Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

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 Iqbal[1]

Department of Parasitology, Sindh Agriculture University, Tandojam; [1]Department 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

A B S T R A C T

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/20 kg 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 10^{th} and 14^{th} 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 14^{th} 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 (LD_{50}) 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 LD_{50} 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.

©2014 PVJ. All rights reserved

To Cite This Article: Akhter N, AG Arijo, MS Phulan and Zafar Iqbal, 2014. *In vivo* and *in vitro* studies on the efficacy of anthelmintics against *Haemonchus contortus* in goats. Pak Vet J, 34(3): 329-332.

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 landless 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 22ºC 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

Table 1: Comparative efficacy of Valbazen, Levamisole and Dectomax in goats before and after treatment

Group	Pre-treatment	Post-treatment days							
	0 Day	3rd		7th		10th		14th	
	Mean EPG	Mean EPG	Efficacy (FECRT%)	Mean EPG	Efficacy (FECRT%)	Mean EPG	Efficacy (FECRT%)	Mean EPG	Efficacy (FECRT%)
A	4787.5	3825	20.1	2312.5	51.7	1425*	70.0	543.75**	88.6
B	4512.5	3856.3	14.5	3175	29.6	2125	52.9	750**	83.4
C	4425.0	3631.3	17.9	2381.3	46.2	931.3**	79.0	362.5**	91.8
D	3962.5	3800	4.1	3912.5	1.3	4137.5	-4.4	4262.5	-7.6

Group A, B and C were treated with Valbazen, Levamisole and Dectomax, respectively while group D served as untreated control. *significant (P<0.05), **Highly significant (P<0.01).

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 pharmaco-dynamics 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 88.6 and 83.4%, respectively. This suggests that Dectomax was the more effective against *H. contortus* in goats as compared to Valbazen and Levamisole. The efficacy percentages observed are lower than 95%, WAAVP's recommendation for suspicion of resistance (Coles *et al*., 1992). The results of the present study are in agreement with the findings of several other workers (Waruiru *et al*., 2003; Ram *et al.*, 2007; Jabbar *et al.*, 2008; Chandrawathani *et al*., 2013), who have reported reduced efficacy of anthelmintics against gastrointestinal nematodes of goats. This may be due to the fact that the dose recommended for goat may not be sufficient to treat goats for this parasite species. In addition, the overuse/or misuse of anthelmintics has led to an increase in the incidence of anthelmintic resistance in gastrointestinal nematodes of small ruminants. Variable efficacy of these drugs ranging from 63 to 100 percent has been reported by other workers from different parts of world (Thomas *et al.*, 2001; Keyyu *et al.*, 2002; Waruiru 2002; Munyua *et al.*, 2004; Ram *et al.*, 2007; Godara *et al.*, 2011; Khalid *et al.*, 2013).

The results of the EHA and the corresponding LD50 are presented in Table 2 and Fig. 1. The results revealed that the inhibitory effect of Benzimidazole was dose dependent i.e., higher concentrations induced greater inhibition. The high percentage of egg hatching i.e., 81.2% was observed at the concentration of 0.0058 µg/ml,

Table 2: Percent eggs hatched at different concentrations of Benzimidazole (BZ) in *H. contortus*

BZ (µg/ml)	BZ (ng/ml)	Hatching (%)	Log (BZ)	Probit (hatching)
0.0058	5.8	81.2	0.763428	5.88
0.0117	11.7	77.0	1.068186	5.74
0.0234	23.4	70.2	1.369216	5.52
0.0468	46.8	65.2	1.670246	5.38
0.0937	93.7	57.5	1.971740	5.18
0.1875	187.5	51.0	2.273001	5.03
0.375	375	43.2	2.574031	4.82
0.75	750	38.0	2.875061	4.70
1.5	1500	23.7	3.176091	4.26
3.0	3000	3.5	3.477121	3.12

Log LD50= 2.0771; LD50= 120.22ng/ml

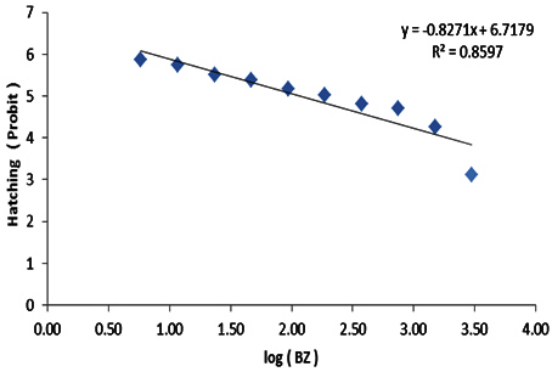

Fig 1: Log-dose probit response line of Benzimidazole for *H. contortus* infection

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 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 harbored 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

Amin S, SU Rahman, I Hussain and G Muhammad, 2013. Seroprevalence of mycoplasma ovipneumoniae among sheep from different districts of Baluchistan, Pakistan. Int J Agric Biol, 15: 1043-1046.

Anonymous, 2013a. Livestock Production Management: Advantages of goat farming/Utility of goats. https://sites.google.com/site/viveklpm/ sheep-and-goat-production-management/advantages-of-goat-farming. Accessed on December 13, 2013.

Anonymous, 2013b. FAO Statistics Division, Live animal production and population, http://faostat.fao.org/. Accessed on December 13, 201.

Akhter N, AG Arijo, MS Phulan, Z Iqbal and KB Mirbahar, 2011. Prevalence of gastro-intestinal nematodes in goats in Hyderabad and adjoining areas. Pak Vet J, 31: 287-290.

Assis LM, CML Bevilaqua, SM Morais, LS Vieira, CTS Costa and JAL Souza, 2003. Ovicidal and larvicidal activity in vitro of Spigelia anthelmia Linn extracts on *Haemonchus contortus*. Vet Parasitol, 117: 43-49.

Bentounsi B, R Trad, N Gaous, K Kohil and J Cabaret, 2006. Gastrointestinal nematode resistance to benzimidazoles on a sheep farm in Algeria. Vet Record, 158: 634-635.

Bhutto B, A Arijo, MS Phullan and R Rind, 2012. Prevalence of fascioliasis in buffaloes under different agro-climatic areas of Sindh Province of Pakistan. Int J Agric Biol, 14: 241-245.

Chandrawathani P, B Premaalatha, R Nurulaini, AL Erwanas and CM Zaini, 2013. Severe anthelmintic resistance in two free grazing small holder goat farms in Malaysia. J Vet Sci Technol, 4: 137.

Chaudary FR, MFU Khan and M Qayyum, 2007. Prevalence of *Haemonchus contortus* in naturally infected small ruminants grazing in the Potohar area of Pakistan. Pak Vet J, 27: 73-79.

Coles GC, C Bauer, FH Borgsteede, S Geerts, TR Klei, MA Tylor and PJ Waller, 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol, 44: 35-44.

Coles GC, F Jackson, WE Pomroy, RK Prichard, G von Samson-Himmelstjerna, A Silvestre, MA Taylor and J Vercruysse, 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol, 136: 11-19.

Farooq Z, S Mushtaq, Z Iqbal and S Akhtar, Shahnaz, 2012. Parasitic helminths of domesticated and wild ruminants in Cholistan desert of Pakistan. Int J Agric Biol, 14: 63-68.

Githigia SM, SM Thamsborg, WK Munyua and N Maingi, 2001. Impact of gastrointestinal helminths on production in goats in Kenya. Small Rum Res, 42: 21-29.

Godara R, RL Sharma and SS Sodhi, 2011. Efficacy of fenbendazole, Levamisole and Ivermactin against gastrointestinal nematodes in Jamunapari goats. J Parasitol Dis, 35: 219-221.

Hamad KK, Z Iqbal, ZuD Sindhu and G Muhammad, 2013. Antinematicidal activity of *Nicotiana tabacum* L. leaf extracts to control benzimidazole-resistant *Haemonchus contortus* in sheep. Pak Vet J, 33: 85-90.

Höglund J, K Gustafsson, BL Ljungström, A Engström, A Donnan and P Skuce, 2009. Anthelmintic resistance in Swedish sheep flocks based on a comparison of the results from the faecal egg count reduction test and resistant allele frequencies of the beta-tubulin gene. Vet Parasitol, 161: 60-68.

Iqbal Z, W Babar, ZUD Sindhu, RZ Abbas and MS Sajid, 2012. Evaluation of anthelmintic activity of different fractions of *Azadirachta indica* A. Juss seed extract. Pak Vet J, 32: 579-583.

Jabbar A, Z Iqbal, HA Saddiq, W Baber, M Saeed, 2008. Prevalence of multiple anthelmintic resistant gastrointestinal nematodes in dairy goats in a desolated tract (Pakistan). Parasitol Res, 103: 29-35.

Keyyu JD, HM Mahingika, HB Magwisha and AA Kassuku, 2002. Efficacy of albendazole and levamisole against gastrointestinal nematodes of sheep and goats. Trop Anim Health Prod, 34: 115-120.

Khalid MM, M Ijaz, AZ Durrani, MA khan, AJ Sabir and MH saleem, 2013. Infection rate and therapeutic trials on various gastrointestinal parasites in sheep and goats in and around Lahore, Pakistan. Pak J Zool, 45: 489-494.

Khan A, R Sharaf, MZ Khan, MK Saleemi and F Mahmood, 2013. Arsenic toxicity in broiler chicks and its alleviation with ascorbic acid: a toxico-patho-biochemical study. . Int J Agric Biol, 15: 1105-1111.

Munyua WK, RM Waruiru and JW Ngotho, 2004. Comparative efficacy of albenzazole, levamisole rafoxanide and ivermectin against naturally acquired gastrointestinal nematodes in goats. Kenya Vet, 27: 5-9.

Murphy GL, 1993. Theories and concept formation. In: Van Mechelen, J Hampton, R Michalski and P Theuns (Eds), Categories and concepts: Theoretical views and inductive data analysis. Academic Press, New York, USA, pp: 173-200.

Nwosu CO, PP Madu and WS Richard, 2007. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north eastern Nigeria. Vet Parasitol, 144: 118-124.

Ram H, TJ Rassol, AK Sharma, HR Meena and SK Singh, 2007. Comparative efficacy of different anthelmintics against fenbendazole-resistant nematodes of Pashmina goats. Vet Res Commun, 31: 719-723.

Sileshi B, H Jamal, RF Hussien, B Kumilachew and T Tadele, 2012. Susceptibility assay of *Haemonchus contortus* to commonly used anthelmintics in Jimma, southwest Ethiopia. Trop Anim Health Prod, 44: 1599-1603.

Tahir MF, MS Mahmood and I Hussain, 2013. Preparation and comparative evaluation of different adjuvanted toxoid vaccines against enterotoxaemia. Pak J Agric Sci, 50: 293-297.

Tariq KA, MZ Chisti, F Ahmad and AS Shawl, 2008. Epidemiology of gastro-intestinal nematodes of sheep managed under traditional husbandry system in Kashmir Valley. Vet Parasitol, 158: 138-143.

Thomas HT, RM Kaplanb, M Larsenc, OM Samplesa, JE Millerd and S Gelayea, 2001. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. Vet Parasitol, 97: 261-268.

Urquhart GM, J Armour, JL Dunncan and FW Jennings, 2000.Veterinary Parasitology, 2nd Edition. Blackwell Science Ltd, London.

Vatta AF, BA Letty, MJ van der Linde, EF VanWijk, JW Hansen and RC Krecek, 2001. Testing for clinical anaemia caused by *Haemonchus* spp. In goats farmed under resource-poor conditions in South Africa using an eye color chart developed for sheep, Vet Parasitol, 99: 1-14.

Varady M, PC Udekova and J Corba, 2007. In vitro detection of benzimidazole resistance in *H. contortus*: Egg hatch test versus larval development test. Vet Parasitol, 149: 104-110.

Waller PJ, 2003. The future of anthelmintics in sustainable parasite control programs for livestock. Helminthologia, 40: 97-102.

Waruiru RM, 2002. Efficacy of closantel plus albendazole combination against naturally acquired and experimentally induced nematode infections in goats. Israel J Vet Med, 57: 113-117.

Waruiru RM, JW Ngotho, MN Mutune and WK Munyua, 2003. Comparative efficacy of ivermectin, albendazole, levamisole and rafoxanide against gastrointestinal nematode infections in goats. Indian J Anim Sci, 73:147-150.

Wolstenholme AJ, I Fairweather, RK Prichard, GV Samsonhimmelst-jerna and NC sangster, 2004. Drug resistance in veterinary helminths. Trends Parasitol, 20: 469-476.

Zaman MA, Z Iqbal, MN Khan and G Muhammad, 2012. Anthelmintic activity of a herbal formulation against gastrointestinal nematodes of sheep. Pak Vet J, 32: 117-121.