Intestinal Protozoa in apprehended New World nonhuman primates

Abstract

The objective of this study was to identify intestinal protozoa in fecal samples of illegally commercialized New World nonhuman primates apprehended by governmental authorities and sent to a Wildlife Screening Center in the municipality of Seropédica, State of Rio de Janeiro, under the administration of Instituto Brasileiro de Meio Ambiente e Recursos Naturais. Feces from marmosets (crossbreeding among Callithrix jacchus and C. penicillata) and brown howler monkeys (Alouatta fusca) were collected and submitted to Richtie’s formaldehyde-ether sedimentation technique. Smears were made with the sediment and submitted to safranin-methylene blue and Wheatley’s modified trichrome stainings. Phenol-auramine staining was used in order to compare positive smears for Cryptosporidium oocysts stained with safranin-methylene blue technique. Three (100%) fecal samples of brown howler monkeys and eight of common marmosets (88.9%) were positive for Cryptosporidium. Blastocystis hominis-like forms were observed in all samples of brown howler monkeys and in 66.7% (6/9) of marmosets. New World nonhuman primates Callithrix crossbreed species and A. fusca can harbor forms similarly identified as potential zoonotic organisms.

Introduction

Increased probability of parasitism is a cost of living in a group\(^1\). Many monkey species (e.g. Alouatta seniculus) have chronic endoparasite infections. Most of these infections are probably asymptomatic, based on low number of parasites per individual monkeys and qualitative measures of health, although, some of the parasite species are potential pathogens\(^2\). Protozoa of the genus Cryptosporidium have been found in feces of many mammalian hosts and it is associated to acute enterocolitis\(^3,4,5\). Cryptosporidium infection is known to be transmitted through person-to-person transmission or through zoonotic transmission\(^6\). The organism Cryptosporidium is an emergent zoonotic agent reported in humans in 1976 at which time it was regarded as a species of animal parasites. Of great concern is that parasitism in wildlife species has been observed to increase in both wild and captive populations, possibly due to stress associated with capture, transport and husbandry of these species. This study aimed to identify intestinal protozoa in fecal samples of illegally commercialized New World nonhuman primates apprehended by governmental authorities and sent to a Wildlife Screening Center in the municipality of Seropédica, State of Rio de Janeiro, under the administration of Instituto Brasileiro de Meio Ambiente e Recursos Naturais.
as a rare opportunistic infection of immunocompromised individuals. Nowadays, cryptosporidiosis is one of the most common second diagnoses in AIDS patients. Cryptosporidium infection is diagnosed by identifying the oocyst stages of the parasites in host feces or biopsy tissue. Oocysts are difficult to demonstrate during routine fecal examination, because their small size (2-6 µm), and are most commonly identified in clinical laboratories by acid-fast staining of fecal smears or concentrates with carbol fuchsin (cold Kinyoun stain, dimethyl sulfoxide-carbol fuchsin, or Ziehl-Neelsen stain), by fluorescence staining with phenol-auramine, or by oocyst flotation on Sheather’s sucrose solution followed by phase microscopy. Other effective methods of oocysts detection are also used, including safranin staining, iodine staining, Hemacolor staining, methylene blue-eosin staining, Giemsa staining, negative staining by periodic acid-Schiff reagent, electron microscopy, and indirect immuno-fluorescence technique.

Blastocystis hominis is an intestinal parasite that has been observed in three distinct forms: vacuolated, granular and ameboid forms. It reproduces by both binary fission and endosporulation. Although, this organisms is generally though of as a common non-pathogenic inhabitant of the intestinal tract, it has been incriminated as a cause of enteric disease and diarrhea in man. The presence of B. hominis in dogs, cats, monkeys, rats, rabbits, mice, guinea pigs, frogs, snakes, earthworms, houseflies and swines is briefly mentioned by Zierdt & Tan, Boreham and Stenzel, Stenzel and Boreham.

This paper aims at to relate enteroparasitosis in two species of illegally commercialized New World nonhuman primates were acquired in a Wildlife Screening Center (CETAS) located in the municipality of Seropédica in the State of Rio de Janeiro, Brazil (22° 43’ S, 43° 42’ W, height: 40 meters). CETAS receives illegally commercialized wild animals apprehended by governmental authorities.

Nonhuman primates examined

Fecal samples were acquired from four brown howler monkeys (Alouatta fusca) and 21 hybrid marmosets (Callithrix spp.). One stool sample was acquired from a posted severely injured newcomer marmoset that died few hours after arriving at CETAS. The indetermination of Callithrix species examined in the present study is due to hybridization commonly found among specimens from this genus (crossbreeding between Callithrix jacchus – common marmoset – and C. penicillata – black tufted-ear marmoset). Newcomer animals are arranged in 40 m³ cages for howler monkeys and 2.5 m³ for marmosets respecting group organization observed when they arrive at the center. Age varied too much, and being apprehended animals, it was virtually impossible to determine it. Nevertheless, animals could be classified into three age groups: juveniles, young adults and elderly animals.

Sample collects and material procedures

It was operationally impossible to proceed to individual collets from all animals housed at CETAS for the following reasons: (i) primates have been lodged into groups and to proceed to individual collets, animals would have to be captured, anesthetized and submitted to anal stimulation to induce defecation; (ii) these procedures conducted to animals that were not submitted to previous feeding stopping, would cause health risks and (iii) submit animals to isolation would induce stressing, which would be a harmful factor to the parasite research and to the study. To avoid
theses factors, clean sheets of paper were distributed over the floor, along the place where the animals are used to defecate. Only feces found over the paper were used as samples, which were collected 24 hr post-floor defense. Approximately ten grams of fresh feces were put in properly labeled, clean wide mouthed containers with tightly fitting lids, fixed in polyvinyl alcohol (PVA) and immediately sent to the Department of Animal Parasitology at the Veterinary Institute in the Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil. At the laboratory, samples were diluted in PBS pH 7.2, drizzled throughout a double-layered gauze and submitted to modified formaldehyde-ether centrifuge-sedimentation technique. One drop of the sediment was transferred to a glass microscope slide, added to another drop of Lugol’s iodine solution (6%), covered with a coverglass and examined in a light microscope. Duplicate sediment smears were done using the material obtained by the previous technique and stained by safranin-methylene blue (S-MB) technique and Wheatley’s trichrome technique to identification of Cryptosporidium oocysts and other intestinal protozoa in light microscope respectively. Fecal smears positive for Cryptosporidium oocysts were submitted to phenol-auramine (P-A) staining and read on an Epi-fluorescence microscope in order to corroborate diagnoses using S-MB technique.

Measurement of Cryptosporidium oocysts
Cryptosporidium oocysts were measured by using a K-15X micrometric ocular (Carl Zeiss Jena) in a tri-ocular microscope (Jena Med, Carl Zeiss Jena) to confirm similarity among specimens identified in the present study and those that are already known to parasite other nonhuman primates.

Results

Health status of the animals
During the period of clinical evaluation, only two juveniles brown howler monkeys had diarrhea. Feces obtained from the cage, where these animals were housed, were weakly molded. Cryptosporidium oocysts (from 6 to 10 oocysts per field) were found in the sediment smear stained by S-MB technique while low infection by Blastocystis hominis-like forms was identified by trichrome staining technique (less than 1 organism per field).

Centrifuge-sedimentation technique
In a total of 12 samples, corresponding to 25 animals included in the present study, in only one of them it became possible to identify some parasite related structure using the centrifuge-sedimentation technique. Blastocystis hominis-like life stages, very similar to granular forms of this organism, were found in wet amount of the fecal sediment from stools collected under a common marmoset cage (Figure 1). No other protozoa or helminth ova were found using this technique.

Safranin - methylene blue technique
Ten stool samples (91.7%) submitted to S-MB technique were positive for Cryptosporidium oocysts (Tables 1 and 3). Oocysts observed were bright, red in color, refractile and round in shape, seen against a blue background. Internal morphology was observed in many of the stained oocysts (Figure 2). They measured from 2.0 to 4.3 µm in diameter. Of 40 oocysts measured, length and width were 3.18 ± 0.52 and 2.80 ± 0.55 µm, respectively. Samples positive for Cryptosporidium oocysts on S-AM staining were also positive on P-A staining (Figure 3).

Trichrome staining technique
Seven fecal samples (75%) were positive for some protozoa parasite when observed on slides stained by Wheatley’s trichrome staining (Tables 2 and 4). Blastocystis hominis-like (Figure 4) forms were visualized on seven slides stained by this technique while Chilomastix mesnili cysts were found in only
Discussion

Through these results, individuals of the species *A. fusca* and *Callithrix* crossbreed species can be infected by the intestinal protozoans *Cryptosporidium*, *C. meleagridi* and *B. hominis*-like. Special attention must be given to the first and the third parasites due to (i) high frequency that these were found in fecal samples and (ii) the possibility that these organisms in being transmitted from monkeys to human. Clinical signs seem not to be obligate in healthy animals similarly to infection in humans. It is not possible to assert if the juvenile howler monkeys evaluated in the present study either had diarrhea due to *Cryptosporidium* infection or had considerably large quantity of oocysts in fecal stool caused by stressing process on account of other factors as housing conditions, food adaptation or reduced activity in captive environment. *Blastocystis* seems to be submitted to same type of doubts.

Another aspect that deserves comments is the fact that none monkey submitted to this study had helminth ova in their feces. It is known that some primates of the genus *Alouatta* have a particular behavior related to defecation sites and group arrangement. This feature seems to make members of the group with low rate of parasite infection and possibly conducts to low rate of parasitism even inside screening facilities.

Some nonhuman primate (NHP)
species have been described as hosts of *Cryptosporidium*, including *Papio hamadryas* (=cynocephalus), *Macaca nemestrina*, *M. mulatta*, *M. radiata*, *M. fascicularis*, *Saimiri* species4,33,34,35,36. *Cryptosporidium* oocysts were detected in feces from *Ateles belzebuth*, *Cercopithecus torquatus lunulatus*, *Cercopithecus aethiops*, *C. campbelli*, *C. talapoin*, *Erythrocebus patas*, *M. mulatta* and *Lemur macaco-mayottensis*, in a study designed at the Barcelona zoo. No clinical sign of cryptosporidiosis was observed35. A study developed with monkeys trapped in the rural area of Kenya revealed that 70.7% of vervet monkeys (*C. aethiops*) and 28.8% of olive baboons (*P. cynocephalus anubis*) had *Cryptosporidium* oocysts in fecal samples38.

McClure 39 related a single case of diarrhea in pig-tailed macaque (*Macaca nemestrina*) where it was found large number of *B. hominis* forms in fecal smears. Beyond this species, *Blastocystis* was also found in feces samples of *M. mulatta* (100% of the samples), *M. fuscata* fuscata (75%), *M. radiata*

<table>
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<th>Species</th>
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<th>Samples examined</th>
<th>Samples positive</th>
<th>Positive rate (%)</th>
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<td><em>Alouatta fuscata</em></td>
<td>Brown howler monkeys</td>
<td>3 (04)</td>
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<tr>
<td><em>Callithrix sp.</em></td>
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<td>8 (17)</td>
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<td>11 (21)</td>
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Number inside parenthesis means animals examined or included in positive samples.

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<td>9 (19)</td>
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<th>More</th>
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<tr>
<td><em>Alouatta fuscata</em></td>
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<td>2 (02)</td>
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<tr>
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<td>3 (09)</td>
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<td><strong>Total</strong></td>
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<td>5 (11)</td>
<td>1 (2)</td>
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Number outside parenthesis means samples included in each intensity cell and inside parenthesis means number of animals

(+): < 1 oocyst per slide field; (+): 1-5 oocysts per field; (++): 6-10 oocysts per field; (+++): > 10 oocysts per field

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<td>5 (12)</td>
<td>4 (07)</td>
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a) Number outside parenthesis means samples included in each intensity cell and inside parenthesis means number of animals

(+): < 1 organism per slide field; (+): 1-5 organism per field; (++): 6-10 organism per field; (+++): > 10 organism per field
(100%) and *Saimiri sciureus* (100%), collected from monkeys bred at the Monkey Care Laboratory of the Primate Research Institute of Kyoto University, revealing high rate of infection by this organism. Fecal smears of common marmosets (*C. jaccus*) were searched for *B. hominis* in animals housed at the same place and in none of them it was found any organism related to this parasite.

Zoonotic transmission of genotype 2 anthropozooophilic *Cryptosporidium parvum* was identified among free-ranging mountain gorillas (*Gorilla gorilla beringei*) and humans who work or live within the Bwindi Impenetrable National Park boundaries. About *B. hominis*, phylogenetic studies reveal relative similarity among strains isolated from *M. fascata fascata* and human. Primate species, especially common marmosets, are used to live in strict contact with humans as pets, an illegal but usual practice. This fact can submit humans to risk in acquiring parasites phylogenetically similar to anthroponotic protozoa and start inducing cross-transmission among nonhuman primates and humans. In other hand, workers that handle these animals, as veterinarians, biologists or general staff in zoos, wildlife screening centers or wildlife preservation parks, have to be informed concerning the risks involved in their jobs and how to avoid or decrease it to the minimum acceptable.

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References


