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Preliminary Report on Gastrointestinal Helminths of Small Mammals (Rodent and Shrew) in an Island Forest Habitat of Lac de Ma Vallée, Kinshasa, Democratic Republic of Congo

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ABSTRACT

Background: infectious diseases are well known threats to wild mammal fauna that can serve as reservoir. With the increasing risk of zoonotic transmission it is necessary to conduct epidemiological surveys in wildlife. **Objective:** we sampled 9 small wild mammal species (*Congosorex polli*, *Myosorex* sp., *Lophuromys flavopunctatus*, *Hylomyscus stella*, *Mastomys natalensis*, *Mus minutoides*, *Praomys jacksoni*, *Rattus rattus*, and *Gerbilliscus validus*) at Lac de Ma Vallée in Democratic Republic of Congo to determine the diversity and prevalence of gastrointestinal helminths. Thirty-nine individual fecal samples were collected between June and October 2015. Coprological analysis has been realized by direct wet mount examination and the details of internal structure were observed under microscope for parasite identification. **Results:** we recorded 15 helminth taxa including three trematodes (*Mesostephanus* sp., *Apophallus* sp., and *Fasciola hepatica*), 11 nematodes (*Strongyloides ratti*, *Trichuris muris*, *Nippostrongylus brasiliensis*, *Apisculuris tetraptera*, *Paraspidodera uncinata*, *Toxascaris* sp., *Heligmosomoides polygyrus*, *Physocephalus* sp., *Ancylostoma* sp., *Syngamus* sp., and *Trichostrongylus retortaeformis*), and one cestode (*Hymenolepis nana*). The highest prevalence ($\geq$ 50%) in small mammals sampling was *N. brasiliensis* (100%), *H. nana* (50-100%), and *Apophallus* sp. (50%). **Conclusion:** we recorded six new hosts for *Apophallus* sp., *F. hepatica*, *Toxascaris* sp., *Physocephalus* sp., *Ancylostoma* sp., and *Syngamus* sp. reported in small mammals. The present study can be used as a baseline for future monitoring of the dynamics and ecological factors of host-parasite relationships and to evaluate the transmission risks.

INTRODUCTION

To date, there is a lack of studies on diversity and ecology on gastrointestinal parasites of African wild small mammals (rodents and shrews) in comparison to available data from Europe (Ibrahim *et al.*, 1984; Malekani, 1996; Behnke *et al.*, 2000; Lutermann *et al.*, 2014) and most of these studies on gastrointestinal parasites were focused on *Rattus* spp. and *Mus* spp. (Raharivololona *et al.*, 2007; Kataranovski *et al.*, 2008; Coomansingh *et al.*, 2009; Sumangali *et al.*, 2012; Kim *et al.*, 2015).

Wild animals, in general, and small mammals, in particular, are naturally infected by parasites that are infectious and pathogenic to human (Hunkeler, 1974; Luis *et al.*, 2013). These parasites cause different levels of damage to tissues and organs, morbidity and even death in small mammals or other vertebrates including human

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(Grézel, 2006; Mayer and Neumayr, 2015). Therefore, it is necessary to conduct epidemiological surveys in wildlife to control and prevent the increasing risk of zoonotic transmission to help bettering management and conservation of these animals.

The helminth fauna of small mammals in the Island forest habitat of Lac de Ma Vallée (LMV) in Democratic Republic of Congo (DRC) has not been yet studied. Thus, the present study aims to monitor helminth taxa parasite of small mammals, especially those of public health concern.

MATERIALS AND METHODS

Ethics statement:

This research was conducted according to the protocol approved by the ethic committee of the Biology Department of the University of Kinshasa, DR Congo.

Study site:

This study was conducted in the Lac de Ma Vallée (LMV, 04° 29’ 31.5’’ S and 15° 19’ 49.4’’ E) in Mont-Ngafula Municipality, at 30 km south-western of Kinshasa province in DR Congo. We conducted four monthly visits during the periods between June-July 2015 and September-October 2015. The forest of LMV covers approximately 244 ha of rainforest surrounding an artificial lake of 6 km contour. The climate is characterized by two rainy seasons (mid-September to mid-January and March to mid-May) and two dry seasons (mid-May to mid-September and mid-January to February). The average annual rainfall is 1500 mm and average daily temperature is around 25 °C. The vegetation is a remain secondary forest dominated by *Pentaclethra eetveldeana* and *Musanga cecropioides*.

Small mammals sampling:

We sampled 41 specimens from the 9 small mammal species. Animals were trapped with sherman, tomahawk (catch-alive small mammal traps), museum special and victor (catch-die small mammal traps). The traps were left open for three consecutive nights. We randomly set 7 trap lines in the study site. Each trap line (250 m distance) consisted of 50 traps positioned 5 m from each other. Traps were placed on ground and trees. Traps were checked twice a day in the morning (6:00 to 8:00) and in the afternoon (15:00 to 17:00) when the catch-alive traps were re-baited with a mixture of peanut butter, bananas, cassava, and sweet potatoes (Rautenbach *et al*., 2013).

Necropsies were carried out soon after capturing, immediately upon coming back from the field station; animals were anaesthetized with halothane by inhalation. Hosts species were identified using the African mammal guide from Kingdon (2010). All host specimens were deposited as voucher specimens in the Musée d’Histoire Naturelle of Faculty of Science at the Kinshasa University.

Coprological analysis:

Euthanized animals were dissected and the entire gastrointestinal tract was fixed in sterile vials with 10% formalin solution. We collected a total of 39 individual gastrointestinal tracts because two individuals were degraded. Parasitological analyses were performed at the Department of Biology at the University of Kinshasa, DRC. Ritchie method was used to analysis each sample by wet mount examination, to diagnose helminth eggs (Thienpont *et al*., 1979; Chartier *et al*., 2000). The parasitological identification of eggs was based on morphological characteristics such as shape, size, color, internal structure, larvae, and cysts (Thienpont *et al*., 1979; WHO, 1982).

Data analyses:

We used Fisher’s exact test to compare parasite prevalence between male and female hosts using PAST version 2.17c (package for education and data analysis). Statistical test significance was set at $P < 0.05$. The Shannon diversity index was used to measure diversity of parasites species in small mammals. Helminth infestation rate was estimated in counting the total number of eggs contained in 1 g of gastrointestinal tract (EPG) material per individual. We have also determined parasite prevalence which is the ratio of infected individuals to examined individuals expressed in percentage.

RESULTS AND DISCUSSION

We recorded in total 15 helminth taxa belonging to 3 classes (Table 1). Of 39 individual gastrointestinal tracts of small mammals examined, 37 (94.87%) were infected with one or more parasites. Only, *Congosorex polli* was not infected. Two trematodes (*Apophallus* sp., and *Fasciola hepatica*) and four nematodes (*Toxascaris* sp., *Physocephalus* sp., *Ancylostoma* sp., and *Syngamus* sp.) were reported in rodents and shrews for the first time. Nematodes infections were the most abundant 11 (73.33%) followed by trematodes 3 (20%), and cestode 1

(6.67%). This difference may be due to direct life cycles of parasites (Raharivololona *et al*., 2007) and in addition, to consumption modes of small mammals such as the geophagy (Okeke *et al*., 2008). The preference of parasites to infect their hosts might depend on host abundance, spatiotemporal scale, or host identity (Sears *et al*., 2012). It is important to understand risks of infectious diseases which can affect small mammals' health for a better *in situ* conservation. The ongoing habitat fragmentation of LMV island forest, due to human activities, has affected the demographic characteristics of small mammal populations of the site and may be responsible of contact augmentation between humans and wild mammals and has increased their exposure to parasites (Loehle, 1995; Ezenwa, 2003). The highest parasitic prevalence (≥ 50%) of small mammals infected was recorded for *H. nana* (50-100%), followed by *N. brasiliensis* (100%) and *Apophallus* sp. (50%) (Table 1). We found no significant differences in prevalence of parasite taxa detected among host species. We found no significant difference in prevalence between male 17/26 (65.38%) and female 9/13 (69.23%) infected (P = 0.508). *Hymenolepis nana* was the helminth that has the most parasitized small mammals with an infestation rate of 56.86% (P<< 0.0001). This obsevation is certainly related to the nature of indirect life cycle of this cestode, its adaptation to different habitats occupied by small mammals, and to the host species sympatry (Ezenwa, 2003).

Table 1: Helminth taxa from fecal samples of small mammals (Prevalence (%), Helminth infestation rate [EPG]: number of eggs found per gram of gastrointestinal tract per individual) in Lake de Ma Vallée

Classes	Small mammals sampling								
Helminth taxa	*M. minutoides* n = 2	*C. polli* n = 2	*P. jacksoni* n = 15	*H. stella* n = 5	*R. rattus* n = 4	*L. flavopunctatus* n = 6	*M. natalensis* n = 2	*G. validus* n = 1	*Myosorex* sp. n = 2
Trematode									
Mesostephanus sp.	0	0	0	0	(25%, 1 EPG)	0	0	0	0
Apophallus sp.	(50%, 1 EPG)	0	0	0	0	0	0	0	0
Fasciola hepatica	0	0	(6.67%, 3 EPG)	0	0	0	0	0	0
Cestode									
Hymenolepis nana	0	0	(20%, 17 EPG)	(60%, 6 EPG)	(75%, 9 EPG)	(83.33%, 14 EPG)	(100%, 9 EPG)	(100%, 1 EPG)	(50%, 2 EPG)
Nematode									
Strongyloides ratti	0	0	(13.33%, 4 EPG)	0	0	0	0	0	0
Trichuris muris	0	0	(6.67%, 10 EPG)	0	0	(16.67%, 4 EPG)	0	0	0
Nippostrongylus brasiliensis	0	0	(13.33%, 5 EPG)	(20%, 1 EPG)	(25%, 1 EPG)	0	0	(100%, 1 EPG)	0
Apisculuris tetraptera	0	0	(6.67%, 2 EPG)	0	0	0	0	0	0
Paraspidodera uncinata	0	0	0	0	(25%, 1 EPG)	0	0	0	0
Toxascaris sp.	0	0	0	(20%, 1 EPG)	0	0	0	0	0
Heligmosomoides polygyrus	0	0	(6.67%, 3 EPG)	0	0	0	0	0	0
Physocephalus sp.	0	0	0	0	(25%, 1 EPG)	0	0	0	0
Ancylostoma sp.	0	0	(6.67%, 1 EPG)	0	0	0	0	0	0
Syngamus sp.	0	0	0	0	0	(16.67%, 2 EPG)	0	0	0
Trichostrongylus retortaeformis	0	0	0	0	(25%, 1 EPG)	(16.67%, 1 EPG)	0	0	0

Table 2: Parasite richness and Shannon's diversity index of small mammals in Lake de Ma Vallée

Small mammal species	Parasite richness (%)	Diversity index (*H*)	Equitability index (*E*)
G. validus	(2/15) 13,33%	1	1
H. stella	(3/15) 20%	1,061	0,6696
L. flavopunctatus	(4/15) 26,67%	1,378	0,6889
M. minutoides	(1/15) 6,67%	0	-
M. natalensis	(1/15) 6,67%	0	-
Myosorex sp.	(1/15) 6,67%	0	-
P. jacksoni	(8/15) 53,33%	2,518	0,839
R. rattus	(6/15) 40%	1,770	0,6846

However, the diversity indices of gastrointestinal helminths in small mammal species (Table 2) from LMV showed relatively high diversity and an equitable distribution of helminths. This is due to the parasite specificity to infect a host.

Among helminth taxa found in this study, seven are zoonotic (*Fasciola hepatica*, *Apophallus* sp., *Hymenolepis nana*, *Strongyloides ratti*, *Paraspidodera uncinata*, *Toxascaris* sp., and *Ancylostoma* sp.), three epizootic (*Mesostephanus* sp., *Physocephalus* sp. and *Syngamus* sp.), and five enzootic (*Trichuris muris*, *Nippostrongylus brasiliensis*, *Apisculuris tetraptera*, *Heligmosomoides polygyrus*, and *Trichostrongylus retortaeformis*). The high number of zoonotic parasites is due to close cohabitation with humans. Indeed, humans by defecating in the nature eliminate in their stool eggs and larvae; small mammals are infected by feeding on contaminated food, and by consuming small mammals humans are infected in turn. In addition, the quality of meat appreciated by peri-urban habitants from the study site would increase the exchange of parasites between human and animal (in case of zoonotic parasites). This spatial proximity between small mammals and humans increases zoonotic transmission as described by Cibot *et al*. (2015). Moreover, LMV contains a wide diversity of wildlife species (mammals, reptiles, birds, amphibians, insects, and fish) that could promote exchanges of parasites between species at different levels in the food chain (in case of epizootic parasites). In addition, the densities of small mammals fluctuating regularly in relationship with seasonal changes can increase the transmission risk of parasites within species or wild animal communities (in case of enzootic parasites). But epizootic and enzootic parasites can be extended to humans causing significant zoonotic transmissions, even lethal infections (Luis *et al*., 2013), as in case of *Trichuris* spp. (Kouassi *et al*., 2015).

Conclusion:

For future studies, coprological analyses (microscopy) must be combined with molecular analyses to avoid miss identification of parasites and to compare the prevalence level between different techniques, and to understand the host-parasite evolution at the spatiotemporal scale. Nevertheless, the present study may be used as a baseline for future monitoring researches on helminth species diversity from small mammals encountered in LMV and their transmission risk of zoonotic diseases to human.

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