Veterinary Integrative Sciences 2024; 22(3): 895 - 905 DOI; 10.12982/VIS.2024.061

Faculty of Veterinary Medicine
Chiang Mai University

Vet Integr Sci
Veterinary Integrative Sciences
ISSN; 2629-9968 (online)
Website; www.vet.cmu.ac.th/cmvj

Research article

Zoonotic helminth infections in rodents and shrews from southern Philippines: Unforeseen One Health hazards

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Abstract

Rodents are known reservoirs of different zoonotic parasites. This study aimed to determine the occurrence of zoonotic helminths in rodents from different habitats in selected municipalities in Cotabato Province, Philippines. A total of 120 rodents and shrews were collected using live traps from two habitats (i.e., agricultural and residential areas) in the municipalities of Kabacan and Matalam. Formalin Ethyl Acetate Concentration Technique (FEACT) and microscpy revealed that 98/120 (81.67%) were infected with zoonotic helminths. Nine parasitic helminths were identified: six species were nematodes (Ascaris spp., *Gonglyonema* spp., *Nippostrongylus* spp., *Strongyloides* spp., *Angiostrongylus* spp. and *Capillaria* spp.); two species were trematodes (*Echinostoma* spp. and *Schistosoma japonicum*); and one cestode species (*Hymenolepis* spp). This is the first report of rodent gongyglonemiasis and schistosomiasis in the southern Philippines. Rats and shrews from agricultural areas had a higher diversity of infectious zoonotic helminths but infection rates were significantly higher in those captured from residential areas. This study confirmed that rats and shrews from the two municipalities are infected with varying zoonotic helminths which pose a significant but overlooked One Health threat to people, other animals, and their shared environment.

Keywords: Land Use, One Health, Public Health, Zoonoses

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Article history; *received manuscript: 9 October 2023,*
revised manuscript: 4 December 2023,
accepted manuscript: 4 January 2023,
published online: 19 January 2024

Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

Rodents are a highly successful and widely distributed group of animals, characterized by a rapid reproduction rate and an ability to adapt to various environments (Parshad, 1999). They thrive in human-populated agricultural, commercial, and urban areas and can cause harm to crop and residential spaces (Claveria et al., 2004). However, rodents are not just merely a nuisance to humans, they also serve as reservoirs for zoonotic parasites (Tenter et al., 2000; Paramasvaran et al., 2005). These parasites can cause harmful diseases, such as toxoplasmosis, leishmaniasis, hymenolepiasis, trichinellosis, echinococcosis, and capillariasis (Davis and Calvet, 2005; Asante et al., 2019). The symptoms induced by these parasites are often self-limiting, potentially causing human infections to go unnoticed (Claveria, 2004; Eduardo et al., 2009). Despite the potential for rodent-borne zoonotic parasites to pose a significant One Health concern, this aspect is frequently overlooked.

The increase in zoonotic illnesses in recent years is attributed to habitat alteration, overcrowding, and mass migration, as humans encroach on remaining natural habitats and come into closer contact with rodents and their diseases (Luniak, 2004). In many countries, rodents are commonly eaten by people, particularly in farming communities as a cultural practice (Suwannarong et al., 2015; Douno et al., 2021). However, this practice increases the risk of parasite transmission to humans (Wongsawad et al., 2014; Tujan et al., 2016). Moreover, infected rodents in agricultural areas pose an occupational hazard to farmers and livestock raisers (Aplin et al., 2003; Eduardo et al., 2008). Similarly, the anthropophilic nature of rodents heightens the risk of disease transmission when they habituate in and infest human settlements (Davis and Calvet, 2005; Rabiee et al., 2018).

Despite the prevalence of rodents and their potential negative impacts on human health, few studies have been conducted on rodent-borne zoonotic parasitoses in Southeast Asia (Blasdell et al., 2011). Moreover, limited research exists on rodent parasitic infections in the Philippines (Luyon and Salibay, 2007). Therefore, this study aimed to provide valuable information on the zoonotic helminths infecting rats and shrews as well as the potential risk they pose to human health in Cotabato Province. In general, this study sought to determine the infection rate of zoonotic helminths in rats and shrews from different habitats in two municipalities in Cotabato Province. Additionally, the research investigated the effect of land use (i.e., agricultural vs. residential) on the occurrence of rodent-borne zoonotic helminths.

MATERIALS AND METHODS

Study Site

The study was conducted in selected communities within the municipalities of Matalam and Kabacan in Cotabato Province (Figure 1). Cotabato Province is situated in central Mindanao, on the eastern edge of Region 12. Covering a significant land area of 656 590 hectares, the province consists of seventeen (17) municipalities and one (1) city, with five hundred forty-three (543) legally established communities also known as *barangays*. Kabacan spans a total land area of 44 809 hectares (7.1072° N, 124.8403° E) and has a population of over 77 000 citizens. Matalam, with a land area of 47 600 hectares (7.2088° N, 124.9286° E), has a population exceeding 81 000. Both municipalities are recognized as agricultural hubs of Cotabato Province.

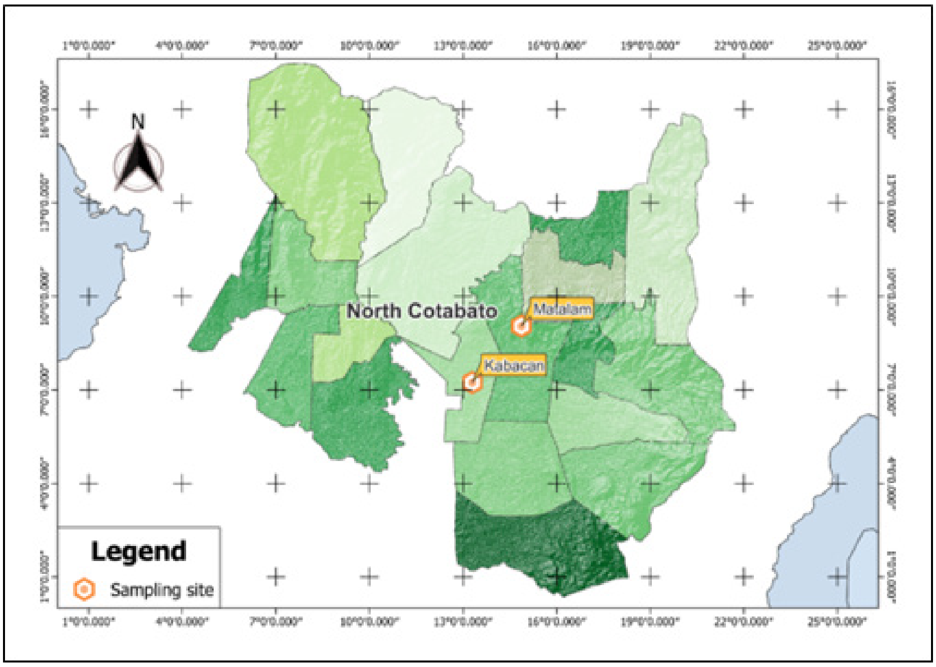

Figure 1 Map of Cotabato Province showing the two municipalities sampled (i.e., Kabacan and Matalam).

Collection and Identification of Rodents

Rodents and shrews were trapped in the communities of Poblacion, Katidtuan, and Osias in Kabacan and in Kilada, Kabulacan, and Dalapitan in Matalam. A total of 120 rodents were collected, with 10 samples each from the agricultural and residential or commercial areas. Rectangular live traps, spaced approximately 5 meters apart, were employed to capture rodents and shrews in agricultural areas. In residential areas, traps were strategically placed where rodents were frequently observed. Baits such as peanut butter and dried fish were utilized to attract and capture rats and shrews. Following the provisions of the Philippine Department of Agriculture Administrative Order No. 40 series of 1999, captured rodents and shrews were humanely sacrificed. Identification of rodents and shrews conducted using the keys provided by Aplin et al. (2003).

Rodents Dissection, Parasite Isolation, and Preservation

Rodents were necropsied to identify the presence of endoparasites. Rodents were dissected starting with a slit on the ventral surface of the body to expose the gut area to the anus. Gut contents were examined in search of adult parasites. Adult parasites were picked out and placed in 5 mL plastic tubes. Each tube was added with 10% formalin to preserve the parasites. Gut contents, including mucosal scrapings and stool, were subjected to Formalin Ethyl Acetate Concentration Technique (FEACT) to concentrate the eggs and larvae of helminths. Processed samples were observed under a light microscope. Each parasite was photo-documented and identified to the genus and species level, if possible, using keys provided by Eduardo et al. (2008), Faltýnková et al. (2015), da Costa Cordeiro et al. (2018), Marchiondo et al. (2019), World Health Organization (2019).

FEACT

An applicator stick was used to homogenize 7mL of formalin-preserved rat gastrointestinal tract contents. The suspension was put into a 10mL test tube after being filtered through three layers of surgical gauze. Three (3) mL of ethyl acetate was added to make a 10 mL solution. The tube's mouth was securely taped and vigorously shaken 20 times. Afterward, the samples were centrifuged for 5 minutes at 1500 rpm. Using the applicator stick in a spiral motion, feces on the uppermost layer were loosened. The supernatant and fecal debris were decanted into a collection beaker in a single motion. The sediments at the bottom of the tubes were combined using a glass pipette and the remaining liquid. The cleaned samples were transferred into an Eppendorf tube, sealed with parafilm, and then viewed under the microscope at 100 and 400 magnification.

Statistical Analysis

The infection rate was determined by dividing the number of infected rodents by the number of total rodent samples multiplied by 100%. In order to compare the occurrence of infection in rats from residential and agricultural areas, analysis of variance (ANOVA) was utilized. When a significant difference is detected, a pair-wise comparison was done using Tukey's Honesty significant difference. Statistical significance was retained at $p < 0.05$.

Ethical Considerations

All procedures involving rodents were in accordance with the provisions of the Philippine Department of Agriculture Administrative Order No. 40 series of 1999. Several rodents used in this research were from the previous research conducted by Tenorio (2022) which had ethical approval from the University of Southern Mindanao Research Ethics Committee (USM REC-202112-128). Environmental trapping was granted permission through a gratuitous permit issued by the Philippine Department of Environmental and Natural Resources Region 12 (RXII – 2022 No. 05).

RESULTS

Rodents Analyzed

A total of 120 small mammals were collected from the agricultural (60) and residential or commercial areas (60) in Kabacan and Matalam (Figure 2A and B). Four species of rodents under the genus *Rattus* were identified: *R. argentiventer, R. exulans, R. norvegicus,* and *R. tanezumi. Suncus murinus* was the only shrew species encountered. Of the five (5) species collected, *R. norvegicus* and *R. argentiventer* were the most frequently caught both in agricultural and residential areas (Figure 2A).

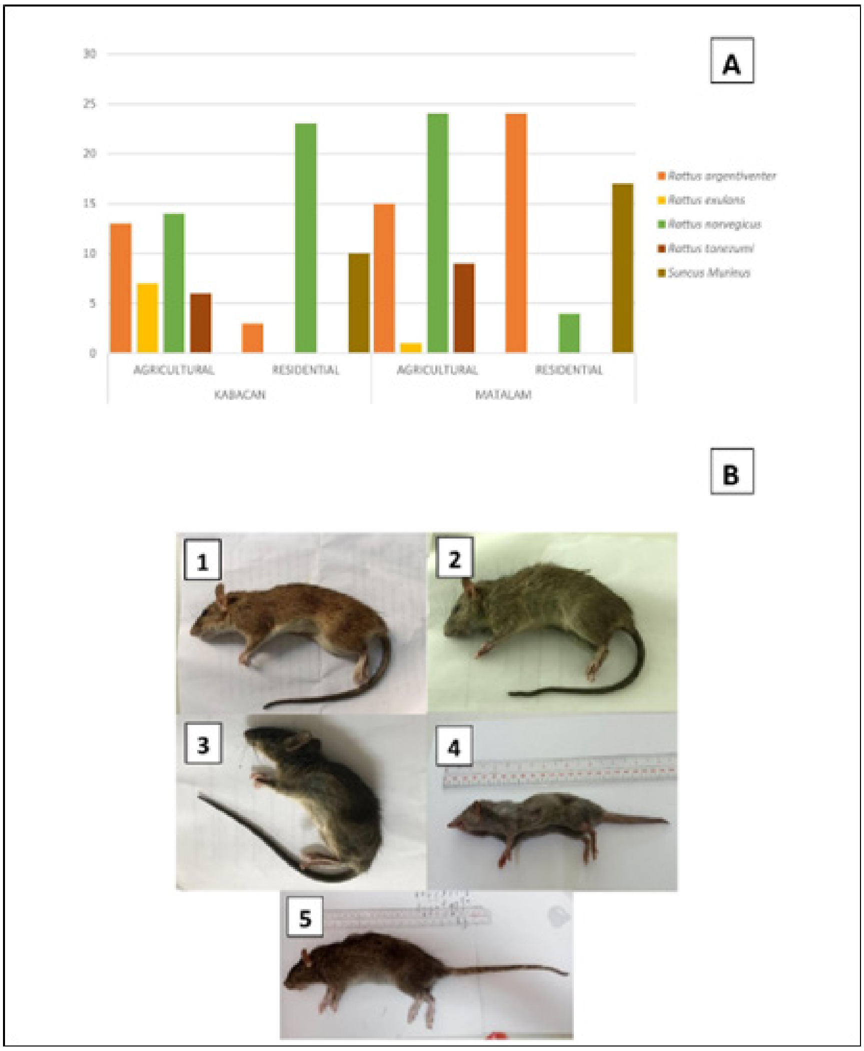

Figure 2 Rodents and Shrews collected in Kabacan and Matalam. A: Rat and shrew species and their distribution. B: Species of Rodents and shrews collected from the study sites: (1) *Rattus argentiventer*, (2) *R. tanezumi*, (3) *R. exulans*, (4) *Suncus murinus*, and (5) *R. norvegicus*.

Zoonotic Helminths

Parasitological examination of rodents revealed that 98 (81.67%) were infected with zoonotic helminths (Figure 3), including nine parasitic helminths with zoonotic potential. Of these, six species were nematodes, two were trematodes, and only one cestode was identified (Figure 4). Table 1 describes the distinct morphological features that were used to identify the parasites. *Hymenolepis* spp., *Echinostoma* spp., and *Gongylonema* spp. were the most frequent endoparasites observed as they were present in agricultural and residential areas of both municipalities (Figure 3). Moreover, *Hymenolepis* spp. had the highest infection rate in residential areas of both municipalities.

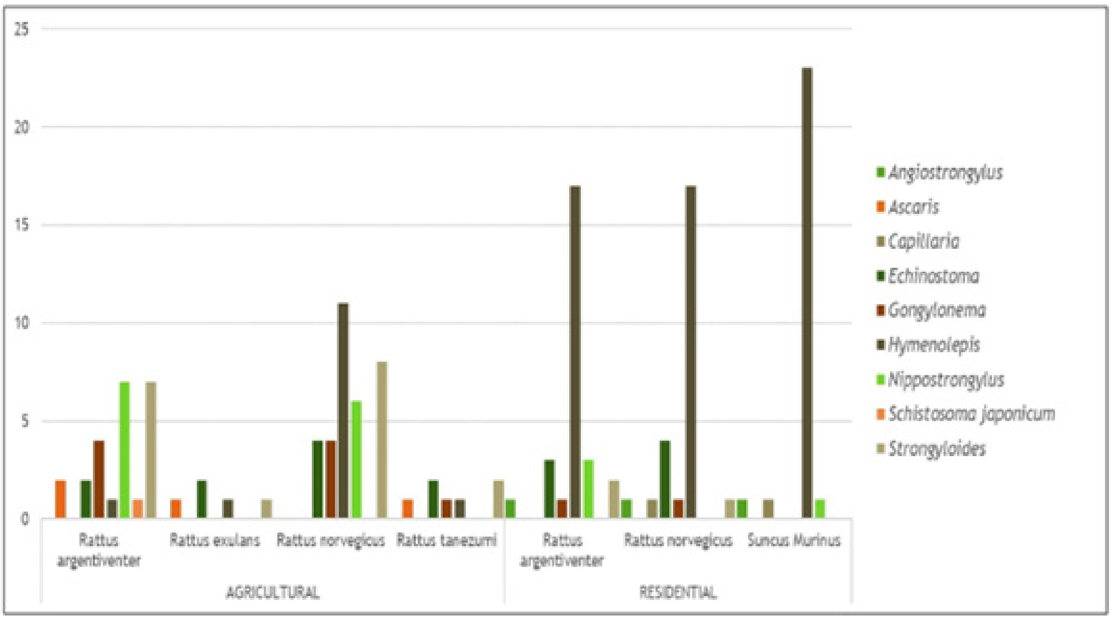

Figure 3 Distribution of zoonotic parasites identified in rodents and shrews from different habitats.

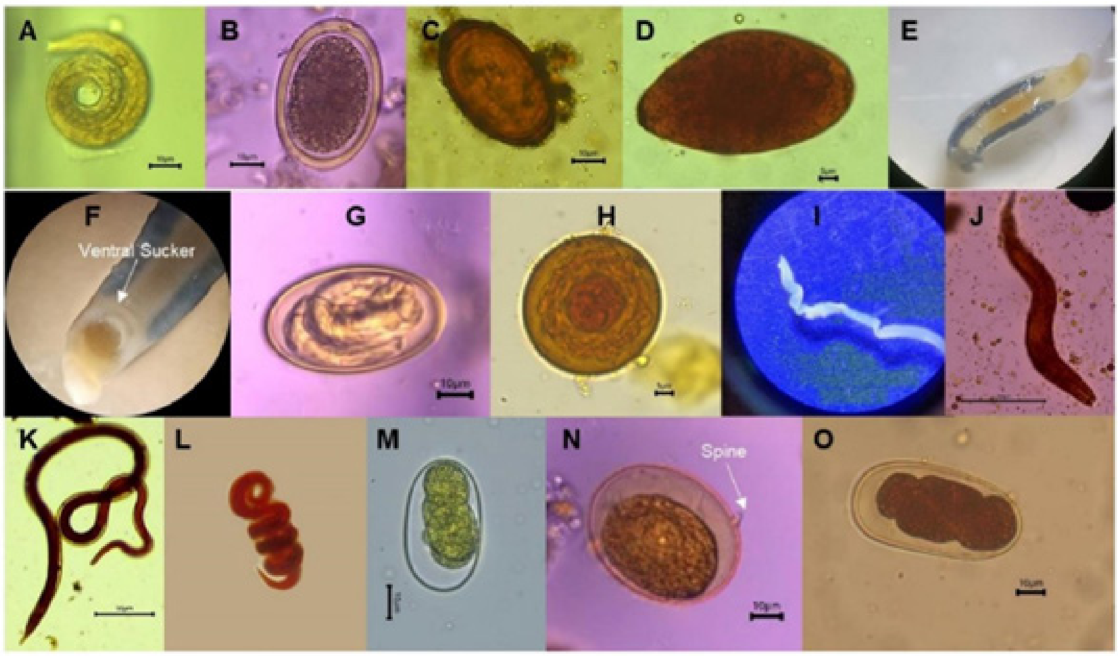

Figure 4 Zoonotic helminth eggs, larvae, and adults were found and identified in this study. A: *Angiostrongylus* spp.; B: *Ascaris* spp.; C: *Capillaria* spp.; D: *Echinostoma* spp. egg, E-F: *Echinostoma* spp.; G: *Gongylonema* spp. egg; H: *Hymenolepis* spp. egg; I: *Hymenolepis* spp.; J and K: Unidentified nematode larvae; L: *Nippostrongylus* spp. egg; M: *Nippostrongylus* spp. larvae; N: Schistosoma japonicum egg; O: *Strongyloides* spp.

Table 1 Morphological characteristics of the eggs, larvae, and adult worms that were used to identify the zoonotic helminths

Parasite Identification	Morphological Feature	Reference Key
Ascaris spp.	A thick-shelled decorticated ascarid egg that does not have the mamillated layer is shown in Figure 4B. The size of the egg is approximately 45 x 30 µm.	World Health Organization (2019)
Gonglyonema spp.	A relatively thick-shelled egg with a visible 1st stage larvae visible inside (Figure 4G). The egg is about 40 x 20 µm	Eduardo et al. (2008) da Costa Cordeiro et al. (2018)
Nippostrongylus spp.	A thin-shelled small ellipsoidal egg with the 1st stage larvae visible inside that measures ~ 50 x 20 µm is presented in Figure 4M. Figure 4N depicts a coiled adult that is red in color with an inflated cuticular expansion.	Marchiondo et al. (2019)
Strongyloides spp.	A large thin-shelled egg with visible 1st stage larvae is depicted in Figure 4O. The size of the egg is about 80 x 50 µm.	Marchiondo et al. (2019)
Angiostrongylus spp.	A small 1st stage larva was found in a coiled position. It is about 8 µm in diameter and nearly 100 µm in length.	Marchiondo et al. (2019)
Capillaria spp.	A thick-walled ellipsoidal egg with a prominent flat polar plug and striated shell (Figure 4C). The size is about 40 x 20 µm.	Eduardo et al. (2008)
Echinostoma spp.	Adult echinostimatid worms were retrieved that were about 8 mm long and had a prominent head collar and uteri that ran along both sides of the length of the body (Figure 4E). A close-up of the head collar and ventral sucker is shown in Fig 4F. Very large trematode eggs with distinct reddish coloration were observed (Figure 4F)	Faltýnková et al. (2015)
Schistosoma japonicum	A thin-shelled ovoid egg with a visible miracidia inside that had the characteristic lateral spine (Figure 4N). Egg size: ~ 60 x 40 µm. *S. japonicum* is the lone schistosome species found in the Philippines.	Eduardo et al. (2008)
Hymonelopis spp.	Figure 4H depicts a spherical, yellowish egg that had a striated internal membrane. A small oncosphere can be seen inside. Egg size: ~ 50 x 40 µm.	Eduardo et al. (2008)

Effect of land use on infection

The analysis revealed that there was a significant difference in terms of infection rate ($p < .001$) of helminth parasites between residential and agricultural areas (Table 2). Rats and shrews from agricultural areas had higher diversity of infectious helminths but infection rates were significantly higher in those captured from residential areas.

Table 2 ANOVA results on infection rate of zoonotic helminths and post-hoc analysis on the difference of infection rate in different sampling site

<table>
<tr><th></th><th>Sum of squares</th><th>dF</th><th>Mean square</th><th>F</th><th>p</th></tr>
<tr><td rowspan="7">Infection Rate</td><td>117607</td><td>3</td><td>39202</td><td>45.7</td><td><.001</td></tr>
<tr><td colspan="5">Comparison of Site</td></tr>
<tr><td colspan="2" rowspan="2">Kabacan: Agricultural Area</td><td colspan="2">Resi-Kabacan</td><td><.001</td></tr>
<tr><td colspan="2">Resi-Matalam</td><td><.001</td></tr>
<tr><td colspan="2" rowspan="2">Matalam: Agricultural</td><td colspan="2">Resi-Kabacan</td><td><.001</td></tr>
<tr><td colspan="2">Resi-Matalam</td><td><.001</td></tr>
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DISCUSSION

To the knowledge of the researchers, this is the first comprehensive report of zoonotic helminths infecting rats and shrews in Cotabato Province. The discovery of at least nine zoonotic helminths spanning various taxa highlights the often-overlooked One Health threat posed by rodent infestations in both residential and agricultural lands.

Rodents in irrigated rice crops and villages are common carriers of zoonotic endoparasites, posing a significant threat to public health (Rabiee et al., 2018; Herawati et al., 2020). These findings align with the study conducted by Islam et al. (2020), documenting the presence of cestodes, nematodes, and trematodes in collected rodents. Moreover, Okorafor et al. (2012) found that the gastrointestinal tract of rodents, particularly the small intestines, harbors the highest number of parasites, mainly nematodes. Helminth infections in rodents negatively impact their health, consequently affecting the ecosystem of the rodent-environment system. Additionally, rodent helminths play a crucial role in undermining the well-being of people, animals, and livestock (Dhaliwal and Juyal, 2013).

Hymenolepis spp. emerged as the most prevalent zoonotic helminth in this research. According to Yang et al. (2017), this parasite is widely distributed globally, with recorded cases in Asia, Southern and Eastern Europe, Central and South America, and Africa. In 2016, Franssen et al. recorded the presence of *Hymenolepis* spp. in *R. norvegicus* in the Netherlands. Meanwhile, Tung et al. (2013) detected infections in commensal rats and shrews in Taiwan. This zoonotic cestode is known to cause hymenolepiasis in humans in various countries (Islam et al., 2020). Despite its global distribution, there have been very few reports of human cases in the Philippines. The most recent of which was identified during the latest national survey on Soil-transmitted helminth (STH) infections in school-aged children; only one out of the 26 107 children tested was positive for *Hymenolepis nana* (Tangcalagan et al., 2022).

Zoonotic helminths found in this study that are also worth highlighting are *Gongylonema* spp., *Schistosoma japonicum*, and ascarids. *Gongylonema* spp. was one of the prevalent helminths observed in this study as it was present in rodents from both agricultural and residential sites within both municipalities. In the previously mentioned study by Tung et al. (2013), *Gongylonema* spp. was also detected in rodents. A study conducted in Bangkok, Thailand found a similar result: *Gongylonema* spp. in rodents caught in urban and residential areas were reported (Paladsing et al., 2020). Currently, there are limited studies about the parasite in the Philippines. Setsuda et al. (2018) molecularly identified *Gongylonema neoplasticum* in two rat isolates from the Philippines; however, the specific geographical origin of the isolates was not mentioned. To the researchers' knowledge, the current research provides the first evidence of gongylonemiasis in rats and shrews from southern Philippines.

Likewise, the presence of schistosome eggs in the samples analyzed in this study indicates the first report of rodent schistosomiasis in Cotabato Province. The province is known to be endemic for schistosomiasis (Tenorio and Molina, 2021). Rats and shrews, along with more than 40 other mammalian species, are known to harbor the trematode and serve as reservoir hosts for human infections (Tenorio et al., 2021). Rodents may play a similar role, along with water buffaloes, in perpetuating schistosomiasis in the province.

Lastly, the presence of ascarid eggs in the rodent samples implicates their potential role as reservoirs of human infections in the province. In the aforementioned national STH survey, Cotabato Province logged a 12.3% *Ascaris lumbricoides* prevalence in school-aged children (Tangcalagan et al., 2022). The role of rodents as potential sources of STH eggs that may contaminate the environment should be further investigated.

The study presented valuable insights regarding rodent-borne zoonotic helminths, highligthing their potential as a One Health threat in southern Philippines. However, the research does have some limitations. The rodent sample size was not calculated due to the absence of studies on infection, the lack of accurate estimates for the rodent population in the area, and the challenges associated with trapping rats and shrews. Nevertheless, the study successfully identified numerous zoonotic helminths in the assessed rodents, and it is believed that these findings accurately reflect the situation in the different sampling sites. The identification of the parasites was conducted morphologically due to a lack of funding to pursue molecular identification at the species level. This limitation presents a research gap for future studies.

CONCLUSIONS

This study unequivocally confirmed that rats and shrews from the two municipalities harbored various zoonotic helminths, thus constituting a substantial yet commonly overlooked One Health threat. The notably high infection rate and variety of zoonotic parasites detected in these anthropophilic animals strongly imply their potential role as sources of zoonotic infections for human inhabitants in the region. Furthermore, the findings point towards a heigthened risk of infection among rodents and shrews in residential areas compared to their counterparts in agricultural settings, underscoring the imperative for targeted interventions aimed at controling and mitigating the risk of rodent-borne parasitoses in residential locales.

AUTHOR CONTRIBUTIONS

Sample collection and data analysis were done by MJHM. Writing of the original manuscript was done by MJHM and JCBT. LFC critically reviewed and revised the manuscript. MJHM is under the supervision of LFC and JCBT. All authors have read and approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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How to cite this article;

Mary Joyce H. Matullano, Jan Clyden B. Tenorio and Lothy F. Casim. Zoonotic helminths infections in rodents and shrews from southern Philippines: Unforeseen one health hazards. Veterinary Integrative Sciences. 2024; 22(3): 895 - 905