Presence and genetic diversity of enteric protists in captive and semi-captive non-human primates in côte d’Ivoire, Sierra Leone, and Peru

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ABSTRACT

Little information is currently available on the occurrence and genetic diversity of pathogenic and commensal protist species in captive and semi-captive non-human primates (NHP) resident in zoological gardens or sanctuaries in low- and medium-income countries. In this molecular-based study, we prospectively collected individual faecal samples from apparently healthy NHP at the Abidjan Zoological Garden (AZG) in Côte d’Ivoire, the Tacugama Sanctuary (TS) in Sierra Leone, and the Quistococha Zoological Garden (QZG) in Peru between November 2018 and February 2020. We evaluated for the presence of pathogenic (Cryptosporidium spp., Entamoeba histolytica, Giardia duodenalis, Blastocystis sp., Enterocytozoon bieneusi, Balantidoides coli) and commensal (Entamoeba dispar, Troglodytella abrassarti) protist species using PCR methods and Sanger sequencing. Giardia duodenalis was the most prevalent species found (25.9%, 30/116), followed by Blastocystis sp. (22.4%, 26/116), and E. dispar (18.1%, 21/116). We detected E. bieneusi (4.2%, 1/24) and T. abrassarti (12.5%, 3/24) only on NHP from AZG. Cryptosporidium spp., E. histolytica, and B. coli were undetected at the three sampling sites investigated here. Sequence analyses revealed the presence of zoonotic sub-assemblages BIII (n = 1) in AZG and BV (n = 1) in TS within G. duodenalis. We identified Blastocystis subtype ST3 (100%, 6/6) in AZG, ST1 (80.0%, 12/15), ST2 (6.7%, 1/15), and ST3 (13.3%, 2/15) in TS, and ST2 (80.0%, 4/5) and ST3 (20.0%, 1/5) in QZG. The only E. bieneusi isolate detected here was identified as zoonotic genotype CAF4. Our PCR-based data indicate that potentially pathogenic protist species including G. duodenalis, Blastocystis sp., E. bieneusi, and B. coli are present at variable rates in the three NHP populations investigated here. The identification of zoonotic genotypes within these species indicates that human-NHP transmission is possible, although the extent and directionality of these events need to be elucidated in future molecular surveys.

1. Introduction

Infections by enteric parasites including helminths and protists are a frequent cause of gastrointestinal disease in captive and semi-captive non-human primates (NHP) globally (Levecke, 2010). Reported clinical manifestations vary greatly from subclinical (asymptomatic)
infections to acute or self-limiting diarrhoea, malabsorption, severe dehydration, granulomatous inflammation, peritonitis, and even death (Johnson-Delaney, 2009). Clinical signs and symptoms are strongly dependent on several factors including the host species considered, the parasite load, the diversity and balance of the diet, the enclosure conditions (confined environments enhance the likelihood of parasitic transmission), or stress-induced immunosuppression conditions, among others (Strait et al., 2012).

Protozoa commonly identified in captive and semi-captive NHP include pathogenic species of veterinary relevance such as the protozoan Cryptosporidium spp., Entamoeba histolytica, and Giardia duodenalis or the microsporidia Enterocytozoon bieneusi. Organisms of uncertain pathogenicity or commensal nature such as the stramenopile Blastocystis sp. and the ciliates Balantidoides coli and Troglodytella abrasarti are also a common finding in the faeces of captive NHP (Johnson-Delaney, 2009; Medkour et al., 2020; Helenbrook and Whipps, 2021). All the above-mentioned protozoan species have been documented in NHP collections (Helenbrook and Whipps, 2021). All the common finding in the faeces of captive NHP (Johnson-Delaney, 2009; Nath et al., 2012; Du et al., 2015; Adrus et al., 2019 Zhao et al., 2020), African (Munene et al., 1998; Pomajbiková et al., 2010a,b), Li et al., 2011; Mbaya and Udeneye, 2011; Ryan et al., 2012; Adetunji, 2014; Debenham et al., 2015; Kouassi et al., 2015), American (Phillips et al., 2004; Wenz et al., 2010; Milozzi et al., 2012; da Silva Barbosa et al., 2015), and European (Levecke et al., 2007; Berrilli et al., 2011; Köster et al., 2021a) countries. These studies revealed marked differences in occurrence rates depending on host species, geographical location, and detection method used. Older epidemiological surveys primarily relied on microscopy examination, a method known to underestimate the true prevalence of enteric parasites due to limited diagnostic sensitivity (Mejia et al., 2013). The advent of molecular-based methods in well-designed studies targeting multiple host species has contributed enormously to overcome this drawback. Additionally, when coupled to Sanger sequencing, PCR assays allows the accurate determination of species/genotypes, an information extremely useful to investigate transmission dynamics including the occurrence and directionality of zoonotic events. For instance, transmission of Blastocystis has been demonstrated between captive NHP and their keepers in a zoological garden in the UK (Stensvold et al., 2009).

Sequence analyses of housekeeping genes including the small subunit (ssu RNA) or the internal transcribed spacer (ITS) region of the ribosomal RNA gene have revealed a high degree of genetic diversity within Cryptosporidium spp., G. duodenalis, Blastocystis sp., and E. bieneusi, leading to the identification of several genotypes/subtypes with marked differences in host specificity and range (Franzén et al., 2009; Stensvold et al., 2012; Li and Xiao, 2019; Garcia-R et al., 2020). Thus, the genus Cryptosporidium encompasses at least 46 valid species, with C. hominis and C. parvum causing most of the infections reported in humans and NHP globally (Ježková et al., 2021; Zahedi et al., 2021). Giardia duodenalis is constituted of eight (A to H) distinct genetic assemblages, of which zoonotic assemblages A and B are able to infect a wide diversity of mammal species including humans and NHP (Ryan and Cacció, 2013). At least 28 subtypes (ST) have been identified within Blastocystis sp. (Higuera et al., 2021; Maloney and Santin, 2021; Maloney et al., 2021) of which ST1–ST5, ST7–ST11, ST13, ST15, and ST19 have been previously reported in NHP (Hublin et al., 2021). More than 500 E. bieneusi genotypes have been defined to date grouped in 11 phylogenetic groups (Li et al., 2019). Of them, at least 78 genotypes have been reported in NHP, with genotype D showing predominance (Zhong et al., 2017; Chen et al., 2019; Li et al., 2019; Karim et al., 2020; Zhao et al., 2020). Comparatively, there is much less information on the genetic variability within other protozoan species including E. histolytica, E. dispar, B. coli, or T. abrasarti (Ponce-Gordo and García-Rodríguez, 2021; Vallo et al., 2012; Weedall et al., 2012). This situation is likely associated to the scarcity of molecular data and the limited range of genetic markers available for typing and subtyping purposes (Gilchrist et al., 2012).

This molecular-based study aims at providing novel data on the occurrence and genetic diversity of enteric protist species of pathogenic and commensal nature in captive and semi-captive NHP in three different epidemiological scenarios in two African (Côte d’Ivoire and Sierra Leone) and one South American (Peru) countries. Information provided here is expected to improve our current knowledge on the epidemiology of these microorganisms and their zoonotic potential in NHP.

2. Materials and methods

Ethical statement

This study was carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (RD 53/2013). This study has been approved by Ethics Committee of the Health Institute Carlos III on December 17, 2018 under the reference number CEI PI-90-2018-v2. Written informed consent was obtained from zookeepers that volunteered to participate in the survey.

2.1. Study area in côte d’Ivoire

We collected faecal samples (n = 24) from captive NHP at the Abidjan Zoological Garden (AZG, Abidjan, Côte d’Ivoire) between April–August 2019. The AZG include 230 individuals belonging to 48 mammalian, reptilian, and avian species. Collected faecal samples belonged to six different genera of NHP including Cercocebus (n = 3), Cercopithecus (n = 1), Chlorocebus (n = 2), Erythrocebus (n = 3), Pan (n = 11), and Papio (n = 4). NHP of the same species were kept in specific enclosures without contact with other NHP.

2.2. Study area in Sierra Leone

We collected faecal samples (n = 67) from captive chimpanzees at Tacugama Sanctuary (TS) in Sierra Leone between November and December 2018. The Sanctuary is placed within the forest of Western Peninsula National Park, but Sanctuary chimpanzees have no contact with the wild ones in the park because they are isolated by fences. The chimpanzees sampled are living in enclosures, with access to forested fenced areas of surfaces smaller than 1 ha. They have daily proximity to humans during feeding (food is brought from the market) and regular veterinarian treatments.

2.3. Study area in Peru

We collected faecal samples (n = 25) from captive NHP at the Quistococha Zoological Garden (QZG, Iquitos, Peru) in February 2020. The QZG extends over 369 ha of secondary rainforest and includes individuals belonging to near 70 mammalian, reptilian, and avian species. Collected faecal samples belonged to seven different genera of NHP including Alouatta (n = 5), Alouatta (n = 1), Cebus (n = 4), Lagotrix (n = 3), Pithecia (n = 1), Saimiri (n = 3), and Sapajus (n = 8). NHP of the same species were kept in specific enclosures without contact with other NHP. Additionally, we collected individual stool samples from three zookeepers in close contact with NHP that volunteered to participate in the study.

2.4. Faecal samples conservation

We collected 5–10 g from the inner core of faeces with sterile plastic spatulas and stored them in 70% ethanol for conservation and transport to the Parasitology Reference and Research Laboratory, Spanish National Centre for Microbiology, Majadahonda (Spain) for further processing and downstream molecular testing. We collected information on
NHP species, enclosure conditions, sampling date, and faecal consistency (diarrheal versus non-diarrheal). No information was available regarding sex and gender, as faecal specimens could not be traced back to individual animals.

2.5. DNA extraction and purification

We isolated genomic DNA from about 200 mg of each faecal specimen by using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions, except that samples mixed with InhibitEX buffer were incubated for 10 min at 95 °C. Extracted and purified DNA samples were eluted in 200 μL of PCR-grade water and kept at 4 °C until further molecular analysis.

2.6. Molecular detection of Cryptosporidium spp

We assessed the presence of Cryptosporidium spp. using a nested-PCR protocol to amplify a 587-bp fragment of the ssu rRNA gene of the parasite (Tiangip and Jongwutiwes, 2002). Amplification reactions (50 μL) consisted of 3 μL of DNA sample and 0.3 μM of the primer pairs CR-P1/CR-P2 in the primary reaction and CR-P3/CR-P6 in the secondary reaction (Table S1). Both PCR reactions were carried out as follows: one step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 50 °C for 40 s and 72 °C for 1 min, concluding with a final extension of 72 °C for 10 min.

2.7. Molecular differential detection of Entamoeba histolytica and Entamoeba dispar

We carried out detection and differential diagnosis between pathogenic E. histolytica and non-pathogenic E. dispar by a qPCR method targeting a 172-bp fragment of the gene codifying the ssu rRNA gene of the E. histolytica/E. dispar complex (Verweij et al., 2003a; Gutiérrez-Cisneros et al., 2010). Amplification reactions (25 μL) consisted of 3 μL template DNA, 0.5 μM of the primer set Ehd-239F/Ehd-88R, 0.2 μM of each TaqMan® probe (Table S1), and TaqMan® Gene Expression Master Mix (Applied Biosystems, CA, USA). Detection of parasitic DNA was performed on a Corbett Rotor GeneTM 6000 real-time PCR system (Qiagen) using an amplification protocol consisting of an initial hold step of 2 min at 55 °C and 15 min at 95 °C followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C.

2.8. Molecular detection and characterization of Giardia duodenalis

We conducted G. duodenalis DNA detection using a real-time PCR (qPCR) method targeting a 62-bp region of the gene codifying the ssu rRNA gene of the parasite (Verweij et al., 2003b). Amplification reactions (25 μL) consisted of 3 μL of template DNA, 0.5 μM of each primer Gd-80F and Gd-127R, 0.4 μM of probe (Table S1), and 12.5 μL TaqMan® Gene Expression Master Mix (Applied Biosystems). Cycling conditions and data analysis were described above for the detection of E. histolytica/E. dispar.

We subsequently assessed G. duodenalis isolates that tested positive by qPCR by sequence-based multi-locus genotyping of the genes encoding for the glutamate dehydrogenase (gdh) (Read et al., 2004), β-giardin (bg) (Lalle et al., 2005), and triose phosphate (tpi) (Sulaiman et al., 2003) proteins of the parasite. We conducted amplifications by semi-nested and nested PCR protocols using specific primer pairs (Table S1).

2.9. Molecular detection and characterisation of Blastocystis sp

We identified Blastocystis sp. by a direct PCR protocol targeting the ssu rRNA gene of the parasite (Scicluna et al., 2006). The assay uses the pan-Blastocystis, barcode primer pair RDS/BRBR and to amplify a PCR product of ~600 bp. Amplification reactions (25 μL) included 5 μL of template DNA and 0.5 μM of each primer (Table S1). Amplification conditions consisted of one-step of 95 °C for 3 min, followed by 30 cycles of 1 min each at 94, 59 and 72 °C, with an additional 2 min final extension at 72 °C.

2.10. Molecular detection and characterization of Enteroctytozoon bieneusi

We conducted E. bieneusi detection by a nested PCR protocol to amplify the ITS region as well as portions of the flanking large and small subunit of the ribosomal RNA gene as previously described (Buckholt et al., 2002). We used the outer EBITs3/EBITs4 and inner EBITs1/E-BITs2.4 primer sets (Table S1) to generate a final PCR product of 390 bp, respectively. PCR reactions (50 μL) consisted of 1 μL of template DNA and 0.2 μM of each primer. Cycling conditions for the primary PCR consisted of one step of 94 °C for 3 min, followed by 35 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and elongation at 72 °C for 40 s), with a final extension at 72 °C for 10 min. Conditions for the secondary PCR were identical to the primary PCR except only 30 cycles were carried out with an annealing temperature of 55 °C.

2.11. Molecular detection of Balantidium coli

We carried out B. coli detection by a direct PCR assay to amplify the complete ITS1-5.8s-rRNA–ITS2 region and the last 117 bp (3’ end) of the ssu-rRNA sequence of this ciliate using the primer set BS5/BS5RC (Ponce-Gordo et al., 2011). PCR reactions (25 μL) consisted of 2 μL of template DNA and 0.4 μM of each primer (Table S1). PCR conditions were as follows: 94 °C for 10 min; 30 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a final extension for 5 min at 72 °C.

2.12. Molecular detection of Troglydytella spp

Hitherto, we aimed to detect the ciliate mutualist Troglydytella spp. by a direct PCR method targeting a 401-bp fragment of the ITS region of the rDNA (ITS1-5.8s-rDNA-ITS2) (Vallo et al., 2012). PCR reactions (25 μL) contained 2 μL of template DNA and 0.8 μM of each primer ssu-end/LSU-start (Table S1). Conditions of PCR for ITS amplification were initial denaturation for 2 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 50 °C, and 90 s at 72 °C, and terminal elongation for 5 min at 72 °C.

2.13. PCR and gel electrophoresis standard procedures

We carried out all the direct, semi-nested, and nested PCR protocols described above on a 2720 Thermal Cycler (Applied Biosystems). Reaction mixtures included 2.5 units of MyTaq™ DNA polymerase (Bioline GmbH, Luckenwalde, Germany), and 5 × MyTaq™ Reaction Buffer containing 5 mM dNTPs and 15 mM MgCl2. The specific DNA primer and probe sequences used in the present study were detailed in Table S1. We routinely used laboratory-confirmed positive and negative DNA samples of human and animal origin for each parasitic species investigated as controls and included them in each round of PCR. We visualized PCR amplicons on 1.5–2% D5 agarose gels (Conda, Madrid, Spain) stained with Pronasafe (Conda) or Gel Red (Biotium, Fremont, California, USA) nucleic acid staining solutions. We used a 100 bp DNA ladder (Boehringer Mannheim GmbH, Baden-Wurttemberg, Germany) for the sizing of obtained amplicons.

2.14. Sequence analyses

We directly sequenced positive-PCR products in both directions using appropriate internal primer sets (Table S1). We conducted DNA sequencing by capillary electrophoresis using the BigDye® Terminator chemistry (Applied Biosystems) on an ABI PRISM 3130 automated DNA sequencer. We visually inspected the obtained chromatograms for
quality control and for detecting the presence of ambiguous (double peak) positions. Sequences obtained in this study were deposited in GenBank under accession numbers MZ501256–MZ501257 (Giardia duodenalis), MZ496540–MZ496545 (Blastocystis sp.), MZ502643 (Enterocytozoon bieneusi), and MZ502644 (Troglydytella abrasarti).

3. Results

3.1. Occurrence of enteric protist species

We report the PCR-based occurrence rates of enteric protist species identified in the captive and semi-captive NHP population investigated in the present study in Table 1. We present the full dataset of the study including diagnostic and molecular genotyping results in Table S2. All participating NHPs had no apparent gastrointestinal clinical manifestations at sampling, and the consistency of their excrements were formed.

At AZG (Côte d’Ivoire), the most common pathogenic protozoa identified was G. duodenalis (29.2%), followed by Blastocystis sp. (25.0%), and E. bieneusi (4.2%). Non-pathogenic E. dispar and T. abrasarti were found in 54.2% and 12.5% of samples, respectively (Table 1). Giardia duodenalis was detected in members of all six NHP genera present at AZG, and Blastocystis sp. in three (Cercopithecus, Pan, and Papiol), whereas we detected E. bieneusi only in a sooty mangabey (Cercopithecus atys). Regarding commensal protists, E. dispar colonized members of all six NHP genera present at AZG, but T. abrasarti was detected in chimpanzees (Pan troglodytes) only (Table S2).

At TS (Sierra Leone), we identified two pathogenic protist species including G. duodenalis (32.8%) and Blastocystis sp. (22.4%) and a single commensal species (E. dispar, 1.5%) infecting or colonizing the chimpanzee population at this sanctuary (Table 1).

We observed a similar diversity of enteric protist species, although at different frequency rates for some of them, in NHP at IZG (Peru). Thus, G. duodenalis (4.0%) and Blastocystis sp. (20.0%) were the only species of pathogenic significance found, and E. dispar (28.0%) the only commensal species identified (Table 1). We detected G. duodenalis in a monk saki (Pithecia monachus), and Blastocystis sp. in four out of the five genera of NHP (Ateles, Callicebus, Lagothrix, and Pithecia) present at IZG. Entamoeba dispar colonized members of three NHP genera including Ateles, Callicebus, and Lagothrix (Table S2). Regarding human stool samples, we identified the presence of Blastocystis sp. in one of the three zookeepers from IZG Peru that participated in the study. The three individuals tested negative for the remaining enteric protist species investigated here.

We did not detect pathogenic Cryptosporidium spp., E. histolytica, and B. coli in any of the three NHP populations surveyed, whereas T. abrasarti was not circulating among NHP at TS (Sierra Leone) and QZG (Peru) (Table 1). Of note, DNA samples from three chimpanzees at AZG (Côte d’Ivoire) cross-reacted with T. abrasarti when tested by PCR for the presence of B. coli (Table S2). Similarly, DNA samples from 10 chimpanzees (nine from TS Sierra Leone and one from AZG Côte d’Ivoire) cross reacted with parasitic protozoan flagellates of the genus Tetratrichomonas when tested by PCR for the presence of T. abrasarti (Table S2).

3.2. Molecular characterization of enteric protist species

The 30 DNA isolates that yielded a positive result for G. duodenalis by qPCR in the present study (seven from AZG Côte d’Ivoire, 22 from TS Sierra Leone, and one from QZG Peru) generated cycle threshold (CT) values ranging from 25.4 to 39.5 (median: 34.4). We successfully amplified only two of them corresponding to chimpanzee samples from AZG Côte d’Ivoire (CT value: 26.6) and TS Sierra Leone (CT value: 25.4), respectively, at the gdh locus, but not at the bg or tip loci. Sequence analyses revealed the presence of sub-assemble BIII (AZG Côte d’Ivoire) and sub-assemble BIV (TS Sierra Leone) (Table 2).

We confirmed a total of 26 isolates as Blastocystis-positive by Sanger sequencing. Of them, six were from NHP (three chimpanzees, two baboons and a mangabey) at AZG Côte d’Ivoire, 15 from chimpanzees at TS Sierra Leone, and the remaining five from NHP (two brown woolly monkeys, a monk saki, a white-bellied spider monkey, and a yellow-handed titi monkey) at QZG Peru. We identified all six samples from AZG Côte d’Ivoire as ST3 (Table 2). Allele 30 was the most common (66.7%, 4/6) genetic variant found within ST3, whereas no allelic information was available for the remaining two ST3 isolates due to insufficient sequence quality.

We identified three Blastocystis STs circulating among chimpanzees at TS Sierra Leone including ST1 (80.0%, 12/15), ST2 (6.7%, 1/15), and ST3 (13.3%, 2/15). We confirmed allele 8 as the most prevalent genetic variant within ST1 (75%, 9/12), followed by mixed infections (determined by the presence of a clear double peak at chromatogram inspection) involving alleles 7 + 8 (25%, 3/12). ST2 and ST3 isolates could not be analysed at the allelic level due to suboptimal sequence quality associated with one or more miscalled nucleotide positions (Table 2).

We found ST2 as the most prevalent Blastocystis subtype present in NHP at QZG Peru (80.0%, 4/5) followed by ST3 (20.0%, 1/5). Alleles 11 (25%, 1/4), alleles 11 + 12 (50.0%, 2/4) were identified within ST2. The allelic form of the fourth ST2 isolate could not be determined due to insufficient sequence quality. The only isolate belonging to ST3 was characterised as allele 34 (Table 2). The zookeeper that tested positive to Blastocystis was colonized by ST1 allele 4, not present in any of the NHP investigated in this zoological garden.

Of note, additional 37 isolates (nine from AZG Côte d’Ivoire, and 28 from TS Sierra Leone) yielded amplicons of the expected size but in the form of faint bands on gel that produced poor quality, unreadable sequences. We conservatively considered these isolates as Blastocystis-negative.

We detected E. bieneusi only in a sooty mangabey from AZG Côte d’Ivoire. Sequence analysis of the ITS region confirmed this isolate as genotype CAF4 (Table 2).

Finally, we identified the presence of T. abrasarti in three captive chimpanzees at AZG Côte d’Ivoire. All three ITS sequences were identical among them but differed by a single SNP (T82C) from reference sequence EU680311 (Table 2).

Table 1

| Species                  | AZG, Côte d’Ivoire (n = 24) | TS, Sierra Leone (n = 67) | QZG, Peru (n = 25) |
|-------------------------|-----------------------------|---------------------------|-------------------|
|                         | Pos. (n) % 95% CI           | Pos. (n) % 95% CI         | Pos. (n) % 95% CI |
| Cryptosporidium spp.    | 0 0.0 –                     | 0 0.0 –                   | 0 0.0 –           |
| E. histolytica          | 0 0.0 –                     | 0 0.0 –                   | 0 0.0 –           |
| E. dispar               | 13 54.2 32.8–74.5           | 1 1.5 0.04–8.4           | 7 28.0 12.1–49.4 |
| G. duodenalis           | 7 29.2 12.6–51.1            | 22 32.8 21.9–45.4        | 1 4.0 0.1–20.4   |
| Blastocystis sp.        | 6 25.0 9.8–46.7             | 15 22.4 13.1–34.2        | 5 20.0 0.1–20.4  |
| E. bieneusi             | 1 4.2 0.1–21.3              | 0 0.0 –                   | 0 0.0 –           |
| B. coli                 | 0 0.0 –                     | 0 0.0 –                   | 0 0.0 –           |
| T. abrasarti            | 3 12.5 26.6–46.7            | 0 0.0 –                   | 0 0.0 –           |
populations investigated in the present study. Additionally, two captive spider monkeys presenting with amoebic dysentery and a mandrill with diarrhoea have been also reported in a zoo in Spain (Koster et al., 2021a) and wild chimps from the Republic of Congo, Guinea Bissau, and Senegal (Gillespie et al., 2009; Gonzalez-Moreno et al., 2013), and at 0–21% by PCR in Senegal and Tanzania (Parsons et al., 2015; Renelies-Hamilton et al., 2019; Koster et al., 2021b). No studies have been conducted on the occurrence of Cryptosporidium spp. in Peruvian NHP populations to date. Remarkably, molecular epidemiological surveys carried out in the last decade have demonstrated that Cryptosporidium infections in NHP are primarily caused by *C. hominis*, the species most reported in human cryptosporidiosis cases globally (Widmer et al., 2020).

As in the case of Cryptosporidium spp., *E. histolytica* infections were undetected in the three NHP populations investigated. It is still unclear to which extent NHP are suitable hosts for *E. histolytica* and, if yes, what the actual pathogenic significance of the infection is. Our PCR-based data agreed with the negative results obtained in similar studies conducted in zoo-kept NHP in Spain (Koster et al., 2021a) and wild chimpanzees in Senegal and Tanzania (Jirků-Pomajbková et al., 2016; Koster et al., 2021b). These surveys showed that wild chimpanzees harbour *Entamoeba* species (e.g. *E. dispar*) similar to those occurring in humans, but no pathogenic species (e.g. *E. histolytica*) were detected. Opposite results have also been reported: a PCR-based prevalence of *E. histolytica* of 11–34% has been observed in apparently healthy chimpanzees and baboons in Tanzania (Deere et al., 2019), whereas a recent study using a next generation sequencing approach revealed the presence of the parasite at low proportions in free-living chimpanzees and gorillas in Cameroon (Vickova et al., 2018). Although a clear link between *E. histolytica* and the occurrence of clinical manifestations could not be established in those surveys, other studies have confirmed outcomes similar to those of human invasive amebiasis in laboratory chimpanzees (Miller and Bray, 1966) and diarrhoeal episodes in infected wild lemurs from Madagascar (Ragazzo et al., 2018). Additionally, two captive spider monkeys presenting with amoebic dysentery and a mandrill with diarrhoea have also reported in a French zoo (Verweij et al., 2003c). In this study, non-pathogenic *E. dispar* was detected at variable (28–54% in zoo NHP, 1.5% in semi-captive chimpanzees) rates. These data are well in agreement with the figures reported in other captive (Regan et al., 2014; Koster et al., 2021a) or free-living (Lau et al., 2013; Jirků-Pomajbková et al., 2016; Koster et al., 2021b) NHP populations globally.

### Table 2

| Species                     | Genotype | Sub-genotype | No. isolates | Locus | Reference sequence | Stretch | Single nucleotide polymorphisms | GenBank ID |
|-----------------------------|----------|--------------|--------------|-------|-------------------|---------|-------------------------------|------------|
| *Giardia duodenalis* B     | B        | BILL         | 1⁷           | gdh   | AF069059          | 41–428  | C87T, G215A, A414G             | M2501256   |
| *Blastocystis* sp. ST1     | Allele 8 | 9⁷           | ssu rRNA     | MZ182327 | 32–606 | None                | M2501257   |
| *Blastocystis* sp. ST1     | Alleles 7 + 8 | 3⁷ | ssu rRNA | MZ2182327 | 5–606 G176R | M2501254 |
| *Blastocystis* sp. ST2     | Allele 11 | 1⁷           | ssu rRNA     | MZ69067 | 1–604 None         | M2501254 |
| *Blastocystis* sp. ST2     | Alleles 11 + 12 | 2⁷ | ssu rRNA | MZ669067 | 53–580 G176R, C476G | M2501254 |
| *Enteroctosynus bienesi*   | Unknown  | 1⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Troglodytella abrassarti* | Unknown  | 1⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Cryptosporidium* spp.     | Allele 30 | 4⁷           | ssu rRNA     | MN238079 | 4–593 A260T | M2501254 |
| *Cryptosporidium* spp.     | Allele 34 | 1⁷           | ssu rRNA     | KY929101 | 13–533 None       | M2501254 |
| *Cryptosporidium* spp.     | Unknown  | 2⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Cryptosporidium* spp.     | Unknown  | 2⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Enterocytozoon*           | Unknown  | 1⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Enterocytozoon*           | Unknown  | 3⁷           | ssu rRNA     | –     | –                 | –       | –                             | –          |
| *Trophrynula abrassarti*   | –        | –             | ssu rRNA     | –     | –                 | –       | –                             | –          |

Gdh: Glutamate dehydrogenase; ITS: Internal transcribed spacer; ssu rRNA: Small subunit ribosomal RNA.

⁷ AZG Côte d’Ivoire.
⁶ TS Sierra Leone.
⁵ QZG Peru.

### Discussion

Present molecular-based epidemiological study provides novel data on the occurrence of eukaryotic intestinal (including parasite and commensal) species in captive and semi-captive NHP populations in two low-income (Côte d’Ivoire and Sierra Leone) and one middle-income (Peru) countries. Although a clear link between the occurrence of *Giardia duodenalis* in Africa and South American NHP populations is scarce (Squire and Ryan, 2017; Rivero et al., 2020; Fantinati et al., 2021), the parasite has been reported at prevalences of 2–67% in free-living gorillas from Central African Republic, Rwanda, and Uganda (Grazczyk et al., 2002; Sak et al., 2013; Hogan et al., 2014), colobus monkeys from Ghana and Uganda (Teichroeb et al., 2009; Johnston et al., 2010), and wild chimpanzees from the Republic of Congo, Guinea Bissau, and Senegal (Gillespie et al., 2009; Sá et al., 2013; Renelies-Hamilton et al., 2019). In South American countries, *G. duodenalis* has been identified in 40–76% of semi-captive and wild howler monkeys in Argentina and Brazil (Volotão et al., 2008; Kowalewski et al., 2011). No information on the occurrence of the parasite is currently available in NHP from Peru. As in the case of our study, most of the above-mentioned surveys did not report the occurrence of clinical manifestations (including diarrhoea) in the investigated animals, suggesting that *G. duodenalis* infections were primarily asymptomatic. In the present study, *G. duodenalis* was more prevalently found in African (29–33%) than in Peruvian (4%) sites, probably reflecting differences in host species, infection sources and/or management procedures of captive/semi-captive animals. Regarding molecular data, we found zoonotic sub-assemblies BIII and BIV in two resident chimpanzees at AZG Côte d’Ivoire and TS Sierra Leone. Both *G. duodenalis* sub-assemblies have also been reported in mountain gorillas in Rwanda (Hogan et al., 2014) and wild howler monkeys and captive NHP in Brazil (Volotão et al., 2008; Soares et al., 2011). The low genotyping success rate (6.7%, 2/30) obtained here can be explained by the limited sensibility of the single-copy genes (gdh, bg, and sp) used for that purpose, which is in sharp contrast with the high sensitivity associated with the multi-copy ssu rRNA gene used in qPCR detection. This fact is consistent with the low parasite burden (indicated by high qPCR Ct values) and apparent absence of clinical manifestations (suggested by the formed consistency of the faecal material) typically found in the surveyed NHP populations.

We found *Blastocystis* sp. at a remarkably similar prevalence rates (20–25%) in the three NHP populations investigated here. This finding would suggest that *Blastocystis* is a common component of the NHP gut microbiota regardless of the host and geographical area. The infection/colonization rates found in the present study are in the lower range of those typically reported in African NHP populations including...
chimpanzees, gorillas, and colobus and vervet monkeys. Blastocystis sp. has been documented at a prevalence of 22% by conventional microscopy in Cameroon (Drakulovski et al., 2014), and of 6–100% by PCR in Senegal, the Republic of Congo, and Tanzania (Petrášová et al., 2011; Renelies-Hamilton et al., 2019; Koster et al., 2021b; Menu et al., 2021). Regarding genetic diversity, ST1 has been demonstrated as the most prevalent Blastocystis subtype circulating in free-living NHP in Africa, accounting for 80–100% of the isolates characterised in wild chimpanzees in Senegal (Renelies-Hamilton et al., 2019; Koster et al., 2021b) and Tanzania (Petrášová et al., 2011), although in the former country few animals carried also ST2 and ST3. Interestingly, a recent molecular study has evidenced that approximately nine out of 10 ST1 isolates identified in a sooty mangabey resident at AZG Côte d’Ivoire, where all Blastocystis-positive animals were infected/colonized by ST2 or ST3. These discrepancies in the frequencies of genetic variants may be due to differences in diet quality, management, or exposure to sources of infection including those of anthropogenic nature. Very few studies have described Blastocystis STs in Central and South American NHP populations. Thus, howler monkeys have been found infected/colonized by ST4 in Colombia (Ramírez et al., 2014), and by ST8 in Ecuador (Helenbrook et al., 2015a). However, all captive NHP resident at QZG Peru with a Blastocystis-positive result harboured ST2 and ST3, a pattern more closely related to the one found at AZG Côte d’Ivoire. Of interest, the only zookeeper that tested positive to Blastocystis in this institution was infected/colonized by ST1 allele 4, a genetic variant not detected in any of the NHP investigated in this zoological garden.

Enteroxozoon bieneusi, the dominant member of the pathogenic microsporidian species, may cause diarrhoea and other gastrointestinal manifestations in humans and domestic and wild animals (Santín and Fayer, 2011). Reported prevalences for such organism in captive NHP populations are in the range of 4%–68%, with most studies coming from China (reviewed in Leśniak and Perec-Matsyiska, 2017). Very little information is currently available on the epidemiology of E. bieneusi in African or South American NHP. The parasite has been reported in 12.3% (29/235) of captive, apparently healthy olive baboons (Papio anubis) in Kenya, with juvenile individuals harbouring the bulk of the infections (Li et al., 2011). In the present study, E. bieneusi was detected in a sooty mangabey resident at AZG Côte d’Ivoire (overall prevalence: 4.2%). Recent phylogenetic analysis of valid E. bieneusi genotypes at the ITS locus have allowed the recognition of 11 major genetic groups (Groups 1 to 11) of which Groups 1 and 2 include genotypes with potential for zoonotic or cross-species transmission (Li et al., 2019). Near 78 E. bieneusi ITS genotypes have been reported from various NHP species to date (Leśniak and Perec-Matsyiska, 2017; Chen et al., 2019; Li et al., 2019; Karim et al., 2020; Zhao et al., 2020). Most of E. bieneusi genotypes identified in NHP belong to Group 1 (D, EbP4, EbPc, Type IV, O, Peru11, etc.) and Group 2 (BB4, BB6, I, J, etc.). In addition, other genotypes included in Group 5 (KB-6 and PbEb XII), Group 6 (Gorilla 3 and KB-5) and Group 7 (CM18 and XH) have been found only in NHP, suggesting that these genetic variants seem to be adapted to this host (Li et al., 2019). Our quantitative analysis revealed that the presence of diarrhea or the presence of the parasite at the intestinal level detected in a zoo mangabey was caused by the extremely rare genotype CAFA4. Indeed, CAFA4 has only been identified to date in four HIV-positive patients in Gabon and five HIV-negative individuals in Cameroon (Breton et al., 2007). Genotype CAFA4 belongs to Group 5 of E. bieneusi, which contains genotypes adapted to humans and NHP (Li et al., 2019). Genotype CAFA4 was thought to be human-specific, but this study confirms its presence in a new host. These data strongly suggest that CAFA4 may represent a zoonotic genotype circulating among human and NHP populations in Western Africa. More research is needed to elucidate the epidemiology of E. bieneusi in low-income countries, including the identification of suitable hosts and transmission pathways.

Balantiooides coli is largely considered a non-pathogenic gut commensal, although fatal ulcerative colitis by this ciliate has been described in captive gorillas (Lankester et al., 2008). This parasite is fairly common in captive and semi-captive great apes, with prevalence rates ranging 14–68% in chimpanzees and gorillas living in sanctuaries in Cameroon and Kenya (Pomajbiková et al., 2010b), or 9% in captive chimpanzees in Nigeria (Mbaya and Udendeye, 2011). In captive and wild-trapped olive baboons, vervets and sykes in Kenya, prevalence ranged from 23 to 53% (Munene et al., 1996). Surprisingly, B. coli was not detected in the chimpanzee populations investigated in our study; at present we do not have a clear explanation for this result. In relation to the non-detection of the parasite in New World NHP, it is an expected result, since B. coli is considered rare or absent in New World monkeys (Levecke et al., 2007). Low prevalences of B. coli cysts have been reported in captive Saimiri sciureus, Leontopithecus sp., Cebus albifrons and Aotus nigriceps (Guerrero et al., 2012; da Silva Barbosa et al., 2015), and in wild Alouatta palliata (Helenbrook et al., 2015b).

The colonic mutualist T. abrassarti is believed to specifically colonize African great apes as most records are from chimpanzees, bonobos, and gorillas (Goussard et al., 1983; Vallo et al., 2012). However, the finding of this ciliate protist in lesser apes of the genus Hylobates suggests that the range of competent host species may be wider than initially anticipated (O’Donoghue et al., 1993). Microscopy-based epidemiological studies have previously reported the presence of T. abrassarti at rates ranging from 0% to 100% and 60–100% in captive and wild chimpanzees, respectively (Muehlenbein, 2005; Pomajbiková et al., 2010a). The fact that T. abrassarti does not form environmentally resistant cysts and rapidly degrading trophozoites are the transmissible stage present in faeces has important diagnostic consequences. PCR-based methods could not be the best choice for the detection of T. abrassarti if genomic DNA from fresh faecal material is not readily available. This limitation may explain the low occurrence rates, or even apparent absence, of T. abrassarti typically reported in molecular studies investigating captive or wild NHP (Koster et al., 2021a,b). This is also the case of the present survey, where T. abrassarti was found at relative low rates (12.5%) in captive chimpanzees at AZG Côte d’Ivoire but was absent in semi-captive chimpanzees at TS Sierra Leone. The later result is in contrast with previous microscopy-based results showing that T. abrassarti is naturally circulating among the resident chimpanzee population in that very same location (Irbis et al., 2008).

5. Conclusions

This is, to date, the first molecular-based epidemiological survey investigating the presence and genetic diversity of protist parasite and commensal species in captive and semi-captive NHP populations conducted in Côte d’Ivoire, Sierra Leone, and Peru. Among well-recognized pathogens, G. duodenalis was found at high occurrence rates in the African sites, but not in the Peruvian one. In contrast, Blastocystis sp. was present at similar rates in the three NHP populations investigated here. Cryptosporidium spp., E. histolytica, and B. coli were undetected in the three sites. Enteroxozoon bieneusi genotype CAFA4 was only detected in a captive sooty mangabey in Côte d’Ivoire, being the first report of this genetic variant in NHP. The identification of zoonotic genetic variants (sub-assemblages BIII and BIV within G. duodenalis, ST1-ST3 within Blastocystis sp., and CAFA4 within E. bieneusi) suggest that human-NHP transmission is possible, although the frequency, extent, and directionality of these events remain to be elucidated in subsequent molecular epidemiological surveys.

Declaration of competing interest

The authors declare that they have no known competing financial
interests or personal relationships that could have appeared to influence the work reported in this paper.

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