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Original Contribution

Intestinal Helminths of Wild Bonobos in Forest-Savanna Mosaic: Risk Assessment of Cross-Species Transmission with Local People in the Democratic Republic of the Congo

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Abstract: Phylogenetic and geographic proximities between humans and apes pose a risk of zoonotic transmission of pathogens. Bonobos (*Pan paniscus*) of the Bolobo Territory, Democratic Republic of the Congo, live in a fragmented forest-savanna mosaic setting, a marginal habitat for this species used to living in dense forests. Human activities in the forest have increased the risk of contacts between humans and bonobos. Over 21 months (September 2010–October 2013), we monitored intestinal parasites in bonobo ($n = 273$) and in human ($n = 79$) fecal samples to acquire data on bonobo parasitology and to assess the risk of intestinal helminth transmission between these hosts. Coproscopy, DNA amplification, and sequencing of stored dried feces and larvae were performed to identify helminths. Little difference was observed in intestinal parasites of bonobos in this dryer habitat compared to those living in dense forests. Although Strongylids, *Enterobius* sp., and *Capillaria* sp. were found in both humans and bonobos, the species were different between the hosts according to egg size or molecular data. Thus, no evidence of helminth transmission between humans and bonobos was found. However, because humans and this threatened species share the same habitat, it is essential to continue to monitor this risk.

Keywords: parasitology, intestinal helminths, zoonosis, bonobos, DRC

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INTRODUCTION AND PURPOSE

The phylogenetic and geographic proximities between humans and apes can lead to zoonotic transmission (Wolfe et al. 1998; Pedersen and Davies 2010), which poses a threat

to the health and survival of both humans and apes. Several cases of pathogen transmission between humans and wild apes have been reported in the literature, including viruses (especially paramyxoviruses and filoviruses), respiratory and digestive bacteria (*Streptococcus pneumoniae*, *Pasteurella multocida*, *Campylobacter* sp., *Salmonella* sp., *Escherichia coli*), digestive protozoans (*Giardia* sp., *Entamoeba histolytica*), and nematodes (*Capillaria hepatica*, *Oesophagostomum bifurcum*, *Ascaris lumbricoides*) (Wolfe et al. 1998; Graczyk et al. 1999; Ferber 2000; Woodford et al. 2002; Chapman et al. 2005; Goldberg et al. 2007; Köndgen et al. 2008, 2010; Kaur and Singh 2009; Krief et al. 2010; McLennan and Huffman 2012; Ghai et al. 2014). Among pathogens, gastrointestinal parasites can have serious health consequences for wild primates (Roberts and Janovy 2009; Krief et al. 2010; Guillot et al. 2012), especially nodular worms infection (*Oesophagostomum* spp.) that can be lethal for humans and apes (Polderman and Blotkamp 1995; Krief et al. 2010; Guillot et al. 2012).

However, this risk can be modulated by different parameters including the types of habitat and climate. For example, chimpanzees living in savannas (Ugalla, Tanzania and Fongoli, Senegal) harbor less or no parasites sensitive to dry climatic condition (thin-shell eggs, including the lethal *Oesophagostomum* spp.) compared to those living in dense forests (Kalousová et al. 2014; Howells et al. 2011). On the contrary, in fragmented habitats, risk of zoonotic transmission between humans and wildlife may increase due to the more frequent contacts between these host species (Goldberg 2008). In order to improve our knowledge on host-parasite relationships and risk of zoonotic transmission, it is essential to study the same host species in different types of habitats.

Among apes, bonobo (*Pan paniscus*, Schwartz 1929) is the last species described. In certain aspects of its social and sexual behavior as well as for his genome, bonobo appears as close to humans as chimpanzees (Prüfer et al. 2012). Little is known about the parasites of this threatened species in the wild, with only two studies in dense forest that have been published (Hasegawa et al. 1983; Dupain et al. 2000, 2009).

Here, we present the first parasitological study on bonobos from a fragmented and more open habitat comprising a forest-savanna mosaic, a marginal habitat for this species used to living in dense forests. This particular bonobo population appears to live in a drier habitat (Bwanogoy et al. 2010) and isolated from the rest of the bonobo distribution area (Kawamoto et al. 2013). Moreover, traditional activities are regularly practiced in the forest pat-

ches where bonobos live. The two main objectives of this study are (1) to describe the intestinal helminths of bonobos in this more open and potentially drier habitat compared to denser forests of the central locations of the Congo Basin, and (2) to compare intestinal helminths in bonobos and local people to search evidence of parasite transmission between these two host species. Because of climatic and habitat features, we predict that we will not find parasites sensitive to dry habitat (with thin-shell eggs) and to direct sunlight, contrary to those found in bonobos living in dense forest habitat (Hasegawa et al. 1983; Dupain et al. 2009). In addition, because human activities occur in the forest, we predict that evidence of parasite transmission will be found *i.e.*, we expect to find same intestinal parasites in fecal samples from both host species.

METHODS

Approval for fieldwork and biological sampling was authorized by the Congolese Ministry of Environment, Conservation, Nature and Tourism. This research project adhered to the ethics, protocols and legal requirements of the Democratic Republic of the Congo (DRC) and to the National Advisory Committee of France (<http://www.ccne-ethique.fr>).

Study Site

This research was conducted in the Manzano forest (2°38'S, 16°23'E) in an area of about 20 km² (Embirima, Bolobo Territory, DRC, Fig. 1), in the community-based conservation area (formalization in process, Narat et al. in prep) led by the Congolese non-governmental organization, Mbou-Mon-Tour (MMT). This study site represents the southwestern most extent of the geographic range of bonobos and is the closest to Kinshasa (Inogwabini et al. 2008; Narat et al. 2012, 2015a) (Fig. 1). The habitat of the Bolobo Territory comprises a forest-savanna mosaic composed of 60% of lowland tropical rainforest and 40% of savanna (survey on 1993 km², Inogwabini et al. 2008).

Human density is lower than five inhabitants per square kilometer (Perrodeau, pers. com. 2010), and all settlements are located within savanna. Five traditional farms, the homes of one or two families (composed of 2 to 15 people), are settled at the edge of the Manzano forest (Fig. 1). Local people, the Teke ethnic group, respect a traditional eating-taboo with respect to bonobos, which

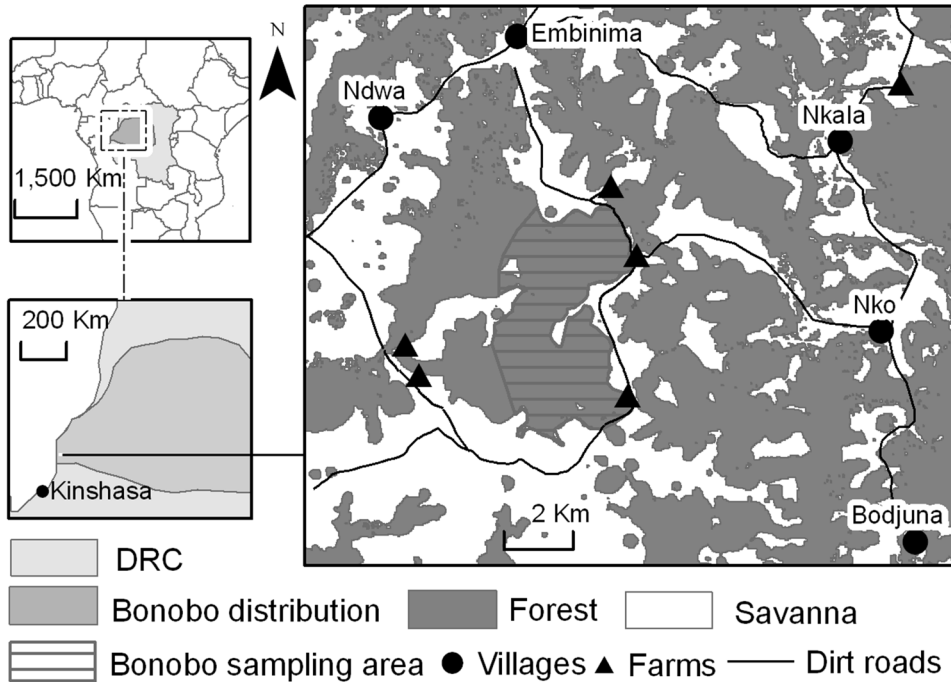


Figure 1. Study site location in the bonobo distribution area (source: IUCN and ICCN 2012).

they consider to be almost human (Narat et al. 2015b). These people are highly dependent on the forest, where they enter almost daily for shifting cultivation (cassava, corn, sugar cane, and bananas make up the majority of crops), to hunt (with rifle, snare, or net), to fish or to forage (Narat et al. 2012).

Over 13 years ago, several villages decided to keep a forested area for bonobo conservation, a decision agreed by the local community councils. In these forest patches, some human activities were forbidden (hunting, shifting cultivation), but human presence was not forbidden during the sampling periods. The Manzano forest is one of these areas.

A bonobo habituation process, led by VN, FP and local field assistants representing MMT, started in 2010 within Manzano forest. Tutin and Fernandez (1991) defined habituation as “the term used to describe acceptance by wild animals of a human observer as a neutral element in their environment.” During this study, the bonobos were semi-habituated and at the end (November 2013), the bonobos accepted the presence of researchers at a 10 m distance during long observation periods; however, continuous daily monitoring from nest-to-nest was rarely possible (Narat et al. 2015a). Based on our observations, the size of Manzano bonobo community is 23 individuals on a 20 km² home range—a community is composed of male and female individuals with closed social bonds and sharing a large home range with fission-fusion social organization (Van Elsacker et al. 1995).

Study Periods

Fecal samples from unidentified bonobos of the Manzano community were collected over a 21-month period, from July to September 2010 and from May 2012 to October 2013. Human fecal samples were collected in June and July 2012.

Fecal Samples

A total number of 273 unidentified fresh bonobo feces (<12 h) were collected early mornings from under bonobos’ night nests ($n = 233$) or during tracking ($n = 40$), and stored in a labeled (N^o, GPS, date, time) hermetically sealed 60 mL flask after macroscopic observation (for mucus, blood, macro-parasites, and consistency).

A total of 79 human volunteers were sampled (38 women, 41 men) in three villages (Embirima, Bodjuna, Nkala; Fig. 1) at a maximum distance of 15 km from the forest patch where the bonobos’ samples were collected. Based on unpublished ethnological data, we know that humans can use forest patches until 15 km around their villages, especially for hunting, fishing or gathering. Each volunteer (or legal representative) signed a consent form after being given information about the study objectives, and was interviewed before collecting the fecal sample (except for one volunteer) to establish if he/she received any previous anthelmintic treatment (curative or prophylactic) and/or if he/she conducted activities in the forest.

The diversity of deworming practices and the small sample size prevented us to analyze treatments' efficiency (molecule and posology) in details. Direct examination of feces was carried out in the following hours and the volunteer was informed of the presence of intestinal parasites. An anthelmintic locally used (levamisol) was systematically proposed, as a compensation, after the microscopic analysis, regardless of the coproscopy results.

For both bonobo and human samples, 2 g of fecal material was fixed with 10% formalin and another 3 g was placed in 95% ethanol for 24 h. The ethanol was removed thereafter and the fecal material was placed in gauze and dried on silica gel beads for storage (Nsubuga et al. 2004).

Coproscopy Analyses and Coprocultures

Coproscopy analyses were made at the Parasitology Department of National Veterinary School of Alfort, France. Two direct smears ($2 \times 50 \mu\text{l}$) of all formalin samples were examined microscopically at 100–400 \times magnification after homogenization. Helminth eggs and ciliates were identified as accurately as possible according to their size, color, and shape and were counted (eggs per gram, EPG). EPG were corrected according to fecal consistency to obtain the Corrected Parasitological Load (CPL) value (Herberg et al. 1986; Krief et al. 2005; Masi et al. 2012): dry feces, $\text{CPL} = \text{EPG} \times 0.5$; soft feces, $\text{CPL} = \text{EPG} \times 2$; liquid feces, $\text{CPL} = \text{EPG} \times 3$. The CPL average was calculated for parasite-positive samples only. Two eggs from humans and bonobos with similar morphology but with different egg size were considered as different parasite species.

After direct examination in the field, fresh stools from stronglylid-positive samples were incubated as coprocultures during the campaign from May to July 2012. After 10–15 days of incubation with vermiculite and active carbon mixed in petri dishes, larvae were collected using the Baermann technique (Greiner and McIntosh 2009) and preserved in 95% ethanol for molecular analyses on mixed larvae. Identifications of larvae stored in Ethanol were not possible because this solvent hinders to observe the shape of intestinal cells, normally used for identification at the genus level (as for *Oesophagostomum*).

Molecular Analyses

Molecular analyses were carried out at the Eco-anthropology and ethnobiology research unit (UMR7206), National

Museum of Natural History, Paris, France. DNA analyses were performed on mixed larvae (coprocultures) and on the corresponding dried feces (22 bonobo fecal samples, 13 human fecal samples). For each sample, two DNA subsamples were analyzed, one from mixed larvae and one from dried feces. DNA from mixed larvae was extracted with a QIAamp DNA Mini kit Tissue (Qiagen, Venlo NL) and DNA from dried feces was extracted with a QIAamp DNA stool kit (Qiagen, Venlo NL) according to the manufacturer's instructions with modifications (supplementary information). Internal transcribed spacer 2 region (ITS2) of rRNA genes was PCR amplified with NC1 and NC2 primers, as described in Gasser et al. (1999). Direct sequencing was performed on the PCR-positive amplified samples and chromatograms were cleaned with 4Peaks[®] software. A basic local alignment search tool (BLAST, National Center for Biotechnology Information, accessed 2014) analysis was performed on the clean sequences, and sequence alignments between them and various reference sequences obtained using BLAST analysis were performed using MEGA6 software (<http://www.megasoftware.net/>).

Statistical Analyses

In order to compare egg size between humans and bonobos, we first used a Spearman test to assess the correlation between eggs length and width for each helminth type. When correlations were detected, we performed a Mann–Whitney *U* test to compare egg length between humans and bonobos. The effect of the presence or absence of prophylactic practices was tested with a Mann–Whitney *U* test to compare the parasitological load and a Chi-Squared test to compare the prevalence. Statistical analyses were performed with R software (R core team 2012) (<http://www.r-project.org/>).

RESULTS

Coproscopy Analyses and Coprocultures

Bonobos

Macroscopic examination shows that most of the feces had normal consistency (87%), 3% were dry, 9% were soft, and 1% diarrhetic. No samples containing blood, mucus, or macroparasites were obtained. Direct microscopic examination of bonobo samples revealed the presence of at least one helminth parasite species in 170 samples (62.3%). Most

Table 1. Positive Sample Rate, Corrected Parasitological Load (CPL, in Eggs Per Gram) and Egg Size (μm) in Human and Bonobo Fecal Samples (Direct Smears on Formalin).

	Bonobos ($n = 273$)			Humans ($n = 79$)		
	Rate (%) of positive samples (n)	CPL (epg)	Size L(min-max) \times l(min-max), μm	Rate (%) of positive samples (n)	CPL (epg)	Size L(min-max) \times l(min-max), μm
<i>Trogodytella</i> sp.	93.8 ($n = 256$)	4790 (100–42500)	(146–159) \times (76–78), $n = 6$	–	–	–
Strongylids	44.0 ($n = 120$)	208 (50–1000)	(60–94) \times (31–50), $n = 90$	43.0 ($n = 34$)	503 (50–2800)	(48–91) \times (32–45), $n = 103$
<i>Enterobius</i> sp.	12.8 ($n = 35$)	261 (50–2200)	(48–56) \times (23–28), $n = 24$	2.5 ($n = 2$)	200 (100–300)	(56–63) \times (28–33), $n = 12$
<i>Capillaria</i> sp.	8.1 ($n = 22$)	150 (100–600)	(46–51) \times (23–24), $n = 5$	1.3 ($n = 1$)	200	57–58 \times 29
<i>Strongyloides</i> sp.	2.6 ($n = 7$)	157 (100–200)	(46–63) \times (28–45), $n = 15$	0	–	–
<i>Ascaris lumbricoides</i>	0	–	–	29.1 ($n = 23$)	10257 (100–58000)	(60–88) \times (47–54)
<i>Toxocara</i> sp.	0.4 ($n = 1$)	100	–	–	–	–
Dicrocoelids	4.4 ($n = 12$)	117 (100–200)	(38–48) \times (23–28), $n = 3$	–	–	–
Taeniidae	–	–	–	1.3 ($n = 1$)	100	35 \times 35
Total helminths	62.3 ($n = 170$)	265 (50; 2200)	–	55.7 ($n = 44$)	5766 (50–58000)	–

The dash is used to give minimum and maximum values.



Figure 2. Photos of the main helminths found in the bonobo and human samples.

of the bonobo samples contained only one helminth parasite (one species: 44.3%; 2–16.5%; 3–1.5%). Four types of nematodes were identified in the samples: strongylida fam. gen. sp. (44.0%), *Enterobius* sp. (12.8%), *Capillaria* sp. (8.1%), and *Strongyloides* sp. (2.6%). Trematodes (*Dicrocoeliidae* gen. sp.) were detected in 4.4% of the samples (Table 1) (Fig. 2, photographs). Ciliates (*Troglodytella* sp.) were detected in 93.8% of the samples. CPL average varied between 100 and 261 EPG for each species or parasite type.

Egg size, percentage of positive samples, and corrected parasite load are summarized in Table 1.

Humans

Macroscopic examination shows that most feces had normal consistency (86%), 4% were dry, 8% soft, and 2% diarrheic. One sample contained mucus, but no samples contained blood, or macroparasites. Direct microscopic examination of human samples revealed the presence of at least one helminth in 44 samples (55.7%). Most helminth-positive samples contained only one species (1–34.2%; 2–20.3%; 3–1.3%). Four types of nematodes were identified: strongylida fam. gen. sp. (43.0% of samples), *A. lumbricoides* (29.1%), *Enterobius* sp. (2.5%), and *Capillaria* sp. (1.3%). One cestode egg (*Taeniidae*, 1.3%) was identified. No ciliates were found. All helminth CPL mean values were between 100 and 10257 EPG. Egg size, percentage of positive samples, and corrected parasite load are summarized in Table 1.

Interviews

Almost all human volunteers included in the present study had entered the forest (96%) at frequencies varying from once a day to once a week. A total of 14 different human activities—including shifting cultivation and several techniques of hunting, fishing, and gathering—were recorded and 65% declared to enter the forest for at least four types of activity. Most of them had taken a dewormer at some point in their lives (95%), while 69% used western drugs (levamisole, albendazole, mebendazole), 9% used traditional medicine, and 17% used both. A prophylactic deworming was used by 31% of people at variable frequency (from once a month to once a year). There were no statistical differences in prevalence and CPC between humans with prophylactic practices compared to those without prophylactic practices.

Comparisons of Egg Size in Bonobo and Human Samples

Capillaria sp., *Enterobius* sp., and strongylids were found in fecal samples from bonobos and humans. Only one human fecal sample contained *Capillaria* sp. eggs, which were larger (57–58 × 29 µm) than the *Capillaria* sp. eggs detected in the bonobo fecal samples ($n = 5$, 46–51 × 23–24 µm). In both hosts, the length and width of *Enterobius*

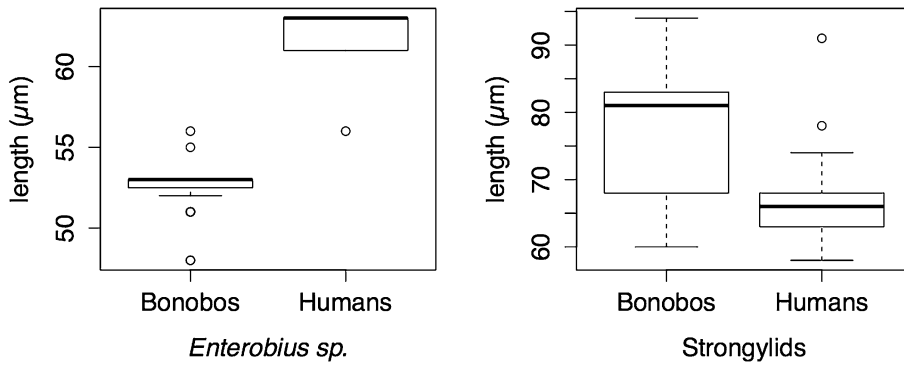


Figure 3. Box plot of length (μm) of *Enterobius* sp. and Strongylids from bonobo and human fecal samples. The median (black line), the 25th and the 75th percentiles are shown. Outliers are plotted individually.

Table 2. Number of Samples from Bonobos and Humans (larvae and Dried Feces) and the Type of Molecular Analysis (DNA Extraction, PCR, Sequencing) Used for Species Identification.

Number	Bonobos			Humans		
	Feces sample	DNA sub-sample from mixed larvae	DNA sub-sample from dried feces	Feces sample	DNA sub-sample from mixed larvae	DNA sub-sample from dried feces
DNA extraction	22	22	22	13	13	13
Positive PCR	14	9	9	10	9	3
Readable sequences	11	6	5	9	8	2
<i>O. stephanostomum</i> (KF250653.1) (% of identity)	7 (99%)	4	3	0	0	0
<i>Nematodirus</i> sp. (HQ844230.1) (% of identity)	3 (87%)	1	2	0	0	0
<i>Necator americanus</i> (AB793528.1) (% of identity)				8 (100%)	7	2
<i>Necator</i> sp. (AB793535.1) (% of identity)	1 (96%)	1	0			

BLASTn: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

Results were obtained by BLASTn.

sp. were strongly correlated ($n = 36$, Spearman test: $r = 0.71$; P value < 0.0001). The average length of *Enterobius* sp. was significantly higher in humans ($n = 12$) than in bonobos ($n = 24$) (Mann–Whitney test, $U = 0.5$, $P < 0.0001$), and there was no overlap between *Enterobius* egg size from humans and bonobos (Fig. 3). The length and width of strongylid eggs were also correlated ($n = 192$, Spearman test: $r = 0.66$; P value < 0.0001). Average length of strongylid eggs was significantly lower in humans ($n = 102$) than in bonobos ($n = 90$) (Mann–Whitney test, $U = 1654$, P value < 0.0001) and there was an overlap between human and bonobo egg size (Fig. 3).

Molecular Analyses

A summary of the molecular analyses performed on the bonobo and human fecal samples is shown in Table 2.

Out of 44 DNA subsamples obtained from 22 bonobo fecal samples (dried feces and larvae), 18 were PCR amplified and sequenced. The low rate of successful DNA amplification for the subsamples probably resulted from PCR inhibitors in the fecal samples. Readable sequences ($n = 11$) obtained by direct sequencing indicated the presence of *Oesophagostomum stephanostomum* (100% homology based on 245 bp of ITS2, GenBank accession number: KF250653.1) in seven bonobo stool samples. In three bonobo stool samples, the closest sequence identified was that of *Nematodirus* sp. (88% homology for two sequences based on 398 bp of ITS2 and 87% homology for one sequence based on 248 bp of ITS2, GenBank accession number: HQ844230.1), while in one bonobo stool sample *Necator* sp. (96% homology on 332 bp of ITS2, GenBank accession number: AB793535.1) was identified.

Table 3. Percentage of Parasite-Positive Samples Obtained from Wamba (Hasegawa et al. 1983), Lomako (Dupain et al. 2009) and this Study.

Site	Wamba (<i>n</i> = 390)	Lomako (<i>n</i> = 87)	Manzano (<i>n</i> = 273) (this study)		
Method	Sedimentation	Sedimentation	Direct smears		
Habitat	Continuous rainforest	Continuous rainforest	Forest-savanna mosaic		
Season	Wet	Wet	Wet <i>n</i> = 129	Dry <i>n</i> = 144	Total <i>n</i> = 273
<i>Troglodytella</i> sp.	99.0	74.7	93.8	93.8	93.8
Strongylida fam. gen. sp.	21.0 + 17.9 (<i>Oesophagostomum</i> sp.)	16.1 + 50.6 (<i>Oesophagostomum</i> sp.)	38.0	49.3	44.0
<i>Strongyloides</i> sp.	52.9	35.6	4.7	0.7	2.6
<i>Capillaria</i> sp.	21.0	0	7.0	9.0	8.1
Oxyuridae gen. sp.	6.2	0	17.1	9.0	12.8
<i>Trichuris</i> sp.	3.3	2.3	0	0	0
<i>Ascaris</i> sp.	0	3.4	0	0	0
<i>Toxocara</i> sp.	0	0	0	0	0.4
Dicrocoeliidae gen. sp.	45.1	3.4 + 1.1	5.4	3.5	4.4

The rainfall threshold between a wet and dry month is 100 mm.

Out of 26 DNA subsamples obtained from 13 human stools (dried feces and larvae), 12 were amplified by PCR and sequenced. All workable sequences (*n* = 9) were *N. americanus* (100% of similarity on 325 bp, GenBank Accession Number: AB793528.1).

DISCUSSION

This study is the first using both microscopy and molecular analyses to explore gastrointestinal parasites in wild bonobos and the risk of zoonotic transmissions between local people and bonobos. Whereas bonobos of Manzano live in a dryer habitat compared to those living in dense forests, helminths with thin-shell eggs were found (strongylids). Moreover, despite of the frequent and diverse human activities in the forest, no evidence of helminth transmissions between humans and bonobos were observed.

Intestinal Parasites of Bonobos Living in Forest-Savanna Mosaic

Discussions on helminths identifications found in bonobos are given in the supplementary information. Contrary to our prediction, parasites with thin-shell eggs were found in bonobos of Manzano (strongylids, *Strongyloides* sp.).

However, we did not find whipworms (*Trichuris* sp.)—as in bonobos living in dense forest (Wamba and Lomako, Table 3)—also known to be sensitive to direct sunlight and requiring moist and shady soil for its development (Roberts and Janovy 2009). Thus, our results do not show a clear pattern related to the effect of drier/open habitat on parasite diversity. Except for whipworms, our results suggest that there are little difference in intestinal parasites between bonobos living in forest-savanna mosaic and bonobos in dense forests (Table 3). We suggest that bonobos spend most of their daily activities in forest patches and use savannas only for traveling between forest patches or for short feeding sessions at the edge, as it was recorded recently with the improvement of the habituation process. According to the Wamba results (Hasegawa et al. 1983), inter-community variations in the same geographical area seem important. Further analyses of identified fecal samples considering individual differences need to be performed on more bonobo communities across our study area to assess inter- and intra-community variations where two other habituated communities far from 10–15 km are available (Nkala and Mpelu).

Human-Bonobo Helminth Transmission

Three helminth types were found in humans and bonobos: *Capillaria* sp., *Enterobius* sp., and strongylids. Justine

Table 4. Risk of Cross-Species Transmission of Helminths in Humans and Bonobos.

Helminths (compatible species)	Transmission mode	Risk of cross-species transmission?	Reference
<i>In bonobos</i>		<i>For humans</i>	
<i>Oesophagostomum stephanostomum</i>	Soil-transmitted (ingestion of larvae)	Yes	Guillot et al. (2012)
<i>Necator</i> sp.	Soil-transmitted (transcutaneous penetration of larvae)	Yes	Hasegawa et al. (2014)
<i>Strongyloides</i> sp. (<i>S. fuelleborni</i>)	Soil-transmitted (transcutaneous penetration of larvae)	Yes	Hasegawa et al. (2010)
<i>Enterobius</i> sp. (<i>E. anthropopithecii</i>)	Direct oro-anal transmission	Never described in humans	
<i>Capillaria</i> sp. (<i>C. brochieri</i>)	Unknown	?	Justine (1988)
<i>In humans</i>		<i>For bonobos</i>	
<i>Necator americanus</i>	Soil-transmitted (transcutaneous penetration of larvae)	Yes	Orihel (1971)
<i>Enterobius</i> sp. (<i>E. vermicularis</i>)	Direct oro-anal transmission	Yes, but unlikely in the wild	Hasegawa and Udono (2007)
<i>Ascaris</i> sp. (<i>A. lumbricoides</i>)	Soil-transmitted (ingestion of eggs)	Yes	Dupain et al. (2009), Stam (1960)

(1988) proposed a potential zoonotic role for *C. brochieri*. In our study, the *Capillaria* sp. eggs found in humans were larger than those found in bonobos and were, therefore, probably a different species (*C. hepatica*, spurious infection, Fuehrer et al. 2011).

Enterobius eggs were also significantly larger in humans than in bonobos. *E. anthropopithecii* is a known oxyurid of the *Pan* genus, while *E. vermicularis* is specific to humans. A mixed species infection containing *E. anthropopithecii* (egg size: 55 × 27 µm) and *E. vermicularis* (egg size: 63 × 30 µm) has been reported only once from a captive chimpanzee (Hasegawa and Udono 2007). In the current study, even if the egg morphology was not sufficient to determine the species of *Enterobius* (Hasegawa 2009) found in humans and bonobos, the species nevertheless differed between the two hosts. *E. vermicularis* can be fatal for chimpanzees in captivity (Murata et al. 2002). *Enterobius* is transmitted directly by the oro-anal route, resisting few days in the environment (eg Hugot et al. 1999; Baker 2007) and has a strong genus-specificity (Cameron 1929; Inglis 1961; Brooks and Glen 1982; Sorci et al. 1997; Hugot 1999; Hugot et al. 1999). This strong specificity of the oxyurid species to the host-genus probably limits the risk of transmission between humans and bonobos in the wild.

Contrary to previous studies on human intestinal helminths in Africa (Polderman and Blotkamp 1995; Pit

et al. 1999; Guillot et al. 2012), our molecular analysis did not reveal *Oesophagostomum* sp. in human samples. In their preliminary study at Lomako (dense forest), Dupain et al. (2000) have found strongylids, *Trichuris* sp., and *Ascaris* sp. both in humans and bonobos, without conclusion possible due to the lack of precise identification. In this study, the molecular data suggest that strongylids were not the same species in the two host species. We did not find *Trichuris* sp. neither in humans nor in bonobos and whereas 29% of the human samples were *Ascaris*-positive, we did not find *Ascaris* sp. in the bonobo samples.

In summary, contrary to our prediction, we did not find evidence of helminths cross-transmission between humans and bonobos during the study period, despite various human activities distributed in bonobos home range. These findings may be explained by low human density in the study area and the eating-taboo toward these apes. This taboo includes a more general avoidance of the bonobos: local people consider that encountering bonobos in the forest is a bad omen (Narat et al. 2015b). However, this behavioral avoidance does not limit the risk of being infected by the soil-transmitted parasites such as strongylids, which have an environmental stage of evolution. For public health concern and for bonobo survival and preservation, health monitoring of bonobos and people is of major importance. Among the participants involved in

this study, the use of dewormer as a prophylactic treatment (once a month to once a year) does not seem efficient enough efficient to decrease significantly prevalence and total parasitological load. The diversity of practices and the lack of data did not allow us to analyze in detail the efficiency of the different molecules used in this area and the posology of prophylactic treatment. The World Health Organization advised for this region to treat one to three times a year all infants and adults exposed to risks with albendazol or mebendazol (WHO 2008). For people working with bonobos, besides the prophylactic treatment (a dewormer twice a year, alternating between molecules), strict regulations are applied during field work (feces are buried 30 cm deep in the ground, wash hands with hydroalcoholic gel) to decrease the risk of parasite transmission. With the habituation process for long-term studies on Manzano bonobos, encounters between humans and bonobos are more and more frequent, and the distance between observers and bonobos is reduced (respecting a minimal distance of 8 meters). Intestinal parasites, especially strongylids, will continue to present a potential risk for field assistants, scientists and bonobos (Table 4). Indeed, the stress engendered by the monitoring might lead to immunodeficiency in bonobos, potentially increasing the number of parasite infections (Woodford et al. 2002). Moreover, Zommers et al. (2013) showed that trail networks laid by researchers influenced chimpanzee ground use, resulting in more parasite infections and greater infections intensity. Other studies have shown the influence of the presence of researchers or tourists on human-ape disease transmission (Wallis and Lee 1999; Nunn and Al-tizer 2006; Goldberg et al. 2007).

CONCLUSION

To the best of our knowledge, the present study is the first to compare intestinal parasites in wild bonobos and humans in a fragmented habitat and to provide results for the risk of zoonotic disease transmission. This kind of investigation improves our knowledge on primate parasitology in different contexts (geographic area, habitat and the local socio-cultural context). The same intestinal helminths were found in bonobos of the forest-savanna mosaic as those recorded in two other studies on intestinal parasites of bonobos in a continuous rainforest. There was no evidence of intestinal helminth cross-species transmission between humans and bonobos despite their overlapping habitat use

and the high frequency of human activities in the forest patches (including the home range of Manzano bonobos). Further analyses must be performed to monitor the human-ape transmission risk over time and to establish the degree of pathogen diversity, especially the protozoans and respiratory pathogens that can parasitize these hosts. Pedersen and Davies (2010) have identified the Congo Basin as an area at higher risk of emerging infectious diseases in the world, which means that researchers in this region, especially those working on great apes, should also focus on health surveillance.

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