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Evaluation Commonly Used Anthelmintics Efficacy in Gastrointestinal Nematodes through Fecal Egg Count Reduction Test in Adaberga Dairy Farm, West Shewa Zone, Central Ethiopia

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Abstract

This study was conducted in Adaberga dairy farm West Showa Zone, from November, 2014 to April 2015 to evaluate commonly used anthelmintics efficacy against gastrointestinal nematodes. An experimental study design and purposive sampling procedure were employed to select 36 naturally infected jersey breed cattle from source population. And study populations were randomly allocated into three groups, twelve in each; the first group was treated with albendazole, the second with tetraclozan and the last group was left untreated (control). Fecal samples were collected from each cow before and after treatment and modified McMaster method was used to count eggs. Third stage larvae (L3) were recovered from the fecal cultures by the Baerman technique to identify gastrointestinal nematodes. The efficacy of each anthelmintic was determined by Fecal Egg Count Reduction Test (FECRT). SPSS Windows version 16.0 was used for data analysis. Descriptive statistics (means, standard error of mean and reduction percentages) were calculated to manage data. Means were compared among groups through analysis of variance (ANOVA) and difference between treatments was compared using least square method of multiple comparisons. The percentage reduction in mean fecal egg count, after 10 days of treatment, for Albendazole and tetraclozan were 95.51% and 98.18% respectively. There was no statistically significant difference (p=0.262) among the egg count of control, albendazole treated and tetraclozan treated groups before treatment. Statistically egg counts were not different (p=0.85) between treatment groups but there were strict differences (p=0.00) between treatment and control groups on the post-treatment. Generally, these findings indicate that albendazole and tetraclozan are effective against gastrointestinal nematodes in the study area. But, appropriate use of these anthelmintics is credible to prevent future occurrence of resistance.

Keywords: Anthelmintics; Efficacy; Gastrointestinal nematodes

Introduction

In Western Oromiya region of Ethiopia, agriculture is the mainstay of the smallholder farmers. Mixed crop-livestock production system is largely practiced in this part of the country [1]. Cattle production is an important component and they are kept under traditional management within this farming system. The animals depend mostly on grazing natural pastures for feed sources with scanty supplements and minimum health care interventions [2].

Livestock diseases are one of the major production constraints frequently observed in the region among which helminthes parasites are the biggest causes of production losses. In general, Gastrointestinal (GI) nematode parasites remain one of the most prevalent and important diseases affecting large ruminants worldwide. They are responsible for both direct and indirect major losses [3]. Losses occur through mortalities, reduced production due to subclinical parasitism and direct costs associated with control [4].

Globally, parasitic and other endemic diseases continue to be a major constraint on profitable livestock production. They are rarely associated with high mortality and easily identifiable clinical signs and their effects are usually characterized by lower outputs of animal products, by- products, manure and traction all contributing to production and productivity losses. However, parasitic diseases are repeatedly identified by livestock owners, particularly small ruminant producers, as constraints to animals reaching their full production potential. It is generally accepted that the cost of control of most parasitic and endemic diseases is the responsibility of the animal owner [5].

Ruminants are prone to infection with helminthes parasite throughout the world inflicting heavy economic losses in ruminant industry due to high mortality in addition to reduction in productivity [6]. Parasitic worms infect livestock and crops, affecting food production with a resultant economic impact and they are also important in the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programs [7].

Therapeutics are concerned with the application of drug in the treatment of the disease such details as the choice of drug, the route of administration, the form in which the drug is applied and the frequency of administration. Most of the anthelmintics used for ruminant are identical, with exception, and vary with dose only. The control of parasitic helminths in domestic animals relies largely on the use of anthelmintic drugs [8]. Therapeutic efficacy and anthelmintic drug resistance will allow detection of changing patterns of parasite susceptibility and timely revision of national and global parasite treatment policies [9].

Most of the nematodes of domestic animals possess the capacity to develop resistance to anthelmintic drugs. Resistance to antiparasitic

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drugs in ruminant is rapidly increasing, particularly in warm and humid climatic regions, probably due to frequent dosing and adoption of common management, nutritional, and therapeutic strategies [10].

In livestock production throughout the world, the use of antiparasitic drugs to control internal and external parasites is a widespread practice. The number of domestically available broad spectrum anthelmintic drugs has increased since 1960s. Several anthelmintics with different modes of action are available in the market for the control of helminthosis. Currently, failure of anthelmintics efficacy due to anthelmintic resistance in ruminant becoming a wide-spread threat all over the world. Resistance to anthelmintics has become a major problem in veterinary medicine, and threatens both agricultural income and animal welfare [11].

Gastrointestinal helminth infections are very common in many parts of Ethiopia and their control is almost exclusively based on anthelmintic treatment [12]. In Ethiopia, the use of anthelmintics has been practiced for a long time, taking a considerable share in drug costs spent by the country in the control of animal diseases. Smuggling and misuse of veterinary drugs involving anthelmintics is a widespread practice in the country. Despite the high use of common specific anthelmentic substances in Ethiopia there are scarce reports on the efficacy of theses anthelmintics against economically important parasites [13].

The prevalence and impact of helminth parasites as well as their sensitivity to the commonly used anthelmintics have been studied in small ruminants in many parts of the country [13-15]. On the other hand, no systematic surveys have been carried out to evaluate anthelmintic efficacy in cattle. Therefore, the objectives of this study were; to evaluate the current efficacy of commonly used antheliminthic drugs against GIT nematodes of cattle, and isolate and identify resistant worms.

Materials and Methods

Study area

The study was conducted from November 2014 to April 2015 in Adaberga dairy farm; Central Ethiopia, West Showa Zone of Oromia Regional State, located around 72 km west of Addis Ababa. The area lies at longitude 38° 30' E and latitude 9° 3' N and includes highland and midland agro-ecologies with an altitude of at about 2600 meters above sea level. The site is characterized by cool sub-tropical climate with mean maximum and minimum temperatures of 26°C and 10°C, respectively with mean relative humidity of 59%. The mean annual rainfall ranges from 800 to 1400 milliliters [16].

Study animals and treatments

Total 36 naturally infected female jersey breed cows of four to five years of age with uniform size and weight were used for the study. The cows which have been bred in Adaberga dairy farm were purposively selected to evaluate commonly used anthelmintic efficacy against gastrointestinal nematodes. Animals which have not been treated in the previous 8 to 12 weeks were considered for the study and grouped into control and treatment groups. A control (untreated) group had been used to allow for monitoring of natural changes in egg counts during the test period. The quality of the drug were evaluated by Ethiopian veterinary drug authority for the presence of required active ingredient, and then Animals were treated with the respective anthelmintic dosage as per the recommendations of the manufacturers according to the weight of animal (Table 1).

Generally each animal under the study was identified by ear tag and were randomly allocated into three groups (twelve in each). The first group was treated with albendazole, the second with tetraclozan and the last group was left untreated (control).

The history of the farms indicated that all animals received regular treatments with anthelmintics twice a year at the beginning and end of the long rainy season in June and November, respectively. Additionally, farmers also experienced to treat with anthelmintics based on individual animal exhibiting clinical parasitism. However, this treatment regimen could lack precision in determining the appropriate dosages. The records available indicated that the types and sources of anthelmintics used on the farms included mainly albendazole 2500 mg as well as tetraclozan (Tetraclozan QK Cattle) for cattle were all available on the local markets.

Study design and methodology

An experimental study design was conducted from November 2014 to April 2015 to investigate the commonly used anthelmintics efficacy in GIT nematodes through fecal egg count reduction test in naturally infected cattle of governmental dairy farm (Adaberga dairy farm) in Adaberga district.

Sampling technique

Purposive sampling technique was used as sampling technique to select the experimental study animals based on their age, size and body weight uniformity as well as egg count whereby animals were selected in Adaberga dairy farm and randomly allocated into three groups.

Sampling procedures and laboratory investigation

Faecal samples were collected from each cows for pre-screening of animals for sufficient egg counts, a minimum of 5 gm of faeces was collected from each animal directly from the rectum using rubber glove. The same procedure was followed at the post-treatment sampling. Samples were placed in individually sealed containers and labeled with specific identification mark then returned rapidly to the Holeta livestock research center parasitology laboratory (Addis Ababa) for egg counts. The post-treatment collection of faecal samples from the experimental cows was 10 days after treatment according to Coles et al. [17]. Waiting for at least 10 days after the treatment allows worms that had been impacted but not removed by the drug to reach full egg production again. If the second set of samples is collected more than 10 days after treatment, worms that infected the animals after they were treated would have a chance to mature and start producing eggs [18].

Fecal egg counts and faecal egg count reduction test

For the process of Fecal egg counts modified McMaster method was used according to FAO [5]. The most commonly used field

Generic name	Trade name	Manufacturer	Route of administration	Recommended dose
Albendazole	Albentong 2500	Chongqing fantong animal pharmaceutical co., Ltd, China	Bolus/oral	1 bolus/350 kg or 7.14 mg/kg
Tetraclozan	Tetraclozan QK Cattle	Chengdu QiankumVet Pharma China	Bolus/oral	1 bolus/150 kg or 22.7 mg/kg

Table 1: Detail information about anthelmintic used in the treatment.

detection method for anthelmintic resistance is the Fecal Egg Count Reduction Test (FECRT). This method can be adapted for use as a screening agent for Veterinarians and producers to identify less than desired clearance of the parasites after anthelmintic treatment [19]. The procedure compares the pre-treatment parasite level with the parasite levels after treatment. The efficacy of each anthelmintic was determined by comparing the fecal egg count reduction percentage from a group of animals before and after treatment.

The differences between the two tests were then calculated and reported as a reduction percent. Arithmetic means of pre-treatment and post-treatment fecal egg counts of control and treated groups were used to calculate the percentage efficacy using the following formula according to Coles et al. [17]: FECRT%=(T1-T2)/T1 × 100 where T1 is pre-treatment egg count and T2 is post- treatment egg count. The 95% confidence limits were calculated by using a software program RESO [20]. Anthelmintic resistance was declared to exist when the FECR% was less than 95% and the lower 95% confidence limit for the reduction was less than 90%. If only one of the two criteria was met, resistance was suspected [17].

The FECRT detects clinical cure rather than the total elimination of the parasites [17]. However, there is no direct relationship between the FECRT and the number of resistant worms Whether or not to continue using a drug once a FECRT indicates that a substantial population of resistant worms may be present depends on the situation [21].

Larval identification

About 10 gm faecal samples from each cow were collected on each sampling day (before and after treatment) and composite faecal cultures were made for each group. Small amount of water was added to moisten, and the samples left for 14 days at room temperature in a Petri dish [22], adding small amounts of water as necessary. Third stage larvae (L3) were recovered from the cultures by the Baerman technique and identified according to Hansen et al. [23].

Data management and analysis

While collecting fecal samples from study animals, all data were recorded with pre-designed format and entered in to computer using Microsoft excel spread sheet. All data were analyzed using Statistical Package for Social Sciences (SPSS) version 16 statistical software. Descriptive statistics (means, standard error of means and reduction percentages) were calculated. pre-treatment and post-treatment faecal egg counts were transformed to the natural logarithm and means were compared among groups through analysis of variance (ANOVA) and difference between treatments was compared using least square method of multiple comparisons Arithmetic means of pre-treatment and post-treatment fecal egg counts of control and treated groups were used to calculate the percentage efficacy of anthelimentics by using fecal egg count reduction test (Table 2).

Results

Mean faecal egg counts and percent reduction after treatment

The reduction in mean fecal EPG, after 10 days of post treatment, for Albendazole and tetraclozan were 95.51% and 98.18% respectively. The pre-treatment, post-treatment egg count mean, standard error of mean and the percent reduction in the fecal egg counts are present in Table 3.

There was no statistically significant difference (p=0.262) between the egg count of control and treated groups as well as between the two treated groups, albendazole treated and tetraclozan treated, before treatment.

Statistically post-treatment egg counts and percentage reduction of the drugs were not different (p=0.85) between treatment groups but there were strict differences (p=0.00) in net egg count between treatment and control groups on the post-treatment.

Survivor parasite after treatment

Fecal cultures were conducted parallel to fecal egg count to differentiate strongly type of eggs both in before and after treatments in each group. In Albendazole treated animals the percentage reductions for *Haemonchus* and *Trichuris* were 92.85% and 90.47% respectively and for other parasites 100%. The only survivor parasite in Tetraclozan treated group was *Trichuris* with percentage reduction of 90.65 (Table 4).

Discussion

The result showed that the mean FECR value of Albendazole and tetraclozan were 95.51 and 98.18 percent with the lower 95% confidence interval of 94.29 and 96.4 respectively. Consequently, FECR test indicated that the anthelmintic resistance was not found for any of the tested anthelmintic drugs. Both albendazole and tetraclozan had good drug efficacy in gastrointestinal nematodes in Adaberga dairy farm. As per World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines Coles et al. [17], Resistance is considered if the percentage reduction in egg counts is less than 95 percent and /or the lower 95 percent confidence level is less than 90 percent. If only one of the two criteria is met, resistance is suspected.

Group	Anthelmintics	Pre-treatment coproculture	Post-treatment coproculture
1	Albendazole	*Trichuris, ascaris, Haemonchus, bunostomum, nematodirus*	*Haemonchus, trichuris,*
2	Tetraclozan	*Trichuris, ascaris, Haemonchus, bunostomum, nematodirus*	*Trichuris*
3	Untreated control	*Trichuris, ascaris, Haemonchus, bunostomum, nematodirus*	*Trichuris, ascaris, Haemonchus, bunostomum, nematodirus*

Table 2: Larval composition (L3) of faecal cultures from experimental animals before and after treatments with anthelmintics.

AH	Mean FEC ± SEM		Reduction (%)			95% Confidence interval	
	Pre treatment	Post treatment	Mean ± SEM	Minimum	Maximum	Lower	Upper
ALB	650.00 ± 104.76	29.17 ± 14.38	95.51 ± 1.46	85.00	100.00	94.29	100
TR	683.33 ± 92.34	12.50 ± 8.97	98.18 ± 1.02	88.24	100.00	96.40	100
NRX	808.33 ± 110.75	835.42 ± 106.09	NA	NA	NA	NA	NA

AH: Anthelmintics; ALB: Albendazole; TR: Tetraclozan; NRX: Untreated control; FEC: Faecal Egg Count; SEM: Standard Error of the Mean; NA: Not Applicable.

Table 3: Mean faecal egg count and percent reduction after treatment of cattle using different anthelmintics.

Group	Anthelmintics	Survived parasite
1	Albendazole	*Haemonchus, trichuris,*
2	Tetraclozan	*Trichuris*
3	Untreated control	*Trichuris, ascaris, Haemonchus, bunostomum, nematodirus*

Table 4: Survivor parasite after treatment with anthelmintics.

The result of FECR test showed that the efficacy of albendazole in this finding was similar with the studies done by Demeler et al. [24] in Germany on monitoring the efficacy of ivermectin and albendazole against gastro intestinal nematodes of cattle North Europe.

Though, anthelmintic resistance is widely accepted in small ruminants based upon the general assumption that cattle are usually less frequently dosed than small ruminants, there are anthelmintic resistance in gastrointestinal nematodes of cattle according to the study done by Gasbarre et al. [19] in US on the identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US.

Most research works regarding to anthelmintic resistance and drug efficacy for GIT nematodes in Ethiopia and most other world have been concerned in small ruminants. The finding of this study is in contrast with that of Bersisa and Abebe [25] observations who reported the presence of resistance in nematodes of small ruminants owned by Hawassa and Haromaya Universities. The disagreement with my report may be highly depending on species difference, drug usage strategies, quality of the available drug and under dosing the anthelmintic drugs.

Conclusion and Recommendations

In general the current finding indicated that both albendazole and tetraclozan were found to be effective in gastrointestinal nematodes in Adaberga dairy farm. Tetraclozan was more efficient than albendazole against gastrointestinal nematodes.

A possible approach could be Targeted selective treatment, only a part of the animal group is treated with anthelmintics, contrary to the current manner to treat the whole group to prevent future existing resistance. To prevent future development of anthelmintic resistance in this area, the following practices were recommended:

- Producers should use drugs to treat their animals from reliable source.
- Frequent and unnecessary anthelmintic treatments should be avoided,
- Optimizing strategic deworming.
- Avoid under dosing of animals
- Further studies, are needed to determine the anthelmintic resistance status of the different species of gastrointestinal nematodes in cattle in different areas of Ethiopia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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