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A Report of Neglected Zoonotic Helminth Infections among House Rats in Selected Areas of Metro Manila and Region IV-A CALABARZON, Philippines

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Abstract

Soil-transmitted helminthiasis remains to be a major global health issue predominantly in tropical and sub-tropical regions including the Philippines. People living in poor areas without adequate sanitation are at high risk of contracting these diseases. Rodents and other mammalian species living near humans can serve as potential reservoir hosts and accelerate the spread of these parasitic infections. However, there are only few data and studies on environmental contamination, animal reservoirs, and the zoonotic potential of these parasites. The aim of this study was to determine the faunal diversity of helminths among house rats in selected areas of Metro Manila and CALABARZON, Philippines. This information will serve as baseline data for the development of long-term and effective approaches to prevent and control the zoonotic transmission in the country. A total of 60 house rats were caught and grossly examined. The overall prevalence of soil-transmitted helminth infections was 96.67% (58/60). Of the cases, 51.67% (31/60) were reported in Metro Manila while 45.00% (27/60) were reported in CALABARZON. *Capillaria hepatica* was the most dominant endoparasite with the highest prevalence and intensity of infection. The four identified helminths namely *Capillaria hepatica*, *Taenia taeniaeformis*, *Hymenolepis diminuta,* and *Raillietina* spp. transmitted by these *Rattus* species namely *Rattus norvegicus* and *Rattus tanezumi* pose a health risk to the public as they are important reservoirs of zoonotic parasites that are capable of transmitting infection to humans. Therefore, it is necessary to regularly monitor and control rodent populations to reduce the spread of parasites in urban and rural areas.

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Introduction

Soil-Transmitted Helminthiasis (STH), caused by intestinal helminths is recognized by the World Health Organization as the second most devastating Neglected Tropical Disease (NTD) worldwide. An estimate of 24% or about 2 billion of the world's population is infected with STH. Majority of infections are recorded in school-age children who reside in geographical areas where these parasites are intensively distributed [1]. In the Philippines, STH caused by *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms are widely prevalent and endemic in 80 provinces where poor hygiene, lack of sanitation, and clean water supplies are observed [2].

Recent studies have also revealed that some of the soil-transmitted helminths including nematodes, tapeworms, and hookworms have exhibited zoonotic capabilities within a very wide host range of about 40 different mammalian species including rodents which allows the transmission of these helminth infections from animals to humans [3]. The zoonotic transmission can be through ingestion of food and water with helminths' eggs or larvae or through exposure to contaminated environment with helminths' eggs or larvae [4].

In addition, the conversion of lands from rural to urban has led to the destruction of natural habitats which have increased the risk of zoonotic infections due to an increased contact between humans and wild animals [5]. The zoonotic potential of these soil-transmitted helminths presents a major issue in health due to the close relationship between animals and humans, particularly dogs, cats, carabaos, and rats which serve as pets, companions in agricultural work, their presence as pests, and even their natural occurrence in the environment. Zoonotic infections may also result to the interruption of animal production of food and other by-products.

Rats are one of the most important but understudied animals in rural and urban areas. Due to their negative health and economic consequences, *Rattus* spp. infestations are growing public health concerns in cities around the world. Rats are a threat to public health because they carry a variety of zoonotic infections and regularly bring highly infectious organisms in contact with humans and other animals. Urbanization, climate change, and ineffective rat control have resulted in an increase in rodent-related risks [6].

Despite the important role played by these animals in the transmission of soil-transmitted helminth infections, the zoonotic transmission and relative significance of various animal reservoirs to the overall burden of these parasitic diseases in humans remains to be unclear. Precise and updated data on neglected tropical diseases are important to design and improve the national control strategies [7]. This study aimed to determine the faunal diversity of helminths among house rats (*Rattus* spp.) in selected areas in Metro Manila and CALABARZON.

Materials and methods

Description of collection sites

The collection sites covering the Metro Manila were Pasig City (14°35'8.39" N 121°03'24.00" E), Quezon City (14° 40' 34.3488'' N 121° 2' 37.8996'' E), Manila City (14.5995° N, 120.9842° E), and Muntinlupa City (14° 24' 29.278" N 121° 2' 29.28" E) while region IV-A CALABARZON was represented by Binangonan, Rizal (14.4765° N, 121.1957° E), Imus, Cavite (14.4064° N, 120.9405° E), and Biñan, Laguna (14.3036° N, 121.0781° E). Rats were collected in residences near dumpsites, rivers, swamps, and rice fields.

The selection of Metro Manila and Region IV-A CALABARZON areas was carefully chosen based on the published articles and reports of parasitic diseases in city health offices, as well as the feasibility of sample collection in the study site despite the ongoing community quarantine measures implemented by the government due to the pandemic caused by COVID-19. This study was conducted in the field from November 2020 to February 2021 until the target sample population of rats has been met.

Collection of samples

Randomly selected households in each study area were identified and twenty [20]. Rat traps were numbered and positioned with food baits. A total of 60 rats, 29 from rural and 31 from urban areas weighing between 150 g to 450 g were captured, placed in a pre-labelled wire cage, and were provided with food pellets and water upon transport to the laboratory for further examination [8]. Data such as sex and weight of rats were recorded and identification of *Rattus* species was accomplished by assessing their morphological characteristics.

Laboratory examination

Chloroform was used as inhalant anaesthetic to euthanize the rats before necropsy. This was performed using open drop method, a clean cotton was placed at the bottom of the container with an ample amount of chloroform. Each rat was then placed inside the container and the lid was tightly closed. After a few minutes, the rats were checked for the signs of anaesthetic efficacy such as slower pulse, breathing, and absence of movement and consciousness [9]. Dissection was initiated once the rat was completely anesthetized [10].

Post-mortem evaluation and organ collection

A systematic inspection of selected visceral organs such as liver, small intestine, and large intestine of each rat was conducted right after euthanasia. The abdominal cavity was opened to expose the gastrointestinal and thoracic viscera. Organs were immediately examined using a stereoscope to determine the presence of soil-transmitted helminths. Parasitic cysts that were found in the excised gastrointestinal tract and liver were collected and placed in 10% formalin [11]. Identification of parasites was done at least to genus level using taxonomic references and published reports. All the important findings were photo documented using a light microscope with built in camera (Nikon Eclipse).

Prevalence rate and intensity of infection

The prevalence rate of soil-transmitted helminths was expressed using non-parametric statistical analysis. The significant observations related with the quantity and intensity of endoparasitic infection identified per rat was determined utilizing an adapted scoring system from Claveria *et al*. (2005) as follows: Low 1-2; Moderate: 3-6; Heavy: ≥ 7*(12)*. Data were presented in tables and figures.

Ethical considerations

Prior to the implementation of this study, permit from the Local Government Units (LGU) and city veterinarian offices of the selected areas were secured and obtained. Municipal and barangay health workers were informed, and approval was gained

prior to conduct of sample collection. All animal experiments done in this study were conducted in full accordance with the ethical guidelines for the use of animal samples acceptable by the Research Ethics Office (REO) of De La Salle University. All rats were anesthetized before they were sacrificed.

Results

In the gross examination of the livers of the two *Rattus* spp., *Capillaria hepatica* infected rats had 0.1 to 0.5 mm wide curled white to yellowish tracts with indistinct borders that were scattered in different sections of the liver (Figure 1A). *Taenia taeniaeformis* infected livers were composed of variable numbers of deeply embedded cysts with sizes ranging from 2.0 to 10.0 mm and color variation of white to grayish white (Figure 1B and C). The cysts contained coiled cestode of white viable larvae that were approximately 15 cm to 30 cm long, with a long neck and prominent scolex that were embedded in a white turbid fluid (Figure 1D and E).

In addition, the intestines of both *Rattus norvegicus* and *Rattus tanezumi* species in rural and urban areas were positive with light to heavy *Hymenolepis diminuta* and *Raillietina* spp. infections. Infected rats had shown a worm chain with head (bracket), neck and strobila with proglottids (Figure 2C). While *Raillietina* spp. exhibited trapezoidal proglottids (Figure 2D). The distinctive features of adult *Hymenolepis diminuta* were its craspedote form of the proglottids by which the anterior proglottid overlaps in the next posterior one and its size measured about 35 mm as compared to the adult *Raillietina* spp. that measured approximately 60 mm with abundant trapezoidal proglottids that becomes rounder in shape towards the posterior region of the strobila.

The overall prevalence rate of endoparasitic infections was 96.67% (58/60). Of the cases, 51.67% (31/60) were recorded in Metro Manila while 45.00% (27/60) were observed from CALABARZON. The liver of 49 (81.67%) rats: 25 (41.67%) from Metro Manila and 24 (40.00%) from CALABARZON were infected with either single or co-infection with *Capillaria hepatica* and *Taenia taeniaeformis*. On the other hand, the intestines of 37(61.67%) rats were infected with either single or co-infection with *Hymenolepis diminuta* and *Raillietina* spp. Of these, 38.33% (23/60) were recorded in Metro Manila whereas 23.33% (14/60) were noted in CALABARZON.

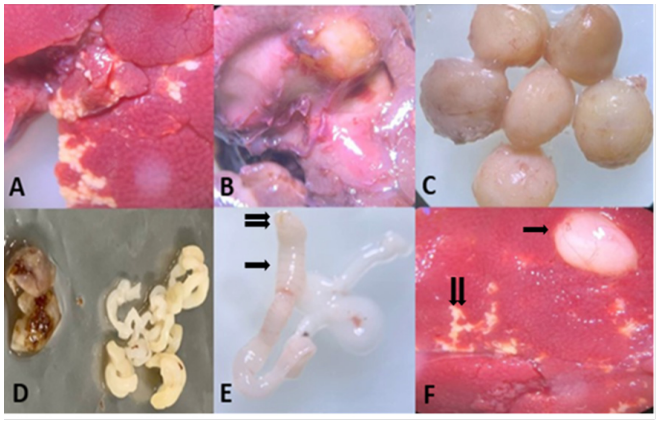

Figure 1: Endoparasites recovered in the liver. **A.** *Capillaria hepatica* showing whitish tracts, **B.** *Taenia taeniaeformis* embedded in the liver, **C.** White cysts containing T. Taeniaeformis larva, **D.** Removed coiled *T. Taeniaeformis* larva from cyst, **E.** White viable *T. Taeniaeformis* larvae with long neck (arrow) and prominent scolex (double arrow), **F.** Co-infection of *T. Taeniaeformis* (arrow), and *C.* hepatica (double arrow) embedded in liver.

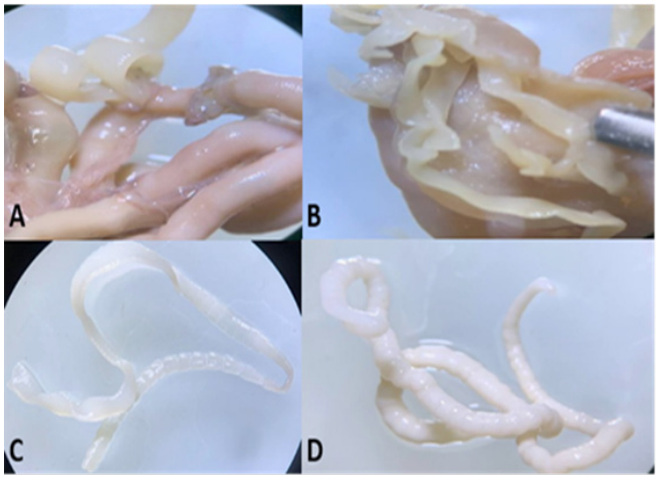

Figure 2: Endoparasites recovered in small and large intestines. **A.** Tapeworm infected intestine, **B.** Excised intestine showing a tapeworm, **C.** *Hymenolepis diminuta*, and **D.** *Raillietina* spp.

Capillaria hepatica had the highest prevalence in the liver of all Rattus species with 71% in Metro Manila and 82.8% in CALABARZON. *Rattus norvegicus* exhibited higher *Capillaria hepatica* infection than Rattus tanezumi in all sites followed by *Taenia taeniaeformis* with 58.1% and 55.2% prevalence rates in Metro Manila and CALABARZON, respectively. In the small and large intestines, *Hymenolepis diminuta* was found to be the most prevalent soil-transmitted helminth with 71% followed by *Raillietina* spp. (16.1%) in Metro Manila. Lower prevalence rates of soil-transmitted helminths were observed in the *Rattus* spp. captured in CALABARZON. *Hymenolepis diminuta* remained to be the most prevalent in the intestines with 48.4% as compared to *Raillietina* spp. with 6.9% (Table 1).

Table 1: Prevalence of soil-transmitted helminths among *Rattus* spp. in Metro Manila and CALABARZON.

Soil-transmitted Helminths	Helminths and No. of rats (% infection)		Total n=60 (%)
METRO MANILA			
	Rattus norvegicus	*Rattus tanezumi*	
	n=15	**n=16**	**n=31**
Capillaria hepatica	13(86.7)	9(56.3)	22(71.0)
Taenia taeniaeformis	9(60.0)	9(56.3)	18(58.1)
Hymenolepis diminuta	9(60.0)	13(81.3)	22(71.0)
Raillietina spp.	2(13.3)	4(25.0)	5(16.1)
CALABARZON			
	Rattus norvegicus	*Rattus tanezumi*	
	n=5	**n=24**	**n=29**
Capillaria hepatica	5(100)	19(79.2)	24(82.8)
Taenia taeniaeformis	2(40.0)	14(58.3)	16(55.2)
Hymenolepis diminuta	3(60.0)	11(45.3)	14(48.4)
Raillietina spp.	0(0.0)	2(8.3)	2(6.9)

The intensities of infection were also recorded based on the density level of parasites. In Metro Manila, all 22 rats with *Capillaria hepatica* had displayed heavy infections followed by *Hymenolepis diminuta* (22/60) with 1 light, 9 moderate and 12 heavy infections, *Taenia taeniaeformis* (18/60) with 1 light, 2 moderate and 15 heavy infections and *Raillietina* spp. (6/60)

with 2 moderate and 4 heavy infections. On the other hand, in CALABARZON, with *Capillaria hepatica* (24/60) infected rats had 2 moderate and 22 heavy infections followed by *Taenia taeniaeformis* (16/60) with 2 light, 4 moderate and 10 heavy infections, *Hymenolepis diminuta* (16/60) exhibited 2 light, 7 moderate and 7 heavy infections. *Raillietina* spp. still had the lowest intensity (2/60) with only 2 light infections (Table 2).

Table 2: Summary of the level of intensity of infection based on adult parasite count in *Rattus norvegicus* and *Rattus tanezumi*.

Soil-transmitted Helminths	Helminths and No. of infected rats					
METRO MANILA	***Rattus norvegicus***			***Rattus tanezumi***		
n=31	**n=15**			**n=16**		
	L	**M**	**H**	**L**	**M**	**H**
Capillaria hepatica	-	-	13	-	-	9
Taenia taeniaeformis	1	2	6	-	-	9
Hymenolepis diminuta	-	2	7	1	7	5
Raillietina spp.	-	-	2	-	2	2
CALABARZON	***Rattus norvegicus***			***Rattus tanezumi***		
n=29	**n=5**			**n=24**		
	L	**M**	**H**	**L**	**M**	**H**
Capillaria hepatica	-	-	5	-	2	17
Taenia taeniaeformis	-	2	-	2	2	10
Hymenolepis diminuta	-	1	2	2	6	5
Raillietina spp.	-	-	-	2	-	-

Discussion

House rats can act as reservoir host harbouring several zoonotic endoparasites that could potentially infect humans. Zoonotic helminth diseases that are transmissible from animals to humans account for various cases of the novel pathogen outbreaks worldwide [13]. Several factors such as overpopulation, urbanization, habitat modification, modernization of agriculture and livestock farming, climate change, destruction of wild animal habitat and mass migration may have contributed to the increase of emergence of zoonotic diseases to humans [14].

Residential areas, particularly in urban cities, are at risk with the emergence of zoonotic diseases. Urban areas offer favourable habitats to diverse species of wild animals that eventually result to regular and increased interaction with humans [15]. Among all the animals found in urban areas, rats (*Rattus* spp.) pose an utmost danger due to their zoonotic potential, high reproductive capacity, and propensity of close association with humans [16]. All four species of endoparasite detected in this study were found to have zoonotic potential namely, *Capillaria hepatica* (nematodes), *Hymenolepis diminuta*, *Raillietina* spp, and *Taenia taeniaeformis* (tapeworms). The prevalence of three zoonotic parasites were significantly high (>50%) in all sites regardless of the habitat being rural or urban area. Rodents living in both environments in proximity to humans reformed habitats that harbor various zoonotic endoparasites pose a threat to health and increases the possibility of obtaining rodent-borne zoonoses [17].

Capillaria hepatica and *Taenia taeniaeformis* are the most common liver endoparasites in rodents which cause hepatic diseases [18]. The detection of C*. hepatica* and *T. taeniaeformis* was performed through macroscopic inspection of the liver to search for distinctive lesions. Both are found in this study wherein both obtained 50%-100% prevalence among all rat species in all study sites which coincides with the reported prevalence ranging from 7.9% to 88.0% for *C. hepatica* and *T. taeniaeformis* infection in rodents [19]. In addition, both parasites have zoonotic potential to transmit infection in approximately more than 40 mammalian species, particularly rodents, dogs, cats, and humans. The pathogenesis of hepatic diseases caused by these two endohelminths is unique and distinct relative to their life cycles.

Following the gross examination of the liver, the gastrointestinal tract, particularly the sections of the small and large intestines, were evaluated to determine the presence of endohelminths. *Raillietina* spp., and *Hymenolepis diminuta* are the most prevalent soil-transmitted helminths of rodents [20]. Similar to other studies, high prevalence of endoparasites was observed in Metro Manila, particularly *Hymenolepis diminuta* with 75% prevalence in *Rattus tanezumi* and 53.3% in *Rattus norvegicus* while it is also highly prevalent in CALABARZON with 41.7% and 60.0% in *Rattus tanezumi* and *Rattus norvegicus*, respectively. On the other hand, *Raillietina* spp. showed lower prevalence of 13.3% in *Rattus norvegicus* and 25.0 % in *Rattus tanezumi* species in the urban areas of Metro Manila. In the rural areas of CALABARZON, macroscopic *Raillietina* spp. helminths were absent in *Rattus norvegicus* and only 8.3% prevalence was recorded in *Rattus tanezumi*.

Cestodes, particularly those of the genus *Hymenolepis,* are known to represent a major health risk in urban settings. Hymenolepis diminuta and *Hymenolepis nana* are the most prevalent rat parasites, infecting roughly 175 million humans globally and affecting around 21 million individuals in tropical and subtropical areas [21]. In this study, *Hymenolepis diminuta* was shown to be the most common among all house rats across all study sites. These cestodes are zoonotic helminths that may be transmitted to humans via the fecal-oral route or by the ingestion of *Tribolium confusum*, a flour beetle that serves as an intermediary host containing cysticercoids from contaminated cereal grains. Reports in the country revealed 8% prevalence of hymenolepiasis diminuta in children less than 3 years old with primarily *Raillie tina garrisoni* as etiologic agent. Infections in humans caused by *Hymenolepis* spp. are typically asymptomatic, although headache, abdominal pain, diarrhea, and weakness may occur [22].

Overall, the endoparasite species richness observed in this study corroborated earlier studies conducted in the country together with Iran, Indonesia, Serbia, and The Netherlands. Their studies have revealed about 3 to 13 different species of parasites infecting wild rats [23,24,25,26]. The high parasite load observed in this study was also similar to previous studies in Malaysia, but the present study differs in the variation of parasites which may be attributed to the geographical locations and environmental conditions in which rats were collected [27,28].

The impoverished condition in some of the study sites particularly in Muntinlupa City (urban) and Laguna (rural) that are inhabited largely by relocated poor families as evidenced by population overcrowding, poor hygiene, lack of sanitation facilities and abundance of stray animals, may have contributed to the higher endoparasite infection rates among house rats in the present study. In contrast, rats caught in non-congested residential houses in villages particularly located in Binangonan and Cavite have revealed lower prevalence rates. The distribution range of soil-transmitted helminths may have also been modified due to the expansion of appropriate habitat and in-

troduction to their intermediate and definitive hosts [29]. If this unceasingly occur, it may lead to a greater risk of spreading of parasitic infection from wildlife animals to other species and communities including humans [30].

The public awareness regarding the risks involved substantially impacts policymaking in the field of health. Widespread outbreaks of zoonotic diseases are not sporadic, particularly as the diseases are often not documented as zoonotic at the outset and may spread unnoticed for some period of time. In any case, the immediate impact on health of these new, emerging, or re-emerging zoonotic disease has been insignificant as compared to other infectious diseases affecting humans. The insignificant knowledge of the role of rats and other animals in the spread of parasitic infection in the country due to lack of updated research indicate the necessity of further investigations. This kind of study is essential to be carried out for a longer time frame to gather further extensive pool of information on several aspects particularly in finding more evidence on host-parasite relationship, biology, and ecology. Findings of these studies will provide a baseline information that could help develop innovative strategies and policies necessary to control and eliminate these parasitic diseases. Updated data regarding these parasitic diseases will also serve as a reminder of the unexpected existence of infectious diseases in new locations and animal species. These zoonotic parasitic diseases have also contributed to the new paradigms of food and water safety policies for the protection of public health. Therefore, more research is necessary to validate that rat age, gender, season and locations have affected the mean worm abundance and prevalence of parasite species to better understand the patterns and drivers of the burden of parasitic infections and in urban and rural areas in the country.

Conclusion

The overall prevalence rate of the endohelminth infections was 96.67% (58/60). Of these, 51.67% (31/60) were recorded in Metro Manila while 45.00% (27/60) prevalence was detected in CALABARZON. The four identified zoonotic parasites harbored by these *Rattus* species pose a threat in public health as they are important reservoirs of zoonotic helminthiasis that can cause the spread of infection to humans. The prevalence rates and variation of zoonotic parasites are greater among house rats captured in urban areas of Metro Manila than in CALABARZON which pose potential threats of rodent-borne disease transmission to more human populations. The significant risk factor identified that is associated in high prevalence and density is the environmental and impoverish condition.

Rodents like rats play an important role in both direct and indirect transmission of zoonotic diseases since these immigrants themselves could be host to a variety of diseases. Through this study, it can be recommended that repetitive screening of wild rodents is essential for integrated pest management through the cooperation among the local government, pest control division and residents is required. Therefore, there is a necessity to promote awareness on the role of rodents in the spread of these zoonotic diseases to establish strategies for control and prevention of rodent populations not only in the study areas but also in other places where majority of human populations live in close contact with rats and other animals.

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Data availability: Data supporting these findings are available within the article or upon request.

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