TRICLABENDAZOLE RESISTANCE INVOLVING *Fasciola hepatica* IN SHEEP AND GOATS DURING AN OUTBREAK IN ALMIRANTE TAMANDARE, PARANA, BRAZIL

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ABSTRACT: - OLIVEIRA, D.R.; FERREIRA, D.M.; STIVAL, C.C.; ROMERO, F.; CAVAGNOLLI, F.; KLOSS, A.; ARAÚJO, F.B.; MOLENTO, M.B. **Triclabendazole resistance involving *Fasciola hepatica* in sheep and goats during an outbreak in Almirante Tamandare, Paraná, Brazil**. [Resistência da *Fasciola hepatica* ao Triclabendazole em ovinos e caprinos durante um surto ocorrido em Almirante Tamandaré, Paraná, Brasil]. *Revista Brasileira de Parasitologia Veterinária*, v. 17, supl. 1, p.149-153, 2008. Laboratory of Parasitic Diseases, Departmento de Medicina Veterinária, Universidade Federal do Paraná, Rua dos Funcionários, 1540, Curitiba, PR 80050-035, Brasil. E-mail: molento@ufpr.br

Fasciolosis is a disease of extreme importance, occurring throughout Brazil, with great economic losses to the animal industry. This study aims to determine the effectiveness of treatment against *Fasciola hepatica* on a sheep and goat farm during an outbreak of fasciolosis, in which a high proportion of deaths occurred. The farm harbored 33 sheep and 60 goats of mixed breeds with varying weights and ages. Parasite control was based on suppressive, monthly treatments with moxidectin. Over the course of the study, assessments with Famacha method, body condition score, faecal exam (EPG), coproculture and the egg sedimentation technique were made on seven occasions. At the time of the first visit 81% of the goats and 100% of the sheep were positive for *F. hepatica*. Salvage treatment with triclabendazole failed to relieve the heavy infection due to resistance, with an efficacy of only 66.3% being obtained against the *F. hepatica* population consensual in sheep and 57.3% in goats. There was no record of triclabendazole having been administered previously on the farm. A low level of correlation was found between the Famacha values and the incidence of anemia due to clinical fasciolosis (C<0.5). There is an urgent need to investigate the correct use of available drugs against *F. hepatica* and the implementation of alternative control strategies in endemic areas in order to ensure optimum sustainability of the efficacy of available fasciolicides.

KEY WORDS: Sheep, goats, *Fasciola hepatica*, triclabendazole.

RESUMO

A Fascilose é uma doença de extrema importância com ocorrência no território do Brasil trazendo grandes perdas econômicas na produção animal. Este estudo tem por objetivo determinar a eficácia do tratamento contra a *Fasciola hepatica* na criação de ovinos e caprinos afetados em surto de fasciolose no qual ocorreram muitos óbitos. A propriedade era composta por 33 ovinos e 60 caprinos mestiços, com variação em idade e peso. O controle parasitário era baseado em tratamento supressivo mensal com Moxidectin. Foram realizadas sete visitas a propriedade com avaliações pelo método Famacha, escore corporal, exames de fezes (OPG), coprocultura e sedimentação. Foi determinado que 81% dos caprinos eram positivos para F. hepática enquanto que essa percentagem nos ovinos era de 100% na primeira avaliação. O tratamento com Triclabendazol falhou para combater a grande infecção devido à resistência, com uma eficácia de apenas 66.3% contra a população de *F. hepatica* em ovinos e 57.3% em caprinos. Não houve registro de triclabendazol ter sido administrado anteriormente na propriedade. Uma baixa correlação foi encontrada entre os valores de Famacha e a incidência de anemia devido a fasciolose clínica (C<0,5). Existe uma necessidade urgente para investigar a utilização correta dos medicamentos disponíveis contra *F. hepatica* e a implantação de estratégias alternativas

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de controle em áreas endêmicas a fim de garantir a sustentabilidade da eficácia de fasciolicidas disponíveis.

PALAVRAS-CHAVE: Ovinos, caprinos, Famacha, fasciolose, resistência.

INTRODUCTION

Fasciolosis is a parasitic disease caused by *Fasciola hepatica* (Linnaeus, 1758), a trematode of the Fasciolidae family, which affects the liver and biliary tract of many species of wild and domestic animals, and humans (DITTMAR, 2003). Serious economic losses in production occur due to condemnation of livers, as well as reduced weight gain, quantity and quality of milk, low fertility and death in heavy infections. While acute fasciolosis results from the migration of the larvae, leading to liver trauma and hepatitis, the chronic disease leads to emaciation, anaemia and condemnation of the liver (FOREYT, 2005).

The availability of a good habitat for the intermediate snail of the genus *Lymnaea* (intermediate host) to develop and the presence of the metacercariae in the environment increase the chance of animal contamination by ingestion of water or plants. In Brazil, different species of snails occurs mainly in the Southern and Southeastern States (UETA, 1980; SERRA-FREIRE; NUERNBERG, 1992; SERRA-FREIRE, 1995; BRUNO et al., 1995; ABILIO; WATANABE, 1998; LESSA et al., 2000; PILE et al., 2001; GOMES et al., 2002). Although an alarming observation is that *F. hepatica* is spreading along the Brazilian territory without proper control methods or technical notification (SIERRA-FREIRE, 1995).

The Famacha method aims to identify individuals that are resistant, resilient or sensitive to bloodsucking parasites (*Haemonchus contortus*) through the color of the conjunctiva, linking to clinical anemia. This would allow the usage of partial selective treatment, reducing the use of anthelmintics (MOLENTO et al., 2004). Although anemia is also a major clinical sign in fasciolosis, the examiner must be aware of other events that are occurring at the farm level, such as multiple parasite infections (VAN WYK, 1997).

Parasite control has become increasingly difficult due to the development of resistance to most parasiticides. Triclabendazole has been the drug of choice against *F. hepatica* due to its high efficacy against both juveniles and adults, but the heavy reliance on a single drug put treatment strategies of fasciolosis at risk (BRENNAN et al., 2007), with several records of resistance to field isolates (COLES et al., 2000; OVEREND et al., 1995; FAIRWEATHER, 2005; ÁLVAREZ-SANCHEZ, 2006). The objective of this study was to determine the effectiveness of triclabendazole against concurrent infections in sheep and goats after a serious outbreak of *F. hepatica*.

MATERIAL AND METHODS

Local Conditions

The property is located in Almirante Tamandaré, Paraná. It has 12 paddocks, with a forest-protected area and lowland flooded area. The 33 sheep and 60 goats, of mixed breed, were treated monthly with moxidectin over the last five years. Sheep and goats tended to frequent different grazing grounds according to the habit of each species, with the goats preferring higher pastures and the sheep, lower and flooded areas. During periods of low pasture availability the nutrition of the animals was supplemented with both protein and minerals.

Clinical Conditions

The owner sought the help of the UFPR Veterinary Hospital services because there were six animals that died in a short time and because of poor productive performance of the flock. Fecal samples were collected at seven to 12-day interval on seven occasions from each animal, with 6 samplings from sheep, and 3 from goats. Clinical examinations revealed signs of anemia, diarrhea and low body score in most of the animals. Initially haemoncosis was suspected, but feces were submitted to laboratory for both nematode and trematode fecal egg counts, respectively using the McMaster technique (GORDON; WHITLOCK, 1939). In addition coproculture and differential nematodes larval counts were performed (HOFFMANN, 1934).

In the first occasion, Abamectin plus Triclabendazole (A+TrBZ) was administrated to all the animals because of the presence of *F. hepatica*. In the following visits, the Famacha method was used for diagnosing haemoncosis and to select animals that required treatment, which was permormed using Ivermectin plus Abamectin (I+A). Animals with Famacha score equal to 3, 4 and 5 were treated. The body condition score was measured using a scale of 1 to 5. *Fasciola* sp. Eggs were determined in pool exams of for animals after treatment.

Statistical analysis was performed using Tukey t test and the coefficient of correlation was calculated using CORREL function in Excel spreadsheet (Microsoft).

RESULTS AND DISCUSSION

Specimens of *Lymnaea* sp. were identified and found to contaminate the areas located at the lowest part of the farm. Laboratory analysis at the first sampling found that 93 to 100% of the samples were positive with *F. hepatica* eggs constituting an outbreak of fasciolosis at the property. No animals were taken to necropsy.

Results in goats

Table 1 shows the results after drug treatment, the implementation of partial selective treatment and the correlation between clinical data. The first sampling matches to the date of application of A+TrBZ in all animals. In the following visits, selective treatment with I+A was performed avoiding losses by *H. contortus*.

However, the EPG account kept elevated in the following occasions, probably because the parasite population was re-establishing to its initial population. Also, Fasciolosis persisted in goats, which was an unexpected finding. The animals had to be monitored after A+TrBZ treatment showed an

Table 1. Average number of Strongyle eggs, EPG (Standard Deviation), Famacha score (percentage of the population, %), prevalence of *Fasciola hepatica* (%), correlation between Famacha (FMC) and EPG and Famacha (FMC) and *F. hepatica* eggs count in goats.

Data	03/28/07	04/05/07	04/21/07
EPG	1291 (1288)	898 (896)	1344 (1051)
Famacha *	3, 4, 5 (40)	3, 4 (30)	3, 4 (19)-5 (1.6)
F. hepatica eggs (%)	100	33.3	52
FMC vs. EPG	0.41	0.36	0.47
FMC vs. *Fasciola*	0.11	0.11	0.07

* Famacha categories, (percentage of animal).

effectiveness of only 57.3% against *F. hepatica*. Specific exams and partial selective treatments were necessary when 52% of the samples contained eggs of *F. hepatica*. However, as there were no more deaths and body condition scores improved in the flock there was also an overall recovery in clinical condition. This was achieved by combining the initial whole-herd treatment and latter with the partial selective treatment using Famacha. These may be due to the increase of animal's resistance to the fluke after a reduction of the parasitic abundance in the area or a reduction of the host infection rate (ALASSAD et al., 2007).

The Famacha score and EPG had a low correlation (C<0.43), suggesting that the Famacha method was efficient to diagnose hemoncosis only. Untreated animals that showed high EPG counts might manifest a false-negative Famacha score and may have contributed to the spread of unselected parasites in refugia (EJLERTSEN et al., 2006). The correlation between Famacha and Fasciolosis was also considered low (C<0,1).

The effectiveness of A+TrBZ against Strongylidae in goats was 21% indicating a serious condition for resistance against *Trichostrongylus sp.* and *Haemonchus contortus*.

Results in sheep

Table 2 shows the compile data from all sampling dates in sheep. The correlation between the Famacha method and the signs of fasciolosis was bellow 0.25 which is considered a low correlation.

It was already described by other authors that caution should be taken under conditions where other anemia-causing parasites are present, as the liver fluke, because it can decreases the accuracy of the Famacha method (EJLERTSEN et al., 2006).

There was no significant correlation between the EPG and Famacha in sheep (C<0.3). A high EPG, as was described, could help refugia, so a treatment based on EPG is not good enough in prolonging the lifespan of drugs and avoiding the development of resistance. The effectiveness of the treatment with A+TrBZ against liver fluke was 66.3%.

At first, 60% of the animals that harbor Strongylidae nematodes presented Famacha scores 3 or 4 and 20% of the animals showed score 5 soon after. Once using the selective treatment strategy, this percentage decreased gradually to 40%. The effectiveness of LEV against Strongylidae was 65% and of A+TrBZ was 20% indicating resistance to both compounds in sheep.

The EPG had a scattered distribution in both sheep and goats. This was due by the fact that individuals may respond differently under different conditions of contamination, ranging between them in relation to the parasite population and, consequently, the values of EPG (quantitative measurement) and health conditions (clinical evaluation).

Resistance occurred in the two flocks assuming that they share the same field strains of *F. hepatica* and the Strongylidae. This occurs due to abusive use of anthelmintics, but in this case resistance to triclabendazole was observed even after an unknown period of restrain (more than five years) in the property indicating that the *F. hepatica* population was retained its resistance to the drug. The mechanisms of resistance to TrBZ remains far from complete (BRENNAN et al., 2007), and the use of drugs with a different mode of actions to that of triclabendazole does not result in reversion of resistance, which is more difficult in trematodes than in nematodes (BORGESTEEDE, 2005; ÁLVAREZ-SÁNCHEZ, 2006).

In trematodes, which undergo asexual multiplication in the intermediate host, selection for resistance and the elimination

Table 2. Average number of Strongile eggs, EPG (Standard Deviation), Famacha score (percentage of the population, %), prevalence of *Fasciola hepatica* (%), correlation between Famacha (FMC) and EPG and Famacha (FMC) and *F. hepatica* eggs count in sheep.

	03/16/07	03/28/07	04/05/07	04/16/07	04/28/07	05/05/07
EPG	542 (1048)	546 (751)	-	2604 746 (672)	1652 (3495)	(2759)
Famacha*	3, 4 (60%)	3, 4 (80%) 5 (20%)	1 (30%)	1, 2 (70%) 5 (3%)	2,3 (71%) 3, 4 (60%)	1, 2 (60%)
F. hepatica eggs (%)	81%	75%	-	55%	33%	27,3%
FMC vs. EPG	-0,18	-	-	0,14	0,38	0,47
FMC vs. *F. hepatica*	0,22	-	-	0,18	0,29	0,19

* Famacha categories (percentage of animal).

of genes for susceptibility may develop more rapidly (BORGSTEEDE et al., 2005). All of these show the importance of the correct use of anthelmintics, and the importance for selecting the animals to be treated.

Alternative strategies such as biological control could also reduce pasture contamination and the contact with the intermediate host reducing the losses caused by the persistency of the parasite in the field.

CONCLUSION

A fasciolosis outbreak was confirmed in the property along with resistance against triclabendazole. The Famacha method may be used with caution once there is the concurrent infection with other bloodsucking parasites.

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