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Short Communication

Prevalence of Mouse and Rat Parasites in Resource Recovery Plants, Farms and Housing Areas of Southern Selangor: Implication for Public Health

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ABSTRACT

Parasites of small mammals living in human areas cause a tremendous burden of vector borne disease. Small mammals infected with parasites can readily facilitate parasitic transmission to humans and other susceptible animal hosts. The objective of this study was to determine the presence of parasites in small mammals (rats and mice) from the Semenyih Recovery Plant (RESBS) and to compare this with parasites from a plantation farm, Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM), and a housing area, Sri Serdang housing area (SSHA), Selangor, Malaysia. The methods employed in this study was to trap small mammals in the resource recovery plant, the plantation farm and the housing area. All the captured small mammals were examined microscopically for the presence of endo and ectoparasites. The brine gravity floatation method was used to determine the presence of parasitic eggs in the feces of the small mammals. The staining method of Semichon's acetic carmine was employed to find the parasites infesting the internal organs of the mammals. Twenty-three small mammals were trapped alive in the study. *Rattus sabanus* (10) and *Suncus murinus* (2) were captured in the Semenyih resource recovery plant; *Rattus argentiventer* (7) in the farm area of Ladang Pertanian Bersepadu UPM and *Mus musculus* (1) and *Suncus murinus* (3) species in the Sri Serdang housing area. *R. sabanus, R. argentiventer* and *M. musculus* were found to be heavily infected with *Echinolaelaps echidinus* (mites). *Polyplax spinulosa* (lice) infected the *R. sabanus* species. The intestinal parasite, cestode *Hymenolepididae* tapeworm, was found to have invaded the small mammals

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from Semenyih, the resource recovery plant and the farm area of Ladang Pertanian Bersepadu UPM. The nematodes were also found in the small mammals from RESBS. The small mammals captured from RESBS were found to be heavily infected with both external and internal parasites including *E. echidinus*, *P. spinulosa*, cestode tapeworms and nematodes compared to the farm and housing areas. Therefore, the existence of small mammals carrying parasites found mainly in waste disposal areas needs to be addressed to prevent serious disease that can cause harm to human health.

Keywords: Parasites, *E. echidinus* (mites), *P. spinulosa* (lice), cestode tapeworms, small mammals, rats, mice

INTRODUCTION

Municipal waste management in Malaysia is managed by the Ministry of Housing and Local Government and with the involvement of the private sector. The quantity of waste is expected to increase to 31,000 tonnes by the year 2020. The Malaysian National Solid Waste Management Policy has aimed to assure the public that public health will not become a concern as a result; however, problems to health are often caused by small mammals carrying parasites that are found mainly in waste disposal areas (Manaf *et al.*, 2009).

The parasites invade small mammals as their hosts and serve as reservoirs for diseases that infect humans. The modes of transmission to host can occur through various parasitic life cycles such as spores, eggs, cysts and juveniles (Roberts & Janovy, 2006). The unhygienic conditions of *Rattus* spp living in an impoverished areas can allow for the transmission of parasites to other susceptible hosts including humans (Claveria *et al.*, 2005). Studies have shown that the ectoparasites (lice and mites) of rats that live on external surface or skin or attached to hair follicles (Roberts & Janovy, 2006) can be transmitted to humans and cause diseases (Hoopman & Baron, 2007; Beck & Folster-Holst, 2009).

Infestations with lice often lead to sub-clinical diseases. The infections may cause anemia, weight loss, dorsum alopecia and even pruritus and cutaneous lesions in severe cases (Hoppmann & Barron, 2007; Beck & Folster-Holst, 2009). The lice species found globally in domestic rats act as vectors to transmit murine hemobartonellosis bacterial disease (Durden, 2002). Mites that live in host bedding usually feed on the host's lachrymal secretions, blood or serous exudates from abraded skin during the night and cause discernible lesions. Moreover, the mite is the vector of *Hepatozoon muris* (blood-protozoan parasites) that infest rats. Small mammals are commonly infected by internal parasites such as cestode tapeworms. The endoparasites that live in the subcutaneous tissue, lymphatics, muscles, trachea, lungs, heart, esophagus, liver, spleen, kidneys, bladder, brain and other internal structures are transmissible to humans (Roberts & Janovy, 2006).

In this study, our intention was to investigate the presence of parasites in mice and rats from Semenyih Recovery Plant (RESBS) and to compare them with

the parasites of mice and rats from a farm, Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM) and Sri Serdang housing area (SSHA), Selangor, Malaysia. The researchers also postulated that there is a difference in parasite species in all three entrapment areas and their impact on human populations.

METHODOLOGY

Small mammal sampling was conducted at three locations i.e. (1) the resource recovery plant, Recycle Energy Sdn Bhd Semenyih (RESBS), (2) the plantation farm, Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM) and (3) the Sri Serdang housing area (SSHA), Selangor, Malaysia. The sampling was done from May 2007 to May 2008. The RESBS is a resource recovery centre or waste-to-energy plant that processes 1,000 metric tonnes of municipal solid waste per day from Kajang municipality and neighbouring areas. The second site, a vegetation farm, is located in the Ladang Pertanian Bersepadu Universiti Putra Malaysia and the third site, a residential area, is located in Sri Serdang. The ethical approvals were obtained from the Department of Biology, Faculty of Science, Universiti Putra Malaysia and RESBS. Rodents were captured in wire cages that were baited with coconut flesh or cheese and placed in the entrapment areas. Trapped rodents were transported to the Parasitology Laboratory of the Faculty of Science, Universiti Putra Malaysia.

Examination for ectoparasites

Individual hosts were killed with an overdose of chloroform and examined for parasites. Screening for ectoparasites involved a thorough examination of the body of the small mammals including the eyes, nose, mouth, ears, body, anus and vulva or penis. The skin of each rodent was examined grossly for ectoparasites. Those rodents with parasites were identified and recorded. Samples of the observed parasites were removed with a camel hair brush and transferred to a vial containing 70% alcohol. They were fixed on a microscopic slide using a drop of formalin and mounted with DPX or Hoyers medium. The specimens were examined under a bright-field microscope. All the *images were captured* at *100x magnification.*

Examination for endoparasites

Every rodent was dissected, and different internal organs were macroscopically examined for the presence of any parasites. All parts of organ or tissue specimens were placed into separate petri dishes containing normal saline. The hollow organs and duct openings were rinsed with physiological saline and the saline solution was poured through a sieve to retain any parasitic worms. The contents of the esophagus, gall bladder, liver passages, lungs, pancreatic ducts, urinary bladder, brain and other organs were carefully examined under a dissecting microscope. Any clumped parasitic worms were gently teased apart from the lining of these organs with a scalpel and placed in a larger vessel.

The worms were gently rinsed with water to remove any remaining fixative prior to staining. Staining time varied depending on the size of the specimens from 1 to 8 hours. A few drops of 0.5 to 1% hydrochloric acid were added to decolourise the internal structures of the parasites. Next, the specimens were passed through a series of alcohol 70%, 85%, 95% and 100% allowing at least for 30 minutes in each solution. The endoparasites were stained with Semichon's acetic carmine and mounted with DPX or Hoyers medium. The specimens were examined under a bright-field microscope at *100x magnification* and the *images were captured*.

Examination of Feces for Worms, Eggs and Larvae

The feces was mixed with 1 to 2ml of brine solution in a test tube and stirred until the solution took on a pasty consistency. Then the mixture was allowed to stand undisturbed for 30 to 60 minutes. A wire loop or brim of a lipless test tube was used to transfer some of the surface film to a clean slide. All the slides were examined under a low power microscope for the presence of worms, eggs or larvae in the feces.

RESULTS

Twenty-three rodents belonging to four species were live-trapped during the study period. Species of hosts trapped were as follows: *Rattus argentiventer, Mus musculus*, *Suncus murinus* and *Rattus sabanus.* The highest number of rodents was from RESBS (N=12; 52.2%), followed by LPB UPM (N=7; 30.4%) and SSHA (N=4; 17.4%). The species of *R. sabanus* was captured from RESBS, *R. argentiventer* from LPB UPM and *M. musculus* from SSHA. The *S. murinus* species was captured in both RESBS and SSHA.

In the present study, captured rodents were identified to be infested with two ectoparasites, *Echinolaelaps echidinus* (mite) and *Polyplax spinulosa* (louse). Among all *R. sabanus* species, 20% were found to be infested with *E. echidinus* and 40% were infested with *P. spinulosa.* The ectoparasites found in each species are summarised in Table 1 and 3.

About 33.3% of captured rodents from RESBS and 28.6% from LPB UPM were infested with cestode tapeworms and no endoparasites were found in rodents from SSHA. Nematode endoparasites were also found in rodents from RESBS (8.3%). The endoparasites found in each species are presented in Table 2 and 3.

The rodents from the LPB UPM were infected with *E. echidinus* and cestode tapeworms whereas the rodents from SSHA were infected with *E. echidinus* parasites only**.** The images of these parasites are shown in Fig.1 and Fig.2.

DISCUSSION

A total of 23 rodents comprising four species were trapped from the three habitats. The captured species of rodent varied from different localities. The different medium and high density residential areas, social

TABLE 1
Number of Host Species Caught in Each Entrapment Area and the Number of Host Positive for Ectoparasites

Location	(n)	Host	Ectoparasites	No. of host (+) Ectoparasite
RESBS	2	House shrews *S. murinus*	Nil	Nil
	10	Long-tailed giant rat *R. sabanus*	Louse *P. spinulosa*	4
			Mite *E. echidinus*	2
SSHA	1	Mouse *M. musculus*	Mite *E. echidinus*	1
	3	House shrews *S. murinus*	Not found	Nil
LPB UPM	7	Rice field rat *R. argentiventer*	Mite *E. echidinus*	5

Recycle Energy Sdn Bhd Semenyih (RESBS); Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM) and Sri Serdang housing area (SSHA), Selangor, Malaysia

TABLE 2
Number of Host Species Caught in Each Entrapment Area and the Number of Host Positive for Endoparasites

Location	(n)	Host	No. of host (+) Endoparasites (Cestode)	No. of host (+) Endoparasites (Nematode)
RESBS	2	House shrews *S. murinus*	2	Nil
	10	Long-tailed giant rat *R. sabanus*	2	1
SSHA	1	Mouse *M. musculus*	Nil	Nil
	3	House shrews *S. murinus*	Nil	Nil
LPB UPM	7	Rice field rat *R. argentiventer*	2	Nil

Recycle Energy Sdn Bhd Semenyih (RESBS); Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM) and Sri Serdang housing area (SSHA), Selangor, Malaysia

TABLE 3
Comparison Between Ecto and Endoparasites According to the Species of Host and Locations

Host species	Location	*P. spinulosa*	*E. echidinus*	*Cestode*	*Nematode*
R. sabanus	RESBS	+	+	+	+
R. argentiventer	LPB UPM	-	+	+	-
M. musculus	SSHA	-	+	-	-
S. murinus	1.RESBS	-	-	+	-
	2. SSHA	-	-	-	-

Recycle Energy Sdn Bhd Semenyih (RESBS); Ladang Pertanian Bersepadu, Universiti Putra Malaysia (LPB UPM) and Sri Serdang housing area (SSHA), Selangor, Malaysia

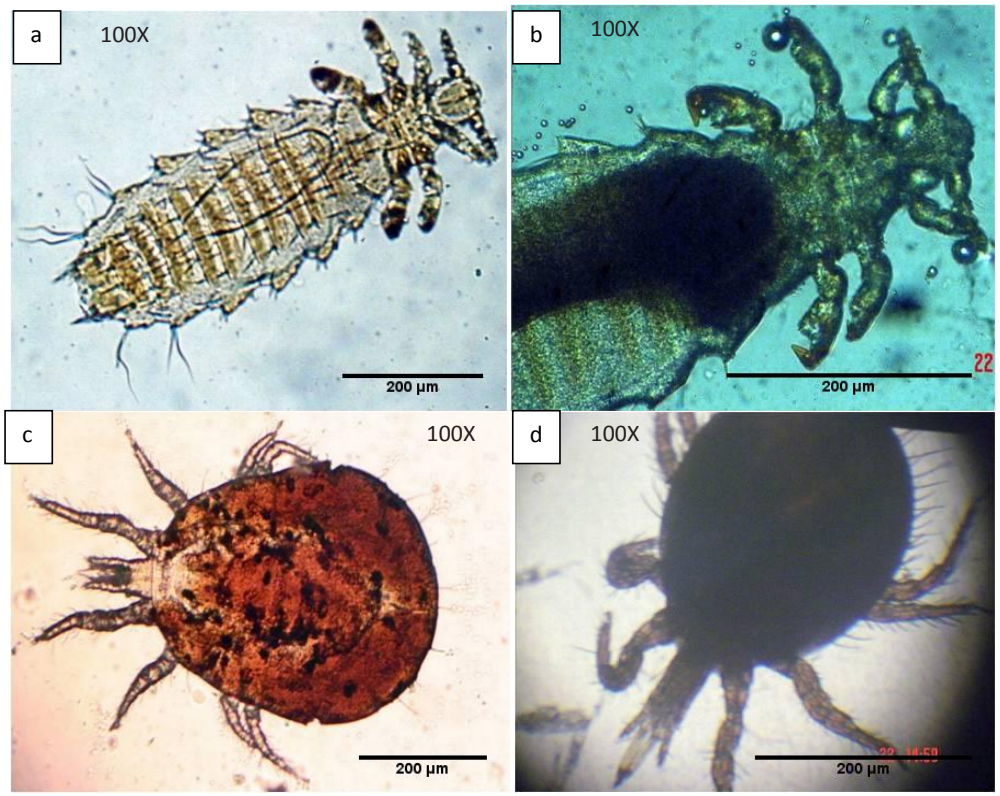

Fig.1: Ectoparasite Anoplura, parasitic (a) *P. spinulosa* morphology mounted with DPX (b) and Hoyers medium. (c) *E. echidinus* (mite) mounted with DPX and (d) Hoyers medium.

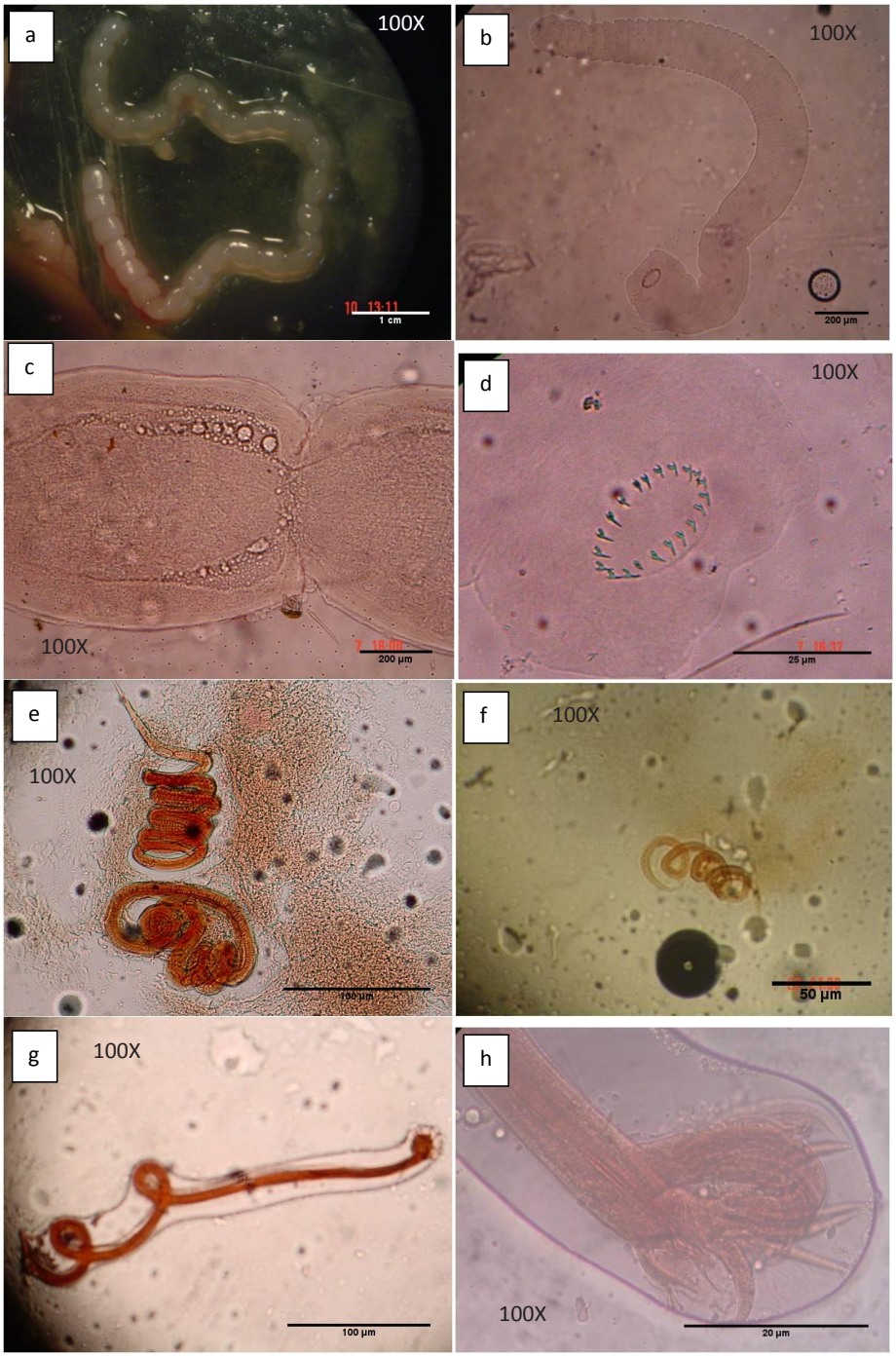

Fig.2: Endoparasites cestode tapeworm from intestine of rodents showing vesicle and proglottids (a, b, c) and scolex with 21 hooks (d), Endoparasites nematode (complete structure) (e, f, g) and bursa of nematode (h).

status and food availability might be the key factors that determined the types of rodents living in different habitats.

Rattus sabanus and *S. murinus* were the two species live rodents trapped from RESBS. *R. sabanus* species are found in Malayan mainland forests (Hoi-Sen, 1970) and it is known as the long-tailed giant rat (Lee, 1965). In the present study *R. sabanus* species were captured only in the RESBS and not in LPB UPM and SSHA. RESBS was only visited by *R. sabanus* that probably came from the bushes surrounding the resource recovery plant because the species mainly live in forested areas or near the bushes. *S. murinus* may have also originated from the same area.

The RESBS is regularly cleaned and the waste disposal does not support the breeding place for rats as the waste is constantly shifted in order to be separated for recovery processes. Even though the *R. sabanus* were found to be heavily infected by ecto and endoparasite that are transmissable to humans, this particular species does not come into human residential areas as it prefers to live in forested areas. Fortunately, there was no captured house rat, *Rattus r. diardi,* in RESBS and this indicated that the house rat from the housing area had not visited the recovery plant.

The long-tailed giant rats (*R. sabanus)* from RESBS were found to be infested with *E. echidinus* (mites), *P. spinulosa* (lice), cestode tapeworms and nematodes. Similarly, Claveria *et al.,* (2005) also reported that rats (*Rattus* spp.) that were trapped from wet markets in Quiapo, Manila and Balayan, Batangas were found to be infested with the same ectoparasites. Infection of the cestodes tapeworms and nematodes in the *R. sabanus* species may be presumably transmitted by consumption of insects from the resource recovery plant.

The rice field rats (*R. argentiventer*) were found in LPB UPM. These rodents prefer to live in a vegetation field and are mainly found in the dikes with a cover of weeds, which provides a good shelter for them to survive (Jakel *et al.*, 2006). In Malaysia they are abundantly present in 55 to 60 organisms per hectare before the rice sowing season (Wood, 1971). Rats from the farm are more vulnerable to ectoparasites infestation. In the present study, 71.4% of rice field rats were infected with *E. echidinus.* The two most commonly reported epidermal parasites of rats were *E. echidinus* and *P. spinulosa* (Soliman *et al.*, 2001). The cestode tapeworms in liver cysts were also found in these rice field rats.

Mus musculus and *S. murinus* were the two species captured in SSHA. The *M. musculus*, known as house mice, was found to be abundantly distributed in eastern Europe and Asia (Pocock *et al*., 2005). The *S. murinus* or house shrews were believed to be transported from India by humans as the species lived in close contact with humans (Meegaskumbura *et al.*, 2010). These house shrews were also captured in RESBS. The *M. musculus* and *S. murinus species* were less infected with the parasites compared to other species.

The *E. echidinus* (mites) classified under the family of Laelaptidae was found in

all three entrapment areas. The adult female mites give birth to live larvae and the larvae usually do not feed until they develop to the protonymphs and deutonymphs stage before becoming adults in three weeks. It has been reported that the ectoparasites, *Laelaps echidninus* (spiny rat mites) belonging to the family Laelaptidae is occasionally found in the *Rattus* species such as Black, Norway rats, house mice and other domestic and wild rodents (Mullen & O'Connor, 2002).

Polyplax spinulosa (spined rat louse) belongs to the family Polyplacidae (Lance & Musser, 1994). *P. spinulosa* is a blood-sucking louse that is easily found by the skin scraping method (Tamura, 2010). Host specific *P. spinulosa* lice are the most common parasites found in rats. The existence of these blood-sucking lice parasites are more in young, neglected and under-nourished rats (Hoppmann & Barron, 2007). The *R. sabanus* from RESBS was the only species infected with *P. spinulosa* compared to the rodents in the two other localities.

The cestode tapeworms found in the study are classified as phylum Platyhelminthes, class Cestoda, known as flatworms (Columbia Encyclopedia, 2007). These cestode tapeworms live in the bodies of vertebrates during their juvenile stage of development and in the digestive tract of the host in the adult stage. The two species of tapeworms found in the study were probably the dwarf cestode tapeworms, *Hymenolepis nana* or rat cestode tapeworms, *Hymenolepis diminuta* (DPDx, 2008). Cestode tapeworms live in the small intestine by anchoring their scolex into the intestinal wall of the host (Williams *et al*., 2011).

A cestode tapeworm requires at least two hosts in order to survive and complete its life cycle. The primary host often carries the larvae that live in the tissue of the host; they are then transmitted to the secondary host, developing to adult stage in the intestine of this final host. Recent studies have shown that *H. nana* and *H. diminuta* are commonly found in rats and mice, respectively (Chai, 2013). Many cestode tapeworms are host-specific and some may only require one host to complete their whole life cycle (Heyneman & Baron, 1996).

The species of roundworm nematodes that was found in the study is probably the *Angiostrongylus sp*. The natural hosts for these roundworms are rodents such as *Rattus norvegicus* and *Rattus rattus*. The intermediate hosts for these roundworms are molluscs. Humans become infected by ingestion of intermediate or paratenic hosts; in humans, infestation can cause eosinophilic meningitis in the brain (Thiengo *et al*., 2013).

Unfed parasites are able to live long without a blood meal. Therefore, careful examination of parasites should be performed not only among the rodents or infected patients but also thorough examination of surrounding areas; this is rarely performed (Beck & Folster-Holst, 2009).

The host animals live in the same general niche and consume similar types of food such as plant materials and insects. They may directly transmit their parasites

to another host. This transmission occurs by burrowing or nesting of parasites in the host and normally happens in conditions of low-host densities in the complex habitat. Similar transmission occurs in the many species of parasites that infest a variety of animals in Malaysia. The process occurs in two stages, firstly through the food and secondly, through the physiological level. All the host species, especially rats, in the previous studies are well known to feed on insects (Hurd, 2003). There are some indications that animal protein is necessary for the survival of some species of rat in Malaysia (Wood & Fee, 2003).

The diversity in rat parasites points to their adaptability as well as the enormous capability of the host to support parasites' behavioral, physiological or nutritive and developmental needs. Despite heavy infection with mites, lice and cestode tapeworms, the rodents still appeared healthy and active, reflective of a well-established and presumably successful rat host-parasite interrelationship. The limitations of this study include the disturbance in laying out the grid and food disposal surrounding the entrapment areas; this may have affected the entrapment of the rodents.

CONCLUSION

In summary, the rodents captured from RESBS were heavily infected with parasites including *E. echidinus*, *P. spinulosa* and cestode tapeworms compared to rodents from the farm and housing areas. Therefore, rodents carrying parasites found mainly in RESBS need to be addressed to prevent parasitic infections in humans.

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