RESEARCH ARTICLE

In vivo and In vitro Studies on the Efficacy of Anthelmintics against Haemonchus contortus in Goats

Nasreen Akhter*, AG Arijo, MS Phulan and Zafar Iqbal1

Department of Parasitology, Sindh Agriculture University, Tandojam; 1Department of Parasitology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: nasreenakhter58@gmail.com

ARTICLE HISTORY (13-299)

Received: July 02, 2013
Revised: December 20, 2013
Accepted: February 17, 2014

Key words:
Anthelmintics
Dectomax
Egg hatch assay
Fecal egg count reduction test
Haemonchus contortus
Levamisole
Valbazen

ABSTRACT

A study was conducted to assess the efficacy of Valbazen, Levamisole and Dectomax against Haemonchus contortus, using fecal egg count reduction test (FECRT) and egg hatch assay (EHA). Thirty two goats with naturally acquired H. contortus infection were divided into four groups of 8 goats each. Group A was administered Valbazen (@1ml/20kg BW) while B & C groups were treated with Levamisole and Dectomax @ 5ml/15kg (oral) & 1ml/33kg BW (sub cut), respectively. The D group served as untreated control. Faecal samples were collected on day 0 before treatment and on day 3, 7, 10 and 14 post treatment. The overall mean percent of fecal egg counts (FECs) on day 10th and 14th post treatment showed significant decrease (P<0.05) in fecal egg count. About 91.8% efficacy against H. contortus was recorded in goats with Dectomax followed by Valbazen (88.6%) and Levamisole (83.4%) on day 14th post treatment suggesting Dectomax as the most effective of the three anthelmintics. The Benzimidazole (pure) was evaluated for its ability to inhibit egg hatching in H. contortus and lethal dose (LD50) was calculated from linear regression. The eggs (200/1ml) were incubated with different concentrations (3.0, 1.5, 0.75, 0.375, 0.1875, 0.0937, 0.0468, 0.0234, 0.0117 and 0.0058 µg/ml) at 22ºC for 48 h. The inhibition of egg hatching at different concentrations ranged from 3.5 to 81.2% and LD50 was 0.1202 µg/ml which is higher than the recommended 0.1 µg/ml. The present data suggest that all the three anthelmintics used in present study showed reduced efficacy against H. contortus in goats than WAAVP level of 95%. Further detailed studies are necessary to clarify the current status of efficacy of three anthelmintics used in the present study.

INTRODUCTION

Goat is a multi-purpose animal producing meat, milk, fiber, leather and manure. The goats not only provide highly nutritious and easily digestible milk, but are also a regular source of additional income for poor and landlords or marginal farmers. Capital investment and feeding costs are low. Goats can be successfully reared in areas where fodder resources are limited and dairy cattle do not thrive. The goat farming is highly profitable and returns on capital investment ranges between 50 to 70% (Anonymous, 2013a). The goat population in Pakistan is 61.4 million heads which produces 0.76 million tons of milk and over 0.28 million tons of mutton (Farooq et al., 2012; Anonymous, 2013b; Khan et al., 2013; Tahir et al., 2013; Amin et al., 2013).

The parasitic infections in general and helminthiasis in particular are the most common problems associated with reduced weight gain, production loss and mortality in small ruminants (Chaudhary et al., 2007). Haemonchus contortus is a predominant, economically important and highly pathogenic nematode of sheep and goats especially in warm and humid climates (Waller, 2003; Nwosu et al., 2007; Tariq et al., 2008; Bhutto et al., 2012; Hamad et al., 2013). It sucks blood which causes low productivity, blood loss and decrease in hemoglobin that can lead to death in heavily infected animals (Githigia et al., 2001; Vatta et al., 2001; Zaman et al., 2012). It has been estimated that each worm sucks about 0.05ml of blood by
seepage or ingestion from lesions per day (Urquhart et al., 2000; Iqbal et al., 2012).

Pharmaceutically derived anthelmintics have been used for the control of helminth infections in domestic animals as a main option in many parts of the world (Keyyu et al., 2002). Indeed, synthetic and semi-synthetically produced anthelmintics have long been considered as the only effective method of controlling helminthiasis. Since there is no practice of rotating anthelmintics, continuous use of the same anthelmintic in a given area may lead to progressive loss of activity due to the development of resistance and that may result in the failure of parasite control in farm animals. Anthelmintic resistance has been reported among the gastro-intestinal nematodes of sheep and goats from different parts of the world (Wolstenholme et al., 2004; Bentounsi et al., 2006).

In Sindh, Pakistan, few studies have been carried out to determine the infection rate and intensity of gastrointestinal nematodes of goats (Akhter et al., 2011). No study has been conducted on the comparative efficacy of the common anthelmintics being used in the field as prophylactic and therapeutic agents, though there are numerous complaints by farmers regarding the ineffectiveness of the common drugs (personal communication). Therefore, the present study was designed to investigate the efficacy of various anthelmintics under local conditions.

**MATERIALS AND METHODS**

**Study area and animals:** In this study, efficacy of three most commonly used anthelmintics i.e., Valbazen, Levamisole and Dectomax was evaluated. The field trials were conducted at Livestock farm, Sindh Agriculture University, Tandojam and small goat farms at Tandojam and surrounding areas, Sindh, Pakistan.

**In vivo studies:** Thirty two goats of either sex, 06 months to 1 year old with naturally acquired *H. contortus* infection were used for this study. Thirty two goats were equally divided in four groups, i.e., A, B, C and D. Pretreatment faecal egg count (FEC) was performed on each goat using modified McMaster technique (Urquhart et al., 2000). Goats with a FEC of more than 200 eggs per gram were selected for experimental trials and were marked by ear tags. The group A was treated orally with Valbazen at a dose rate of 1ml/20kg BW (Pfizer, Pakistan Pvt. Ltd), while group B and C were treated with Levamisole (ICI, Pakistan PVT. Ltd) and Dectomax (Pfizer, Pakistan Pvt. Ltd) at the dose rate of 5 ml/15 kg and 1 ml/33 kg BW, respectively. Group D served as control. The rectal fecal samples were collected from each goat and eggs per gram (EPG) of the goats of all four groups were counted at day 0 (pre-treatment) and day 3, 7, 10, 14 (post-treatment). Samples were examined within 2-3 hours of collection for *H. contortus* eggs using saturated salt solution with 1.2 specific gravity as a floatation solution. Faecal egg count (FEC) was performed for each sample by the modified McMaster egg counting technique. The efficacy of the each anthelmintic was calculated according to the field controlled FECRT (Coles et al., 2006).

**In vitro Egg Hatch Assay (EHA):** The EHA was conducted using eggs collected directly from female worms or from the feces of the naturally infected goats. Gastrointestinal tracts infected with nematodes were collected from slaughter houses of Hyderabad. Female worms were separated, washed in PBS (pH 7.2), transferred to 0.9% normal saline and incubated at 37°C for 24 hours. After 24 hours the worms were removed from incubator and normal saline. Eggs laid were collected by using Standard Floatation Technique, and adjusted to a known density in water using McMaster Technique.

Four goats naturally infected with *H. contortus* were selected and kept indoor at experimental farm, Sindh agriculture university, Tandojam. These goats served as source for eggs. The EHA was employed as described by Murphy (1993). One ml of BZ serial dilutions (0.0058-3.0 µg/ml) dissolved in dimethyle sulfoxide (DMSO) was placed into each well of 24-well flat bottom micro titration plate. Eggs (200/0.1 ml distilled water) were added to 1 ml BZ solutions and the control well and were incubated at 22°C for 48 hours. The plates were sealed to prevent evaporation of water. A drop of dilute Lugol’s iodine solution was added to each well for termination of the experiment. Unhatched eggs and first stage larvae were counted in each well under x 40 magnification in duplicate and percent hatch was calculated.

**Statistical analysis:** The efficacy of anthelmintics was calculated using the guide lines laid down for the FECRT by the World Association for the Advancements in Veterinary Parasitology (WAAVP). Results of FECR were expressed as mean, and differences among intervals were analyzed through ANOVA while Tukey’s test was applied to determine the differences between means. The data from EHA were transformed by probit transformation and plotted against the logarithm of concentration. The lethal dose (LD50) values for Benzimidazole were calculated graphically from linear regression. A log dose probit line for egg mortality was derived from the data and LD50 was derived from the graph.

**RESULTS**

The mean FEC and reduction percentage in FEC after treatment of goats with recommended doses of Valbazen, Levamisole and Dectomax are presented in Table 1. There was gradual decrease in FEC and resulted in significantly (P<0.05) decreased FEC on day 10 and 14 post treatment compared to the control group. However, no statistically significant difference was observed on day 3 and 7 post treatment. The highest recorded FEC reduction was 91.8% on day 14 with Dectomax at recommended dose. Treatment of goats with Valbazen and Levamisole also showed significant reduction (P<0.01) on day 14 post treatment as compared to control group. The average reduction in FEC was 88.6 and 83.4% with Valbazen and Levamisole, respectively.

The results of the EHA are presented in Table 2. The higher concentrations showed higher inhibition rate as compared to lower concentrations and control. In general, the egg hatching ranged from 3.5 to 81.2% at different
concentrations. LD50 values calculated by probit analysis are shown in Fig II. LD50 for egg hatch inhibition of Benzimidazole was 0.120µg/ml which is higher than the WAAVP guideline of susceptibility range (LD50<0.1 µg/ml).

**DISCUSSION**

Efficacy of the drug is one of the most important steps in establishing and maintaining the effective parasite control of nematode parasites of livestock particularly for small ruminants. This can be done in vivo using the FECRT and in vitro using EHA. The FECRT is generally accepted as test of choice, whereas the EHA measures the effect of drug directly on hatching, development and motility of parasites without interfering with internal physiological functions of the host and the pharmacodynamics and pharmacokinetics of the drug (Assis et al., 2003). The in vitro experiments were designed to screen and evaluate efficacy of Benzimidazole on egg hatching of gastrointestinal nematodes of goat.

The results of FECRT were interpreted according to WAAVP recommendations (Coles et al., 1992) for efficacy evaluation of the anthelmintics. The maximum reduction of 91.8% was recorded with Dectomax at the recommended dose. Similarly, the reduction percentages of FECs with Valbazen and Levamisole were recorded as recommended dose. Similarly, the reduction percentages of FECs with Valbazen and Levamisole were recorded as 81.2% was observed at the concentration of 0.0058 µg/ml, while lowest percentage of egg hatching was observed at the concentration of 3.0 µg/ml. The calculated LD50 was 0.12022 µg/ml, which is higher than the WAAVP guideline of susceptibility range (LD50<0.1 µg/ml). This is in consistent with the findings of other researchers (Varady et al., 2007; Hoglund et al., 2009; Sargison et al., 2011; Sileshi et al., 2012). This may be due to the fact that the registered anthelmintics are used by poor farmers to treat only those animals showing clinical signs. These farmers do not follow the scientific guide lines for the use of anthelmintics and to cut down cost, often uses low doses and discontinue treatment once clinical signs subside. The immediate objective of the poor farmer is to subside. The immediate objective of the poor farmer is to eliminate the symptoms and prevent death. This approach may indicate that when such drugs are administered to infected animals, all parasites are not eliminated. Depending on the drug’s degree of efficacy, only a certain percentage of the worms harvested are killed. The sub population of the parasites remaining in animals at the end of the treatment may develop resistance to the drug that was used.

**Conclusion:** Present study suggested that compared to Valbazen and Levamisole, Dectomax was more effective...
against *H. contortus*. However, the efficacy of all three anthelmintics used was lower than the WAAVP recommended level of 95 percent, which may indicate resistance. This was further supported by EHA where LD50 of Benzimidazole was 0.12022 µg/ml, which is also greater than 0.1 µg/ml, suggested by WAAVP. Further studies may confirm the reasons/mechanism of reduced efficacy to anthelmintics in goats.

REFERENCES


