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High prevalence of fasciolosis and evaluation of drug efficacy against *Fasciola hepatica* in dairy cattle in the Maffra and Bairnsdale districts of Gippsland, Victoria, Australia

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ABSTRACT

Liver fluke (*Fasciola hepatica*) is a common parasite amongst grazing livestock in the south-eastern region of Australia and is responsible for significant production losses in the beef and dairy industries. Gippsland in Victoria is a major region for dairy production but no fluke prevalence data in livestock has been obtained in this region since the late 1970s prior to the introduction of Triclabendazole (TCBZ). TCBZ resistance is also now widespread in cattle in south east Australia. In this study, we evaluated the prevalence and intensity of liver fluke infections in dairy cattle in Gippsland and assessed the efficacy of TCBZ and other drenches against *F. hepatica* on one farm. We obtained 30 individual faecal samples from each of 15 different farms and, using the liver fluke coproantigen ELISA, tested bulk faecal samples pooled from each farm. Any farm that returned a positive bulk sample had all of the samples tested individually to assess the intra-herd prevalence. One farm in the Maffra district also had a coproantigen reduction test and faecal egg count reduction test to assess the efficacy of TCBZ, Clorsulon (CLOR) and Oxyclozanide (OXY). The coproantigen ELISA proved to be a highly sensitive test for liver fluke with a high correlation ($R^2 = 0.8849$) observed between ELISA data from bulk samples and individual samples, suggesting that future larger scale screening on farms for fasciolosis could use the bulk analysis technique. The ELISA data revealed that animals on six of the 15 farms were infected with *F. hepatica* and the herd prevalence of the infected herds ranged from 47 to 100% (mean 81%) which exceeds the prevalence value for production losses of 25%. The intensity of fluke infection in cattle varied considerably both within and between herds with a proportion of animals exhibiting a positive control value in the coproantigen ELISA of 50–88%. We also confirmed that TCBZ resistance was present on one farm but that CLOR or OXY can be used to remove the adult stage of the TCBZ-resistant parasites. We conclude that fasciolosis is a significant disease and a likely cause of production losses in dairy cattle in the irrigation zones of Gippsland and that TCBZ resistance is a serious threat to fluke control. We suggest that more work needs to be performed in Gippsland to further define the extent of fasciolosis and drug resistance and to ensure that effective chemical and non-chemical methods of fluke control are incorporated on farms in order to improve animal welfare and reduce financial impacts on producers.

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1. Introduction

Liver flukes (*Fasciola hepatica* and *Fasciola gigantica*) are flatworm helminths which cause the disease fasciolosis in livestock and humans (Mas-Coma et al., 2005). Infection occurs due to the ingestion of metacercariae, the migration of juvenile and immature flukes through the liver and maturation to adult flukes in the bile ducts. In Australia, an estimated 40 million sheep and 6 million cattle graze fluke (*F. hepatica*) infested pastures, resulting in economic losses of approximately A$10 million/year due to treatments and a further A$50–80 million/year on production losses (Boray, 2007). Global production losses due to fasciolosis exceed US$3 billion/year (Piedrafita et al., 2010). In dairy cattle, milk production losses due to fluke infection vary according to the intensity of infection and level of animal nutrition, but fasciolosis has been estimated to reduce milk yield in dairy cattle by 3.8% to 15.2% (Schweizer et al., 2005; Charlier et al., 2014) and perhaps as high as 30% (Hope-Cawdery, 1984). Fasciolosis also impacts fertility in dairy cattle due to the delayed onset of puberty in young stock or an increase in the number of artificial inseminations required in adult stock (Schweizer et al., 2005; Charlier et al., 2012, 2014). A herd prevalence rate of >25% in cattle, or an intensity of 30–40 flukes/animal, is considered indicative of production losses in cattle (Hope-Cawdery et al., 1977; Vercruysse and Claerebout, 2001). Charlier et al. (2008) suggest the threshold for production losses in dairy cattle may be as low as 10 flukes.

In terms of the prevalence and intensity of fasciolosis in Australia, earlier studies reported the overall prevalence of liver fluke in cattle in Victoria of 41% with a prevalence in Gippsland of 31–55% (Watt, 1977, 1979), and up to 98% with mean fluke burdens of 24 parasites (McCausland et al., 1980). Tracer infection studies in sheep in irrigation regions of the Goulburn–Murray region in Northern Victoria revealed fluke intensities of up to 72 flukes/animal (Meek and Morris, 1979). Analysis of fluke infections in cattle in Queensland showed prevalence ranging from 0.4 to over 50% (Roberts, 1982; Baldock and Arthur, 1985; Molloy and Anderson, 2006). Since the 1980s control of fasciolosis in Australia has relied on the use of Triclabendazole (TCBZ) (Boray et al., 1983) but resistance to this drench, first reported in Victoria (Overend and Bowen, 1995), has now been identified in livestock in south-eastern Australia (Brockwell et al., 2014), the United Kingdom, Europe and South America (Alvarez-Sanchez et al., 2006; Daniel et al., 2012; Gordon et al., 2012; Sargison, 2012; Ortiz et al., 2013; Robles-Pérez et al., 2013). Burdens of drug-resistant flukes of 20–34 were observed in cattle following treatment with TCBZ in Australia (Brockwell et al., 2014). Liver fluke is also zoonotic and human infections have been reported in Australia and globally, including one case of infection with TCBZ-resistant liver fluke in the Netherlands (Hughes et al., 2003; Mas-Coma et al., 2005; Winkelhagen et al., 2012).

Victoria's raw milk production in 2010–2011 was valued at A$2.48 billion (Department of Environment and Primary Industries, 2012) with 22% being produced in the Gippsland region (Dairy Australia, 2008). The area is suitable for dairy production due to good soil fertility, predictable rainfall and, in some areas, irrigation. These factors are also suitable for the *Lymnaea* species of freshwater snail which is the liver fluke's intermediate host. The risk of liver fluke infection in dairy cows in this region is high and is a serious concern due to the effect on animal health and production (Watt, 1979; McCausland et al., 1980). This study was performed in dairy herds in Gippsland to determine the current prevalence of fasciolosis, to provide an estimate of the intensity of fluke burdens in dairy cattle and evaluate the efficacy of several commonly used drenches since TCBZ resistance was recently reported in one dairy herd in the Maffra district in Gippsland (Brockwell et al., 2014). The liver fluke coproantigen ELISA (BIOK 201, BIO-X Diagnostics, Belgium) was used to test all samples, as this test has been validated for use in cattle (Charlier et al., 2008, 2014; Brockwell et al., 2013, 2014; Palmer et al., 2014). It has been shown that there is a good correlation between coproantigen ELISA results and liver fluke burden in the animal, suggesting that this ELISA can be used to indicate the intensity of fluke infections in cattle (Mezo et al., 2004; Charlier et al., 2008; Brockwell et al., 2013).

2. Materials and methods

2.1. Site location and sample collection for the prevalence study

A total of 450 individual faecal samples were collected for liver fluke testing from 30 lactating cows on each of 15 farms in Gippsland, Victoria, in the Leongatha, Maffra and Bairnsdale districts (Fig. 1). The samples were taken in 2013 during disease investigations by the Department of Environment and Primary Industries in dairy herds that had reported anaemia and the presence of theileriosis; the fluke status and treatment history of the herds was unknown at the time of sampling. Individual 2 g aliquots were taken from all 30 samples and two 10 g bulk samples were made by pooling 2 g from the first 10 samples collected (samples 1–5, termed bulk A; and samples 6–10, termed bulk B) for each farm. The bulk sample was thoroughly mixed and a 2 g sample taken for coproantigen ELISA analysis. Any farm that had a positive bulk sample then had all of the individual samples analysed by coproantigen ELISA in duplicate. A bulk faecal coproantigen ELISA analysis has been shown to detect one positive sample diluted with four negative samples in an experimental fluke infection (Brockwell et al., 2013). All faecal samples were stored frozen at −20 °C until analysis.

2.2. Liver fluke egg count and coproantigen ELISA

The FlukeFinder kit® (www.flukefinder.com) was used to determine the presence or absence of fluke eggs for all animals at all time points in the resistance study. The protocol supplied with the kit was followed, using 2 g of faecal matter which was sieved and washed back into an 11 ml column of water in a 15 ml falcon tube. The suspension was allowed to settle for 2 min; the supernatant was poured off and then refilled to 11 ml. This was repeated three times and the product was poured off into a small, marked dish and two drops of 1% methylene blue dye were added as a