Short Report: Efficacy of a Single Oral Dose of Oxfendazole against *Fasciola hepatica* in Naturally Infected Sheep

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Abstract. The efficacy of a single oral dose of 30 mg/kg of oxfendazole against *Fasciola hepatica* was evaluated in a controlled study in naturally infected sheep. Sheep were diagnosed by stool microscopy after sedimentation, and positive animals were randomized to oxfendazole ($N = 20$) or no treatment ($N = 20$). A new stool exam was performed 10 days after treatment. All stool microscopies were performed masked to the treatment group. No side effects were noticed. All sheep in the control group remained infected with similar counts of eggs per gram of stools. None of the animals in the treatment group showed *Fasciola* eggs in stools after 10 days of treatment. A single dose of oxfendazole is highly effective against *F. hepatica*, providing a new drug alternative for the control of fascioliasis or integrated zoonosis control.

INTRODUCTION

Fascioliasis is an infection caused by the trematode *Fasciola hepatica*, commonly found in the liver and biliary system of farmed ruminants.¹ This infection has a cosmopolitan distribution and it is the main parasitic disease of livestock in terms of economic losses. Fascioliasis causes chronic human liver disease and is an emerging public health problem.^{2,3} Fascioliasis is endemic in the Peruvian highlands, with infection rates up to 100% in sheep and cattle, and prevalence in humans ranging from 9% to 16%.^{4,5} Control of fascioliasis is crucial for animal and human health, and rests solely on chemotherapy.^{6,7} Triclabendazole (TCBZ) is the current drug of choice; however, TCBZ resistance has already been reported in livestock farms across Europe, Australia, and some countries of South America.^{8–12} Our group has extensive experience using a single oral dose of 30 mg/kg of oxfendazole to treat porcine cysticercosis (*Taenia solium*). To the best of our knowledge, this scheme has not been tested for trematode infections in field conditions. We evaluated the efficacy of 30 mg/kg of oxfendazole against *F. hepatica* in naturally infected sheep.

MATERIALS AND METHODS

Study area and animals. The study was conducted in two sheep farms (A and B) located in Occobamba community, an endemic area to *F. hepatica*. Both farms were similar in terms of environmental settings and animal handling conditions. Occobamba is an agricultural and pecuary community located in the Andes at 4,000 m above the sea level in Cusco, Peru. Animal husbandry is based on raising sheep, cattle, and South American camelids.

Study design and treatment. The sheep farms had a total of 50 and 60 sheep up to 1 year of age. All animals from the farms had a stool sample collected for coproparasitological and screened for *Fasciola* one day before the beginning of the study (Day 0). Stool samples were collected directly from the rectum of each sheep, placed into labeled new polyethylene bags, transported to the local laboratory, and processed for microscopy examination after sedimentation¹³ to detect

Fasciola-positive animals and determine their baseline fecal egg output. Only animals with fecal *Fasciola* egg counts equal or higher than two eggs per gram (*epg*) were included in the study. All included sheep also showed parasite eggs compatible with nematode and cestode infections in their baseline stool samples. The sample size was calculated using the Snedecor and Cochran¹⁴ formula to estimate a difference between proportions, considering a minimal difference between groups of at least 30% to justify treatment of infected animals.

After screening, 20 sheep from each farm were randomly selected; 20 sheep (14 females and 6 males) from farm A were considered as the control group, and 20 sheep (15 females and 5 males) from farm B were considered as the treatment group. The control group did not receive treatment, whereas the treatment group was treated orally with a single dose of 30 mg/kg of body weight of oxfendazole (Synanthic, Fort Dodge, Mexico), adapted from the studies from Gavidia and others¹⁵ and Gonzalez and others.¹⁶ A drench dosing gun was used for oxfendazole administration, with the animal in standing position. After treatment the animals were released and allowed to continue grazing in the area. The median was used to express the parasite burden (number of eggs per gram of feces) on each group. The data were analyzed using a non-parametric approach with two-sample Mann-Whitney test.

Sampling and sample processing. Fecal samples were taken from each animal 1 day before treatment and 10 days after treatment. The samples were analyzed individually with a sedimentation method. An original sieve technique was used for the quantitative assessment of the presence of eggs of *F. hepatica* in the feces. For this technique, 3 g of fecal samples were homogenized with 15 mL of tap water, slowly passed through a sieve, and then slowly washed under running water. Material filtered on the sieve was transferred to an edged Petri dish and examined under microscope at 100 $\times$. The sensitivity of this method is very high allowing counting one egg per gram.¹³

Ethical Approval. This study was approved by the ethical committee of animal welfare of the Veterinary School from San Marcos University.

RESULTS

At Day 0 the sheep had a median of 4 (range 2–28) *epg*, with similar egg counts between groups (Table 1). Ten days

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TABLE 1

Median and range of eggs before and after treatment with oxfendazole in sheep

	Control	Treatment
	N = 20	N = 20
Male	6	5
Age (in year)	3.5 (1–5)	3.8 (2–5)
Weight (kg)	35.6 (28–40)	36.6 (29–41)
Median baseline egg count	5 (2–28)*	4 (2–22)*
Post treatment egg count	2 (1–37)†	0†

*Mann-Whitney test demonstrated no difference between control and treatment at baseline.

†Significant difference between control and treatment (Mann-Whitney test, $P < 0.01$).

after treatment, we observed a statistical difference between control and treatment; the stool samples from all animals in the control group were positive for *Fasciola* eggs (median = 2), whereas none of the samples from treated animals had eggs ($P < 0.01$) (Table 1). Likewise, at baseline all sheep were positive for nematode eggs (median 450, range 100–2,800 *epg*), and three sheep from the control group and seven sheep from the treatment group were also positive for cestode eggs. On post-treatment follow-up the control group had a median of 400 (range 0–1,700) nematode *epg* and 5 sheep from the control group were positive for cestode eggs. In the treatment group all animals were negative for helminth parasites.

DISCUSSION

This study provides proof of concept of feasibility and efficacy of treating *F. hepatica* and other gastrointestinal helminthes in sheep, with a single oral dose of 30 mg/kg of oxfendazole, under field conditions.

Benzimidazoles are a group of broad-spectrum anthelmintic drugs with good efficacy against nematode, cestode, and trematode, commonly used in human and veterinary medicine.¹⁷ For fascioliasis the most used benzimidazoles are albendazole and TCBZ. Albendazole is recommended for the control of *F. hepatica* in domestic animals¹⁸; however, its anthelmintic effect is only for chronic fascioliasis (adult stages) and is not effective to acute and sub-acute fascioliasis (immature stages).^{19,20} Currently, fascioliasis is mainly treated using the TCBZ because of its ability to act in both stages, immature and mature *F. hepatica*.²⁰ This drug has been used since the 80s and it has shown good activity against liver fluke for many years.^{21–23} However, in recent years *F. hepatica* has been reported to present resistance to TCBZ in various countries.^{8–12,24} This study demonstrated that oxfendazole at 30 mg/kg acts efficaciously against *F. hepatica* in naturally infected sheep. Although the study was not designed to evaluate the safety or pharmacokinetics of this dosage of oxfendazole in sheep, the animals did not show any signs of toxicity.

Oxfendazole has been previously evaluated against other important zoonotic tissue helminths. Gonzalez and others¹⁶ showed that oxfendazole is effective against *T. solium* cysticercosis in naturally infected pigs. Likewise, Gavidia and others¹⁵ showed that oxfendazole in combination with praziquantel is effective against hydatid disease (*Echinococcus granulosus*) in naturally infected sheep. Oxfendazole has also been previously used for the treatment of fascioliasis. Faser and others²⁵ realized a survey about antiparasitic drug used in a sheep farm from England, and found that a farm used oxfendazole in the treatment of fascioliasis, but no mention of the dose or efficacy

of oxfendazole are provided. Likewise, Furmaga and others²⁶ evaluated oxfendazole against sheep parasitosis at doses of 5 and 15 mg/kg of body weight, reporting an efficacy for fascioliasis of 14% to 20%.

Oxfendazole is stored in the rumen of ruminants and it is detectable for up to 7 days after administration.^{27,28} Therefore, it is not necessary to dose the animals twice a week.²⁹ We selected 30 mg/kg based on the literature and our experience in sheep, and also in pigs that are monogastric. Whether higher or lower doses could be used with similar efficacy and no toxicity remains to be studied.

Our study could have potentially overestimated cure rates if the coproparasitological examination was insensitive, although there is no reason to assume lack of sensitivity in the diagnosis. Follow-up egg counts in the controls were always positive and the egg counts were in similar ranges (Mann-Whitney test, $P = 0.11$). On the other hand, we cannot exclude the possibility of a temporary effect, if *Fasciola* infections were only partially damaged and surviving larvae begin to produce eggs again after 2 or 3 weeks. For logistical reasons, we could not prolong our stay in the region for more time.

The data presented here shows that a single dose of oxfendazole is safe and effective against *F. hepatica* in sheep. Oxfendazole is not expensive, effective in a single dose, and available worldwide. It should be considered as a new alternative drug for the control of fascioliasis, and eventually for integrated zoonosis control.

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