



Short communication

Seasonal differences in the efficacy of pour-on formulations of triclabendazole and ivermectin or abamectin against late immature liver fluke (*Fasciola hepatica*) in cattleR.M. Sargent^{a,*}, M. Chambers^b, T. Elliott^b^a Intervet Schering-Plough Animal Health, 26 Artisan Road, Seven Hills 2147, NSW, Australia^b Veterinary Health Research, Private Bag, West Armidale, NSW 2350, Australia

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ABSTRACT

Three controlled studies were conducted to determine the efficacy against late immature (6 weeks) *Fasciola hepatica* of two currently available fasciolicides (Genesis[®] Ultra and Coopers[®] Sovereign[®]) which are applied externally to cattle. Efficacy of the two products was assessed when application was made under winter, spring and summer conditions. Efficacies for winter, spring and summer respectively, based on arithmetic mean total fluke counts, were 78.9%, 91.7% and 99.6% for Coopers[®] Sovereign[®] and 73.4%, 89.7% and 99.6% for Genesis[®] Ultra. Seasonal differences with treatment efficacy were indicated. The studies also confirmed previous observations that liver fluke egg counts overestimate the efficacy of fasciolicides and that total fluke counts is the most reliable method for assessing efficacy of such products.

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1. Introduction

The efficacy of triclabendazole against the various stages of liver fluke, *Fasciola hepatica*, when administered orally to cattle, has been well documented (Boray, 1982; Richards et al., 1990; Ibarra-Velarde et al., 2001). Until relatively recently, the concurrent treatment of liver fluke and nematodes in cattle required the administration of separate products. An oral combination product containing triclabendazole plus ivermectin (Fasimec[®] Cattle, Novartis Animal Health, North Ryde, NSW) has since been available in Australia (Stevenson et al., 2002). More recently, pour-on products containing triclabendazole plus abamectin or ivermectin have been introduced in Australia. One product, Genesis[®] Ultra (5 g/L abamectin + 300 g/L triclabendazole) (Ancare Australia, Kingsgrove, NSW), has a solvent-based formulation whilst the other, Coopers[®] Sovereign[®] (15 g/L

ivermectin + 240 g/L triclabendazole) (Schering-Plough, North Ryde, NSW), is a micellar formulation.

Seasonal differences in the efficacy of a pour-on anthelmintic formulation containing levamisole have been previously reported in Australia (Forsyth et al., 1983) although a later study conducted in the United States of America failed to repeat the findings (Seibert et al., 1986). There have been no published reports on the efficacy of pour-on formulations containing the fasciolicide triclabendazole against late immature liver fluke in cattle, especially when applied under differing seasonal conditions. Three controlled pen studies have been conducted to examine the efficacy of Genesis[®] Ultra and Coopers[®] Sovereign[®] against late immature (6 weeks old) liver fluke of cattle when applied under different seasonal conditions.

2. Materials and methods

2.1. Animals and treatments

All three studies were conducted at Armidale, NSW, Australia using mixed breed heifers and steers (Hereford

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and Hereford X) artificially infected with 500 triclabendazole susceptible *F. hepatica* metacercariae. The *F. hepatica* metacercariae are referred to as the “Sunny Corner” strain and were isolated >10 years ago by Dr Joe Boray and is a known triclabendazole susceptible reference strain. This strain has been maintained in donor animals and snails under laboratory conditions. Cattle were free of adult (patent) liver fluke infections as assessed via individual animal liver fluke egg counts (LFECs) 2 days (winter study) and 6 days (spring and summer studies) prior to application of artificial infections.

Cattle were then treated 42 days later with Genesis[®] Ultra or Coopers[®] Sovereign[®] at the recommended dose rates of 5 mL/50 kg (0.5 mg/kg abamectin + 30 mg/kg triclabendazole) and 5 mL/50 kg (1.5 mg/kg ivermectin + 24 mg/kg triclabendazole) respectively. Coopers[®] Sovereign[®] was applied percutaneously along the dorsal midline as a broad strip between the centre of the back and the tailhead. Genesis[®] Ultra was also applied percutaneously along the dorsal midline but as a narrow strip from the withers to the tailhead. Cattle were maintained under overhead cover in separate group pens during treatment and for the next 5 days to prevent precipitation interfering with the study. They were then relocated to separate open grazing paddocks and exposed to ambient weather conditions. Cattle had *ad lib* access to feed and water. Group 1 remained untreated as the Negative Control, Group 2 was treated with Coopers[®] Sovereign[®] and Group 3 treated with Genesis[®] Ultra.

2.1.1. Study 1 (winter)

Thirty (30) heifers and steers weighing 65–184 kg were allocated to three treatment groups of 10 cattle each. Temperature at the time of treatment was 0.5 °C as measured at 9:00 a.m. at the Armidale weather centre. Treatment occurred in early winter (June). Mean minimum–maximum temperatures for the first 28 days following treatment were 0–10 °C with a total rainfall of 25 mm. Cattle were exposed to overnight temperatures of below 0 °C on at least 13 occasions during this 28-day period.

2.1.2. Study 2 (spring)

Eighteen (18) heifers and steers weighing 122–224 kg were allocated to three treatment groups of 6 cattle each. Temperature at the time of treatment was 11 °C as measured at 9:00 a.m. at the Armidale weather centre. Treatment occurred in late spring (November). Mean minimum–maximum temperatures for the first 28 days following treatment were 11–24 °C with a total rainfall of 100 mm. Cattle were exposed to overnight temperatures of less than 5 °C at only one time during this 28-day period.

2.1.3. Study 3 (summer)

Thirty (30) heifers and steers weighing 137–215 kg were allocated to three treatment groups of 10 cattle each. Temperature at the time of treatment was 18 °C as measured at 9:00 a.m. at the Armidale weather centre. Treatment occurred in mid-summer (February). Mean minimum–maximum temperatures for the first 28 days following treatment were 13–27 °C with a total rainfall of 121 mm. Cattle were not exposed to overnight

temperatures of less than 5 °C at any time during this 28-day period.

2.2. Liver fluke egg counts

Individual faecal samples were collected from all cattle at sacrifice on days 84–85 (Studies 1 and 3) and days 95–97 (Study 2). Individual LFECs were then performed using a method based on that of Taylor (1964) and Dunn (1969). Six grams of cattle faeces was weighed into a labelled hexagonal sample bottle and filled with water. The sample was immediately mixed with a mechanical stirrer and poured through a 177 µ sieve into a 250 mL sedimentation flask. This step was repeated. The sample within the sedimentation flask was then left to stand for 3 min following which the water was slowly poured off. The sediment was then washed into a 15 cm test tube and filled to the top. This sample was allowed to stand for a further 3 min (minimum) allowing the final solution to sediment. The supernatant was carefully removed (down to 2 cm), stained with 1% methylene blue and shaken. The contents of the tube was washed into a perspex counting tray and mixed thoroughly. The sample was examined under a stereo microscope at 15× magnification. This method has a reported sensitivity of 66.7% and specificity of 100% with an overall accuracy of 73.9% with positive and negative predictive values of 100% and 45.5% respectively (Anderson et al., 1999).

2.3. Total fluke counts

All cattle were sacrificed on days 97–99 (Study 2) or days 84–85 (Studies 1 and 3) post-infection and their livers, intact gall bladders and associated small intestines removed. Whole intact livers were collected immediately following sacrifice, into pre-labelled plastic bags. The immediate small intestine remnants were occluded 15–20 cm each side of the liver and retained attached to the liver. Total fluke counts were conducted using an adaptation of the method in the guideline by Wood et al. (1995). Small intestine contents were emptied and examined for liver fluke. The liver main bile duct leading from the liver to the gall bladder, and the gall bladder itself, were incised and any fluke present removed. The liver was then cut (one strip at a time) into strips of 1–1.5 cm thickness, starting from one edge. As each strip was separated from the liver, the strip and the adjacent portion of liver were squeezed with moderate pressure. All adult and immature liver fluke thus exposed were gently removed with a pair of forceps and deposited in a clean area. Pieces of fluke were matched to ensure correct counts.

On completion of liver sectioning and visible fluke retrieval the sections were covered in warm saline overnight (within a 10 L approximate plastic bucket). Contents (saline solution and liver segments) were then passed through a mesh sieve and rinsed with running water. Liver sections were discarded (after careful inspection to remove any remaining fluke) and remaining fluke removed from the sieve. Adult and immature fluke thus

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