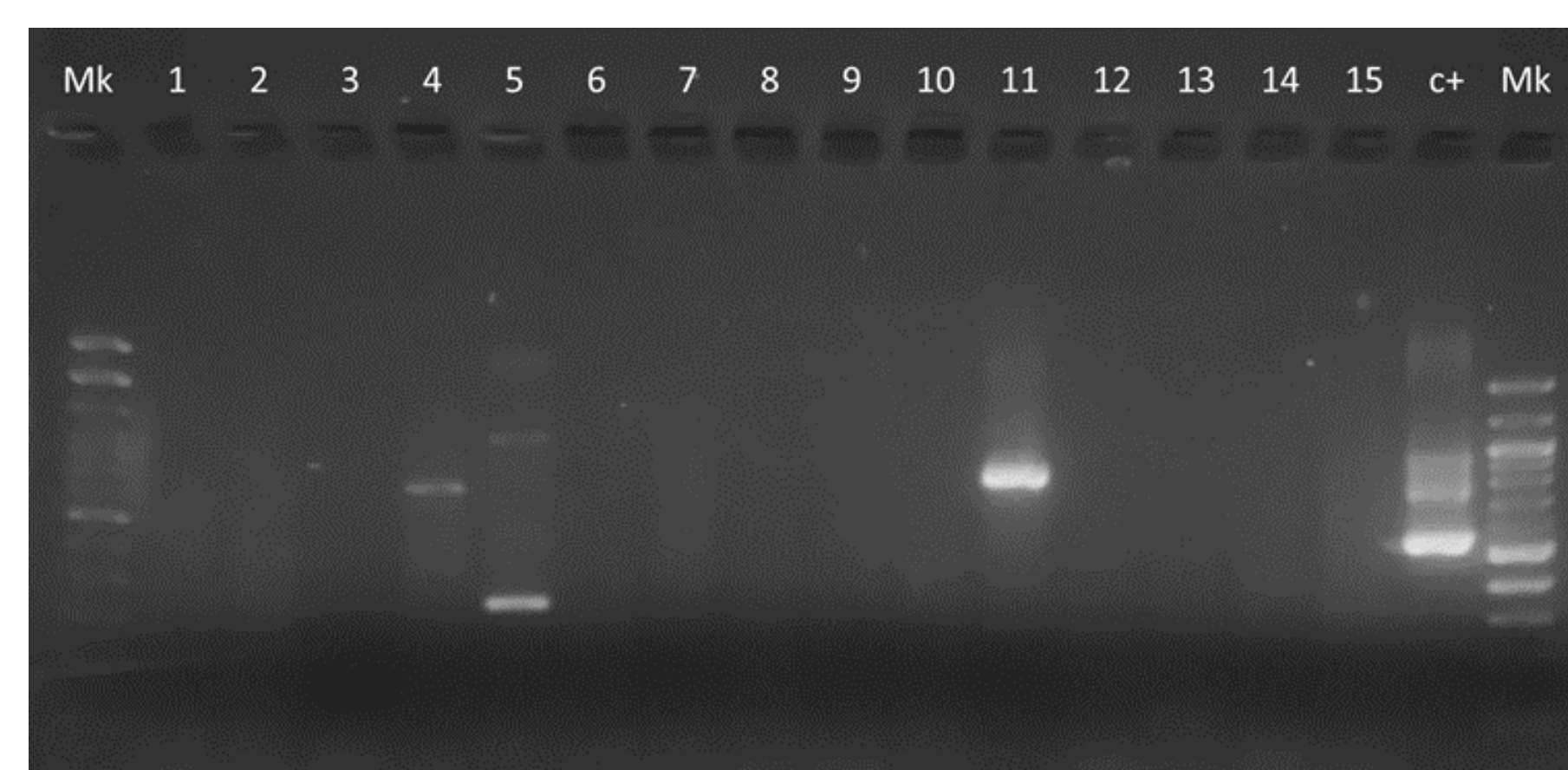


Figure 01: Photographic record of agarose gels revealing positivity for *E. intestinalis*.



Figure 02: Photographic record conducted by the Flags and Highways Project of the individuals testing positive for *E. intestinalis*.

Discussion:

The anteaters are located in a Cerrado area where extensive livestock farming occurs in the sample collection regions, representing a risky situation due to close contact between domestic and wild animals. The microsporidium *E. bieneusi* has previously been found in 17.5% of bovine fecal samples in the state of Rio de Janeiro [3], revealing an important epidemiological situation in Brazilian cattle farming. It could be hypothesized that cattle might have been the source of infection for these anteaters. *Myrmecophaga tridactyla*, like *Priodontes maximus*, constantly suffers from the accelerated loss of its habitat [4], leading it to be listed as a vulnerable species to extinction by the IUCN (2022) and at risk of extinction in most Brazilian states (ICMbio 2018). The microsporidium is an emerging pathogen of global interest, occurring in all biomes and easily transmitted through the consumption of contaminated water, food, and animals [5]. Understanding its circulation in the environment is necessary for adopting mitigating measures for One Health.