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RESEARCH ARTICLE

Comparative Efficacy of Six Anthelmintic Treatments against Natural Infection of Fasciola Species in Sheep

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ABSTRACT

The objective of this study was to assess the flukicidal effect of various anthelmintics against naturally occurring infection of Fasciola, in sheep. Fasciola positive female (n=175) of Kajli breed at Livestock Experiment Station, Khizerabad, district Sargodha were randomly assigned to seven groups, including an untreated control group that received normal saline as placebo. The six groups were treated with four anthelmintics alone and in combination. The faecal egg count reduction test (FECRT) was performed to determine the chemotherapeutic efficacy of anthelmintics. The FECRT for the candidate anthelmintics showed significant ($P<0.05$) reduction in eggs per gram of feces of treated groups compared to untreated control group. The highest efficacy was found of a combination therapy of oxyclozanide and oxfendazole (97.50%) treated group; whereas, levamisole treated group showed lowest efficiency (65.67%). Time dependent response of each treatment was determined on 7th, 14th, 21st and 28th days post treatment. In groups treated with combination of drugs, 28th day while, in groups treated with single drug 21st day was found the most efficacious time dependent response against natural Fasciola infection. It can be concluded that combination of oxyclozanide and oxfendazole holds potential as part of an integrated management plan for the control of Fasciola in Pakistan.

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INTRODUCTION

Parasitism is one of the important issues all over the globe and reducing the productivity of animals (Vercruysse and Claerebout, 2001; Solcan *et al*., 2015; Asif *et al*., 2016). The importance of helminth infection is enhanced several times in third globe nations like Pakistan where the economy of peoples is mainly dependant on their livestock (Chowdhury *et al*., 1994). Among helminths, genus Fasciola is very important due to extensive variety of specified hosts such as, cattle, buffalo, sheep, goat and human beings (Rondelaud *et al*., 2001), and causes acute as well as chronic infection. In acute cases, due to direct or indirect loss of blood, anemia and hypoalbuminemia occur which can lead to the death of animals. While, chronic cases can lead to the lower productivity in terms of milk, meat and wool production by reducing the feed conversion ratio. In Pakistan, reported epidemiological studies of fascioliasis revealed an estimated prevalence ranging from 14.7 to 25.6% in different regions of Punjab (Khan *et al*., 2009; Zaman *et al*., 2015; Shamim *et al*., 2016).

Control of fascioliasis is crucial for the health of animal at farm level to get optimum productivity (Gomez-Puerta *et al*., 2012). As there is antigenic variation in helminths, so no vaccines are available for their effective control and the only choice is chemotherapeutic control that is globally recommended (McManus and Dalton, 2006). In view of the management constraints of the small farming system in the developing countries, chemotherapy remains the cornerstone for the control of helminths generally and flukes specifically (Boray, 2005). Different chemotherapeutic drugs have been used for the control of fascioliasis in small as well as large ruminants worldwide (Fairweather, 2005). Chemotherapy is the heart of any controlling campaign because it is a cost-effective way of treating parasitic disease. The possible yearly rotation of a

single or combination of two or more anthelmintic compound with less frequent strategic treatment is very effective against both immature and adult flukes (Parr and Gray, 2000). Most of the anthelmintic compounds which are used for the control of parasitic diseases are imported without proper test and registration, which may have an effect on their efficacy (Atnafe and Melaku, 2012). The development of anthelmintic resistance in field animals is due to use of traditional treatment, inadequate dose level, low protein diet, environmental toxicity and poor efficacy of anti-parasitic agent (Sindhu *et al*., 2014). In Pakistan, most of the farmer use hit and trial method against parasitic infection. Other than this under dosing, use of drugs having lower efficacy and provision of low protein diet to their animals are major factors for the development of resistant worms. Keeping in view the importance of this disease, a controlled trial of the efficacy of several anthelmintic compounds as a single and combined therapy in the treatment of Fasciola in naturally infected sheep was carried out. This study provides solid information of effectiveness or development of resistance against trial compounds. The outcome of this study will be helpful for the farming community of Pakistan for the effective control of fasciolosis.

MATERIALS AND METHODS

Study area: The study was carried out at the livestock experiment station, Khizerabad, district Sargodha, situated at 32°5′1″N 72°40′16″E. It lies between 100-200 meters above sea level, bounded on the west and east by Jhelum and Chenab rivers, respectively. In Sargodha, mean temperature in summer is 41ºC, whereas in winter it becomes as low as 6ºC. The average rainfall in district Sargodha ranges from 3-84 mm with an average of 26.75 mm. The climate in the area is rainy summer and wet autumn and dry winter. The district has marshy areas which are suitable for snails, the intermediate host of *Fasciola* spp. The district has been reported having the most favorable circumstances for the settlement, growth and propagation of Fasciola and snails.

Experimental animals: A total of 384 sheep were selected randomly for faecal sample collection to determine the Fasciola infection. Faecal samples were collected directly from the rectum and preserved in properly labeled wide-mouthed close plastic bottle by using 10% formalin solution (1:3). These samples were brought to the Department of Parasitology for coproscopic examination using sedimentation technique (Tsotetsi *et al*., 2013). Among animals harbouring the Fasciola infection, 175 animals with similar age, sex and breed were selected for efficacy trial.

Fecal egg count reduction test: The animals harbouring the Fasciola infection were selected and randomly divided into seven groups according to WAAVP guidelines (Wood *et al*., 1995), group A thru G each comprised of 25 animals. Group wise allocation of treatments with their dose rates is given in the Table 1. The efficacy of the drugs was evaluated by measuring egg shedding. For each animal, faecal samples were collected on day 0, 7th, 14th, 21st and 28th post treatment. For detection of egg count per gram of feces (EPG), the sedimentation technique was conducted, as mentioned by Mooney *et al*. (2009). Briefly, 2 g of feces were taken and mixed with 28mL of sedimental solution (33% $ZnSO_4$). The mixture was sieved to remove large piece of debris. Each chamber of McMaster was filled with sample and examined under light microscope at 10X. Eggs in all lines were counted and egg per gram (EPG) was calculated by using following formula:

$$EPG = \frac{N}{\text{Number of chambers}} \times \frac{60\ mL/5g}{0.15\ mL}$$

The efficacy of the drugs was assessed by the reduction of mean egg excretion at each measurement point mentioned above, following the formula described by Foreyt (1988). The anthelmintic efficacy percent was calculated by the equation:

$$FECR = \frac{\text{EPG of control group} - \text{EPG of treated group}}{\text{EPG of control group}}$$

According to WAAVP guidelines (Wood *et al*., 1995), the drug was considered effective if FECR was more than 95% and the lower limit of the 95% confidence interval was more than 90%.

Statistical analysis: Microsoft Excel, 2010 was used to calculate the descriptive statistics. A complete randomized design was used for chemotherapeutic trial. To find out *in-vivo* efficacy of fasciolicides, analysis of variance (ANOVA) was used. Variables having $P<0.05$ were considered significantly different. All analyses were carried out by using SPSS 16.0 software package at 95% confidence interval.

RESULTS

Out of 384 examined sheep, 192 were found positive for Fasciola infection. The FECRT for the candidate anthelmintics showed significant ($P<0.05$) reduction in EPG of treated groups compared to control group. The mean reduction in EPG of each treated group has been compared with the control group (Table 2). The overall highest efficacy was found in group G (97.50%) whereas, group C showed lowest efficiency (65.67%). The efficacy (%) of each treated group at day 7th, 14th, 21st and 28th is given in Fig. 2. The highest mean post-treatment reduction in EPG on day 28th was noted in group G (Oxfendazole and Oxyclozanide) followed by groups F (Triclabendazole and Levamisole), B (Triclabendazole), E (Oxfendazole), D (Oxyclozanide), and C (Levamisole).

DISCUSSION

Among helminth diseases, fasciolosis is one of the most important health threats for livestock in many parts of the world. However, the chronic infection of Fasciola in sheep was responsible for causing a major economic impact due to reductions in weight gain, milk yield, fertility and liver damaging (Boray, 2005). In the current study, six different treatments were used to determine the most effective drug against Fasciola, which help to control the infection very effectively.

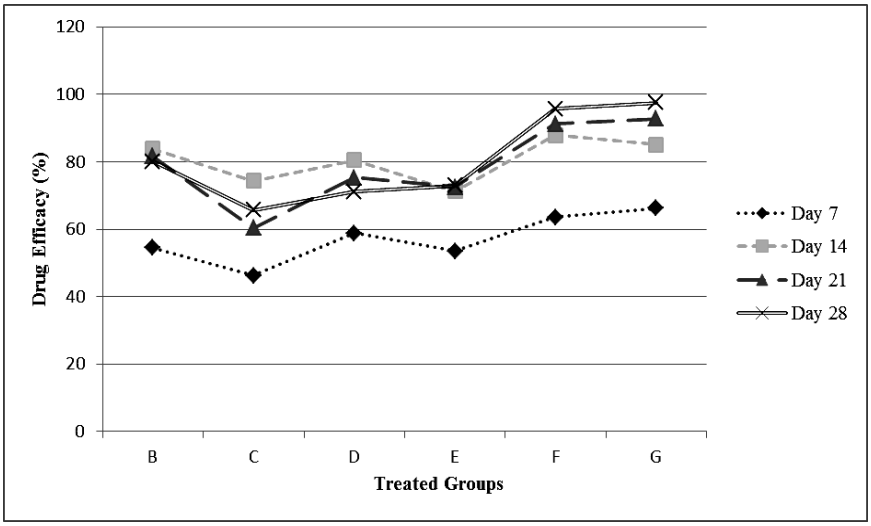

Fig. 2: Day wise efficacy (%) of each treated group of selected sheep population at Livestock Experimental Station, Khizerabad, district Sargodha.

Table 1: Protocol for chemotherapeutic trial in sheep naturally infected with *Fasciola* spp. at Livestock Experimental Station, Khizerabad, district Sargodha

Groups	Treatment	Dose rates (mg.kg^{-1} BW)	EPG Schedule (Days)
A Control	Normal saline	25	0, 7, 14, 21, 28
B	Triclabendazole	10	0, 7, 14, 21, 28
C	Levamisole	10	0, 7, 14, 21, 28
D	Oxyclozanide	16.6	0, 7, 14, 21, 28
E	Oxfendazole	22.65	0, 7, 14, 21, 28
F	Triclabendazole + Levamisole	50 + 37.50	0, 7, 14, 21, 28
G	Oxfendazole + Oxyclozanide	22.65 + 62.50	0, 7, 14, 21, 28

Table 2: Comparison of mean reduction EPG±SE of each treated group with control group of selected sheep population at Livestock Experimental Station, Khizerabad, district Sargodha

Groups	Experimental Days					P Value
	0	7	14	21	28	
Control (A)	0±0	12±04	24±07	32±08	40±07	P>0.05
Triclabendazole (B)	0±0	172±28	264±25	260±30	256±26	P<0.05
Levamisole (C)	0±0	162±36	246±33	235±27	226±31	P<0.05
Oxyclozanide (D)	0±0	215±29	284±35	278±34	278±32	P<0.05
Oxfendazole (E)	0±0	182±22	240±30	245±28	249±25	P<0.05
Triclabendazole + Levamisole (F)	0±0	196±20	272±23	284±27	296±22	P<0.05
Oxfendazole + Oxyclozanide (G)	0±0	223±24	283±29	298±26	320±23	P<0.05

The efficacy of triclabendazole (TRI) treated group was recorded 83% against Fasciola infection in this study. A comparable result was reported by Moll *et al*. (2000), and Elitok *et al*. (2006). In contrast to our results, a higher efficacy (100%) was reported by Maes *et al*. (1990). Because *Fasciola* spp. are not a host species specific parasite and cattle and sheep graze the same pastures, it is likely that resistant flukes have also established in sheep (Moll *et al*., 2000). Tubulin (one of several members of a small family of globular proteins) is the target for TRI action. As a result, initial thoughts about the mechanism of resistance to TRI centered on possible mutations in the tubulin molecule (Flanagan *et al*., 2011). Regarding the result of levamesole (LEV) treated group, the result indicated that the efficacy of LEV not more than 74.32% in this study. However, the current results are not in agreement with the finding of Grade *et al*. (2008). It is thought that the development of resistance in parasites against levamesole is due to reduction in acetylcholine receptors or due to a change in binding characteristics of drug (Boulin *et al*., 2011).

In oxyyclozanide (OXY) treated group, 80.40% efficacy was recorded which is compatible to the results of Babiker *et al*. (2012), but not compatible with the result of Boray (2005), Athar *et al*. (2011) and Shokiera *et al*. (2013). Because of the fact that in this study OXY treated group had no effect, it is highly indicative that resistance of *Fasciola* spp. against OXY is present (Moll *et al*., 2000). The development of resistance in parasites is a potential drawback of any oppressive treatment system. Resistant strains of *Fasciola* spp. have been reported by various scientists in different parts of the world against a variety of anthelmentic compounds. The development of resistance in sheep has been implicated due to frequency of treatment and contributes to the development of resistant *Fasciola* spp (Atnafe and Melaku, 2012). This alarming condition may be avoided by the adoption of strategic dosing for control of fasciolosis and involving the treatment of healthy stock with little liver damage.

The efficacy of oxfendazole (OXF) treated group was recorded 72.85% against Fasciola infection in this study. Comparable results were recorded by Athar *et al*. (2011), while, Gomez-Puerta *et al*. (2012) results showed higher efficacy. The OXF is a broad spectrum anthelmintic belonging to benzimidazoles group. This drug has more efficacy against the nematode infection than that of *Fasciola* spp. The combination of drugs (separately have different action) may develop synergistic effects between the drugs. In order to treat mixed helminths infection, the combinations of drugs are commonly used to control parasitic infection. The combination of drugs play important role to slow down the development of resistance.

Along with this it is mainly for convenience and ease of administration (Sangster, 2001). In the present study, two different combinations (TRI + LEV and OXF + OXY) were used to determine their efficacy against the natural Fasciola infection. The effectiveness of TRI along with LEV against Fasciola was recorded 95.71% in the current study, which is not very different from those of Yüksek *et al*. (2007).

A novel combination of flukicide *i.e.* OXF along with OXY was also used in this study. This combination might have served to extend the useful life of the individual drugs, increasing the pharmacokinetics of both the compounds and the possibility of using lower concentrations of drugs in treatment regimens (Lifschitz *et al*., 2009).

Conclusions: The combination therapy of the OXF and OXY might have led to the binding of ß-tubulin as well depriving the parasite from energy through disruption of the ATP synthesis simultaneously which resulted in an effective control of natural Fasciola infection in the sheep. Hence, in the light of above discussion, a combination of OXF and OXY is recommended in the small holder livestock population of Pakistan in order to control the Fasciola infection in sheep. However, such kind of chemotherapeutic trials should be replicated in large ruminant population, too before recommending this novel combination in large ruminant fasciolosis in Pakistan.

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Author's contribution: MNK, MSS and AQ planed, designed and supervised the experiment. MR helped in the collection of the faecal samples. HMR and MKK processed samples. RZA helped in the identification of parasites and analyzed the data. All authors read and approved the manuscript.

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