

## Parasites bared in *Rattus norvegicus* and *Rattus tanezumi*

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### Abstract

This paper documents the richness of parasites inhabiting *Rattus tanezumi* and *Rattus norvegicus*. *Rattus* spp. revealed six ectoparasites, namely: mites genus Chirodiscoides, and *Radfordia ensifera*, *Laelaps nutalli* and *Ornithonyssus bacoti*, fleas *Xenopsylla cheopis*, and lice *Polyplax spinulosa*. While infestation with *L. nutalli* was heavy in 15 (100%) and 13 (86.7%) *R. norvegicus* and *R. tanezumi*, respectively, only *R. tanezumi* harbored Chirodiscoides and *P. spinulosa* at 80.0%. Endoparasites identified were two kinds of nematodes belonging to genus *Nippostrongylus* (Class Secernentia) and *Capillaria hepatica* (Class Adenophorea), and tapeworms identified as genus *Raillietina*, and *Hymenolepis*, and *Taenia taeniaformis*. While all 30 rats showed 100% parasitism with ecto- and endoparasites, *Babesia* infection was detected only in eight rats (26.6%), with seven cases recorded in male rats. Most dominant endoparasites were *Nippostrongylus* in *R. norvegicus* at 73.3%, and *R. tanezumi* at 100.0%; while *R. norvegicus* and *R. tanezumi* revealed 86.6% and 66.6% prevalence with *C. hepatica*, respectively. Interestingly, *Taenia taeniaformis* and *Raillietina* were detected only in *R. norvegicus* at 80.0% and 20.0% infection, respectively. While both species of rats examined revealed susceptibility to different kinds of parasites, the heavier *R. norvegicus* seemed to nurture and support greater parasite species richness and density. In view of the argument of a clear accumulation of helminth diversity and species burden with increasing age of rat species and in the absence of information of the ages of rats used in the present survey, studies to cover an expanded rat population in the Philippines is recommended.

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### Introduction

In the Philippines, there are several recognized rat species including the most widespread *Rattus norvegicus* and *Rattus rattus* and are considered pests owing to the damage they cause to farms and farm produce (Rabor, 1977; Sanchez and Benigno, 1985; Singleton et al., 2008). But more than being pestiferous, they carry ectoparasites that transmit viruses, microbes and protozoan parasites, and are also hosts to different endoparasites (Pratt and Good, 1954;

Miyata and Tsukamoto, 1975; Oshima et al., 1978; Morsy et al., 1994; Fedorko, 1999; Soliman et al., 2001; Dagny et al., 2002; Paramasvaran et al., 2005). Many of these parasites are zoonotic and are transmissible to humans (Jueco, 1983; Davis et al., 2005). In the Philippines, there are a few documents on parasitism of rats with mites, lice, tapeworms, acanthocephalans and nematodes (Claveria et al., 2004; Salibay and Luyon, 2007). Through inoculation of mice with blood from residential, commercial and agricultural rats that were tested seropositive for anti-



*Toxoplasma gondii* antibodies, Salibay and Claveria (2005) provided the first evidence of the existence of virulent, moderately virulent and avirulent strains of *T. gondii* in the country. Considering other documented zoonotic nature of many rat-borne parasites (Namue and Wongsawad, 1997. Paramasvaran et al., 2009; Belizario and De Leon, 2015; Chaisiri et al., 2015), potential human exposure becomes a significant public health concern, particularly because parasites generally induce self-limiting symptoms, such that infections may remain unnoticed. This paper documents species richness of parasites inhabiting *R. norvegicus* and *R. tanezumi* in a Philippine setting.

## Materials and Methods

### Collection site and identification of rat species

Fifteen rats were collected from rice fields in Barangay Tulat, San Jose, Nueva Ecija, Philippines. Using traps, 15 rats were captured from a semi-commercial residential area close to a university and various food chains and commercial buildings along Fidel A. Reyes Street, Malate, Metro Manila, Philippines. Captured rats were housed individually in wired cages and provided water prior to their transport to the animal facility of De La Salle University. Rats were provided bread and rice grains, and water. Based on body morphometrics and other morphological features of rats like foot pads, tail and nose, rice-field and semi-commercial residential area collected were identified as *Rattus tanezumi* and *Rattus norvegicus*, respectively (Rabor 1977; Sanchez and Benigno, 1985). *Rattus tanezumi* measured 244mm± 46mm and weighed 165g±24g; while *R. norvegicus*, measured 327mm ± 70mm long and weighed 257g ± 66g (Fig. 1).

### Rat dissection, parasite isolation and identification

Rats were appropriately handled thru slow anesthetization using cotton balls wet with chloroform. When the rats became unconscious, each rat was moved to rest individually on a paper-lined dissecting pan. Using fine combs, the entire body surface including those of the legs and tail was examined for the presence of ectoparasites. Parasites were collected and preserved in 70% ethanol, and kept until further examination for proper identification. Rats were dissected starting with a slit from the end shoulder ventral surface of the body to expose the heart, from where few drops of blood were obtained for smear preparation. The body was further dissected

down to the posterior end for the examination of tissue and gut/organ-dwelling parasites. For blood parasite detection, smears were prepared, fixed in 70% ethanol and stained with Giemsa. Parasites that were exenterated from the different organs and tissues were transferred to individual properly-labelled Petri dishes with mammalian phosphate buffer solution (PBS), examined under a stereomicroscope (20x-100x), and then preserved in 5% formalin solution. Parasites were identified down to the genus and species level (Claveria et al., 2004; Salibay and Luyon, 2007). Overall prevalence and percent infection with parasitic agent identified and parasitemia or parasite density were computed and compared between the rat species.



**Figure 1:** *Rattus norvegicus* (A) showing brown to gray dorsal fur (B) with bi-colored dark fur in tail (C), possessing an elongated inner metatarsal tubercle on the foot pad (D), and snout (E). *Rattus tanezumi* (F) with an olive brown dorsal fur (G) along with a very dark gray tail (H), a longer and much narrower foot (I) and snout (J).

### Data analysis

Overall prevalence of parasitic infection was compared between *R. norvegicus* and *R. tanezumi*, including differences in occurrence between male and female rats, following the formula: number of rats parasitized over total number of rats examined per

species/ gender multiplied by 100. Considering the low rat population examined, prevalence data were not statistically analyzed. As an alternative, other valuable observations associated with density/burden/level of parasitism per etiologic agent identified was determined using a modified scoring system adopted from Daynall Teaching Laboratory Liverpool School of Tropical Medicine (1997), and modified as follows: for number of ectoparasites per rat: Low: <10; Moderate  $\geq 10$  - <50; and Heavy:  $\geq 50$ ; for number of endoparasites per rat: Low: 1-2; Moderate: 3-6; Heavy:  $\geq 7$ .

## Results and Discussion

All 30 rats examined showed 100% parasitism with ecto- and/or endoparasites, while protozoan *Babesia* infection was detected only in eight rats (26.6%), with seven cases in male rats (Table 1; Fig.2). Ectoparasites identified were mites belonging to genus *Chirodiscoides*, *Radfordia ensifera*, *Laelaps nutalli* and *Ornithonyssus bacoti*; fleas, *Xenopsylla cheopis* and lice, *Polyplax spinulosa*. While infestation with *L. nutalli* was heavy and noted in 15 (100%) and 13 (86.7%) *R. norvegicus* and *R. tanezumi*, respectively, only *R. tanezumi* had infestation with *Chirodiscoides* and *P. spinulosa* at 80.0% (Tables 2 and 3).

Endoparasites identified were two kinds of nematodes belonging to genus *Nippostrongylus* (Class Secernentia) and *Capillaria hepatica* (Class Adenophorea) (Schmidt and Roberts, 1985) (Fig. 3), and tapeworms *Raillietina*, *Hymenolepis* and *Taenia taeniaformis* (Fig. 4). As shown in Table 4, most dominant endoparasites were *Nippostrongylus* nematodes detected in 12 (80.0%) *R. norvegicus* and 15 (100.0%) *R. tanezumi*; while infection with *C. hepatica* was recorded in 13 (86.6%) *R. norvegicus* and 10 (66.6%) *R. tanezumi*. Interestingly, tapeworms *Taenia taeniaformis* and *Raillietina* were recorded only in *R. norvegicus* at 80.0% and 20.0% infection, respectively. While both rat species examined revealed vulnerability to different kinds of parasites, *R. norvegicus* revealed a more diverse pool of parasites (Table 4). Worth mentioning is the high density of *Nippostrongylus* in both *R. norvegicus* and *R. tanezumi*, and high infection rate in both species

with *C. capillaria*, nonetheless of low parasite burden (Tables 4 and 5).

The parasites detected in the present study collaborate earlier reports (Linardi et al., 1994; Soliman et al., 2001; Antolin et al., 2006; Claveria et al., 2004; Salibay and Luyon, 2007; Zain et al., 2012). *Hymenolepis diminuta* and *Raillietina garrisoni* are common parasitic tapeworms of rodents (Hinz, 1985). In the Philippines, there are documents of 8.0% prevalence of hymenolepsiasis *diminuta* and infection of children <3 years of age with *R. garrisoni* (De Leon and Solon, 1998; Jueco, 1975). In Thailand, there are reported similar observations of passing out young worms of *R. garrisoni* in 2-5 years old children (Chandler and Pradatsundarasar, 1957).

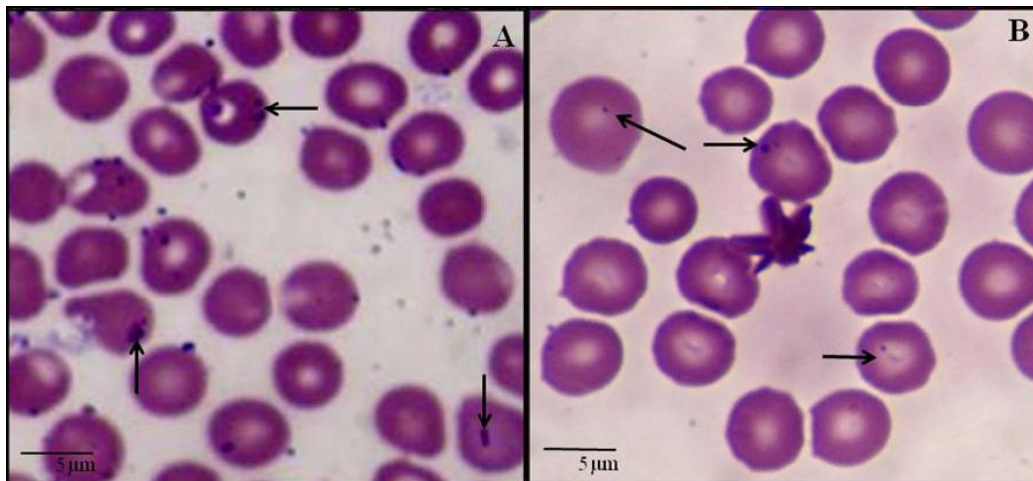
In a study on wild-caught murid rodents from forest, non-flooded land, irrigated land, and human settlement in seven localities of Thailand, Cambodia, and Lao PDR, Chaisiri et al. (2015) concluded that among the rodents examined, *R. tanezumi* plays a central role as reservoir of seven zoonotic helminth species, including *Raillietina* spp. and *Hymenolepis* spp. In the present study, *R. tanezumi* had infection with *Hymenolepis* only and none of *Raillietina*, and considering the greater species diversity of helminth parasites in *R. norvegicus*, *R. norvegicus* seems highly likely to play a bigger role in zoonosis, a supposition that agrees with the findings of Namue and Wongsawad (1997) of very high level of susceptibility of *R. norvegicus* to 10 helminths including *Raillietina* sp., *Taenia* sp. (cysticercus), *Nippostrongylus* sp. and *C. hepatica*.

Maizels et al. (2004) and Behnke (1987) opined that parasitic helminths can readily evade host immunity and thus, cause persistent or chronic often non-symptomatic infections. Interestingly, in assessing the dynamics of urban rodent communities alongside rodent infections, Zain et al. (2012) observed a clear accumulation of helminth diversity and species richness/burden with increasing age of urban *R. rattus* and *R. norvegicus*. While this is thought-provoking argument, in the present study however, despite greater species diversity of endoparasites in *R. norvegicus*, in the absence of data on rat ages, further work needs to be carried out to validate if such observation is true to Philippine *Rattus* spp.



**Table 1: Comparison of prevalence of parasitism in *Rattus* spp. examined**

Gender (# rats)	Ectoparasites	Blood Parasites	Endoparasites
<i>R. norvegicus</i>			
♂ (8)	+	-	+
♀ (3)	+	-	+
♂ (4)	+	+	+
<i>R. tanezumi</i>			
♂ (7)	+	-	+
♀ (4)	+	-	+
♂ (3)	+	+	+
♀ (1)	+	+	+
Prevalence (%)	100	26.7	100.0



**Figure 2: Blood smears of *R. norvegicus* (A) and *R. tanezumi* (B) showing *Babesia* infected erythrocytes (arrows)**

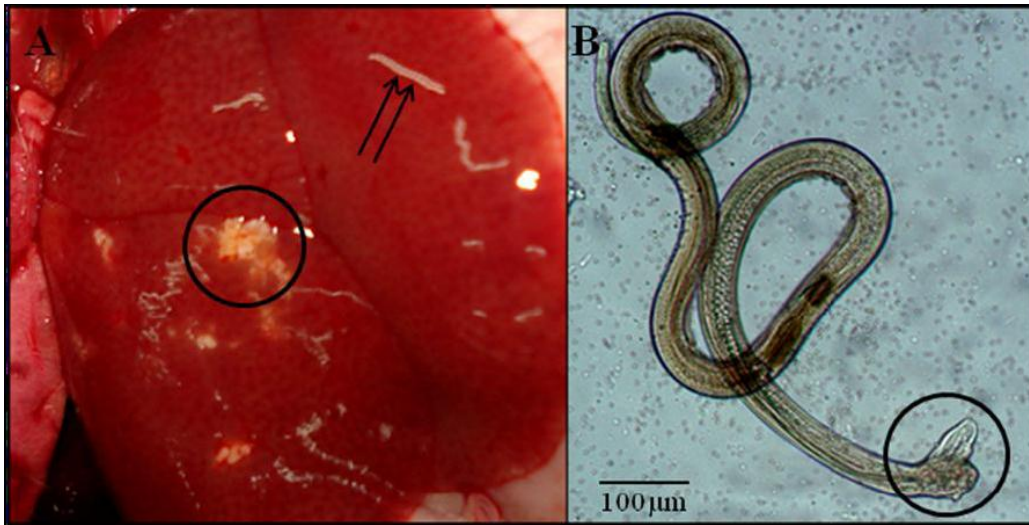
**Table 2: Comparison of infestation between *Rattus* spp. examined with ectoparasitic arthropods**

Species	# Rats (% infestation)		Total (%)
	<i>R. norvegicus</i>	<i>R. tanezumi</i>	
Chirodiscoides	0 (0.0)	12 (80.0)	16 (53.3)
<i>R. ensifera</i>	1 (6.7)	1 (6.7)	2 (6.7)
<i>L. nutalli</i>	15 (100.0)	13 (86.7)	28 (93.3)
<i>O. bacoti</i>	0 (0.0)	1 (6.7)	1 (3.3)
<i>X. cheopis</i>	2 (13.3)	0 (0.0)	2 (6.7)
<i>P. spinulosa</i>	0 (0.0)	12 (80.0)	12 (40.0)

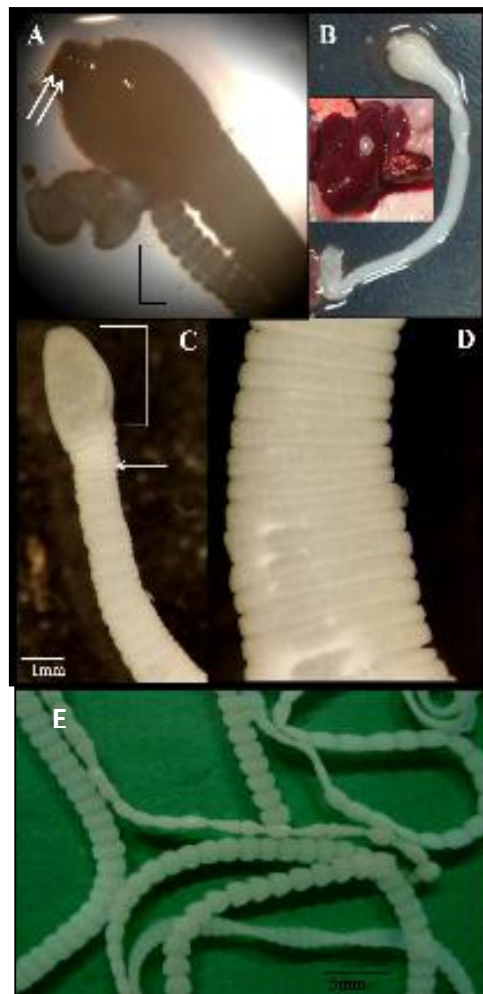
**Table 3: Summary of the number of rats that showed different level of parasite counts: Comparison between *Rattus* spp. examined**

Species	<i>R. norvegicus</i>			<i>R. tanezumi</i>		
	L	M	H	L	M	H
<i>Chirodiscoides</i>	-	-	-	1	-	-
<i>R. ensifera</i>	1	-	-	1	-	-
<i>L. nutalli</i>	8	7	-	10	2	1
<i>O. bacoti</i>	-	-	-	1	-	-
<i>X. cheopis</i>	2	-	-	-	-	-
<i>P. spinulosa</i>	-	-	-	5	6	1

Legend: Parasite density determination: Low: <10; Moderate ≥10 - <50; and Heavy: ≥50



**Figure 3:** A. Whitish spots in rat liver containing eggs (encircled) and adult worms (double arrows) of *C. hepatica*. B. Genus *Nippostrongylus* with bursa (encircled)



**Figure 4:** A and B. *Taenia taeniaformis* showing scolex (double arrows) and excysted larva (B) (inset: liver with encysted larvae [arrow]). C and D. *Hymenolepis* showing head (bracket), neck (arrow) and strobila with proglottids (D). Proglottids of *Raillietina* (E)

**Table 4: Comparison of helminthic infections between *Rattus* spp. examined**

Species	# Rats (% Infection)		Total (%)
	<i>R. norvegicus</i>	<i>R. tanezumi</i>	
<i>Nippostrongylus</i>	11 (73.3)	15 (100.0)	26 (86.7)
<i>C. capillaria</i>	13 (86.7)	10 (66.7)	24 (76.7)
<i>T. taeniaformis</i>	12 (80.0)	0 (0.0)	12 (40.0)
<i>Hymenolepis</i>	7 (46.7)	3 (20.0)	10 (33.3)
<i>Raillietina</i>	3 (20.0)	0 (0.0)	3 (20.0)

**Table 5: Summary of the number of infected rats that manifested different levels of parasite counts/burden (=parasitemia): Comparison between *Rattus* spp. examined**

Species	# Rats Examined					
	<i>R. norvegicus</i>			<i>R. tanezumi</i>		
	L	M	H	L	M	H
<i>Nippostrongylus</i>	-	-	11	-	-	15
<i>C. capillaria</i>	13	-	-	10	-	-
<i>T. taeniaformis</i>	7	5	1	-	-	-
<i>Hymenolepis</i>	-	5	2	-	-	3
<i>Raillietina</i>	-	3	-	-	-	-

Legend: Parasite density determination: Low (L): 1-2; Moderate (M): 3-6; Heavy (H): ≥7

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