



# Efficacy of Locally Available Anthelmintics against Helminths of Dairy Cows in Gazipur, Bangladesh

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**Abstract** | Parasitic diseases are crucial factors in reducing dairy production. To find out the efficacy of locally available anthelmintics against helminths, anthelmintic treatments were given to dairy cows in five different farms of Gazipur, Bangladesh and the helminth parasitic loads of dairy cows were determined before and after treatment by Modified Stoll's Dilution Technique using a McMaster counting chamber. A total of 85 faecal samples from five different dairy farms were examined. The overall infection rate of helminths in dairy cows is 37.65%. A total of five different types of nematodes were observed in all the investigated dairy farms. The highest infection rate (11.76%) and the lowest infection rate (4.71%) was found for *Neoscaris* spp. (*Toxocara* spp.) and *Fasciola* spp., respectively. *Hemonchus* spp. were fully (100%) susceptible to Ivermectin, Fenbendazole, and Levamisole. *Neoscaris* spp. were shown 33.33% and 16.67% resistant against Ivermectin and Fenbendazole, respectively. The *Fasciola* spp. was 66.67% resistant against Triclabendazole and the *Trichuris* spp. was the highest resistant against Ivermectin injection (100%) in all the investigated dairy farms. These findings are crucial for the selection of anthelmintics for the treatment of various types of nematodes in dairy cows and might be crucial in preventing anthelmintic resistance in dairy cows.

**Keywords** | Anthelmintic resistance, Dairy cows, Effectiveness, Helminth infections, Local anthelmintics

**Received** | October 21, 2022; **Accepted** | November 15, 2022; **Published** | November 30, 2022

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**Citation** | Islam MA, Sumon SMMR, Rahman ANMA, Khatun F (2022). Efficacy of locally available anthelmintics against helminths of dairy cows in Gazipur, Bangladesh. *Adv. Anim. Vet. Sci.* 10(12): 2630-2640.

**DOI** | <http://dx.doi.org/10.17582/journal.aavs/2022/10.12.2630.2640>

**ISSN (Online)** | 2307-8316



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## INTRODUCTION

Dairy is an essential part of the global food circulation system and it plays a crucial role in the sustainability of rural areas in particular. Globally, about one billion people are living on dairy farms (Shamsuddin et al., 2007). The dairy industry actively contributes to the economies of many communities, regions, and countries (IDF, 2013; Islam et al., 2018). The dairy sector can play an important role in providing jobs for rural communities (Islam et al.,

2019; Haque et al., 2021). Dairy production and processing provide employment, not only to people who work on dairy farms or in dairy plants but also to the whole sector, from upstream (inputs and services providers...) to downstream (marketing of finished products...) (Shamsuddin et al., 2007; Islam et al., 2020a). The dairy sector is a dynamic global industry, with growing production (since 2000 on an average +2.2% growth annually) which is forecast to continue in the long-term (IDF, 2013). Consumption of dairy products is consequently expected to increase by 20%

in future, according to FAO and OECD (DLS, 2008). In this context, dairy production and dairy processing appear as industries of utmost importance in contributing to the global challenge of today's food security and for decades to come (IDF, 2013; Islam et al., 2019a).

Although the dairy sector has lots of potentials, these dairy animals are infected with lots of infectious, metabolic, nutritional, and parasitic diseases. During the last decade, evidence has been generated that gastrointestinal helminth negatively affects the performance of adult dairy animals (Vercruysse and Claerebout, 2001). The effects of parasitism can be separated into two types – subclinical and clinical (Islam et al., 2020). Losses in animal productivity (milk production, weight gain, altered carcass composition, conception rate, etc.) are subclinical effects; whereas, visible, disease-like symptoms (roughness of coat, anaemia, oedema, diarrhoea) are clinical effects (Charlier et al., 2009). Many trials have estimated the effects of gastrointestinal helminths by measuring the association between infection levels and production parameters or by assessing the effect of anthelmintic treatment on production traits (Vercruysse and Claerebout, 2001; Smith, 1997). The distribution of parasites in adult dairy cows is overdispersed (Agneessens et al., 2000; Borgsteede et al., 2000; Islam et al., 2015) and the prevalence of gastrointestinal helminths in dairy cows were determined previously in different areas of Bangladesh (Alim et al., 2012; Islam et al., 2015; Rahman et al., 2018). It is evident from either artificial infection or anthelmintic control studies that helminths have several adverse impacts on dairy cows, including effects on milk production, suckler calf weaning weight and fertility (Hawkins, 1993). The subclinical effects are of major economic importance to the producer and can induce a decrease in milk production of up to and even over 11% and could be responsible for chronic and insidious economic losses in adult dairy cows (Charlier et al., 2009; Gross et al., 1999; Sanchez et al., 2004).

Due to the negative effects of parasites on dairy cows, it is essential to control parasitic diseases in dairy animals. In the absence of other alternatives, parasite control continues to rely on anthelmintics because of their high performance and their use is likely to continue in the foreseeable future as the first and foremost line of defence against parasites (Martin, 1985; Rahman et al., 2018). Because of the haphazard and extensive use of anthelmintics, the helminth parasites become resistant to commonly used anthelmintics and ultimately treatment failure occurs (Besier, 2007). Thus, the determination of anthelmintic resistance against helminths is crucial for the development and application of new helminth control methods. Furthermore, the determination of the underlying mechanisms for the development of anthelmintic resistance in dairy cows is also crucial

(Cotter et al., 2015).

There is a scarcity of studies to determine the resistance of locally available anthelmintics against helminths in Bangladesh (Rahman et al., 2018; Hoque et al., 2003). By considering the above facts, the present work has been undertaken to obtain detailed information on the parasitic infestation in dairy cows and evaluate the efficacy of some locally available anthelmintics and the degree of resistance (if any) against helminth parasites in some selected dairy farms of Gazipur district, Bangladesh. The first objective is to determine the common gastrointestinal helminths infecting small-scale dairy cows in the Gazipur district of Bangladesh. The second objective is to treat infected dairy cows *in vivo* using locally available specific anthelmintics against helminths to determine the efficacy of commonly used anthelmintics against helminths to interpret the degree of anthelmintic resistance (if any) at Gazipur, Bangladesh. The findings are essential in the selection of effective anthelmintics for the treatment of helminths infections in dairy cows. The findings might be crucial for the prevention of anthelmintic resistance in dairy cows globally.

## MATERIALS AND METHODS

The present study is designed to determine the efficacy of locally available anthelmintics against helminths, anthelmintic treatments, which given to dairy cows in five different dairy farms.

### STUDY AREA AND PERIOD

The research was undertaken on 5 (five) different dairy farms in the Gazipur district of Bangladesh. Gazipur is the industrial city of Bangladesh and is considered a highland city of Bangladesh. The dairy farms in Gazipur are mostly small-scale and consist of 5–10 dairy cows. The Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) dairy farm is established for teaching and research purposes. The small-scale dairy farms are owned by the farmers. All the selected farms were fed dairy cows mostly rice straw and concentrate consisting of rice polish, wheat bran, maize crush, and oil cake (Table 1). The cows were supplied with limited green grass with zero grazing in the pasture land. The age of all the cows was 2–4 years. Among all the tested farms, only the BSMRAU dairy farm was routinely dewormed by different types of locally available anthelmintics to all the dairy cows without identifying parasite infected cows. Before conducting the field experiment, the BSMRAU dairy farm treated dairy cows 3 (three) times subsequently at 3–4 months intervals. Except for the BSMRAU dairy farm, the other 4 (four) farms did not routinely deworm dairy cows (Table 1). All the cows of all five farms were apparently healthy and did not show any visible signs of helminth infections. The study was con-

ducted from July 2015 to June 2016.

### QUESTIONNAIRE

Each dairy cattle farm owner was asked basic questions regarding the use of anthelmintics for deworming dairy cows, their properties, specifically, which anthelmintics had been used and the policy determining when the dairy cows were treated. Information about feeding and grazing was also gathered from the record book (Table 1).

### SAMPLE COLLECTION AND STORAGE

About 10–20g of fresh faecal samples were collected from every animal by inserting the hand directly into the animal's rectum. The faecal samples were then put into a sample collection vial with 10% formalin solution and shifted to the laboratory & kept refrigerated until examination (Islam et al., 2020). All the cows of all the dairy farms were sampled for gastrointestinal helminths.

### SAMPLE EXAMINATION

The quantitative estimation of helminth eggs was done by employing 'Modified Stoll's Dilution Technique' by using a McMaster counting chamber as described previously (Soulsby, 1982). In short, three grams of well-mixed faecal sample was put into a 100 (hundred) mL beaker containing 42 (forty-two) mL of zinc sulfate solution with a specific gravity of 1.25–1.30. Then some glass beads were added to the beaker. Then the mixture was thoroughly mixed with a stirrer. The mixture then was strained with a coffee strainer, shaken well and 0.15 mL was taken using 1 (one) mL special pipette and put on two chambers of the McMaster slide and allowed the counting chambers to stand for 5 (five) minutes. Care was taken to avoid bubble formation during putting the sample on the slide. The slide was then examined under a compound microscope at 10 × 10 magnification. The eggs of specific helminths were identified based on morphological features and counted (Soulsby, 1982; Hendrix, 2006; Valero et al., 2009). The counts were made from each sample and the total number of eggs of parasites found in the slide was multiplied by 100 to get the eggs per gram of faeces (EPG). The EPG was calculated using Eq. (1).

$$EPG = \frac{n}{N} \times 100 \quad (1).$$

Where n is the number of eggs on a smear and N is the times/frequency of 0.15 mL samples examined (1–7 smears in this study). A subsequent 7 (seven) smears were examined before considering a sample as a negative sample.

### ANTHELMINTIC TREATMENT

Anthelmintic resistance tests were conducted *in vivo* only with slight modifications from previous studies (Coles et al., 1992; Coles et al., 2006). The weight of the individual

dairy animals were measured using the scale. After identifying infected dairy cattle, the infected cattle were divided into four groups and treated by using commonly used local anthelmintics (Rahman et al., 2017) and are as follows:

- (i) Oral Fenbendazole at 7.5 mg per kg body weight (Trade name: Peraclear bolus: Techno drugs) for nine infected dairy animals. Each bolus contains Fenbendazole 250 mg.
  - (ii) Injectable Ivermectin subcutaneously at 0.2 mg per kg body weight (Trade name: Vermic injection: Techno drugs Ltd., Ivermectin 10 mg/mL) for eight infected dairy animals.
  - (iii) Oral Levamisole and Triclabendazole combination at 6.75 mg per kg body weight (Trade name: Levex bolus: ACME Laboratories Ltd) for 10 infected dairy animals. Each bolus contains Levamisole 600 mg and Triclabendazole 900 mg.
- In the local market, Levamisole orally at 8 mg per kg body weight (Trade name: Levavet bolus: ACME Laboratories Ltd., Each bolus contains Levamisole 600 mg) were used for the treatment of nematodes and Triclabendazole 12 mg/kg body weight (Trade name: Fasinex bolus: Novartis, Bangladesh Ltd., Each bolus contains Triclabendazole 900 mg) were used for the treatment of *Fasciola* spp. During the experiment period, due to the unavailability of the Levavet bolus (Levamisole only) and Fasinex bolus (Triclabendazole only), Levex bolus (combination of Levamisole and Triclabendazole) is used for the treatment of both nematodes and *Fasciola* spp. Anthelmintic treatments were done as per the recommendation of the company.
- (iv) The control group (untreated). Five infected dairy animals from five different dairy farms.

Except for the control group, all the positive 27 (twenty seven) cows were treated with various types of anthelmintics. After 14 (fourteen) days of treatment, again faecal samples were collected, stored (Flanagan et al., 2011) and examined by the same technique described previously (Soulsby, 1982) and the efficacy of different anthelmintics were compared by using faecal worm egg count reduction test (FWCRT) using the Eq. 2.

$$Efficacy = \left( \frac{FWC_0 - FWC_{14}}{FWC_0} \right) \times 100 \quad (2).$$

Where  $FWC_0$  is= the faecal worm egg count before anthelmintic treatment (0 days) and  $FWC_{14}$  is the faecal worm egg count after anthelmintic treatment (at 14 days).

### INTERPRETATION OF RESISTANCE

The reduction percentage of eggs of specific helminths after treatment were determined by using Eq. 2. If the egg reduction after treatment is < 95%, then the parasite is considered to be resistant to that specific anthelmintic



**Table 1:** Descriptive characteristics and management factors of the dairy farms

Name of dairy farms	Total number of dairy animals	Grazing in the pasture land	Roughage supplied	Concentrate supplied	Anthelmintic treatment	Supplied vitamin-mineral premixes
Hedayet dairy farm	13	No	Rice straw, limited green grass	Rice polish, wheat bran	No anthelmintic treatment	Table salt with concentrate daily orally
BSMRAU dairy farm	41	No	Rice straw, sufficient green grass	Rice polish, wheat bran, maize crush, oil cake	Regular anthelmintic treatment at 3–4 months intervals using locally available anthelmintics	Parenteral multivitamin-mineral premix at 3–4 months intervals and oral table salt & vitamin-mineral premix with concentrate daily
Porabari moddho para dairy farm	11	No	Rice straw, limited green grass	Rice polish, wheat bran	No anthelmintic treatment	Oral table salt with concentrate daily
Abu Taleb dairy farm	09	No	Rice straw, limited green grass	Rice polish, wheat bran	No anthelmintic treatment	Table salt with concentrate daily orally
Mojibor dairy farm	11	No	Rice straw, limited green grass	Rice polish, wheat bran	No anthelmintic treatment	Table salt with concentrate daily orally

as described previously (Cotter et al., 2015). Data analysis were performed in MS Excel in Windows version 10.

## RESULTS

A total of 85 (eighty five) samples were collected from 5 (five) different dairy farms in the Gazipur district, Bangladesh. On ‘Modified Stoll’s Dilution Technique’ ova of 5 (five) different types of helminths were found. The EPG (egg per gram) of different helminths was 20–100. The mean ± SD of EPG of Hedayet dairy farm, BSMRAU dairy farm, Porabari moddho para dairy farm, Abu Taleb dairy farm, and Mojibor dairy farm were 25.75 ± 5.38, 34.05 ± 20.47, 29.67 ± 10.80, 22.50 ± 2.89, and 22.50 ± 2.89, respectively. Among 85 samples, 32 samples were positive for different helminths. The overall infection of different types of helminths was 37.65% (Table 2). The infection rate of *Neoscaris* spp., *Hemonchus* spp., *Trichuris* spp., *Capillaria* spp., and *Fasciola* spp. was 11.76, 8.24, 7.06, 5.88 and 4.71%, respectively in different farms (Table 2). A total of 27 treatments were given in different 5 (five) farms (leaving five positive animals as a positive control on five farms) using different types of local anthelmintics against five different types of helminths (Table 2). The mean efficacy of Ivermectin injection against *Hemonchus* spp., *Neoscaris* spp., *Trichuris* spp., and *Capillaria* spp. were 100, 66.67, 0, and 100%, respectively and the mean resistance of Ivermectin injection against *Hemonchus* spp., *Neoscaris* spp., *Trichuris* spp., and *Capillaria* spp. were 0, 33.33, 100, and 0%, respectively. The mean efficacy of Fenbendazole tablets against *Hemonchus* spp., *Neoscaris* spp., and *Tri-*

*churis* spp. were 100, 83.83, and 100%, respectively, and the mean resistance of Fenbendazole tablets against *Hemonchus* spp., *Neoscaris* spp., and *Trichuris* spp. were 0, 16.17, and 0%, respectively. The mean efficacy of Levamisole bolus against *Neoscaris* spp., *Hemonchus* spp., *Capillaria* spp., and *Trichuris* spp., were 100, 100, 100, and 100%, respectively and mean resistance of Levamisole bolus against *Neoscaris* spp., *Hemonchus* spp., *Capillaria* spp., and *Trichuris* spp., were 0, 0, 0, and 0%, respectively (Table 2). The mean efficacy of Triclabendazole against *Fasciola* spp. was 33.33% and mean resistance of Triclabendazole against *Fasciola* spp. was 66.67%. Except for the BSMRAU dairy farm, all other 4 (four) farms were fully susceptible to all the tested anthelmintics. The highest resistance (66.67%) was observed for Triclabendazole against *Fasciola* spp. in all the investigated dairy farms (Figure 1). The BSMRAU dairy farm alone was shown 75, 100, and 25% resistant Ivermectin, Triclabendazole, and Fenbendazole, respectively against different types of helminths e.g., *Neoscaris* spp., *Trichuris* spp., and *Fasciola* spp. (Figure 2).

## DISCUSSION

At present, anthelmintic and/or antimicrobial drug resistance-free livestock production is one of the critical challenges of sustainable animal agriculture production (Islam et al., 2021; Haque et al., 2020; Naide et al., 2018). Effective surveillance, specific diagnosis, and accurate treatment are prerequisites for the control of gastrointestinal helminths in dairy cattle to boost production from dairy animals. The overall infection rate of gastrointestinal helminths in the

**Table 2:** Farm wise distribution of different helminths and the efficacy of different anthelmintics against helminths in Gazipur, Bangladesh

Name of dairy farm	Total samples		Infection (%)	Name of parasites	Anthelmintic treatment			EPG		Control sample	Anthelmintic efficacy/reduction (%)
	Exam-ined	Positive for parasites			Vermic injection	Peraclear tablet	Levex bolus	Before treatment	After treatment		
Hedayet dairy farm	13	4	30.77	<i>Hemonchus</i> spp.	✓	×	×	25	0	<i>Capillaria</i> spp. (EPG 20)	100
				<i>Hemonchus</i> spp.	×	✓	×	25	0		100
				<i>Neoscaris</i> spp.	×	×	✓	33	0		100
BSMRAU dairy farm	41	14	34.15	<i>Neoscaris</i> spp.	×	✓	×	33	0	<i>Trichuris</i> spp. (EPG 25)	100
				<i>Neoscaris</i> spp.	×	✓	×	100	50		50
				<i>Hemonchus</i> spp.	×	✓	×	33	0		100
				<i>Neoscaris</i> spp.	✓	×	×	25	0		100
				<i>Hemonchus</i> spp.	×	×	✓	33	0		100
				<i>Trichuris</i> spp.	✓	×	×	20	17		15
				<i>Neoscaris</i> spp.	×	✓	×	50	0		100
				<i>Fasciola</i> spp.	×	×	✓	33	20		39
				<i>Capillaria</i> spp.	×	×	✓	20	0		100
				<i>Trichuris</i> spp.	×	×	✓	20	0		100
				<i>Fasciola</i> spp.	×	×	✓	25	17		32
				<i>Trichuris</i> spp.	✓	×	×	33	17		50
<i>Neoscaris</i> spp.	✓	×	×	33	17	50					
Porabari moddho para dairy farm	11	6	54.55	<i>Hemonchus</i> spp.	×	×	✓	50	0	<i>Hemonchus</i> spp. (EPG 25)	100
				<i>Fasciola</i> spp.	×	×	✓	33	0		100
				<i>Neoscaris</i> spp.	×	✓	×	25	0		100
				<i>Trichuris</i> spp.	×	✓	×	25	0		100
				<i>Hemonchus</i> spp.	✓	×	×	20	0		100

Abu Taleb dairy farm	9	4	44.44	<i>Neoscaris</i> spp.	✓	×	×	25	0	<i>Fasciola</i> spp. (EPG 20)	100
				<i>Neoscaris</i> spp.	×	✓	×	25	0		100
				<i>Capillaria</i> spp.	×	×	✓	20	0		100
Mojibor dairy farm	11	4	36.36	<i>Neoscaris</i> spp.	×	✓	×	25	0	<i>Capillaria</i> spp. (EPG 20)	100
				<i>Trichuris</i> spp.	×	×	✓	25	0		100
				<i>Capillaria</i> spp.	✓	×	×	20	0		100
Total/range	85	32	37.64	6 = <i>Hemonchus</i> spp. 10 = <i>Neoscaris</i> spp. 5 = <i>Trichuris</i> spp. 3 = <i>Fasciola</i> spp. 3 = <i>Capillaria</i> spp.	8	9	10	20–100	0–50	20–25	15–100

✓ Indicates treatment and × indicates no treatment

present study is 37.65% (Table 2) which is considered a moderate infection. Previously helminth infections in dairy cattle were also reported (Keyyu et al., 2006; Epherem, 2007; Alim et al., 2012) which supports our present study. This moderate infection could be considered an insidious enemy of dairy cows because the moderate infection sometimes goes unnoticed without any clinical signs. This type of moderate infection deprives nutrition from dairy cows and reduces the production of meat, milk, etc. from dairy cows (Khatun et al., 2021). Thus, moderate helminth infection might cause huge economic losses for the dairy industry due to reduced earnings from dairy farms.

The EPG of different parasites in the present study (20–100) is comparatively lower than in previous studies (Islam et al., 2015; Geurden et al., 2015). This is because of the differences in geography (high land), management (zero-grazing in pasture land with limited green grass), and age of the cows (Hoste et al., 2005; Pfukenyi and Mukaratirwa, 2013; Tulu and Lelisa, 2016). The geography, management, nutrition, and age of cows, etc. have profound effects on the load of the parasites (Talukder et al., 2015; Islam et al., 2020b). Before establishing infection, adult dairy cows expel parasites that are ingested and usually acquire immunity (Dunn, 1978) that's why the EPG in the present study might be lower.

At present, dairy cows were infected predominantly with *Neoscaris* spp. (11.76%) (Table 2). Previously the higher infection rate of dairy cows with *Neoscaris* spp. was also reported (Rajakaruna and Warnakulasooriya, 2011) which supports our present study. *Neoscaris* spp. are very critical helminth parasites for dairy animals. They might be responsible for producing diarrhoea, biliary and intestinal obstruction, poor performance, and death of dairy cows. Besides these the migrating larvae of *Neoscaris* spp. damages many internal organs of dairy cows including the lungs and might predispose them to secondary bacterial infections. They can transmit to the dairy calves through milk (Khatun et al., 2021). Along with diarrhoea, colic, inappetence, and weight loss, these helminths could cause intestinal obstruction and death of dairy calves. So effective preventive measures are essential for the prevention of *Neoscaris* spp. infection in dairy cows.

The infection caused by *Hemonchus* spp., *Trichuris* spp., and *Capillaria* spp. in the present study were considered moderate. These helminths are crucial for the maintenance of the health of adult dairy cows. They could cause diarrhoea, inappetence, rough coat due to loss of nutrition, and ultimately reduced production from dairy cows. A relatively lower rate of infection (4.71%) was observed in dairy cows with *Fasciola* spp. which is almost similar to the findings

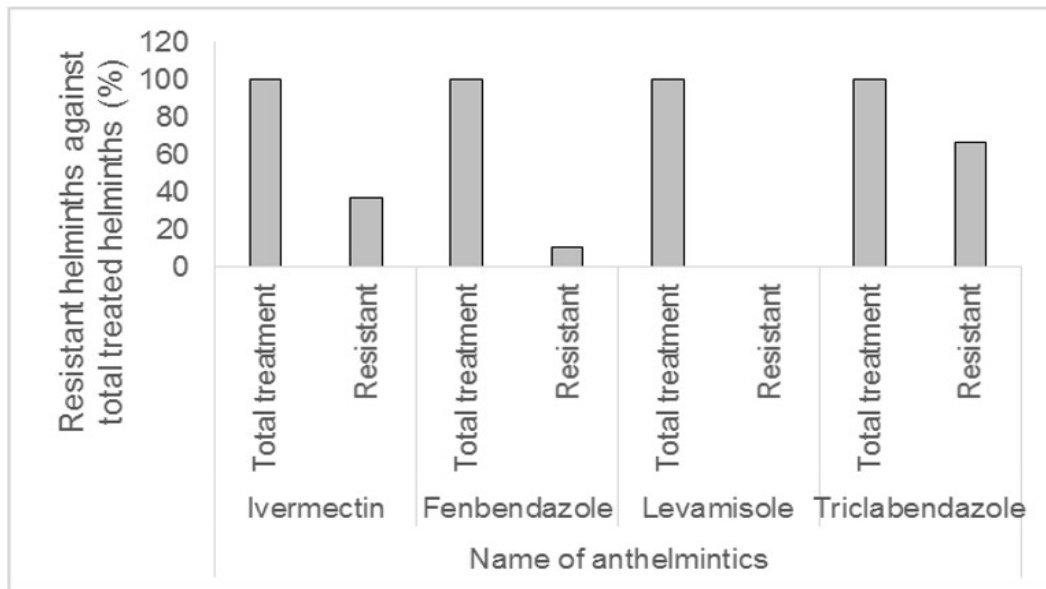


Figure 1: Overall resistance pattern of different anthelmintics in all the five investigated dairy farms.

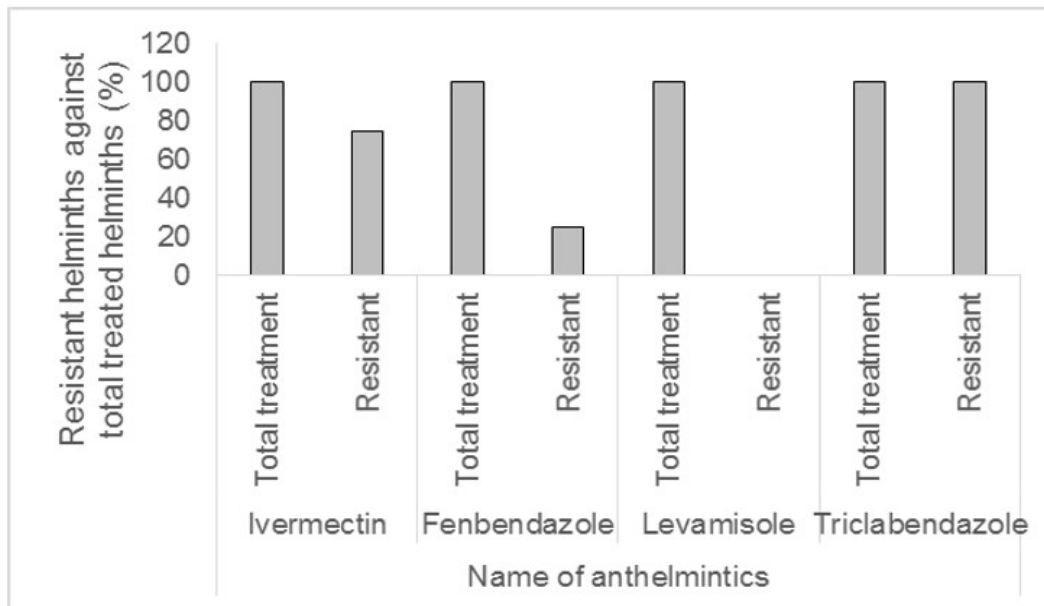


Figure 2: Resistance pattern of different anthelmintics in BSMRAU dairy farm only.

of a previous study (Höglund et al., 2010). This lower rate of infection does not mean that *Fasciola* spp. infection in small-scale dairy cattle could be overlooked. *Fasciola* spp. is an important trematode in dairy cows that affects the liver of the animals and is responsible for global production losses from dairy animals and negatively affects the world economy (Beesley et al., 2018; Schweizer et al., 2005). Furthermore, it was reported that *Fasciola* spp. infected cattle are more prone to *Salmonella dublin* infection (Vaessen et al., 1998). So measures should be taken to prevent infection of *Fasciola* spp. in dairy cows.

Among all the 5 (five) investigated farms, except the BSMRAU dairy farm, all the cows of the other 4 (four) farms were fully susceptible to all the tested anthelmintics (Table

2). Thus Fenbendazole, Ivermectin, Triclabendazole, and Levamisole are cent percent effective against *Hemonchus* spp., *Neoscaris* spp., *Capillaria* spp., *Fasciola* spp., and *Trichuris* spp. in naïve dairy cows. This indicates that there was no development of anthelmintic resistance against used anthelmintics in the other four farms (Table 2) except the BSMRAU dairy farm. The most likely reason is that the farmers of those 4 (four) farms had not used any anthelmintics for the routine treatment of the dairy cows (Table 1) maintaining an adequate level of helminth refugia (proportion of helminth population that are not exposed to used anthelmintics of this study). Thus *Hemonchus* spp., *Neoscaris* spp., *Capillaria* spp., *Fasciola* spp., and *Trichuris* spp. of four farms were not exposed to Fenbendazole, Ivermectin, Triclabendazole, and Levamisole previously. As a

result, the anthelmintic resistance genes were not developed in helminths of other 4 (four) farms as evidenced by a previous study (Shalaby, 2013). As a result, these helminths of 4 (four) farms (except BSMRAU dairy farm) are fully susceptible to anthelmintics used in the present study.

Among all the 5 (five) farms, the BSMRAU dairy farm showed < 95% egg reduction of *Neoscaris* spp., *Trichuris* spp., and *Fasciola* spp. against Fenbendazole & Ivermectin, Ivermectin, and Triclabendazole (Table 2 and Figure 2) respectively. These findings indicate that there is the development of anthelmintic resistance helminths in the BSMRAU dairy farm. This form of anthelmintic resistance against different helminths could be considered as a limitation for the effective control of these nematodes in dairy cows. The probable reason is that this farm was dewormed three times to all the dairy cows routinely (3–4 months interval) before our experiment without identifying parasite-infected cattle (Table 1). From the present findings we can conclude that a subsequent third-time indiscriminate treatment in dairy cows using different types of anthelmintics might cause the development of resistant *Neoscaris* spp., *Trichuris* spp., and *Fasciola* spp. against Fenbendazole, Ivermectin, and Triclabendazole, respectively in dairy cows significantly. Previously development of anthelmintic resistant helminths (Geurden et al., 2015; Kelley et al., 2020; Cristel et al., 2017) and antibiotic-resistant bacteria (Sumon et al., 2018; Islam et al., 2008; Islam et al., 2011) due to indiscriminate use of anthelmintics and antibiotics, respectively have also been reported which supports our present findings.

This type of indiscriminate use of anthelmintics will create serious conditions for the maintenance of the health of dairy cows along with public health implications. For overcoming this problem, detection of infected cattle and specific anthelmintic treatment of the infected cattle are suggested (Vercruyse et al., 2009). Anthelmintic treatments using a combination of two or three anthelmintics might delay the development of anthelmintic resistance in dairy cows as evidenced by previous studies (Leathwick and Besier, 2014; Leathwick et al., 2012; Bartram et al., 2012; Dobson et al., 2011). Besides these, the development and application of non-anthelmintic methods of helminth control could be considered as one of the best time demanding approaches to avoid indiscriminate treatment using various anthelmintics and subsequent avoidance of anthelmintic and/or drug resistance in farm animals.

The resistant helminth against Levamisole in BSMRAU dairy farm was not developed after a third time indiscriminate treatment using different types of anthelmintics which indicates that Levamisole resistance develops at a slower rate than Fenbendazole, Ivermectin, and Triclabendazole

in dairy cows. The probable reason for the different findings on Levamisole might be due to the immune stimulant properties of Levamisole (Mansour, 2018). These findings indicate that Levamisole is still effective for the treatment of dairy cows in Gazipur, Bangladesh. Previously it was also reported that Levamisole was fully effective against *Cooperia* spp. in dairy cows in Western Australia (Cotter et al., 2015), Europe (Demeler et al., 2009), and Argentina (Suarez and Cristel, 2007) which is similar to our present findings.

Anthelmintic resistance is a global problem in the livestock industry with severe consequences in emerging countries because of indiscriminate use and huge consumption of anthelmintics in livestock farming, lack of education, lack of awareness, compromised immunity, inability to purchase more effective but costly anthelmintics, underdosing, undernutrition, and a habit of self-administration of anthelmintics to the livestock by farmers (Rahman et al., 2018). Previously resistance of *Fasciola* spp. against triclabendazole was also reported (Kelley et al., 2020; Brockwell et al., 2014) which supports our present findings. Resistance to macrocyclic lactone compounds such as Ivermectin and benzimidazoles has also been reported in dairy cattle to nematodes previously (Sutherland and Leathwick, 2011; Geurden et al., 2015; Cristel et al., 2017). So, the choice of these resistant anthelmintics for the treatment of helminths of dairy cows is not judicious. This might cause treatment failure and ultimately create more severe consequences. Choice of alternate anthelmintics, limited use of anthelmintics following strict regulations, educating the farmers and health care professionals about the negative effects of anthelmintics might reduce anthelmintic resistant helminths in the livestock production systems.

One of the limitations of the present study is the fewer samples. Future studies should be directed to finding the effectiveness of the local anthelmintics in dairy cows using huge samples. Determination of anthelmintic resistance using faecal worm egg reduction count alone is prone to uncertainty in cattle (Geurden et al., 2015). Factors such as the quality of anthelmintic drugs, determination of the weight of the animal for the selection of accurate dose to avoid underdosing, and loss of anthelmintics during drenching are also crucial. But the authors believe the findings and views expressed in this paper are crucial in reducing anthelmintic resistance in dairy farming systems. But every nation should undertake and effectively implement an anthelmintic efficacy testing program following standard guidelines to detect new anthelmintic resistant helminths to prevent the spread of resistant helminths globally through animals, animal products such as milk, meat, and the environment.



Zero grazing in pasture land and limited green grass supply from highland in small-scale dairy farming causes moderate helminth infections in dairy cows. Naive dairy cows are fully susceptible to Ivermectin, Fenbendazole, Levamisole, and Triclabendazole therapy. A subsequent third-time indiscriminate treatment using anthelmintics did not develop Levamisole-resistant helminths in dairy cows. But a subsequent third-time indiscriminate use of different types of anthelmintics for the treatment of helminths might cause the development of Ivermectin, Fenbendazole, and Triclabendazole resistant *Neoscaris* spp., *Trichuris* spp., and *Fasciola* spp. significantly in dairy cows. Thus, non-anthelmintic robust and sensitive approaches to helminth control are crucial to developing anthelmintic free dairy production systems and to avoiding the emergence of anthelmintic resistant helminths in dairy cattle globally.

## FUNDING

This research was funded by Research Management Committee (RMC), Bangabandhu Sheikh Mujibur Rahman Agricultural University, grant number RMC Project No. UGC/RMC/49 (section 7) 2015-16" and "special allocation for R&D projects supported by the Ministry of Science and Technology, Government of the People's Republic of Bangladesh, grant number 39.00.0000.009.06.009.20-1331/150BS (08 December 2020) for the fiscal year 2020-2021.

## DATA AVAILABILITY STATEMENT

All data of this manuscript are included herewith the manuscript.

## ACKNOWLEDGMENTS

The support from dairy farmers and their attendants during the field experiment was greatly acknowledged.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## AUTHOR CONTRIBUTIONS

Conceptualization, M.A.I.; methodology, M.A.I.; software, M.A.I.; validation, M.A.I., S.M.M.R.S., and F.K.;

formal analysis, M.A.I.; investigation, M.A.I.; resources, M.A.I.; data curation, M.A.I.; writing—original draft preparation, M.A.I.; writing—review and editing, M.A.I., S.M.M.R.S., and A.N.M.A.R.; visualization, M.A.I.; supervision, M.A.I.; project administration, M.A.I.; funding acquisition, M.A.I. All authors have read and agreed to the published version of the manuscript.

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