



Enteric protists in wild western chimpanzees (*Pan troglodytes verus*) and humans in Comoé National Park, Côte d'Ivoire

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Abstract

The western chimpanzee (*Pan troglodytes verus*), a subspecies of the common chimpanzee, is currently listed as Critically Endangered. Human-driven habitat loss and infectious diseases are causing dramatic chimpanzee population declines and range contractions that are bringing these primates to the brink of extinction. Little information is currently available on the occurrence of diarrhoea-causing enteric protist species in chimpanzees in general, and in western chimpanzees in particular, or on the role of humans as a potential source of these infections. In this prospective molecular epidemiological study, we investigated the presence, genetic variability, and zoonotic potential of enteric protists in faecal samples from western chimpanzees ($n=124$) and humans ($n=9$) in Comoé National Park, Côte d'Ivoire. Parasite detection and genotyping were conducted by using polymerase chain reaction (PCR) and Sanger sequencing. The protist species found in the chimpanzee samples were *Entamoeba dispar* (14.5%), *Blastocystis* sp. (11.3%), *Giardia duodenalis* (5.8%), *Troglodytella abrassarti* (2.5%) and *Cryptosporidium hominis* (0.8%). The protist species found in the human samples were *G. duodenalis* (22.2%) and *Blastocystis* sp. (11.1%). *Entamoeba histolytica*, *Enterocytozoon bieneusi*, and *Balantioides coli* were undetected in both chimpanzee and human samples. Sequence analyses revealed the presence of *Blastocystis* subtype (ST) 1 (alleles 4 and 8) and ST3 (allele 24) in chimpanzees, and ST3 (allele 52) in humans. ST1 allele 8 represents a chimpanzee-adapted *Blastocystis* genetic variant. Cross-species transmission of pathogenic enteric protists between chimpanzees and humans might be possible in Comoé National Park, although the frequency and extent of zoonotic events remain to be fully elucidated.

Keywords Enteric protists · Wild chimpanzees · Humans · Polymerase chain reaction · Genotyping · Conservation · Zoonoses · Transmission

Introduction

Wild western chimpanzee (*Pan troglodytes verus*) populations in West Africa decreased by 80% between 1990 and 2014 as a direct consequence of anthropogenic activities (agriculture, poaching, extractive industries, infrastructure

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development, human–chimpanzee interactions, lack of law enforcement) and infectious diseases (Kühl et al. 2017). It is estimated that there are currently 35,000 western chimpanzees remaining in the wild, but a projection of the population decline observed over the past three generations suggests that there will be an additional 99% decline in their abundance over the next two generations (Kühl et al. 2017). Consequently, the western chimpanzee, a subspecies of the common chimpanzee, is currently classified as Critically Endangered (Humble et al. 2016).

After habitat loss, infectious diseases are the most important threat to the survival of all the chimpanzee subspecies in the wild (Wilson et al. 2020). Respiratory disease (including bacterial infections by *Streptococcus pneumoniae* and *Streptococcus pyogenes*) appears to be the most common cause of death from illness in chimpanzees (Williams et al. 2008). Ebola outbreaks in particular are taking a great toll on western chimpanzee populations (Formenty et al. 1999). In comparison to ebola, much less information is available on the occurrence of diarrhoea-causing viral, bacterial and parasitic pathogens in different subspecies of chimpanzees in the wild. Among parasitic pathogens, the protozoans *Cryptosporidium* spp., *Entamoeba histolytica* and *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*), and to a lesser extent the stramenopile *Blastocystis* sp. and the microsporidian *Enterocytozoon bieneusi*, are the species of greatest veterinary significance for chimpanzees (Strait et al. 2012; Medkour et al. 2020).

Genotyping and sub-genotyping analyses are essential to ascertain sources of infection, transmission pathways, and the zoonotic potential of pathogens. In recent years, an important effort has been made to develop informative genotyping methods and ascertain the genetic diversity of *Cryptosporidium* spp., *G. duodenalis*, *Blastocystis* sp., and *E. bieneusi*. To date, at least 48 valid species and more than 100 genotypes have been described for the genus *Cryptosporidium* (Ježková et al. 2021). Among them, *C. hominis* is the species most frequently reported in non-human primates (NHP) and humans globally (Widmer et al. 2020). *Giardia duodenalis* is the only *Giardia* species known to infect primates. The genotypes of this species have been classified into eight (A–H) genetic assemblages with marked differences in host range and specificity, of which zoonotic assemblages A and B are responsible for most of the infections identified in NHP and humans (Ryan and Cacciò 2013). At least 27 subtypes (ST) have been identified within *Blastocystis* sp. (Maloney et al. 2021) of which ST1–9 and ST15 have been previously reported in captive and free-ranging NHP (Hublin et al. 2021). Out of the more than 500 *E. bieneusi* genotypes identified to date, no less than 78 have been found in NHP, many of them with zoonotic potential (Li et al. 2019). In comparison to these species, there is much less

information on the molecular diversity of protist species with limited pathogenic potential or that are commensal, such as the ciliates *Balantioides coli* and *Troglodytella abrossarti* (Ponce-Gordo et al. 2011; Vallo et al. 2012).

In this prospective molecular-based epidemiological study, we aimed to describe, for the first time, the diversity, genetic variability, and zoonotic potential of pathogenic and commensal enteric protist species in endangered western chimpanzees inhabiting Comoé National Park in Côte d'Ivoire. To assess the role of humans as a potential source of chimpanzee infections, the same methodological approach was adopted for personnel (rangers, researchers, students and assistant staff) working on site who volunteered to be part of this study.

Materials and methods

Study site

Comoé National Park is the biggest protected area in Côte d'Ivoire. It covers an area of 11,500 km² comprising mostly woody savannah habitats, although 13% of the park, mostly in the southern part, is covered by a dry tropical forest mosaic. Created in 1968 in the northeastern corner of Côte d'Ivoire, close to the border with Burkina Faso, the park has suffered from fluctuating law enforcement and intense poaching, especially during the Ivorian politico-military crisis (2002–2011). Despite this, it still harbours quite well-preserved natural habitats that shelter a still viable population of western chimpanzees and a rich sympatric fauna including more than 130 mammal species (Lapuente 2021). Improvements in conservation measures, including those achieved with the collaboration of the Comoé Chimpanzee Conservation Project (CCCP), allowed the park, which is a World Heritage Site, to be removed from the UNESCO List of World Heritage in Danger in 2017. However, there is still negative human pressure on the park in the form of poaching, illegal fishing and disturbance due to gold mining that affects, among other species, the chimpanzees living there, which enter occasionally into contact with the uncontrolled human intruders who undertake these activities. The area in which the CCCP works, in the southwestern part of the park, covers most of the present range of the chimpanzees, and the permanent presence of researchers and local assistants, together with patrols carried out by rangers from the Office Ivoirien des Parcs et Réserves, helps to reduce these illegal activities, but exposes the chimpanzees to humans at proximity, even though this is controlled and hygienic measures are taken to prevent any spread of disease to the chimpanzees.

Sampling

We opportunistically collected 124 fresh (<24 h old) chimpanzee faecal samples in Comoé National Park between October 2016 and March 2019. Samples were identified by experienced field guides and collected non-invasively in the forest from the ground under the chimpanzee night nests early in the morning or along paths during follows. We took a 5- to 10-g sample from the core of the faeces and stored the samples in 70% ethanol ($n=47$) or in Total-Fix stool collection kit vials ($n=77$) (Durviz; Valencia, Spain) at room temperature.

We also collected stool samples ($n=9$) from rangers, researchers, students, and assistant staff working at Comoé National Park who volunteered to participate in the survey. All staff members complied with strict personal hygiene practices such as taking a daily shower and hand washing before meals, and with field hygiene procedures. Following recommended guidelines (Gilardi et al. 2015), staff members never defecate, spit or urinate in the forest and, in an emergency, go into the savannah, dig a deep hole and cover it over with soil following urination/defecation; they also avoid touching anything that might be handled by chimpanzees, clean all work tools with hydroalcoholic solution, and change their clothing after fieldwork. The human stool samples were preserved in 70% ethanol at room temperature.

All the chimpanzee and human faecal samples collected were analysed and corresponded to types 2–4 of the Bristol Stool Scale, which is commonly used to measure intestinal transit in human patients (O'Donnell et al. 1990). Samples were shipped to the Parasitology Reference and Research Laboratory, National Centre for Microbiology, Majadahonda, Spain for processing and downstream molecular testing.

DNA extraction and purification

We thoroughly washed the faecal samples in phosphate buffered saline to remove an excess of preserving (ethanol, Total-Fix) solutions. 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, with the exception that the samples mixed with InhibitEX buffer were incubated for 10 min at 95 °C.

Molecular detection of protist species

We assessed the presence of *Cryptosporidium* spp. using a genus-specific nested-polymerase chain reaction (PCR) protocol to amplify a 587-base pair (bp) fragment of the small subunit ribosomal RNA (*SSU* rRNA) gene of the parasite (Tiangtip and Jongwutiwes 2002). The detection

and differential diagnosis between pathogenic *E. histolytica* and non-pathogenic *Entamoeba dispar* was carried out using a real-time (qPCR) method targeting a 172-bp fragment of the *SSU* rRNA gene of the *E. histolytica/E. dispar* complex (Verweij et al. 2003a; Gutiérrez-Cisneros et al. 2010). *Giardia duodenalis* DNA was detected using a qPCR method targeting a 62-bp region of the *SSU* rRNA gene of the parasite (Verweij et al. 2003b). We subsequently assessed *G. duodenalis* isolates that tested positive by qPCR by sequence-based multi-locus genotyping of fragments of the glutamate dehydrogenase gene (*gdh*; 432 bp) (Read et al. 2004), β -giardin gene (*bg*; 511 bp) (Lalle et al. 2005), and triose phosphate gene (*tpi*; 530 bp) (Sulaiman et al. 2003) of the parasite.

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 primers to amplify a PCR product of ~600 bp. *Enterocytozoon. bieneusi* was detected by a nested PCR protocol used to amplify the internal transcribed spacer (ITS) region as well as portions of the large and small subunits of the rRNA gene to generate a final PCR product of 390 bp (Buckholt et al. 2002). We attempted to detect *B. coli* 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 (Ponce-Gordo et al. 2011). We used a *Troglodytella*-specific direct PCR method targeting a 401-bp fragment of the ITS1–5.8S-rRNA–ITS2 region to detect species of this ciliate genus (Vallo et al. 2012).

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 mixes included 2.5 units of MyTAQ DNA polymerase (Bioline, Luckenwalde, Germany) and 5× MyTAQ Reaction Buffer containing 5 mM deoxynucleoside triphosphates and 15 mM MgCl₂. The specific DNA primer and probe sequences used in the present study are given 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, CA) nucleic acid staining solutions.

Sequence analysis

We directly sequenced positive-PCR products in both directions using appropriate internal primer sets (Table S1). We conducted DNA sequencing by capillary

Table 1 Occurrence of enteric parasitic and commensal protist species identified in faecal samples from wild western chimpanzees and humans in Comoé National Park, Côte d'Ivoire

Protist species	Chimpanzees (n = 124)			Humans ^a (n = 9)		
	Positive (n)	%	95% CI	Positive (n)	%	95% CI
<i>Cryptosporidium</i> spp.	1	0.8	0.02–4.4	0	0.0	–
<i>Entamoeba histolytica</i>	0	0.0 ^b	–	0	0.0	–
<i>Entamoeba dispar</i>	18	14.5	8.8–22.0	0	0.0	–
<i>Giardia duodenalis</i>	7 ^b	5.8	2.4–11.6	2	22.2	2.8–60.0
<i>Blastocystis</i> sp.	13	10.5	5.7–17.3	1	11.1	0.3–48.3
<i>Enterocytozoon bieneusi</i>	0	0.0	–	0	0.0	–
<i>Balantioides coli</i>	0 ^b	0.0	–	0	0.0	–
<i>Troglodytella abrossarti</i>	3 ^b	2.5	0.5–7.1	0	0.0	–

CI Confidence interval

^aVolunteer rangers, researchers, students, and assistant staff working at Comoé National Park

^bThree chimpanzee samples were not tested due to an insufficient amount of genomic DNA. Prevalence rates were calculated over 121 samples

electrophoresis using BigDye Terminator (Applied Biosystems) chemistry on an ABI PRISM 3130 automated DNA sequencer. Representative sequences obtained in this study were deposited in GenBank under accession numbers MZ813317 (*C. hominis*), MZ813318–MZ813321 (*Blastocystis* sp.), and MZ855892 (*T. abrossarti*).

Results

Occurrence of enteric protist species in wild western chimpanzees

The PCR-based occurrence rates of pathogenic and commensal protist species found in the investigated chimpanzee faecal samples are shown in Table 1.

Overall, one in four (31/124) of the investigated faecal samples harboured DNA from at least one enteric protist species. Among well-recognized pathogenic protists, *G. duodenalis* was the most common species found [5.8%; 95% confidence interval (CI) 2.4–11.6], followed by *Cryptosporidium* spp. (0.8%; 95% CI 0.02–4.4). The protozoan *E. histolytica*, the microsporidian *E. bieneusi*, and the ciliate *B. coli* were not detected in any faecal samples of the surveyed chimpanzee population. Based on the consistency of the faecal material collected, neither *G. duodenalis* nor *Cryptosporidium* seemed to be associated with diarrhoea in the infected chimpanzees.

We found the protist of uncertain pathogenicity *Blastocystis* sp. in 10.5% (95% CI 5.7–17.3) of the investigated

faecal samples, and the commensal species *E. dispar* and *Troglodytella* spp. in 14.5% (95% CI 8.8–22.0) and 2.5% (95% CI 0.5–7.1) of the samples, respectively. Infections/colonisations by single protist species were more common (67.8%; 21/31) than mixed ones involving two (29.0%; 9/31) or three (3.2%; 1/31) protist species (Table 2). The most frequent co-infections/colonisations were *E. dispar* with *Blastocystis* sp. (12.9%; 4/31) and *E. dispar* with *G. duodenalis* (9.7; 3/31). A single faecal sample (3.2%; 1/31) harboured DNA from three protist taxa: *E. dispar*, *Blastocystis* sp. and the genus *Troglodytella*.

Occurrence of enteric protist species in humans

Nine individuals (two rangers, one researcher, four field students and two assistant staff) working in Comoé National Park volunteered to participate in the study. The rangers and assistant staff were locals, whereas the researchers/students were European or American citizens. The male/female ratio was 8.0, and the mean age 31 years (range 20–50 years). All of the participants were apparently healthy and did not declare that they were suffering from any gastrointestinal disorders at the moment of sampling or that they had suffered from any in the previous week. *Giardia duodenalis* was found in two individuals (overall infection rate: 22.2%; 95% CI 2.8–60.0). A third individual (a male ranger) harboured *Blastocystis* sp. (overall colonisation rate: 11.1%; 95% CI 0.3–48.3). No mixed infections by two or more enteric protist species were detected.

Table 2 Single and multiple enteric protist infections/colonisations detected in faecal samples from wild western chimpanzees in Comoé National Park, Côte d'Ivoire

Protist species	Faecal samples (n)	Relative frequency (%)
<i>Entamoeba dispar</i> only	9	29.0
<i>Blastocystis</i> sp. only	7	22.6
<i>Giardia duodenalis</i> only	3	9.7
<i>Cryptosporidium</i> spp. only	1	3.2
<i>Troglodytella</i> spp. only	1	3.2
<i>Entamoeba dispar</i> and <i>Blastocystis</i> sp.	4	12.9
<i>Entamoeba dispar</i> and <i>Giardia duodenalis</i>	3	9.7
<i>Entamoeba dispar</i> and <i>Troglodytella</i> spp.	1	3.2
<i>Giardia duodenalis</i> and <i>Blastocystis</i> sp.	1	3.2
<i>Entamoeba dispar</i> , <i>Blastocystis</i> sp. and <i>Troglodytella</i> spp.	1	3.2
Total	31	100

Table 3 Diversity, frequency, and molecular features of *Cryptosporidium hominis*, *Blastocystis* sp. and *Troglodytella abrassarti* isolates from wild western chimpanzees and humans in Comoé National Park, Côte d'Ivoire

Protist species	Genotype	Subgenotype	No. isolates	Locus	Reference sequence	Stretch	Single nucleotide polymorphisms	GenBank identifier
Chimpanzees								
<i>Cryptosporidium hominis</i>	Family I	Unknown	1	<i>SSU</i> rRNA	AF108865	687–872	None	MZ813317
<i>Blastocystis</i> sp.	ST1	Allele 4	1	<i>SSU</i> rRNA	MZ396317	1–390	C374A, A375C	MZ813318
	ST1	Alleles 8	11	<i>SSU</i> rRNA	MZ182327	70–606	None	MZ813319
	ST3	Allele 24	1	<i>SSU</i> rRNA	DQ232790	1–530	None	MZ813320
<i>Troglodytella abrassarti</i>	–	–	3	ITS	EU680311	1–418	T82C	MZ855892
Humans								
<i>Blastocystis</i> sp.	ST3	Allele 52	1	<i>SSU</i> rRNA	MF581574	17–530	G52C	MZ813321

SSU rRNA Small subunit ribosomal RNA, *ITS* internal transcribed spacer

Genetic diversity of enteric protist species in wild western chimpanzees

Sequence analysis of the only faecal sample that tested positive for *Cryptosporidium* spp. by *SSU*-PCR revealed the presence of *C. hominis*. The generated sequence was identical to reference sequence AF108865 (Table 3). Repeated attempts to amplify this sample at the gene coding for the 60-kDa glycoprotein (*gp60*) of the parasite failed, so the genotype family of this isolate could not be determined. Because of the large genetic diversity of *gp60*, this marker is widely used for the genotyping and sub-genotyping of *Cryptosporidium* species (including *C. hominis*) in human and NHP populations (Widmer et al. 2020). A substantial number of *SSU*-PCR reactions ($n = 11$) yielded amplicons of a size compatible with that expected for *Cryptosporidium*

spp. However, sequence analyses confirmed that these originated from uncultured eukaryotes ($n = 4$), freshwater green algae of the genus *Desmodesmus* ($n = 1$), or were unspecific (unreadable) sequences.

All 18 samples that tested positive for *E. dispar* by qPCR generated cycle threshold (Ct) values ranging from 27.1 to 42.6 (median 34.1, SD 4.5). No attempts were made to determine the genetic diversity of this commensal protozoan, as our laboratory does not have the required genotyping protocols in place. All seven samples that tested positive for *G. duodenalis* by qPCR generated Ct values ranging from 33.2 to 37.7 (median 35.3, SD 1.4). None of these could be genotyped at the *gdh*, *bg*, or *tpi* loci, so their genetic diversity in terms of assemblages and sub-assemblages remains unresolved.

Blastocystis DNA was confirmed in 13 chimpanzee faecal samples. Sequence analyses revealed the presence of ST1 (92.3%; 12/13) and ST3 (7.7%; 1/13). Within ST1, allele 8 was the most frequent genetic variant (91.7%; 11/12), followed by allele 4 (8.3%; 1/12). The only sample characterised as ST3 was assigned to allele 24 of the protist (Table 3). An additional eight samples yielded faint bands on gel electrophoresis which were associated with unreadable sequences. Because of the lack of confirmation by Sanger sequencing, these samples were conservatively considered as negative for *Blastocystis* sp.

Finally, all three ITS sequences for *T. abraxarti* were identical but differed by a single nucleotide polymorphism (T82C) from reference sequence EU680311 corresponding to a western chimpanzee from Limbe Wildlife Centre in Cameroon (Table 3).

Genetic diversity of enteric protist species in humans

The two human stool samples positive for *G. duodenalis* by qPCR yielded Ct values of 33.6 and 38.1. As was the case for the chimpanzee faecal samples, attempts to amplify them at the *gdh*, *bg*, and *tpi* loci failed. Therefore, the *G. duodenalis* assemblages/sub-assemblages circulating in these humans remain to be elucidated. Sequence analysis of the only human stool sample positive for *Blastocystis* sp. by SSU-PCR confirmed the presence of ST3 allele 52 (Table 3).

Discussion

We present here, to the best of our knowledge, the first molecular-based epidemiological study on the presence and molecular diversity of pathogenic and commensal enteric protists in Critically Endangered western chimpanzees living in Comoé National Park, Côte d'Ivoire. *Cryptosporidium* spp. were present in less than 1% of the chimpanzee faecal samples investigated. Similar low prevalence rates (0–1%) have been reported previously in free-ranging central chimpanzees (*Pan troglodytes troglodytes*) in the Republic of Congo by using conventional microscopy (Gillespie et al. 2009) and in western chimpanzees living in a protected area in Senegal by using PCR (Renelies-Hamilton et al. 2019; Köster et al. 2021b; Menu et al. 2021). Much higher PCR-based prevalence rates of 9–21% have been documented in eastern chimpanzees (*Pan troglodytes schweinfurthii*) living in Tanzania (Gonzalez-Moreno et al. 2013) and in the Greater Gombe Ecosystem in the same country (Parsons et al. 2015). *Cryptosporidium* infections in wild chimpanzees are mainly caused by *C. hominis* (Parsons et al. 2015; Köster et al. 2021b). *Cryptosporidium hominis* has a large

genetic diversity, with at least 14 *gp60* genotype families, named Ia to Ii (Widmer et al. 2020). Lack of genotyping data at the *gp60* locus and the absence of the parasite from the limited number of human volunteers participating in the present study prevented us from fully elucidating whether the only *C. hominis* infection detected here in a chimpanzee was of human origin or not.

We did not detect the presence of *E. histolytica* in the chimpanzee and human populations under study. In other studies using PCR as a detection method, this protozoan parasite was also apparently absent from western chimpanzees living in Senegal (Köster et al. 2021b) and Tanzania (Jirků-Pomajbíková et al. 2016). In contrast, a prevalence rate as high as 34% was found in apparently healthy eastern chimpanzees in the Greater Gombe Ecosystem in Tanzania (Deere et al. 2019). In chimpanzees, non-pathogenic *Entamoeba* species including *E. dispar*, *Entamoeba coli*, and *Entamoeba hartmanni* are detected far more frequently than *E. histolytica* when PCR is used for differential diagnostic purposes (Jirků-Pomajbíková et al. 2016; Renelies-Hamilton et al. 2019; Köster et al. 2021b). This was also the case in the present study.

Information on the occurrence and genetic diversity of *G. duodenalis* in wild chimpanzee populations is scarce. This protozoan parasite has been identified by conventional/immunofluorescence microscopy at prevalence rates of 6% in the Republic of Congo (Gillespie et al. 2009) and in the Republic of Guinea Bissau (Sá et al. 2013). Lower infection rates of 1–2% have been found by PCR in Senegal (Renelies-Hamilton et al. 2019; Köster et al. 2021b; Menu et al. 2021). These previous molecular studies failed to genotype the *G. duodenalis* isolates. This was also the case in the present study: *G. duodenalis* was observed in 6% and 22% of the chimpanzee and human faecal samples examined, respectively, but none of them could be genotyped. These findings strongly suggest that *G. duodenalis* primarily causes light, asymptomatic infections in both western chimpanzees and humans in endemic areas. Although we cannot accurately assess to what extent the *G. duodenalis* infections detected in the chimpanzees here may be a consequence of anthropogenic activities in this area, this is a plausible possibility that deserves consideration. For instance, a higher *G. duodenalis* infection rate (9% vs. 3%) and mean intensity of infection (1833 cysts/g vs. 1000 cysts/g) were observed in resident central chimpanzees in disturbed (logged) forests than in undisturbed forests in the Republic of Congo (Gillespie et al. 2009).

Blastocystis sp. is commonly found in the faeces of both captive and free-living NHP (Hublin et al. 2021). In studies on different chimpanzee populations, *Blastocystis* sp. has been detected by conventional microscopy at a prevalence rate of 13% in *P. troglodytes verus* in Nigeria (Mbaya and Udendey 2011), 49% in the same subspecies in the

Republic of Guinea-Bissau (Sá et al. 2013), and 22% in *P. troglodytes troglodytes* in southeast Cameroon (Drakulovski et al. 2014). Reported prevalence rates determined using PCR were 6–98% for *P. troglodytes verus* in Senegal (Renelies-Hamilton et al. 2019; Köster et al. 2021b; Menu et al. 2021) and 71% for *P. troglodytes schweinfurthii* in Tanzania (Petrášová et al. 2011). The *Blastocystis* carriage rate found in the chimpanzees here was comparatively lower (10%), but very similar to that found in the humans (11%). However, our genotyping analyses revealed marked differences in subtype diversity and frequency among them. *Blastocystis* ST1 accounted for 92% of the *Blastocystis* subtypes circulating in the chimpanzees in the present study and, interestingly, was also the most prevalent subtype in western chimpanzee populations in Senegal [82–100% (Renelies-Hamilton et al. 2019; Köster et al. 2021b)] and in eastern chimpanzees in Tanzania [100% (Petrášová et al. 2011)]. Furthermore, in the survey presented here and in previous molecular surveys, most of the ST1 isolates belonged to allele 8, a NHP-adapted genetic variant of this protist which is rarely seen in humans (Köster et al. 2021b). This finding seems to indicate that *Blastocystis* cross-species transmission does not frequently occur in the specific epidemiological situation examined here.

Information on *E. bienersi* in wild chimpanzees is particularly scarce. In a previous molecular-based survey in Senegal, *E. bienersi* could not be detected in free-ranging western chimpanzees (Köster et al. 2021b). This was also the case in the present study. In contrast, low prevalence rates (<5%) of *E. bienersi* were found in semi-captive resident chimpanzees in Cameroon and Kenya, and the infections were caused by zoonotic genotypes EbpA, PigEBITS5, and D (Sak et al. 2011). Furthermore, *E. bienersi* genotype CAF4 was recently identified in a captive mangabey in Abidjan Zoological Garden in Côte d'Ivoire (Köster et al. 2021a). The genotype CAF4 has also been found in HIV-positive patients in Gabon and apparently healthy individuals in Cameroon (Breton et al. 2007), suggesting that cross-species transmission between humans and NHP sharing habitats in West Africa is possible.

Few epidemiological studies have investigated the occurrence of *B. coli* in chimpanzee populations. This parasite has been previously reported, in studies using coproscopy, at a prevalence rate of 68% (58/86) in semi-captive chimpanzees in Cameroon and Kenya (Pomajbíková et al. 2010). In contrast, in studies using the same diagnostic methodology, *B. coli* was not detected in chimpanzee populations in Senegal [$n = 132$ (Howells et al. 2011)], Tanzania [$n = 254$ (Kooriyama et al. 2012)] or from several sites across Africa [$n = 699$ (Pomajbíková et al. 2010)]. A *B. coli* prevalence rate of 0.9% ($n = 114$) was documented in southeast Cameroon (Drakulovski et al. 2014), and 0–10% ($n = 153$) in

different sampling years at Gombe National Park in Tanzania (Gillespie et al. 2010). Using PCR, *B. coli* was not found in Senegal [$n = 234$ (Köster et al. 2021b)] or in the present study ($n = 124$). Interestingly, a microscopy-based prevalence of 5% ($n = 362$) was found in eastern chimpanzees inhabiting degraded forest fragments amid farmland and villages in Uganda (McLennan et al. 2017), suggesting that extensive overlap between a chimpanzee population's range with areas in which humans and livestock are present may be a source of chimpanzee infection by *B. coli* (Lilly et al. 2002).

Chimpanzees are regarded as the most common host of *T. abrasarti* (Vallo et al. 2012). When using conventional microscopy, *T. abrasarti* prevalence rates in chimpanzees are typically reported to be over 60%. These figures are in line with those reported in wild apes for other mutualist/commensal ciliate species such as *Troglocorys cava* (Pomajbíková et al. 2012). *Troglocorys cava* has been reported in chimpanzees at prevalences of 44% in Cameroon (Drakulovski et al. 2014), 62% in Guinea-Bissau (Sá et al. 2013), 72–96% in Tanzania (Kaur et al. 2008; Gillespie et al. 2010; Kooriyama et al. 2012), 65% in Senegal (Howells et al. 2011), and 80% in Uganda (McLennan et al. 2017). Surprisingly, PCR-based studies report a much lower prevalence of *T. abrasarti* (0–6%) in chimpanzees in Senegal (Köster et al. 2021b) and Côte d'Ivoire (present study), and in resident semi-captive western chimpanzees in Sierra Leone (Köster et al. 2021a).

Conclusions

To our knowledge, this is the first molecular-based epidemiological study to assess the presence, genetic variability, and zoonotic potential of common enteric protist species in Critically Endangered western chimpanzees inhabiting Comoé National Park in Côte d'Ivoire. In this study, we have shown that chimpanzees are infected/colonized by diverse enteric protist species, including well-known diarrhoea-causing agents such as *C. hominis* and *G. duodenalis*. Although we could not confirm the occurrence of zoonotic transmission events, the possibility that these do occur cannot be underestimated.

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Author contributions PCK, JL and DC designed the study. PCK, JL, AD, BB, ASM, DGB and DC collected and curated the data and performed the analyses. PCK and DC wrote the first draft of the

manuscript. PCK, JL, DGB, RCB, FPG and DC finalized and edited the final draft of the manuscript. All the authors approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for International Organizations of Medical Sciences and the International Council for Laboratory Animal Science (RD 53/2013). This research complied with the Guidelines of Best Practice for Field Primatology of the protocols of the International Primatological Society. This study was approved by the Ethics Committee of the Health Institute Carlos III on 17 December 2018 under reference number CEI PI_90_2018-v2. Written informed consent was obtained from the rangers, researchers, students and assistant staff of Comoé National Park who volunteered to participate in the survey.

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