



Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole

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ARTICLE INFO

Article history:

Received 1 November 2012

Received in revised form 3 April 2013

Keywords:

Fasciola hepatica

Liver fluke

Coproantigen ELISA

Faecal egg count

Cattle

Triclabendazole

ABSTRACT

Three methods of diagnosing *Fasciola hepatica* (*F. hepatica*) infection (a coproantigen ELISA, Bio-X Diagnostics, Belgium, Faecal Egg Count (FEC), and a serum IgG ELISA, Bio-X Diagnostics, Belgium) were evaluated in artificially infected cattle, with and without drug treatment. Specifically, the potential value of the coproantigen ELISA in the quantitation of *F. hepatica* infection was sought. Twelve steers were each infected with 100, 200 or 500 metacercariae ($n = 4$ cattle/group). On day 84, post infection (PI), 2 animals from each group were treated orally with triclabendazole (TCBZ). Faecal and blood samples were collected weekly after infection from all animals, as well as over 5 consecutive days (days 105–109 PI) for the six animals remaining infected to determine the repeatability of these assays. Cattle were killed 126 days PI and the coproantigen, FEC and IgG levels were compared with the number of fluke recovered. Animals first tested positive for infection with the serum ELISA, with 11/12 animals positive on day 28, and IgG responses increased to day 42 PI. The coproantigen ELISA was first positive on day 42 (3/12 animals), with all animals positive by day 56 PI. The first *F. hepatica* egg was detected on day 49 from an animal infected with 500 metacercariae; however only on one occasion (day 84) did all animals return positive FEC. Within one week of treatment with TCBZ, all six treated animals had returned to negative status by coproantigen ELISA and FEC whereas IgG levels persisted. Weekly variation in both coproantigen level and FEC was evident throughout the trial. Results from the consecutive daily collections varied greatly between days for both methods, with 2–6-fold differences in coproantigen levels and 2–4-fold variation in FEC. Strong correlations were observed between fluke burdens (day 126) and day 125 coproantigen levels ($R^2 = 0.8718$) and FEC ($R^2 = 0.8368$). The coproantigen ELISA was more sensitive than FEC (FEC displayed false negatives) and detected infection earlier. This ELISA showed good correlation to fluke burdens in these cattle and has promise as a test for detecting low fluke burdens.

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1. Introduction

The liver fluke, *Fasciola hepatica* (*F. hepatica*), is a common parasite of ruminants, and causes significant economic losses (>US\$3B per annum) to the livestock industries worldwide (Spithill et al., 1999). A major concern

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for producers is the emergence of flukes resistant to one of the most commonly used drugs for control, triclabendazole (TCBZ). Resistance was first reported in naturally infected sheep in Australia (Overend and Bowen, 1995) and is now more widespread with resistance in cattle and sheep reported in Ireland, the UK and the Netherlands (Brennan et al., 2007; Fairweather, 2009).

Infection with liver fluke begins with excystment of metacercariae in the small intestine and migration of juvenile flukes to the liver where subsequent parasite development in the liver parenchyma induces antibody responses and causes extensive tissue inflammation and necrosis. While development rates are variable in cattle the acute phase of disease occurs during the first five to eight weeks of infection in sheep; after 8–10 weeks flukes mature, migrate to the bile ducts and begin shedding eggs and antigens into the bile and faeces. This chronic phase of infection is characterised by liver fibrosis, anaemia and subsequent production losses.

Assessment of parasite drug resistance has traditionally relied on faecal egg count reduction tests (FECRT); however, standard fluke egg counts using 10 g faecal samples may be unreliable (Rapsch et al., 2006). Egg counts are poor indicators of infection levels when the parasite burden is low or when immature fluke are migrating; therefore FECRT only provides an indication of drug efficacy during patency and cannot detect resistance within immature stages (Gaasenbeek et al., 2001). Techniques for faecal egg count (FEC) are also laborious, require skills in identifying eggs and are unsuitable for large scale testing.

Detection of serum antibodies to fluke antigen by ELISA, whilst very useful for the early detection of infection, is of no value in field resistance testing because the antibodies persist long after treatment (Gaasenbeek et al., 2001; Sánchez-Andrade et al., 2001). Recently, a coproantigen ELISA test has been demonstrated to be highly specific, sensitive and simple for the diagnosis of acute and chronic *F. hepatica* infections in sheep and cattle (Mezo et al., 2004; Charlier et al., 2008; Valero et al., 2009; Flanagan et al., 2011a,b). However, this ELISA does not detect immature stages of fluke before week 5 PI (Mezo et al., 2004; Valero et al., 2009; Flanagan et al., 2011a).

Confirmation of drug resistance in liver flukes is achievable by passaging parasites and testing efficacy in a controlled infection (Gaasenbeek et al., 2001). However, there remains a need for a reliable, cost-effective diagnostic tool for use as a routine test for the confirmation of drug resistance in *F. hepatica* populations. The coproantigen test has been evaluated with more or less success for use in the detection of drug efficacy in sheep (Flanagan et al., 2011b; Gordon et al., 2012; Novobilsky et al., 2012). The aim of this study was to evaluate the *F. hepatica* coproantigen ELISA (Mezo et al., 2004) by determining the time of *F. hepatica* antigen detection and its disappearance after treatment of cattle, as well as the relationship of the coproantigen ELISA results to fluke burden. It was compared with antibodies in sera and faecal egg count (FEC). This study also allowed us to determine the repeatability of these assays, the specificity with respect to paramphistome infection and whether this test is a useful tool for the quantitative assessment of responsiveness to drug

treatment in cattle, especially in the examination of pooled samples.

2. Materials and methods

2.1. Animals and experimental infection

This research was conducted under Charles Sturt University's Animal Care and Ethics Committee protocol number **09/104**. Twelve yearling Angus X Wagyu steers were screened and found negative by FEC, coproantigen and sera antibody ELISAs. Animals were weighed and treated for internal parasites including paramphistomes with Nilzan® oral drench (active constituents: 150 g/L oxy-clozanide; 75 g/L levamisole, Coopers, Australia) at a dose rate of 5 mL/45 kg liveweight, and were kept on dryland pasture for the duration of the trial. On day 0 the animals were experimentally infected orally via a capsule gun with metacercariae of the triclabendazole-susceptible Sunny Corner isolate of *F. hepatica* (Veterinary Health Research, Armidale, NSW). Twelve animals within groups of 4 were infected at the following rates. Group 1: 100 metacercariae; Group 2: 200 metacercariae; Group 3: 500 metacercariae. On day 84, two animals from each group were treated orally with triclabendazole (Flukare C®, Virbac, active constituents: 120 g/L triclabendazole; 1 g/L selenium) at a dose rate of 2.5 mL/25 kg liveweight and two were left untreated. This design was adopted to provide a range of burdens so that correlations could be robustly tested and because a range of infection intensities would approximate natural infection patterns. Throughout the trial, faeces were collected per rectum and animals were tail bled using vacutainers containing clot activator. Animals were bled and faeces collected weekly from day 28 until day 112 PI. Faecal samples were also collected on days 105 through to 109 PI from untreated animals to evaluate the daily variation in FEC and coproantigen ELISA values. One additional faecal sample was taken day 125 PI the day prior to slaughter.

2.2. Fluke recovery

Animals were slaughtered at a commercial abattoir 126 days PI. The livers and gall bladders were collected for dissection and fluke recovery. Gall bladders were opened, the bile was collected then the bladders were opened out and flushed with PBS. The major bile ducts were opened, obvious fluke were collected with blunt forceps and the livers then palpated to aid further fluke removal and collection. Livers were then sliced into strips approximately 1 cm thick and squeezed with any fluke collected. Strips were then soaked in a bucket of warm water before further rinsing through a 300 µm sieve to recover any remaining parasites. To calculate the total fluke count, the number of whole flukes was added to the number of anterior or posterior cut portions, whichever was greater.

2.3. Sample analysis

For Serum antibody ELISA, blood was allowed to clot at room temperature before being centrifuged at 1000 × g for 5 min at room temperature. Supernatant was then stored

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