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BRIEF REPORT

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Resistance to triclabendazole in *Fasciola hepatica* on a commercial sheep farm in Taranaki, New Zealand

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ABSTRACT

Aims: To investigate potential resistance to anthelmintics containing triclabendazole in naturally acquired *Fasciola hepatica* (liver fluke) present on a sheep and beef farm in New Zealand.

Materials and methods: A necropsy study was conducted with three groups of mixed-age Romney ewes naturally infected with *F. hepatica*. Ewes were either left untreated as controls or treated with a single oral dose of either a combination of 0.2 mg/kg moxidectin and 10 mg/kg triclabendazole or 10 mg/kg triclabendazole alone, both at the manufacturer's recommended dose. At necropsy, 49 days later, livers were collected for total adult liver fluke counts.

Results: All ewes in the trial had live liver fluke (min 1, max 148 flukes/liver). The efficacy was 30% for moxidectin plus triclabendazole and 21% for triclabendazole alone, confirming triclabendazole resistance on this farm.

Conclusion: This is the first reported case of triclabendazole-resistant liver fluke in New Zealand.

Clinical relevance: The finding of triclabendazole-resistant liver fluke in New Zealand should alert farmers and veterinarians to consider triclabendazole resistance as a potential cause of treatment failure, and adapt the farm liver fluke management and treatment regimens. In addition, resistant fluke could be widespread around New Zealand due to livestock being sold from this farm.

Abbreviations: FEC: Faecal egg count

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Triclabendazole; *Fasciola hepatica*; liver fluke; resistance; sheep

Introduction

In New Zealand, liver fluke (*Fasciola hepatica*) are commonly found in sheep and cattle, predominantly in Northland, Taranaki, Manawatū and the East Coast of the North Island, as well as the West Coast and upper parts of the South Island (Charleston and McKenna 2002). In most affected regions, liver fluke infection typically does not cause severe clinical disease and generally does not require the same intensive control measures as gastrointestinal parasites; however, it may impact growth rates (Dowling 2023).

Methods of anthelmintic control of fasciolosis have not been widely surveyed but, based on limited reports, liver fluke control is common in dairy cattle in Northland (McKay 2008) and the West Coast (Dowling 2023). For sheep, there is only anecdotal evidence of frequent usage of trematodicides in some areas. Nevertheless, heavy infections capable of causing weight loss and mortality in sheep can occur. It is widely reported that triclabendazole has the highest efficacy against liver fluke as it can control all stages of fluke from 2-week-old immature stages through to adult (Boray *et al*. 1983). However, cases of liver fluke becoming resistant to triclabendazole have been reported in many parts of the world (Kelley *et al*. 2016).

There have been no reports of lack of efficacy to any flukicide in New Zealand to date. In June 2010, owners of a mixed sheep and beef farm in Taranaki contacted their local veterinarian as they were suspicious that liver fluke (*F. hepatica*) was not being adequately controlled in their sheep. It was noted that despite treatment with flukicide drench containing 100 g/L triclabendazole, numerous ewes exhibited clinical signs such as oedema of the lips, failure to thrive, poor weight gain and liver fluke detected at slaughter. Liver fluke infections were historically a concern on this farm, with ewes regularly suffering from clinical fasciolosis. Adult ewes and replacement hoggets were commonly drenched three to four times a year, depending on assessed need. This brief report describes the evaluation of triclabendazole in two different formulations

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against liver fluke in sheep on this farm in New Zealand in 2011. It is being reported now due to recent publications on the impact of liver fluke in New Zealand (Dowling 2023; Dowling *et al*. 2025) in order to alert veterinarians to the possibility of triclabendazole resistance.

Materials and methods

Animal ethics approval was granted by the Kaiawhina Animal Ethics Committee (55275).

Flock history

The farm under study is located in the Taranaki region of New Zealand. It is a mixed sheep and beef breeding and finishing property of 1,000 hectares of rolling and steep country with many areas of bush in the gullies as well as streams, swamps and ponded areas. In 2010, the farmer became suspicious that triclabendazole (Fasinex 10; 100 g/L triclabendazole, 1 mL/10 kg; Elanco, Auckland, NZ) was not working effectively. Triclabendazole-treated hoggets and two-tooths, in poor condition, did not gain weight and their mucous membranes remained pale.

Following several incidents of failure to thrive despite treatment, the efficacy of triclabendazole on this farm was evaluated by the farmer and local veterinarian in 2010. To that effect, 13 poorly conditioned, two-tooth Romney sheep, with clinical signs consistent with fasciolosis were enrolled in this study; six were treated with 10 mg/kg oral triclabendazole (Fasinex 10); four were treated with a combination oral drench containing 1 mg/mL abamectin and 50 mg/mL closantel (Genesis Ultra, Boehringer Ingelheim, Auckland, NZ) at a dose of 1 mL/5 kg; three remained untreated. Sheep were assigned to groups randomly as they came up the race and weights were estimated at 65 kg. They were dosed by the veterinarian according to label instructions. The sheep came from a cohort of 500 same-aged sheep and were managed separately until slaughter in a mob of about 50 other poorly conditioned ewes. Four weeks after treatment, the sheep were humanely slaughtered in accordance with the NZ Animal Welfare Act 1999 (Anonymous 1999) by a single gunshot to the head followed by exsanguination. Their livers were inspected by the veterinarian, and the liver fluke present were observed; however, the actual numbers of fluke were not recorded. Three of four sheep in the abamectin and closantel treatment group had no live fluke present. All three untreated control animals had what was considered a large number of live fluke present, confirming a fluke infestation on this farm. Three of six animals in the triclabendazole group had small numbers of fluke present. Based on this initial evaluation, it was concluded that triclabendazole was still effective on the farm.

In July 2011, ewes were treated orally with 0.2 mg/kg moxidectin plus 10 mg/kg triclabendazole (Cydectin Plus Fluke Oral Solution for Sheep; Zoetis, Auckland, NZ). One month later, two ewes were slaughtered and necropsied on-farm by the farmer. Both animals had live adult liver flukes, indicating potential treatment failure.

Around the same time, 10 faecal samples were collected from the ground from the mob that had been treated with Cydectin Plus Fluke and sent to New Zealand Veterinary Pathology (now IDEXX Laboratories, Palmerston North, NZ) for a liver fluke faecal egg count (FEC), which was conducted as follows. The sample was washed using a repeated sedimentation process. Four grams of faeces were mixed with tap water, then passed through a coarse sieve (tea strainer), and the sieved material was suspended in a 100 mL measuring cylinder and filled with water. This was left to sediment for 5 minutes, and the supernatant removed with a suction device. The cylinder was again filled with tap water, mixed and allowed to sediment. This was repeated until the supernatant was clear, usually 3–4 times. The sediment was then collected, and the eggs were counted using direct microscopy. Eggs per gram (epg) were calculated by the weight used. Eight of the 10 samples were positive for fluke eggs, with counts ranging from 5–210 epg. An investigation was planned to determine the efficacy of triclabendazole alone and in combination with moxidectin on that farm.

Evaluation of triclabendazole efficacy in October 2011

At tailing in October 2011, 60 wet-dry ewes (ewes that have lambed but then lost the lamb or stopped lactating) or dry-dry ewes (ewes that did not lamb and are not lactating) from the mob previously treated in July with Cydectin Plus Fluke Oral Solution were ear tagged and rectal faecal samples were taken by the veterinarian for FEC. The 60 ewes were selected based on their poor condition and perceived probability of being infected with liver fluke. Of these, 55 had positive FEC (i.e. at least one liver fluke egg). After excluding clinically unhealthy animals, 42 of the remaining sheep with the highest FEC were selected for the study. Ewes were deemed clinically unhealthy if they were in very poor body condition (< 1.5; scale 1–5), had submandibular oedema, or were very pale or weak. They were moved to a neighbouring, fluke-free farm where they were unlikely to become reinfected and grazed for 3 weeks prior to treatment. This farm did not have bogs and streams to the same extent as the study farm, and liver fluke had not been seen in necropsied animals nor reported via slaughter premises. The period of 3 weeks was allocated both for management reasons and to allow the immature flukes that were ingested within 7 days prior to leaving the study farm to mature and reach a stage at which they would be susceptible to triclabendazole. While triclabendazole has efficacy against all stages of fluke, efficacy against early immature flukes (< 1 week) is minimal. Due to the 49-day withdrawal period of Cydectin Plus Fluke, newly ingested fluke would have matured in this time, potentially confounding the efficacy of the treatments. Three weeks after moving to the new pasture, the ewes were blocked on FEC (0–20, 21–100, 101–326 epg) and, within blocks, randomly assigned to one of three treatment groups, using a commercial random number generator (Microsoft Excel; Microsoft, Redmond, WA, USA): group T1, untreated control (n = 16); group T2, 0.2 mg/kg moxidectin, 10 mg/kg triclabendazole (Cydectin Plus Fluke Oral Solution for Sheep) (n = 10); and group T3, 10 mg/kg oral triclabendazole (Fasinex 24 for cattle; Elanco) (n = 10). On Day 0, all animals were individually weighed and dosed as described.

In addition, on Day 0, four animals were randomly selected for blood sampling. Samples were transported on ice to the New Zealand Veterinary Pathology Laboratory (now IDEXX Laboratories; Palmerston North, NZ). Samples were pooled (an equivalent volume of serum from each animal was mixed) to determine the level of fluke infection using ELISA (IDEXX Fasciolosis Verification Test; IDEXX, Hoofdorp, Netherlands).

Four weeks after treatment, five faecal samples were taken from each of the two treated groups (T2 and T3) for FEC (as described above). This was to confirm that treated animals were still positive for liver fluke and that continuation of the trial was justified.

Nine weeks after treatment, five uninfected sentinel sheep from the fluke-free property, where the trial animals were grazing, were blood sampled and tested individually by ELISA for antibodies to assess likelihood of reinfection in the trial animals. These sheep had been present on this farm since birth.

Approximately 9 weeks after treatment, all animals in the three trial groups were sent for slaughter. Whole livers were collected, individually identified and sent to IDEXX for total adult liver fluke counts. Adult flukes were counted by incising and examining the major and minor bile ducts. The gall bladder, common bile duct and interlobar ducts in all liver lobes were incised with 125-mm straight, sharp surgical scissors. Compression was manually applied to liver lobes to extrude any adult fluke from smaller intact bile ducts into the larger incised ducts. The contents were flushed into a tray with water, sieved, rinsed, and the number of adult fluke counted manually in a separate tray. The pathologist who performed the liver fluke counts (GO) was blinded to the treatment groups.

Arithmetic mean efficacy was calculated using the formula:

$$Efficacy = \left(\frac{control - treated}{control}\right) \bullet 100$$

No CI are reported as this was a total fluke count.

Results

The ELISA result for the trial animals showed a moderate level of fluke infection. Four of the five sentinel animals tested to assess infection on the "fluke-free" farm were negative for fluke antibodies on the ELISA, suggesting reinfection of the trial animals was unlikely and therefore results were unlikely to be compromised. However, one animal was positive, indicating exposure may have occurred and/or this farm was not totally fluke-free.

The FEC conducted 4 weeks after treatment were positive for fluke eggs, confirming persistent fluke infestation in groups T2 and T3 (Table 1). No analysis of efficacy was made using FEC, as no samples were taken from the control animals (group T1) on Day 27 post-treatment.

Fluke counts showed that all animals in both treatment groups (T2 and T3) had live liver fluke present, with counts ranging from 1–148 adult fluke per liver (Supplementary Table 1; summarised in Table 1). No immature flukes were recorded in any animals, confirming the unlikely reinfection of liver fluke while on the fluke-free farm, in these animals.

Table 1. Mean faecal egg counts, liver fluke (*Fasciola hepatica*) counts and drench efficacy from a controlled efficacy trial of drenches containing triclabendazole on a single sheep farm in New Zealand with a history suggestive of emerging triclabendazole resistance.

Group	Treatment	Faecal egg counts (epg)		Liver fluke count (Day 57)	Efficacy (%)
		Day −34	Day 27		
T1 (n = 16)	Control	93.6	NT	43.1	
T2 (n = 10)	Moxidectin (0.2 mg/kg) and triclabendazole (10 mg/kg)[a]	68.3	24.9	29.8	30.8
T3 (n = 10)	Triclabendazole (10 mg/kg)[b]	61.9	17.5	33.9	21.3

[a]Cydectin Plus Fluke Oral Solution for Sheep (Zoetis, Auckland, NZ)
[b]Fasinex 24 (Elanco, Auckland, NZ)
NT = not tested

Drench efficacy based on arithmetic mean fluke counts was 30% in the group treated with triclabendazole in combination with moxidectin (T2) and 21% in the group treated with triclabendazole alone (T3). The results showed that on this farm, the efficacy of triclabendazole, regardless of formulation, was markedly lower than > 92% previously reported by Boray *et al*. (1983), confirming resistance of triclabendazole on this farm.

Discussion

To the best of our knowledge, this is the first report of lack of efficacy of triclabendazole against *F. hepatica* on New Zealand sheep farms.

The ewes on this Taranaki farm were historically known to suffer from clinical fasciolosis. During the 10-year period prior to the study in 2011, triclabendazole had been the only flukicide used in both sheep and cattle on the farm. It is highly probable that the frequency of drenches given, solely with the same active, had intensified the selection pressure on the liver fluke, resulting in this resistant population. Resistant fluke may also have potentially been introduced to the property with bought-in sheep or cattle. Of interest, cattle from this farm were used for another study in 2017 where it was observed at slaughter that 28/29 mixed-age cows and 6/8 steers had adult flukes in their livers, although the mean number of flukes overall in these cattle was low (Dowling 2023). In that study, there was a negative relationship between fluke burdens and weight at slaughter. This provides further evidence that liver fluke was an issue on this farm.

In the 14 years since the initial finding of lack of efficacy of triclabendazole, liver fluke control on the farm has changed. No triclabendazole has been used since the study in 2011. Instead, the choice of flukicide has changed to products containing closantel for sheep and clorsulon for cattle. Ewes typically receive two treatments per year (down from 3–4 in 2011) in January and May, due to many demonstrating clinical signs of fasciolosis. Replacement ewe lambs receive a closantel product in May only. Cows are given one clorsulon treatment at scanning in May/June, with replacement heifers receiving a closantel drench at this time. The remaining weaners are sold “store”, for further fattening, in autumn. Lambs and cattle weaners, which are all sold store in May, are not drenched for fluke before leaving the farm. In addition to the change in drench active and frequency, farming practices on this property have been altered to help manage fluke infection levels. Stock numbers have reduced from 4,200 ewes in 2011 to 3,000 ewes in 2024, allowing the farmer to avoid grazing marginal marshy land suitable for the transmission of *F. hepatica*. Despite the decrease in stock numbers, production has increased. Ewe pregnancy scanning percentages were approximately 185% in 2023 and 2024, an increase from the 2010 average of 165%. However, while animals may be performing better due to reduced ingestion of metacercariae and better feed quantity as a result of 25% fewer ewes on the property, there is still high selection pressure on the flukes. The only refugia on the farm are undrenched youngstock. These receive a treatment in May or leave the farm at that time, so that there is only a short period where unselected liver fluke can complete their lifecycle. It should also be noted that this farm typically sells many young stock to other farmers to purchase and finish, so it is highly likely any resistant flukes will have been distributed away from this property.

This study was not conducted in a controlled pen study environment and, as such, absolute confirmation of efficacy cannot be assumed. Liver fluke egg counts in the control animals were also lower than VICH guidelines (100–200 epg; VICH 1999), although efficacy calculations were not made on FEC but on adult fluke. However, in conjunction with the anecdotal evidence from previously treated ewes still harbouring fluke infestations, this is further evidence that the efficacy of triclabendazole, in two different formulations, is highly compromised on this farm, which is likely to be due to parasite resistance mechanisms.

This report of anthelmintic failure should prompt others to consider the efficacy of flukicide treatments on their farms, and potentially adapt their treatment regimes. Anthelmintic efficacy could be investigated either by FEC reduction test or liver fluke counts in necropsied animals. Information can be found in the the VICH guidelines (VICH 1999). Consideration should be given to the possibility of reinfection with new metacercariae or advancement of parasite stages if long meat withholding periods are required, depending on the active ingredient used, as some, such as closantel, only kill adult stages and developing immature stages could confound the results. This report, together with those of Dowling (2023) and Dowling *et al*. (2025), demonstrates the presence of *F. hepatica* in New Zealand livestock and underscores the importance of accurately diagnosing triclabendazole resistance to inform effective management strategies.

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Disclosure statement

V Chapman is an employee of Zoetis New Zealand Ltd.

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