

# Flukicide efficacy against *Fasciola hepatica* of Triclabendazole and Nitroxynil in cattle of the central valley of Chile

Avaliação da eficácia de triclabendazol e nitroxinil para o tratamento da infecção por *Fasciola hepatica* em bovinos do Vale Central do Chile

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## Abstract

On a farm with permanent history of fasciolosis a study was performed aimed to know the efficacy of triclabendazole (TCBZ) and then to contrast with that of nitroxynil. Thirty-nine cattle naturally infected with *Fasciola hepatica* were randomly allocated into 4 experimental groups: Group 1 (control) was left untreated. Group 2 was treated with 12 mg/kg body weight (bw) of TCBZ by oral route. Group 3 treated with 24 mg/kg bw TCBZ orally. Group 4 was treated with 10 mg/kg bw of nitroxynil subcutaneously. The anthelmintic efficacy was calculated as the percentage of reduction in faecal egg count (FEC) at 14 and 28 d post-treatment. Results indicated that there were no significant differences in the percentage of FEC reduction between control group and the groups treated with 12 or 24 mg/kg of TCBZ. On the contrary, the treatment with nitroxynil significantly reduced the FEC and decreased the percentage of positive animals. In conclusion, *Fasciola hepatica* is reported for first time as resistant to TCBZ in Chile, which highlights the need of rotating drugs and assessing the efficacy of the administered drug in order to avoid the selection of resistant worms.

**Keywords:** Anthelmintic, fasciolosis, liver fluke, resistance, flukicide.

## Resumo

Em uma fazenda com histórico de fasciolose permanente, foi realizado um estudo com o objetivo de conhecer a eficácia do triclabendazol (TCBZ) e depois contrastar com o do nitroxinil. Trinta e nove bovinos naturalmente infectados com *Fasciola hepatica* foram distribuídos aleatoriamente em 4 grupos experimentais: Grupo 1 (controle), sem tratamento. O grupo 2 foi tratado com 12 mg/kg de peso vivo (PV) do TCBZ por via oral (VO). Grupo 3 tratado com 24 mg/kg de PV TCBZ por VO. O grupo 4 foi tratado com 10 mg /kg de PV Nitroxinil via subcutânea. A eficácia anti-helmíntica foi calculada comparando a percentagem de redução na contagem de ovos fecais (FEC) 14 e 28 dias pós tratamento. Não houve diferença significativa na porcentagem de redução FEC entre o grupo controle e os grupos tratados com 12 ou 24 mg/kg de TCBZ. Entretanto, o tratamento com nitroxinil reduziu significativamente o FEC e diminuiu a porcentagem de animais positivos. Em conclusão, a *Fasciola hepatica* é relatada pela primeira vez como resistente ao TCBZ no Chile, o que destaca a necessidade de realizar uma rotação em relação aos medicamentos anti-helmínticos e avaliar a eficácia do mesmo, a fim de evitar a seleção de vermes resistentes.

**Palavras chave:** Antihelmíntico, fasciolose, resistência, fasciolíctica

Fasciolosis is an important parasitic disease that significantly affects the ruminant production by reducing growth, conversion rate, milk production, quality and quantity of meat, as well as reproduction (MARTÍNEZ-VALLADARES et al., 2010).

For several years, fasciolosis has been considered a major problem in ruminant livestock production, but recently largely due to climate change, there has been a worrying upsurge in the

incidence and spread of the disease (HALFERTY et al., 2008). Considered as a secondary zoonotic disease until mid-1990s, human fasciolosis is at present emerging and re-emerging in many countries, including increases of prevalence and intensity and geographical expansion. Research in recent years has justified the inclusion of fasciolosis in the list of the significant human parasitic diseases (MAS-COMA, 2005).

Fasciolosis is endemic in areas dedicated to breeding cattle and sheep in most South American countries (CARMONA & TORT, 2017). In Chile, the disease is widely distributed throughout the country, exception made to the Magallanes Region (ALCAINO &

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APT, 1989). Although there are no epidemiological studies about the prevalence of the disease in the country, it is known that at slaughterhouse level the disease produces a high rate of discard of livers infected with *F. hepatica* (MORALES et al., 2000). According to that study, more than 32% of the total cattle slaughtered in the country is positive to liver flukes. On the other hand, in the regions of Central Valley of the country more than 80% of liver infected with *F. hepatica* are discarded (MORALES et al., 2000).

Triclabendazole (TCBZ), a benzimidazole derivative, is one of the most widely used drugs to control fasciolosis worldwide due to its high activity against both adult and immature flukes (BORAY et al., 1983). This high activity against immature flukes is significant because this is the most damaging stage of infection since this is when the flukes are in migratory and tissue invasion stage. However, resistance of *F. hepatica* to TCBZ has already been reported in a number of countries indicating that resistance exists (MOONEY et al., 2009; MEHMOOD et al., 2017). Because of the absence of new drugs against fluke infections, it is necessary to prove and compare the efficacy of the anthelmintics in order to prevent resistance development (MARTÍNEZ-VALLADARES et al., 2010). This study was aimed to know the efficacy of TCBZ at two levels of doses and then, to contrast it with the efficacy of nitroxynil.

The study was conducted between August and September 2015 on a milk farm located in the province of Ñuble, Bío Bío Region, Chile, area in which the infection with *F. hepatica* is endemic. The farm had a history of permanent fasciolosis and the farmer had relied solely on regular treatments with TCBZ to the whole stock.

A total of thirty-nine naturally infected non-lactating cattle, between 1 and 6 years old, with faecal egg counts positive to *F. hepatica* were selected for the study. Individual faecal samples were collected *per rectum* from all animals 7 days before treatment (T0) and then after 14 and 28 days' post-treatment. Each individual faecal sample was submitted to a modified sedimentation AMSIII method. In short: 1.0 g of fecal sample was filtered (60 mesh filter) with 15 ml of water, centrifuged at 2,000 rpm during one minute and the supernatant was discarded. Then, 14 ml of AMS medium (water solution of HCl 6,3% v/v, Na<sub>2</sub>SO<sub>4</sub> 4,8% w/v), four drops of Tween 80<sup>®</sup>, and 6 ml of ether were added to the sediment, and vigorously mixed. After that, the mixture was centrifuged at 2000 g during 2 minutes and then an aliquot of sediment was examined in a slide under binocular microscope.

The animals were randomly allocated into 3 experimental groups: Group 1 (control, n = 10) was left untreated and no fasciolicide drug was administered, Group 2 (n = 14) was treated with an oral dose of 12 mg/kg body weight (bw) of TCBZ (Faxicur<sup>®</sup>, solution 10%, Intervet, Chile) and Group 3 (n = 15) was treated with 24 mg/kg bw of TCBZ orally (OLAECHEA et al., 2011). Taking into account that 28 days after treatment, all animals treated with TCBZ remained with high faecal egg counts of *F. hepatica*. A fourth experimental group of 15 cows (composed by 5 animals from the initial control group more 10 positives to *F. hepatica* previously treated with TCBZ) was treated with a subcutaneous dose of 10 mg/kg bw of Nitroxynil (Dovenix<sup>®</sup>, Merial, Chile), as a positive control (Group IV).

The percentages of efficacies, in terms of faecal egg counts reduction test (FECRT) were determined using the formula:

$$\text{FECRT} = 100 \times (1 - \text{T}_x / \text{T}_0)$$

where: T<sub>0</sub> = arithmetic mean egg on day 0;

T<sub>x</sub> = arithmetic mean egg on 14 or 28 days.

The individual faecal counts eggs were transformed to natural logarithms and compared by analysis of variance associated to a multiple comparisons test of Student-Newman-Keuls.

The mean values of faecal egg count and the percentage of cow positives to *F. hepatica* before and after treatment with TCBZ or Nitroxynil are presented in Table 1. As shown, there were no significant differences between the control group and the cows treated with 12 or 24 mg/kg of TCBZ. Moreover, both groups of TCBZ treated cows remained 100% positives to the *F. hepatica* faecal egg count. On the contrary, the treatment with nitroxynil significantly reduced the faecal egg count and decreased the number of positive animals to values of 1/15 (6.66%) at 14 days and 2/15 (13.3%) at 28 days after treatment.

Low levels of efficacy were observed after treatment with a therapeutic dose of TCBZ and remained low after the double dose of the anthelmintic was administered (Table 2). On the other hand, the treatment with nitroxynil significantly increased the efficacy, indicating values of 99.9 and 99.8% at 14 and 28 days' post-treatment, respectively (Table 2).

Resistance is usually defined *in vivo* by a reduction in the expected efficacy of an anthelmintic (MOONEY et al., 2009). The main method used to identify resistance in the field has been the FECRT with the recommended post-treatment sample collection time point at 21 days (DANIEL et al., 2012; KELLEY et al., 2016). The 21-day period between pre and post-treatment testing is used to avoid the problem associated with retention of fluke eggs in the bile ducts even after the adult flukes has been killed (FLANAGAN et al., 2011). In the current research the efficacy of TCBZ was equal or lower than 50% (Table 2) in both 14 and 28 days sampling periods.

Confirmation of the diagnosis of fasciolicide anthelmintic resistance is not straightforward. Furthermore, lack of anthelmintic efficacy is not necessarily synonymous with anthelmintic resistance and might arise following under dosing, brought about by: underestimation of animals' body weight, failure to administer the correct dose rate, poor product storage, host pharmacokinetic effects, or the use of poor quality products (FAIRWEATHER, 2011; SARGISON, 2012). In order to avoid the effect of underdosing over the rate of efficacy, a double dose of an internationally well-known trade mark of TCBZ was used. Thus, our results show that a strong resistance of *F. hepatica* against TCBZ is present in this farm.

When TCBZ was launched, the efficacy (98%) was achieved even at doses of 2.5 mg/kg against adult *F. hepatica* (BORAY et al., 1983). The resistance of *F. hepatica* to TCBZ in the field was first reported in Australia by Overend & Bowen (1995). After that, resistance has been studied and reported in other places around the world, as in Europe, America, Asia and Oceania (KELLEY et al., 2016; MEHMOOD et al., 2017). In Chile, resistance to TCBZ has been reported only in four humans (GIL et al., 2014).

In the studied farm, there are antecedents of a long usage of TCBZ twice a year without parasitic studies, which has been described as causes of resistance to antiparasitic drugs. This activates the need of assessing the efficacy of drugs after treatments in

**Table 1.** Mean values of faecal egg counts of and percentage of cow positives to *F. hepatica* before and after treatment with TCBZ or nitroxylinil.

Time	Group 1 Control	<i>Fasciola hepatica</i> Positives	Group 2 TCBZ 12 mg/kg	<i>Fasciola hepatica</i> Positives	Group 3 TCBZ 24 mg/kg	<i>Fasciola hepatica</i> Positives	Group 4 Nitroxylinil 10 mg/kg	<i>Fasciola hepatica</i> Positives
(Days)	(hpg)	(%)	(hpg)	(%)	(hpg)	(%)	(hpg)	(%)
-7	35.1 ± 42.8*	100	39.6 ± 39.9a	100	32.5 ± 29.0a	100	54.9 ± 39.6a <sup>A*</sup>	100 <sup>A</sup>
14	23.1 ± 27.0a	100	19.5 ± 14.2a	100	25.4 ± 15.0a	100	0.07 ± 0.25b <sup>B</sup>	6.6 <sup>B</sup>
28	18.6 ± 18.5a	100	23.6 ± 24.4a	100	22.9 ± 19.6a	100	0.13 ± 0.35b <sup>B</sup>	13.3 <sup>B</sup>

\*a,b = P < 0.05, different letters in horizontal direction indicate statistically significant differences; \*A,B = P < 0.05, different letters in vertical direction indicate statistically significant differences.

**Table 2.** Efficacy of triclabendazole (TCBZ) at the recommended dose (Group 2, 12 mg/kg) double the recommended dose (Group 3, 24 mg/kg) and nitroxylinil (Group 4, 10 mg/kg) on the egg output of natural infections with *F. hepatica*.

Time	Group 2 TCBZ 12mg/kg	Group 3 TCBZ 24mg/kg	Group 4 Nitroxylinil 10 mg/kg
(Days)	% Efficacy	% Efficacy	% Efficacy
14	50.7a*	21.3a	99.9b
28	23.6a	29.5a	99.8b

\*a,b = P < 0.05, different letters in horizontal direction indicate statistically significant differences.

order to decide whether it is necessary to change the drug to be used. The appearance of the resistance to TCBZ means an important issue since TCBZ-resistance of *F. hepatica* seems not to be reversible (BORGSTEEDE et al., 2005). Thus, resistance of *F. hepatica* to TCBZ means a loss of a drug in the therapeutic arsenal against this parasite.

On the other hand, there is no antecedent of Nitroxylinil usage in the farm, which explains the high susceptibility of *F. hepatica* to this drug. This result is similar to that observed in Northern Ireland where *F. hepatica* was resistant to TCBZ and sensible to Nitroxylinil and Closantel (HANNA et al., 2015). In Spain, a higher resistance to TCBZ than to Nitroxylinil was also observed (MARTÍNEZ-VALLADARES et al., 2010).

Considering previously published literature reporting resistance of *F. hepatica* to other drugs has included resistance to Albendazole (NOVOBILSKÝ et al., 2016). In addition, isolates resistant to Albendazole and sensible to TCBZ have also been described (SANABRIA et al., 2013). A third drug considered here is Closantel, which has been reported with both resistance and sensibility to *Fasciola hepatica* isolates (HANNA et al., 2015; NOVOBILSKÝ & HÖGLUND, 2015). All of the above underlines the need to study in each location the sensibility of the parasites to drugs administered in order to stop or at least slow down the process of resistance selection.

The resistance to TCBZ by *F. hepatica* is also important from the public health point of view since TCBZ is one of the most widely used drug for the human infection, and given that resistance to this drug has also been observed in human infection (WINKELHAGEN et al., 2012; GIL et al., 2014).

New combinations of drugs have been tested in order to potentiate the action of TCBZ against *F. hepatica*. For instance, the co-administration of Ketoconazole seems to be a good alternative (DEVINE et al., 2012), although a failure in a human patient (WINKELHAGEN et al., 2012) suggests to be aware of the efficacy in each case.

It is concluded that *F. hepatica* is reported for first time as resistant to TCBZ in Chile, which highlights the need of rotating drugs and assessing the efficacy of the administered drug in order to avoid the selection of resistant worms and the emerging of the resistance to other drugs.

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