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DETECTION OF PINWORMS IN CONVENTIONALLY MAINTAINED LABORATORY MICE

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ABSTRACT

The laboratory mice (*Mus musculus*) are commonly utilised for research purposes. Despite strict biosecurity, they potentially harbour parasites which may compromise the experimental study. Parasite intensity differs among strains of mice. This study aims to identify the presence of parasites between two strains of laboratory mice. A total of 48 mice (n = 48) obtained from the UPM Animal Resource Unit (ARU), consisting of 24 animals for each group of inbred strain Bagg Albino (BALB/c) and outbred Institute Cancer Research (ICR) mice were used for detection of helminths, ectoparasites and blood parasites. Based on parasitological distinct characteristics, *Syphacia obvelata (S. obvelata)* and *Aspiculuris tetraptera (A. tetraptera)* were detected. Both helminths were seen in 8.33% of BALB/c and 20.83% of ICR mice, respectively. Single infection by *S. obvelata* was detected in 33.33% of BALB/c mice while 12.5% of ICR mice were manifested merely by *A. tetraptera.* The findings revealed an optimal method to identify *S. obvelata* through perianal tape test while *A. tetraptera* is best detected by the faecal flotation technique. Statistically, the type of helminth was significantly associated with the strains of mice (P=0.043). Overall, there were low amounts of opportunistic helminths and ova with the absence of ectoparasites and blood parasites for both strains of laboratory mice which is suggestive of appropriate management practised.

Keywords: laboratory mice, parasitological methods, pinworms, strains

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INTRODUCTION

The laboratory mice (*Mus musculus*) are used widely in research and testing due to the economic choice for the studies. Besides, they are well known to have identical characteristics to those of human biological functions. They may harbour low amounts of parasite species in the skin and gastrointestinal tract but do not compromise the health status of animals overtly. However, some parasites may influence certain experimental results, especially during a high worm burden. Parasites could be detected in animals through various diagnostic techniques. The knowledge of early diagnosis of parasitic infestation would be of great importance in the process of preventing the transmission of diseases. Previous studies have revealed that the prevalence of helminths infection in laboratory animals were variously based on the host's age, strain, health status (Taffs, 1976), stocking density (Ain-Fatin *et al*., 2020 a) and environmental conditions (Ain-Fatin *et al.*, 2021). Different strains of laboratory mice produce different responses (Muhammad-Azam *et al.*, 2019; Tuttle *et al.*, 2018).

There are some identifiable helminths and ectoparasites documented in the laboratory mice (Medeiros, 2012). The most common parasites in laboratory mice are nematodes that are known as pinworms. They are non-pathogenic parasites and clinical signs are rare unless heavy loads of infection were observed (Baker, 1998). These parasites are opportunistic pathogens and their presence are expected in laboratory mice at low levels. Parasitic infection is transmitted through the faecal-oral route where the ingestion of embryonated egg shed in faeces can occur (Perec-Matsyiak *et al.*, 2006). A high burden of worms may result in enteritis, dehydration and pruritis at the perianal region and possibly lead to impaction, colonic intussusception, or rectal prolapse (Medeiros, 2012). Similar to other species, ectoparasites identified in laboratory mice are comprised of ticks, fleas, mites, and lice. The two most commonly found ectoparasites are the fur mites; *Myocoptes musculinis* and *Myobia musculi*. Low infestation of these parasites is usually subclinical but heavy infection causes irritation and pruritus where hair loss and scabs could also be seen on the host.

Various parasitological methods have been described in identifying parasites. The perianal tape test method is commonly used to detect *Syphacia* spp. using a cellophane tape at the perianal region of the animal (Baker 2007; Eguiluz *et al.*, 2001; Taffs, 1976) whereas, the faecal concentration methods are commonly used to detect *A. tetraptera* infection (Baker, 2007; Phillipson, 1974; Taffs, 1976). For ectoparasites, various methods could be used to detect the infestation such as skin

scraping, tape impression, fur pluck, and observation. Fur pluck and tape impression are efficient methods that could be done quickly although minimal stress induced to the animal but still result in high accuracy most of the time (Burdett *et al.*, 1997).

The prevalence and intensity of parasites are influenced by certain strains of mice (Chan and Kopilof, 1958; Chen *et al.*, 2011). Due to limited documentation based on strains difference of laboratory mice, this study aims to identify parasites using various parasitological methods between inbred and outbred animals housed in the conventionally maintained animal facility. It is crucial to provide a baseline animal health status before the animal is used as an experimental model to ensure validation of the research outcome. Besides, the contamination level of the animal facility can be assessed through the parasitic load. The identification of common parasites enables an accurate diagnosis of a particular parasitic infection.

MATERIALS AND METHODS

Ethical approval and experimental animals: All study was undertaken following criteria approved by the Universiti Putra Malaysia (UPM) Institutional Animal Care and Use Committee (IACUC) with the approval codes of UPM/ACUC/AUP-U008/2018 and UPM/ACUC/AUP-U048/2018. The study was conducted in the year of 2018 to 2019. A total of forty-eight (48) adult animals with 24 for each; inbred BALB/c and outbred ICR mice were randomly selected from the conventionally maintained animal facility, UPM Animal Resource Unit (ARU). Management of the experimental animals was consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The study was conducted in Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine (FPV), UPM. They were anaesthetised with ketamine 100 mg/kg and xylazine 10 mg/kg intraperitoneally before blood collection by intracardiac puncture followed by euthanasia for whole carcass immersion and gastrointestinal contents examination.

Endoparasites examination: All parasitological techniques were performed as shown by Parkinson *et al.*, 2011. Detection of helminths was conducted by perianal tape test, direct faecal smear, faecal flotation, and direct examination of gastrointestinal contents. Perianal tape test was done by applying tape at the perianal region of mice for few times to ensure the possible parasites stick to the tape before placing it on a glass slide. Faecal smear was done by directly smearing the faeces onto a glass slide. The faecal flotation technique was carried out by mixing about 1 g of fresh faeces samples with 40 mL of sodium nitrate solution with a specific gravity of 1.3. The mixture was filtered before pouring it in a vial until the meniscus is formed on top of the vial to examine helminth's ova and larval stages, and coccidian oocysts. Gastrointestinal contents were exposed before the thorough examination was carried out under a dissecting microscope for helminths detection. Helminths morphology on the glass slides was examined and identified microscopically using a compound microscope at the magnifications of 100x and 400x (Baker, 2007; Owen, 1992).

Blood parasites were detected by thin and thick blood smear methods. The blood samples were spread onto the glass slides and stained with Giemsa's staining for 30 to 60 minutes and then rinsed through the running tap water before being examined at X1000 magnification for blood parasites identification.

Ectoparasites examination: Fur pluck, fur tape impression and whole-body immersion techniques were performed for ectoparasites identification. Animal's fur was grasped using hemostats and gently plucked from the areas of the mouse's scapular, cervical region, axillary, inguinal, and dorsal rump before a drop of mineral oil was applied onto a glass slide. On the same sites, sellotape was also applied to perform a fur tape impression test. Whole-body immersion was done by soaking the carcass into the specimen bottles containing 75% alcohol for further examination. The microscopic examination was conducted to identify ectoparasites using a compound microscope at the magnifications of 100x and 400x (Baker, 2007; Owen, 1992).

Statistical analysis: Statistical analysis was carried out using the software Statistical Packages for the Social Sciences (SPSS) version 24. The association between the type of helminths infection with the laboratory mice strains were analysed using Pearson's Chi-Square method and it was considered significant when $P < 0.05$. The independent variable of the test study is the mice strains (BALB/c or ICR) and the dependant variable is the type of parasitic infection observed.

RESULTS

In this study, conventional parasitological methods were used for the detection of helminths and their ova. From the direct faecal smear technique, adult worm and the larva recognised in infected mice were *S.oblevata* and *A. tetraptera* as shown in Figure 1. Both pinworms were identified based on their distinct characteristics on the anterior and posterior anatomical structure as well as the ova morphology. The ova of the *S. obvelata* could be recognised as pointed ovals measuring an average of 134 x 36 µm that is flattened on one side while *A. tetraptera* ova are ellipsoidal and symmetrical in shape with an average size of 86 x 37 µm (Pritchett, 2007). Although ova's pinworm could not be observed through the faecal smear method, the

characteristics could be identified by the faecal flotation technique as illustrated in Figure 2.

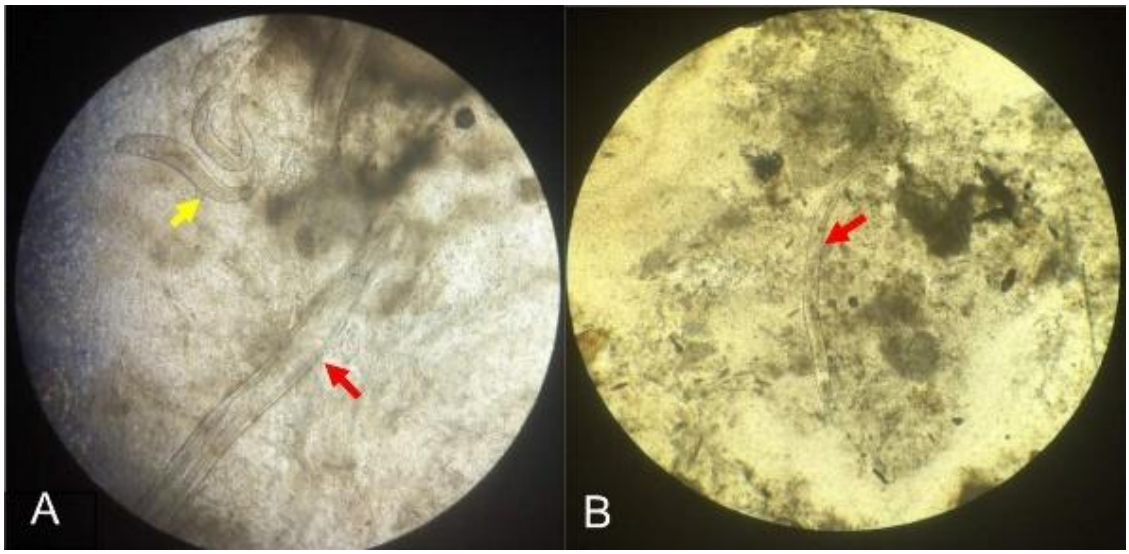

Figure 1. A - Direct faecal smear technique showed an adult worm of *Syphacia obvelata* (red arrow) and its larva (yellow arrow); B - Evidence of an adult worm of *Aspiculuris tetraptera* (red arrow) by the same technique under 100x magnification.

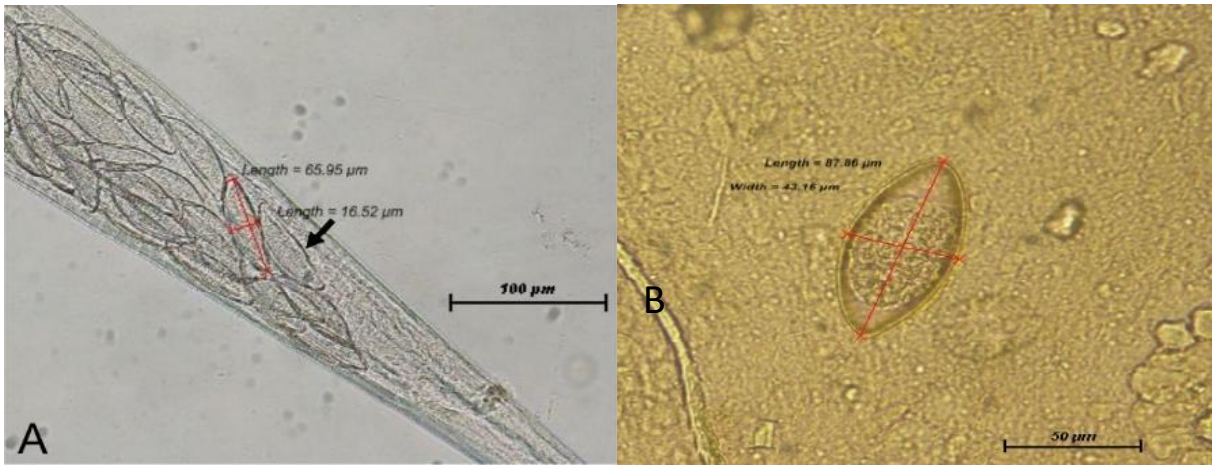

Figure 2. Pinworm eggs by faecal flotation technique. A - Flatten shape ova on one side within the gravid female of *Syphacia obvelata* (arrow) at 200x; B - Symmetrical ovoid and ellipsoid-shaped ova of *Aspiculuris tetraptera* (red arrow) under 400x magnification.

Our study demonstrated that an adult of *S. obvelata* is characterised by an average length of 3.4 to 6 mm with a subtle cervical alae, round oesophageal bulb, long pointed tail and its vulvar is located at the anterior body (Figure 3). *Aspiculuris tetraptera* is distinct from *S. obvelata* in terms of morphology and size. Generally, they have a body length of 3 to 4 mm with prominent cervical alae (female). Differing from *S. obvelata,* the female *A. tetraptera* has a conical tail with a vulvar located at the mid of the body as shown in Figure 3.

The numbers of BALB/c and ICR mice infected with helminths, ectoparasites and blood parasites were summarised in Table 1. Examinations of the mice revealed that 33.33% of BALB/c mice were infected with a single infection of *S. obvelata* but it was 0% or absent in ICR mice. While BALB/c mice showed no single infection of *A. tetraptera*, 12.5% of ICR mice were presented with it. However, 8.33% of BALB/c and 20.83% of ICR mice were mix-infected with both pinworms together. Meanwhile, fur pluck, fur tape impression, and whole carcass immersion tests revealed no infestation of ectoparasites. Similarly, negative results were also obtained for blood parasites throughout the study (Table 1).

Table 1. Helminths, ectoparasites and blood parasite in BALB/c and ICR mice (n = 48).

Mice	N	Helminths			Ectoparasites	Blood parasite
		Syphacia obvelata	*Aspiculuris tetraptera*	Mixed both		
BALB/c	24	8	0	2	0	0
ICR	24	0	3	5	0	0

Note: Minimal amounts of helminths and ova of *Syphacia obvelata* and *Aspiculuris tetraptera* for each infected mouse were lesser than 60 and 30, respectively.

Based on the parasitological techniques performed, the amount of BALB/c and ICR mice that had been infected with helminths and ova were summarised in Table 2. Through the simple flotation method, *A. tetraptera's* ova was detected in 18.75% of animals with 16.67% contributed by ICR mice. This study also demonstrated that 25% of mice were found to be positive for *S. obvelata* but all animals were negative for *A. tetraptera* by perianal tape test. Hence, *A. tetraptera* could be most likely detected by faecal flotation technique and *S. obvelata* was best to be identified by perianal tape test. Direct examination of the gastrointestinal tract revealed 6.25% of mice were positive for *S. obvelata* and 6.25% of mice had *A. tetraptera*. Only a single BALB/c mouse was infected with *A. tetraptera* and the other two were positive for *S. obvelata*. In contrast, two ICR mice were infected by *A. tetraptera* with one of them was mix-infected with *S. obvelata*. Therefore, it showed that the amount of both pinworms residing on the gastrointestinal tract are not specific for any strains.

Table 2. Comparison of parasitological methods to detect the presence of helminths and ova; *Syphacia obvelata* and *Aspiculuris tetraptera*, in BALB/c and ICR mice (n = 48).

		Parasitological methods							
		Direct faecal smear		Faecal flotation		Perianal tape test		Gastrointestinal examination	
		BALB/c	ICR	BALB/c	ICR	BALB/c	ICR	BALB/c	ICR
Helminths	*Syphacia obvelata*	0	0	1	0	8	4	2	1
	Aspiculuris tetraptera	0	0	1	8	0	0	1	2

***Note*:** No finding was observed by direct faecal smear method in any groups of mice.

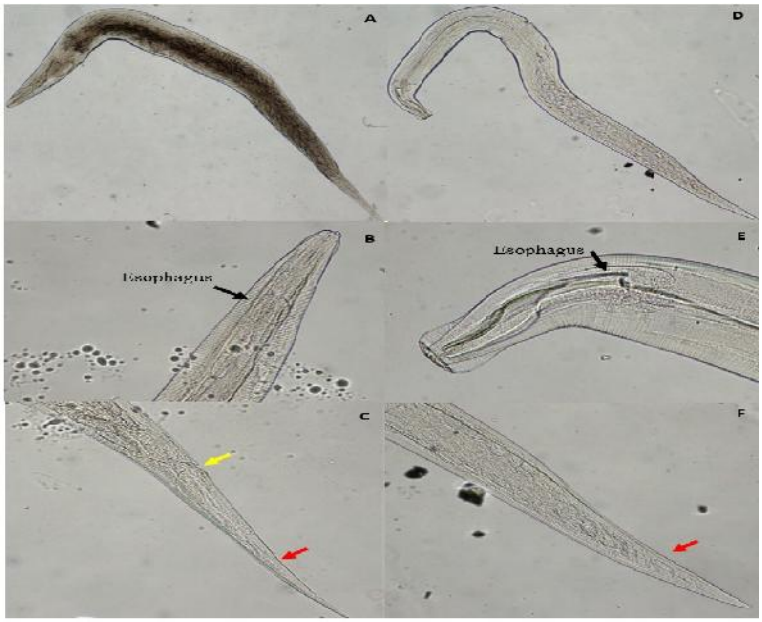

Figure 3. A gravid female of *Syphacia obvelata* (A) under a light microscope at 200x magnification. It was characterised by a round oesophageal bulb (B: arrow), subtle cervical alae with a long and vulvar opening (C: yellow arrow), and a pointed tail (C: red arrow) at 400x; A gravid female *Aspiculuris tetraptera* (D) at 200x was observed with the oval oesophageal bulb (E: arrow) and conical tail (F: red arrow) at 400x magnification.

Our findings showed that there was a preference of *S. obvelata* towards inbred strain; BALB/c mice as observed in Table 1. Nevertheless, a higher mixed infection was observed in the outbred, ICR mice than those in BALB/c mice. Statistical analysis by Pearson's Chi-Square proved that there is an association between the type of helminths with the laboratory mice strains since the value obtained was P = 0.043.

DISCUSSION

This particular study revealed that there was a low burden of helminths in laboratory mice and the most commonly found helminths are pinworms belonging to the family of Oxyuridae; *Syphacia obvelata* and *Aspiculuris tetraptera.* They are opportunistic pathogens that frequently infect laboratory animals in conventional, semi-open animal facilities (Bazzano *et al.*, 2002). Overall, only 18 out of 48 mice (37.5%) were infected with these helminths at low levels with a lesser than 60 eggs count for *S. obvelata* and not more than 30 eggs count for *A. tetraptera*. According to Chan (1952), *S. obvelata* females deposit an average of 350 eggs at the perianal area, which develops into the infective third-stage larvae within 20 to 24 hours. The *A. tetraptera* female releases an average of 17 eggs daily (Phillipson, 1974). According to a study conducted by Perec-Matysiak *et al.* (2006), the number of animal facilities with positive infection by *S. obvelata* and *A. tetraptera* were 14% and 7%, respectively.

These data indicated that the presence of opportunistic parasites in laboratory mice are very common but their influence should not be neglected as infected animals are unsuitable for any critical work such as nutritional and blood values as experimental results may be affected by the parasitic infestation. Although non-pathogenic, pinworms trigger a Th2-associated immune response that hinders research outcomes (Grencis, 1997). Heavily infected rodents by pinworms showed signs of rectal prolapse, faecal impaction, rough hair fur, and weight (Baker, 1998). Additionally, there were significant hematopoietic changes characterized by increased myelopoiesis and erythropoiesis and altered sensitivity to IL-17 in laboratory mice infected with *S. obvelata* (Bugarski *et al.*, 2006). This demonstrates that endoparasites infection may significantly alter the hosts' hematopoietic response leading to interference with the experimental settings and thus altering the final results.

Our study revealed that there was a correlation between the types of helminths and strains of laboratory mice. Although the animals were chosen from the same age in a group, housed in the same environmental settings, and maintained by the same management and personnel, there was still a difference in the presence of helminths depending on their strains. Our findings also showed specific trends of helminths for different types of strains as there was a preference of *S. obvelata* infection towards BALB/c strain. The strains of laboratory mice influence the prevalence of *S. obvelata* infection (Taffs, 1976). Similarly, Chan and Kopilof (1958) also mentioned that certain inbred strains of mice, Columbia strain contribute to a higher prevalence as compared to CF1 strain, an outbred mice. According to the study, the Columbia mice harboured an average of 52 worms per mouse than CF1 strain with an average of 9 worms per mouse. They suggested that the genetic factor influence the resistance of pinworms to the infection where suitable flora is necessary for the worm survival. But, it differs from a previous study that revealed inbred mice harboured smaller parasite loads (Goncalves *et al.*, 1998). Hence, there is a variation on the parasitic level depending on the specific strains. It is also supported by another study where a similar pattern of resistance was observed between multiple groups of inbred and outbred strains when comparing to each other (Derothe *et al.*, 1997).

In the current study, mixed infection by both pinworms was seen in both BALB/c and ICR mice. Mixed infection by *A. tetraptera* and *S. obvelata* are common (Taffs, 1976) although the site of infection differs as *A. tetraptera* is mainly found in the colon whereas, *S. obvelata* is commonly seen in the caecum. Direct examination of caecal and colonic contents has been described as the 'gold standard' for detecting pinworms with the major drawback of the animals required to be euthanized beforehand (Feldman and Bowman, 2007). An earlier study revealed that infections due to a single species were detected in 62% of the laboratory mice in comparison to only 16% of multiple species (Bazzano *et al.*, 2002). Generally, a heavy burden of these helminths in clinically healthy conventional laboratory mice may require treatment.

Our findings revealed that the best parasitological method to identify *S. obvelata* is the perianal tape test than the other methods. It has been used widely and effectively to detect eggs of *Syphacia* spp. most likely due to the behaviour of laying eggs at the perianal region (Eguiluz *et al.*, 2001). The sensitivity of the test to detect *S. obvelata* is up to 85.5% (Hill *et al.*, 2009). This study showed that *A. tetraptera* is best diagnosed by faecal flotation test, as also mentioned previously (Sharp and Villano, 2012). Our finding also proved that a direct faecal smear test is an ineffective method as not reveal any ova or helminths. This is further proven for eggs demonstration by this technique is the least dependable with only 3.2% of positive results (Sasa *et al.*, 1962). Methods of pinworm screening such as direct faecal smear, faecal flotation and perianal tape test are significantly less sensitive than the direct examination of gastrointestinal content for the adult helminths (West *et al.*, 1992). Gastrointestinal contents examination is considered to be the 'gold standard' to diagnose

pinworms (Dole *et al.*, 2011). The gastrointestinal examination has also been proven to be effective in detecting pinworms as compared to the other methods (Ain-Fatin *et al.*, 2020 b). Our results showed only certain mice were identified with worms despite being positive by the other methods. It is similar to a previous study where they had worm-positive with FCC or PCR but undetectable by traditional methods of direct worm detection or faecal flotation. Therefore, optimal pinworm detection could be achieved by combining PCR analysis with the intestinal content examination (Garwin *et al.*, 2017). Possible factors to these events are stipulated to be ingestion and elimination of unembryonated eggs, recent expulsion of worms, and failure to detect an infection.

Meanwhile, no ectoparasites infestation to be identified throughout the experimental period most likely due to proper laboratory animal husbandry and management practised by the facility personnel. In another study by Bazzano *et al.*, 2002, adequate adopted procedures and qualified working personnel provide barriers against parasitic infestation. Therefore, proper facility management should be practised to maintain biosecurity within an animal facility. Maintaining laboratory animals in proper environmental conditions should also be considered by guidelines settling previously (National Research Council, 2011). Furthermore, routine health screening of laboratory animals in an animal facility every 6 to 12 months helps to monitor the parasitic load over time. Therefore, this could prevent direct transmission of ectoparasites in the laboratory mice population. However, a further sensitive diagnostic method such as skin scraping should be implemented in a future study to increase the chances of ectoparasites detection (Perec-Matsyiak *et al.*, 2006). Expectedly, microscopic examination of the animal's blood smear revealed an absence of any blood parasites or protozoa. The presence of blood parasites was very rare in laboratory rodents as reported previously (Pritchett-Corning and Clifford, 2012). It proved that they are unlikely to be infected with blood parasites where proper husbandry and management are met in a conventionally maintained animal facility. Therefore, integrated control strategies and preventive measures should be implemented to control parasitic infection in laboratory mice.

Conclusion: In conclusion, a higher infection of *S. obvelata* in inbred BALB/c strain and greater mixed infection of both pinworms in outbred ICR mice with a very low amount of helminths and their ova for each infected animal. Throughout the study, the absences of ectoparasites and blood parasites in conventional laboratory mice are maintained by appropriate husbandry and management practised. Our data also showed an association between parasites and the strains of the laboratory mice. In addition, there is an opportunity to further broaden the study by carrying out a higher sensitivity parasitological method such as PCR testing for characterisation of parasites to provide better insight into parasitic identification.

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Conflicts of interest: The authors declare that there is no conflict of interest.

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