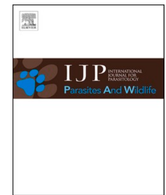
International Journal for Parasitology: Parasites and Wildlife 16 (2021) 199–207

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw



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

Jane Lamb [a,*], Emma Doyle [a], Jamie Barwick [a,b], Michael Chambers [c], Lewis Kahn [a]

[a] University of New England, Armidale, NSW, 2351, Australia
[b] Precision Agricultural Research Group, University of New England, Armidale, NSW, 2351, Australia
[c] Invetus Pty Ltd, Locked Bag 6865, West Armidale, NSW, 2350, Australia

ARTICLE INFO

Keywords:
Fasciola hepatica
Prevalence
Macropods
Livestock production
Australia

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 *Echinoccocus 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., 1987; 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 higher liver fluke prevalence (73%) than those originating from forest areas (24%) (Spratt and Presidente, 1981) highlighting the potential risk.

The liver fluke intermediate snail host, *Austropeplea* spp. (Boray, 1978; Boray et al., 1985), is predominately found in higher rainfall areas (>600 mm annually) of eastern Australia (Boray, 1964; Barger 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

* Corresponding author. School of Environmental & Rural , University of New England, Armidale, NSW, 2351, Australia.
E-mail address: jbowles2@myune.edu.au (J. Lamb).

https://doi.org/10.1016/j.ijppaw.2021.10.006
Received 6 September 2021; Received in revised form 10 October 2021; Accepted 11 October 2021
Available online 13 October 2021
2213-2244/© 2021 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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,

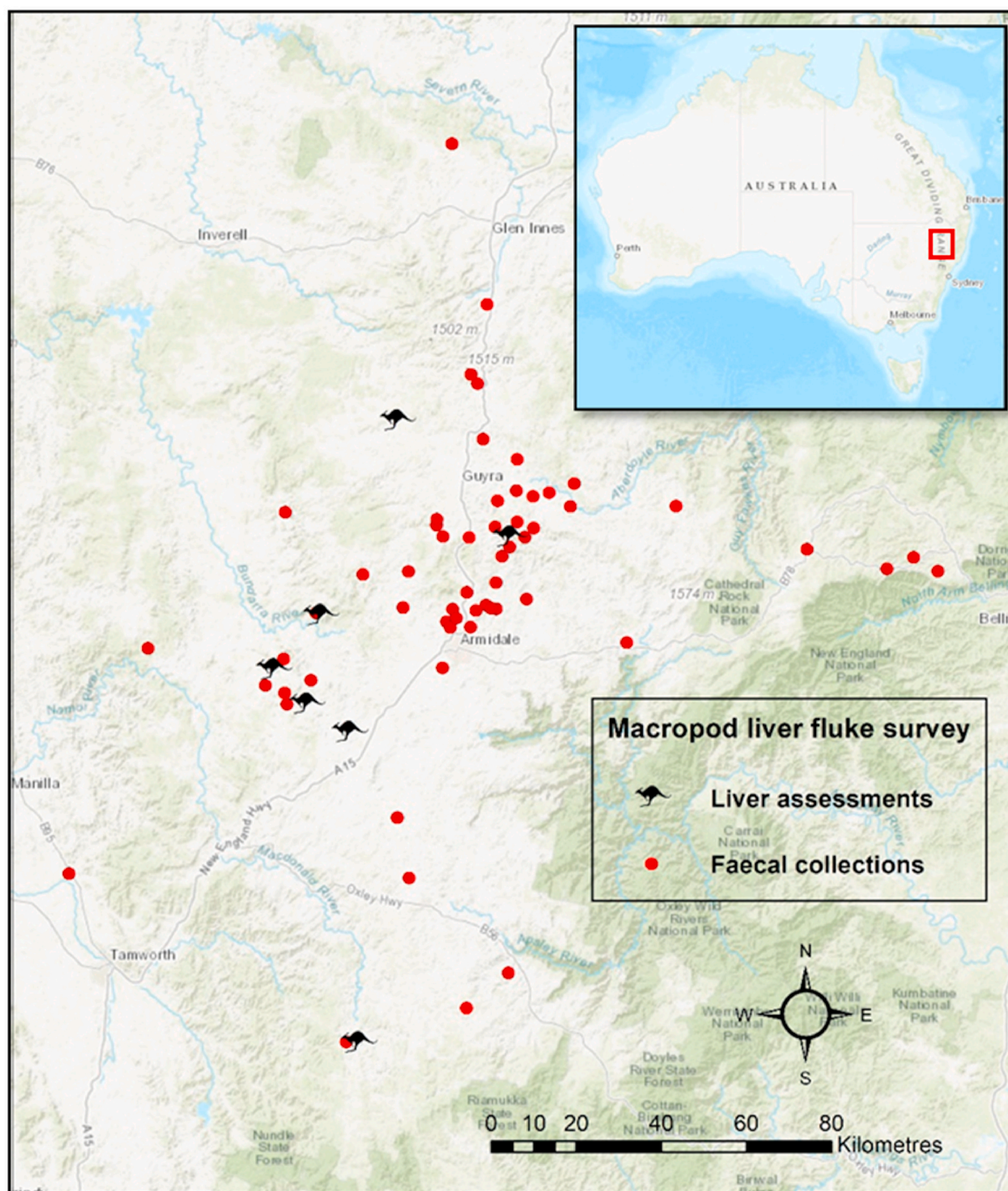


Fig. 1. Geographical location of livestock farms (A–G) surveyed in the Northern Tablelands region of NSW, Australia, to assess liver fluke prevalence in Macropods (ArcGIS 10.4.1 software, 2018).

245 Macropods (181 Eastern grey kangaroo and 64 Common wallaroo) cohabiting on seven livestock farms were examined for the presence of liver fluke via total fluke (TFC) and fluke egg counts (FEC), cELISA and gross pathology. Macropods were humanely euthanised by commercial harvesters or by landholders with a NSW Government Licence to Harm Kangaroos. Permission was obtained from the NSW National Parks and Wildlife Service (NPWS) to opportunistically collect samples after euthanasia (NSW NPWS Office of Environment & Heritage Scientific Licence, SL102153). This study was conducted with approval from the University of New England Animal Ethics Committee (AEC18-101).

In Phase 2, Macropod faecal samples were collected from 60 farms (distinct from those in Phase 1) to assess liver fluke prevalence via fluke egg counts (FEC).

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, 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 was 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 H_20), 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 (x^2) 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 ± 0.03) with a FEC range of 0–195 epg (lsm 0.16 ± 0.03). Lower burdens (all <12 flukes) were recovered from the Common wallaroo (lsm 0.1 ± 0.05) with FEC range of 0–82 epg (lsm 0.12 ± 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 ($R^2 = 0.9$, $p < 0.001$). Infections were of mixed age however 92% of Macropods infected with liver fluke had adult fluke whilst the remaining Macropods had immature fluke which were identified throughout all seasons.

The livers of Eastern grey kangaroo were significantly ($p = 0.001$) heavier than for the Common wallaroo (lsm 430.0 g ± 10.4 and 359.2 g ± 17.9 respectively). Livers weights were also significantly ($p < 0.001$) heavier for male Macropods than for females (lsm 487.6 g ± 16.9 and 301.7 g ± 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 ± 4.4% (range 0–96%).

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 (≥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.

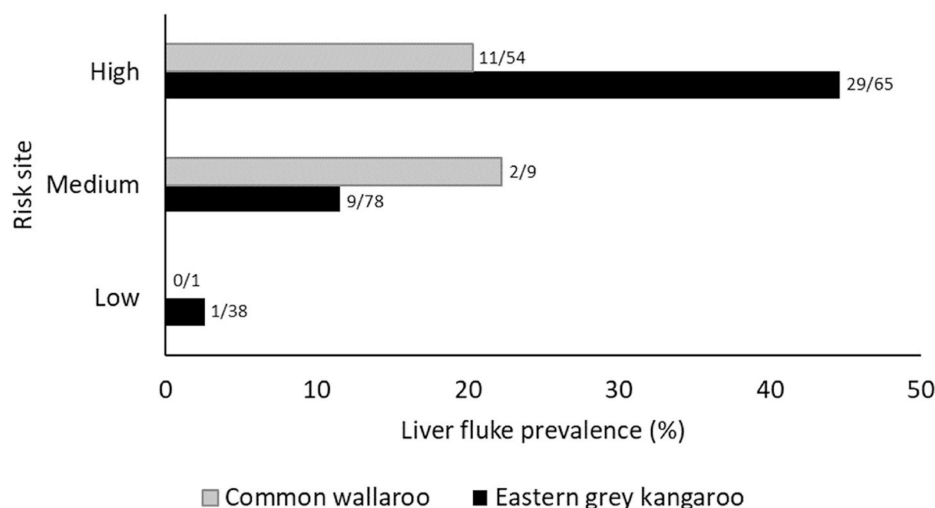


Fig. 2. Liver fluke prevalence in Macropods (infected/total sampled) cohabiting farms in the Northern Tablelands region of NSW, Australia. Number of farms by risk site: low – 2 farms, medium – 3 farms, high – 2 farms.

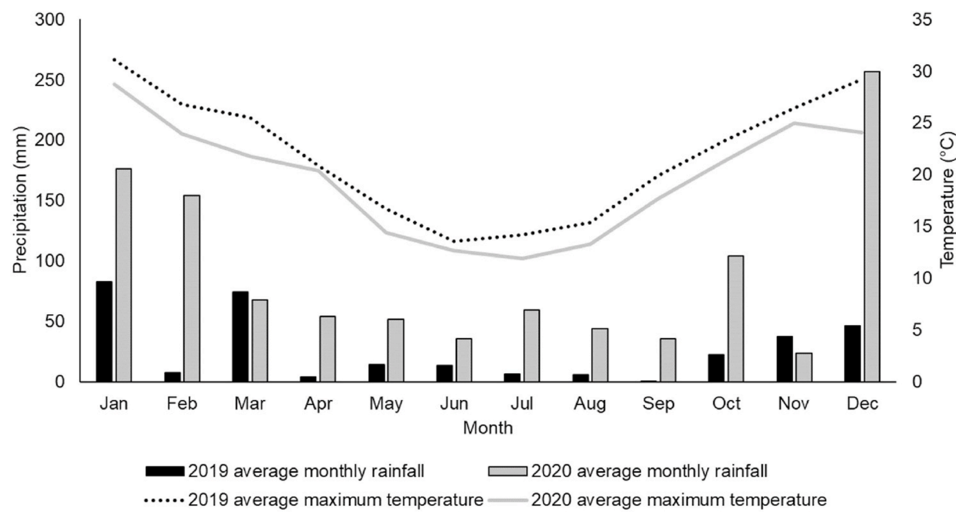


Fig. 3. Rainfall and temperature data throughout 2019–2020 recorded at the Armidale airport NSW, Australia (Australian Government of Bureau of Meteorology, 2019, 2020).

Table 1
Back transformed least square means ± s.e. (range) describing liver fluke infections in the Eastern grey kangaroo and Common wallaroo cohabiting livestock farms in the Northern Tablelands region of NSW.

Species	No. with liver fluke (prevalence)	Sex (F/M)[a]	Kidney fat Index (%)	No. with pouch young	Liver pathology score (0–5)	Liver weight (g)	Total fluke count	Fluke egg count (gall bladder)	Fluke Egg count (epg)[b]
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 (224–777)	6.0 ±1.3 (1–122)	454.2 ±1.9 (0–94,400)	7.0 ±1.4 (0–195)
		17 M	11.8 ± 4.1	N/A	1.3 ± 0.3	647.8 ±29.7 (398–890)	4.0 ±1.3 (1–26)	404.5 ±2.0 (0–83,200)	3.7 ±1.4 (0–87)
Common wallaroo	13 of 64 (20%)	7 F	38.0 ± 6.0	6 of 7 (86%)	1.0 ± 0.5	292.8 ±46.3 (194–338)	2.6 ±1.5 (1–7)	289.2 ±3.0 (3–13,500)	5.5 ±1.8 (0–82)
		6 M	14.0 ± 6.5	N/A	1.4 ± 0.5	549.6 ±50.0 (417–692)	3.4 ±1.5 (1–12)	6960 ±3.3 (355–73,080)	2.4 ±1.8 (0–19)
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.

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 lsm 12.2 ± 1.1, Common wallaroo lsm 16.0 ± 1.9) however KFI was higher ($p < 0.001$) in female Macropods (lsm 18.3 ± 1.2 female, 9.9 ± 1.8 male). In Macropods with liver fluke, Eastern grey kangaroo had a significantly lower (p = 0.04) KFI than Common wallaroo (lsm 14.9 ± 2.7, 26.0 ± 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 (Spearman's p = 0.55, $p < 0.001$) and FEC (Spearman's p = 0.61, $p < 0.001$). No significant differences (p = 0.304) were observed in coproantigens by species (Eastern grey kangaroo lsm 1.2 ± 0.1, Common wallaroo lsm 1.0 ± 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 =

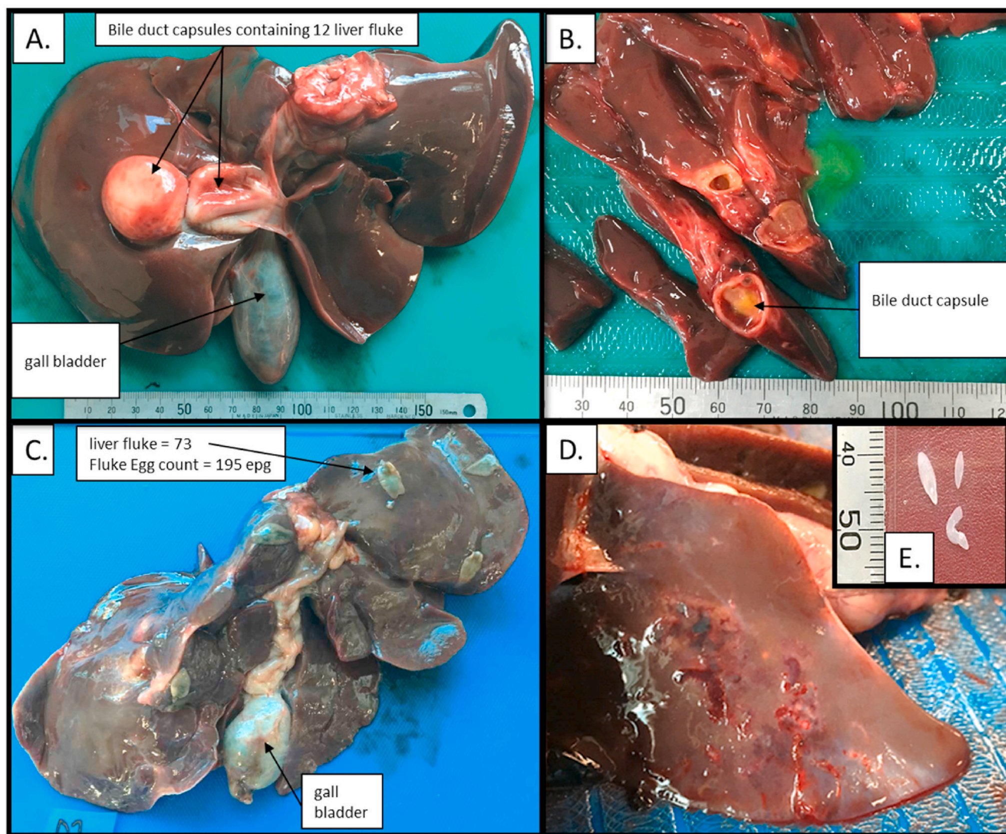


Fig. 4. A. Common wallaroo liver (visceral surface) with prominent fibrotic capsules. B. Liver cross-section of fibrous capsules. C. Eastern grey kangaroo liver (visceral surface) with irregular form, hepatomegaly, fibrotic lesions and bile duct hyperplasia. D. Necrotic tracks generated by immature fluke.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

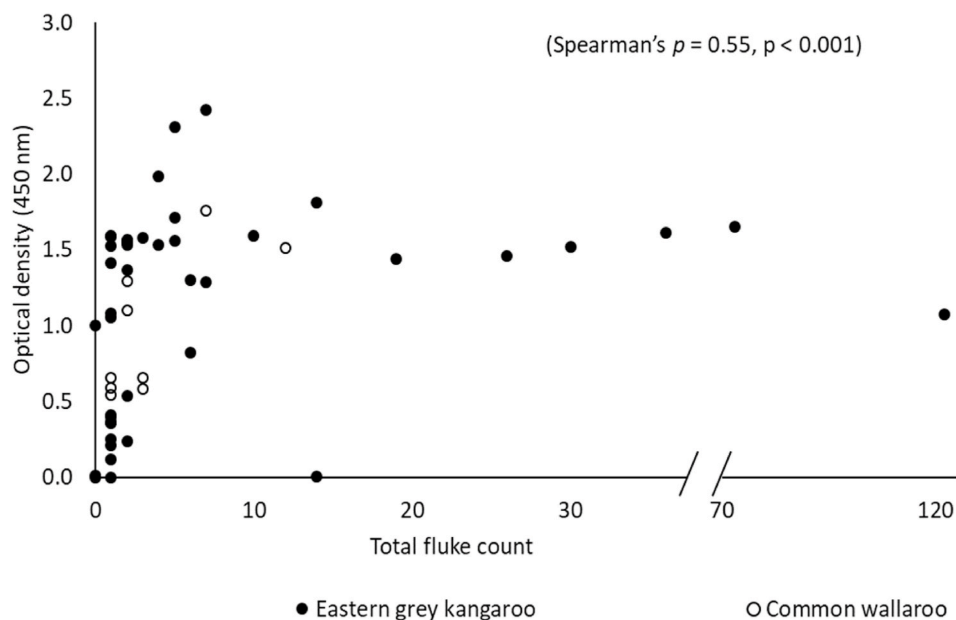


Fig. 5. Scatter plot of *Fasciola hepatica* coproantigen concentration (optical density, 450 nm) and total fluke count in Macropods.

0 epg).

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

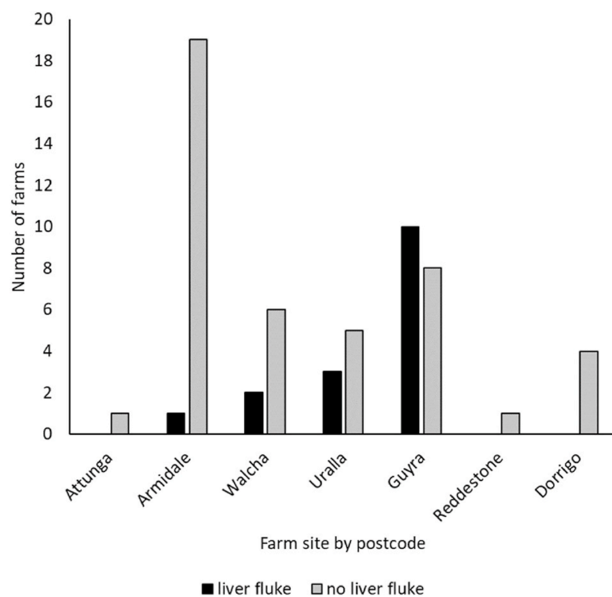


Fig. 6. Livestock farms in the Northern Tablelands region of NSW, Australia, with Macropods harbouring liver fluke infections (December 2018–June 2021).

(Fig. 6). No significant differences were detected in prevalence by season or year across farms.

8. Discussion

This study confirmed liver fluke in Eastern grey kangaroo (22%) and Common wallaroos (20%) cohabiting livestock farms in the Northern Tablelands region of NSW with prevalence as high as 45% in Eastern grey kangaroo across high risk sites. Macropods harbouring low fluke burdens (<12 flukes) had pathological lesions limited to small areas of the liver and flukes were frequently found encapsulated in fibrous capsules. Extensive lesions were observed in Macropods with higher fluke burdens ranging from 12 to 122 flukes. The detection of liver fluke in Macropods by cELISA proved to be a reliable assessment with high specificity (100%) and sensitivity (98%), detecting low fluke burdens (1–2 flukes) of mixed aged however pathology in the liver may limit correlation of coproantigens with fluke burden.

Liver fluke prevalence in Macropods would largely reflect their frequency of grazing the habitats of the intermediate snail host. The home range of Macropods is estimated to be 20–300 ha (Caughley et al., 1987; Hill, 1982; Jarman and Taylor, 1983; Grice et al., 1988; Croft, 1991; Viggers and Hearn, 2005). Free-roaming Macropods with unrestricted access to wetlands, supporting green pastures, would preferentially graze these areas when forage is limited or when rainfall is low as experienced in 2019 (40% below the long term average annual rainfall). Within liver fluke endemic regions, Macropods may pose risks to livestock production by maintaining the liver fluke life cycle in the grazing environment and limiting fluke control. Moreover, Macropods cohabiting farms with anthelmintic resistance may also be vectors (Walker et al., 2011; Chintoan-Uta et al., 2014) for dispersal of resistant strains to neighbouring farms.

Results suggest Eastern grey kangaroo had a higher susceptibility to infection. These results are consistent with earlier findings by Spratt and Presidente (1981) who reported Eastern grey kangaroo with the highest liver fluke prevalence (59%) compared to all other mammals examined. Habitat and behaviour may account for species susceptibility. Eastern grey kangaroo inhabit a diverse range of habitats including open pastures on livestock farms (Taylor, 1984). The Common wallaroo are comparatively sedentary within their habitat (Croft, 1991) preferring mountain and rocky landscapes (Taylor, 1984) which are areas less likely to support the habitat of the intermediate snail host. Furthermore, 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 $pH_{CaCl2} > 4.5$ 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 ($pH_{CaCl2} < 4.5$) 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 kangaroo 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., 2020; Lamb et al., 2021) 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 have also been identified with similar pathology in the liver and this was also thought to contribute to a lower sensitivity of cELISAs assessments in that species (French et al., 2016). Nevertheless, we cannot rule out that daily fluctuations in the release of coproantigens may play a role in the limited sensitivity as coproantigens were recently reported to fluctuate 2.6–8.9 fold in dairy cattle and correlated higher with fluke burden when cELISAs were conducted on faecal samples collected before midday (a.m.) (Kelley et al., 2021). Lastly, the one Macropod with no liver fluke, but positive for coproantigens and fluke eggs in the faeces and gall bladder, most likely occurred as fluke can easily be missed during visual inspection or liver collection given that culling of Macropods was predominately conducted at night.

9. Conclusion

Eastern grey kangaroo and Common wallaroos have been identified as definitive hosts and reservoirs for liver fluke on livestock farms located on the northern Tablelands of NSW. Sustaining large Macropod populations may pose risks to livestock production by maintaining the liver fluke life cycle in the grazing environment and limiting the effectiveness of control measures. Monitoring infections in Macropods could be achieved using the commercial Bio-X Diagnostic cELISA kit to provide informed decisions on disease management and populations.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to acknowledge the support of the Australian Commonwealth Government through the award of the Australian Government Research Training Program Scholarship (awarded to Jane Lamb) along with Animal Health Australia, WoolProducers Australia and Sheep Producers Australia for support in funding. The authors would also like to thank all landholders, commercial harvesters and livestock producers for their support on this project and providing access to farms for sample collection.

References

Australian Government Bureau of Meteorology BOM, 2019. Annual Climate Statement 2019. Retrieved. http://www.bom.gov.au/climate/current/annual/aus/2019/. (Accessed 1 September 2021).
Ali, T.S., Zarichehr, V., Reza, T.M., Amroallah, B., Hossin, T., Amir, M., Akbar, T., Hossin, H., Tourag, R., Hassan, E., 2011. Prevalence of liver flukes infections in slaughtered animals in Kashan, Isfahan province, central Iran. IIOAB J. 2, 14–18.
Andrews, S.J., 1999. The Life Cycle of *Fasciola hepatica*. Fasciolosis. Dalton JP CABI, Wallingford, pp. 1–20.
Australian Government Bureau of Meteorology BOM, 2020. Annual Climate Statement 2020. Retrieved. http://www.bom.gov.au/climate/current/annual/aus/2020/. (Accessed 1 September 2021).
Barger, I.A., Dash, K.M., Southcott, W.H., 1978. Epidemiology and Control of Liver Fluke in Sheep. The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia, pp. 65–74.
Best, E.C., Seddon, J.M., Dwyer, R.G., Goldizen, A.W., 2013. Social preference influences female community structure in a population of wild eastern grey kangaroos. Anim. Behav. 86, 1031–1040.
Beveridge, I., Arundel, J.H., 1979. Helminth parasites of grey kangaroos, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest), in eastern Australia. Wildl. Res. 6, 69–77.
Beveridge, I., Emery, D., 2015. Australasian animal parasites: inside and out. Aust. Society for Parasitol. 186.
Beveridge, I., Chilton, N.B., Johnson, P.M., Smales, L.R., Speare, R., Spratt, D.M., 1998. Helminth parasite communities of kangaroos and wallabies (*Macropus* spp. and *Wallabia bicolor*) from north and central Queensland. Aust. J. Zool. 46, 473–495.
Boray, J.C., 1964. Studies on the ecology of *Lymnaea tomentosa*, the intermediate host of *Fasciola hepatica*. 1. History, geographical distribution, and environment. Aust. J. Zool. 12, 217–230.
Boray, J.C., 1978. The potential impact of exotic *Lymnaea* spp. on fascioliasis in Australasia. Vet. Parasitol. 4, 127–141.
Boray, J.C., Fraser, G.C., Williams, J.D., Wilson, J.M., 1985. The occurrence of the snail *Lymnaea columella* on grazing areas in New South Wales and studies on its susceptibility to *Fasciola hepatica*. Aust. Vet. J. 62, 4–6.
Brandimarti, M.E., Gray, R., Silva, F.R., Herbert, C.A., 2021. Kangaroos at maximum capacity: health assessment of free-ranging eastern grey kangaroos on a coastal headland. J. Mammal. 1–15. https://doi.org/10.1093/jmammal/gyab022.
Brockwell, Y.M., Spithill, T.W., Anderson, G.R., Grillo, V., Sangster, N.C., 2013. Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. Vet. Parasitol. 196, 417–426.
Brown, G., Coleman, G., Constantinoiu, C., Gasser, R., Hobbs, R., Lymbery, A., Handly, O. R., Phalen, D., Pomroy, W., Rothwell, J., Sangster, N., 2014. Australasian animal parasites inside & out. The Aust. Society for Parasitol 401–405.
Cairns, S.C., Bearup, D., Lollback, G., 2020. A report to the Biodiversity and conservation Division. In: Industry and Environment on the Consultancy: Design and Analysis of Helicopter Surveys of the Kangaroo Populations of the Northern Tablelands Kangaroo Management Zones. New South Wales Department of Planning, 2019.
Caminade, C., McIntyre, K.M., Jones, A.E., 2019. Impact of recent and future climate change on vector-borne diseases. Ann. N. Y. Acad. Sci. 1436, 157.
Catchpole, H., 2007. What scat is that? Retrieved. https://www.abc.net.au/science/articles/2007/09/26/2044094.htm. (Accessed 1 September 2021).
Caughley, G., Shepherd, N., Short, J., 1987. Kangaroos: Their Ecology and Management in the Sheep Rangelands of Australia. Cambridge University Press, Cambridge, UK, pp. 1–253.
Chintoan-Uta, C., Morgan, E.R., Skuce, P.J., Coles, G.C., 2014. Wild deer as potential vectors of anthelmintic-resistant abomasal nematodes between cattle and sheep farms. Proc.Royal Soc. B: Biol. Sci. 281, 20132985.
Cripps, J.K., Wilson, M.E., Elgar, M.A., Coulson, G., 2011. Experimental manipulation of fertility reveals potential lactation costs in a free-ranging marsupial. Biol. Lett. 7, 859–862.
Croft, D.B., 1991. Home range of the euro, *Macropus robustus erubescens*. J. Arid Environ. 20, 99–111.
Fairweather, I., McShane, D.D., Shaw, L., Ellison, S.E., O'Hagan, N.T., York, E.A., Trudgett, A., Brennan, G.P., 2012. Development of an egg hatch assay for the diagnosis of triclabendazole resistance in *Fasciola hepatica*: proof of concept. Vet. Parasitol. 183, 249–259.
Finger, S.E., Brisbin, I.L., Smith, M.H., Urbston, D.F., 1981. Kidney fat as a predictor of body condition in white-tailed deer. J. Wildl. Manag. 45, 964–968.
Flanagan, A.M., Edgar, H.W.J., Forster, F., Gordon, A., Hanna, R.E.B., McCoy, M., Brennan, G.P., Fairweather, I., 2011. Standardisation of a coproantigen reduction test (CRT) protocol for the diagnosis of resistance to triclabendazole in *Fasciola hepatica*. Vet. Parasitol. 176, 34–42.
French, A.S., Zadoks, R.N., Skuce, P.J., Mitchell, G., Gordon-Gibbs, D.K., Craine, A., Shaw, D., Gibb, S.W., Taggart, M.A., 2016. Prevalence of liver fluke (*Fasciola hepatica*) in wild red deer (*Cervus elaphus*): coproantigen ELISA is a practicable alternative to faecal egg counting for surveillance in remote populations. PLoS One 11 e0162420.
George, S.D., Vanhoff, K., Baker, K., Lake, L., Rolfe, P.F., Seewald, W., Emery, D.L., 2017. Application of a coproantigen ELISA as an indicator of efficacy against multiple life stages of *Fasciola hepatica* infections in sheep. Vet. Parasitol. 246, 60–69.
Grice, D., Barker, R., Brown, B., Caughley, G., 1988. The edge of range. J. Anim. Ecol. 57, 771–786.
Happich, F.A., Boray, J.C., 1969. Quantitative diagnosis of chronic fasciolosis. 2. The estimation of daily total egg production of *Fasciola hepatica* and the number of adult flukes in sheep by faecal egg counts. Aust. Vet. J. 45, 329–331.
Hernández-Guzmán, K., Molina-Mendoza, P., Olivares-Pérez, J., Alcalá-Canto, Y., Olmedo-Juárez, A., Córdova-Izquierdo, A., Villa-Mancera, A., 2021. Prevalence and seasonal variation of *Fasciola hepatica* in slaughtered cattle: the role of climate and environmental factors in Mexico. J. Helminthol. 95.
Hill, G.J.E., 1982. Seasonal movement patterns of the eastern grey kangaroo in southern Queensland. Wildl. Res. 9, 373–387.
Jarman, P.J., Taylor, R.J., 1983. Ranging of eastern grey kangaroos and wallaroos on a New England pastoral property. Wildl. Res. 10, 33–38.
Jenkins, D.J., Baker, A., Porter, M., Shamsi, S., Barton, D.P., 2020. Wild fallow deer (*Dama dama*) as definitive hosts of *Fasciola hepatica* (liver fluke) in alpine New South Wales. Aust. Vet. J. 98, 546–549.
Kajugu, P.E., Hanna, R.E.B., Edgar, H.W., Forster, F.I., Malone, F.E., Brennan, G.P., Fairweather, I., 2012. Specificity of a coproantigen ELISA test for fasciolosis: lack of cross-reactivity with *Paramphistomum cervi* and *Taenia hydatigena*. Vet. Rec. 171, 502, 502.
Kajugu, P.E., Hanna, R.E.B., Edgar, H.W., McMahon, C., Cooper, M., Gordon, A., Barley, J.P., Malone, F.E., Brennan, G.P., Fairweather, I., 2015. *Fasciola hepatica*: specificity of a coproantigen ELISA test for diagnosis of fasciolosis in faecal samples

from cattle and sheep concurrently infected with gastrointestinal nematodes, coccidians and/or rumen flukes (*paramphistomes*), under field conditions. Vet. Parasitol. 212, 181–187.

Kelley, J.M., Stevenson, M.A., Rathinasamy, V., Rawlin, G., Beddoe, T., Spithill, T.W., 2021. Analysis of daily variation in the release of faecal eggs and coproantigen of *Fasciola hepatica* in naturally infected dairy cattle and the impact on diagnostic test sensitivity. Vet. Parasitol. 298, 109504.

King, W.J., Goldizen, A.W., 2016. Few sex effects in the ontogeny of mother-offspring relationships in eastern grey kangaroos. Anim. Behav. 113, 59–67.

Lamb, J., Doyle, E., Barwick, J., Chambers, M., Kahn, L., 2021. Prevalence and pathology of liver fluke (*Fasciola hepatica*) in fallow deer (*Dama dama*). Vet. Parasitol. 293, 109427.

Mazeri, S., Sargison, N., Kelly, R.F., Bronsvoort, B.M.D., Handel, I., 2016. Evaluation of the performance of five diagnostic tests for *Fasciola hepatica* infection in naturally infected cattle using a Bayesian no gold standard approach. PLoS One 11, e0161621.

Mezo, M., González-Warleta, M., Carro, C., Ubeira, F.M., 2004. An ultrasensitive capture ELISA for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). J. Parasitol. 90, 845–852.

Mezo, M., González-Warleta, M., Castro-Hermida, J.A., Manga-González, M.Y., Peixoto, R., Mas-Coma, S., Valero, M.A., 2013. The wild boar (*Sus scrofa* Linnaeus, 1758) as secondary reservoir of *Fasciola hepatica* in Galicia (NW Spain). Vet. Parasitol. 198, 274–283.

Miller, K., 1987. Nutrition and immunity. Nutr. Bull. 12, 32–40.

NSW Government Department of Planning Industry and Environment, 2021. Quota Report. NSW Commercial Kangaroo Harvest Management Plan 2017 – 2021. Retrieved. https://www.environment.nsw.gov.au/-/media/OEH/Corporate-Site/Documents/Animals-and-plants/Wildlife-management/Kangaroo-management/commercial-kangaroo-harvest-management-plan-2017-2021-quota-report-200485.pdf. (Accessed 1 September 2021).

Palmer, D.G., Lyon, J., Palmer, M.A., Forshaw, D., 2014. Evaluation of a copro-antigen ELISA to detect *Fasciola hepatica* infection in sheep, cattle and horses. Aust. Vet. J. 92, 357–361.

Poole, W.E., Carpenter, S.M., Wood, J.T., 1982. Growth of grey kangaroos and the Reliability of age Determination from body measurements I. The eastern grey kangaroo, *Macropus giganteus*. Wildl. Res. 9, 9–20.

Portas, T.J., Taylor, D., 2015. Clinicopathologic correlates of fascioliasis in two eastern grey kangaroos (*Macropus giganteus*). J. Zoo Wildl. Med. 46, 953–956.

Presidente, P.J.A., Beveridge, I., 1978. Cholangitis associated with species of *Progamotaenia* (Cestoda: *anoplocephalidae*) in the bile ducts of marsupials. J. Wildl. Dis. 14, 371–377.

Pybus, M.J., Butterworth, E.W., Woods, J.G., 2015. An expanding population of the giant liver fluke (*Fascioloides magna*) in elk (*Cervus canadensis*) and other ungulates in Canada. J. Wildl. Dis. 51, 431–445.

Sargent, R.M., Chambers, M., Elliott, T., 2009. Seasonal differences in the efficacy of pour-on formulations of triclabendazole and ivermectin or abamectin against late immature liver fluke (*Fasciola hepatica*) in cattle. Vet. Parasitol. 161, 133–137.

Siddle, H.V., Sanderson, C.E., Deakin, J.E., Belov, K., 2010. Genetic architecture of the macropodid immune system. In: Macropods: the Biology of Kangaroos, Wallabies and Rat-Kangaroos. CSIRO Publishing, Melbourne, pp. 13–23.

Sissay, M.M., Uggla, A., Waller, P.J., 2007. Prevalence and seasonal incidence of nematode parasites and fluke infections of sheep and goats in eastern Ethiopia. Trop. Anim. Health Prod. 39, 521–531.

Spratt, D.M., Presidente, P.J., 1981. Prevalence of *Fasciola hepatica* infection in native mammals in southeastern Australia. Aust. J. Exp. Biol. Med. Sci. 59, 713–721.

Stannard, H.J., Miller, R.D., Old, J.M., 2020. Marsupial and monotreme milk—a review of its nutrient and immune properties. PeerJ 8, e9335.

Taylor, R.J., 1983. The diet of the eastern grey kangaroo and wallaroo in areas of improved and native pasture in the New England Tablelands. Wildl. Res. 10, 203–211.

Taylor, R.J., 1984. Foraging in the eastern grey kangaroo and the wallaroo. J. Anim. Ecol. 65–74.

Upjohn, B., Fenton, G., Conyers, M., 2005. NSW Department of primary industries. Soil acidity and liming. AgFacts AC 19, 1–24, 3rd edition.

Valero, M.A., Panova, M., Mas-Coma, S., 2001. Developmental differences in the uterus of *Fasciola hepatica* between livestock liver fluke populations from Bolivian highlands and European lowlands. Parasitol. Res. 87, 337–342.

Valero, M.A., Panova, M., Mas-Coma, S., 2005. Phenotypic analysis of adults and eggs of *Fasciola hepatica* by computer image analysis system. J. Helminthol. 79, 217–225.

Viggers, K.L., Hearn, J.P., 2005. The kangaroo conundrum: home range studies and implications for land management. J. Appl. Ecol. 42, 99–107.

Walker, S.M., Johnston, C., Hoey, E.M., Fairweather, I., Borgsteede, F.H.M., Gaasenbeek, C.P.H., Prodohl, P.A., Trudgett, A., 2011. Potential role of hares in the spread of liver fluke in The Netherlands. Vet. Parasitol. 177, 179–181.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone Jr., J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World Association for the Advancement of Veterinary Parasitology (WAAVP) of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol. 58, 181–213.

Woodgate, R., Cassidy, T., Love, S., 2016. Laboratory detection of *Fasciola hepatica* in live sheep. In: District Veterinarians of NSW Annual Conference. Retrieved. http://www.flockandherd.net.au/sheep/reader/fasciola-detection-live-sheep.html. (Accessed 1 September 2021).