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Rats and their helminth parasites: Potential zoonosis threats of land use change in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines

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Summary

The continuous challenges of land use change have brought potential threats to biodiversity and the spread of zoonotic diseases. In this study, synanthropic rodents and their helminth parasites were used as sentinels to assess the potential impact of land use on zoonosis. Rats were collected in different ecosystems, namely agricultural, agroforest, and residential areas in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines. Three (3) species of rats were captured, namely, *Rattus tanezumi, Rattus norvegicus,* and *Rattus exulans*. Of the total 180 rats collected, 92.7 % were found infected with helminth parasites, namely *Hymenolepis diminuta, Hymenolepis nana, Taenia pisiformis,* and *Strobilocercus fasciolaris* (cestodes); *Angiostrongylus cantonensis, Nippostrongylus brasiliensis*, *Strongyloides ratti, Capillaria hepatica, Trichuris muris*, and *Rictularia* sp. (nematodes); and *Echinostoma ilocanum* (trematode). Of these 11 species, nine (9) were considered zoonotic. This study provides important information on the helminth parasites of rats in the northeastern sub-watersheds of Mount Makiling and the potential threat of zoonotic transmission due to increasing land use change and urbanization in the area. Moreover, urbanization can provide favorable eco-epidemiological conditions for rodent-borne pathogens, such as parasites, that are seriously threatening agricultural settings and human settlements in these areas.

Keywords: Helminth parasites; Molawin-Dampalit sub-watershed; rats; zoonosis

Introduction

Rodents have an important place in many food webs, but they are also well-known agricultural and residential pests. For species that are synanthropic, their close association with human habitation could pose threats to public health due to zoonotic transmission. Many of these rodents are carriers of pathogens such as bacteria, viruses, and parasites (Gravinatti *et al.*, 2020; Mihalca *et al.*, 2012), and the spread of rodents into human habitats and vice versa can lead to an increase in spillover risk (Ecke *et al.*, 2022). Studies have shown that rats from different ecosystems in Southeast Asia may serve as reservoirs of some zoonotic helminth species (Chaisiri *et al.,* 2015; Fagir & El-Rayah, 2009; Mohd Zain *et al.,* 2012; Sumangali *et al.,* 2012).
In the Philippines, *Rattus tanezumi* (Asian house rat), *Rattus argentiventer* (ricefield rat), *Rattus exulans* (Polynesian rat), and *Rattus norvegicus* (brown rat) are known to occur in agroecosystems and have been recognized as pests of crops such as rice and sugarcane (Stuart *et al.*, 2008). Numerous parasitic infections have also been recorded in rodents collected in the Philippines, such as echinostomiasis, paragonimiasis, capillariasis, schistosomiasis, and angiostrongyliasis (Castillo & Paller, 2018; Eduardo, 1991; Estaño, 2023; Estaño *et al.*, 2020; Estopa & Estopa, 2016). Consequently, there could be a potential effect on the dynamics

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of pathogen transmission associated with rats and their changing environments. Rodent species that are known to be disease reservoirs are more abundant in anthropogenically modified habitats (Mendoza *et al.*, 2020), and the spread of rodent-borne disease due to land use change is likely to increase in the future (García-Peña *et al.*, 2021). In the Philippines, urban land conversion is expected to grow continuously (Johnson *et al.*, 2021; Mishra *et al.*, 2021), and, in Mount Makiling specifically, built-up areas have already increased by 117 % between 1992 and 2015 (Soriano *et al.*, 2019).

Unfortunately, there are limited studies in the country investigating rat-borne pathogens, particularly endoparasites, and their distribution in rat host populations in relation to host occurrence in different land use types. This study aimed to determine the distribution of rats and their helminth parasites in the changing landscape of Mt. Makiling. With the dearth of epidemiological data on many zoonotic pathogens, there is a need to do more reliable surveillance and elucidate how land use change can influence the risk of disease transmission.

Materials and Methods

Study Area

Mount Makiling Forest Reserve (MMFR) is located in southern Luzon Island, Philippines, between 14°6' to 14°11' N and 121°09' to 121°15' E. It covers a total land area of 4,244 hectares within the Municipalities of Los Baños and Bay, and the City of Calamba in Laguna Province, and the Municipality of Santo Tomas in Batangas Province. As stipulated in Republic Act No. 6967, MMFR is currently under the exclusive jurisdiction of the University of the Philippines Los Baños as a laboratory for forest research, protection, and development. This protected area hosts a range of aquatic and terrestrial ecosystems that contain numerous habitats for endemic and non-endemic terrestrial species (Macandog *et al.,* 2012). The faunal biodiversity of its terrestrial ecosystem includes 15 terrestrial snail species, 45 species of mammals, 120 species of birds, 52 species of reptiles, and 25 species of amphibians (Gonzalez, 2000; Mallari *et al.,* 2001). Due to its high biodiversity in flora and fauna, MMFR offers a wide range of ecosystem goods and services, providing farming and livelihood opportunities for those residing within and around the peripheries of the watershed. However, the changing landscape due to various threats, such as human settlements, tourism, and agricultural activities, has posed environmental threats in this area (Macandog *et al.,* 2011).

MMFR has four sub-watersheds, the largest being Molawin-Dampalit, followed by Cambantoc, Greater Sipit, and Tigbi sub-watersheds, each supplying low-lying municipalities with surface water (Lapitan *et al.,* 2011). The Molawin-Dampalit and Cambantoc sub-watersheds are found on the northeast slope of the mountain, while the Greater Sipit and Tigbi sub-watersheds are on the west to southwest slope. The northeastern sub-watersheds (Fig. 1) make up a critical landscape with very high biodiver-

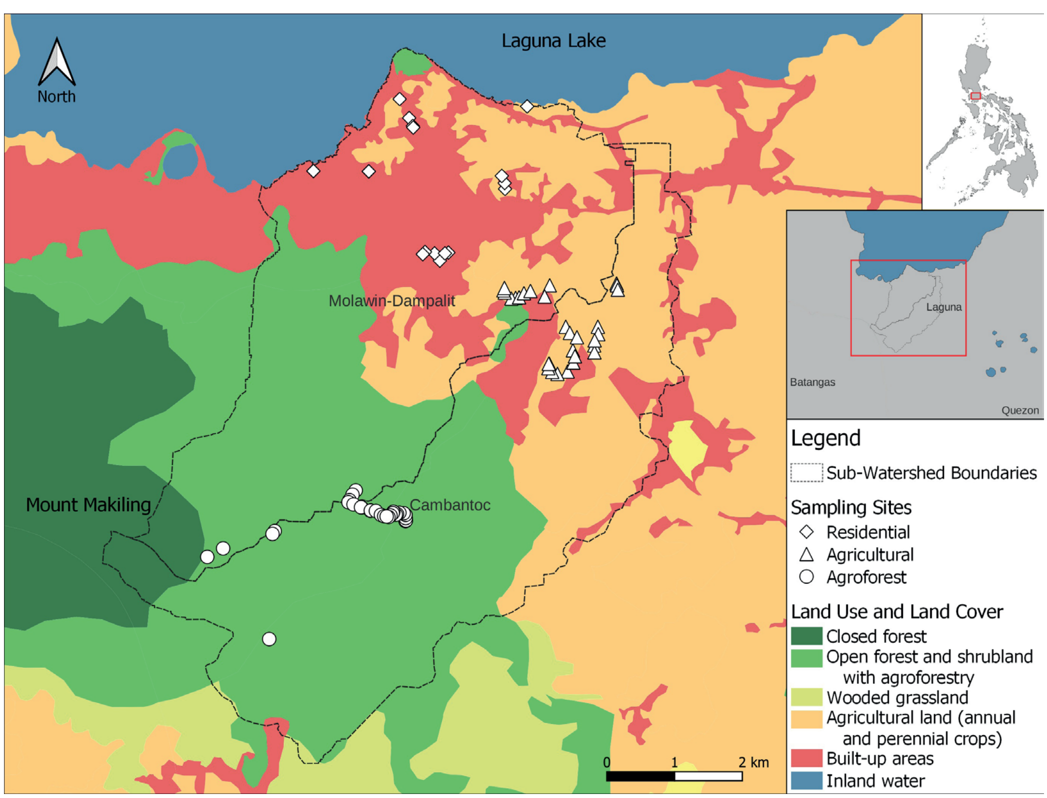

Fig. 1. Spatial distribution of rats (*Rattus* spp.) and land cover types in Molawin-Dampalit subwatershed, Laguna, Philippines.

sity, both flora and fauna, that offer various ecosystem services. It covers areas that feature different terrestrial ecosystems, such as forest, agroforest, agricultural, and residential lands, exhibiting different ecological patterns and processes (Soriano *et al.*, 2019).

Sampling Design and Collection of Rat Samples

Before collecting samples, the estimated sample size was determined using GPower 3 Statistical Software with a predicted prevalence of 80 % and a confidence interval of 95 % (Faul *et al.*,2009). Three (3) sampling sites in the northeastern sub-watersheds of Mount Makiling were chosen to represent each of the following land use types: residential, agricultural, and agroforest (Fig. 1). These land use types were determined using the 2010 Philippine land use and land cover map (NAMRIA, 2010) with ground-truthing conducted at each trap location. The collection of rat samples was done using purposive sampling from July to December 2017 and from January to June 2018, during wet and dry seasons. Standard methods for trapping small non-volant mammals from Heaney *et al.* (2006) were used. Ten sampling points in each site were randomly selected, and 3-5 steel wire box traps baited with dried fish and fried coconut with peanut butter were placed in each sampling point. The traps were set for two trap nights each, with each trap being checked after the first trap night for the presence of rats and re-baited for the second night (Estaño *et al*., 2020). A total of 180 non-native rat samples (*Rattus* spp.) were collected, with 60 individuals collected from each land use type.

Processing of Samples

Collected rat individuals were sedated with 5 % isoflurane via drop jar method and were euthanized by cervical dislocation following the approved Institutional Animal Care and Use Committee (IACUC) protocol. Morphometrics such as tail length, head-body length, foot length, ear length, and weight were recorded coupled with morphological characteristics to identify the rat species (Heaney *et al.,* 2006; Heaney *et al.,* 2010). Age classes of rats were classified into juvenile and adult based on a combination of morphometric and reproductive characteristics, particularly the presence of lactating teats in females and testes in males (Aplin *et al.,* 2003; Estaño *et al.,* 2020).

Thereafter, the rat samples were dissected. The gut region (stomach, small and large intestines, and caecum) was cut open and observed under a stereomicroscope (Leica, China). Organs such as kidneys, muscles, intestine, liver, brain, heart, and lungs were also collected and subjected to artificial tissue digestion. Each organ was cut into pieces and placed separately in a beaker with pepsin solution (1000 ml distilled H20+ 1 ml HCl + 1g pepsin). Samples were then placed in a hot plate with a magnetic stirrer set at 37°C for 30 minutes to one hour to complete digestion. After this, the solution was filtered to remove large debris and washed with 0.96 % NaCl twice to recover parasites. Samples were examined under a stereomicroscope for helminth parasite identification.

Helminth Parasite Identification and Preservation

The collected nematodes from rats were soaked in 0.96 % NaCl solution, washed in Phosphate Buffer Solution (PBS), and then preserved in 70 % ethanol. Collected cestodes were soaked in warm water for a couple of minutes to relax and strengthen the worms before they were preserved in 70 % ethanol. Trematodes were also relaxed in warm water to allow the specimens to expel eggs that might otherwise obscure some organs before they were preserved in 70 % ethanol. Collected helminth parasites were stored in adequately labeled vials. Helminth parasites were mounted on slides for identification. All prepared permanent slides were observed under a compound microscope (Nikon, Tokyo) and a trinocular stereo zoom microscope (Leica, China). Measurements of the specimens were taken using stage, ocular micrometers, and an optic camera (OptixCam, OCS-SK2-5.2X, China; ToupView software). The parasites were identified using standard references (Baker, 2007; Khalil *et al.,* 1994).

Data Analysis

Parasite load was calculated by counting the total number of parasites recovered in each individual rat. The prevalence rates of helminth parasites in rats and co-infections were calculated with 95 % confidence level. To evaluate the statistical differences in helminth parasite prevalence among habitat, rat species, sex, and age groups, the Chi-square test of independence was used. Shapiro-Wilk test was used to assess the normality distribution of the data. To evaluate the parasite mean intensity among sex and age groups, Mann-Whitney U test was performed. Kruskall-Wallis test was performed to evaluate the parasite mean intensity among habitat and species groups. Pearson correlation was used to determine the association between parasite load and rat morphometrics (length and weight). Statistical computations were done using the following software for Windows: Quantitative Parasitology (QP) version 3.0. and SPSS v. 20.0.

Ethical Approval and/or Informed Consent

Prior to the conduct of study, the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños approved the experimental protocol with assigned protocol number IBS-201701.

Results

Helminth Fauna in Rats

Three (3) species of non-native rats, namely *R. tanezumi, R. norvegicus,* and *R. exulans*, were captured from three (3) land use types: residential, agricultural, and agroforest (Table 1). *R. tanezumi* was found in all types of habitats, while *R. norvegicus* was only found in residential areas and *R. exulans* in agricultural areas. Of 180 individual rats trapped, 167 of them were infected with at

Table 1. Summary of prevalence (%) of helminth parasites in non-native rats (*Rattus* spp.) collected from various land use types in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines.

Parameter	No. of Sample	No. of Infected	Prevalence Rate (%) Confidence Interval (CI**)
Habitat			p = 0.718
Agricultural	60	55	91.7 (84.76-98.66)
Agro-forest	60	55	91.7 (84.76-98.66)
Residential	60	57	95 (89.49-100)
Species			p = 0.264
R. exulans	18	15	83 (66.12-100)
R. norvegicus	32	30	93.8 (85.36-100)
R. tanezumi	130	122	93.8 (89.72-97.98)
Sex			p=0.142
Female	103	93	90.3(84.57-96.01)
Male	76	74	96.1 (93.77-100)
Age			p=0.013*
Adult	135	129	95.6 (92.08-99.03)
Juvenile	45	38	84.4 (73.86-95.03)
Season			p=0.400
Dry	109	99	90.82 (85.41-96.24)
Wet	71	68	95.77 (91.1-100)

*Significant at P < 0.05.
**95% CI.

least one (1) parasite with a 92.7 % overall prevalence. Eleven (11) helminth parasites species were identified, including four (4) cestodes (*Hymenolepis diminuta, Hymenolepis nana, Taenia pisiformis,* and *Strobilocercus fasciolaris)*, six (6) nematodes (*Nippostrongylus brasiliensis*, *Strongyloides ratti, Capillaria hepatica, Trichuris muris, Angiostrongylus cantonensis,* and *Rictularia* sp.), and one (1) trematode (*Echinostoma ilocanum).*

Of these parasites, *N. brasiliensis* showed the highest prevalence (50.56 %; 95 % CI 43.25-57.86), followed by *H. nana* (38. %; 95 % CI 32.84-47.16), *H. diminuta* (36.11 %; 95 % C1 31.77-46.01), *S. fasciolaris* (35.3 %; 95 % CI 28.03-41.97), *C. hepatica* (23.88 %; 95 % CI 22.79-36.1), *A. cantonensis* (18.33 %; 95 % CI 12.68-23.99), *S. ratti* (11.11 %; 95 % CI 6.076-15.04), *T. muris* (7.22 %; 95 % CI 3.44-11), *E. ilocanum (4.44* %; 95 % CI 1.05-6.54), *T. pisiformis* (1.67 %; 95 % CI 0-3.54), while *Rictularia sp*. (1.11 %; 95 % CI 0-2.64) recorded lowest prevalence (Table 2).

This study recorded co-infections of helminth parasite in rats. Four (4) cases of multiple infections with six (6) species of helminth parasite were observed. One (1) rodent was co-infected with seven (7) helminth parasites namely: *H. diminuta, H. nana, S. fasciolaris, N. brasiliensis, C. hepatica, T. muris,* and *A. cantonensis* (Table 3). Out of the four cases of multiple infections with six (6) species, three (3) cases were associated with residential areas and one (1) with agroforest habitats. The rodent individual co-infected with seven (7) species of helminth parasites was also associated with residential habitats. All cases of multiple helminth co-infections in residential habitats were observed in *R. norvegicus* while the case reported in agroforest habitats were observed in *R. tanezumi*.

In addition, it was observed that most helminth parasites species were found in all land use types sampled (residential, agricultural, and agroforest) except *T. pisiformis* (residential areas only), *Rictularia* sp. (residential and agricultural areas only), and *E. ilocanum* (agricultural areas only). All helminth parasite species were recovered from *R. tanezumi*, while in *R. norvegicus,* the cestode *T. pisiformis* was not recovered, and in *R. exulans*, *T. pisiformis* and the nematodes *Rictularia* sp. and *T. muris* were not recovered. Among the rat species, *R. tanezumi* and *R. norvegicus* showed the highest prevalence with 93.8 % (95 % CI 89.72-97.98), and 93.8 % (95 % CI 85.36-100) respectively, followed by *R. exulans* with 83 % (95 % CI 85.36-100). Statistical analysis revealed no significant difference (p = 0.264) in prevalence among rat species. Meanwhile, results showed that adult rats had a significantly higher (p = 0.013) infection rate at 95.6 % (95 % CI 92.08-99.03), than juveniles at 84.4 % (95 % CI 73.86-95.03). There was no significant difference (*p* = 0.142) in the prevalence of helminth parasites between male (96.1 %; 95 % CI 93.77-100) and female (90.3 %; 95 % CI 93.77-100) rats. Prevalence during the dry season (90.82 %; 95 % CI 85.41-96.24) and wet season (95.77 %; 95 % CI 91.1-100) were

Table 2. Prevalence (%) of helminth parasites per species of non-native rats (*Rattus* spp.) collected from various land use types in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines.

Parasites	*R. exulans* (n=18)	*R. tanezumi* (n = 130)	*R. norvegicus* (n = 32)	Overall (N = 180)	*p-value
H. diminuta	27.78	37.69	50	38.89	0.00
H. nana	27.78	31.31	71.88	36.89	0.00
S. fasciolaris	38.89	53.08	50.00	51.11	0.44
N. brasiliensis	50.00	51.54	46.88	50.56	0.66
S. ratti	0.00	8.46	25.00	10.56	0.08
E. ilocanum	11.11	4.62	0.00	4.44	0.00
T. muris	0.00	3.85	25.00	7.22	0.00
A. cantonensis	44.44	16.92	9.38	18.33	0.00
Rictularia sp.	0.00	0.77	3.13	1.11	0.60
T. pisiformis	0.00	0.77	6.25	1.67	0.05
C. hepatica	2.23	9.14	12.46	23.88	0.02

*Significant at P < 0.05.

not significantly different (p = 0.40). In addition, Pearson's correlation analysis showed moderate positive correlations between parasite load and the body weight (r = 0.30, p = 4.4e-05) and body length (r = 0.23, p = 0.0017) of the rat host (Fig. 2).

Discussion

This study records three (3) rat species and 11 helminth parasite species in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines. The presence of *R. tanezumi* in all land use categories may suggest that *R. tanezumi* can act as a bridge species, carrying parasites across different habitats (Estaño *et al.,* 2020). *R. norvegicus* is opportunistic and highly adaptive to a wide range of conditions, enabling them to inhabit and survive in human-modified habitats successfully (Estaño, 2023). *R. exulans* is a significant agricultural pest in many parts of Southeast Asia, and it is reported to destroy farm crops such as coconut, palm, rice, sweet potatoes, and maize (Wood & Singleton, 2015). Moreover, this study revealed that rats from residential areas have higher prevalence rates of helminth parasites (Table 1), but rats from agricultural and agroforest sites reported helminth parasites with more complex life cycles that require intermediate hosts. In particular, lowlands or irrigated rice fields are the preferred habitats of helminth parasites, such as *Angiostrongylus cantonensis* and *Echinostoma ilocanum*, which require gastropods as intermediate hosts.

This study revealed an overall prevalence of *A. cantonensis* in rats at 18.33 %. This parasite is a leading cause of human eosinophilic meningitis and infects a wide range of animal hosts, including snails, which plays a significant role in zoonotic transmission (Hochberg *et al.*, 2007). The highest prevalence rate was significantly observed in agricultural and agroforest areas but low in residential areas. Estaño *et al.* (2020) also reported the same trend due to intermediate host snails abundant in rice fields and swamps. Studies in the Philippines by Tujan *et al.* (2016) and Castillo & Paller (2018) also reported *R. tanezumi* and *R. norvegi-*

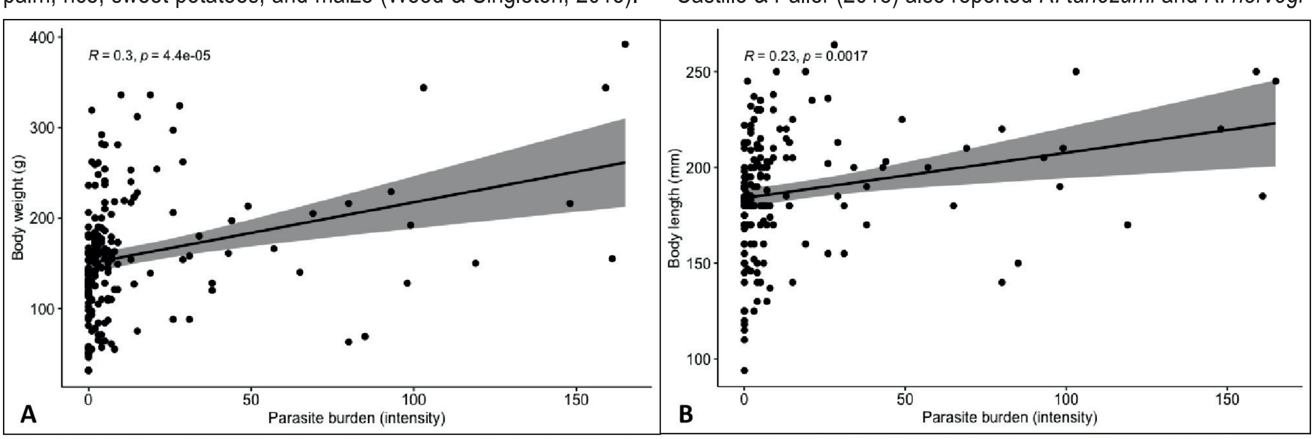

Fig. 2. Relationship between parasite load and body (A) weight and (B) length of rats (*Rattus* spp.) in northeastern sub-watersheds of Mount Makiling, Laguna, Philippines.

Table 3. Prevalence of co-infection of helminth parasites in non-native rats (*Rattus* spp.) collected from various land use types in the northeastern sub-watersheds of Mount Makiling, Laguna, Philippines.

——	Agricultural (% ; n=60)	Agroforest (% ; n=60)	Residential (% ; n=60)
H. diminuta + *H. nana* + *C. hepatica* + *T. muris* + *T. pisiformis*	0.00	0.00	1.70
H. diminuta + *H. nana* +*N. brasiliensis* + *A. cantonensis*	1.70	0.00	0.00
H. diminuta + *H. nana* + *N. brasiliensis* + *C. hepatica*	0.00	0.00	5.00
H. diminuta + *H. nana* + *N. brasiliensis* + *Rictularia* sp.	1.70	0.00	0.00
H. diminuta + *H. nana* + *N. brasiliensis* + *Strongyloides* + *C. hepatica* + *T. muris*	0.00	0.00	1.70
H. diminuta + *H. nana* + *S. fasciolaris* + *A. cantonensis*	0.00	0.00	1.70
H. diminuta + *H. nana* + *S. fasciolaris* + *C. hepatica*	0.00	0.00	10.00
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis*	1.70	3.30	0.00
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis* + *C. hepatica*	0.00	0.00	8.30
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis* + *C. hepatica* + *T. muris* + *A. cantonensis*	0.00	0.00	1.70
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis* + *Strongyloides* sp. + *C. hepatica*	0.00	0.00	1.70
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis* + *S. ratti* + *T. muris*	0.00	1.70	0.00
H. diminuta + *H. nana* + *S. fasciolaris* + *N. brasiliensis* + *T. muris*	0.00	0.00	1.70
H. diminuta + *H. nana* + *S. ratti* + *C. hepatica* + *A. cantonensis*	0.00	0.00	1.70
H. diminuta + *S. fasciolaris* + *C. hepatica* + *A. cantonensis*	0.00	0.00	1.70
H. diminuta + *S. fasciolaris* + *N. brasiliensis* + *C. hepatica*	0.00	0.00	3.30
H. diminuta + *S. fasciolaris* + *N. brasiliensis* + *S. ratti* + *C. hepatica* + *T. muris*	0.00	0.00	1.70
H. diminuta + *S. fasciolaris* + *S. ratti* + *A. cantonensis*	0.00	1.70	0.00
H. nana + *N. brasiliensis* + *C. hepatica* + *A. cantonensis*	0.00	1.70	0.00
H. nana + *N. brasiliensis* + *S. ratti* + *A. cantonensis*	0.00	1.70	0.00
H. nana + *N. brasiliensis* + *S. ratti* + *C. hepatica* + *T. muris*	0.00	0.00	1.70
H. nana + *S. fasciolaris* + *N. brasiliensis* + *C. hepatica*	0.00	0.00	3.30
H. nana + *S. fasciolaris* + *N. brasiliensis* + *S. ratti* + *C. hepatica* + *T. pisiformis*	0.00	0.00	1.70
N. brasiliensis + *S. ratti* + *C. hepatica* + *A. cantonensis*	1.70	0.00	0.00
N. brasiliensis + *S. ratti* + *C. hepatica* + *T. muris* + *Rictularia* sp.	0.00	0.00	1.70
S. fasciolaris + *N. brasiliensis* + *C. hepatica* + *A. cantonensis*	1.70	0.00	0.00
S. fasciolaris + *N. brasiliensis* + *E. ilocanum* + *A. cantonensis*	1.70	0.00	0.00
S. fasciolaris + *N. brasiliensis*+ *S. ratti* + *T. muris* + *A. cantonensis*	0.00	1.70	0.00

cus to be 100 % co-infected with *A. cantonensis* in Nueva Ecija, Philippines, a notable rice-growing province. Meanwhile, Estaño (2023) recently reported the infection of this helminth parasite in *R. tanezumi* and *R. norvegicus* collected in agricultural areas in the Southern Philippines.

C. hepatica was also recovered in this study with an overall prevalence of 23.88 % and was found highest in *R. norvegicus* at 12.46 % collected in residential sites and lowest at 6.7 % in *R. exulans* collected in agricultural site. *C. hepatica* is a zoonotic parasite, and human infection is primarily due to unsanitary practices, poor hygiene, and an abundance of rats. Urbanization in residential areas increases the amount of garbage produced, which favors the proliferation of these rats (Mohd Zain *et al.,* 2012). Transmission in humans occurs via ingestion of embryonated eggs commonly found in the environment (Horiuchi *et al.,* 2013). A higher incidence in children was reported, which may be due to soil-hand mouth practice (Fuehrer *et al.,* 2011; Juncker-Voss *et al.,* 2000; Ochi *et al.,* 2017; Wang *et al.,* 2011). Quilla and Paller (2020) further reported the histopathological effects of the parasites in rat liver collected from Mt. Makiling and adjacent areas. No human infection has been reported in the Philippines, but this could be due to misdiagnosis or unreported cases.

Rats in this study were also found infected with four cestode species, namely, *H. diminuta, H. nana*, *T. pisiformis*, and *S. fasciolaris*. All cestodes were found to be present in agricultural, agroforest, and residential areas except for *T. pisiformis*, which was observed only in rats from residential areas. *H. nana* showed the highest prevalence, which could be attributed to the ability of the parasite to be transmitted and complete its life cycle even without the presence of an intermediate host, like insects. This parasite is commonly found in tropical and subtropical areas (Gitonga *et al.,* 2016; Iliev *et al.,* 2017). Interestingly, these two species of *H. diminuta* and *H. nana* are zoonotic, causing hymenolepiasis. Infection of other mammalian hosts is acquired by ingesting an intermediate host, like insects carrying the larvae. Likewise, human infections occur by accidental ingestion of infected intermediate hosts in precooked cereals or other food items. Direct ingestion from the environment via oral exploration can also cause infection in children. Mild to moderate infection may be asymptomatic; however, heavy infection may cause dizziness, anorexia, abdominal distress, and diarrhea (Chaisiri *et al*., 2015; Hancke & Suarez, 2015; Rohela *et al.,* 2012).

On the other hand, *E. ilocanum* was only recovered in agricultural areas with a 4.4 % prevalence rate. *E. ilocanum* is an intestinal parasitic fluke in mammals and birds and has an indirect life cycle with aquatic snails as intermediate hosts. Among them, *Gyraulos* and *Hippeutis* are the first intermediate hosts, while *Pila*, *Viviparus*, *Vivipara*,*Thiara*, *Planorbis*, and *Lymnaea* act as second intermediate hosts in the Philippines (Chaisiri *et al.,* 2015; Eduardo, 1991). The occurrence of *E. ilocanum* in rats in agricultural habitats might be due to the presence of intermediate host snails of the parasite (Paller *et al.,* 2019). The lower prevalence of this parasite can be attributed to its life cycle requiring an intermediate host, unlike that of other helminth parasites with direct life cycles, which may have higher chances of dispersion. This parasite can cause Echinostomiasis in humans, infecting the intestine and bile duct (Belizario *et al.,* 2007). Regarding distribution, *E. ilocanum* is common in South Korea and Southeast Asia, especially in the Philippines. Infection in humans was reported in Southern parts of the Philippines (Eduardo, 1991). Infection of this parasite in wild murid rodents was also recorded in the Indo-Chinese Peninsula, where *Echinostoma malanayum* and *E. ilocanum* were recovered (Chaisiri *et al.,* 2015). The presence of infection of *E. ilocanum* poses a threat to public health, especially in agricultural communities.

Among the parasites recovered, *N. brasiliensis* showed the highest prevalence and was found in all habitats. High prevalence among rats in different habitats was expected since the transmission is direct and does not require an intermediate host (Gomez Villafañe *et al.,* 2008; Paramasvaran *et al.,* 2009). Moreover, *N. brasiliensis* infection can cause lung damage by disrupting the alveolar architecture due to parasite migration and can result in pulmonary emphysema (Marsland *et al.,* 2008; Hoeve *et al.,* 2009). *S. ratti (*10.56 %), on the other hand, is also known to cause strongyloidiasis in humans, resulting in coughing, nausea, vomiting, abdominal pain, and weight loss; however, it is usually asymptomatic. Other parasites recorded were *Rictularia* sp. (1.11 %) and *T. muris* (7.2 %), which were not zoonotic.

Results on the multiple helminth co-infections in rats reveal some evidence of greater parasite species richness and cases of co-infections in residential habitats similar to previous reports (Chaisiri *et al.,* 2010). The higher number of co-infection cases can be attributed to a greater host density in residential areas as well as the host-susceptibility of *R. norvegicus* to helminth parasites (Bordes *et al.*, 2011; Morand *et al.*, 2015). With greater host density in residential areas, there is a greater chance of transmission of generalist parasite species within the same host species population. Multiple papers, however, have reported contradicting results but have noted the possible impact of rodent assemblages and community structure in the habitat sampled (Hurtado *et al.*, 2021; Palmeirim *et al.*, 2014;Werner *et al.,* 2020).

This study also attempted to associate the occurrence of parasites with the morphometric characteristics of the hosts. Results revealed a positive correlation between the rat host body size and parasite infection. This could be age-related, so older hosts are more exposed to environmental parasite transmission. Larger hosts can support higher parasite biomass (Poulin & George-Nascimento, 2007) and are also more susceptible because of active behavior related to foraging (Winternitz *et al.,* 2012). Heavier hosts are also more likely to engage in breeding activities, exposing them to chronic infections (Perrin *et al.,* 1996; Schwanz, 2008).

With regards to sex, the prevalence between male and female rats was not significantly different. Although both sexes are susceptible to infections, studies reported that males were more active and had a more extensive home range than females, making them more likely to get infected. This distribution requires male rats to forage more, having higher chances of encountering infective stages of parasites in the environment (Heaney *et al.,* 1999; Soliman *et al.,* 2001; Tew & Macdonald, 1994). Ferrari *et al.* (2007) mentioned that male rats, especially older males, harbor a substantial portion of parasites in a host population, releasing disproportionately more infective stages into the environment than females. Furthermore, males and females can constitute different environments for parasites. Factors like sex hormones, immune-competence, mobility, body size, home range, and dispersal rates differ between sexes, rendering male and female hosts different habitats for parasites. In addition, it was observed that in agricultural areas and agroforest settings, male rats tend to travel to alternative habitats, whereas breeding females continue to forage in rice fields in order to meet the nutritional requirements needed to raise litter (Estaño *et al.,* 2020). In some cases, male and female rats harbor different parasite loads due to differences in spatial behavior. It is interesting to note that there was no significant difference in infection rates of rats during wet and dry seasons. The climate conditions do not quite vary in tropical countries, and infective stages of parasites are likely to be present in the environment throughout the year.

Infected rats that harbored infection were found primarily in agroforest and agricultural areas. This could be attributed to the presence and broader distribution of the parasites' intermediate hosts. For instance, *A. cantonensis* and *E. ilocanum* require mollusks as intermediate hosts, which abound in agricultural and agroforest areas. However, the increasing land use change and urbanization in the areas could also lead to changes in the dynamics of host behavior, such as the movement of rats to human settlements. Given the evidence of increased transmission in urban-adapted hosts such as rats, the potential zoonosis risk could pose public health threats. Urbanization can provide favorable eco-epidemiological conditions for rodent-borne pathogens, becoming an emerging risk and a severe threat in these areas.

Conflict of Interest

Authors state no conflict of interest.

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