Identification and Diagnostic Procedure of Gastrointestinal Nematode Egg Based on EPG Method on Cattle, Sylhet, Bangaladesh

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A study was conducted to determine Gastrointestinal nematode (GIN) egg count in faeces based on McMaster technique (EPG method) in naturally infected cattle around Sylhet Agricultural University. About 60 cattle were selected and divided into three groups of 20 animals each: the 1st group was treated with albendazole, the 2nd group was treated with levamisole and the 3rd with ivermectin. Faecal samples were collected on day 0 before treatment and again day on 12 post treatment. Pre Treatment Mean EPG±SE for albendazole group was 850±18.137, levamisole group was 650±22.064 and ivermectin group was 550±16.623. After post treatment we got Mean EPG±SE for albendazole group was 100±8.885, levamisole group was 50±8.885 and ivermectin group was 200±17.770. After treatment with albendazole, levamisole and ivermectin it reduced EPG count significantly ($p<0.001$). Further detailed investigations are necessary to clarify the current status in different agro ecology, species of animals and management systems.

Keywords: Gastrointestinal nematode (GIN), McMaster Technique (EPG Method).

1. INTRODUCTION

Gastrointestinal nematode (GIN) infections in cattle are of considerable economic importance, causing clinical disease and mortalities, but more importantly, by causing subclinical chronic production losses as a result of weight loss, reduced weight gain, and reduced milk production. In addition to the impact of these parasites on food production, quality and safety, these infections are also of major animal welfare concern. Gastrointestinal nematode infections cause diarrhoea, emaciation and abdominal pain in affected animals. Helminth infections are an important cause of lost productivity in livestock world-wide, often necessitating anthelmintic treatment. GIN infections have been observed to affect younger cattle more than adults, with the super family *Trichostrongyloidea* having the biggest impact, leading to clinical manifestations including pale mucous membranes due to anemia, poor body condition and reduced immunity [1]. In Africa, a study carried out in Ouagadougou, Burkina Faso, on the prevalence of GIN in cattle showed that *Cooperia* was most prevalent (89.4%), followed by *Haemonchus contortus* (66%), and *Oesophagostomum radiatum* (42.6%), whereas *Haemonchus* became predominant in the rainy season as it was able to withstand harsh

climatic condition through arrested development in the larval stage [2]. However, a large part of the anthelmintics are used indiscriminately because the parasite levels are too low to justify treatment or because the treatments are not correctly programmed, resulting in under treatment or overtreatment. Control of these parasites relies heavily on treatment with anthelmintics. A study done in Zimbabwe on 16,264 communally grazed cattle, by [3], showed the prevalence of GIN to be 43%. Another study carried out in Ngorongoro District of Tanzania on pastoral cattle found the prevalence of GIN to be 20% [4]. The milk yield after anthelmintic treatment on pastured dairy cattle in the Netherlands was estimated to increase by 1 kg/cow/day [1]. There is limited information on the economic impacts of GIN on milk production in Africa.

Anthelmintics are routinely administered to animals on several occasions during the perceived period of parasite threat to livestock. Effective control of these infections is hampered by several factors. Climate change and changing animal husbandry methods are thought to significantly alter the epidemiology of helminth infections. In addition, anthelmintic resistance can reduce the efficacy of the commonly used anthelmintic drugs.

In our research, we aim to understand the impact of helminths on production of cattle and to diagnose the gastrointestinal nematode infection based on McMaster technique (EPG method).

2. OBJECTIVES

1. To understand the impact of helminths on production of cattle.
2. To diagnose the gastrointestinal nematode infection based on McMaster technique (EPG method).

3. MATERIALS AND METHODS

3.1. Study area

The current study was conducted from June 2014 to December 2014 on Cattle of some local farms around Sylhet Agricultural University, Tilagor, Sylhet-3100. The area is elevated actually Tilla based and is characterized by sandy loam soils covered with scattered acacia trees and bushes. The main rainy season extends from April to September EMA [5]. The average minimum and maximum temperatures of the area are 17 and 39°C, respectively. Cattle constitute the major livestock of the area and are managed under traditional husbandry.

3.2. Study animals

Total 60 local cattle of different age were used for the study. The study cattle were taken from some local farms around Sylhet Agricultural University. In addition, each cattle was individually marked with a numbered ear tagged, body weight and body condition score were determined for each cattle as per the methods of AIGR [6]. None of the cattle received any anthelmintic before the start of the study.

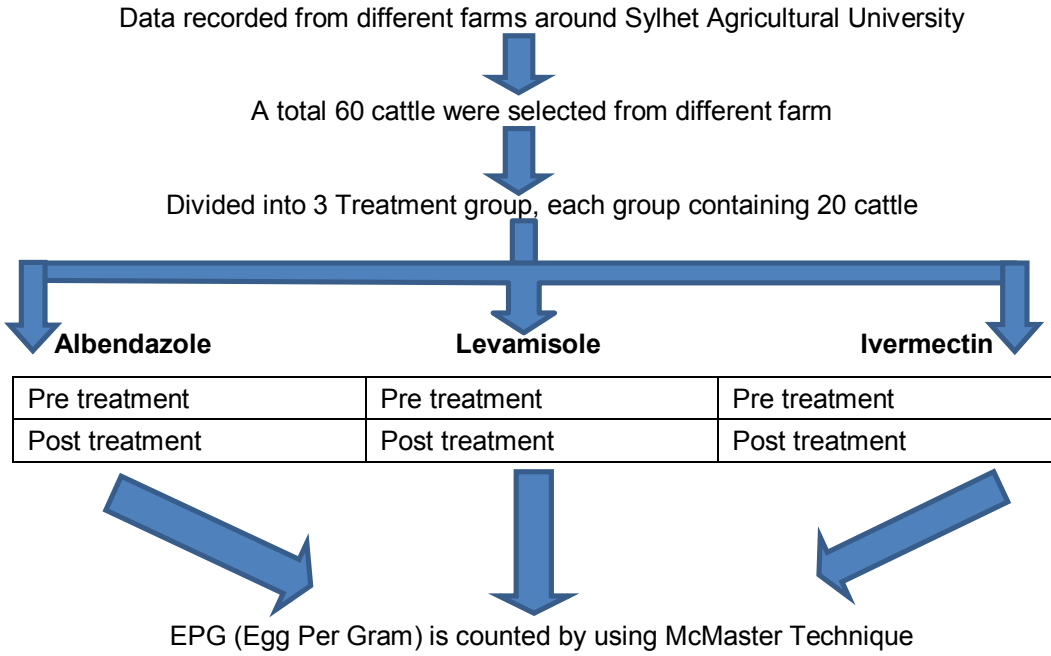
3.3. Questionnaire survey

A questionnaire survey on the use of anthelmintics for cattle was performed on a total of 60 cattle and 3 veterinary drug venders in the study area. Information on the widely used anthelmintics, selection criteria, application interval and sources of drugs, methods of dosage determination and rotation of anthelmintic family were all collected and complied.

3.4. Data analysis

Data was analyzed by SPSS (Statistical Packages for Social Science) version 20.0. Sign rank test was performed to analyze the data set.

3.5. Experimental Design



3.6. Anthelmintics used

Generic Name	Trade name	Dose and Route of administration	Company
Albendazole	Aldazole	1bol/41-75 kg body weight orally	Techno
Levamisole	Levavet	1bol/41-75 kg body weight orally	Acme
Ivermectin	Acimec	1ml/50 kg body weight, S/C	ACI

3.7. McMaster Technique

The McMaster technique uses a counting chamber which enables a known volume of faecal suspension (2 x 0.15 ml) to be examined microscopically.Thus, if a known weight of faeces and a known volume of flotation fluid are used to prepare the suspension, then the number of eggs per gram of faeces (e.p.g.) can be calculated.The quantities are chosen so that the faecal egg-count can be easily derived by multiplying the number of eggs under the marked areas by a simple conversion factor.

The McMaster chamber has two compartments, each with a grid etched onto the upper surface. When filled with a suspension of faeces in flotation fluid, much of the debris will sink while eggs float to the surface, where they can easily be seen and those under the grid counted in Figure 1.

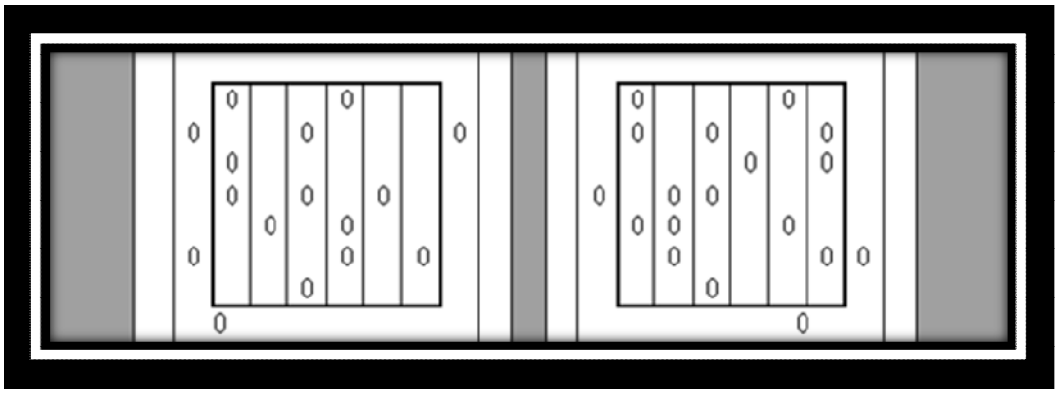


Fig. 1: McMaster slide.

3.7.1 Diagnosis of gastrointestinal nematode based on McMaster technique

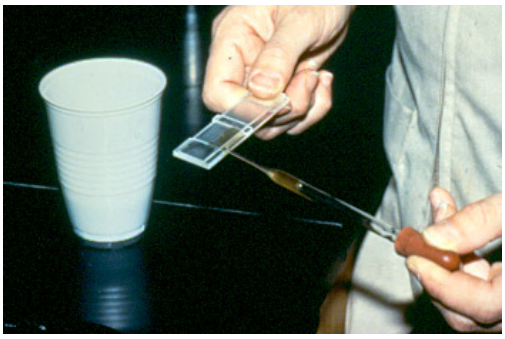
1. Weigh 3 grams of faeces and place into a container.

2. Add 42 ml of NaOH as flotation fluid.

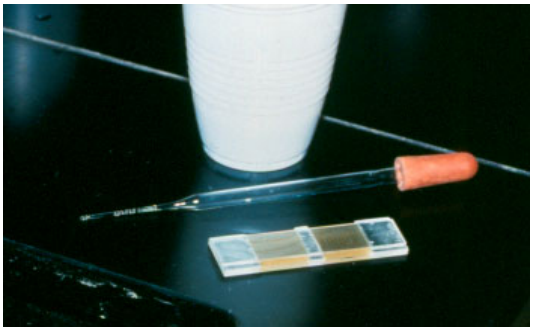
3. Stir the contents of the beaker thoroughly with a spatula.

4. Filter the faecal suspension through a tea strainer.

5. Stir fluid and fill first compartment of the McMaster counting chamber with the sample. Stir fluid again and fill second chamber with the sample.

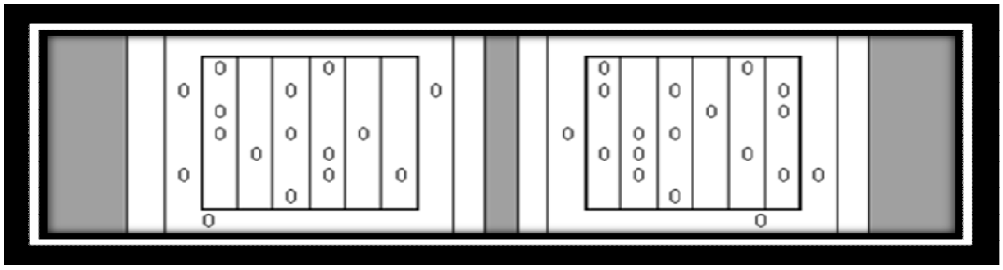


6. Allow the counting chamber to stand for 5 minutes. It is important to leave the chamber to stand to allow the eggs to float to the surface and the debris to go to the bottom of the chamber.



7. Examine the subsample of the filtrate under the compound microscope at 10 x 10 magnifications.

8. Identify and count all eggs within the engraved area of both chambers.



9. 12 eggs seen in chamber 1 and 15 eggs seen in chamber 2

So EPG = (12+15) x 50 = 1350.

4. RESULTS AND DISCUSSIONS

In first grazing season calves, a minority of susceptible animals is shedding high numbers of GI nematode eggs, causing most of the pasture contamination with infective larvae. Treatment of these calves early in the grazing season could protect the whole herd against GI nematode-related production losses. Although the susceptibility of calves to GI nematode infection is genetically determined [7], no genetic markers are currently available to identify these animals. Faecal egg counts early in the FGS may be used as a phenotypic marker for resistance against GI nematodes [8]. In Western Europe, the peak egg output occurs around two months after turnout [9,10].

The current study showed that GIN-infection was associated with 1.5 liters per cow per day less milk [11], after controlling for farm clustering and significant confounders. As milk is the most important output in a dairy enterprise, GINs lead to direct loss in production and income.

When interpreting McMaster results, it must be remembered that a number of factors can influence the occurrence, recognition or numbers of helminth eggs found in a faecal sample. In particular, the number of eggs is not necessarily indicative of the number of worms present. Reasons for this include: Eggs are produced only by fertile adult female (or hermaphrodite) worms and will, therefore, be absent in immature or single sex infections. The daily output of eggs by fertile females is influenced by host-physiological factors such as stress or lactation (increased) or immunity (decreased).

Chemotherapy can also affect egg-production e.g. corticosteroids (increased) or sub-lethal anthelmintic doses (decreased). Some food-stuffs may have similar effect e.g. tannin-rich forages (decreased). The concentration of eggs (per gram of faeces) is influenced by the daily volume of faeces being produced by the host, the rate of passage by the ingesta through the intestine, and the distribution of eggs throughout the faecal mass. Some types of eggs are heavier than others and may not float well in solutions of lower specific gravity (e.g. *Fasciola*)

Some eggs from different species are indistinguishable (particularly trichostrongylids and strongylids). This complicates clinical interpretation as some species (e.g. *Haemonchus*) produce many more eggs per day than others (e.g. *Ostertagia*).

In our study we have collected data from 3 treatment group of both pretreatment and post treatment. We collected faeces then examined it by using McMaster technique (EPG method). In the Albendazole group the pretreatment EPG was 850 and post treatment EPG reduced to 100, in Levamisole group pretreatment EPG was 650 and post treatment EPG reduced to 50 and in Ivermectin group pretreatment EPG was 550 and post treatment EPG was 200.

The result of Mean EPG±SE in both pretreatment and post treatment based on McMaster technique are given in Table1 as:-

Table 1: Mean EPG±SE in both pretreatment and post treatment.

Faecal egg count / **Treatment Group**	**Pre Treatment (Mean EPG ± SE)**	**Post Treatment (Mean EPG ± SE)**
Albendazole	850±18.137	100±8.885
Levamisole	650±22.064	50±8.885
Ivermectin	550±16.623	200±17.770

This tabulated data can be described in bar graphs as given in Figure 2.

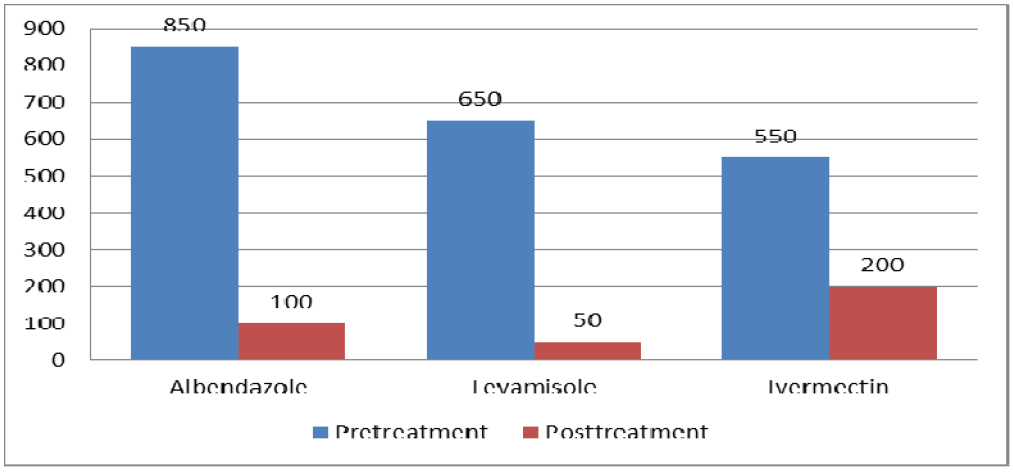


Fig. 2: Average faecal Gastrointestinal nematode (GIT) nematode egg count before and after treatment.

After treatment with albendazole, levamisole and ivermectin it reduced EPG count significantly ($p<0.001$).

5. SUMMARY

Gastrointestinal nematode infection was relatively common (14 to 20%), with a higher prevalence in rainy season, and among calves as compared to adults. The most prevalent genus was *Haemonchus*. Gastrointestinal nematode infection was associated with 1.5 kg per cow per day less milk (W. M. Kabaka, et al., 2013). To avoid such losses in production, efforts should be directed towards gastrointestinal nematode control in groups of animals that are considered at higher risk of infection. Pre Treatment we got Mean EPG±SE for albendazole group was 850±18.137, levamisole group was 650±22.064 and ivermectin group was 550±16.623. After post treatment we got Mean EPG±SE for albendazole group was 100±8.885, levamisole group was 50±8.885 and ivermectin group was 200±17.770. After treatment with albendazole, levamisole and ivermectin it reduced EPG count significantly ($p<0.001$). Further detailed investigations are necessary to clarify the current status in different agro ecology, species of animals and management systems.

REFERENCE

[1] J. Charlier, J. Hoglund, G. von Samson-Himmelsjerna, P. Dorny and J. Vercruyss; “Gastointestinal nematode infection in adult dairy cattle: Impact on production, diagnosis and control”, Vet. Parasitol., Vol. 164(1), pp. 70-79, 2009.

[2] A.M.G. Belem, O.P. Ouedrago and R. Bessin; “Gastro-intestinal nematodes and cestodes of cattle in Burkina Faso”, Biotechnol. Agron. Soc. Environ., Vol. 5(1), pp. 17-21, 2001.

[3] D.M. Pfukenyi, S. Mukaratirwa, A.L. Willington and J. Monrad; “Epidemiological studies of parasitic gastrointestinal nematodes, cestodes and coccidia infections in cattle in highveld and lowveld communal grazing area of Zimbabwe”, Onderstepoort J. Vet. Res., Vol. 74(2), pp. 129-142, 2007.

[4] E.S. Swai, P.F. Mtui, A.N. Mbise, E. Kaaya, P. Sanka and P.M. Loomu; “Prevalence of gastro intestinal parasite infections in Maasai cattle in Ngorongoro District, Tanzania”, Vet. Parasitol., Vol. 117, pp. 117-120, 2006.

[5] EMA (Ethiopian Mapping Authority); “National Atlas of Ethiopia”, Berhanena Selam Printing Press, Addis Ababa, pp. 77, 1988.

[6] AIGR (American Institute for Goat Research); “Body Condition Score Description”, Goat Research and Extension, Langston University, Langston, pp. 73050, 2000.

[7] L.C. Gasbarre, E.A. Leighton and C.J. Davies; “Genetic control of immunity to gastrointestinal nematodes of cattle”, Vet Parasitol., Vol. 37(3-4), pp. 257-272, 1990.

[8] E.A. Leighton, K.D. Murrell and L.C. Gasbarre; “Evidence for genetic control of nematode egg-shedding rates in calves”, J. Parasitol., Vol. 75(4), pp. 498-504, 1989.

[9] D.J. Shaw, J. Vercruysse, E. Claerebout, J. Agneessens and P. Dorny; “Gastrointestinal nematode infections of first-season grazing calves in Belgium: general patterns and the effect of chemoprophylaxis”, Vet Parasitol., Vol. 69(1-2), pp. 103-116, 1997.

[10] D.J. Shaw, J. Vercruysse, E. Claerebout and P. Dorny; “Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis”, Vet Parasitol., Vol. 75, pp. 115-131, 1998.

[11] W.M. Kabakaa, G.K. Gitaub, P.M. Kitalaa, N. Maingic and J.A. Van Leeuwen; “The prevalence of gastrointestinal nematode infection and their impact on cattle in Nakuru and Mukurweini districts of Kenya”, Ethiop. Vet. J., Vol. 17(1), pp. 95-104, 2013.