Efficacy of Albendazole and Tetramisole Anthelmintics Against *Haemonchus contortus* in Experimentally Infected Lambs

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ABSTRACT

The efficacy of albendazole and tetramisole was evaluated against Ogaden isolate of *Haemonchus contortus* in experimentally infected lambs. Thirty Arsi breed experimental lambs were randomly divided into 4 treatment groups, 1 positive control group, and 1 negative control group. The treatment groups received on Day 35 post-infection either of the following compounds: albendazole (Exiptol, ERFAR Pharmaceutical Laboratories, Greece), albendazole 300-mg bolus (Star Laboratories, Pakistan), tetramisole (ERFAR Pharmaceutical Laboratories, Greece), and tetramisole (Duxamintic, Star Laboratories, Pakistan). The efficacy of the drugs was evaluated in vivo by fecal egg count reduction test (FECRT) and controlled anthelmintic efficacy test. In vitro egg hatch assay was performed using different concentrations of albendazole on eggs of *H contortus*, Ogaden isolate, and the result was compared with eggs from known susceptible and resistant reference strains of *H contortus*. All the drugs were found to possess a 100% efficacy against Ogaden isolate of *H contortus* at the dose recommended by manufacturers using the FECRT and controlled anthelmintic efficacy evaluations tests. The LD_{50} values obtained for known susceptible and resistant reference strains of *H contortus* were 0.08 µg/mL and 1.28 µg/mL, respectively, while for that of Ogaden isolate, the LD_{50} value of concentration of albendazole was 0.06 µg/mL, further validating the susceptibility of our local isolate to benzimidazole compounds. The efficacy of the evaluated anthelmintics can only be maintained and conserved by wise and better utilization of the existing drugs to prevent the inevitable emergence of anthelmintic resistance as the consequence of anthelmintic usage.

INTRODUCTION

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 the introduction of thiabendazole in the early 1960s. Several anthelmintics with different modes of action

are available in the market for the control of helminthosis; however, intensive and indiscriminate use of these drugs to suppress infestation has resulted in rapid selection for resistance.[1]

Currently, failure of anthelmintics efficacy due to anthelmintic resistance in sheep and goat nematodes is becoming a widespread threat in Europe, Australia, and South America and is of increasing importance in certain African countries like South Africa and Kenya.[2] Waller[3] pointed out that most of the nematodes of domestic animals posses the capacity to develop resistance to anthelmintics. Resistance to antiparasitic drugs in sheep and goats is rapidly increasing, particularly in warm and humid climatic regions, probably due to frequent dosing and adoption of common management, nutritional, and therapeutic strategies. Of the many species of helminths so far reported to develop resistance to a number of anthelmintics, *Haemonchus contortus* is the most common and frequently reported nematode in many parts of the world.

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 albendazole and tetramisole substances in Ethiopia there are scarce reports on the efficacy of theses anthelmintics against economically important parasites such as *H contortus*. The purpose of this study is therefore to investigate and compare the efficacy of albendazole and tetramisole against Ogaden isolate *H contortus* in experimentally infected lambs.

MATERIALS AND METHODS

Animals and Experimental Design

A total of 30 male Arsi breed lambs age 4 to 6 months were purchased from open markets and used as experimental animals. All the lambs were treated with ivermectin at 0.25 mg/kg live weight to remove any burden of parasites; 4 successive fecal egg counts were performed on the first, second, third, and fourth weeks during the first month of adaptation period. All animals were housed in an isolation area in concrete-based units, and were fed on concentrates and locally dried straw throughout the adaptation period to preclude any accidental parasitic infections. At the end of the adaptation period, the animals were ear-tagged and allocated randomly to 6 groups of 5 animals each: Group 1, infected and treated with albendazole (Exiptol, ERFAR Pharmaceutical Laboratories, Greece); Group 2, infected and treated with albendazole 300-mg bolus (Star Laboratories, Pakistan); Group 3, infected and treated with tetramisole (ERFAR Pharmaceutical Laboratories, Greece); Group 4, infected and treated with tetramisole (Duxamintic, Star Laboratories, Pakistan); Group 5, infected and non-treated positive control; and Group 6, non-infected negative control.

All except group 6 were orally infected on Day 0 with 4000 infective third-stage larvae of Ogaden isolate of *H contortus*. Except for the positive and negative control groups (Group 5 and 6), all other animals were treated on Day 35 post-infection with their respective drugs indicated above and according to the manufacturer's dosage recommendation. On Day 42 post-infection, all animals were slaughtered. The abomasums were separately ligated and the contents examined to count for the number of adult and immature parasites using classical counting procedures indicated in MAFF,[4] Hansen and Perry,[5] and Wood et al.[6]

Fecal Egg Count Reduction Test (FECRT)

Fecal samples were collected from each group of animals on the day of treatment and on the 10th day after treatment (post-treatment). The efficacy of each anthelmintic was determined by comparing the FECRT from a group of animals before and after treatment.[7] Arithmetic means of

pre- and post-treatment fecal egg counts of control and treated groups were used to calculate the percentage efficacy using the following formula:

$$FECRT\ \% = (T_1\text{-}T_2)/T_1 \times 100,$$

where T_1 is pre-treatment and T_2 is post-treatment arithmetic mean of egg per gram (EPG) of feces measured using modified MacMaster egg counting techniques.[8] Failure of efficacy or resistance in Ogaden isolate of *H contortus* to the evaluated anthelmintics is confirmed when the value of FECRT % is less than 95% and the lower 95% confidence limit of reduction is less than 90%. If only one of the conditions is met, resistance is suspected.[6,9]

Controlled Anthelmintic Efficacy Test

All lambs were slaughtered 10 days after treatment and the number of worms present in the lumen and mucosa of the abomasums recovered and counted. Efficacy percentage was calculated as the difference between the geometric mean worm counts in the untreated control and the treated groups expressed as a percentage of the geometric mean worm counts in the control group.[7]

In Vitro Egg Hatch Assay

In the present study, the egg hatch assay was conducted and interpreted as outlined in the WAAVP recommendations.[6,9] Pretreatment samples were pooled for all lambs, and undeveloped eggs were recovered using saturated magnesium sulphate as a flotation fluid from freshly collected feces within 2 hours before processing. Magnesium sulphate was removed from eggs with excess tap water. The recovered eggs were adjusted at 50-100 eggs in 100 µL of water and were incubated for 48 hours at 23°C in serial concentrations of albendazole Greece dissolved in 1% DMSO ranging from 0 to 8.96 µg/mL.[10] The control was prepared in 5 replicates whereas each of the different concentrations were prepared in triplicates. Lugol's iodine was used to stop further hatching, and all eggs and larvae at each albendazole concentration were counted as dead, embryonated, or hatched to L1.

Known susceptible and resistant reference strains of *H contortus* (generously donated by Dr. Jacques Cabaret, INRA de Tour, France) were used to compare with the response of the test isolate. The percentage of eggs that hatched, were embryonated, or died at each concentration was determined by counting the whole content of each labeled tube under a microscope. Natural mortality was corrected from the percentage of eggs that hatched in the controls, and the percentage death value of each tube was plotted against each different concentrations of the anthelmintic under evaluation.[7] The estimation of the ED_{50} values were performed using a logit model by GenStat for Windows, 6th edition (VSN International Ltd., Herts, United Kingdom).

RESULTS

Reduction in Fecal Egg Count

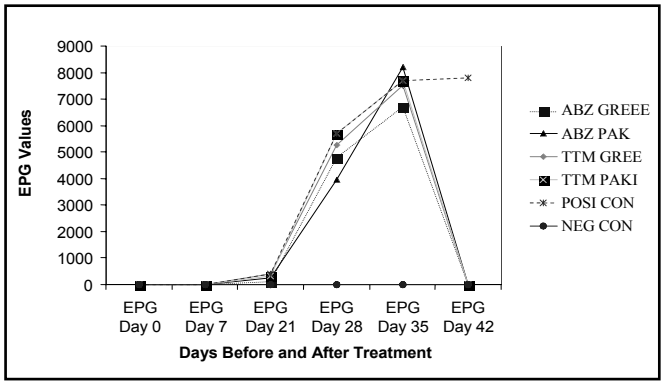
Follow-up of the infection on the basis of a weekly determination of fecal egg count indicated that all animals were positive on Day 21 post-infection and the fecal egg count was seen to dramatically rise in the subsequent weeks in all infected groups (Figure 1). Following treatment with the different types of drugs on Day 35 as per the experimental design, all the treated groups were observed to be totally negative for EPG count a week after treatment. Thus, compared to the control group, which remains still positive, and as per the formula of calculation used to estimate the FECRT, all 4 anthelmintics reduced the fecal egg counts by 100% suggesting that the Ogaden isolate of *H contortus* is susceptible to all the 4 tested anthelmintics.

Reduction in Worm Burden

Slaughter of all animals on Day 42 post-infection and examination of the abomasums unveiled that all the treated groups of animals, including the negative control groups, were negative for either larval or adult stage *H contortus*, whereas the posi-

tive control group was harboring an average of 630 adult worms of *H contortus* in their abomasums. As a result of using the calculations indicated for controlled anthelmintic efficacy test based on worm count reduction, it was possible to confirm that Ogaden isolate of *H contortus* remained fully susceptible to both albendazole and tetramisole produced either by ERFAR Pharmaceutical Laboratories, Greece, or Star Laboratories, Pakistan. The percentage efficacy values calculated for all the anthelmintics were thus 100%, further supporting the result calculated by FECRT.

Figure 1. EPG Values Before and After Treatment up to the Day of Slaughtering (Day 42) of the Experimental Lambs (n = 30). ABZ GREE = albendazole Greece group; ABZ PAK = albendazole Pakistan group; TTM GREE = tetramisole Greece group; TTM PAK = tetramisole Pakistan group; POSI CON = positive zzcontrol group; NEG CON = negative control group.



In Vitro Egg Hatch Assay

The LD_{50} values obtained using a logit model by GenStat for Windows, 6th edition, of albendazole (Exiptol) evaluated on Ogaden isolate of *H contortus*, known susceptible and resistant strains of *H contortus* were 0.06 µg/mL, 0.08 µg/mL, and 1.28 µg/mL, respectively, and the results were analyzed after correcting for natural mortality in control dilutions (Figure 2).

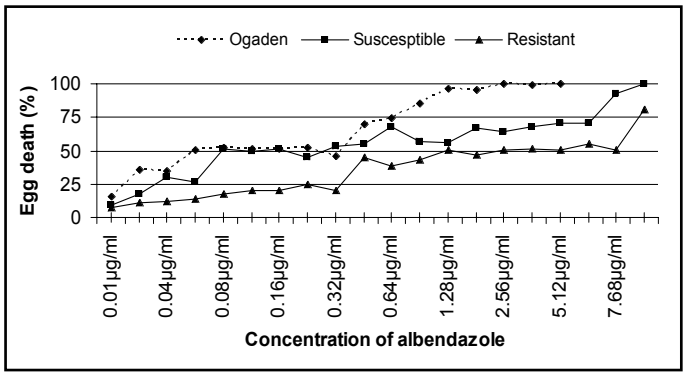
DISCUSSION

The efficacy results of the FECRT carried out and interpreted according to the WAAVP recommendations[6,9] provided evidence of susceptibility of Ogaden isolate of *H contortus* to the 4 tested anthelmintic drugs: albendazole (Exiptol, ERFAR Pharmaceutical Laboratories, Greece), albendazole 300-mg bolus (Star Laboratories, Pakistan), tetramisole (ERFAR Pharmaceutical Laboratories, Greece), and tetramisole (Duxamintic, Star Laboratories, Pakistan). The results of the controlled anthelmintic efficacy tests based on reduction in worm burden for each group of all experimental lambs and the calculated efficacy percentages of all 4 anthelmintics based on FECRT were 100%. According to Coles et al,[9] Results of the FECRT below 90% can only be regarded as strongly suggesting the presence of anthelmintic resistance. The FECRT finding is also supported by the necropsy analysis in which all the evaluated anthelmintics at the manufacturers' recommended dose rate have completely removed all the internally established Ogaden isolate of *H contortus* from all infected lambs, further validating the 100% efficacy of all tested drugs. All the uninfected (negative) control group lambs were found to be negative for *Haemonchus* worms confirming all the experimental lambs were kept in conditions that precluded any accidental infection during the whole experimental period.

In our attempt to further validate the absence of resistance in Ogaden isolate of *H contortus*, the eggs extracted from pooled fecal samples were subjected to in vitro egg hatch assay. The results of the egg hatch assay in comparison with known susceptible and resistant reference strains showed that the Ogaden isolate of *H contortus* was highly susceptibility to benzimidazole drugs (Figure 2). The LD_{50} values obtained for known susceptible and resistant reference strains were 0.08 µg/mL and 1.28 µg/mL, respectively, while for that of Ogaden iso-

late, the LD_{50} value of concentration of albendazole was 0.06 µg/mL. The LD_{50} of 0.06 µg/mL albendazole for the Ogaden isolate of *H contortus* is within the range of values indicative of susceptibility reported elsewhere for benzimidazole susceptible strains.[11] Le Jambre[11] reported that nematode strains resistant to benzimidazoles generally had an LD_{50} of more than 0.12 µg/mL.

Figure 2. Comparative Findings of Egg Hatch Assay Using Ogaden Isolate, and Known Susceptible and Resistant *Haemonchus contortus* Strains.



The results of the present study clearly indicate that the 3 methods of efficacy evaluation techniques were observed to match in confirming the susceptibility of our Ogaden isolate of *H contortus* to albendazole and thus benzimidazole compounds. This is in accordance with the recommendations of WAAVP guidelines that require validation of the result of FECRT by egg hatch assay.[9] Johansen[12] also indicated that the egg hatch assay validated very well and can distinguish benzimidazole susceptible and resistant nematodes reliably.

Some of the previous studies made at field level in Ethiopia suggest the susceptibility of GIT parasites to benzimidazole and imidazothiazole compounds. Kasahun[13] reported susceptibility of GIT parasites to albendazole and tetramisole in Woliata Sodo, Hussein et al[14] stated the good efficacy of albendazole, and Daniel[15] also reported susceptibility of different species of parasites to oxfendazole and tetramisole at a Sebeta farm.

The susceptibility of Ogaden isolate of *H contortus* to all tested drugs observed in this study is indicative of the absence of development of anthelmintic resistance in the area of study. One of the probable reasons is due to the very low frequency of anthelmintic treatment practiced in the area. In most cases, animals are treated only when they get sick and as a result there are large numbers of parasites as refugia in the study area.

It is also worth mentioning that in spite of the considerable variation in cost and preferences by the professionals and user society in Ethiopia based on the origin of drugs circulating in the market, the results obtained in this study clearly confirmed that the evaluated anthelmintics irrespective of the country of origin retained a high level of efficacy (100%) against Ogaden isolate of *H contortus*, which is one of the most pathogenically important parasites of small ruminants. In our study, the 2 drug origins were taken to accommodate the concern of most professionals whereby drugs coming from developing countries like Pakistan are considered inefficacious despite the low relative costs of the drugs on Ethiopian market. This piece of work therefore disproves the irrational bias we have towards drugs coming from developing countries. However, this finding should not be a guarantee for all and it is always advisable to undertake regular quality check-ups for all drugs circulating in open markets to protect any undesirable effects, a practice which is absent under the current Ethiopian condition. The high efficacy of all the studied drugs observed in the experimental study can only be conserved and the effective field life of these drugs be prolonged by relying on better use of the available anthelmintics that avoids all the factors that select resistant strains of parasites. Hence, all the evaluated anthelmintics

of the different chemical groups can alternatively be used to control this isolate of parasite in the study area, provided these drugs are used appropriately and wisely to conserve their susceptibility by avoiding all the advantages that select resistant strain to these drugs. It should be noted that resistance genes are present in unexposed populations, albeit at very low frequency, and serve as a primer in the evolution of resistance when effective selection pressure is applied.[16] This means that resistant individuals are already present in a population of parasites and resistance can be brought about when the selection pressure of resistant genes is increased.

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