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Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle

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ABSTRACT

Triclabendazole (TCBZ) is the drug of choice for *Fasciola hepatica* control and reports of *F. hepatica* resistant to this drug from a wide range of geographic regions are very concerning. This study investigated the presence of TCBZ resistance in *F. hepatica* in naturally infected Australian beef and dairy cattle herds and evaluated methods of measuring the levels of resistance. Faecal egg count and coproantigen reduction tests (FECRT and CRT, respectively) were conducted on 6 South-eastern Australian beef properties and one dairy property where treatment failure by triclabendazole (TCBZ) was suspected. The CRT was conducted on an additional beef property. On each property 15 animals were treated with an oral preparation of TCBZ at the recommended dose and 15 animals remained as untreated controls. Fluke eggs in faeces were counted and coproantigen levels were measured before treatment and 21 days after treatment and in the untreated control animals. These data were evaluated using three different methods to calculate % reductions compared with controls. Resistance (<90% reduction) was detected on the dairy property using both FEC and CRT, and on 3/6 beef properties using FECRT and 4/7 beef properties using CRT. Using the FECRT, reductions of 6.1–14.1% were observed in dairy cattle and 25.9–65.5% in beef cattle. Using the CRT, reductions of 0.4–7.6% were observed in dairy cattle and 27.0–69.5% in beef cattle. Live flukes were recovered at slaughter following TCBZ treatment of 6 cattle from 3 of the beef properties, confirming the TCBZ resistance status of *F. hepatica* in these cattle. This is the first report of *F. hepatica* resistant to TCBZ in cattle in Australia and the results suggest that resistance is widespread in the South-eastern region. The CRT is shown to be a robust alternative to the FECRT for evaluation of TCBZ resistance in *F. hepatica* in cattle.

1. Introduction

In Australia, livestock production losses attributed to the common liver fluke *Fasciola hepatica* were estimated to be A$50 to 80 million per annum in 1999 and annual fluke treatment alone costs A$10 million (Boray, 2007). Over 6 million cattle graze at-risk pastures with most stock concentrated in South-eastern Australia where there is a suitable habitat for the intermediate snail host (MLA, 2005), especially along watercourses and in irrigation zones. The epidemiology of fasciolosis is similar to other countries.

Due to its efficacy against both immature and mature adult stages of *F. hepatica* within the mammalian host, triclabendazole (TCBZ) has been the drug of choice for parasite control. The emergence of resistance to TCBZ now threatens fluke control in livestock in several parts of Europe (Fairweather, 2009). TCBZ-resistant *F. hepatica* were first reported from sheep in Victoria, Australia, in 1995 (Overend and Bowen, 1995) and resistance has now been reported in several countries both in sheep (Mitchell et al., 1998; Moll et al., 2000; Thomas et al., 2000; Gaasenbeek et al., 2001; Mooney et al., 2009; Sargison and Scott, 2011; Gordon et al., 2012), and cattle (Moll et al., 2000; Olaechea et al., 2011; Ortiz et al., 2013). Recently, a case of TCBZ-resistant *F. hepatica* was reported in a human from the Netherlands. The patient did not respond despite several treatments with the drug, highlighting the serious zoonotic threat posed by fasciolosis especially that of resistant parasites (Winkelhagen et al., 2012).

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Anthelmintic resistance in nematode parasites is commonly detected by the use of parasite faecal egg count reduction tests (FECRT). Although FECRT have not been validated for fluke (Coles et al., 2006), this method has been applied to evaluate treatment failure and indicate the existence of possible drug resistance in *F. hepatica* populations. A commercial coproantigen ELISA is available for the detection of *F. hepatica* infection in ruminant livestock (Mezo et al., 2004). Trials using sheep (Flanagan et al., 2011a,b; Gordon et al., 2012; Novobilsky et al., 2012) and cattle (Brockwell et al., 2013) show that this coproantigen ELISA can be used to demonstrate survival of fluke following treatment and thus in identifying resistant populations. The recent work of Brockwell et al. (2013) has demonstrated that this test reflects fluke burdens in cattle and that coproantigen levels fall within 7 days after successful treatment suggesting that this test has utility as a method for measuring reductions due to treatment. This opens the way for a coproantigen reduction test (CRT) to be used for measuring the level of TCBZ resistance in *F. hepatica* in cattle.

In this study, we aimed to identify, for the first time, resistant fluke isolates in cattle in Australia and to evaluate and compare the FECRT and CRT as methods for measuring the level of drug resistance in *F. hepatica*. We used the same coproantigen ELISA test as used by others (Flanagan et al., 2011a,b; Gordon et al., 2012; Novobilsky et al., 2012) and compared the three methods described by Pook et al. (2002) for assessing reductions in FEC and coproantigen ELISA values. We show that the RESO technique, which compares post-treatment arithmetic means of treated and control groups, was favoured because its derivation generates less statistical error, relies on post treatment results only and is cheaper for field application.

2. Materials and methods

2.1. Tests

2.1.1. FEC

Pre and post treatment *F. hepatica* faecal egg counts (FEC) were performed by Para-Site Diagnostic Service, Benalla, Victoria on fresh faeces sent by overnight courier, using the Sedimentation test for Liver Fluke©, Western Australia Department of Agriculture and Food (WADAF). The procedure was to homogenize 10 g of faeces with 100 mL of water and pour the slurry through a sieve stack with sequentially smaller aperture sizes (150, 90 and 45 μm). The homogenate was washed through the sieves with a stream of tap water followed by washing of each of the lower two sieves after removing the sieve above. The filtrate collected on the 45 μm sieve was washed into a graduated flask and diluted to 100 mL with water and allowed to settle for 6 min. The supernatant was reduced to 20 mL using a vacuum pump, diluted again to 100 mL with water and allowed to settle for 6 min. The sediment was suspended in 10 mL and one drop of methylene blue added. After 5 min, the material was transferred to a viewing chamber and eggs counted under an inverted microscope using 40× magnification.

2.1.2. Coproantigen test

To measure faecal antigen levels, aliquots of 2 g of the same faecal samples were stored at −20 °C for up to 3 days, and for several months in the case of the Nimmitabel samples, until analysis with a commercial ELISA kit for the detection of *F. hepatica* faecal antigen (BIO K 201, BIO-X Diagnostics, Belgium). The protocol was optimised for use in our laboratory as described (Brockwell et al., 2013). Coproantigen values are expressed as a percentage of the positive control antigen and corrected to allow for a zero value by subtracting the negative cut-off value of 1.3%. This negative cut-off value was determined by the mean plus 3 times the standard deviation taken from 103 FEC negative field samples. The specificity of the coproantigen ELISA has been established in several studies against natural infections of gastrointestinal nematodes, *Moniezia, Dicrocoelium* and *Echinococcus* (Mezo et al., 2004) and *Paramphistomum cervi* (Kajugu et al., 2012; Brockwell et al., 2013).

2.2. On-farm trials

This research was conducted with approval by Charles Sturt University's Animal Care and Ethics Committee. The beef cattle properties were selected for preliminary screening following reports of suspicion of treatment failure by veterinarians in the Livestock Health and Pest Authority (NSW) and the Department of Primary Industries (Victoria). The dairy property was selected on the advice of a local veterinarian. The properties identified in this trial were located at Parrots Nest, Irvington and Caniaba in North-eastern NSW; at Numbugga, near Bega in the far South-eastern area of NSW; Nimmitabel in the Monaro region of South-eastern NSW; Gireke near Berrigan in the southern Riverina irrigation area of NSW; and in the Tallangatta Valley region of North-eastern Victoria. The dairy property was located near Maffra in the Gippsland region of eastern Victoria (see Fig. 1). The brief history of fluke control on these properties is as follows. All properties surveyed had been using TCBZ exclusively for longer than 5 years and treated animals orally. Only the Caniaba property indicated that cattle were treated using a pour on as well. None indicated using what could be considered excessive treatments (>3 treatments per year). Most treated cattle once annually, with only the Numbugga property manager stating he treated when he thought the animals showed evidence of disease. Nimmitabel reported no treatment of cattle but twice yearly oral treatment of sheep co-grazing with cattle. Parrots Nest and Irvington reported 2–3 treatments per year. Scales to weigh animals were used only on the Numbugga and Tallangatta Valley properties with all animals dosed at the rate applicable for the heaviest weight obtained. On all other properties the animal's weight was estimated for dose calculation.

On each property 30 animals of no specific age or gender were enrolled in the trial. Animals were randomly allocated to either a treatment or control group (n = 15/group). The mean body weights (kg ± SD) of the control and treated animals, respectively, on each property were: Parrots Nest: 396 ± 34; 444 ± 23; Irvington: 435 ± 31; 387 ± 32; Caniaba: 552 ± 18; 502 ± 13; Nimmitabel: 314 ± 8; 298 ± 9; Numbugga: 375 ± 9; 375. ± 11; Gireke: 447 ± 15; 421 ± 19; Tallangatta Valley: 252. ± 7; 242 ± 4; Maffra: 211 ± 6; 200 ± 5. Fifteen treated cattle were dosed orally with a commercial TCBZ drench (Flukare C®, Virbac Animal Health) at the manufacturer's recommended dose rates (12 mg/kg based on individual body weight) using a drenching hook. A second group of 15 animals remained untreated as controls. Per rectum faecal collection for FEC and coproantigen ELISA were performed on each animal prior to treatment and 21 days post-treatment. The exceptions to this protocol were: (i) for the Nimmitabel property poor weather delayed the post-treatment sample collection until day 24; (ii) on the Maffra property the 'untreated control' group were subsequently treated on day 21 and then retested for coproantigen on day 42. There was no untreated control group for comparison with this treated group. On some properties only 13 or 14 animals were available on the day of testing as shown in Tables 1 and 2.

2.3. Statistical analysis

Anthelmintic resistance was declared when the calculated TCBZ efficacy was <90% (APVMA, 2001). FEC were determined as