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ANTHELMINTIC RESISTANCE TO CATTLE GASTROINTESTINAL NEMATODES IN SELECTED DAIRY FARMS OF MYMENSINGH AND SIRAJGANJ DISTRICTS OF BANGLADESH

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ABSTRACT

Anthelmintic resistance (AR) to commonly used dewormers is one of the major world-wide constrain in livestock production. The present study was investigated the status of AR in BAU dairy farm, Mymensingh and Talukder dairy farm, Sirajganj. Faecal egg count reduction test (FECRT) was applied to assess AR in cattle of two dairy farms during January to June 2017. The anthelmintics tested were Albendazole (ABZ), a benzimidazole anthelmintic (Almex®, Square Ltd.) and Ivermectin (IVM) (Vermic®, Techno drugs Ltd.), administered at the doses recommended by the manufacturers. In each farm, cattle were divided into treatment and control (not treated) group based on faecal egg counts (FEC), that is at least 200 eggs/g. At 14 days after treatment, faecal samples were collected for post-treatment FEC, which is compared between treatment and control group. Resistance was defined if there was <95% reduction, with lower 95% confidence limit (CL) <90% in the FEC. AR was present in both the dairy farms involved in this study. The FECRT using ABZ revealed 79.7% (95% CL 87.9, 65.8) reduction and 95.8% (95% CL 98.7, 87.1) reduction of FEC in BAU and Talukder dairy farms, respectively. Also, FECRT using IVM revealed 77.9% (95% CL 97.7, 85.5) and 94.2% (95% CL 97.7, 85.5) reduction of FEC in BAU and Talukder dairy farms, respectively. Our study suggest that AR is present in both selected dairy farms and further extensive studies are required to determine the extent of AR in different cattle farms of Bangladesh.

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INTRODUCTION

Anthelmintic resistance (AR) in gastrointestinal (GI) helminth population is now widely recognized emerging problem in many parts of the world. Anthelmintics offer a simple, cheap, cost-effective method of controlling nematodes. They kill existing parasites and reduce the production of eggs. Therefore they can prevent disease in infected animals and reduce the intensity of future infection in infected animals and their offspring. There are three major classes of drugs used to control nematodes in livestock: benzimidazoles (ABZ), nicotinic agonists (levamisole) and macrocyclic lactones (IVM). Unfortunately, resistance is evolving in nematode populations to all three classes of anthelmintic (Bartley et al. 2004).

Animals raised under grazing conditions are highly susceptible to various parasites and the primary control method is through the use of broad-spectrum anthelmintics (Waller, 1997). This continual use has led to the selection of populations of drug resistant worms worldwide. Most producers generally use anthelmintics liberally without any rational strategy. This situation has led to the development of AR. Prichard (1980) defined resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable. AR has been well documented in the small ruminant industry, in which GI nematodes resistant to all classes of anthelmintics have been used (Waller, 1997; Stafford and Coles, 1999; Pomroy, 2006). Alongside, several surveys indicate widespread resistance to one or more of the broad spectrum anthelmintics in cattle also (Coles, 2002a; Kaplan, 2004; Le Jambre, 2006). Sutherland and Leathwick (2011) reported resistance in cattle to both benzimidazole (ABZ) and macrocyclic lactone compounds (IVM). There have been increasing circumstantial reports of anthelmintic drug resistance in cattle to benzimidazoles in New Zealand (Vermunt et al. 1995; Hosking et al. 1996), to IVM in Great Britain (Stafford and Coles, 1999), to both IVM and ABZ in Argentina (Anziani et al. 2004), and to both benzimidazoles and IVM (Sutherland and Leathwick, 2011) in New Zealand.

The present study conducted faecal egg count reduction test (FECRT), which, remains the only proven way of detecting AR in the field. FECRT could be used as a potential method to treat helminths effectively. In this method animals are treated on the basis of mean faecal egg count which is correlated with worm burden (Demeler et al. 2009). There is lack of investigation on AR in Bangladesh, only a single report has been made (Hoque, 2003). Hence, the objective of the present study was thus to investigate AR to GI nematodes in cattle, and, also to study of safe therapeutic use of anthelmintics against parasitic infestation.

MATERIALS AND METHOD

Sample collection and processing

Faecal samples were collected directly from the rectum of each animal in pre-labelled vials containing 10% formalin at the first farm visit. The samples were processed in the laboratory of Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, within 24 h after faecal sample collection and examined by using a modified McMaster technique (Coles et al. 1992).

Fecal egg count reduction test (FECRT)

The study followed the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al. 1992, 2006). On day 0, Cattle with faecal egg counts (FEC) >200 eggs per gram (EPG) of feces were randomly distributed into 3 treatment (1-3) groups and 1 control (untreated) group. Group-1 was treated with Albendazole, Group-2 was treated with Ivermectin and Group-3 was untreated (Control). The anthelmintics tested were Albendazole (ABZ), a benzimidazole anthelmintic (Almex-Vet®, Square Pharmaceuticals Ltd.) and Ivermectin (IVM) (Vermic®, Techno Drugs Ltd.), administered at the doses recommended by the manufacturers. At 14 days post-treatment, faecal samples were collected for determining the FEC for calculating the faecal egg count reduction (FECR).

Statistical analysis

Pre-treatment and post-treatment arithmetic mean of FEC was used to calculate the FECR % using the following formula:

FECR % = 100 (1− $\bar{x}_t$ / $\bar{x}_c$), where $\bar{x}_t$ represents arithmetic mean of FEC of treatment group and $\bar{x}_c$ represents arithmetic mean of FEC of control group.

Interpretation of results

Resistance was defined if there was <95% reduction, with lower 95% confidence limit (CL) <90% in the FEC. If only one of the two criteria was met out, the resistance was classed as suspected, but if, both criteria were positive, then it strongly indicates the presence of AR (Coles et al. 1992).

RESULTS

The FECRT (conducted with ABZ and IVM) results for the cattle of BAU and Talukder dairy farm in Mymensingh and Sirajganj are presented in Table 1-2. The study indicated the presence of AR in both selected dairy farms.

Faecal egg count reduction test (FECRT) ABZ

The FECR % for ABZ revealed 79.7% (95% CL 87.9, 65.8) reduction and 95.8% (95% CL 98.7, 87.1) reduction of FEC in BAU dairy farm, Mymensingh and Talukder dairy farms, Sirajganj, respectively. ABZ had high level of resistance in BAU dairy farm, Mymensingh, but, in Talukder dairy farm, ABZ resistance was suspected because only lower level of CL was <90% (Table 1).

Faecal egg count reduction test (FECRT) IVM

The FECR % for IVM revealed 77.9% (95% CL 86.1, 64.9) and 94.2% (95% CL 97.7, 85.5) reduction of FEC in BAU dairy farm, Mymensingh and Talukder dairy farms, Sirajganj, respectively. IVM showed high level of resistance in both the dairy farms (Table 2).

Table 1. Faecal egg count reduction with percentage reductions and 95% confidence limits estimating the status of albendazole resistance in cattle

Name of farms	FECR %	95% CL	Status
BAU dairy farm, Mymensingh	79.7	(87.9, 65.8)	Resistance
Talukder dairy farm, Sirajganj	95.8	(98.7, 87.1)	Suspected resistance

Legend: FECR % = Faecal egg count reduction percentage, CL= confidence limits

Table 2. Faecal egg count reduction with percentage reductions and 95% confidence limits estimating the status of ivermectin resistance in cattle

Name of farms	FECR %	95% CL	Status
BAU dairy farm, Mymensingh	77.9	(86.1, 64.9)	Resistance
Talukder dairy farm, Sirajganj	94.2	(97.7, 85.5)	Resistance

Legend: FECR % = Faecal egg count reduction percentage, CL= confidence limits

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). Additionally, from different reports it has been found that, repeated sub-therapeutic treatment with anthelmintics in cattle and sheep can lead to develop resistant nematode populations (Bhinsara et al. 2018; Van Zeveren et al. 2007).

In the current study, AR was present in both the dairy farms. The current finding was supported by Hoque et al. (2003) who also reported AR in BAU dairy farm, Mymensingh. However, the estimated prevalence of AR varied by region, anthelmintic class and host. Most of the cases of AR in bovine nematodes were due to macrocyclic lactone type (e.g. Ivermectin) anthelmintics (Coles, 2002b). Although, resistance to ABZ (Bhinsara et al. 2018, McKenna 1991, 1996) and co-resistance to IVM (Geurden et al. 2015; Anziani et al. 2004) was also reported. IVM resistance (92% and 100%) was most common in cattle nematode than the ABZ (76% and 54%) in New Zealand and Australia (Waghorn et al. 2006; Rendell 2010). In addition, in Brazil, bovine nematodes are mostly resistant to IVM (96%) but lower levels of resistance was found in case of ABZ (24%) (Soutello et al. 2007). Moreover, in Northern Europe, IVM resistance was observed in 70% of 20 farms tested (Demeler et al. 2009). Hoque et al. 2003 first reported anthelmintic resistance in Bangladesh, although report of such resistance problems has been made frequently from adjacent country like India (Verma et al. 2018; Yadav et al. 1995; Dhirendra et al. 1995; Gill, 1996). The level and type of AR in the GI nematodes in different farms appeared to be associated with the type and frequency of anthelmintic used and the management practices followed in the farms.

FECRT alone was used to study anthelmintic resistance in this study without use of confirmatory or supplementary controlled in vivo study and in vitro tests (e.g. hatch assay, larval development assay, tubulin binding test, etc.). Many authors used in vitro tests or controlled in vivo study as confirmatory or supplementary to FECR tests (Wong and Sargison, 2018; Varady and Corba, 1999). FECRT only measures effects on egg production of mature worms, and the output of eggs does not sufficiently correlate with the actual worm burden (Eysker and Ploeger, 2000). Moreover, interpretation of FEC on cattle is difficult due to mixed populations of adult and immature worms of different species and varying egg production over time (Jackson et al. 2006). Martin et al. (1989) demonstrated that the FECRT only detects BZ-resistance in *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in sheep. Advanced PCR tools may be used to underpin extent of resistance, in combination with conventional parasitological techniques (e.g., FECRT) (Roeber et al. 2012a, 2012b).

CONCLUSION

Constant monitoring for anthelmintic resistance is essential both on organized and unorganized farms to determine the effectiveness of anthelmintics before their use, where resistance has not already emerged. Molecular study is also an effective technique for the detection of anthelmintic resistance to GI nematodes in ruminant. Therefore, resistance of anthelmintics against GI nematodes in cattle needs to be assessed throughout the Bangladesh to maintain the efficacy of anthelmintics and to identify risk factors for the development of AR.

COMPETING INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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