

Case Study

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Evaluation of Anthelmintic Resistance of Benzimidazole and Levamisole on Gastrointestinal Nematode Parasites of Cattle

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ABSTRACT

Gastrointestinal nematodes (GINs) resistant to anthelmintics have been reported in several regions of India which may be associated with economic losses for the cattle industry. This study aimed to evaluate the anthelmintic resistance (AR) for gastrointestinal nematodes from naturally infected cattle in Assam to several commercially available anthelmintics like fenbendazole, albendazole and levamisole. The faecal samples were collected per rectum from 325 cattle to screen for detection of GINs and examined qualitatively by floatation and sedimentation technique and quantitatively by Stoll's egg counting method. The parasites found positive were *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Cooperia* and *Mecistocirus* genera. To evaluate AR by FECRT, 24 animals were randomly selected irrespective of sex and age having EPG >200 then divided into four groups consisting of six animals in each group before treatment. The first, second and third group was treated with fenbendazole (@ 5 mg/kg), albendazole (@ 7.5 mg/kg) and levamisole (@ 7.5 mg/kg) body weight, respectively and the fourth group was kept as infected untreated control. Faecal samples were collected per-rectally on 0th day (pre-treatment) and 10 and 14th day post-treatment from animals of all groups and individual faecal egg counts were determined by Stoll's egg counting technique. The study revealed FECR% of 100.00, 85.00 and 90.00% in animals of first, second and third group, respectively. The study indicated that the GINs of cattle have developed BZ resistance and there is need to control it at an earliest with levamisole.

Keywords

Anthelmintic resistance, Benzimidazole, Levamisole, FECRT, Cattle

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Introduction

Trichostrongylids, the gastrointestinal nematodes (GINs) play a significant task in production and productivity of cattle by plummeting their milk and milk production due to poor health condition, anorexia, anaemia, diarrhoea etc (Choudhury *et al.*,

2018a). Among Trichostrongylids, *Haemonchus*, an abomasal nematode plays an important role in immense fiscal losses to livestock through morbidity, mortality, causing decreased reproductive performance, a low growth rate, weight loss, and poor food conversion (De Graef *et al.*, 2013; Bulbul *et al.*, 2015; Bulbul *et al.*, 2016; Allaie *et al.*,

2018). The favourable weather condition and availability of larval stages on pasture act as predisposing factor for gastrointestinal parasitism throughout the year (Bulbul *et al.*, 2011). The highly efficacious BZ class of anthelmintic (Dorny *et al.*, 1995; Hafiz *et al.*, 2011) and levamisole (imidazothiazoles) (Mason *et al.*, 2006; Choudhury *et al.*, 2018b) are widely used to control the GINs more or less throughout the globe. The non-judiciously use of BZ drugs used in field condition ensuing in AR throughout the world (Kaplan, 2004; Bulbul *et al.*, 2018) has been detected in parasitic nematodes of livestock since the early 1960s (Conway, 1964; Drudge *et al.*, 1964).¹ However, it has been reported from many states in India in sheep, goat and cattle *viz.* Haryana (Yadav *et al.*, 1993; Singh *et al.*, 1995), Tamil Nadu (Arunachalam *et al.*, 2005) and Karnataka (Dhanalakshmi *et al.*, 2003) and in cattle (Yadav and Verma 1997; Choudhury *et al.*, 2018b). Thus spread of anthelmintic resistance has directed to major problems in livestock farming industries worldwide (Kaplan, 2004; Cabaret, 2000; Papadopoulos *et al.*, 2012; Torres-Acosta *et al.*, 2012).

Anthelmintic drug resistance has led to the search for alternatives in controlling helminth infections (Ramos *et al.*, 2016). Among the various methods used to detect the AR in livestock (Prichard *et al.*, 1980; Coles *et al.*, 1992; Coles *et al.*, 2006), the faecal egg count reduction test (FECRT), egg hatch assay (EHA) and larval development assay (LDA) have proven to be suitable tests for detecting BZ resistance (Le Jambre, 1976; Coles *et al.*, 1992). FECRT, the commonest nematicide evaluation means (Coles *et al.*, 1992; Coles *et al.*, 2006), has some shortcomings primarily due to the fact that egg count does not correspond to worm burdens. The FECRT can however be standardized by randomization of animals into treatment groups for even dispersion of egg

counts and drug effectiveness, accurate dosing to make sure no administered drug is lost, and proper tagging of animals in each treatment group to ensure correct sampling. To effectively manage roundworm parasites infection in cattle the study was carried out to evaluate the AR against naturally infected GINs in cattle employing FECRT.

Materials and Methods

A total of 146 cattle were selected irrespective of age and sex from organized farm of Assam to screen the GIN infections. The collected faecal samples were brought to the laboratory and proceed to record the prevalence of GINs by the floatation technique and sedimentation technique by qualitatively and quantitatively by Stoll's technique (Soulsby, 1982). After ascertaining the parasitic load (EPG), the therapeutic study was performed as per below mentioned.

Therapeutic study

To evaluate the anthelmintic resistance the animals were then divided into four groups irrespective sex and age having EPG ≥ 200 consisting of six animals in each group before treatment. The first group was treated with fenbendazole @ 5 mg/kg body weight; second group was treated with albendazole @ 7.5 mg/kg body weight; third group was treated with levamisole @ 7.5 mg/kg body weight and the fourth group was kept as infected untreated control. The drugs were given orally in first three groups of animals. Faecal samples were collected per-rectally on the 0th day (pre-treatment) and 10 and 14th day of post-treatment from animals of all groups and faecal egg counts of an individual sample were determined by Modified McMaster technique. The FECR% of the drugs and AR were performed by employing the FECRT as per the standard method (Coles *et al.*, 1992; Coles *et al.*, 2006).

Results and Discussion

The parasitic ova found positive were *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Cooperia* and *Mecistocirus* genera based on egg characteristics. The prevalence of GINs in cattle in organised farm was found to be 41% (58 out of 146). The worldwide distributed GI nematodosis also occurs in Assam (Choudhury *et al.*, 2018a) due to the hot and humid sub-tropical climate which always favours to develop and survive the pre-parasitic stages of nematodes throughout the year especially in pre-monsoon, monsoon and post-monsoon (Bulbul *et al.*, 2016) hence infective larvae are available on the pasture (Bulbul *et al.*, 2011) as a source of infection (Das *et al.*, 2016). *Strongyloides*, *Trichostrongylus*, *Haemonchus*, *Oesophagostomum*, *Cooperia*, *Bunostomum* and *Mecistocirrus* are the most important genera prevalent in cattle of North-eastern India (Latha *et al.*, 2013; Das *et al.*, 2015) including other parts of the country (Bulbul *et al.*, 2014; Allaie *et al.*, 2018) like our observations in the present study.

Faecal egg count reduction test

In the first group mean EPG before treatment was found to be 300. After 10 and 14 days of treatment mean EPG was found to be 0. The FECRT (%) was found to be 100 after treatment of 10 and 14 days. In the second group mean EPG before treatment was found to be 267. After 10 and 14 days of treatment mean EPG was found to be 50. The FECRT (%) was found to be 85 after treatment of 10 and 14 days. From the data calculated according to the formula of FECRT, it was found that the first group that was found to be 100% effective where as the second and third group that are treated with albendazole and levamisole had found to develop resistance. When administered BZs, it inhibit the

polymerisation of α and β -tubulin dimmers to microtubules (Sangster *et al.*, 1985) and restrain the glucose uptake leading to death of worms (Lacey, 1988). Therefore, the BZs are administered to exert their effect on the parasites by binding to the tubulin protein and preventing its polymerization into microtubules (Lacey and Gill, 1994). The use of chemotherapeutic drugs to control internal and external parasites is a widespread practice in livestock production and several broad-spectrum anthelmintics are available in the market for the control of helminthosis. Currently, failure of anthelmintics and reduced efficacy due to resistance of nematodes in cattle are becoming a threat in some countries and are of increasing importance in Indian sub-continent (Bulbul *et al.*, 2018). According to Waller (1987) most of the nematodes of domestic animals have shown resistance to common anthelmintics especially in warm and humid parts of the world and this has been suspected to be due to frequent dosing and poor therapeutic strategies (Waller, 1999; Danquah *et al.*, 2012).

Levamisole, an imidazothiazole derivative, was a good alternative for the treatment of gastrointestinal nematodes at some farms, in line with reports by (Gasbarre, 2014; Choudhury *et al.*, 2018b). Simultaneously, in the third group mean EPG before treatment was found to be 250. After 10 days of treatment mean EPG was found to be 50 and after 14 days of treatment mean EPG was found to be 90. The FECRT (%) was found to be 80 and 64 after treatment of 10 and 14 days. While farmers reported knowledge of levamisole in the present study, it was frequently used, indicating high selection pressure. The levamisole compounds act as depolarising neuro-muscular blocking agents in both nematodes and their host. Levamisole acts selectively as a cholinergic agonist at the synaptic and extra synaptic nicotinic

acetylcholine receptors on nematode muscle cells which cause spastic paralysis of susceptible nematode by selectively gating acetylcholine receptor ion channels on nerve and muscle.

The fourth group which consists of infected untreated control showed the EPG in increasing trends towards the successive days. On 0 day, the mean EPG was found to be 350 and after 10 days it was found to be 433 and after 14 days 483 as previously observed by Choudhury *et al.*, (2018b).

Anthelmintic drugs such as albendazole, ivermectin, and levamisole are used to treat parasitic infection in cattle. The regular use of these drugs and sometimes improper use make the issue of resistance inevitable (Bulbul *et al.*, 2018). According to Shalaby (2013), it has been observed that frequent use of the same group of anthelmintic, use of anthelmintics in suboptimal doses, prophylactic mass treatment of domestic animals, and frequent and continuous use of a single drug have contributed to the widespread development of anthelmintic resistance in helminths as observed in our present investigation.

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