Middle East Journal of Applied Science & Technology (MEJAST)
Volume 8, Issue 2, Pages 65-73, April-June 2025

Effectiveness of different deworming drugs on Intestinal parasites in laboratory mice (*Mus musculus*) in Department of Medical Research

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DOI: https://doi.org/10.46431/MEJAST.2025.8207

Article Received: 07 April 2025 *Article Accepted: 15 June 2025* *Article Published: 19 June 2025*

ABSTRACT

Rodents, as mice and rats are the most common laboratory animals used in research and testing. The parasite infections can affect investigations by inducing physiological and immunological alterations in the hosts, increasing or diminishing host susceptibility to experimental stress, inducing tissue damages stimulating abnormal tissue growth competing with the host for nutrients, decreasing the volume of host's blood and body fluids and by mechanical interference. Therefore, the study aimed to determine the effects of different de-worming drugs on intestinal parasites in laboratory mice (*Mus musculus*) from August 2023 to January 2024. Before experiment, mice groups were checked and detected parasite's eggs by the taping method. Intestinal parasites index 4 plus positive 30 males and 30 female mice and 10 each male and female were selected from colony and housed in cages in breeding rooms separately. Before treatment of de-worming drugs, 10 each of male and female mice will be housed in 8 cages separately according to de-worming drugs except control cages in the testing room at 22±2°C, RH 65±5% and good air ventilation. All the tested mice were given to free access the diet and tap water during the experimental period. Treatment regimens as Mebendazole, Albendazole and Praferan for mice were used orally administration of 10mg/kg/day for 10 consecutive days. After the de-worming test all the tested mice were checked and examined the intestinal parasites weekly till to fine reinfection. Results revealed that highest number of mice were Pinworm positive followed by Ascariasis. After intervention by three de-worming drugs on 4plus parasites positive mice were cleaned all kinds of intestinal parasites till 15 weeks expect Mebendazole. Albendazole and Praferan were found most effective de worming drugs for mice population. After 14weeks or during 15 weeks, Mebendazole treated mice were found 10% of reinfected by pin worm infection. Albendazole and Praferan are suitable de-worming drugs for laboratory mice. Intestinal parasite or worms are transmitted through fecal-oral contact (injection of embryonated eggs). Therefore, cages and its utensils of de-wormed mice should be cleaned with detergent daily and need to treat three monthly monitoring of parasite control plan in mice husbandry colony.

Keywords: Deworming Drugs; Laboratory Mice; *Mus musculus*; Effectiveness; Albendazole; Mebendazole; Praferan; Parasites; Taping Method; Positivity; Treatment; Clean.

1. Introduction

Pinworms are most prevalent in laboratory mice and rats [1,2]. Rats are usually infected with *Syphacia muris* and mice are infected with *Syphacia obvelata* and *Aspiculuris tetraptera* and rats can be incidental hosts of *Syphacia obvelata* and mice can be incidental hosts of *Syphacia muris* [3]. And also, rats are infected with *Aspiculuris tetraptera* [4]. Pinworms are nematode parasites (Family Oxyuridae) that have simple, direct life cycles and are frequent contaminants of both specific pathogen free (SPF) and conventional colonies of laboratory rats and mice. Pinworms are transmitted through the ingestion of embryonated eggs. Two species of pinworms as *Syphacia obvelata* and *Aspiculuris tetraptera* that commonly infect laboratory rats and mice. The prevalence of pinworms in an infected rodent population depends on many factors, including parasite environmental load, gender, age, strain, and immune status. Males tend to have higher burdens than females, while young animals tend to have higher worm burdens than older animals. Mice and rats are the most common laboratory animals used in research and testing of many kinds of traditional and pharmaceutical research. The parasite infections can affect investigations by inducing physiological and immunological alterations in the hosts, increasing or diminishing host susceptibility to experimental stress, inducing tissue damages, stimulating abnormal tissue growth, competing with the host for nutrients, decreasing the volume of host's blood and body fluids and by mechanical interference [3]. Still little is known about the effects of environmental changes on the biological variation in experimental results [5]. In the

mouse and rat caecum and colon, *Qspicularis tetraptera* may be found together with *Syphacia muris* or *Syphacia abvelata* [6].

The life cycle of *Syphacia muris*, *Syphacia obvelata* and *Aspiculuris tetraptera* is direct and completed in 7 to 8 days, [7] making this particular pinworm ideal for epidemiologic study. Adult worms are inhabiting in the cecum and colon, and female worms migrate to the anus and deposit all their eggs on the perianal region of the host before dying. Within a few hours, the eggs are developed to embryonate and they are considered infective [5].

Infection is believed to occur via 3 modes: (1) direct ingestion of the eggs; (2) ingestion of food or water contaminated with the eggs; and (3) retro infection [8]. Ingestion of eggs is considered to be the primary mode of infection, and the eggs are reported to remain viable within the environment for as long as 4 weeks [9,10]. Antemortem diagnosis traditionally is made by identification of these eggs on a perianal cellophane tape, given the ease of collection and interpretation, although direct examination of the cecum postmortem is considered the most dependable method for *Syphacia muris*, *Syphacia obvelata* and *Aspiculuris* diagnosis [11].

Ascariasis prevalence was found in humans and animals Kindem. *Ascaris suum* is mostly infected to rats, mice, swine and pigs. *Ascaris suum* infection is established orally by third stage larvae after their development from embryonated eggs. The third stage larvae invade the small intestine of host migrate into the lever and lung, and finally reach to the cecum and/or proximal colon, where they develop into adult worms [12]. At the same time, to fine out the effectiveness of deworming drugs on helminth parasites in laboratory mice colonies in DMR. Therefore, the objective of the study was to fine out the effectiveness of different deworming drugs on intestinal parasites in laboratory mice (*Mus musculus*) in Department of Medical Research.

2. Materials and Methods

2.1. Study Area

The study was conducted on laboratory mice in Laboratory Animal Service, Department of Medical Research, Ministry of Health, Yangon, Myanmar.

2.2. Study period

The study period was one year, from April, 2023 to March, 2024.

2.3. Study Design

Laboratory based descriptive study design was used.

2.4. De-worming drugs

Required de-worming drugs as Mebendazole, Albendazole and Praferan were purchased from Medicinal shops from New Bo Gyoke market, Yangon.

2.5. Treatment ratio and duration of treatment

All the treated de-worming drugs, Mebendazole, Albendazole and Praferan were prepared for mice were used orally administration of 10mg/kg/day for 10 consecutive days.

2.6. Study procedure

Before experiment, mice groups were checked and detected parasite's eggs by the taping method [14]. Intestinal parasites index 4 plus positive 30 males and 30 female mice were selected from colony and housed in cages separate cages and parasites negative 10 male and 10 female mice were housed in another two cages as control in the breeding rooms separately. Before treatment of de-worming drugs, 10 each of male and female mice were housed in 8 cages ((250 mm x 170 mm x 100 mm) separately according to de-worming drugs except control cages, in the testing room, was maintained at 22±2°C, RH 65±5% using an exhaust fan and air-conditioner were provided for good air ventilation. All the tested mice were given to free access the diet and tap water during the experimental period. Treatment regimens as Mebendazole, Albendazole and Praferan for mice were used orally administration of 10mg/kg/day for 10 consecutive days. After the treatment of deworming drugs all the tested mice were checked and examined the intestinal parasites weekly till to fine reinfection by the above mention method [14] for the preventive periods of de-worming drugs.

2.7. Analysis of Data

The mean and standard deviation of each parameter, was calculated by standard statistical method. Positive rate was calculated in percent by the excel software and parasite density was calculated by parasite/100 microscopic fields.

3. Results

Table 1 shows that percentage density of intestinal parasites in laboratory mice. All total 563 laboratory mice (288 (51.16%) males and 275(48.8%) females were examined, of these 279(49.56%) males and 262(46.54%) females were positive for intestinal parasites. Within Male and female positive cases males (51.57%) were infested than Female mice (48.43%). Of which 3Plus parasite positivity rate was found highest of the intestinal parasites positivity 224(39.78%) followed by 4plus parasite positivity rate 219(38.89%) and lowest parasite positivity rate was observed 1plus parasite positivity 11(1.95%).

Table 1. Positivity of intestinal parasites according to parasites density in mice population

Parasite density	Total exam (male + female)	Male mice Parasite positivity	Female mice Parasite positivity	% of Positivity (According to parasites density)
4+	219	106	113	219 (38.89%)
3+	224	125	99	224 (39.78%)
2+	87	43	44	87 (15.45%)
1+	11	5	6	11 (1.95%)
Neg	22	9	13	22 (3.91%)
Total Exam	563	288 (51.16%)	275 (48.85%)	563 (100%)
Parasite Positive rate		279 (49.56%)	262 (46.54%)	563 (100%)
Male and female positivity rate		279 (51.57%)	262 (48.43%)	541 (100%), (96.09%)

Figure 1 shows that diagnosis of different types of intestinal parasites in mice population and found that a total of 5 different intestinal parasite such as Pin Worm (*Enterobius spp*), *Ascariasis spp*, F= *Faciolar spp*, T=*Trichuris spp*,

S=*Schistosome spp*. Of these, single Pin worm positivity was found highest in laboratory mice followed by Pin worm + *Ascariasis spp* positivity and lowest positivity was observed *Trichuris spp* species in mice population. Singal Pin worm. Ascariasis, Faciolar, Trichuris and Schistosome were found 241(42.81%),142(7.10%) 10(1.78%), 3(0.53%) and 0% , Two parasite mixed as Pinworm + Ascariasis, Pin worm + Faciolar, Pinworm + Trichuris and Pin Worm + Schistosome were found 142(25.22%), 30(5.33%), 20 (3.55%), 2.(0.36%)and 3 parasite mixed as Pinworm + Ascariasis + Faciolar, Pinworm +Ascariasis +Trichuris and Pinworm+ Ascariasis + Schistosome were found 40(7.10%), 25(4.44%), 0% respectively and very small number of 4 kinds of intestinal parasites mixed were found in mice population.

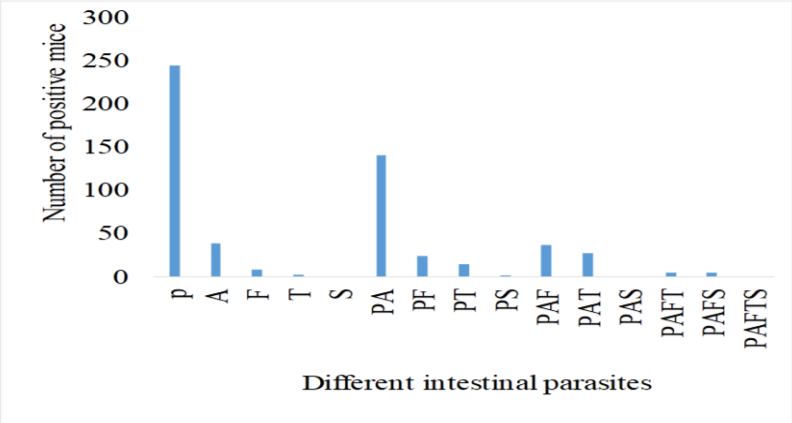

Figure 1. Diagnosis of different types of intestinal parasites species in laboratory mice population (Single and more species infections) P=Pin Worm (*Enterobius spp*), A = *Ascariasis spp*, F= *Faciolar spp*, T=*Trichuris spp*, S=*Schistosome spp*

Figure 2 shows that condition of laboratory mice before and after administration of 3 de-worming drugs and found that before intervention with 3 de-worming drugs, all the test mice were positive with 4 plus intestinal parasites and control mice were negative with intestinal parasites. After intervention of 1 week, all intestinal parasites were destroyed by 3 de-worming drugs and mice were free from parasites. Weekly examination of intestinal parasites preventive period was observed 14 weeks for Mebendazole and 15 weeks for Albendazole and Praferan in laboratory mice population.

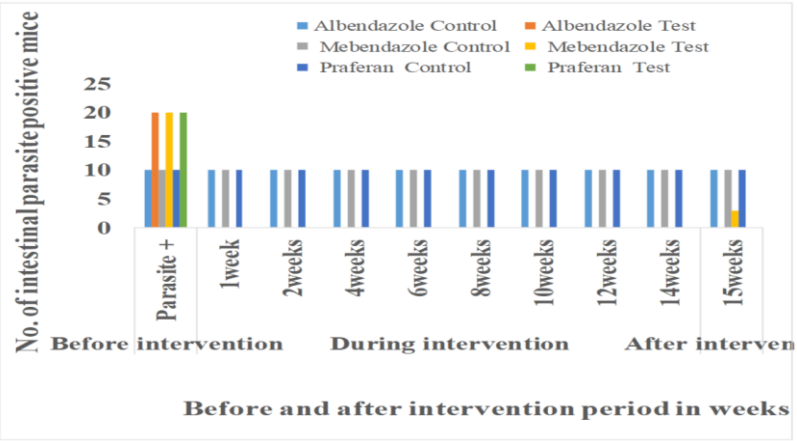

Figure 2. The condition of laboratory mice before and after administration of 3 de-worming drugs till parasites seen again in tested mice

4. Discussion

Mice and rats are very useful animal in different kinds of research in the laboratory. Some are useful for cancer research, some are useful for snake bite research, some are useful for bone healing research and some are useful for toxicity research. Therefore, healthy laboratory animals are needed to access accurate and good results. In the present study before the treatment of deworming drug, laboratory mice were found pinworm *(Syphacia muris*) and *Ascaris suum* eggs, Faciolar spp, Trichuris spp, Schistosome spp, were positive by the examination of tapping method under the compound microscope with 40X lance. All total 563 laboratory mice (288 (51.16%) males and 275(48.8%) females were examined, of these 279(49.56%) males and 262(46.54%) females (total 541(96.09%) mice were positive for intestinal parasites. Of which 3Plus parasite positivity rate was found highest of the intestinal parasites infection rate followed by 4plus parasite positivity rate and lowest parasite positivity rate was observed 1plus. In single intestinal parasite positivity (as only Pinworm, Ascariasis, Faciolar spp, Trichuris spp, Schistosome spp), Pin worm was found the highest parasite positivity 42.81%, followed by Ascariasis 7.10% and lowest was observed Trichuris spp 0.53%. In 2 parasites mixed infection was found highest in Pinworm+ Ascariasis followed by Pinworm +Faciolar spp and lowest was found Pinworm+ Schistosome spp. In 3 and 4 parasite species mixed, the highest percentage was found Pinworm + Ascariasis + Faciolar spp, followed by Pinworm + Ascariasis + Trichuris spp were observed. Same results were found in laboratory rats' population, in laboratory rats' population, mixed infection (Pinworm + Ascariasis) was higher than single parasite positivity [15].

Other researchers revealed that the prevalence of pinworms in an infected rodent population depends on many factors, including environmental load, gender, age, strain, and immune status. Males tend to have higher parasite burdens than females, while young animals tend to have higher worm burdens than older animals. Laboratory mice tend to be more resistant to experimentally induced infection than wild mice. Athymic mice, as might be expected, have an increased susceptibility to infection [16]. In the present study, Present study on laboratory mice were found high risk of pinworm infection followed by Ascariasis infection. A study reported that laboratory rats were infected with Ascaris suum in high density, which is morphologically different from Ascaris lumbricoides [16,17]. Recent study have revealed that *Ascaris suum* of swine-origin can develop in humans, indicating its zoonotic importance [18]. Although numerous studies have been carried out thus far to characterize the two species of parasites on a morphological basis, species discrimination between *Ascaris lumbricoides* and *Ascaris suum* has been controversial [19,20]. The prevalence of pinworms in an infected mouse colony depends on many factors, including environmental load, gender, age, strain and immune status. Male mice tend to harbor more of the parasites than female mice, and young mice tend to harbor more of them than older mice. Laboratory mice tend to be more resistant to infection than wild mice. Athymic nude mice, as might be expected, are abnormally susceptibility to infection [21].

After treatment of de worming drugs of Mebendazole, Albendazole and Praferan drugs on 4 plus intestinal parasites positive male and female mice were found parasites negative, after one week by the diagnosis of taping method. And also found that all the de-worming drugs prevent long period as 14 weeks and in 15 weeks, Mebendazole treated mouse were found Pinworm positive during 15 weeks. Meade and Watson [16] revealed that Egg hatching after treatment with chlorine dioxide was significantly reduced as compared with that of unexposed control eggs (P

< 0.01). Eggs exposed to 400 mg/L chlorine dioxide gas hatched at a rate of 0.3%. Biologic indicators supported efficacy of the gaseous treatment. Furthermore, these eggs showed morphologic differences in the appearance of the capsule, as compared with control eggs. Liquid forms were significantly ($P < 0.01$) less effective at preventing hatching than the gaseous form of chlorine dioxide. On the basis of his data, he recommended that perianal tape testing should occur as close as possible to the peak egg-shedding time of 1400, to maximize the sensitivity of this particular diagnostic test.

In the present study all the infected mice were found free from all species of intestinal parasites as pin worm and ascariasis eggs, *Faciolar* spp, *Trichuris* spp, *Schistosome* spp, after treatment of deworming drugs, although other researchers informed that eggs may contaminate ventilation ducts [22] or shared equipment or procedure areas [23] and can re-contaminate a colony after the completion of treatment. Knowledge of egg longevity in the environment is important to determine the need for environmental decontamination, but specific data are unavailable. Aspiculuris *tetraptera* eggs are thought to be long-lived in the environment, remaining dormant for several months at 4°C [5]. In a study to determine methods to inactivate viable *Syphacia muris* eggs, 100% inactivation occurred only with temperatures of 100°C for 30 minutes and ethylene oxide, although high killing rates with formaldehyde and chlorine dioxide suggested that these chemicals could be successful with adjustments to the protocol [10]. Huerkamp and colleagues [24] reported the eradication of *S. muris* without environmental decontamination, suggesting that the eggs in the environment may not have outlived the treatment period (fenbendazole in feed every other week for five treatments). *S. obvelata* eggs appear to be unstable, they are reported to survive only 42 hours under ideal conditions, and may be inactivated by drying or immersion in liquids [25]. As noted above, *Syphacia muris* eggs are resistant to most common disinfectants [10], and it is assumed that Aspiculuris *tetraptera* eggs have similar properties. In the present study after treatment of Mebendazole, Albendazole, Paferan, deworming drugs, all the mice were found parasites negative, and a 100 % reduction was observed in all groups of mice. All the deworming drugs were found very effective to control all intestinal parasites infection. Monthly treatment and weekly washing the cages with detergent is needed to control or eliminate the intestinal parasites reinfection in laboratory animals.

5. Conclusion

Pinworms and ascariasis remain prevalent in laboratory mice, rats, swine, and pigs. Mice with *Syphacia obvelata* and *Aspiculuris tetraptera as well as Ascaris suum* in both mice and rats in laboratory. Therefore, it was need to eliminate the worm infection from laboratory rats. Four plus parasites positive thirty males and thirty females of laboratory mice (*Mus musculus*) from DMR were randomly selected and 10 each were housed in cages separately according to different deworming drugs in test room. Three deworming drugs as Mebendazole, Albendazole and Paferan) were applied for selected groups of mice. Results found that 541 (96.09%) out of 563 were positive for Pinworm eggs, Ascariasis eggs, Faciolar spp, Trichuris spp, Schistosome spp. Of these the highest density of Pinworm (*Syphacia muris*) infection followed by Ascariasis worms were positive and lowest positivity was observed *Trichuris spp* species in mice population. 4 plus intestinal parasites positive 30 males and 30 females were treated with deworming drugs. After treatment of deworming drugs, all the mice were free from intestinal parasite and 100 % reduction was observed in all groups of mice till 15 weeks expect Mebendazole treated mice

was found 10% pinworm positive during 15 weeks. Deworming drugs are very effective to control all intestinal parasites infection and need monthly treatment of deworming drugs and weekly washing the cages with detergent to prevent from intestinal parasites reinfection in laboratory animals. Other laboratory reared rats, rabbits, guinea pigs should be tested intestinal parasites. Additionally, de worming drugs should be treated to remaining laboratory reared animal to become healthy animals.

Declarations

Source of Funding

This study was financially supported by the DMR grant from Department of Medical Research.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Ethical Approval

Based on institutional guidelines.

Informed Consent

Not applicable.

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