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Efficacy of anthelmintics against naturally infected helminths in rabbits

Md. Delwar Hossen[1], Suman Kumer Paul[2], Dilruba Parvin[1], Md. Shahadat Hossain[1], Sharmin Shahid Labony[1], Anisuzzaman[1], Ausraful Islam[1,3]*

[1]Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Microbiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Program Officer, Mennonite Central Committee, Bangladesh (MCC,B)

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Corresponding Author

Ausraful Islam
rajibdvmpara@gmail.com

ABSTRACT

In Bangladesh, rabbit is not only reared as pets but also rabbit farming is expanding to supplement the animal protein. Rabbits are reared in semi-intensive manner where they have limited access to the yards and allowed to graze, which acts as source of helminth infection. Here, we report gastrointestinal helminth (GITH) infection in domesticated rabbits along with anthelmintic efficacy at Modhupur in Tangail district, Bangladesh. Through coprological examination, we identified ova of three helminths such as *Graphidium* sp., *Trichostrongylus* sp., and *Strongyloides* sp. We evaluated three commercially available anthelmintics such as ivermectin (IVM), piperazine (PPZ) citrate, and fenbendazole (FBZ) in three groups of naturally infected rabbits, each consisting of five animals. An untreated infected group of animals served as a control. Our study revealed that IVM was 100% effective against all nematodes and provided protection up to 30 days. Piperazine showed 100% efficacy against *Trichostrongylus* sp. and *Strongyloides* sp. but FBZ was 100% effective against all helminths up to 15 days. Rabbits treated with IVM, PPZ, and FBZ were free from *Trichostrongylus* sp. and *Strongyloides* sp. up to more than 75 days on average. The rabbits treated with FBZ were free from any helminths for the longest duration, up to 72 days. The rabbits treated with IVM or PPZ were free from helminths up to 67 days and 64 days, respectively. In conclusion, these three drugs can be used against common nematode infections in rabbits at two months interval.

INTRODUCTION

Rabbits are herbivorous animals and a colony of rabbits consisting of 10 to 20 heads can easily be reared in backyard with very little investment as compared to livestock and poultry. Additionally, rabbits are more resistant to common infectious diseases that affect and hinder livestock and poultry production. Small-scale rabbit farming is becoming popular in Bangladesh and may play a vital role in improving the lifestyle of low-income families (Vietmeyer, 1985). Faster growth, high prolificacy, good market value, and low rearing cost make rabbit farming popular to the people in Bangladesh (Amin et al., 2011).The rabbit production system in rural areas of Bangladesh is mainly forage-based.

The most common diseases of rabbits include gastrointestinal disorders, respiratory infections, skin disorders, and ectoparasitic infestation. Gastrointestinal disorders are mainly of unknown etiology. However, gastrointestinal tract (GIT)-dwelling helminths may have a significant rule. Due to scavenging nature, rabbit reared in Bangladesh easily get infected with GIT-nematodes (GINs). In fact, globally, very little attention has been paid to identifying the parasitic fauna inhabiting the GIT of rabbits. Also, the prevalence of parasites of rabbits and the anthelmintic efficacy is yet to be studied. This study was conducted to identify GIT-helminths affecting rabbits in Bangladesh and to estimate the efficacy of commercially available anthelmintics against infection with GINs.

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MATERIALS AND METHODS

Sampling and coprological examinations

A coprological survey was conducted at Madhupur Upazila in Tangail district, Bangladesh (Figure 1). Fecal samples were randomly collected from 50 domesticated rabbits. Samples were collected directly from the rectum using a smooth-edged glass rod and preserved in 10% formalin. Samples were initially screened by the direct smear technique. The eggs were identified according to the keys and descriptions given by Soulsby (1982) and Thienpont et al., (1979) and EPG (egg per gram of feces) was estimated using McMaster technique as described previously (Thienpont et al., 1979).

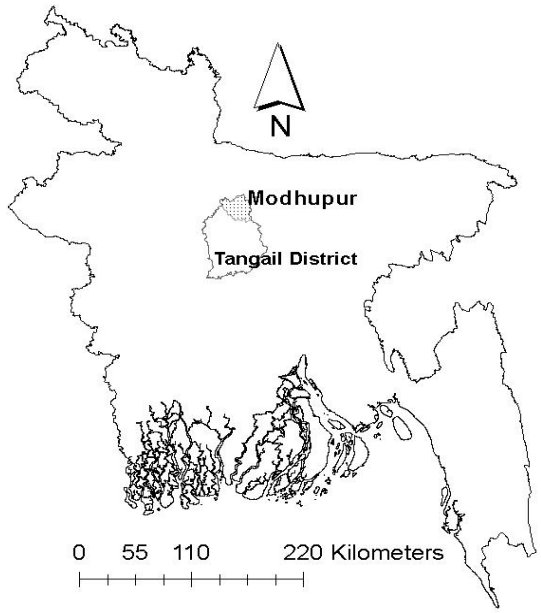

Figure 1: Study site, Tangail district of Bangladesh

Estimation of anthelmintic efficacy

To study the efficacy of different anthelmintics, 20 rabbits were selected on the basis of EPG (animals with 200-400 EPG) and concurrent infection with all three species, and marked with tagging. Then the selected rabbits were grouped into three groups such A, B, and C consisting of five animals in each groups. Prior to anthelmintic treatment, day-0 EPG of each animals was estimated and recorded. The selected 20 rabbits were apparently healthy with nearly similar age and body weight. Group A was treated with ivermectin (IVM) @ 1ml/50 kg body weight through subcutaneous injection. Group B and C were treated orally with piperazine (PPZ) citrate @ 1g/5kg and fenbendazole (FBZ) @ 10 mg/kg body weight, respectively, and group D was kept as untreated control. Animals of all groups were maintained under the same condition by providing food and water *ad libitum* and they had free access to the yard for scavenging. We collected fecal samples at day15, day 30, day45, day60, and day75 of post treatment (PT). Efficacy (E) of anthelmintics was calculated using the formula mentioned below.

E= [(Mean EPG before treatment-Mean EPG after treatment)/(Mean EPG before treatment)] ×100

Statistical analysis

A completely randomized design (CRD) with equal replications was employed to know whether there existed any significant difference among the effects of the anthelmintics against the helminths of rabbits from individual and widespread parasitic infection (Zar, 2002). The results were compared by the least significant difference (LSD) test to identify the best anthelmintic (Gomez and Gomez, 1984).

RESULTS

All the rabbits examined were infected with helminthes

To estimate the infection rate of gastro-intestinal helminths (GIH), we collected fresh faecal sample from 50 rabbits and examined as described in the Materials and Method section. Our study revealed that all the animals examined were infected with GIH. During routine coprological examination, we identified ova of three species of helminths such as *Graphidium* sp., *Trichostrongylus* sp., and *Strongyloides* sp (Figure 2). Then, we processed fecal sample to estimate EPG as described above. Based on estimated EPG, the parasitic burden of *Trichostrongylus* sp. was the highest (100±73.86), followed by that of *Graphidium* sp (66.67±65.13) and *Strongyloides* sp (25±45.22).

Table 1: Mean EPG at pretreatment and post treatment

Mean EPG	Drugs used		
	Ivermectin	**Piperazine**	**Fenbendazole**
Graphidium sp.			
Pretreatment	100	75	25
15 days post treatment	0	0	0
30 days post treatment	0	50	0
45 days post treatment	50	100	0
60 days post treatment	125	150	50
75 days post treatment	200	175	50
Trichostrongylus sp.			
Pretreatment	75	75	150
15 days post treatment	0	0	0
30 days post treatment	0	0	0
45 days post treatment	0	0	0
60 days post treatment	0	0	0
75 days post treatment	0	0	0
Strongyloides sp.			
Pretreatment	25	25	25
15 days post treatment	0	0	0
30 days post treatment	0	0	0
45 days post treatment	0	0	0
60 days post treatment	0	0	0
75 days post treatment	0	0	50

Table 2: Comparison of effects of anthelmintics for protecting the rabbits from each of the parasitic infection

Name of parasites	Relapsing time in day (mean±SE) of the various treatment			F-value
	Ivermectin	**Piperazine**	**Fenbendazole**	
Graphidium sp.	56.25±3.75	52.50±9.62	63.75±11.25	0.42
Trichostrongylus sp.	75±0	75±0	75±0	
Strongyloides sp.	75±0	75±0	75±0	

Efficacy of anthelmintics

Since commercial rabbit rearing is increasing and infection rate with GIH is very high, therefore, we planned to determine the efficacy of commonly used and commercially available anthelmintics. In group A, the efficacy of IVM was 100% against all nematodes and provided 100% protection up to 30 days. But at day-45PT, the EPG (50) of *Graphidium* sp. gradually increased. After 60 days, it was not effective against *Graphidium* sp. After 75 days, EPG of *Trichostrongylus* sp. and *Strongyloides* sp. was zero (Table 1).

In group B, we used PPZ citrate, and it showed 100% efficacy against *Trichostrongylus* sp. and *Strongyloides* sp. and EPG remained at basal level up to 75 days, however, it was 33% effective against *Graphidium* sp. on 30 days PT. At day-45, PPZ was not effective against *Graphidium* sp. (Table 1).

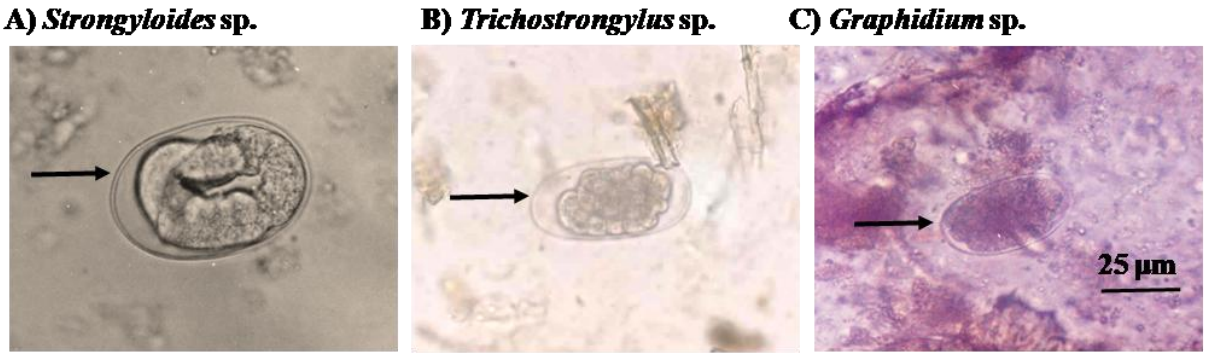

Figure 2: Helminth ova detected during microscopic examination (x40)

In group C, we used FBZ, which was 100% effective up to 45 days against all three nematodes. After 60 days, it was not effective against *Graphidium* sp. However, on the day 75 PT, EPG of *Trichostrongylus* sp. was still zero but, was not effective against *Strongyloides* sp. (Table 1). Rabbits treated with IVM, PPZ, and FBZ were free from *Trichostrongylus* sp. and *Strongyloides* sp., on an average, up to >75 days (Table 2).All three anthelmintics were effective against *Graphidium* sp. at least up to 45 days. The rabbits treated with FBZ were free from any helminths for the longest duration up to at least 60 days (Table 3). The rabbits treated with IVM and PPZ were free from helminths up to 60 days. In the contrary, neither overall EPG nor parasite-specific EPG declined in untreated control groups.

Table 3: Comparison of overall effects of anthelmintics for protecting the rabbits from parasitic infection

Treatment group	Relapsing time (Mean±SE)	F-value
Ivermectin	67.50±2.31	
Piperazine hydrochloride	64.50±3.28	0.10
Fenbendazole	72±2.33	

DISCUSSION

Rabbits are litter-bearing, rapidly growing animals, which can be domesticated very easily. However, they prefer free access at least to a limited space like yard rather than being captive. Due to their very scavenging nature, rabbits become frequently exposed to parasitic infections, particularly to the soil-tansmitted helminths (STH). Additionally, rabbits are coprophagic animals, which make them more prone to STH infections since they mainly follow oro-faecal route (Anisuzzaman and Tsuji, 2021). Here, we describe the diversity of GIH in domesticated rabbits reared under semi-scavenging condition along with anthelmintic efficacy against those parasites.

We found that 100% rabbits were infected with STH with variable worm burdens. We could not compare the data due to paucity of relevant literature. Hot and humid weather of Bangladesh with very long summer but short winter provide a very conducive environmental condition for propagation, development and survival of helminths. Additionally, due to coprophagic nature, if one animal of a rabbit colony become infected then the other animals get the infection very easily, and the infection spread to all the animals very rapidly. Therefore, very high infection rate of STH is not unexpected.

We estimated the efficacy of some commercially available and commonly used anthelmintics against naturally infected GINs. We found that all three drugs such as IVM, PPZ and FBZ drastically reduced EPG of all GINs recorded and provided protection for variable duration. We could not compare our estimated anthelmintic efficacy of IVM, PPZ, and FBZ against the mentioned parasites of rabbits due to the paucity of relevant literatures. However, the effectiveness of the drugs has been evaluated against other helminths

affecting rabbits or other rodents. Anantaphruti et al., (1992) studied the efficacy of IVM against advanced third-stage larvae of *Gnathostoma spinigerum* in rabbits. They found that the drug reduced worm load up to 74.2%. Also, the efficacy of IVM against *Trichostrongylus* sp. in mountain hares (*Lepustimidus* sp.) up to six months was reported from Scotland (Newey et al., 2004). IVM is a macrolid (macrocyclic lactones) derivative of the avermectin group and is effective as a broad-spectrum anthelmintic against internal and external parasites. IVM binds to the glutamate-activated Cl^- channel receptors in the neurons and muscle cells of the parasite and irreversibly activate these channels. As a result, neuronal activity and muscular contractility are inhibited, which causes flaccid paralysis and eventually the death of the parasite (Kotze et al., 2014; Gyatt et al., 1997).

Fetisov (1964) administered 500-750 mg PPZ per kg body weight for two consecutive days to eliminate *Passalurus* sp. infection in rabbits and found higher efficacy. Moreover, the efficacy of PPZ against nematodes of different animals has been reported (White et al., 2007; Praslicka et al., 1997; Steffan et al., 1988). PPZ blocks acetylcholine at the myoneural junction leading to flaccid paralysis in parasites. Thus, the worm can not maintain its position in the gut and is expelled by normal peristalsis (Gyatt et al., 1997).

Kirsch and Diiwel (1980) reported high efficacy of FBZ against adult and immature stages of *Aspiculuris tetraptera* in artificially infected mice. Düwel and Brech (1981) reported FBZ as an effective drug against adult *Passalurus ambiguous* of rabbits. FBZ is an anthelmintic of benzimidazole group that inhibits microtubule synthesis in nematodes. It also irreversibly impairs glucose uptake by inhibiting the fumarate reductase enzyme and preventing ATP production. Finally, the parasites die and clear off the gut (Gyatt et al., 1997).

We did not find any sign of intoxication, drug hypersensitivity, idiosyncrasy, and/or untoward effects during our study. Based on this study, we recommend use of any of the three anthelmintics to control the GIH/STH infection in rabbits at an interval of two months. Additionally, cages or houses of rabbits as well as the premises must be cleaned properly and proper disposal of feces are very essential to prevent reinfections.

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