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Monitoring of nematodes hosted by endangered Bonobo (*Pan paniscus* Schwartz 1929) at Lola ya Bonobo Sanctuary (Kinshasa, DRC).

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ABSTRACT

Subject description: Infectious diseases are now recognized to have a significant impact on some populations of wildlife. Although many infectious agents are species-specific, several pathogenic organisms can cross the species barrier and cause severe clinical diseases in new hosts. It is critical to understand the role played by emerging infectious diseases and zoonoses transmitted between humans and great apes.

Objective: The objective pursued by this study is to contribute to the sustainable conservation of bonobos (*Pan paniscus*), an endemic species of DRC that faces various threats including diseases such as parasites.

Methods and Results: The lab analyses were carried out at the Central Veterinary Laboratory in Kinshasa based on two main methods including qualitative and quantitative coproscopy by flotation and counting the number of eggs per gram (OPG) by the McMaster method.

In total 72 samples were analysed, 46 samples (64%), were infested by five species of nematodes, including *Ankylostoma spp*., *Trichostrongylus spp., Ascaris lumbricoides*, *Trichuris trichiura* and *Oesophagostomum sp.* The infestation with *Trichostrongylus spp*. was found to be higher compared to other nematodes. In addition, subadults were more infected compared to specimens of other age groups.

Conclusion and application of results: In this study, nematodes hosted by endangered bonobos were monitored in a relatively large number of 72 bonobos samples. The study found that individuals that were more central in the social interaction such as Subadults had higher chances of contracting parasites. These results indicate that for bonobos, social behaviour and age influence the risk of contracting parasites. Two factors that can be taken into account when managing diseases outbreaks in captivity and regular monitoring and treatment of individuals must be done to protect the species.

Keywords: Bonobo, Zoonotic, Nematodes, Parasites, Sanctuary, Democratic Republic of Congo.

INTRODUCTION

Human-animal-environment interaction plays a major role in understanding the spread of infectious agents such as nematodes to humans (Morse *et al.,* 2012). The complexity of this interaction continues to increase, due to globalization, and in the face of this phenomenon the appearance of the "One Health" concept. Understanding human-animal-environment interactions today requires the study of genetic material, effective and sufficient, in order to carry out relevant studies and establish an assessment of the state of health of a given population (Wolfe *et al.,*2005; Levinson *et al.,*2013). This is the case, in particular, for great apes (Gorillas, common Chimpanzees) (Mossoun *et al.,* 2015) which have been the cause of major endemics (HIV) (Karesh *et al.,* 2012) or epidemics (Ebola) (Anthony *et al.,* 2015). Nowadays, endemic regions where great apes are found are characterized by phenomena such as extensive agriculture, mining, deforestation, and the emission of toxic substances such as mercury and cyanide (Micheletti *et al.,* 2018); this leads to a decrease in primate populations and their population density (Odeniran *et al.,*2018). Sharing the same environment as well as increased contact between humans and primate populations encourage exchanges and the dispersal of pathogens (Narat *et al.,*2018); therefore, it is essential to continue to identify pathogenic microorganisms in these primates; necessary to know their cycle, their virulence and their viability to better understand infectious diseases in primates and thus implement strategies to combat these diseases (Woolhouse and Gaunt, 2007) and the Democratic Republic of Congo is home to one of the largest forests in the world, rich in biodiversity with numerous wild animal species including a large number of endemic species such as the Okapi (*Okapia johnstoni*), the Congolese peacock (*Afropavo congolensis*) and Bonobo (*Pan paniscus*) discovered in 1929 which is one of the species threatened with extinction, according to the International Union for Conservation of Nature (Fruth *et al.,*2016). Its survival is threatened with extinction, particularly following the demographic explosion which is putting increasing pressure on natural resources, the illegal trade in bush meat, poaching, armed conflicts, and the destruction of their natural biotope, illegal use in traditional medicine and diseases (de Waal, 1997; Isabelle and François, 2005). The trade of small orphaned Bonobos as pets also compromises their survival in their natural habitat. This practice is particularly disastrous because, to obtain the juvenile bonobo that will be sold, poachers must kill their mothers (Isabelle and François, 2005). Some authors have studied bonobos, in their publications as possible reservoirs of certain pathogens. Each study focused on the search for a specific pathogen and bonobos were found to be positive for human respiratory syncytial virus, *Streptococcus pneumoniae*, primate T cell lymphotropic virus (Grützmacher *et al.,* 2018; Ahuka-Mundeke *et al.,* 2016), Herpesviridae (Lavergne *et al.,* 2014), a Papillomavirus (Hoffmann *et al.,* 2019), the hepatitis E virus of bonobos in captivity (Spahr *et al.,* 2018), Adenoviruses (Vitelli *et al.,* 2013). The encephalomyocarditis virus was also detected in two dead bonobos at the Lola ya Bonobo Sanctuary and there has been research on bacteria sharing (Jones *et al.,* 2011) such as Shigella (Rothschild *et al.,* 2005), parasites such as *Plasmodium malariae*, *P. vivax and P. ovale* (Kaiser *et al.,* 2010), Balantidium coli (Pomajbíková *et al.,* 2010) and *Plasmodium lomamiensis* (Liu *et al.,* 2017). At Lola ya Bonobo Sanctuary, certain bonobos, such as orphan juveniles under six years old, lives in permanent contact with humans like surrogate mums for their mental rehabilitation and this contact creates an ideal

framework not only for their training but also, unfortunately, can contribute for the exchange of certain microorganisms such as ubiquitous nematodes which can be among the cause of diarrhoea cases often observed without the principal cause being clearly determined. This work aims to evaluate the level of infestation of bonobos in this sanctuary, especially by gastrointestinal parasites such as nematodes which constitute the largest group of parasitic worms in mammals.

MATERIAL AND METHODS

Study Area: Lola ya Bonobo is a sanctuary for protection, rehabilitation and reintroduction of orphaned bonobos located in Kinshasa (about 25 km from the city center). The sanctuary was founded by Mrs. Claudine André in 1994. She is also at the origin of the Ekolo ya Bonobo nature reserve reintroduction site, a part of tropical forest of 20,000 hectares, dedicated to the conservation of animals and plants in the Equateur province.

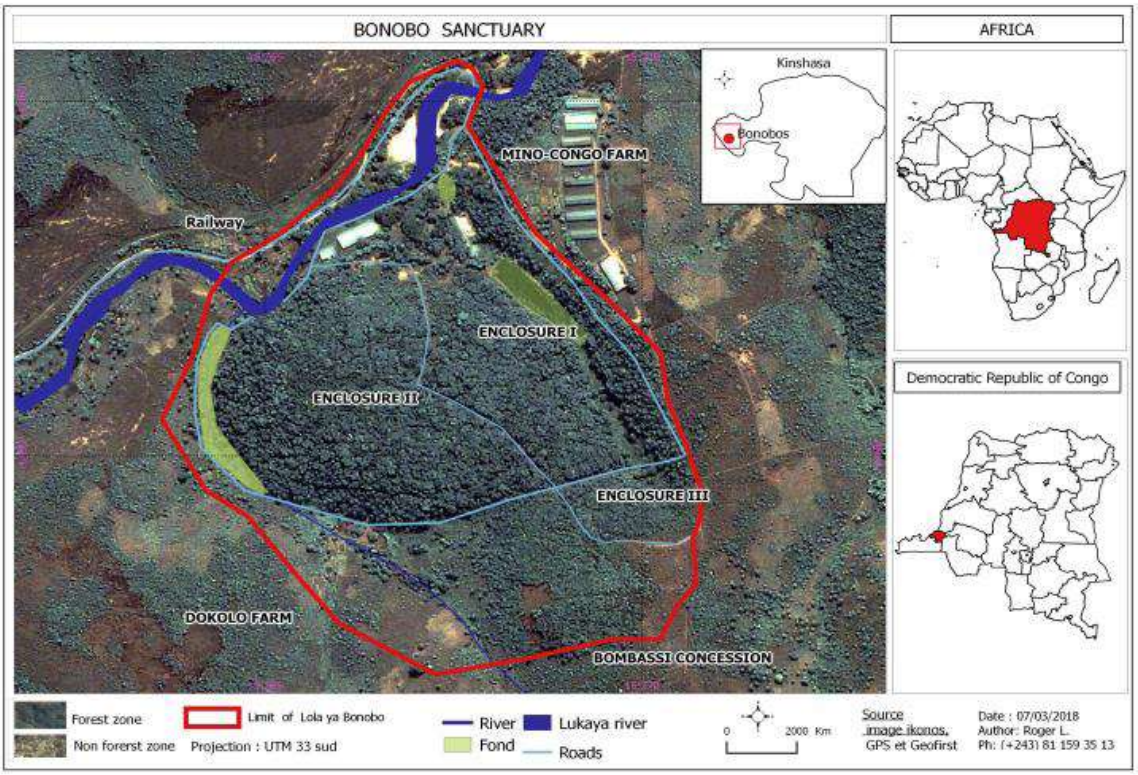

Figure 1. Geographic location of Lola ya Bonobo Sanctuary (Roger L., 2018)

Material: The study was carried out using faecal samples from 72 bonobos living in semi-freedom in this sanctuary with health monitoring and food intake. However, no bonobo-invasive method of collecting samples has been authorized by the sanctuary Management. Regulations and restrictions aim to protect these primates against any potential damage to their physical health and safeguard their well-being.

Methods

Samples collection: For each sample, fresh stools (approximately 5 g) from identified individual were collected early in the morning after defecation in the night shelter using a spatula from the upper part of the

defecated material not yet in contact with the soil. Then, this quantity of stool was put in a sterile plastic bottle fitted with a cap with gloved hands in order to limit any contamination.

Transportation of samples: The samples collected were transported in an isothermal container containing cold accumulators to the Central Veterinary Laboratory of Kinshasa where they were to be transferred to a refrigerator at 4°C to be analysed there in less than 24 hours.

Samples analyses

Macrocoproscopic examination: This examination consisted of seeing if the parasites were visible macroscopically or even certain unusual elements such as whole leaves or blood, to assess the colour and finally the appearance of the faeces which was "soft, mouldy or diarrhoeal".

Direct microcoproscopic examination: The technique consisted of placing two drops of physiological water on the object slide, then taking a small quantity of faecal matter using a wand (toothpick) adding a drop of Lugol's solution afterward, well mixed, then covering it with a coverslip and analysed under a microscope using a 10x objective followed by a 40x objective. Eggs were identified based on microscopic morphology.

A microcoproscopic examination after enrichment: This technique consists of concentrating parasite eggs or larvae found in faeces so that, even in small numbers, they can be detected using the Willis flotation technique using 40% brine (400 g of table salt (NaCl) in water (1 litre). This brine (density of 1.19 at 20°C) is suitable for rapid detection of nematodes and cestodes. It is recommended in practice in poorly equipped laboratories (Fischer and Say, 1989). The analysed solution was obtained from 2 g of faecal matter per 100 ml of brine (Talvik *et al.,* 2006).

A microcoproscopic examination after flotation in a McMaster cell: Originally developed in Australia, the McMaster method is the most widely used for counting nematode eggs (Bosco *et al.,* 2014). This technique is used to assess the degree of infestation by a parasitic worm in an animal or human specimen. This infestation rate is estimated as follows:

OPG= Number of eggs in both cells x 50.

Identification of Nematodes: The eggs or larvae were identified using identification boards present in the Laboratory as well as various books containing identification keys (Thienpont *et al.,* 1979).

Statistics: Calculated averages and percentages using Excel.

RESULTS

In total 72 (100%) bonobos constituting the total number of individuals at the sanctuary, 46 (64%) were infested by nematodes of five different species (Table I) and their faeces were mainly moulded (75%) followed by soft (15%) and diarrheal (10%) and that the presence of blood was not noted in any sample.

Trichuris trichiura egg

Ascaris lumbricoidis egg

Oesophagostomum spp egg

Pan paniscus animal hosted

Table 1. Number of eggs counted per gram of faeces (OPG) between different age groups

			Parasites						
Groups	**Samples number**	**Infected**	***Ankylostoma spp***	***Trichostrongylus spp***	***Ascaris lumbricoidis***	***Trichuris trichiura***	***Oesophagostomum spp***	*Total*	%
Juveniles (0-5 years)	19	11(58 %)	1400	7200	700	200	-	9500	28
Subadults (6-13 years)	30	21(70 %)	7100	1900	1500	1800	400	12700	38
Adults (13 + years)	23	14(61 %)	4000	6800	600	-	-	11400	34
Total	72	46(64 %)	12500	15900	2800	2000	400	33600	-
%	-	-	38	47	8	6	1		100

Main nematodes isolated are: *Ankylostoma spp*., *Trichoctrongylus spp*., *Ascaris lumbricoides*, *Trichuris trichiura* and *Oesophagostomum sp.* Subadults were most infected (38%), followed by adults (34%) and lastly juveniles (28%). Infestation with *Trichostrongylus spp* was the most frequent (47%), then *Ankylostoma spp* infestation (38%) followed by *Ascaris lumbricoides* (8%), *Trichuris trichiura* (6%) and, finally, *Oesophagostomum spp* (1%). The immune system and behaviour towards soiled objects between individuals can be considered as factors that influence the infection rate between different groups (Moureaux, 2005). Added to this is the shortage of diversity of plants and fruits for bonobos in semi-freedom, which can cause a state of nutritional stress. From there, imposing an increase in physiological needs could influence the susceptibility of bonobos to nematodes (Krief *et al.,* 2005).

DISCUSSION

In this study, five nematodes were identified at the occurrence, (*Ankylostoma spp, Trichostrongylus spp, Ascaris spp, Trichirus trichiura, and Oesophagostomum spp*). While, a study carried out by (Koto *et al.,*2018) on bonobo parasites in the same sanctuary highlighted the presence of *Ankylostoma duodenale* with a very high level of infestation, 19,600 eggs out of (86.3%), followed by *Trichuris trichiura*, 2900 eggs (12.8%) and *Strongylus sp.,* 200 eggs (0.9%). Although some nematodes are also identified in the present study, we note some differences with another study carried out in 2018 by Koto *et al.,* which gave the following results*: Trichostrongylus spp*. with 15900 eggs (47*%)* followed by *Ankylostoma spp.* with 12500 eggs (38%)*, Ascaris lumbricoides* with 2800 eggs (8%), *Trichuris Trichiura* with 2000 eggs (6%) and *Oesophagostomum spp.* with 200 eggs (1%). These differences can be explained by the difficulty of identifying different species of nematodes which can lead to confusion or because our study took place a few years after the first study justifying a possible introduction of other nematodes and study populations of the two studies are not constituted in the same way but also the different period between two studies. The coproscopic prevalence rates of different parasitic species are generally higher in our study than in the previous study, except *Ankylostoma spp*. which revealed a prevalence of 86% in the previous study compared to 38% in ours. We also note that three nematodes (*Ascaris lumbricoides, Trichostrongylus spp*., and Oesophagostomum *spp*.) were only isolated in our study, this could be explained by the highest number of samples taking into account the entire bonobo population of the sanctuary in our study.

All parasites' eggs isolated in our study from bonobos in this sanctuary have already been isolated from other great apes in Africa like in chimpanzees and gorillas living either in captivity, as habituated wild populations or not. The same nematodes were isolated from Chimpanzees by (Ashford *et al.,* 2000; Dupain *et al.,* 2009; Huffman *et al.,* 1997, 2009; Krief *et al.,* 2005; McGrew *et al.,* 1989; Modry´ *et al.,* 2009; Nejsum *et al.,* 2006). Wild chimpanzees are not widely infected by parasites (Lilly *et al.,*2002; Murray *et al.,*2000;), they have been isolated from three wild chimpanzee populations in Gombe National Park in Tanzania and another study (Landsoud-Soukate *et al.,*1995) in Lope, Gabon put forward hypotheses according to which chimpanzees were accidentally contaminated by other mammals or by other environmental factors. A high prevalence of *Oesophagostomum sp*. during the rainy season was recorded in the groups of chimpanzees of Mahale and Budongo

(Huffman *et al.,* 1997, 2009) also, in wild bonobos habituated to Lomako (Dupain *et al.,* 2002). Kalema *et al.,*2004, in Uganda, showed that gorillas habituated for tourism had a large number of eggs counted for these different parasites too (*Oesophagostomum spp., Trichostrongylus, Ascaris Lubricoides and Trichuris trichiura*). All habituated and non-habituated gorillas were infected with *Ascaris lumbricoides*, but only non-habituated gorillas were infected with *Trichuris trichiura* and all nematodes' eggs identified in this study had previously been isolated from humans. Two cases of *Trichostrongylus* whose two eggs were similar to those of *Oesophagostomum* had been isolated from gorillas in Bwindi National Park in Uganda; they were instead *Paralibyostrongylus kalinae* and *Tricostrongylus kigeziensis* (Durrette-Dusset *et al.,* 1992). Kalema *et al,* in their study in 2004, faced the problem of the resemblance of the eggs of certain nematodes, for example, that of differentiating the eggs of *Ascaris lumbricoides* and *Ascaris suum* which are morphologically indistinguishable. It can be noted that *A. lumbricoides* infects humans and primates more than other animals while *A. suum* is rarely found in them (Anderson, 1995). Also, the polar shape of the eggs of *Trichuris trichiura* which particularly infect humans and which are also morphologically indistinguishable from the eggs of *Trichuris suis* which do not affect humans. Nematodes isolated in this study have been isolated in previous studies (*Ankylostoma spp., Trichostrongylus spp., Ascaris lumbricoides, and Trichuris trichiura*), respectively from mountain gorilla populations in Bwindi National Park in Uganda and Virunga National Park in the DRC (Rothman *et al.,* 2004) and these worms can be found in humans behave like zoonotic parasites.

CONCLUSION AND APPLICATION OF RESULTS

As environmental changes exacerbate the threat coming from infectious diseases in wild mammal species, monitoring their health and gaining a better understanding of the immune functioning at the species level have become critically important. Our study confirmed that bonobos at Lola ya Bonobo Sanctuary are hosted by nematodes such as *Ankylostoma spp, Ascaris lumbricoides,* and *Oesophagostomum spp*. Combined, these results highlight a joint role of social behaviour and age in increased risk of contracting nematodes, two factors that could be taken into account for future welfare management of the species in captivity.

Author contributions statement

Mungongo Mayama: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation, Manuscript revision. Tshikung Kambol: Manuscript Revising, Formal analysis, Writing- Review & Editing. Diafuka Saila-Ngita: Data curation, Manuscript revision, Investigation. Madimba Kapanga: Data curation, Manuscript revision, Investigation. Kandu-lelo Clement: Language revising, Investigation. Lufiulusu **Nzotuvuidi**: Language revising, Manuscript revising. Masuku Masky: Investigation. Malekani Mukulire: Conceptualization, Supervision, Manuscript revising, Editing.

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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