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Article

Status of anthelmintic resistance of gastrointestinal nematodes in organized sheep and goat farms

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Abstract: Anthelmintic resistance has become a global problem that threatens livestock production worldwide. The present study was investigated the status of anthelmintic resistance in gastrointestinal (GI) nematodes of small ruminants in two organized sheep and goat farms in two different areas of Bangladesh by fecal egg count reduction test (FECRT) for albendazole, levamisole and ivermectin. In each farms, naturally infected animals were divided into four groups of 10 animals. Fecal samples from each group were collected on day 0 and day 14 of post treatment to measure the eggs per gram of feces (EPG). The fecal samples of each group before and after treatment were also considered for culture to identify resistant parasite. In case of organized sheep farm, the result of FECRT of albendazole was 90.17, 95% confidence with upper and lower limit was 97.82 and 55.68, respectively. The result of FECRT of levamisole and ivermectin was 98.25 and 96.77, 95% confidence with upper and lower limit was 99.79, 85.12 and 99.11, 88.31, respectively. In case of organized goat farm, the result of FECRT of albendazole was 100, 95% confidence with upper and lower limit was 0 and 0, respectively. The result of FECRT of levamisole and ivermectin was 97.99 and 100, 95% confidence with upper and lower limit was 99.59, 90.28 and 0, 0 respectively. The results revealed that gastrointestinal nematodes were found to be resistant to albendazole in organized sheep farm and suspected to be resistant to levamisole and ivermectin. In organized goat farms, the GI nematodes found to be susceptible to all anthelmintics used for this study. Coproculture revealed that *Haemonchus* spp. were resistant parasite in sheep farm. This seems to be the first documentation of anthelmintic resistance against GI nematodes in organized sheep and goat farms in Bangladesh. Appropriate measures need to be taken to overcome the situation.

Keywords: gastrointestinal nematodes; anthelmintic resistance; FECRT; sheep and goats; coproculture

1. Introduction

Gastrointestinal parasitic infection is a serious threat to small ruminant production systems. In fact, most of the economic losses caused by internal parasites are actually due to associated production losses in terms of decreased milk and wool production, poor hair coat, cost of prevention, cost of treatment and the death of infected animals (Gordon, 1974). The degree of parasitism or worm burden greatly depends on the management and hygienic conditions of the area. Anthelmintic resistance (AR) is becoming a significant concern in the world and this phenomenon severely threatens the potential control strategy of GI nematodes. Sheep and goat rearing has been a major source of income especially to the marginal farmers of the country. Control of gastrointestinal parasites is mainly achieved by the use of anthelmintic drugs and it will continue to remain, as there seems to be no other alternative for helminth control in small ruminants. Different classes of anthelmintics are available to control these helminth infections. There are three major classes of drugs used to control nematodes in livestock: benzimidazoles (albendazole), nicotinic agonists (levamisole) and macrocyclic lactones (ivermectin). The widespread use, incorrect dosing and increased frequency of treatment have led to the development of resistance (Coles, 1986; Waller, 1986) against anthelmintics in GI nematodes of sheep and goat in organized farms (Kumar and Yadav, 1994). Multiple resistances to most of the anthelmintics against gastrointestinal nematodes have also been detected in many countries. There have been several reports of anthelmintic resistance from different states in India, mainly from organized sheep and goat farms (Meena and Singh, 2005). Reports of anthelmintic resistance in GI nematodes have been made from South Africa, Australia, NewZeland, Malaysia, Spain, France, Denmark, UK, Brazil and the United States (Mortensen *et al*., 2003). The increasing occurrence of AR in helminth populations threatens the sustainability, as well as the efficiency of livestock production. Detection of anthelmintic resistance requires sensitive tools which can enable diagnosis of AR in a very early stage. At present scenario in vivo, in vitro and molecular tests are in use for detecting AR (Coles *et al*., 1992; Le Jambre, 1976). In vivo tests consist of fecal egg count reduction test, critical anthelmintic test. In case of in vitro technique egg hatch assay, larval paralysis test, larval development assay, larval migration inhibition assay, micro motility meter test, larval feeding assay are used for detection of AR. Among the in vivo tests, faecal egg count reduction test (FECRT) can best be used to evaluate anthelmintic efficacy in commercial flocks and herds (Kumar *et al.,* 2013). The simple and practical method for detection of AR is FECRT. It is used to detect AR to all drug classes against nematodes **(**Denwood *et al.,* 2010). The FECRT is still gold standard for detection of AR in GI nematodes of small ruminants (Demeler *et al.,* 2009). Anthelmintic resistance in Bangladesh has not yet been investigated established data in organized sheep and goat farms as well as in veterinary field condition. Therefore, the present study was done to find out the existence of anthelmintic resistance in organized sheep and goat farms.

2. Materials and Methods

2.1. Experimental animals

The study was carried out on an organized sheep farm of Tarail upazila at Kishoregonj district and an organized goat farm of Gopalpur upazila at Tangail district. The farm had a history of irregular deworming and no fixed regimes of deworming were followed. At the time of the start of the experiment there had been no history of deworming in the previous 12 weeks.

2.2. Fecal egg count reduction test (FECRT)

FECRT was performed according to the guidelines of World Association for Advancement of Veterinary Parasitology (Coles *et al*., 1992). The study was conducted on a total of forty animals in each farm. All selected animals had fecal egg counts of more than 200 per gram of feces (epg). The animals were divided into four groups (Groups A, B, C and D) of ten animals each, irrespective of age, sex and weight. Group A, B and C were treated with albandazole (Almex® 600mg, Square Pharmaceuticals) at a dosage rate of 7.5mg/kg body weight orally, levamisole (Endex®, Elanco Pharmaceuticals) at a dosage rate of 7.5 mg/kg body weight orally and ivermectin (Inj. Vermic®, Techno Drugs Pharmaceuticals) at a dosage rate 0.2mg/kg body weight subcutaneously, where Group D was kept as the untreated control. Fecal samples were collected from each experimental animal individually in separate plastic containers on day 0 and day 14 post-treatment. The samples were brought to the laboratory and processed for EPG determination using a modified McMaster technique (Coles *et al.*, 1992). Resistance to a particular classes of anthelmintic was considered to be present if the percentage reduction in egg count was less than 95% and also the 95% confidence was less than 90%. If only one of the two criteria was met out, the resistance was classed as suspected. The data were analyzed statistically as described by (Coles *et al.,* 1992). Pooled fecal culture samples from each group considered for culture on days 0 and 14 post treatment and larvae were collected using the standard Barman’s apparatus. The larvae were identified according to the keys and description of Taylor *et al*. (2007).

3. Results

In the present study, the efficacy was evaluated of the three most commonly available and frequently used anthelmintics for sheep and goats farms in two distinct region of Bangladesh. On organized sheep farm, the percentage reduction in FEC with albendazole, levamisole and ivermectin was 90.17, with a 95% CI of (97.82-55.68), 98.25 with a 95% CI of (99.79-85.12) and 96.77 with a 95% CI of (99.11-88.31) respectively. In organized goat farm, the percentage reduction in FEC recorded with albendazole, levamisole and ivermectin, was 100 with a 95% CI of (0-0), 97.99 with a 95% CI of (99.59-90.28) and 100 with a 95% CI of (0-0) respectively. The results revealed that gastrointestinal nematodes were found to be resistant to albendazole (Group A) in organized sheep farm and suspected to be resistant to levamisole (Group B) and ivermectin (Group C). In organized goat farm, it was susceptible to all althelmintics. The status of resistance was assessed by considering the percentage reduction of fecal egg count and 95% CI value (Table 1). Before treatment, the fecal culture revealed *Haemonchus* spp. as the predominant parasite with an occurrence rate of 85% followed by *Trichostrongylus* spp. (7%), *Oesophagostomum* spp. (5%), *Bunostomum* spp. (2%) and *Strongyloides* spp. (1%) (Table 2). After 14 days of treatment, only *Haemonchus* spp. were detected as the predominant and resistant species in organized sheep farm.

Table 1. Result of FECRT estimating the status of AR in organized sheep and goat farm.

Name of Farm	Anthelmintic used	% FECR	95% C I upper limit	95% C I lower limit	Status
Sheep Farm, Tarail, Kishoregonj	Albendazole	90.17	97.82	55.68	R
	Levamisole	98.25	99.79	85.12	SR
	Ivermectin	96.77	99.11	88.31	SR
Goat Farm, Gopalpur, Tangail	Albendazole	100	0	0	S
	Levamisole	97.99	99.59	90.28	S
	Ivermectin	100	0	0	S

FECR: Fecal egg count reduction, CI: Confidence interval, R: Resistance, SR: Suspected resistance, S: Susceptible.

Table 2. Gastrointestinal nematodes larvae in cultures obtained from sheep and goat farms before and after treatment.

Name of farm	Before treatment in culture	After treatment in culture
Sheep farm, Tarail, Kishoregonj	*Haemonchus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichostrongylus* spp., *Bunostomum* spp.	*Haemonchus* spp.,
Goat farm, Gopalpur, Tangail	*Haemonchus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichostrongylus* spp., *Bunostomum* spp.	*Haemonchus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichostrongylus* spp., *Bunostomum* spp.

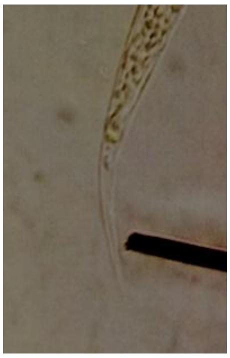

Figure 1. Identified resistant larva (*Haemonchus contortus*).

4. Discussion

Anthelmintics are used traditionally as an integral part of helminth control strategies for grazing livestock to prevent production losses from parasitic infections. The indiscriminate and frequent anthelmintic treatments, use of anthelmintics with a similar mode of action for several years are thought to contribute to the development of AR in livestock species (Verma *et al.,* 2018; Coles and Roush, 1992). The reduced efficacy of albendazole and levamisole drugs against GI nematode parasites in small ruminant has been well documented by (Yadav and Uppal, 1992; Yadav *et al.*, 1995; Ram *et al.,* 2007). Contrary to these, ivermectin was found to be most effective against GI nematodes in both sheep and goat farms in the present study. This might be attributed to the less frequent use of ivermectin for deworming in the studied farm. However, albendazol was found to be less effective and resistance against GI nematodes in one of the studied farm. Levamisole was also less effective in both studied farms. The highest potency of albedazole and levamisole against gastrointestinal nematode parasites in goats was recorded by Gill (1996) and (Ram *et al.,* 2007). This difference in results may be due to the past higher frequency of use of albendazol in the studied farm for deworming which causes the initiation of resistance in parasites. There are comparatively fewer reports of resistance against ivermectin in domestic animals from the Indian sub-continent (Vieira *et al.,* 1992; Miller and Barras, 1994; Ranjan *et al.*, 2002; Makawana and Singh, 2009). However, there seems to be no earlier documentation of ivermectin resistance in goats against gastrointestinal nematodes from the Indian sub-continent. Although full resistance has not been reported, nevertheless, the present study may act as a frontier in the field of anthelminthic resistance against albendazol, levamisole and ivermectin in gastrointestinal nematodes of sheep and goats from private sector in Bangladesh. The level and type of anthelmintic resistance in the parasites on two farms in the present study appeared to be the frequency of anthelmintic used with the management practices on the farms. The role of management practices and the frequent use of anthelmintics are very important factors for the development of resistance (Barton, 1980; Martin *et al.,* 1982). The selection pressure exerted by regular use of anthelmintic is responsible for the development of anthelmintic resistance. The occurrence of anthelmintic resistance against the commonly used anthelmintics due to their frequent use has also been reported previously in sheep (Green *et al.*, 1981; Yadav *et al.,* 1995; Sing and Yadav, 1997) and in goats (Uppal *et al.,* 1992; Singh and Yadav, 1997; Waruiru *et al.,* 2003). Another factor which may have contributed to the high worldwide prevalence of anthelmintic resistance in small ruminants is the common use of the sheep dosage of these products in both sheep and goats (Conder and Campbell, 1995). In this situation, resistance nematodes may have been transmitted from goats to sheep, if they were grazed together or sequentially on the same pasture during the same year or in the following years. Coles (1997) recommended that goats require higher dosage of anthelmintics than sheep to achieve similar efficacy against GI nematodes. In the present study, sheep and goats of both two farms were grazed together or sequentially on the same pasture and received similar dosages of anthelmintics. Hence, constant monitoring for anthelmintic resistance is essential in both sheep and goat farms to determine the effectiveness of anthelmintics before their use, where resistance has not already emerged. This in turn is expected to help in taking timely measures to be taken to prevent or to delay the occurrence of anthelmintic resistance based on minimum anthelmintic usage.

5. Conclusions

The prevalence of anthelmintic resistance of GI nematodes in small ruminants continues to increase. The present study was performed to assess the status of AR of GI nematodes in sheep and goat farms by FECRT. Multiple anthelmintic resistances was found in sheep farm and *Haemonchus* spp. was the resistance parasites. The present study suggested that rotational use of different groups of anthelmintics would be helpful to prevent AR. Hence, there is need for the use of molecular technologies particularly PCR-based detection of AR dealing with identification of particular alleles may help to provide clear information on AR.

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Conflict of interest

None to declare.

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