International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2020; 5(5): 23-26

www.veterinarypaper.com
Received: 18-07-2020
Accepted: 20-08-2020

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Comparative efficacy of five anthelmintic drugs against a naturally acquired *Fasciola* species infection in sheep in Jumla, Nepal

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DOI: https://doi.org/10.22271/veterinary.2020.v5.i5a.298

Abstract
Fascioliasis is one of the important parasitic diseases in tropical and subtropical countries which limit productivity of ruminants. The aim of present study was to evaluate the efficacy of five different anthelmintics against liver fluke in a naturally infected sheep flock at Sheep and Goat Research Program, Guthichaur, Jumla. Altogether 30 sheep positive with *Fasciola* eggs were selected for the study. These 30 sheep were divided into six groups including control one and each group consisted five animals. Sheep of Group I, II, III, IV and V were treated with different anthelmintics *viz*. Albendazole, rafoxanide, oxyclozanide, oxyclozanide and levamisole combination, and triclabendazole respectively. Faecal samples were obtained from each animal per rectum on the day of treatment and again 1, 3, 7, 14 and 28 days post-treatment. The efficacy of the used anthelmintics was determined by faecal egg count reduction percent in which the fecal egg was investigated by sedimentation technique. The results for oxyclozanide, oxyclozanide and levamisole combination and triclabendazole indicated that these drugs were highly effective with faecal egg count being reduced by 100% by day 14 post-treatment. However, the results for albendazole and rafoxanide groups yielded lower efficacy levels, with faecal egg count reductions 92.8% and 91.8% respectively, over the period 28 days post-treatment. These results are highly indicative for albendazole and rafoxanide resistant *Fasciola* in sheep on this farm.

Keywords: Five anthelmintics, efficacy, fascioliasis, sheep and Jumla

1. Introduction
The common liver fluke (*Fasciola species*) causes significant production disease in sheep and cattle worldwide. Control of fascioliasis is based on treatment with flukicides that differ in chemical structure and mode of action, but also in their efficacy against different liver fluke developmental stages [1]. Triclabendazole and albendazole are common benzimidazoles used against liver fluke infection [2]. Triclabendazole is effective against both adult and juvenile flukes from the age of 2 days, and is the drug of choice for both sheep and cattle, whereas albendazole is effective only against adult flukes (from 12 weeks) [1]. Albendazole is a commonly used flukicide around the world. Unlike in many other European countries it has long been used as drug of first choice against liver flukes in sheep in Sweden [3].
Resistance of liver flukes to flukicides had not been noted until the first report of triclabendazole resistance in Australia in 1995 [4]. Since then, numerous cases of triclabendazole resistance have been reported in Australia [5], Europe [6, 7, 8, 9] and South America [10]. There is no standard recommended protocol for determination of flukicide efficacy/resistance [11]. Faecal egg count reduction test (FECRT) [6, 9, 12], coproantigen reduction test (CRT) [5, 13], detection of fluke DNA in faeces by polymerase chain reaction (PCR) [14], post-mortem fluke counts [15] and histology of flukes exposed *in vivo* [16] have all been used for detection of flukicide efficacy in ruminants. In common veterinary practice, a combination of FECRT and CRT seems to be the most promising methods for detection [5, 8, 17, 18].
Fasciola is one of the most important helminth parasites of livestock in many countries of the world and often causes severe economic loss. Triclabendazole, a benzimidazole derivative, is one of the most widely used drugs to control fascioliasis worldwide due to its high activity against both adult and immature fluke [19] but in Nepal it is used sometimes only. It has been shown that closantel, nitroxynil, rafoxanide and oxyclozanide are effective against adult

triclabendazole-resistant fluke [6, 12, 15]. Resistance is usually defined *in vivo* by a reduction in the expected efficacy of an anthelmintic.

Farm of Sheep and Goat Research Program, Guthichaur, Jumla recorded prevalence of ovine fascioliasis 34% in fecal test, 6.7% in slaughtered sheep and 23.5% in post mortem examinations [20]. Although in earlier, few studies have already reported the prevalence of fascioliasis in different animals from many parts of Nepal [21, 22, 23, 24]. Generally, sheep of the farm were treated with albendazole, oxyclozanide against liver fluke. But there is limited authorized data and published literature about efficacy of anthelmintics against liver fluke. Hence this study wants to explore about efficacy of different flukicides in sheep.

The aim of the present study was thus to evaluate the efficacy of five different classes of anthelmintic drugs (albendazole, rafoxanide, oxyclozanide, oxyclozanide+levamisole and triclabendazole) against a naturally acquired *Fasciola species* infection in sheep flock in the farm using a faecal egg count reduction (FECR) test.

2. Materials and methods

2.1 Animal management

The experiment was conducted at Sheep and Goat Research Program, situated at Guthichaur Rural Municipality ward no. 2, Jumla District, Karnali Province, Nepal and is located at 19 km far from District head quarter (Khalanga) Jumla. The flock of farm comprised of 315 animals including goats. Study location was situated in mountainous region with the altitude ranges from 2500-3000 (avg 2700) meter above sea level. Latitude and longitude of the site is $29^0$17'72.5" N and $82^0$40'83.4"E respectively. Sheep and goats remain as intensive housing system during snowfall (December to March). Both the animals were supplied with hay and concentrates during this period. Sheep were taken to high altitude Majhpatan (3300 meter above sea level) and other pasture lands for grazing in summer and rainy seasons as free range system.

About 100 sheep were examined for presence of *Fasciola* eggs by microscopic examination of faeces. This experiment was carried out on reared sheep in an endemic zone by fascioliasis. The farm has a history of fascioliasis and ewes are routinely treated with a fasciocidal anthelmintic [20]. The selected sheep flock (n = 30 ewes) used for the present study had not received anthelmintic treatment for at least three months prior to the commencement of the study.

The farm area located at Sheep and Goat Research Program, Guthichaur and three sides of this farm was surrounded by marshy land, water-logged areas. Also, the animals were fed regularly from hays and grasses that grow in river basin at the time of examination. Thirty females of Baruwal sheep, having age of one to one and half years, and weighing from 20 to 30 kg (mean: 25 kg) were selected for inclusion in the study. During the experiment duration for 28 days in May-June 2020, all animals were kept in the same conditions: intensive system of housing and ration (dry ration).

2.2 Experimental design and procedure

Before starting of drug application, fecal examination was carried out to report the egg count using the sedimentation test. The 30 animals represent the sum of the experimental groups ($n = 25$) and the control group ($n = 5$). The groups were divided randomly to 5 experimental groups (5 animals each) and treatment regimens were described in Table 1. The control group ($n = 5$) received no treatment. Individual faecal samples were collected on days 0, 1, 3, 7, 14 and 28 days post-treatment. Each faecal sample was taken per rectum for all animals. The fecal egg count of *Fasciola* eggs per gram (epg) was decided using a sedimentation technique.

Table 1: Anthelmintic treatment regimens administered to sheep

Group	Anthelmintic	Route of use	Dosage: mg/kgbw (body weight)	Trade name
I	Albendazole	Oral	15mg/kg bw	Aben
II	Rafoxanide	Oral	10mg/kgbw	Ranide
III	Oxyclozanide	Oral	15 mg/kgbw	Clozan
IV	Oxyclozanide+Levamisole	Oral	22.5 mg/kgbw	Clozan-L
V	Triclabendazole	Oral	10 mg/kgbw	Fasinex

2.3 Sedimentation technique

Fecal samples were collected per rectum from the sheep. Each samples of 5 g of fecal material was collected in clean polythene bag containing 10% formalin as preservative. These samples were stored at 4^0C in individual self-seal polythene bags labelled with the management number of the corresponding animal, until the number of *Fasciola* eggs per gram of faeces was determined using a modified sedimentation technique as outlined below.

For each individual faecal sample, 3 g of faeces was added to 42 ml of water in a graduated cylinder. The contents were then mixed thoroughly in mortar and pestle, using a glass rod it was poured through a tea strainer to remove large debris. The solution was then further passed through a sieve (mesh aperture 210 µm) into a conical flask and water was run through the sieve to ensure no eggs remained attached to the sieve. The filtrate was then allowed to sediment for 3 minutes after which the supernatant was siphoned off taking care not to disturb the precipitated matters. The latter was stained with two drops of methylene blue and the entire sediment placed on slide covered with a cover slip and viewed under a dissecting microscope (Labomed). The number of *Fasciola* eggs observed was counted and from this the number of epg was calculated [25].

2.4 Evaluation of drug efficacy

The efficacy of the drugs was evaluated by measuring egg shedding. For each animal, faecal samples were collected on day 0, 1, 3, 7, 14 and 28 days (end of the study) after treatment. For detection of egg count per gram of feces (epg), the sedimentation technique was conducted, as previously mentioned above, according to Mooney *et al.* [25]. The efficacy of the drugs was assessed by the reduction of mean egg excretion at each measurement point. A number of mathematical formulae can be used to calculate anthelmintic efficacy and the choice of formulae can influence whether or not a farm is declared resistant [26]. The efficacies of the anthelmintic were determined from the faecal egg count reduction (FECR) using following formula:

The anthelmintic efficacy percent was calculated by the equation: {Eggs number in untreated control group (T_0) - eggs number in the treated one (T_x)} / Eggs number in untreated control group (T_0) x 100

OR, FECR=100 x $\{(T_0-T_x)/T_0\}$

2.5 Statistical analyses

The eggs per gram feces were statistically analysed by using SPSS 20.0. Differences were considered significant for $p < 0.01$. The eggs per gram of faeces were uniformly distributed according to the Kolmogorov–Smirnov's Normality Test and were expressed as the mean ± SEM (standard error of the mean).

3. Results

In day zero, the results showed that, the mean epg between different groups (treated and control non-treated) was non-significant (before treatment). Also there was no difference between all groups in the epg feces count on first day after treatment in the treated groups. But it was found that epg count began to decrease from third day.

In the 7th day after treatment, the fecal egg count in the treated groups began to decrease abruptly. At the day 14 post-treatment, the egg count per gram feces in the albendazole and rafoxanide groups decreased significantly ($p < 0.01$) than the control one. So the efficacy of these drugs (albendazole and rafoxanide) was 92.8% and 91.8% respectively.

On the other hand, the group III (oxyclozanide), group IV (oxyclozanide and levamisole combination) and group V (triclabendazole) showed a significant ($p < 0.01$) reduction in the egg count from the day 7 post-treatment in the treated animals (Table 2). Although the efficiency of the used therapeutics was observed on days 14 and 28 in all the treated groups but group III, IV and V were the most effective drugs in reducing the mean epg during the same period of the study to zero. Finally, in these three treated groups, the eggs disappeared from feces after 14 days post-treatment and did not return again till the end. So, the efficiency of drugs of group III, IV and V was 100% reduction of eggs depending on the last reading of egg count at the end of the experiment (28 days post-treatment) (Table 2).

Table 2: Means of fecal egg count/3 g of feces before and after administration of the five anthelmintic drugs used

Observation day	Albendazole Gr I	Rafoxanide Gr II	Oxyclozanide Gr III	Oxyclozanide+Levamisole Gr IV	Triclabendazole Gr V
Zero day	35 ± 1.7	35.8 ± 1.8	33.0 ± 1.4	34.4 ± 0.9	35.9 ± 1.8
One day	36.5 ± 1.8	35.7 ± 1.8	32.2 ± 1.3	34.4 ± 0.9	35.9 ± 1.8
3 Days	23.5 ± 1.2	24 ± 1.2	21.8 ± 0.7	21.1 ± 0.9	21 ± 1.2
7 Days	6.2 ± 0.6*	9.5 ± 1.0*	6.3 ± 0.5*	5.6 ± 0.3*	6.2 ± 0.6*
14 Days	3.5 ± 0.5*	5.5 ± 1.0*	0*	0*	0*
28 Days	2.5 ± 0.5*	2.1 ± 0.8*	0*	0*	0*
Efficacy	92.8%	91.8%	100%	100%	100%

Note: * indicates a value that is statistically significant different from the corresponding value in "zero day" means significant with zero day.

4. Discussion

Fascioliasis remains one of the most important helminthic diseases of livestock in many countries in the world. However, the sheep infection was chronic causing a major economic impact due to reductions in weight gain, reduce lamb birth, milk yield and fertility and to liver damage.

Triclabendazole and rafoxanide were used first time in this farm while others were used time to time against fascioliasis.

It is clear from the results that triclabendazole, oxyclozanide and combination of oxyclozanide and levamisole are highly effective compounds for the treatment of *Fasciola spp* on this farm.

Previous field studies on the use of triclabendazole against mixed-age fluke infections in sheep and experimental studies show that a 100% reduction in egg count (efficacy) would be expected after 3 weeks [27] and 100% efficacy in cattle [28]. Triclabendazole is effective against both adult and immature fluke due to its high activity on them [19]. This high activity against immature fluke is significant because this is the most damaging stage of infection since this is when the fluke is in the migratory and tissue invasion stage. Regarding Oxyclozanide efficacy, this study is in line with previous report [25]. But higher efficiency level of triclabendazole (100%) found in our finding than other report (66%) [25].

We observed in the present study that albendazole and rafoxanide were the treatments that had a limited effect on fascioliasis, as their administration decreased the egg count number but the eggs did not disappear. The low efficacy obtained in this study indicates the presence of albendazole (92.8%) and rafoxanide (91.8%) resistant *Fasciola spp* in sheep on this farm. This may be referred to that albendazole and rafoxanide affect only the adult stages of *Fasciola* species while the immature stages developed to reach adult stages so that it may be explained why eggs still discharge in this treated group. Even though, the treatment by albendazole was done, most treated cases gave eggs in feces after treatment; this may be attributed to the presence of resistance to this drug. Albendazole was used common in Nepal from many years and it applied in random manner by farmers. Use of albendazole in treatment and prophylaxis against gastrointestinal helminths was repeated in manner may be lead to give strain resistant to it by time.

From this study we conclude that shedding of *Fasciola* eggs disappears in 14 days of treatment with triclabendazole or oxyclozanide or combination of oxyclozanide with levamisole in case of sheep. The interval needs to be long enough to allow the expulsion of eggs, which, in the case of *Fasciola spp*, may be stored in the gall bladder even after adult fluke have been removed [29], but short enough to precede the development of new patent infection.

5. Conclusions

Among five anthelmintic drugs used against fascioliasis in sheep in this study, oxyclozanide, oxyclozanide+levamisole and triclabendazole are found highly effective in comparison to albendazole and rafoxanide. *Fasciola* eggs disappeared on 14 day post -treatment in case of oxyclozanide, oxyclozanide+levamisole and triclabendazole. It is recommended that triclabendazole should be used against liver fluke infestation if available.

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