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Zoonotic parasites of brown rats in selected ricefields surrounding Lake Mainit, Philippines

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ABSTRACT

Zoonotic parasites in brown rats (*Rattus norvegicus*) can be transmitted through environmental contaminations and cause diseases to humans. This study was conducted to determine the prevalence and intensity of zoonotic parasites among *R. norvegicus* in selected ricefields surrounding Lake Mainit, Philippines. A total of 50 brown rats were trapped in metal cages baited with bread or fish or both and subjected to parasite examination under the dissecting microscope. The results documented two species of ectoparasites and five species of endoparasites recovered from brown rats. The prevalence rate of infection was recorded highest in the hookworm *Nippostrongylus brasiliensis* (74%) and the rat tapeworm *Hymenolepis nana* (34%). Results also show that *N. brasiliensis* obtained the highest Mean Intensity (MI) of infection (MI=40). Among ectoparasites, the sucking louse *Polyplax* spp. and the mite *Laelaps* spp. were also infesting brown rats with a prevalence of 48% and 17%, respectively. A positive weak significant association (r=0.331; *p*=0.035) between the parasite's intensity and the rat's weight was recorded. Barangay Matin-ao holds the highest MI of infection, while Barangay San Isidro has the lowest (*p*=0.001). Risk factors associated with parasite infection varied from one place to another. The existence of these rats carrying parasites represents a potential risk to nearby residents. Hence, it is essential to address environmental and public health awareness against future zoonotic transmission through ubiquitous brown rats.

Keywords: *Lake Mainit, Brown rats, Zoonotic parasites, Rat parasites*

1 Introduction

Parasitic zoonoses are common and broadly disseminated in the Southeast Asian region (Dorny et al. 2009). Later biomedical overviews conducted in the Philippines show that parasitic infections are still considered a vital health issue in the country (Harhay et al. 2010). Rats are among the carriers of these pathogenic parasites, which play a critical role in distributing various pathogens, including zoonotic parasites (Himsworth et al. 2013). In rural areas, the close association between wild rats and humans facilitates zoonotic transmission. Thus, the presence of wild rats has serious implications for environmental and public health (Johnson et al. 2015).

The zoonotic parasites of wild rats in the Philippines were already recorded to cause many problems, including environmental contamination (Eduardo et al. 2008; Tujan et al. 2016). Brown rats tend to be a reservoir of some pathogenic parasites transmitted to humans by consuming various food leading to disease outbreaks associated with high morbidity and high mortality (Dhama et al. 2013).

Lake Mainit is considered one of the Key Biodiversity Areas in the Philippines and is the habitat of some endemic and threatened wildlife species, making the lake and its nearby surroundings of high ecological value (Demetillo et al. 2016; Paylangco et al. 2020). Lake Mainit is known for its tourism potential; however, safety and awareness from parasitic infection are not well studied. A report from Jumawan and colleagues (2016) revealed that several gastropod fauna in Lake Mainit might be vectors of zoonotic parasites. Parasitosis was evident in this area from infected snail vectors of *Schistosoma japonicum* and bovine feces, with zoonotic helminths were contaminating the area (Abao-Paylangco et al. 2019; Jumawan and Estaño 2021). However, the distribution of zoonotic parasites in *Rattus norvegicus* or brown rats in ricefields surrounding the lake has not been documented.

This study was conducted to help widen the scope and knowledge about rat parasite fauna in the Philippines. The study results will help raise environmental and public health awareness on zoonosis linked to brown rats.

2 Materials and Methods

Study area

The study was conducted in ricefields surrounding Lake Mainit, namely, Magpayang, San Isidro, Matin-ao, Roxas, and Alegria (Figure 1). The lake is located in the northeastern part of Mindanao and is a shared resource by Surigao del Norte and Agusan del Norte provinces. Some physical attributes such as the observed presence of other suitable and reservoir hosts roaming in the study areas were also initially assessed for a risk factor to zoonotic transmission (Himsworth et al. 2013; Eduardo et al. 2008).

Collection of rat samples

A Wildlife Gratuitous Permit (No. R13-2018-44) was issued by DENR Regional Office XIII, approving the collection and number of collected *R. norvegicus* individuals for the study. A total of 50 brown rats were randomly sampled between June to November 2018 and examined for parasites. Ten individuals in each selected barangay were trapped in metal cages baited with bread or fish or both to increase the capture rate. The cages were placed strategically at least 50 m intervals within the ricefields alongside ditches. Captured brown rats were transported to the laboratory for processing, classification, and parasite examination. Before the parasite examination, weight (g) and length (cm) were obtained from each individual. Rats were euthanized, and the lungs and gastrointestinal tract were dissected for zoonotic parasites (Estaño et al. 2021).

Isolation and identification of parasites

Ectoparasites were collected by thoroughly combing the rat's fur using a fine-tooth comb onto plain white paper. Forceps were utilized to remove ticks and mites if it is difficult to remove them by combing. Collected ectoparasites were preserved in 70% alcohol. Ectoparasites from each rat were stored in separate vials (Castillo et al. 2018; Quilla and Paller 2020).

Rat dissection followed for endoparasite examination. The body cavity was slit open from the throat to the anus to expose its internal organs. It was dissected separately under a dissecting microscope for helminth examination. The collected lungs and intestines were fixed in 70% alcohol. The

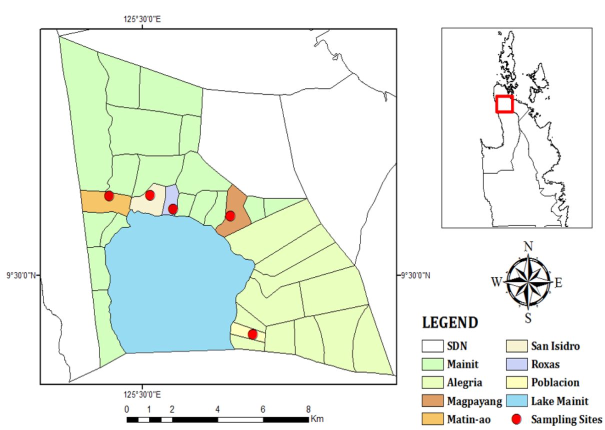

Figure 1. The location of Lake Mainit, Philippines showing the five sampling sites.

contents of the internal organs were also examined carefully for parasitic infection. Clumped parasitic worms were removed gently from the lining of the organs with a scalpel and placed in a larger vial for identification. The collected parasites were then examined under a compound microscope from 40x-100x magnifications (Paller et al. 2017).

Data analyses

The mean intensity (MI) and prevalence (%) of the recovered parasites were computed based on the following epidemiological formula (Estaño et al. 2021).

$$\text{Mean Intensity (Parasite per host)} = \frac{\text{Total number of recovered parasites}}{\text{Total number of positive samples}}$$

$$\text{Prevalence (\%)} = \frac{\text{Total number of positive samples}}{\text{Total number of samples}} \times 100$$

Statistical analysis was done through the use of SPSS version 20. Spearman Rho correlation analysis was used to test the relationship between the parasite's mean intensity and the rat's body weight and length measurements. The Kruskal-Wallis Test was used for the comparison of parasite's mean intensity across sampling areas. If the p-value is equal to or less than 0.05, or at 5% alpha, the results are significant.

3 Results and Discussion

Recovered parasites in brown rats

The study results documented five species of endoparasites under the Class/ Order Trematoda, Cestoda, Rhabditida, and Oxyurida (Figure 2) and two species of ectoparasites under Class Insecta and Arachnida (Figure 3; Table 1). All 50 examined brown rats harbor zoonotic parasites. The morphometric measurements of the collected brown rats are shown in Table 2.

The intestinal fluke *Echinostoma* sp. (Figure 2-A) are hermaphroditic worms characterized by a prominent ventral sucker larger than its oral sucker. The testes are positioned high in the half of the posterior of the trematode's body. Echinostomes usually use various gastropod mollusks species for their first and secondary developmental stages (Keeler and Huffman 2009). Their length varies from 1 mm to 12 mm, and their shapes are leaflike, piriform, spatulate, or lanceolate (Mahanty and Cross 2011).

The rat lungworm *Angiostrongylus cantonensis* (Figure B.1-B.2) is a zoonotic helminth that causes angiostrongylosis. The primary cause of eosinophilic meningitis in humans is accidental ingestion of the intermediate host, particularly in Indo-Pacific locations where it is endemic. The animal-human natural interface of *A. cantonensis* is troublesome to survey as it changes effortlessly with the influence of the environment. Its life cycle involves wild rats as definitive hosts and mollusks as intermediate hosts. It is also found in shrimps and frogs without further development and remains infectious when consumed. Changes in the ecology and the environment may result in changes in the study of the disease transmission of this parasite. Hence, it is critical to evaluate the parasite's transmission course due to its danger to economic health (Tujan et al. 2016; Estaño et al. 2021).

The dwarf tapeworm *Hymenolepis nana* (Figure C.1-C.3) has a small and slender body (range of 25-40 mm). It has a scolex that bears four suckers and a rostellum with a row of hooks (Roberts and Janovy 2000). This tapeworm can be transferred directly from person to person. This tapeworm also differs from other tapeworms because it can complete its entire life cycle in a single host. Infection follows when eggs are accidentally ingested through contaminated food or water. Rats or humans can be reservoir hosts of this tapeworm (Sadaf et al. 2013). This cestode is more common in areas with a warm climate, a lack of sanitary facilities, and unsanitary conditions, all of which pose a risk of human transmission (Hancke and Suarez 2011).

The gastrointestinal roundworm *Nippostrongylus brasiliensis* (Figure 2-D) was revealed to be the most common parasite infecting the sampled brown rats in study stations surrounding Lake Mainit. Its adult form is slender, while the buccal cavity and mouth are small (Swain et al. 2016). This parasite commonly infects *R. norvegicus* but is not reported to be zoonotic, and its effect in densely populated environments is unknown (Hancke and Suarez 2011).

The adult common rat pinworm *Syphacia muris* (Figure 2) is cylindrical, and its life cycle is basic and direct (Stahl 1963). Rats become infected after ingesting the infective eggs from the perianal locale of an infected host or the contaminated materials within the environment, such as the mucus coating of fecal pellets. The eggs hatch within the rat's upper intestinal tract, and the larvae go to the cecum, where the worm's chief territory is located. Many worms oust their eggs on the rat perianal region (Baker 2006).

The sucking louse *Polyplax* spp. is a wingless insect and known commonly infesting rats. Their mouthparts were specialized for piercing the skin and sucking the hosts' blood (Hopla et al. 1994). The parasitic mite *Laelaps* spp. is an acariform with eight legs and has one actual body segment. Their sizes range from less than 1 - 10 mm. This blood-sucking arthropod is generally non-pathogenic but can be vectors of some parasites such as protozoans (Baker 2006).

Prevalence and mean intensity of the parasites isolated from brown rats

The nematode parasite *N. brasiliensis* was the most prevalent (74%) in sampled brown rats, followed by *Hymenolepis nana* (34%) (Table 3). The *N. brasiliensis* also has the highest mean intensity (40 pph), while *A. cantonensis* holds the

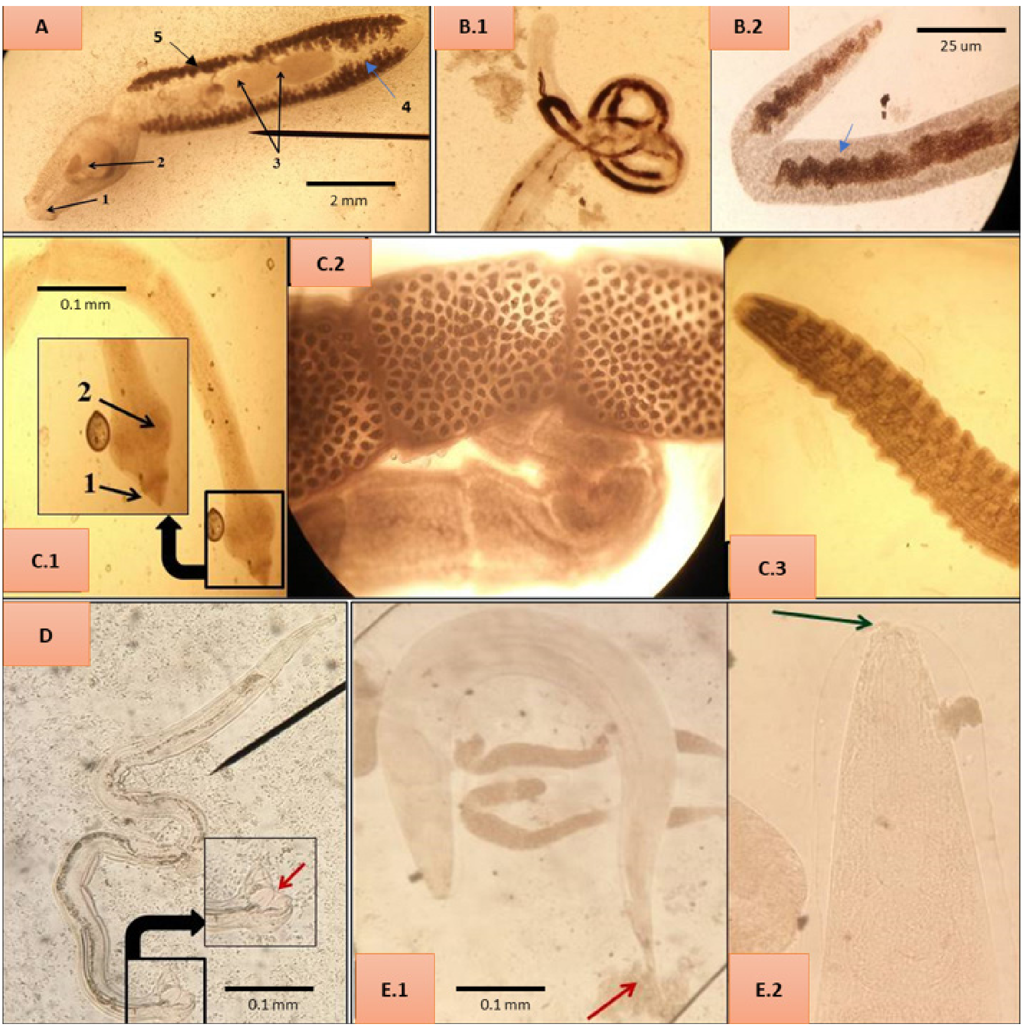

Figure 2. The collected endoparasites from wild rats in ricefields surrounding Lake Mainit, Philippines. (A) *Echinostoma* sp., in the small and large intestines, pointing the (1) oral sucker, (2) ventral sucker, (3) testes, (4) uterus, (5) vitellaria; (B.1-B.2) *Angiostrongylus cantonensis* in rat lungs pointing the intestine; (C) *Hymenolepis nana* in the small intestines, pointing the (C.1) rostellum with hooks (1) and one of the suckers (2), (C.2) the gravid segment, and (C.3) the strobila; (D) *Nippostrongylus brasiliensis* in the small intestines, pointing the male copulatory bursa; (E) *Syphacia muris* in small intestines, pointing the (E.1) tail and the (E.2) distinct head of the parasite.

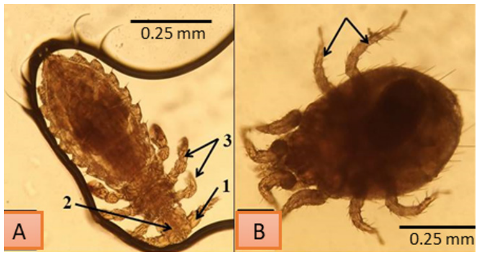

Figure 3. The collected ectoparasites from brown rats in ricefields surrounding Lake Mainit, Philippines. (A) *Polyplax* spp. (100X), pointing the (1) antenna, (2) head and the (3) tibial spurs; (B) *Laelaps* spp. (100X), pointing the legs.

Table 1. The isolated parasites, their microhabitat, common name, and related diseases (zoonotic) from wild rats collected in selected ricefields surrounding Lake Mainit, Mindanao, Philippines.

Parasites	Common Name	Microhabitat	Disease caused
Ectoparasites			
Arachnida			
Laelaps sp.	Mites	Fur	Skin Wounds and Dermatitis (Rat Health Guide 2016)
Insecta			
Polyplax sp.	Sucking Louse	Fur	Anemia and Skin Wounds (Rat Health Guide 2016)
Endoparasites			
Cestoda			
**Hymenolepis nana*	Dwarf Tapeworm	Small and Large Intestine	Hymenolepiasis (Di Yang et al. 2017; Hancke and Suarez 2011; De Leon 2004)
Trematoda			
**Echinostoma* spp.	Intestinal Fluke	Small Intestine	Echinostomiasis (Mahanty and Cross 2011; Belizario 2007)
Nematoda			
**Nippostrongylus brasiliensis*	Rat Hookworm	Small Intestine	Emphysema and Chronic Obstructive Pulmonary Disease (Franssen et al. 2016; Swain et al. 2016; Antolin et al. 2006)
**Angiostrongylus cantonensis*	Lung worm	Lungs	Angiostrongyliasis (Belizario and Trinidad 2004; Tujan et al. 2016)
**Syphacia muris*	Rat Pinworm	Caecum	Pinworm Infection (Jueco and Zabala 1990; Baker 2006; Franssen et al. 2016)

*Zoonotic

Table 2. The morphometric measurements of brown rats collected from selected ricefields surrounding Lake Mainit, Philippines.

Barangays	Head-Body Length (cm)		Tail Length (cm)		Weight (g)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Quezon	17.9	1.1	16	1	125.3	20.1
Matin-ao	17.8	0.9	16	1	141.9	11.9
Alegria	17.2	1.0	14	3	118.8	17.6
San Isidro	18.1	0.8	16	1	133.9	12.6

lowest mean intensity (2 pph) among the parasites. Nematode parasites such as *brasiliensis* and *S. muris* tend to have a monoxenous development (living on only one type/kind of host throughout its life cycle), responsible for nematode species dominating the helminth community found in wild rats (Bellocq et al. 2003). Among the ectoparasites from the sampled rats' fur, *Polyplax* spp. and *Laelaps* spp. yielded a prevalence of 48% and 17%, respectively.

Nippostrongylus brasiliensis was highly prevalent in rats collected from rural areas near ditches (Franssen et al. 2016). These findings suggest that suitable intermediate hosts relevant for parasitic helminths may be present from ditches of the surroundings of farms, rendering rats at farms prone to parasitic infections.

Hymenolepiasis is a neglected zoonotic disease in humans caused by cestodes *Hymenolepis* spp. *Hymenolepis nana* was reported with a 6.1% prevalence in vertebrate hosts, including rodents (Di Yang et al. 2017). As to human cases, more than 175 million hymenolepiasis are caused by *H. nana* has been reported in humans worldwide. The brown rats are the most common host, as they tend to live in less than desirable hygiene conditions (Crompton 1999). In addition, there was a high prevalence (63.3%) of *Hymenolepis* spp. infestation in brown rats from some rice farms in the Philippines (Eduardo et al. 2008).

Intestinal echinostomiasis in brown rats has been documented worldwide. Namue and Wongsawad (1997) reported that trapped brown rats from Chiang Mai Moat were infected with *Echinostoma* sp. (23.68%), *A. cantonensis* (42.10%), and *Nippostrongylus* sp. (34.21%). Moreover, *A. cantonensis* was also reported among brown rats from rice fields in the Philippines (Tujan et al. 2016). Some factors related to the infection rate of *A. cantonensis* could be its complex life cycle because it involves intermediate snail hosts. In this study, the prevalence could be linked to the presence of a suitable snail host in the study area, as reported by Jumawan et al. (2016). *S. muris* was reported high (21%) in rats from all environments (Franssen et al. 2016). *S. muris* is commonly transmitted between rat families. Although *S. muris* is considered non-pathogenic, it has been shown to negatively affect the digestibility of nutrients and consequently the growth of laboratory rats, which could interfere with experiment outcomes (Plachy et al. 2016).

Comparison of parasite intensity across sampling areas (Barangay) was also performed (Figure 4). The results showed that the Barangay Matin-ao holds the highest mean intensity of infection (86 pph), followed by Magpayang (50 pph), Alegria (13 pph), and Roxas (4 pph). The wild rats captured from San Isidro have the lowest parasite mean intensity (6 pph). Some risk factors associated with the parasite intensity could be due to the observed suitable or reservoir hosts in the study area. Domesticated/ livestock animals such as dogs, cats, bovines, and swine were documented in the study areas. These animals can transmit helminth parasites by contaminating soil and water (Jumawan and Estaño 2021).

According to Meerburg et al. (2009), some environmental factors such as sun or rain and temperature are crucial abiotic factors that impact rats' habitat quality and food availability as some pathogens or parasites needed wet or warm circumstances to increase. If the necessities concerning environment quality and food accessibility are met, then rat density will increase. An escalation of rat density proves essential for

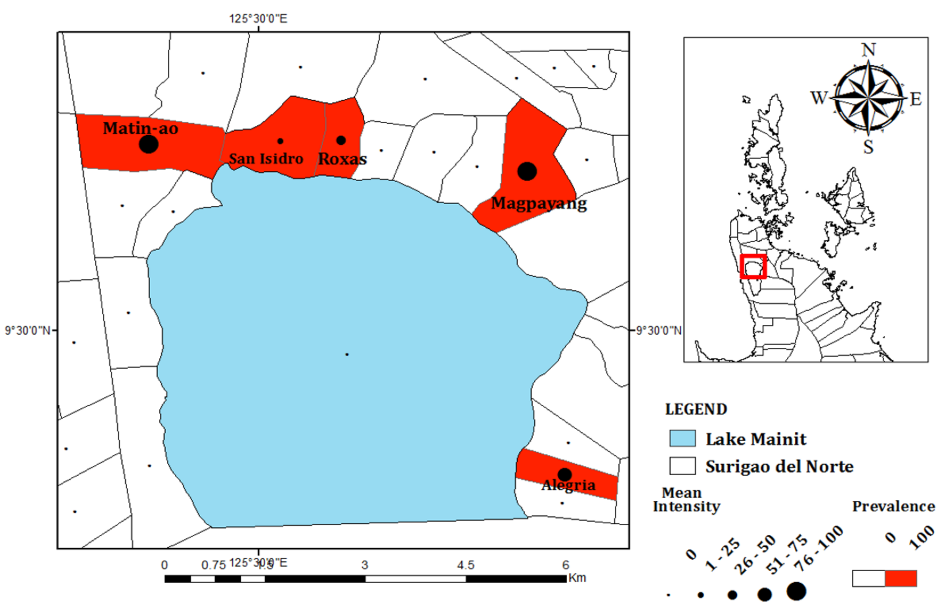

Figure 4. The prevalence and mean intensity of parasites isolated from wild rats in ricefields surrounding Lake Mainit, Philippines.

Table 3. The parasite taxa, total number of infected rats, the mean intensity, and prevalence of parasites from wild rats collected in selected ricefields surrounding Lake Mainit, Mindanao, Philippines.

	Parasites	Mean Intensity (parasite per host)	Prevalence (%)
Ectoparasites			
Arachnida			
	Laelaps sp.	1	17
Insecta			
	Polyplax sp.	2	48
Endoparasites			
Cestoda			
	**Hymenolepis nana*	3	34
Trematoda			
	**Echinostoma spp.*	4	6
Nematoda			
	**Nippostrongylus brasiliensis*	40	74
	**Angiostrongylus cantonensis*	2	4
	**Syphacia muris*	2	4

*Zoonotic

the dynamics of pathogens and parasites (Telfer and Bown 2012). Meanwhile, the variation of distribution among helminth fauna might be attributed to differences in environmental aspects such as temperature, altitude, rainfall, and topography (Bett et al. 2017).

Relationship of rat morphometrics and parasite intensity

This study revealed that only the rat's weight showed a weak significant relationship to parasite intensity ($r=0.331$; $p=0.035$). The fitness of parasites can either positively or negatively correlate with host fitness (Tseng and Myers 2014). Several factors could impact the wild rats' morphometric measurements, such as the climate, availability of food, and the environment's conditions (Singleton et al. 2010). Thus, the parasite number harbored by brown rats does not necessarily influence the morphometric measurements of infected brown rats.

Meanwhile, parasite mean intensity can be influenced by different body weights within rat species. According to Castillo and Paller (2018), regardless of stages, body weight, and length, rodents are vulnerable to infection by parasitic helminths present in the environment because this species is a voracious eater irrespective of their body composition.

Some identified risk factors that are possibly associated with parasite transmission

Several associated risk factors were identified and observed within the selected rice fields, ranging from human activities, the presence of suitable or reservoir hosts, and types of livelihoods. Human activities such as doing laundry near irrigation systems in rice fields, walking barefoot, living in comfortable rooms near rice fields, and using irrigation as a water source for bathing were observed to increase the risk of parasitic infection. Domesticated and livestock animals (dogs, cats, pigpens near the ricefields, chickens, bovines) also increase parasitic infection susceptibility. Moreover, rice farming, fishing, and domestication of animals are common occupations in all of the study areas. Farmers who work in barefooted rice fields increase their risk of contracting zoonotic parasites.

Zoonotic transmission is evident when there is close contact between humans and livestock/ domesticated animals. Farming activities increase infection risks as more close contacts between rats and humans eventually lead to health problems (Meerburg et al. 2009). Wildlife territories are usually converted to agricultural land, yet the original dwellers (e.g., rats) are still there (Hoffman and Cawthorn 2012). Hence, the diseases passed to both humans and livestock could expand. A study has shown that rat colonies near households and farm animals increase the likelihood of severe health problems in the community (Kues and Niemann 2004).

Implications to public health

Rattus norvegicus is known to be a vector of various pathogens responsible for transmitting diseases to humans. Rats inhabit almost all habitats and can rapidly adapt to environmental changes, making them sentinels for the rapid transmission of many infectious organisms, particularly parasites, to humans. To maintain the parasite cycle in nature, these rats often act as reservoirs for zoonotic parasites.

One of the reasons for the high prevalence of parasite infection in the area is the close proximity of livestock and domesticated animals to households. This scenario is similar to those documented in reports of a higher prevalence of parasitic infection in humans with dogs raised in their households (Shrestha et al. 2018) and parasite infection in children who are in close proximity to parasite-infected livestock (Daniels et al. 2015).

The lack of sanitation facilities, proper personal hygiene, and low-level farming practices that include not wearing boots while working on the ricefields were identified as risk factors contributing to parasitic transmission. A high prevalence of parasite infection was recorded among children who do not practice handwashing after defecation (Khanal et al. 2011). Consequently, cases of hymenolepiasis in children with better hygiene practices are low (Erismann et al. 2016).

Soil and water contamination were among the modes of parasitic infection. Drinking water contaminated with rat feces could act as a carrier of parasite eggs. Direct contact with rats is crucial for the exchange of such pathogens and zoonotic parasites. Hence, there is a significant association between the parasite load and the associated risk factors, including walking barefoot, having livestock close to the household (Jiraanankul et al. 2011), and raw meat consumption (Ruankham et al. 2014).

4 Conclusion and Recommendations

The brown rats *Rattus norvegicus* in the selected ricefields surrounding Lake Mainit in the Philippines harbored various forms of zoonotic parasites such as flukes, tapeworms, and roundworms. Ectoparasites were also infesting the brown rats. Health education on the risk of zoonotic transmission and prevention can significantly reduce future infections and infestations. To be successful in this public health awareness campaign, the Local Government Units' cooperation, public health workers, biologists, and veterinarians are highly needed for this effort.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest s regarding the publication of this paper.

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