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ORIGINAL PAPER

Prevalence and climatic associated factors of *Cryptosporidium* sp. infections in savanna chimpanzees from Ugalla, Western Tanzania

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Abstract Studies about parasitization by *Cryptosporidium* in great apes have been scarce and mostly conducted in captivity. The present study reports the presence of *Cryptosporidium* sp. in wild chimpanzees (*Pan troglodytes*) from Ugalla, western Tanzania. Ugalla is one of the driest, most open, and seasonal habitats inhabited by chimpanzees. *Cryptosporidium* sp. was found in 8.9 % of the samples. The presence of the parasite was determined by preserving fecal samples in chemical conventional fixatives (MIF and alcohol absolute) staining them using a modified Zielh-Neelsen technique, and examining them with a light microscope. The number of fecal samples positive for *Cryptosporidium* was significantly higher during the rainy than during the dry season ($p<0.005$). The results showed that feces collected in the rainy season were almost three times more likely to be positive for *Cryptosporidium* than those collected in the dry season (OR=2.81). *Cryptosporidium* detection was significantly negatively affected by highest temperatures (>28.7 °C, $p<0.001$). Cryptosporidiosis can cause serious health problems in humans and its potential effect on Ugalla chimpanzees is discussed.

Cryptosporidium spp. are worldwide intestinal protozoan parasites that infect humans as well as a broad spectrum of domestic and wild hosts including ruminants, carnivores, and primates (e.g., Fayer et al. 2000). The parasite is transmitted by anthroponotic and zoonotic oocysts via a fecal-oral route (Xiao 2010). Intestinal parasitization by oocysts belonging to the genus *Cryptosporidium* has been reported in both wild and captive conditions in a wide spectrum of non-human primates belonging to several species of monkeys and prosimians (Gomez et al. 1992, 2000; Fayer et al. 2000; Muriuki et al. 1997; Hope et al. 2004; Legesse and Erko 2004; Ekanayake et al. 2007; Salzer et al. 2007; da Silva et al. 2003). In apes, the parasite has been reported in captivity in gorillas, orangutans, and chimpanzees (Fayer et al. 2000; Gomez et al. 2000; Lim et

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al. 2008), and in the wild in gorillas (*Gorilla gorilla beringei*, Nizeyi et al. 1999) and chimpanzees (Gonzalez-Moreno et al. 2004; Lonsdorf et al. 2009). The wild gorillas infected by *Cryptosporidium* live in Bwindi Impenetrable National Park in Uganda (Nizeyi et al. 1999). There the parasite was also found in neighboring populations of humans and cattle, and it has been argued that people may act as a reservoir for the anthropozoonotic parasite, further exposing the apes with increased shared habitat usage (Nizeyi et al. 2002a, 2002b; Graczyk et al. 2001).

The present study was conducted in Ugalla, western Tanzania. Ugalla is one of the driest, most open, and seasonal habitats inhabited by chimpanzees (Kano 1972; Itani 1979; Nishida 1989; Moore 1994, 1996; Hernandez-Aguilar 2006, 2009). These apes exhibit population densities estimated to be 1/50th of populations living in forested habitats and home ranges more than ten times larger than their forest counterparts (Moore 1992; Hernandez-Aguilar et al. 2006; Ogawa et al. 2007). This low population density is associated with low density and high seasonality of foods (Hernandez-Aguilar 2006, 2009), features common to other savanna chimpanzee study sites that make them marginal and presumably difficult for the apes (McGrew et al. 1981). Environmental stress has been associated with an increase in the susceptibility to *Cryptosporidium* infections (Chalmers and Davies 2010) and the detection of this parasite in the Ugalla chimpanzees can be a signal of the difficult environmental conditions the apes face in the study area.

The aims of the present study were: (1) to determine the prevalence and intensity of shedding oocysts of *Cryptosporidium* sp. in chimpanzees living in Ugalla and (2) to establish how seasonality (rainfall and temperature) affect prevalence of *Cryptosporidium* infections.

Materials and methods

Study site and subjects

The Ugalla region is located in western Tanzania east of Lake Tanganyika. This 3,300 km^2 region is bordered by the Malagarasi River on the north, the Niamanzi (Ilumba) basin on the South, the Uvinza-Mpanda road on the west, and the Ugalla River on the east. Fecal samples were collected from a single population of chimpanzees living in this region, from two study sites: Issa and Ilumba. Two collection periods (December 2001–June 2003 and October 2008–March 2010) came from the Issa study area. This study area is located in the west of the Ugalla region and comprises >80 km^2, is occupied by a single community of chimpanzees (Rudicell et al. 2011), and it is the location of ongoing research. A third collection period (August 2006) came from the Ilumba study area, ~40 km southeast of Issa.

The vegetation of the Ugalla region is miombo woodland where *Brachystegia* and *Julbernardia* (Fabaceae) are the dominant tree genera, with forest patches comprising less than 2 % (see further description in Kano 1972; Itani 1979; Nishida 1989; Moore 1994; Ogawa et al. 2007; Hernandez-Aguilar 2006, 2009). The climate is divided into rainy and dry seasons, the dry season lasting from May to October. Annual rainfall averaged less than 1,000 mm^3 (Hernandez-Aguilar 2009). Average daily temperatures ranged from 13.2 to 34 °C. It was during the dry season when temperatures were the highest during the day and the lowest during the night. Some parts of Ugalla were inhabited by humans in the past, but these small settlements were shifted out of the area during the government's Ujamaa relocation program in the late 1960s. At present, there are a few small settlements in peripheral areas.

The chimpanzees of Ugalla are not habituated to observers and consequently community size and structure are not directly known. However, recent genetic analyses suggest the community has a minimum size of 67 individuals (Rudicell et al. 2011). Our research complied with the protocols approved by the Animal Care Committee of our home institutions and the legal requirements of Tanzania.

Collection and examination of stool samples

A total of 406 chimpanzee fecal samples were collected: 63 samples in 2001–2003 and 332 in 2008–2009 from Issa, and 11 samples (2006) from Ilumba. Of these, 54 samples were collected from the early rainy, 112 from the late rainy, 92 from the early dry, and 148 from the late dry seasons. The samples were collected soon after defecation (fresh feces) and were fixed in situ using chemical fixatives MIF (merthiolate–iodine–formalin Solution) and alcohol absolute (Golvan et al. 1972). Every sample consisted of approximately 2–4 g of feces, homogenized into 10 ml fixator. Samples were stored in the field under cool conditions. Each sample was marked with an identification number, date, and GPS location. When the samples were collected at a nesting site, tubes were labeled with the nest and nesting tree number. Every sample belonged to a different nest within a nesting party and therefore likely belonged to a different individual. Since our sample size is larger than the number of individuals identified to be part of the community, feces from some of the same individuals must have been collected on different dates. The samples were shipped to the Parasitology Laboratory, Department of Microbiology and Sanitary Parasitology, Faculty of Pharmacy, University of Barcelona in Spain. In the laboratory, fecal samples were individually weighed and re-homogenized with MIF fixator, sieved to remove debris, and centrifuged at 2,500 rpm for 10 min at room temperature to obtain a dense pellet. The resulting supernatant was discarded. The entire area of the smears was examined with a light microscope (Leitz Laborlux K) using a ×100

objective. Oocysts were measured and identified according to their morphology (Fayer et al. 1997).

Samples were considered positive for *Cryptosporidium* spp. when oocysts were found in the three smears analyzed per sample. Rainfall data were classified in three groups: low (<40 mm^3), moderate (40–80 mm^3), and intense (>80 mm^3); minimum temperature (13.2–17 °C, SD=0.3) in two groups: ≤15.33 and >15.33 °C; and maximum temperature (26.6–34 °C, SD=1.6) in two groups: ≤28.69 and >28.69 °C. Seasons were divided in early rainy (November to January), late rainy (February to April), early dry (May to June), and late dry seasons (August to October).

Statistical analysis

Statistical analysis was performed using SPSS 15.0. A descriptive analysis was conducted to describe the distribution of parasitization by rainfall, temperature, and season. Associations among qualitative variables were assessed using chi-square tests. A multivariate logistic regression analysis was performed to determine the effect of these factors on the odds (OR) of presenting a *Cryptosporidium* infection. In addition, were compared the prevalence of *Cryptosporidium* infections between the different study periods at Issa (2001–2003 and 2008–2010). All p values <0.05 were considered significant.

Results

Cryptosporidium sp. oocysts were observed in 36 of the 406 samples studied (8.9 %). No samples examined were diarrheic. Thus, animals showing the presence of oocysts were considered asymptomatic carriers. The oocysts were round or subround in shape and the diameter varied within a range of 3.5–5.8 μm (X=4.14±0.49; N=180). Concentration of *Cryptosporidium* varied from 9,950 to 55,900 oocysts per gram of feces (X=28828±14732.379, N=36). *Cryptosporidium* oocysts were found isolated and in small accumulations (four to 15 oocysts).

Most positive samples (23 out of 36; 63.9 %) were collected during the two rainy seasons (early rainy six samples and late rainy 17 samples), 13 (36.1 %) of the positive samples were collected during the early dry season, and no positive samples were found during the late dry season. See Table 1.

Cryptosporidium infections increased with higher rainfall and presented the highest peak associated with the group of moderate rainfall (40–80 mm^3; p<0.05). The highest number of positive samples was associated with the lowest range of maximum temperatures (≤28.69; p<0.000); no significant differences were found when the two groups of minimum temperatures were compared (p=0.29). See Table 2.

No statistically significant differences were found when the prevalences of *Cryptosporidium* infections between the two study periods in Issa were compared (2001–2003 vs. 2008–2010).

In the multivariate analysis performed by logistic regression (Table 2) two climatic variables (rainfall and maximum temperature) and seasonality were the predictive factors associated with parasitization by *Cryptosporidium*. The rainy season (late wet and early wet) showed an odds ratio OR=2.81 (95 % confidence interval (95 %CI)=1.38–5.72) when the dry season (late dry and early dry) was taken as a reference group. Moderate rainfall (40–80 mm^3) showed an OR=5.50 (95 %CI=1.02–29.67), and intense rainfall (>80 mm^3) an OR=3.12 (95 %CI=1.43–6.85) when low rainfall (<40 mm^3) was taken as reference group. The results of the logistic regression (p=0.29) suggested that minimum

Table 1 *Cryptosporidium* sp. prevalences according to seasonality (rainy and dry seasons), rainfall, and temperature

Factor	Negative	Positive	Sig.	OR	IC (95 %)
Total	370 (91.1 %)	36 (8.9 %)			
Seasonality (2 seasons)	p<0.003				
Wet (late wet + early wet)	143 (86.1 %)	23 (13.9 %)	0.004	2.81	(1.38–5.72)
Dry (late dry + early dry)	227 (94.6 %)	13 (5.4 %)		Ref.	
Rainfall	p<0.005				
<40 mm3	212 (95.1 %)	11 (4.9 %)		Ref.	
40–79 mm3	7 (77.8)	2 (22.2 %)	0.047	5.506	(1.02–29.67)
>80 mm3	111 (86.0)	18 (14.0 %)	0.004	3.125	(1.43–6.85)
Maximum temperature	p<0.000				
≤28.69	186 (86.5 %)	29 (13.5 %)	0.001	11.46	(2.7–48.81)
>28.70	147 (98.7 %)	2 (1.3 %)		Ref.	
Minimum temperature	p=0.29				
≤15.33	162 (93.1 %)	12 (6.9 %)	0.29	0.67	(0.31–1.41)
>15.34	171 (90.0 %)	19 (10.0 %)		Ref.	

Presence and absence of *Cryptosporidium* sp. is compared by Chi-square test; odds ratio for occurrence of parasitization by *Cryptosporidium* sp

Table 2 *Cryptosporidium* sp. prevalences according to seasonality

Factor	Negative	Positive	Sig.	OR	IC (95 %)
Total	370 (91.1 %)	36 (8.9 %)			
Seasonality (4 seasons)	$p<0.000$				
Late wet (Feb–April)	95 (84.8 %)	17 (15.2 %)	0.48	1.43	0.53–3.86
Early dry (May–July)	79 (85.9 %)	13 (14.1 %)	0.61	1.13	0.47–3.7
Late dry (August–October)	148 (100 %)	0	0.95	0	0
Early wet (Nov–Jan)	48 (88.9 %)	6 (11.1 %)	0	Ref.	

Presence and absence of *Cryptosporidium* sp. is compared by Chi-square test; odds ratio for occurrence of parasitization by *Cryptosporidium* sp

temperature did not have an effect on *Cryptosporidium* infections. However, maximum temperature was statistically significantly associated with *Cryptosporidium* detection, the lowest group of maximum temperatures (≤28.69 °C) showed the strongest association effect (OR=11.46; 95 %CI=2.70–48.81) when the highest group of maximum temperatures (>28.69 °C) was taken as a reference group.

Our results suggest that dry and hot environmental conditions could decrease the rate of *Cryptosporidium* transmission. Both conditions peaked during the late dry season, when we did not detect *Cryptosporidium* infections.

Discussion

The prevalence of *Cryptosporidium* infections found in wild chimpanzees in the present study (8.9 %) is lower than those detected in free olive baboons in close contact with humans (32 %, Hope et al. 2004), in free vervet monkeys ranging into human habitats (29.3 %, Legesse and Erko 2004), and in vervet monkeys (*Cercopithecus aethiops*) and olive baboons (*Papio anubis*) in captive conditions (>66 %, Muriuki et al. 1997). Salzer et al. (2007) found *Cryptosporidium* in red colobus (*Pilocolobus tephrosceles*) and in red-tailed guenons (*Cercopithecus ascanius*) living in disturbed forest fragments, but not in individuals of either of the two species living in undisturbed forest. Higher prevalence than that found in the Ugalla chimpanzees was reported for conspecifics living in captive conditions in the Negara Zoo of Malaysia (36.4 %, Lim et al. 2008). The higher prevalences of *Cryptosporidium* in captive animals may be explained by the increased likelihood of parasite transmission in unnatural conditions where animals often live in confined spaces and/or in close contact with one another, as has been noted by Gomez et al. (2000), Gracenea et al. (2002), and Lim et al. (2008). Animals contract *Cryptosporidium* as a result of ingesting food or water in facilities contaminated with positive feces (Alves et al. 2005).

The prevalence of *Cryptosporidium* detected in the present study is similar to that reported for chimpanzees at Gombe, Tanzania (10 %, Lonsdorf et al. 2009), for gorillas at Bwindi Impenetrable National Park (11 %, Nizeyi et al. 1999), and for free olive baboons who range into human habitats in Ethiopia (11.9 %, Legesse and Erko 2004). In the present study, although there has been an increase in human activity and introduction of cows in studied area since the second period of collection, no statistically significant differences were found when we compared the prevalence of *Cryptosporidium* infections between the two study periods. This suggests that the impact of these growing human activities may not yet be considerable.

Fayer et al. (2000) reported the presence of *Cryptosporidium parvum* (or *C. parvum*-like) in 152 animal species belonging to 11 orders. Eight of these orders are present at Ugalla (Artiodactyla, Chiroptera, Insectivora, Lagomorpha, Perissodactyla, Primates, Proboscidea, and Rodentia). Due to this ubiquitous nature of *Cryptosporidium* it is possible that other species besides chimpanzees are carriers of this parasite in Ugalla.

Some authors such as Fayer et al. (1998) and Gracenea et al. (2002) detected statistically significant differences in oocyst shedding between cold and warm periods of the year, finding higher shedding during the cold period. This is consistent with the results of the present study since the majority of positive samples were found in the rainy season when average temperatures during the day are lower than those in the dry season.

No seasonal differences in parasite prevalences were found in Assirik, Senegal, a dry habitat like Ugalla (McGrew et al. 1989), or in individuals reintroduced to the Rubondo Island National Park, Tanzania (Petrzelkova et al. 2010). Parasite prevalences in Gombe tended to be higher during the dry than the rainy season (Bazuka and Nkwengulila 2009). Huffman et al. (1997) found no statistically significant differences between dry and rainy season in prevalences of other parasites besides *Oesophagostomum stephanostomum* in chimpanzees of the Mahale Mountains, western Tanzania. Parasitic infections mainly by *Oesophagostomum* sp. increased in chimpanzees and bonobos during the early rainy season (Kawabata and Nishida 1991; Huffman et al. 1996, 1997; Huffman and Caton 2001; Dupain et al. 2002). Infections by *Cryptosporidium* in Ugalla chimpanzees may follow trends in seasonality similar to those by *Oesophagostomum* sp.

Cryptosporidium infections may affect the chimpanzees similarly to humans. The prevalence of *Cytosporidium* in Gombe chimpanzees was highly associated with diarrhea or gastro-intestinal dysfunction (Lonsdorf et al. 2009). We did not identify age classes in our samples, but as in humans,

infants, older, and immune-compromised individuals of the Ugalla chimpanzee population likely are at higher risk of suffering health problems associated with *Cryptosporidium* infections. All fecal samples from Ugalla were non-diarrheic, including those that came out positive, and thus we have assumed that the chimpanzee carriers were asymptomatic. Asymptomatic humans do not show gastrointestinal symptoms but they may have clinical sequelae (Chalmers and Davies 2010) and this raises the possibility that asymptomatic chimpanzees may also suffer not evident health problems.

Chimpanzees at Gombe lost weight during the dry season likely because of food scarcity (Goodall 1986; Pusey et al. 2005). Bazuka and Nkwengulila (2009) suggested that this low food intake may cause nutritional and immunity problems and stress in the apes and may explain the high prevalences of nematodes they found in Gombe chimpanzees during the dry season. They also suggested that water scarcity during the dry season in Gombe may increase transmission of parasites because chimpanzees and other animals congregate at drinking points. In Ugalla the dry season is a time of food shortage for the chimpanzees (Hernandez-Aguilar 2006, 2008) and indirect evidence suggested that the apes drink standing water from pools used by other animals during the late dry season when sources of running water are scarce (Hernandez-Aguilar 2006, 2009). Thus, it would be expected that the harsher environmental conditions Ugalla chimpanzees face during the dry season would increase vulnerability to infections by *Cryptosporidium*. Contrary to this expectation, however, fewer positive samples were found in the dry season and this requires explanation.

Alternation of sleeping and feeding sites in primates has been seen as an adaptation to avoid parasitization (Freeland 1980; Hausfater and Meade 1982; McGrew et al. 1989; Anderson 1998). Bazuka and Nkwengulila (2009) explained the higher parasitic prevalences in one of the chimpanzee communities in Gombe as a result of more repeated use of sleeping and gathering areas because that community had more individuals. Chimpanzees in Issa reused nesting sites more often during the rainy season (Hernandez-Aguilar 2009) and this may increase the rate of infection by *Cryptosporidium* at this time of the year. *Cryptosporidium* does not survive well under hot climatic conditions and the higher day temperatures in Ugalla during the dry season possibly lower *Cryptosporidium* survival at this time of the year, decreasing the risk of individuals becoming infected upon contact with feces.

In conclusion, we found a significantly higher number of samples positive for *Cryptosporidium* sp. during the rainy than during the dry season at Ugalla. This seasonal difference could by explained by climatic conditions directly affecting the survival of the parasite and by chimpanzee behaviors that would reduce the chance of becoming infected. Obtaining prevalences of *Cryptosporidium* infections for chimpanzees living in savanna (seasonal) and forested (less seasonal) habitats is important for understanding how environmental conditions and social and behavioral factors (e.g., population density, home range use, and reuse of nesting sites) influence the chances of getting infected by this parasite. The findings that *Cryptosporidium* sp. is present in wild chimpanzees (Ugalla and Gombe) are important for broadening our knowledge of the diversity of parasites affecting this ape species.

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