Prevalence and gross pathology of liver fluke in macropods cohabiting livestock farms in north eastern NSW, Australia, and diagnosis using cELISA

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ABSTRACT

Liver fluke (Fasciola hepatica) is a parasite of herbivores including wildlife. Macropods, such as Eastern grey kangaroo (Macropus giganteus) and Common wallaroo (Osphranter robustus), are frequently observed sharing grazing sites with domestic livestock. The impact of Macropods, as reservoirs of infection, on livestock production and risks to cross-species transmission are largely unknown. In Phase 1 of this study, liver and faecal samples were collected from 245 Macropods (181 Eastern grey kangaroos, 64 Common wallaroos) cohabiting livestock farms (n = 7) in the Northern Tablelands regions of New South Wales. Total fluke (TFC) and fluke eggs (FEC) were counted in the liver and faeces, respectively, to assess prevalence. Faecal antigens were also measured using the commercial Bio-X Diagnostic Monoscreen AgELISA Fasciola hepatica kit (cELISA) to assess suitability as a diagnostic tool. In Phase 2, Macropod faecal samples were collected from 60 livestock farms to conduct FEC and assess prevalence by region. Liver fluke was prevalent in 22% of Eastern grey kangaroo and 20% of Common wallaroos with prevalence as high as 45% in the Eastern grey kangaroo. Fluke burdens ranged from 1 to 122 flukes (mean = 9 flukes) with a FEC range of 0–195 eggs per gram (epg) of faeces (mean = 18 epg). Evidence of dead and live flukes trapped within fibrotic capsules confirms the ability of Macropods to resolve infections. cELISA proved highly specific (100%) and sensitive (98%) in liver fluke detection however fibrotic capsules observed in the liver may reduce the correlation of coproantigens with fluke burden. Phase 2 revealed that 27% of livestock farms had Macropods infected with liver fluke. Overall, this study confirmed Eastern grey kangaroo and Common wallaroo are susceptible hosts and potential reservoirs for liver fluke and, monitoring infections in Macropods would assist in livestock disease management.

1. Introduction

Australian Macropods (i.e. kangaroos, wallabies, tree kangaroos and pademelons) harbour a diverse range of parasites however of these, only Fasciola hepatica (liver fluke) and Echinococcus granulosus (hydatid tapeworm) are known to infect domestic livestock (Beveridge et al., 1998; Beveridge and Emery, 2015). Macropods are herbivores and are frequently observed sharing grazing sites with livestock (Caughley et al., 1978; Taylor, 1983) yet the impact of Macropods, as reservoirs of infection, on livestock production is largely unknown as is the risk of cross-species transmission. In an earlier study, Eastern grey kangaroo (Macropus giganteus) cohabiting with livestock were reported with a prevalence of liver fluke (Macropus giganteus) cohabiting (Taylor, 1984) highlighting the potential risk.

The liver fluke intermediate snail host, Austropepleus spp. (Boray, 1978; Boray et al., 1985), is predominately found in higher rainfall areas (>600 mm annually) of eastern Australia (Boray, 1964; Berger et al., 1978). Throughout this region, the Eastern grey kangaroo and Common wallaroo (Osphranter robustus) are also widespread (NSW Government Department of Planning, Industry and Environment, 2021). In the Northern Tablelands region of New South Wales (NSW), a region of eastern Australia and where this study was conducted, the Common wallaroo (136 km−2) and Eastern grey kangaroo (79 km−2) have been detected in higher density on improved agricultural pastures compared to unimproved areas (32 km−2 and 37 km−2 respectively) (Taylor, 1984). Macropods cohabiting livestock farms, within liver fluke endemic areas, may facilitate the liver fluke life cycle and act as reservoirs of infection.
thus reducing the effectiveness of integrated parasite management strategies for liver fluke control.

Fasciolosis in livestock requires diagnosis as clinical signs of infection are nonspecific or often not apparent. Whilst fluke egg counts (FEC) provide a simple diagnostic test for liver fluke detection, FEC can only detect patent infections from 10 to 12 weeks post infection (wpi) with sensitivity ranging from 30 to 81% (Mazeri et al., 2016; Woodgate et al., 2016). An alternative test, measuring faecal *F. hepatica* antigens using the BIOK201-2 coproantigen enzyme linked immunosorbent assay (Bio-X Diagnostic, Belgium) has higher sensitivity and specificity (Mezo et al., 2004; Kajugu et al., 2012, 2015; Brockwell, 2013), detecting liver fluke earlier from 5 to 6 wpi (Flanagan et al., 2011; Brockwell et al., 2013; George et al., 2017). In wild red deer (*Cervus elaphus*) (French et al., 2016) and boars (*Sus scrofa*) (Mezo et al., 2013), cELISA demonstrated higher sensitivity than FEC and the assay was considered a practical diagnostic test to monitor disease in wildlife. In horses (*Equus caballus*) however, cELISA was considered unsuitable having low sensitivity (Palmer et al., 2014). An investigation of liver fluke in Macropods offers the opportunity to examine the suitability of cELISA as a diagnostic tool for use in these species given they harbour a diverse range of parasites which are often in high numbers (Beveridge and Arundel, 1979; Brown et al., 2014).

In order to ascertain the potential of Macropods as reservoirs of liver fluke infection in livestock production, Macropods cohabiting livestock farms in the Northern Tablelands region of NSW were examined to confirm: (i) liver fluke prevalence and; (ii) pathogenicity. Additionally, the BIOK201-2 coproantigen ELISA kit was assessed as a diagnostic tool for liver fluke detection in Macropods.

2. Material and methods

2.1. Experimental design, sampling site and animals

The study consisted of two separate experimental phases. In Phase 1,
2.2. Sampling sites

Phase 1 and 2 samples were collected from Macropods cohabiting livestock farms within a 105 km radius of Armidale, NSW (30.5016° S, 151.6662° E) (Fig. 1). Phase 1 samples (liver and faecal) were collected from seven farms (A-F) throughout November 2018–October 2020. Farms were classified by risk of liver fluke infection based on the number of freshwater springs, identified from farm inspections, that were capable of supporting the habitat of the intermediate snail host;

- Low risk - no or few freshwater springs (<2); farms A and B.
- Medium risk - freshwater springs (1–10) encompassing up to 1% of the area of the property; farms E, D and G.
- High risk: numerous freshwater springs (>10) encompassing 5% or more of the property; farms C and F.

Phase 2 faecal samples were collected from 60 farms throughout December 2018–June 2021, randomly selected within the 105 km radius of Armidale from respondents to a pre-trial grazier survey.

2.3. Sample collection

Macropods were identified by location (A-G) and number within farm. The species, sex and number of pouch young were recorded for each individual and the tail and foot length measured using a metric measuring tape (Poole et al., 1982). The chest cavity was subsequently opened to collect: liver with intact gall bladder, kidneys with surrounding adipose tissue, and a faecal sample. Livers were then weighed, their eggs were identified based on colour, shape and texture (in accordance with French et al., 2016). The kidneys were separated from surrounding adipose tissue and a faecal sample. Livers were then weighed, photographed and the gall bladder removed prior to freezing (-20 °C) the liver pending TFC and gross pathology assessment. The gall bladder and contents were assessed on the day of collection for liver fluke and their eggs. The kidneys were separated from surrounding adipose tissue and weighed separately within 24 h of collection to measure kidney fat index (KFI). Faecal samples were stored at 4 °C and FECs conducted within seven days of collection. A sub-sample of each faecal sample was stored frozen (-20 °C) for the later measurement of coproantigens.

At each farm in Phase 2, up to 20 fresh Macropod faecal samples (as available) were collected from the ground. Fresh Macropod faeces were identified based on colour, shape and texture (in accordance with Catchpole, 2007). Samples were stored at 4 °C and FEC conducted within 7 days of collection.

3. Faecal assessments

3.1. Fluke egg counts

FEC were conducted on duplicate faecal samples (Phase 1: 3 g; Phase 2: 6 g) as described by Lamb et al. (2021). Fluke eggs were recovered from the gall bladder by washing the bile through a 90 μm sieve to a 500 ml conical flask. After eggs were left to sediment for 30–60 min, the supernatant was reduced to 100 ml using a vacuum suction pump. The flask was then re-filled with tap water and the sedimentation process repeated until eggs were clean of bile. Fluke eggs were counted and recorded for each gall bladder using a stereo microscope (Nikon SMZ800N) at 40× magnification. Fluke eggs (100 eggs) were incubated in water at 25 °C for 14 days then exposed to 2 h of artificial light to confirm hatching to viable miracidia. Confirmation of egg development and hatching were based on those described by Fairweather et al. (2012).

3.2. Coproantigen ELISA

Coproantigens were measured in triplicate on faecal samples (0.5 g) collected from all Macropods in Phase 1 that were confirmed positive for liver fluke by TFC or FEC (n = 52), and randomly selected from 30 Macropods with no liver fluke. The commercial cELISA kit (BIOK201-2 Monoscreen AgELISA Fasciola hepatica, Bio-X Diagnostic, Belgium) was used to measure coproantigens with the manufacturer’s guidelines modified to include an overnight soak of faecal samples at room temperature in the supplied dilution buffer prior to centrifugation (Brockwell et al., 2013). Samples were considered positive for F. hepatica if mean optical density was >8% at 450 nm. Coproantigens were measured to assess sensitivity and specificity of cELISA for use in Macropods with whole liver examinations used as a reference standard (French et al., 2016).

4. Liver assessments

4.1. Total fluke counts

TFCs were conducted according to Wood et al. (1995) on thawed livers. The liver was sliced to 0.5–1.0 cm wide strips with each strip examined and bile ducts squeezed to release fluke. Liver slices were subsequently soaked overnight in warm saline (9.0 g NaCl/L H2O), then washed with tap water over a 300 μm mesh sieve to collect any residual fluke. Fluke were counted based on the number of whole fluke and heads recovered from each liver. Adult fluke were distinguished from immature fluke based on the presence of reproductive organs (ovary and testis) and fluke eggs (Valero et al., 2001, 2005). Other parasites cohabiting the liver of Macropods were identified (Presidente and Beveridge, 1978; Beveridge and Emery, 2015) and recorded.

4.2. Liver pathology score

Gross pathology of the liver was assessed and scored (0–5 scale) based on the methodology described by Sargent et al. (2009).

5. Kidney fat index

Kidney fat index (KFI) was used to assess body condition. The fat and kidneys were weighed separately to calculate the mean weight for each individual. KFI was calculated by dividing the mean weight of the fat by the mean weight of the kidney and multiplied by 100 (Finger et al., 1981).

6. Statistical analysis

Data were checked for normality and the homogeneity of variance assumption confirmed using Levene’s test. The statistical software JMP®16 was used to calculate least squares means (LSM) ± standard error (s.e.). Analyses were based on the effects of species, sex, risk rating, season and their possible interactions.

Chi-square tests (χ²) were used to assess the association between prevalence of F. hepatica and species, sex, risk site and sampling season. Correlations conducted on parametric data used linear regression or Pearson’s correlation whilst correlations on non-parametric data used Spearman’s correlation.

Tabulated non-parametric data were presented as back-transformed
least square means. Risk site and pathology score were assessed as ordinal traits. As only one Common wallaroo was examined at the low risk site (Phase 1), this animal was excluded from analysis when assessing prevalence by risk site.

7. Results

7.1. Phase 1 — liver fluke prevalence

7.1.1. Macropod species

Overall, 39 of 181 Eastern grey kangaroos (22%) and 13 of 64 Common wallaroos (20%) were infected with liver fluke \((x^2 = 0.02, p = 0.879)\). No significant effects or interactions for fluke prevalence were observed between species and sex \((x^2 = 2.67, p = 0.446)\).

7.1.2. Risk site

Liver fluke prevalence differed significantly by risk site \((x^2 = 31.3, p < 0.001)\) and was 33.1%, 12.6% and 2.6% for high, medium and low risk sites respectively (Fig. 2). There was a strong suggestion that liver fluke prevalence differed by species across risk sites \((x^2 = 3.64, p = 0.06)\) with Eastern grey kangaroo appearing more sensitive than Common wallaroo to risk rating (Fig. 2). A significant interaction between risk site and sex \((x^2 = 10.6, p = 0.001)\) was also observed with a higher fluke prevalence in female Macropods across the medium and high risk sites.

7.1.3. Season

Rainfall and temperature data recorded at Armidale airport by the Bureau of Meteorology throughout the sample period are detailed in Fig. 3. For both species, liver fluke prevalence was unaffected by season \((x^2 = 4.96, p = 0.174)\).

7.2. Phase 1 — pathogenicity

7.2.1. Total fluke count and fluke egg counts

TFC ranged from 0 to 122 flukes in the Eastern grey kangaroo (lsm 0.2 \(\pm\) 0.03) with a FEC range of 0–195 epg (lsm 0.16 \(\pm\) 0.03). Lower burdens (all \(<12\) flukes) were recovered from the Common wallaroo (lsm 0.1 \(\pm\) 0.05) with FEC range of 0–82 epg (lsm 0.12 \(\pm\) 0.06). No effects or interactions of species, sex or risk site in TFC or FEC were significant. A strong positive correlation was observed between TFC and FEC \((R^2 = 0.9, p < 0.001)\) and pathology score \((R^2 = 0.3, p < 0.001)\). Liver fluke infections in Macropods are summarised in Table 1.

7.2.2. Gross pathology

Significant differences in pathology score \((x^2 = 22.2, p < 0.001)\) were observed by species with Eastern grey kangaroo having greater pathology than the Common wallaroo. No significant interaction was observed between the effects of species and sex for pathology score. Liver pathology score had a strong positive correlation with TFC \((r = 0.9, p < 0.001)\) and FEC \((r = 0.7, p < 0.001)\). Liver fluke were found throughout all areas of the liver. Macropods with low infections \((<12\) flukes) had pathological lesions limited to small areas and livers were dark in colour with regular formation (Fig. 4A and B). Pathological lesions consisted of partial or complete fibrotic capsule formations, encapsulating dead and live flukes.

When liver fluke burdens were higher \((\geq 12\) flukes) or immature fluke were present, pathological lesions were more extensive with haemorrhagic lesions, necrotic migratory tracks, bile duct hyperplasia, cholangitis and fibrotic lesions (Fig. 4C, D, E). Livers ranged from dark to pale in colour with irregular form. Gross pathology extended to the mucosal lining of the gall bladder (hyperplasia) in heavy infestations.

7.3. Phase 1 — other parasites

Progamotaenia festiva (Kangaroo tapeworm) were also found in the liver and in higher prevalence in Eastern grey kangaroo (70%) than Common wallaroo (34%). Infections were detected across all farms but in lower prevalence at sites where liver fluke were detected. In Macropods with liver fluke, 6% (Common wallaroo) and 10% (Eastern grey kangaroo) were also co-infected with Kangaroo tapeworm. Gross pathology attributed to kangaroo tapeworm was considered mild with slight enlargement of bile ducts walls and cholangitis.

Echinococcus granulosus (Hydatid tapeworm) was only found in female Eastern grey kangaroo at the medium and high risk sites and all were co-infected with liver fluke. Livers presented with multiple cysts, ranging up to 50 mm wide, hepatomegaly and disfigurement.

The livers of Eastern grey kangaroo were significantly \((p = 0.001)\) heavier than for the Common wallaroo (lsm 430.0 g \(\pm\) 10.4 and 359.2 g \(\pm\) 17.9 respectively). Livers weights were also significantly \((p < 0.001)\) heavier for male Macropods than for females (lsm 487.6 g \(\pm\) 16.9 and 301.7 g \(\pm\) 12.0 respectively). Liver weight had a weak positive correlation with TFC \((R^2 = 0.3, p < 0.001)\) and pathology score \((R^2 = 0.3, p < 0.001)\). Liver fluke infections in Macropods are summarised in Table 1. The mean fluke egg hatch rate of eggs recovered from the gall bladder of Macropods was 37.4 \(\pm\) 4.4% (range 0–96%).

![Fig. 2. Liver fluke prevalence in Macropods (infected/total sampled) cohabiting farms in the Northern Tablelands region of NSW, Australia. Number of farms by risk site: low – 2 farms, medium – 3 farms, high – 2 farms.](image)
7.4. Phase 1 — morphometric measurements

Macropod tail length had a strong positive correlation ($r = 0.8$, $p < 0.001$) with foot length. TFC also had a positive correlation with tail length ($r = 0.2$, $p = 0.018$) and foot length ($r = 0.1$, $p < 0.05$).

7.5. Phase 1 — kidney fat index

No significant differences ($p = 0.09$) in KFI were detected between Macropod species (Eastern grey kangaroo $l_{sm} 12.2 \pm 1.1$, Common wallaroo $l_{sm} 16.0 \pm 1.9$) however KFI was higher ($p < 0.001$) in female Macropods ($l_{sm} 18.3 \pm 1.2$ female, $9.9 \pm 1.8$ male). In Macropods with liver fluke, Eastern grey kangaroo had a significantly lower ($p = 0.04$) KFI than Common wallaroo ($l_{sm} 14.9 \pm 2.7, 26.0 \pm 4.4$). No significant differences were observed between the interaction of season and sex ($p = 0.08$) for KFI.

7.6. Phase 1 — cELISA

In those Macropods positive for liver fluke by TFC or FEC, 48 of 52 Macropods (92%) were also positive for coproantigens (Fig. 5). A moderate positive correlation was observed between cELISA and TFC ($r = 0.55$, $p < 0.001$) and FEC ($r = 0.61$, $p < 0.001$). No significant differences ($p = 0.304$) were observed in coproantigens by species (Eastern grey kangaroo $l_{sm} 1.2 \pm 0.1$, Common wallaroo $l_{sm} 1.0 \pm 0.2$) or by sex ($p = 0.962$) and no significant differences were observed in the interaction between the effects of species and sex. Coproantigens had a weak positive correlation with pathology score ($R^2 = 0.2$, $p = 0.54$).

One cELISA result which was positive for coproantigens (Optical density at 450 nm = 1.0) had no liver flukes in the liver however fluke eggs were detected in the gall bladder (496 eggs) and faeces (0.2 epg) inferring an active infection.

Of the four Macropods which were negative for coproantigens, three Macropods had resolved infections (no or dead liver flukes) with minimal fluke eggs in the gall bladder (1, 8 and 137 eggs) and faeces (0, 0 and 0.5 epg). The fourth Macropod was a false negative having 14 flukes encapsulated within two fibrotic capsules and fluke eggs in the gall bladder (144 eggs) and faeces (6.5 epg).

In those Macropods with mixed age infections (adult and immature fluke), all were positive for coproantigens including one Macropod with only immature fluke (Optical density at 450 nm = 0.4, TFC = 1, FEC =

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Table 1

| Species                     | No. with liver fluke (prevalence) | Sex (F/M) | Kidney fat index (%) | No. liver pathology score (0–5) | Liver weight (g) | Total fluke count | Fluke egg count (gall bladder) | Fluke egg count (epg) |
|-----------------------------|----------------------------------|-----------|----------------------|---------------------------------|-----------------|------------------|-------------------------------|-----------------------|
| Eastern grey kangaroo       | 39 of 181 (22%)                  | 22 F      | 18.0 ± 3.4           | 19 of 22 (86%)                  | 1.8 ± 0.3       | 401.3 ± 26.1     | 6.0 ± 1.3                    | 454.2 ± 19.9           |
|                             | 17 M                              | 11.8 ± 4.1 | N/A                  | 1.3 ± 0.3                       | 647.8 ± 29.7    | (224–777)        | 4.0 ± 1.3                    | (94,400–1,036)         |
| Common wallaroo             | 13 of 64 (20%)                   | 7 F       | 38.0 ± 6.0           | 6 of 7 (86%)                    | 1.0 ± 0.5       | 292.8 ± 46.3     | 2.6 ± 1.5                    | 289.2 ± 3.0            |
|                             | 6 M                               | 14.0 ± 6.5 | N/A                  | 1.4 ± 0.5                       | 549.6 ± 50.0    | (194–338)        | 3.4 ± 1.5                    | (3–13,500)            |

*p-value (species x sex)  0.091  –  0.174  0.897  0.349  0.087  0.833

*a* F = female, M = male.

*b* epg = eggs per gram faeces.
Macropods (n = 30) which were confirmed negative for liver fluke by TFC and FEC, were also negative for *F. hepatica* coproantigens.

7.7. Phase 2 — liver fluke prevalence on-farm

Of the 60 farms surveyed, 16 farms (27%) had Macropods infected with liver fluke (FEC range 6–22 epg). Liver fluke were detected in Macropods cohabiting farms in Guyra, Uralla, Walcha and Armidale.

Fig. 4. A. Common wallaroo liver (visceral surface) with prominent fibrotic capsules. B. Liver cross-section of fibrous capsules. C. Eastern grey kangaroo liver (visceral surface) with irregular form, hepatomegaly, fibrotic lesions and bile duct hyperplasia. D. Necrotic tracks generated by immature fluke. E. Immature fluke (mm).

Fig. 5. Scatter plot of *Fasciola hepatica* coproantigen concentration (optical density, 450 nm) and total fluke count in Macropods.
The density of Eastern grey kangaroo (26.4 km\(^{-2}\)) in the Northern Tablelands region of NSW is higher than Common wallaroo (7.4 km\(^{-2}\)) (Cairns et al., 2020) which would further increase grazing pressure within their habitat during periods of low rainfall and pasture growth.

The prevalence of liver fluke was also higher in female Macropods. Although reasons for this are unclear, age, immune response (Siddle et al., 2010), nutritional stress (Miller, 1987; Brandimarti et al., 2021), high energy requirements whilst supporting young (Stannard et al., 2020) and grazing behaviour (Cripps et al., 2011) may all be contributing factors. Female macropods are also philopatric, preferring to stay or return to their place of birth (Best et al., 2013; King and Goldizen, 2016), which may increase competition for food and frequency of grazing 'fluky' habitats where they exist.

Macropods with longer morphometric measurements also had higher liver fluke prevalence. As morphometric measurements increase with age (Poole et al., 1982), results suggest either that older Macropods are more susceptible to liver fluke or that they have accumulated greater exposure time to fluke infection. Younger Macropods would have a shorter accumulation period and be grazing less as they are not weaned until 13–18 months of age (King and Goldizen, 2016).

The prevalence of liver fluke on farms in Phase 2 (27%) was comparable to levels of Phase 1 (21%). Liver fluke were detected in Macropods throughout Guyra, Walcha, Uralla and Armidale regions. These areas have high rainfall, basalt soils with freshwater springs ("black springs") and soil pH\(\text{CaCl}_2\) \(> 4.5\) which are all attributes considered suitable for snail habitats (Boray, 1964; Upjohn et al., 2005). The Dorrigo region also has high rainfall but soil pH is comparatively acidic (pH\(\text{CaCl}_2\) \(< 4.5\)) which may deter snail establishment (Boray 1964).

Seasonality patterns of liver fluke prevalence typically observed in livestock (Sissay et al., 2007; Ali et al., 2011; Hernández-Guzmán et al., 2021) were not apparent in Macropods. Without anthelmintic intervention, Macropods may be harbouring persistent infections spanning a number of seasons or years, limiting the effectiveness of control programmes. Immature fluke were also detected in Macropods year-round demonstrating susceptibility in all seasons and the ability of (at least some) metacercariae to survive winters (Caminade et al., 2019), especially the warmer winter of 2019.

Macropods with 1–122 flukes generated a FEC range of 1–195 epg demonstrating their ability to contaminate pastures grazed by livestock. The fecundity of liver fluke in Macropods however appears lower than in sheep, which are capable of shedding 20,000–50,000 eggs per day (Happich and Boray, 1969), suggesting Macropods may be less suitable hosts for liver fluke despite their susceptibility.

To date, there have been mixed reports concerning the pathogenicity of liver fluke in Macropods. For example, Eastern grey kangaroo harbouring up to 95 flukes were reported with no clinical signs of infection (Presidente and Beveridge, 1978; Spratt and Presidente, 1981) whilst Portas and Taylor (2015) reported two Eastern grey kangaroos with 18 and 36 flukes having severe clinical signs of infection requiring euthanasia. In the present study, liver pathology correlated strongly with fluke burden. Some Macropods however had fluke encapsulated in fibrous capsules of the liver, attaining a lower pathology score. Evidence of dead decaying flukes within these capsules confirms their inherent ability to resolve infections. Similar capsule formations, described as "pockets" or "cyst-like lesions", have been identified in red (Cervus elaphus) (French et al., 2016) and fallow (Dama dama) deer (Jenkins et al., 2016) but also elk (Cervus canadensis) infected with Fascioloides magna (Pybus et al., 2015). Having the ability to isolate and restrict fluke migration minimises damage in the liver and would reduce clinical signs of infection. Moreover, the life span of liver fluke in Macropods may be shorter than the life span in sheep where flukes are capable of surviving up to 11 years (Andrews, 1999).

Detection of liver fluke by cELISA demonstrated high specificity and sensitivity, detecting low infections (1 fluke) as well as immature fluke. These results are comparable to that observed in sheep and cattle, also detecting infections of just 1–2 fluke (Mezo et al., 2004). One false
negative however was identified in a Macropod with 14 flukes encapsulated in fibrous capsules of the liver (6.5 epg faeces, 144 eggs gall bladder). These fibrotic capsules may partially or completely impede the release of fluke antigens to the small intestine and limit cELISA sensitivity. In experimentally infected lambs, cELISAs demonstrated a strong correlation between coproantigen and fluke burden (r = 0.89, p < 0.001) with infections of 1–36 flukes (Mezo et al., 2004). When burdens exceeded 14 flukes in Macropods, the correlation weakened. Macropods were also harbouring mixed aged infections, with varying development stages of fibrotic capsule formation, which may have contributed to individual variation despite harbouring similar fluke burdens. Red deer stages of fibrotic capsule formation, which may have contributed to exceeded 14 flukes in Macropods, the correlation weakened. Macropods 0.001) with infections of 1 to farms for sample collection. 

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