Rapid communication

Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in The Netherlands

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Abstract

In the winter of 1998/1999, sheep on a farm in the province of North Holland, The Netherlands, died from subacute and chronic liver fluke disease despite four previous treatments with triclabendazole (TCBZ). Faecal examinations of sheep and cattle on the farm showed high number of liver fluke eggs. In a randomised clinical trial, the fluke egg output was monitored weekly for 3 weeks in sheep which were treated with TCBZ or with closantel; in dairy cows treated with TCBZ or with clorsulon; and in heifers treated with TCBZ or clorsulon. The results showed a significant reduction of 99.7, 98.1 and 99.2%, respectively, in fluke egg output at 21 days in all non-TCBZ treated animals. TCBZ treatment produced percentage decreases of 15.3, 4.3 and 36.6%, respectively. These results are highly indicative of the presence of TCBZ-resistant *Fasciola hepatica* in sheep and cattle on this farm. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Fasciola hepatica*; Cattle-Trematoda; Sheep-Trematoda; Triclabendazole; Drug resistance; The Netherlands

1. Introduction

Triclabendazole (TCBZ) is the most widely used drug for the control of fasciolosis in ruminants. It is highly effective against immature and adult stages of *Fasciola* spp. (Boray, 1982) and frequent treatments within the prepatent period can reduce the fluke infection to a negligible level (Boray et al., 1985; Boray and Rolfe, 1990).

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The first reports of evidence of resistance of liver fluke against an anthelmintic came from Dorsman (1967, 1968) who observed reduced efficacy of hexachlorophene. In his survey of drug resistance in *Fasciola hepatica*, Boray (1990) observed reduced efficacy of rafoxanide with side resistance to closantel. In the laboratory, he was able to select on resistance of liver fluke against luxabendazole and TCBZ, but not against clorsulon. Resistance of liver fluke against TCBZ in sheep under practical conditions was initially reported by Overend and Bowen (1995) in Australia with subsequent reports from Ireland (Lane, 1998; O’Brien, 1998) and Scotland (Mitchell et al., 1998).

In the winter of 1998/1999, there was evidence of resistance of *F. hepatica* against TCBZ on a farm with cattle and sheep in the province of North Holland, The Netherlands. Despite dosing the sheep four times with TCBZ, several had died and post-mortem results demonstrated severe signs of subacute and chronic fasciolosis. Faecal examination of sheep and cattle treated with TCBZ showed high number of liver fluke eggs, although resistance of *F. hepatica* to TCBZ in cattle has never been reported. To investigate if resistance against TCBZ was present on this farm, a randomised clinical trial with different groups of animals was conducted.

2. Materials and methods

2.1. Farm and animals

The study was carried out on a dairy farm situated in the western part of The Netherlands, 20 km northwest of Amsterdam. On an area of approximately 40 ha, divided in 20 paddocks, 41 dairy cows, 14 heifers, 15 calves and 80 sheep, were grazed in autumn 1998. The soil was peaty with a groundwater level being 0–80 cm below the surface. Due to heavy rainfall in 1998, all drainage-furrows contained water during the autumn and winter in 1998. On all pastures, *Lymnaea truncatula* — the intermediate host of liver fluke — was found. The farm had a history of fasciolosis for at least 10 years and regular treatments with TCBZ during the previous years had produced satisfactory control. In November 1998, all cattle were housed and 14 days later heifers and calves were treated with TCBZ. The dairy cows were treated in groups of five animals in November, December and January. In 1998, the sheep were treated in September, October, November and December with TCBZ. The sheep remained on pasture and were only housed around lambing time, in March 1999.

2.2. Experimental procedures

2.2.1. Experiment 1

In January 1999, 20 ewes from the flock of 80 in December 1998 TCBZ treated ewes, were randomly allocated into two equal groups and housed in a sheepbarn. The housed sheep were fed silage ad libitum and concentrates. One group was treated with TCBZ (Fasinex®; Novartis; 10 mg/kg) and the other group with closantel (Flukiver®; Janssen Pharmaceutica; 5 mg/kg).
2.2.2. Experiment 2

From the housed and the November or December 1998 TCBZ treated dairy cows; 10 cows were randomly selected and allocated to two groups. In February 1999, five cows of one group were treated with TCBZ (12 mg/kg) and the other five cows with clorsulon (Ivomec F®; Merial B.V.; 8 mg/kg).

2.2.3. Experiment 3

In March 1999, 14 heifers were randomly allocated to two groups and housed together, one group was treated with TCBZ (12 mg/kg) and the remaining seven with clorsulon (8 mg/kg). During housing, all cattle were provided with silage and concentrates.

2.3. Sampling and clinical observations

Individual faecal samples were taken on the day of treatment Day 0 and on Days 7, 14 and 21. The number of \( F. hepatica \) eggs per gram faeces (epg) was determined using the Dorsman technique (Dorsman, 1965). Necropsy was performed on all animals that died or were euthanised for ethical reasons.

2.4. Statistical analysis

Prior to statistical analysis, observations were checked for unlikely values but no data were excluded for this reason. The percentage efficacies in terms of reduction of the egg counts were determined using the following formula:

\[
\text{Percentage efficacy} = \left[ \frac{\text{epg Day 0} - \text{epg Day 7, 14 or 21}}{\text{epg Day 0}} \right] \times 100
\]

Differences in epg’s from Day 0 were analysed using a paired \( t \)-test after natural logarithm (ln+) transformation (Sokal and Rohlf, 1981). The statistical significance level was chosen at \( p=0.05 \).

3. Results

3.1. Experiment 1 (sheep)

The results of the faecal examinations of the sheep are summarised in Table 1. On Day 0, there was no significant difference between the mean epg in the TCBZ group and the closantel group. On Days 7, 14 and 21, the mean epg in the TCBZ-group did not decrease, whereas the closantel-group showed a significant effect of the treatment (\( p<0.01 \)). Two sheep of the closantel-group died 4 and 6 days, respectively, after treatment. Both sheep showed severe signs of chronic fasciolosis but no \( F. hepatica \) could be detected in the livers, although several adult flukes were found in the gall bladders of the two sheep. The epg’s of the faecal samples taken from these ewes were included with the values on Day 7. One
Table 1
Efficacy of treatment with triclabendazole (TCBZ) and closantel (experiment 1: sheep), and TCBZ and clorsulon (experiment 2: dairy cows; experiment 3: heifers) on the egg output of natural infections with Fasciola hepatica

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCBZ</td>
<td>Closantel</td>
<td>TCBZ</td>
<td>Closantel</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 sheep/group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean epg</td>
<td>1840.0</td>
<td>3034.0</td>
<td>3087.0</td>
<td>129.5***</td>
</tr>
<tr>
<td>Range</td>
<td>350–3330</td>
<td>850–5400</td>
<td>410–12320</td>
<td>0–470</td>
</tr>
<tr>
<td>Efficacy (%)</td>
<td>−67.8</td>
<td>95.7</td>
<td>−13.6</td>
<td>95.0</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Five dairy cows/group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean epg</td>
<td>47.0</td>
<td>54.0</td>
<td>66.0</td>
<td>3.0**</td>
</tr>
<tr>
<td>Range</td>
<td>10–105</td>
<td>15–130</td>
<td>5–220</td>
<td>0–5</td>
</tr>
<tr>
<td>Efficacy (%)</td>
<td>−40.4</td>
<td>94.4</td>
<td>−38.3</td>
<td>92.6</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
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<tr>
<td>Seven heifers/group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean epg</td>
<td>122.9</td>
<td>87.9</td>
<td>102.1</td>
<td>0***</td>
</tr>
<tr>
<td>Range</td>
<td>5–305</td>
<td>15–195</td>
<td>20–215</td>
<td>5–180</td>
</tr>
<tr>
<td>Efficacy (%)</td>
<td>16.9</td>
<td>100</td>
<td>26.8</td>
<td>99.2</td>
</tr>
</tbody>
</table>

* Day 0 is the day of treatment.
* Difference (p<0.05) from mean epg on Day 0.
** Difference (p<0.01) from mean epg on Day 0.
*** Difference (p<0.001) from mean epg on Day 0.

Sheep of the TCBZ-group had an epg of 12320 on Day 7 and died on Day 14 with more than 200 adult living flukes in the liver and gall bladder.

3.2. Experiment 2 (dairy cows)

There was little variation between the epg’s of the dairy cows on Day 0 (Table 1). The epg in the clorsulon group decreased significantly within a week to very low values and remained so till Day 21. In the TCBZ group, the egg output stayed relatively high. There were no severe clinical signs of fasciolosis during the experiment.

3.3. Experiment 3 (heifers)

The treatment of the heifers with clorsulon was very effective. No eggs were found on Day 7 and only very low numbers on Day 14 and 21. On the other hand, treatment with TCBZ was not successful with an efficacy of only 16.9% on Day 7 and 36.6% on Day 21.

4. Discussion

TCBZ has until now proven to be the most effective flukicide against both mature flukes and immature flukes. Boray et al. (1983) found efficacies of 93–98, 99–100 and 100%,
respectively, against early immature, immature and adult flukes at a dosage rate of 10 mg/kg. Efficacy of TCBZ treatment in experimentally and naturally infected sheep was between 96.5 and 100% (Wolff et al., 1983; Rapic et al., 1984; Turner et al., 1984). In field trials, 100% reduction in egg output in sheep was found within 3 weeks (Stansfield et al., 1987; Rapic et al., 1988; Maes et al., 1990). The significantly low efficacies of TCBZ in this study indicate the presence of TCBZ-resistant *F. hepatica* in sheep on this farm.

In this study, the efficacy of closantel in sheep against adult flukes is 95–99.7%. These findings support the results described by Janssen Pharmaceutica (1986) where, in clinical trials with sheep, the efficacy of closantel varied from 84–100 and 95–100% after 1–2 and 3–4 weeks, respectively. Although adult flukes are killed within 1 week after treatment with closantel, it is possible to find eggs in the faeces for a further week (Janssen Pharmaceutica, 1986). In experimentally infected sheep, Maes et al. (1990) found no fluke eggs in the faeces 1 week after closantel treatment, however, in our study we still found some eggs in the faeces 3 weeks after closantel treatment.

In controlled studies with TCBZ treatment in cattle, Rapic et al. (1988) and Richards et al. (1990) showed efficacies of 96.5 and 97.8%, respectively. In naturally infected cattle, efficacies of 90 (Lecuyer et al., 1985) and 100% (Craig and Huey, 1984; Lecuyer et al., 1985; Stansfield et al., 1987) were achieved. Because of the fact that in this study TCBZ treatment in dairy cows and heifers had no effect, it is highly indicative that resistance of *F. hepatica* against TCBZ is present.

Treatment of *F. hepatica* with clorsulon in cattle showed efficacies of 97.3 (Malone et al., 1984) and 100% (Kilgore et al., 1985; Zimmerman et al., 1986). Treatment of the dairy cows and heifers with clorsulon in the present study gave efficacy results within this range.

The regular treatment of sheep on the farm with TCBZ is likely to have selected for resistance. Because *F. hepatica* is not a host species specific parasite and cattle and sheep graze the same pastures, it is likely that resistant flukes have also established in cattle. This study performed on one farm demonstrates the resistance of *F. hepatica* against TCBZ in cattle and sheep. To evaluate the importance of *F. hepatica* TCBZ-resistance in The Netherlands, a study on more farms is needed.

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**References**


