

## **Gastrointestinal parasites in non-human primates from the Wildlife Animal**

### **Screening Center of Vitória da Conquista, Bahia, Brazil**

**Parasitos gastrointestinais em primatas não-humanos do Centro de Triagem de Animais Silvestres de Vitória da Conquista, Bahia, Brasil**

**Parásitos gastrointestinales en primates no-humanos del Centro de Cribado de Animales Silvestres de Vitória da Conquista, Bahía, Brasil**

Received: 01/22/2022 | Reviewed: 01/29/2022 | Accept: 02/02/2022 | Published: 02/04/2022

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### **Abstract**

The investigation of gastrointestinal parasites in primates kept in captivity is important for the management of these animals and for human health, since many of their parasites cause zoonoses. The aim of this study was to evaluate gastrointestinal parasitism in primates kept in captivity at a Wildlife Animal Screening Center in Bahia, Brazil. 135 stool samples from 45 primates were analyzed using the Direct method and the Hoffman method, three times, with an interval of fifteen days. Infections by protozoa were verified, being mild for *Balantidium* sp. and *Entamoeba* sp. and moderate for *Cystoisospora* sp., the latter had the highest sample prevalence among all the parasites found (91.11%). The presence of helminths of *Ancylostoma* sp. and *Strongyloides* sp. was recorded. Infection by gastrointestinal parasites

in captive primates is frequent. With the results of the present work, it is concluded that periodic parasitological evaluations are necessary for better sanitary management of these animals.

**Keywords:** Captive animals; Conservation medicine; Helminths; Protozoa; Stool examination.

### Resumo

A investigação por parasitos gastrintestinais em primatas mantidos em cativeiro é importante para o manejo desses animais e para a saúde humana, pois muitos dos seus parasitos são causadores de zoonoses. O objetivo deste estudo foi avaliar o parasitismo gastrintestinal em primatas mantidos em cativeiro em um Centro de Triagem de Animais Silvestres da Bahia, Brasil. Foram analisadas, pelo método Direto e pelo método de Hoffman, 135 amostras de fezes de 45 primatas, três vezes, com intervalo de quinze dias. Foram verificadas infecções por protozoários sendo de grau leve para *Balantidium* sp. e *Entamoeba* sp. e moderada para *Cystoisospora* sp., este último teve maior prevalência amostral entre todos os parasitos encontrados (91,11%). Foi registrada a presença de helmintos de *Ancylostoma* sp. e *Strongyloides* sp. É frequente a infecção por parasitos gastrintestinais em primatas em cativeiro. Com os resultados do presente trabalho conclui-se que a realização de avaliações parasitológicas periódicas se faz necessária para melhor manejo sanitário destes animais.

**Palavras-chave:** Animais cativos; Medicina conservativa; Helmintos; Protozoários; Exame de fezes.

### Resumen

La investigación de parásitos gastrointestinales en primates mantenidos en cautiverio es importante para el manejo de estos animales y para la salud humana, ya que muchos de sus parásitos causan zoonosis. El objetivo de este estudio fue evaluar el parasitismo gastrointestinal en primates mantenidos en cautiverio en un Centro de Detección de Animales Silvestres en Bahía, Brasil. Se analizaron 135 muestras de heces de 45 primates utilizando el método Directo y el método Hoffman, tres veces, con un intervalo de quince días. Se verificaron infecciones por protozoos, siendo leves para *Balantidium* sp. y *Entamoeba* sp. y moderadas para *Cystoisospora* sp., este último tuvo la mayor prevalencia de muestra entre todos los parásitos encontrados (91,11%). Se registró la presencia de helmintos de *Ancylostoma* sp. y *Strongyloides* sp. La infección por parásitos gastrointestinales en primates en cautiverio es frecuente. Con los resultados del presente trabajo se concluye que son necesarias evaluaciones parasitológicas periódicas para un mejor manejo sanitario de estos animales.

**Palabras clave:** Animales cautivos; Medicina conservadora; Helmintos; Protozoos; Examen de heces.

## 1. Introduction

The trafficking of wild animals consists of the removing act the fauna from its natural habitat and commercializing it. When it comes to monetary movement, this is the third largest illegal activity in the world, behind only drug and arms trafficking (Nassaro et al., 2010; Antonio et al., 2021).

The Wildlife Animal Screening Center (CETAS) are units created with the objective of mitigating the damage caused by the trafficking of wild animals to Brazilian biodiversity, given that Brazil is a megadiverse country and its biodiversity is widely exploited by trafficking (Destro et al., 2012). Among the responsibilities of CETAS are: (i) to receive, (ii) to evaluate, (iii) to recover and (iv) to allocate of the animals - preferably back to nature (Brasil, 2015).

Both animals that are destined to live in captivity, and those sent for release in the wild, need to undergo several clinical and laboratory evaluations, provided for in the regulations that govern the CETAS. One of the planned laboratory tests is the coproparasitological test, which allows the identification of gastrointestinal parasites (Brasil, 2015). The investigation for parasites in animals is a tool for analyzing the health status of the population and the environment quality in captivity, and should be carried out in fauna management projects and in periodic assessments of captivity animals (Catenacci et al., 2004).

Among the nine orders of the Mammalia class that entered the CETAS in Bahia, between 2009 and 2019, Primates are the most frequent (Santos et al., 2021). Primates can be parasitized by both helminths and protozoa (Rodrigues et al., 2021; Silva et al., 2008). The vast majority of parasites are well adapted to their hosts, probably causing few pathological changes. However, some parasites, in addition to facilitating the installation of secondary infections, cause physiological disorders, injuries that weaken the animal, weight loss and even death (Kindlovits, 2009).

The roundworms (Phylum Nematoda) are helminths that have several representatives that inhabit segments of the digestive tract of New World monkeys (NW - non-human primates of the American continent). Some have no adverse effects,

while others may be associated with clinical illnesses characterized by diarrhea and weight loss (Rodrigues et al., 2021; Silva et al., 2008). Species of the genus *Ancylostoma* are parasites of the human digestive tract, but it is also reported in non-human primates, parasitizing the small intestine. (Araújo & Ferreira, 1997; Rodrigues et al., 2021). According to Toft II and Eberhard (1998), in massive infections the clinical signs are similar to those observed in humans. Anemia is the main change observed - due to the blood that helminths remove from the body -, in addition to weight loss, diarrhea with mucus or blood, dehydration and death in severe cases (Kindlovits, 2009).

In addition to helminths, primates are also parasitized by protozoa. Currently, according to the taxonomic review proposed by Burki et al. (2020), the phyla Amoebozoa, Metamonada, Ciliophora and Apicomplexa have representatives that can be parasites. The phylum Amoebozoa comprises protozoa that use pseudopods to move around, being represented by amoebas, responsible for the so-called intestinal amoebiasis. Several species of amoeba have been described in Platyrrhines, most of which are not pathogenic. However, *Entamoeba histolytica* is known to be pathogenic, causing profuse diarrhea and severe depression in certain neotropical primates, mainly in *Ateles*, *Alouatta* and *Lagothrix*, several cases have also been reported in *Cebus* (Kindlovits, 2009).

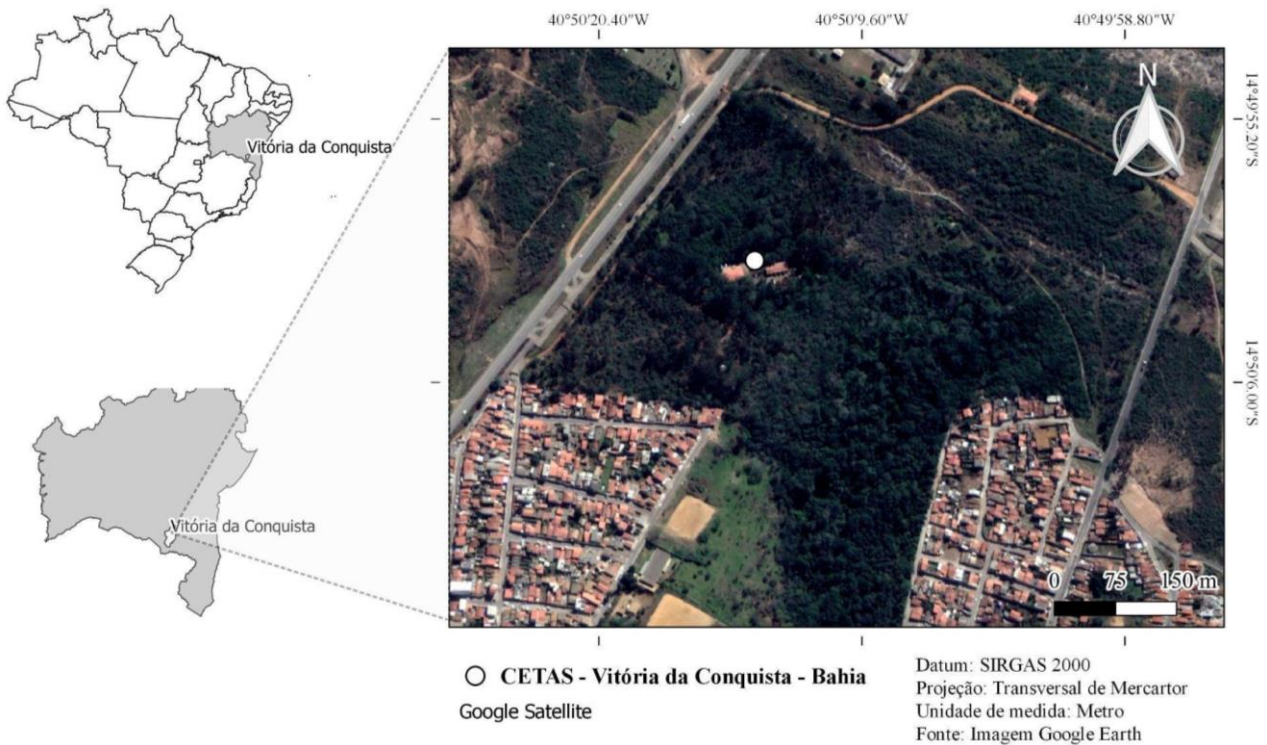
Studying gastrointestinal parasites is important for the management of primates and for maintaining the human's health who work with these animals, as many of these parasites are potent zoonosis causes (Diniz, 1997). This parasites investigation also benefits projects to release primates in the wild, as the presence of endoparasites is quite common in these wild animals, presenting asymptomatic in mild infections, up to severe and lethal clinical manifestations, mainly in animals in captivity that are subjected to high levels of stress, which compromises their immunity, leaving them weakened (Santos, 2005).

Considering the above, the objective of this study was to evaluate gastrointestinal parasitism in primates kept in captivity in a CETAS in Bahia, Brazil.

## 2. Methodology

All procedures described in this study were approved by the Ethics Committee on the Use of Animals (CEUA) of the Multidisciplinary Institute in Health, Campus Anísio Teixeira, Federal University of Bahia (Protocol 007/2013). All the animals analyzed here come from CETAS in Vitória da Conquista-BA (Figure 1) and were submitted to the best management practices, as required by the Brazilian law on the use of animals in research (Law nº 11,794/08).

**Figure 1.** Wildlife Animal Screening Center of Vitória da Conquista, Bahia, Brazil.



Source: Authors.

Figure 1 shows the exact location of the Wildlife Animal Screening Center of Vitória da Conquista, Bahia, Brazil, which is highlighted with a circle with a black border and white filling, approximately in the middle of the image.

The sample of animals consisted of 45 primates, which had their species previously confirmed by Polymerase Chain Reaction (PCR) (Pereira, 2013), namely: (i) 1 of the Atelidae family, species *Alouatta caraya* (Humboldt, 1812) (black howler monkey); (ii) 2 of the Pitheciidae family, both of the species *Callicebus nigrifrons* (Spix, 1823) (guigo); and (iii) 42 from the Cebidae family, being 1 *Sapajus flavius* (Schreber, 1774) (golden capuchin monkey), 18 *Sapajus libidinosus* (Spix, 1823) (yellow capuchin monkey), 5 *Sapajus robustus* (Kuhl, 1820) (crested capuchin monkey) and 18 *Sapajus xanthosternos* (Wied-Neuwied, 1826) (yellow-breasted capuchin monkey). The last two species mentioned are threatened with extinction in the state of Bahia in Brazil, according to the National List of Species of Brazilian Fauna Threatened with Extinction (Brasil, 2003).

All animals evaluated were housed in CETAS in Vitória da Conquista, BA, Brazil ( $14^{\circ}50'01.2''S$   $40^{\circ}50'14.3''W$ ). This CETAS has nineteen enclosures for primates, six enclosures with only one animal, five enclosures with two, five enclosures with three, two enclosures with four and one enclosure with six.

A total of 135 fecal samples were collected fortnightly, making up three collections. As described by Rodrigues et al. (2021), the samples were collected directly from the floor of the enclosures using a shovel and were placed in a transport collector. At each collection, the amount of feces was collected in a number equivalent to the amount of primates allocated in each enclosure, and it was not possible to identify which animal, specifically, each biological material belonged to. Due to this, the results referring to the present research will refer to the total of "infected samples" and not to the number of infected individuals. The collected material was transported in refrigerated thermal boxes to the Zoology Laboratory of the Multidisciplinary Institute in Health, Campus Anísio Teixeira, of the Federal University of Bahia ( $14^{\circ}52'41.0''S$   $40^{\circ}48'43.9''W$ ), where they were analyzed.

In the laboratory, the following procedures were performed: (i) Direct method and (ii) Hoffmann method. The first method consisted of: (i) diluting a small amount of feces in 01 drop of water on the slide; (ii) add 01 drop of lugol and homogenize;

(iii) cover with a coverslip; and (iv) perform the reading under an optical microscope. The second method consisted of: (i) weighing 2g of feces; (ii) dissolving in distilled water; (iii) filter the fecal material in a cup with a gauze, leaving it to rest for two hours; (iv) preparing a lugol stained slide using the filtered material; and (v) perform readings under an optical microscope (Hoffmann, 1987). The samples were identified through identification plates containing photos of primate gastrointestinal parasites. In samples where helminth eggs were found, coproculture was carried out, in order to obtain third-stage larvae, to better identify them (Roberts & O'Sullivan, 1950).

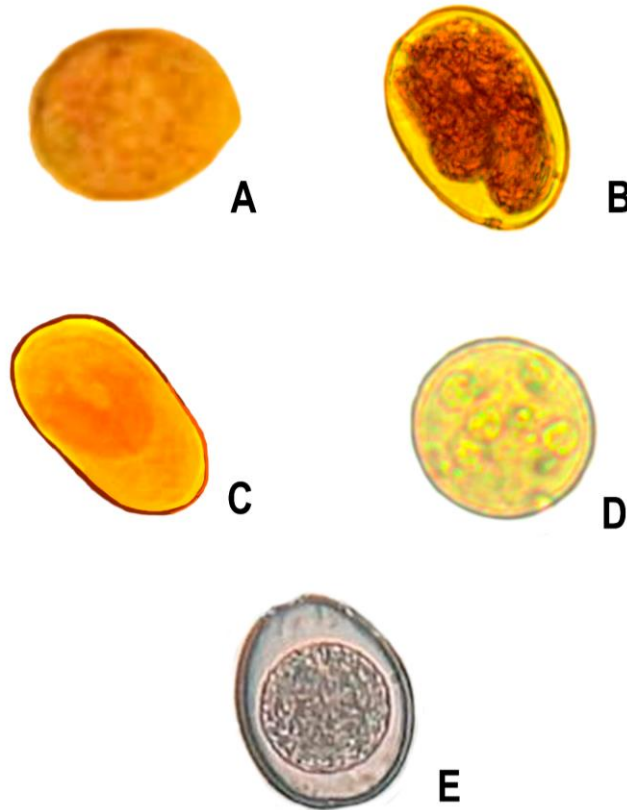
Coproculture consisted of weighing 20g of feces and homogenizing with sterilized sawdust in an equal volume in a flask containing 1mL of distilled water. The container with the mixture material was partially covered with a petri dish, keeping a small opening for the aeration of the culture to occur. This organization was achieved with the help of the inclusion of a string between the petri dish and the container with the material. This material was incubated at room temperature for 7 days. After this period, in order to recover the infective larvae, the culture flask was filled with distilled water, capped with a petri dish and abruptly inverted. The remaining space of the petri dish was filled with 10mL of water and after 4h the contents present in the dish were transferred to a test tube. This material was kept at 4°C/3h. The precipitate was evaluated for the identification/counting of the larvae, examining them between a slide and a coverslip with the addition of lugol, under a microscope (Roberts & O'Sullivan, 1950).

The assessment of the parasite load for protozoan cysts was based on the classification by Gonçalves et al. (1994), who consider mild infection when 1 to 100 cysts or oocysts are found per slide, moderate when 101 to 300 cysts or oocysts are found, and high when more than 301 cysts or oocysts are counted per slide. The assessment of this load was performed using the Hoffmann method, a semi-quantitative method, as presented by Rodrigues et al. (2021).

### 3. Results

Using the Hoffman technique, parasitism by three protozoa was observed: (i) *Balantidium* sp., (ii) *Cystoisospora* sp. and (iii) *Entamoeba* sp.; and two helminths: (i) *Ancylostoma* and (ii) *Strongyloides* (Figure 2 A-E). *Balantidium* sp. had a prevalence of 15.6% in the sample of animals, *Cystoisospora* sp. 91.1%, *Entamoeba* sp. 24.4%, *Ancylostoma* sp. 75.6% and *Strongyloides* sp. 64.4% (Table 1). Only the samples of *S. robustus* and *S. xanthosternos* were infected by all parasites. No parasites were present in all samples simultaneously (Table 2).

**Figure 2.** Protozoa and helminths observed in fecal samples from non-human primates from the Wildlife Animal Screening Center in Vitória da Conquista, BA, Brazil. A-E: Objective 40X, F-G: Objective 4X.



Source: Authors.

Figure 1 shows some of the protozoa and helminths observed in the fecal samples analyzed in the present study. In “A” can be observed a cyst of *Balantidium* sp., in “B” an egg of *Ancylostoma* sp., in “C” an egg of *Strongyloides* sp., in “D” an *Entamoeba coli* cyst and in “E” a *Cystoisospora* oocyst.

**Table 1.** Total number and prevalence of samples infected by each gastrointestinal parasite identified in stool samples collected at the Vitória da Conquista Wildlife Animal Screening Center, BA, Brazil.

Parasite	Parasitized primates	
	n	%
<b>Protozoa</b>		
<i>Balantidium</i> sp.	07/45	15,6%
<i>Cystoisospora</i> sp.	41/45	91,1%
<i>Entamoeba</i> sp.	11/45	24,4%
<b>Helminths</b>		
<i>Ancylostoma</i> sp.	34/45	75,6%
<i>Strongyloides</i> sp.	29/45	64,4%

Source: Research data.

**Table 2.** Number of infected samples by species, for each gastrointestinal parasite identified in stool samples collected at the Vitória da Conquista Wildlife Animal Screening Center, BA, Brazil.

Parasite	Number of parasitized animals					
	<i>A. caraya</i> (n=1)	<i>C. nigrifrons</i> (n=2)	<i>S. flavius</i> (n=1)	<i>S. libidinosus</i> (n=18)	<i>S. robustus</i> (n=5)	<i>S. xanthosternos</i> (n=18)
<b>Protozoa</b>						
<i>Balantidium</i> sp.	-	-	-	-	3/5	4/18
<i>Cystoisospora</i> sp.	1/1	2/2	-	17/18	5/5	16/18
<i>Entamoeba</i> sp.	-	2/2	-	2/18	2/5	5/18
<b>Helminths</b>						
<i>Ancylostoma</i> sp.	-	2/2	1/1	17/18	5/5	9/18
<i>Strongyloides</i> sp.	-	-	1/1	16/18	3/5	9/18

Source: Research data.

Table 1 shows the prevalence of each parasite found over the total sample. Table 2 shows the relationship between Primate species and the number of infected samples by each of the identified gastrointestinal parasites.

The parasite load for protozoan cysts was considered moderate only for infections by *Cystoisospora* sp. in primates *S. libidinosus*, *S. robustus* and *S. xanthosternos* (individuals shared an enclosure with more than 5 primates). On the other hand, *C. nigrifrons* and *A. caraya* (allocated in individual enclosures) showed mild infection of this parasite. The other protozoan infections were considered mild. Only the species *S. flavius* did not show any protozoan infection (Table 2).

Of the 135 samples analyzed, helminth eggs were found in 102. These 102 samples were submitted to coproculture, through which *Ancylostoma* sp. and *Strongyloides* sp., having been identified in 76% of the L3 or filarioid larvae samples (Figure 2 F-G). Only the species *A. caraya* did not show any helminth infection (Table 2).

#### 4. Discussion

Usually works investigating the presence of gastrointestinal parasites in non-human primates report the presence of *Balantidium* spp. (Barbosa et al., 2015; Kouassi et al., 2015; Levecke et al., 2007; Sestak et al., 2003). Studies with New World (NW) Primates show low percentages of sample prevalence, being similar, but lower than what was found here (15.6%). Levecke et al. (2007), for example, did not find monkeys infected with *Balantidium* spp. when they studied captive NW primates. Barbosa et al. (2015) observed *Balantidium coli* in 7% of samples from NW monkeys in one of the evaluated groups.

In the present work, the primate species *C. nigrifrons* and *A. caraya* presented mild infection by *Cystoisospora*, probably because they were in separate enclosures, since, in contrast, the species *S. libidinosus*, *S. robustus* and *S. xanthosternos*, which shared an enclosure with more of five primates, showed a moderate degree of infection. Indicating that the increase of animals per enclosure can interfere with the welfare of the animals, causing stress and providing the emergence of this coccidian. Considering previous studies with non-human primates, which recorded a prevalence of less than 50% for *Cystoisospora* sp., the sample prevalence (91.1%) found here for this parasite can be considered high (Li et al., 2017; Perea -Rodriguez et al., 2010).

Andrade et al. (2002) states that NW Primates are more susceptible to *Entamoeba* infection than Old World Primates (OW- non-human primates from the African and Asian continents), which does not corroborate the results found in the present

study. For *Entamoeba* sp. we obtained a sample prevalence of just over 24%, when this percentage is compared with studies including OW primates, we can consider it a low percentage. Kouassi et al. (2015), investigated gastrointestinal parasites in non-human African primates and found up to 91.96% prevalence of *Entamoeba coli*, not counting two other *Entamoeba* species found. Sestak et al. (2003), evaluating *Macaca mulatta* (OW) observed a prevalence of 90% of amoeboid protozoa in the evaluated sample. Li et al. (2015) stated that they had observed a higher prevalence of infection by *Entamoeba* spp. in OW monkeys. Munene et al. (1998), evaluating gastrointestinal parasites in non-human primates from Africa, found a prevalence of up to 74% of *E. coli* in captive animals.

On the other hand, and strengthening our argument, previous works including NW primates, obtained lower percentages of infection by *Entamoeba* spp., when compared to the studies cited here using OW primates. Levecke et al. (2007), investigating the presence of gastrointestinal protozoa in captive non-human primates, including *Atelidae* and *Cebidae*, found a prevalence of 40% of *Entamoeba* spp. in NW primates. Perea-Rodriguez et al. (2010), recorded a percentage of 23% of the total sample for *Entamoeba* sp. in *Aotus azarai azarai*. Barbosa et al. (2015), in a study in Brazil, obtained, for captive NW primates, a prevalence of 8.7% of *Entamoeba* sp. in one of the evaluated groups. Phillips et al. (2004), observed a prevalence of 4.6% of *Entamoeba* sp. in NW primates.

The helminths found here, *Ancylostoma* sp. and *Strongyloides* sp., are common parasites in non-human primates (Alcântara et al., 2016; Batista et al., 2021; Figueiredo et al., 2020; Kouassi et al., 2015; Li et al., 2017). Previous studies, in most cases, present *Strongyloides* sp. with a lower prevalence percentage than that found here (64.4%), with 3.7% (Li et al., 2015), 5% (Levecke et al., 2007), 6.2% (Li et al., 2007), 6.2% (Li et al., 2015). al., 2017), 15% (Phillips et al., 2004), 23% (Alcântara et al., 2016) and 30% (Perea-Rodriguez et al., 2010). However, other studies have reported a sample prevalence of *Strongyloides* sp. more equivalent to that of this study: 74.42% (Kouassi et al., 2015) and 44.8% (Munene et al., 1998). For *Ancylostoma* sp. the same pattern is repeated, as the prevalence is also generally lower than that found here (75.6%): 0.5% (Li et al., 2017), 8.3% (Michaud et al., 2003) and 45% (Alcântara et al., 2016). However, there are exceptions such as the work by Kouassi et al. (2015), who observed a prevalence of 73.87% of *Ancylostoma* sp., very close to the percentage of the present study. It is believed that the high prevalence of helminths observed in the present work may be linked to two main reasons: (i) the probable low immunity of primates coming from the trafficking of wild animals, since stress impairs animal immunity (Müller et al., 2005), making them susceptible to parasites; and (ii) the agglomeration in captivity, since more than 50% of the animals were allocated in ponds with three or more individuals, the very close contact within the ponds may have provided the rapid transmission and maintenance of these parasites in these populations, through food and water contaminated by the primates themselves.

## 5. Conclusion

As can be seen, infections of non-human primates by helminths and protozoa are not rare, several species of these parasites are normally found in these animals, and in many cases they can reach humans. The results of the present study evidence the need to carry out parasitological evaluations in captive primates. Because, identifying the gastrointestinal parasites that affect the squad helps in the execution of targeted treatment and periodically evaluating allows to verify the efficiency of the medications used. Therefore, it is recommended for the future that similar work be carried out in the long term, aiming to monitor post-treatment gastrointestinal parasites, determining aspects such as the time of parasite elimination. Finally, it is expected that the present work will influence research institutions throughout the country, to dedicate efforts to assist the CETAS in the parasitic evaluation of non-human primates, as well as in the performance of several other tests in all wild species allocated in the Brazilian CETAS.



## Acknowledgments

We thank to the Wildlife Animal Screening Center of Vitória da Conquista-BA and its technicians, biologists and veterinarians, for having opened the doors to carry out this research and for assisting in the collection of biological materials, we especially thank Aderbal Alves, Rosana Ladeia, Gisele Filadelfo and Caio Márcio Rodrigues Santos.

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