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Assessment of Anthelmintic Resistance of *Fasciola* spp. against Flunil-L® and Fasinash®

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Abstract

Fasciolosis caused by *Fasciola* spp. is recognized to be one of the major problems affecting health and productivity of cattle in Bhutan. Various anthelmintic drugs are used to treat and control fascioliasis in the country among which, triclabendazole and oxyclozanide are the most common ones. These drugs have been used for a very long time in the country and possibility of development of resistance to these drugs is high. Also, limited studies had been carried out to test their efficacies in the country. Therefore, this study was done to determine the prevalence of fasciolosis in cattle in Maedwang gewog under Thimphu Dzongkhag and assess the status of resistance of *Fasciola* spp. to Fasinash® and Flunil-L® drugs. A total of 218 faecal samples were collected from cattle and subjected to parasitological test using standard sedimentation technique. The animals positive to *Fasciola* were treated with Fasinash® (triclabendazole bolus) and Flunil-L® (oxyclozanide+levamisole suspension). The faecal eggs were analyzed 14 days after the treatment by Faecal Egg Count Reduction Test and the efficacy was calculated. The overall prevalence of fasciolosis in the study was 32.11% with a prevalence of 28.80% in Namseling and 36.56% in Khasadrapchu. The mean faecal egg count (epg) of *Fasciola* spp. detected in Namseling was 0.65 ± 1.53 *SD* and in Khasadrapchu was 1.03 ± 2.07 *SD*. There was no significant difference in the mean faecal egg counts in the two chiwogs ($p > .05$ at 95% confidence level). The overall efficacy of Fasinash® was 86.96% and for Flunil-L® was 91.38%. Significant difference was observed between pre-treatment and post-treatment in both the treatment groups ($p < .05$ at 95% confidence level). However, the study implies development of resistance of *Fasciola* spp. to triclabendazole in the study area.

Keywords: Anthelmintic resistance, fasciolosis, fasinash®, flunil-L®

Introduction

Livestock is an integral component of the farming system in Bhutan and an important sub-sector of agriculture (Ministry of Agriculture and Forests [MoAF], 2014). It plays important role to human health and poverty alleviation in Bhutan (MoAF, 2014). Cattle constitute most of the livestock population in Bhutan. Government has given the highest development priority to the dairy sector since first five-year plan until today by ensuring enabling policy supports and allocating substantial resources, considering its importance to rural populace (Wangdi *et al*., 2014). Parasitic infestation is recognized to be one of the major problems in

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livestock production affecting the health and productivity of animals (Dorji, 2015). According to a study carried out by Phanchung *et al*. (2002), gastro-intestinal worm infestation is reported as one of the major animal health problems in South-west and East-central Bhutan.

In Bhutan, Fasciolosis is prevalent especially in the paddy growing areas of the country (National Centre for Animal Health [NCAH], 2010). Prevalence of fasciolosis in Bhutan has been reported by several workers. Although the nationwide prevalence of fasciolosis is not known exactly, the limited studies done in the country show that the regional prevalence of fascioliasis in cattle in eastern Bhutan is 2.53% (Dorjee, 2011). However, the estimation of economic loss due to fasciolosis at national or regional level is limited due to lack of accurate estimation of the prevalence of disease in the area.

Fasciola spp. in cattle in Bhutan is controlled by using chemical anthelmintics since the start of livestock development programs in early 1960s (Wangdi, 2015). Triclabendazole and oxyclozanide are used as flukicide and apparently are the most extensively used anthelmintics in Bhutan (NCAH, 2016). These drugs have been used for a very long time and there is possibility that *Fasciola* spp. have developed resistance to these drugs as there are reports of regular incidence of fascioliasis from places where deworming is done using these drugs. According to Sharma (2011), flukes have developed resistance to rafoxanide and oxyclozanide in Bhutan. This study investigated the status of prevalence of fasciolosis and assessed the resistance of *Fasciola* spp. to triclabendazole and oxyclozanide + levamisole suspension in cattle.

Materials and Methods

Study area

The study was carried out in Maedwang gewog under Thimphu Dzongkhag from December to February 2017. It comprises of five chiwogs (Chiwog is a sub-block), 55 villages, 955 households and has a population of 5,136 people as of 2015 (Department of Livestock [DoL], 2015).

Out of five chiwogs in Maedwang gewog, only Tshaluna chiwog is a non-paddy growing area. The prevalence of fascioliasis in cattle in Tshaluna was 0.96% (n = 104) which is relatively low (Dorji, 2015) and hence villages in Tshaluna have not been selected for this study. Therefore, two chiwogs out of four were randomly selected for the study.

Data collection

A randomized controlled clinical trial in eight villages under two chiwogs in Maedwang gewog was done. The sample size was calculated using WinEpiscope 2.0 version (95% confidence level and 5% margin of error). The study area included four villages under Khasadrapchu chiwog and four villages under Namseling chiwog which has a total cattle population of 510 heads (DoL, 2015) and the sample size worked out to 219.

About 40 g of faecal samples were collected directly from the rectum in sterile condition in the morning from the sampling unit in eight villages. The parasitological examination was carried out using standard operating procedure (sedimentation technique) followed by National Centre for Animal Health (NCAH, 2015).

The number of pre-treatment faecal egg positive for each *Fasciola* infested animal was determined and all test results recorded in Microsoft Office Excel 2007. The prevalence of *Fasciola* spp. in the study area was determined and the identified *Fasciola* spp. positive animals were randomly divided into three groups and clinical treatment trials undertaken as below:

Treatment 1: Control

Treatment 2: Triclabendazole bolus (Fasinash®)

Treatment 3: Oxyclozanide + Levamisole suspension (Flunil-L®)

Faecal samples from each cattle were collected and examined using the standard sedimenta-

tion technique. The percentage reduction in the number of eggs after treatment was determined and compared with the control group. Resistance or failure of treatment was established when faecal egg count reduction test revealed an efficacy less than 90% on day 14 post treatment (Fiel *et al.*, 2015; Venturina *et al.*, 2015; Bullen *et al.*, 2016). The efficacies of the anthelmintic compound were determined based on reduction of egg excretion at 14 days post-treatment using the formula below (Young *et al.*, 2000; Venturina *et al.*, 2015).

$$Efficacy = \frac{Pre-treatment\ FEC - Post-treatment\ FEC}{Pre-treatment\ FEC} \times 100$$

Data analysis

The efficacy of drugs was calculated for each group and recorded in the Microsoft Excel 2007 and data were analyzed using statistical package PHstat8. Percentages, means and standard deviations (M ± SD) are presented. Graphs were generated using Microsoft Excel (2007). Paired sample *t*-test was carried out to determine the mean difference within groups and independent sample *t*-test was carried out to determine the differences in mean between groups in the trial.

Results and Discussion

Sample characteristics

A total of 218 faecal samples were collected during the study from two chiwogs under Maedwang gewog in Thimphu involving eight villages and 49 households. Out of 218 samples, 58.72% of the samples were collected from Jersey Cross breed followed by 35.32% from Local breed and 5.96% from Pure Jersey breed. Similarly, 74.77% of the samples were from female and 25.23% were from male animals and 53.67% of the samples were from young animals followed by 41.28% from adults and 5.05% from calves. The chiwog-wise and animal-wise faecal samples collected during the study for pre-treatment analysis is shown in Table 1.

Prevalence of helminthes and protozoa species

Out of 218 faecal samples examined during the study, the overall positivity to various endo-parasites in the study area was 77.98% (n = 170) out of which, 84.71% (n = 144) was positive to helminthes and 15.29% (n = 26) was positive to protozoan species. This study showed higher positivity to gastro-intestinal parasites when compared to similar study conducted in Haa District which reported an overall positivity of 43.96% with only 26.00% positive to helminthes (Tshering and Dorji, 2013).

The overall prevalence of endo-parasites was higher in Namseling Chiwog with 81.6% as compared to Khasadrapchu chiwog which was 73.12%. However, the overall prevalence of *Fasciola* spp. was higher in Khasadrapchu chiwog with 36.56% as compared to Namseling chiwog which was 28.8%. The chiwog-wise overall and species-wise prevalence of gastro-intestinal parasites in the study area is given in Table 2.

Prevalence of Fasciola spp.

The overall prevalence of *Fasciola* spp. in the study area was 32.11% (70 positive out of 218

Table 1: Chiwog-wise and animal-wise faecal samples collected for pre-treatment analysis

Chiwog	Village	H/H	Animal-wise Faecal Samples							
			Breed			Sex		Age		
			n = 218							
			PJ	JX	L	M	F	Calf	Young	Adult
Namseling	4	21	13	75	37	23	102	8	72	45
Khasadrapchu	4	28	0	53	40	32	61	3	45	45

H/H=Households, PJ=Pure Jersey, JX=Jersey Cross, L=Local, M=Male, F=Female, Calf=<1-year age, Young=1-5 years age, Adult=>5 years age

samples) with a prevalence of 28.80% (36 positive out of 125 samples) in Namseling chiwog and 36.56% (34 positive out of 93 samples) in Khasadrapchu chiwog. Although both the chiwogs are paddy growing areas and both follow similar husbandry practices that is stall feeding with paddy straws along with concentrates and grazing in paddy fields, the prevalence of *Fasciola* spp. was higher in Khasadrapchu chiwog compared to Namseling chiwog. Moreover, the *Fasciola* spp. detected in the faecal samples of sick animals in Namseling chiwog was 43.75% (7 out of 16 sick animals) as compared to 40.00% (2 out of 5 sick animals) in Khasadrapchu chiwog. The low prevalence of *Fasciola* in Namseling chiwog as compared to Khasadrapchu chiwog was mainly attributed to the regular sampling, testing and de-worming of animals against fascioliasis carried out by National Centre for Animal Health and District Veterinary Hospital as a national and district level fascioliasis control programs.

The overall prevalence of *Fasciola* spp. in this study was found to be consistent with the results reported by Wangdi (2015) where in the study conducted in Maedwang gewog reported a prevalence of 38.90% in the year 2012 and 25.20% in the year 2014. A similar trend of prevalence (38.90%) was also reported in Chokhor gewog in Bumthang (NCAH, 2010). The overall prevalence of *Fasciola* spp. in this study was higher compared to the studies done in Haa District which was 13.11% (Tshering and Dorji, 2013) and in Tshaluna chiwog under Maedwang gewog which reported a prevalence of 0.96% (Dorji, 2015). The low prevalence rates in these two areas as compared to the present study was because the studies were not focused on the paddy growing areas as was done in this study.

The prevalence and mean faecal egg count of *Fasciola* spp. were compared breed-wise, age-wise, sex-wise and health-wise for the study. The mean faecal egg counts for different breeds, age groups, sexes and health status of

Table 2: Overall and species-wise prevalence of gastro-intestinal parasites

Chiwog	Prevalence of Gastro-intestinal Parasites								
	Fas	**Asc**	**Str**	**Coc**	**Dic**	**Par**	**Stro**	**Bcoli**	**Tri**
Namseling	36	47	23	18	26	2	1	1	2
(*n* = 125)	(28.80)	(37.60)	(18.40)	(14.40)	(20.80)	(1.60)	(0.80)	(0.80)	(1.60)
Khasadrapchu	34	42	4	4	4	2	1	3	0
(*n* = 93)	(36.56)	(45.16)	(4.30)	(4.30)	(4.30)	(2.15)	(1.08)	(3.23)	(0.00)
Overall	70	89	27	22	30	4	2	4	2
(*n* = 218)	(32.11)	(40.83)	(12.39)	(10.09)	(13.76)	(1.83)	(0.92)	(1.83)	(0.92)

Figures in parenthesis show prevalence of different endo-parasites in percentage
Fas=Fasciola, Asc=Ascaris, Str=Strongyle, Coc=Coccidia, Dic=Dicrocoelium, Par=Paramphistomum, Stro=Strongyloides, Bcoli=Balantidium coli, Tri=Trichuris

the animals were then compared using *t*-test (0.5 level of significance and 95% confidence level) and the result is given in Table 4.

The prevalence of fasciolosis was higher (45.45%) in local breed of cattle compared to the improved ones in the area. A significant difference ($p < .05$) was observed between the breeds. This could be due to differences in the management practices of the farmers. The local breeds are reared under traditional husbandry system and farmers give more attention to crossbreed than local breeds because of their production differences (Genet and Derso, 2015). This result was different from what Tshering and Dorji (2013) reported in Haa in which the prevalence of fasciolosis was higher in improved adult cows (9.17%) than the local adult cows (1.67%), however, in Haa a higher prevalence was observed in local younger cows (2.50%) than the improved breeds

(0.0%).

In the study area, the prevalence of fasciolosis was higher in adult cattle (35.56%) than calves (27.27%) and young ones (29.91%), however there was no significant difference in different age groups. Similar results were also reported by Fentene and Addis (2014) with prevalence of 23.1% and 21.8% in young and adult cows respectively.

The prevalence of fasciolosis was higher in male (45.45%) than female (27.60%). A similar result reported by Yehenew (1985) revealed a higher prevalence in male than female. This was probably related to the management system with longer exposure of male outdoor while females are kept indoor during pregnancy and lactation (Genet and Derso, 2015). However, in the present study no significant difference was observed between sex groups.

The higher prevalence of fasciolosis was found in sick animals (42.86%) when compared to healthy animal (30.96%); however, the difference was not significant (Table 3). These findings were different from what Yusuf *et al*. (2016) reported in which the prevalence of fasciolosis in sick animal was significantly higher than healthy animals.

Resistance of Fasciola spp. to selected anthelmintic drugs

After the examination of 218 faecal samples for various endo-parasites, the positive samples for *Fasciola* spp. were randomly selected and divided into three groups. Among these, 27 animals were subjected to treatment using triclabendazole, 21 animals were treated using oxyclozanide + levamisole and 21 were kept as control group. The assessment of faecal egg counts for *Fasciola* spp. in each group was then done using faecal egg count reduction test (FECRT) and the results analyzed.

For treatment 2, the mean faecal egg count for *Fasciola* spp. during the pre-treatment was 2.56 ± 2.33 *SD* (*n* = 27) and 0.33 ± 0.78 *SD* (*n* = 27) for post-treatment. Similarly, for the treatment 3, the mean faecal egg count for *Fasciola* spp. during pre-treatment was 2.76 ± 2.76 *SD* (*n* = 21) and 0.24 ± 0.44 *SD* (*n* = 21) for post-treatment. For the control group, the mean faecal egg count for *Fasciola* spp. during the pre-treatment was 2.05 ± 1.75 *SD* (*n* = 21) and 2.00 ± 1.45 *SD* (*n* = 21) for post-treatment.

The results showed decreasing trends in the faecal egg counts of *Fasciola* spp. in treatment 2 and treatment 3 while there was no significant ($p > .05$) change in the faecal egg counts

Table 3: Prevalence of *Fasciola* spp. breed-wise, age-wise, sex-wise and health status-wise

Category	Types	Prevalence (%)	Mean Faecal Egg Count (epg)	Confidence Interval	*p - value*
Breed	Local (*n* = 77)	45.45	1.30 ± 2.35[a]	0.26-1.24	*p = .00*
	Improved (*n* = 141)	24.82	0.55 ± 1.32[b]		
Age	Calves (*n* = 11)	27.27	0.36 ± 0.67[a]		
	Young (*n* = 117)	29.91	0.81 ± 1.77[a]	-1.52-0.62	*p = .41*
	Adults (*n* = 90)	35.56	0.87 ± 1.90[a]	-1.66-0.64	*p = .38*
Sex	Male (*n* = 55)	45.45	0.74 ± 1.78[a]	-0.17-0.93	*p = .17*
	Female (*n* = 163)	27.60	1.12 ± 1.79[a]		
Health status	Healthy (*n* = 197)	30.96	0.77 ±1.75[a]	-1.28-0.34	*p = .25*
	Sick (*n* = 21)	42.86	1.24 ± 2.10[a]		

Calves=<1yr, Young=1-5yrs, Adults=>5yrs

Means of same letter shown in superscripts for each category compared are not significant at $p < .05$

in the control group. Differences in the pre-treatment and post-treatment faecal egg counts of the different treatment groups can be possibly attributed to the fact that these animals were owned by different farmers (Venturina *et al.*, 2015). It is likely that they have differences in *Fasciola* load in liver and different number of eggs excreted in the feces (Venturina *et al*., 2015)

The pre-treatment and post-treatment mean faecal egg counts of *Fasciola* spp. for three groups were compared using paired sample *t*-test and the result showed that there was significant difference in the means of *Fasciola* spp. egg counts in treatment 2 ($p < .05$) and treatment 3 ($p < .05$) treated groups while there was no difference in the means of pre-treated and post-treated faecal egg counts for control group ($p > .05$).

The efficacies of the triclabendazole and oxyclozanide + levamisole drugs were calculated, and the results were compared with the control group and are shown in Table 4.

The result showed that the efficacy of triclabendazole against *Fasciola* spp. in this study was 86.96% which is less than 90% indicating that triclabendazole drug was ineffective and there is a development of resistance to this drug by *Fasciola* spp. (Coles *et al*., 1992; Wood *et al*., 1995). Resistance or failure of treatment with the tested drug was defined as efficacy of less than 90% based on the standards set by the World Association for the Advancement of Veterinary Parasitologists (WAAVP) (Coles *et al*., 1992; Wood *et al*., 1995; Venturina *et al*., 2015). The development of resistance to triclabendazole in the study area by fasciolosis is suspected since this drug had been in use for a very long time. Similar findings have also been reported by Venturina *et al*. (2015) in buffaloes in Philippines whereby efficacy of triclabendazole was recorded 73.33% after 14 days of treatment. A very low efficacy (31.05%) of triclabendazole was recorded at 14 days post treatment in Peru by Ortiz *et al*. (2013).

The efficacy of oxyclozanide + levamisole suspension in the study was 91.38% which was higher than 90% efficacy indicating that the drug is effective against *Fasciola* spp. and the development of resistance has not yet been established to this drug. Although oxyclozanide suspension had been used in Bhutan to treat fascioliasis since 1980s (Wangdi, 2015), this drug has been replaced in the year 2014 with combined formulation containing Oxyclozanie + Levamisole (DoL, 2016) and thus retaining its higher level of efficacy at present. Similar study done by Shirmali *et al*. (2015) in goats in South Gujarat showed an efficacy of 94.74% for oxyclozanide + levamisole suspension after seven days post-treatment. Arafa *et al.* (2015) also reported 100% efficacy after two weeks post-treatment of Oxyclozanie + Levamisole to *Fasciola* in a study conducted in cattle in Egypt.

Conclusion

Fasciolosis is an economically devastating parasitic disease that causes serious illness and death of animals in serious cases or loss in production due to chronic diarrhoea. Pathological lesions due to fasciolosis cause a considerable economic loss due to condemnation of the affected livers. The present finding showed a high prevalence (32.11%) of *Fasciola* spp. in the study area affecting the health and productivity of animal.

The low efficacy of triclabendazole in this study indicates the presence of triclabendazole resistant *Fasciola* spp. in cattle in the study area. Regular treatment with triclabendazole is likely to have resulted to resistance and it is likely that resistant fluke populations have also been established in cattle. The results show that Oxyclozanide+Levamisole are effective drugs for the treatment of *Fasciola* spp. in the study area. Therefore, the drug Flunil-L is highly recommended for treating fascioliasis in the study area. This study carried out demonstrates the resistance of *Fasciola* spp. to triclabendazole in cattle in Maedwang gewog only. Further research is required to determine

Table 4: Mean FEC and efficacy of selected anthelmintics against *Fasciola* spp.

Drug	Body Weight (kg)	Dose rate	Mean Faecal Egg Counts (epg)		Efficacy (%)
			Pre-Treatment	Post-Treatment	
Triclabendazole	257.81	12 mg/kg BW	2.56 ± 2.33^{a}	0.33 ± 0.78^{b}	86.96
Oxyclozanide + Levamisole	296.56	50 ml/100kg BW	2.76 ± 2.76^{a}	0.24 ± 0.44^{b}	91.38
Control	271.67	No treatment	2.05 ± 1.75^{a}	2.00 ± 1.45^{a}	2.33

Means of the different letters in pre-treatment and post-treatment groups indicate significant difference ($p < .05$)

the extent of anthelmintic resistance across the wider dairy population in the country to validate the present findings and for the department of livestock to change or update the anthelmintics accordingly.

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