Veterinary Parasitology 293 (2021) 109427

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Prevalence and pathology of liver fluke (*Fasciola hepatica*) in fallow deer (*Dama dama*)

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ARTICLE INFO

Keywords:
Fasciola hepatica
Prevalence
Fallow deer
Pathology
Australia

ABSTRACT

A survey conducted on fallow deer (n = 79) in northern New South Wales Australia, aimed to ascertain the prevalence and gross pathology of liver fluke. In total, three deer populations were assessed (1 farmed and 2 wild) across 2 sites (site A and B) by conducting total fluke counts in the liver and fluke egg counts in faecal samples. At site A, 16 of 19 farmed deer (84.2 %) and 9 of 20 wild deer (45 %) had active or resolved infections. At site B, 16 of 40 wild deer (40 %) had active or resolved infections. Deer with active infections had low fluke burdens (1–11 fluke) which were in the adult development stage, shedding eggs with faeces (0–121.7 eggs per gram). Liver pathology score did not exceed 3.5 out of 5 with gross pathomorphological lesions predominately confined to the peripheral regions of the left lobe. Farmed deer, confined within a fluky habitat, attained the highest group mean pathology score, with dense fibrosis and concomitant atrophy of the left lobe (site A: farmed – 1.8, wild- 0.6; site B: wild – 0.3). Well-defined fibrotic capsules captured and restricted fluke migration beyond the peripheral region of the left lobe of the liver. The presence of live and dead fluke within the fibrotic capsules confirms the inherent ability of fallow deer to resolve infections. This survey has highlighted the susceptibility of fallow deer to liver fluke within an endemic region. Recurrent exposure, as seen in the farmed deer confined within a fluky habitat, appears to strengthen tissue response in terms of gross pathology and may impede the release of fluke eggs from the liver. Low fluke burdens and limited lesions suggest fallow deer have a strong level of resistance to liver fluke. Nevertheless, within this endemic region, fallow deer are widespread and clearly facilitating the liver fluke life cycle. Further research is warranted to ascertain the impact of fallow deer on disease transmission in livestock production when cohabiting the grazing environment.

1. Introduction

Liver fluke (*Fasciola hepatica*) is a parasite which infests the liver of herbivores including domestic livestock and wild animals. Herbivores, such as deer, become infected after grazing wet pasture with encysted metacercariae. Ingested metacercariae develop to adult fluke in the liver and shed 20,000–50,000 eggs per day which are expelled in the faeces (Boray, 2017). When eggs in faeces are deposited in the freshwater habitat of the intermediate snail host (*Austropeplea* spp. or *Pseudosuccinea* spp.), they undergo development to free swimming miracidia following an incubation of approximately 1.5–3 weeks. The miracidia then penetrate the snail and undergo development to infective metacercariae (Boray, 2017). Liver fluke infections in domestic livestock, referred to as fasciolosis, can result in considerable production loss due to the parasite-induced liver damage (Roseby, 1970; Schweizer et al., 2005). Fasciolosis prevalence is common worldwide which includes the temperate regions of eastern Australia where the intermediate snail host predominately lives (Barger et al., 1978; Puslednik et al., 2009).

During the early 1800s, deer were introduced to Australia for hunting and aesthetics (Hall and Gill, 2005). The fallow deer (*Dama dama*) is just one of the six introduced species that have established wild populations and are reportedly increasing in number and distribution (Moriarty, 2004; Davis et al., 2016). In the state of New South Wales (NSW), the distribution of wild deer increased from a coverage of approximately 8 % (2009) to 17 % (2016) of the state and with fallow deer recorded in higher density in eastern NSW; the region which also supports the liver fluke snail (NSW Government Department of Primary Industries, 2019).

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https://doi.org/10.1016/j.vetpar.2021.109427
Received 10 February 2021; Received in revised form 8 April 2021; Accepted 9 April 2021
Available online 16 April 2021

Deer are well known vectors for a range of diseases and parasites of livestock including liver fluke (Vengušt and Bidovec, 2003; Böhm et al., 2007; Rijks et al., 2011; Cripps et al., 2019; Barone et al., 2020) yet liver fluke prevalence in fallow deer of Australia remains largely unknown. A recent survey in south-eastern NSW (Jenkins et al., 2020) reported 53 % of wild fallow deer (9 of 17 deer) with active or past (resolved) infections. Fluke burdens ranged from 3 to more than 50 fluke per deer. In an earlier survey of red deer (*Cervus elaphus*), rusa (*C. timorensis*) and fallow deer conducted in south eastern Queensland, McKenzie et al. (1985) reported liver fluke only in red deer and with fluke burdens less than 5 fluke per deer.

Across a number of studies in Europe and the United Kingdom, the prevalence of liver fluke in wild deer has ranged from 10 to 53 % in red deer (Shimalov and Shimalov, 2000; French et al., 2016) and 0–6 % in roe deer (*Capreolus capreolus*) (Shimalov and Shimalov, 2000; Arias et al., 2013). Male red deer (30.8 %) were also reported with a higher prevalence than female deer (18.4 %) (French et al., 2016). Similarly, farmed fallow deer are susceptible to liver fluke infestation. In Slovenia, one study with 43 deer reported 44 % infested with liver fluke (Vengušt and Bidovec, 2003). These studies highlight the potential risk of wild and farmed fallow deer as reservoirs of infection in the grazing environment.

The susceptibility of herbivores to liver fluke however varies with the definitive host and has been shown in a number of experimental studies (Kendall et al., 1967; Ross et al., 1967; Boray, 1967; Hayes et al., 1972; Reddington et al., 1986; Martınez-Moreno et al., 1999; Kolodziejczyk et al., 2005). Experimental studies in deer have also shown apparent differences in susceptibility at the species level. The black-tailed deer (*Odocoileus hemionus columbianus*) are more susceptible to acute fasciolosis than sheep (Kistner and Koller, 1975). Roe deer are more susceptible than red deer (Barth and Schaich, 1973) and the white-tailed deer (*Odocoileus virginianus*) appear to have an inherent resistance to liver fluke (Presidente et al., 1974, 1975). With an increasing trend in wild deer populations, understanding the potential risks that deer species may pose in disease transmission when cohabiting the grazing environment is fundamental to livestock production. The aim of this study was to ascertain liver fluke prevalence and associated gross pathology in wild and farmed fallow deer on two extensive livestock grazing systems located on the Northern Tablelands of NSW. Over the past 5 years, domestic livestock at both sites had been treated frequently for fasciolosis (2–3 times/year).

2. Materials and methods

2.1. Sample site and animals

A total of 79 fallow deer of mixed ages were euthanised during routine pest or herd management programs throughout 2019 – 2020. Permission was gained from landholders and the NSW Government Local Land Services to opportunistically examine and collect samples from deer cohabiting two livestock production systems (site A and site B).

Site A was located east of Guyra, NSW (-30.21865 °N, 151.6718 °E) and situated approximately 1275 m above sea level, with mean annual rainfall of 873 mm and mean daily maximum temperature of 17.9 °C (Australian Government Bureau of Meteorology, 2020). The livestock (cattle and sheep) grazing area consisted of 2,023 ha of heavily timbered elevated terrain descending to low lying cleared grazing pastures with spring-fed gullies. The intermediate snail host was identified in the freshwater springs during late September 2019 to May 2020.

Thirty nine (39) fallow deer were examined at site A. Nineteen (19) of these deer had been farmed within an enclosed 4 ha paddock containing two large spring-fed dams and maintained for up to 5 years at a high stocking rate (8 deer/ha). Sheep and cattle intermittently cohabited this paddock. The remaining 20 deer were wild, roaming unrestricted on the farm. No parasite management in terms of anthelmintics were administered to any deer inhabiting this farm and no supplementary feed was offered during the study.

Site B was located south of Glen Innes, NSW (-29.73618 °N, 151.73596 °E) and situated approximately 1062 m above sea level, with mean annual rainfall of 859 mm and mean daily maximum temperature of 20.2 °C (Australian Government Bureau of Meteorology, 2020). The livestock grazing area consisted of 1520 ha with heavily timbered high terrain, cleared pastures and spring fed gullies. Forty (40) wild fallow deer of mixed sex (5 males and 35 females) and ranging from approximately 1.5–11 years of age were examined at this site.

2.2. Sample collection

The liver with proximal end of the small intestine and a faecal sample were collected from each deer to conduct total fluke (TFC) and fluke egg counts (FEC), respectively. The small intestine was excised and the contents examined for liver fluke. The liver was then weighed, photographed and stored frozen (−20 °C) pending assessment. All images of liver dissections were captured after freeze-thaw. Faecal samples were stored at 4 °C and FECs conducted within 7 days of collection. The right and left kidney were collected from each deer with surrounding fat. Kidneys and fat were stored at 4 °C and weighed within 24 h of collection.

2.3. Total fluke counts

TFCs were conducted according to Wood et al. (1995) on thawed livers. The liver was sliced into 0.5–1.0 cm wide strips. Each strip was examined and bile ducts squeezed in order to release any 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. Total fluke were counted based on the number of whole fluke and heads recovered from each liver. Livers with fluke were classified as ‘active’ infections whilst livers with no fluke but fibrosis were classified as ‘resolved’ infections.

Individual fluke were weighed then flattened between two glass slides to measure length and width (mm). Fluke were then examined microscopically (40 x magnification) to confirm development stage and patency.

2.4. Fluke egg counts

FECs were conducted in duplicate on faecal samples based on a modified sedimentation process (Department of Agriculture and Food Western Australia (DAFWA), 2013). Two 3 g faecal samples were weighed and tap water added to each sub-sample and homogenised to a faecal slurry. The faecal slurry was subsequently washed through a 90 μm sieve stacked over a 34 μm sieve. The filtered washings collected in the bottom sieve (34 μm) were subsequently washed into a graduated flask and diluted to a final volume of 100 mL with water and allowed to settle for 6 min. The supernatant was then reduced to 20 mL using a vacuum suction pump and the sediment was diluted again to 100 mL with water. This procedure was repeated until the sample was relatively clean of faecal matter (approximately 5–6 times). The 20 mL sediment was then transferred to a counting tray with 2 drops of 0.5 % methylene blue. FECs were conducted using a stereo microscope at 40 x magnification and the mean FEC was calculated in eggs per gram (epg) of faeces.

2.5. Liver pathology assessment

Liver pathology was assessed and scored based on the methodology described by Sargent et al. (2009). Pathology score ranged from: 0 for no pathology and no fluke; 1 for mild pathology with < 5% of liver affected; 2 for moderate pathology with 5–10 % of liver affected; 3 for moderate pathology with 10–20 % of liver affected; 4 for moderate pathology with 20–30 % of total liver affected; and 5 for severe and extensive pathology

with > 30 % of total liver affected.

2.6. Kidney fat index

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

2.7. Statistical analyses

Total fluke counts, FECs, liver weight and KFI were collated by group (farmed vs wild) using Microsoft EXCEL (2016). Data were checked for normality and the homogeneity of variance assumption confirmed using Levene's test. Parametric data were compared using group arithmetic means at $p < 0.05$, unless stated, using One Way Analysis of Variance (ANOVA) or paired *t*-test using the statistical software R 3.5.2 (R version, 2018). Group means were compared within ANOVA using Tukey's all pairwise comparison of means. Non parametric data was log 10 transformed in EXCEL using the formula y = Log(x+1). Geometric means were calculated by back-transforming the average of the log values using the formula (10^(average(x_1:x_a))-1 and compared using ANOVA. The correlation (R^2) between groups was conducted using linear regression on parametric data or Spearman's correlation on nonparametric data both in Microsoft EXCEL (2016).

3. Animal ethics

This study was conducted with approval from the University of New England Animal Ethics Committee (AEC18−101).

4. Results

4.1. Liver fluke prevalence

At site A, 11 of 19 farmed deer (57.9 %) and 3 of 20 wild deer (15.0 %) had active liver fluke infections. At site B, only 1 of 40 wild deer (2.5 %) had an active liver fluke infection. In addition to these active infections, deer at both sites (farmed A - 5, wild A – 6, wild B - 15) also presented with gross pathological lesions consistent with active infections however no fluke or fluke eggs were found (resolved infections). Overall, liver fluke prevalence (active and resolved infections) in fallow deer ranged from 40 to 45 % in wild deer and 84 % in farmed deer with significant differences observed between the farmed and wild deer at site A (Table 1).

4.2. Total fluke and fluke egg counts

Total fluke counts (TFCs) were low ranging from 1 to 11 fluke with no significant differences between individuals by group or sex at $p < 0.05$ (Table 2). No significant differences were observed between FECs, however wild deer (27.7 epg) at site A had a higher group mean FEC compared to farmed deer (3.3 epg) despite having a similar fluke burden (wild A – 4.6, farmed A – 3.9). A high correlation between FEC and fluke burden was also observed in both the farmed ($R^2 = 0.76$) and wild deer ($R^2 = 0.80$) at site A. As only 1 deer had an active infection at site B, no comparative analyses was conducted between sites.

All fluke recovered from livers were in the adult development stage and had established patency. Group mean fluke length was significantly different ($p < 0.05$) in farmed deer by sex and between groups (farmed and wild) at site A (Table 3). Significant differences were also observed in fluke weight of farmed deer by sex whilst no significant differences were observed between group mean fluke weight or width at Site A. Only 2 whole fluke (and 2 partial fluke) were recovered from wild deer at site B so no comparative assessment could be conducted between sites.

4.3. Liver weights

Overall, liver weights were significantly higher in male deer compared to female deer ($p = 0.047$). Liver weights were also significantly different between farmed and wild deer by group and sex at site A (Table 4). No significant differences were observed within or between groups ($p < 0.05$) in liver weights of female or male deer with or without fluke.

4.4. Gross pathology

Liver pathology did not exceed a score of 3.5 out of 5 (Fig. 1). Group mean pathology score was significantly higher in farmed deer ($p < 0.001$) compared to the wild deer populations (farmed A – 1.8, wild A - 0.6, wild B - 0.3). One wild deer (site A) attained a liver pathology score of 3.0 as liver fluke and *Echinococcus granulosus* (hydatid tapeworm) cohabited this liver (outlier marked as number 30 in Fig. 1 and pictured in Fig. 5A, C–D). A low correlation within groups was observed between pathology score and TFC ($R^2 = 0.58$ farmed, 0.31 wild A, 0.05 wild B) and between pathology score and FEC ($R^2 = 0.48$ farmed, 0.31 wild A, 0.05 wild B).

Liver morphology ranged from normal shape with even smooth surfaces to irregular shaped with uneven surfaces. At site A, 16 of 19 farmed deer (84 %) had fibrotic lesions and irregular shaped livers. Seven of the 19 livers displayed clear atrophy of the left lobe and

Table 1
Liver fluke prevalence based on active and resolved infections.

Site A					Site B			
Sex	No. deer	Prevalence (%) of active infections (No. infected)	Resolved infections	Prevalence (%) including resolved infections	No. deer	Prevalence (%)of active infections (No. infected)	Resolved infections	Prevalence (%) including resolved infections
Wild deer					Wild deer			
Male	7	0 (0)	1	14.3	5	0 (0)	2	40.0
Female	13	23.1 (3)	5	61.5	35	2.9 (1)	13	40.0
Group	20	15.0 (3)	6	45.0[b]	40	2.5 (1)	15	40.0[b]
Farmed deer								
Male	13	53.8 (7)	3	76.9				
Female	6	66.7 (4)	2	100.0				
Group	19	57.9 (11)	5	84.2[a]				

[a],[b] Columns with different superscripts are significantly different at $p < 0.01$. No. = number.

Table 2
Total fluke count and FECs in deer with active fluke infections ± standard deviation.

Group	Mean total fluke count				Mean fluke egg count (epg)			
	Farmed A	Wild A	Wild B	p-value	Farmed A	Wild A	Wild B	p-value
Arithmetic mean(range)	4.8± 3.5(1–11)	5.0± 2.6(3–8)	4.0± 0.0(4)	–	5.4± 6.4(0–17.7)	62.4± 59.8(2.0–121.7)	4.3± 0(4.3)	–
Geometric mean	3.9[a]	4.6[a]	4.0[a]	0.935	3.3[a]	27.7[a]	4.3[a]	0.082
No. Infected	11 of 19	3 of 20	1 of 40	–	11 of 19	3 of 20	1 of 40	–

[a] Rows with the same superscripts are not significantly different at $p < 0.05$.

Table 3
Group mean liver fluke morphometric data (range) ± standard deviation.

Deer	Fluke length (mm)	Fluke width (mm)	Fluke weight(g)
Wild A	24.3[a](19.0–30.0)± 3.3	10.8[a](10.0–15.0)± 1.4	0.18[a](0.13–0.23)± 0.03
Wild B	37.5(35.0–40.0)± 3.5	12.0(11.0–13.0)± 1.4	0.26(0.23–0.29)± 0.04
Farmed A	28.0[b](21.0–45.0)± 4.7	11.6[a](5.0–21.0)± 3.2	0.21[a](0.10–0.38)± 0.07
p-value	0.01	0.43	0.16
Male (Farmed A)	29.3[a]±4.9	11.1 [a]± 2.5	0.07[a]± 0.02
Female (Farmed A)	26.6[b]± 4.0	12.1[a]± 3.8	0.09[b]± 0.3
p-value	0.0392	0.2597	0.0295

[a],[b] Columns with different superscripts are significantly different at $p < 0.05$.
Analyses based on whole fluke recovered only - farmed A – 49 fluke, wild A - 12 fluke; wild B – 2 fluke.

Table 4
Group mean liver weights (g) ± standard deviation.

Deer	Farmed A(6 F, 13 M)	Wild A(13 F, 7 M)	Wild B(35 F, 5 M)	p-value
Female	522.1[a]	753.6[b]	667.5[ab]	0.008
Male	599.0[a]	998.5[b]	966.8[ab]	0.011
Group(range)	574.7[a](250.5–1246.8)± 235.1	839.3[b](397–1581.9)± 267.0	704.9[ab](408.2–1198.3)± 181.3	0.001

[ab] Rows with different superscripts are significantly different at $p < 0.05$. F = female, M = male.

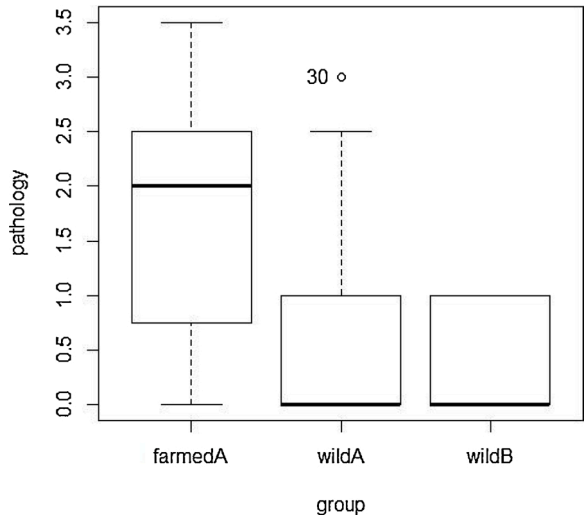

Fig. 1. Group mean liver pathology score.

compensatory hypertrophy of the right lobe (Fig. 2A, B, C). Only 5 of 60 wild deer (8.3 %) had livers of irregular shape and 40 % displayed mild fibrotic lesions at both sites (wild A - 8 of 20, wild B - 16 of 40) with no defined atrophy of the left lobe (Fig. 2D, E, F). Deer with resolved infections also displayed fibrotic lesions predominately on the left lobe which were consistent with those deer infested with liver fluke.

Rust or grey-white coloured plaques were occasionally observed in farmed deer on the diaphragmatic surface of the Glisson's capsule (left lobe) producing an uneven granular appearance (Fig. 3). Fibrotic lesions were predominately confined to the peripheral regions of the left lobe whilst the right lobe was largely unaffected or with small areas of fibrosis limited to the peripheral regions of the lobe (Fig. 4A). Liver fluke (live and dead) were found in the fibrotic regions, encapsulated within a thick-walled capsule filled with dark brown exudate (Figs. 4B and 5B). No fluke were found beyond the falciform ligament in the right lobe and no immature fluke were recovered from livers however residual migratory tracks were observed. No visible signs of Black disease (*Clostridium novyi*) were observed in any deer infested with liver fluke.

4.5. Kidney fat index

All deer appeared visually healthy with no signs of infection. Based

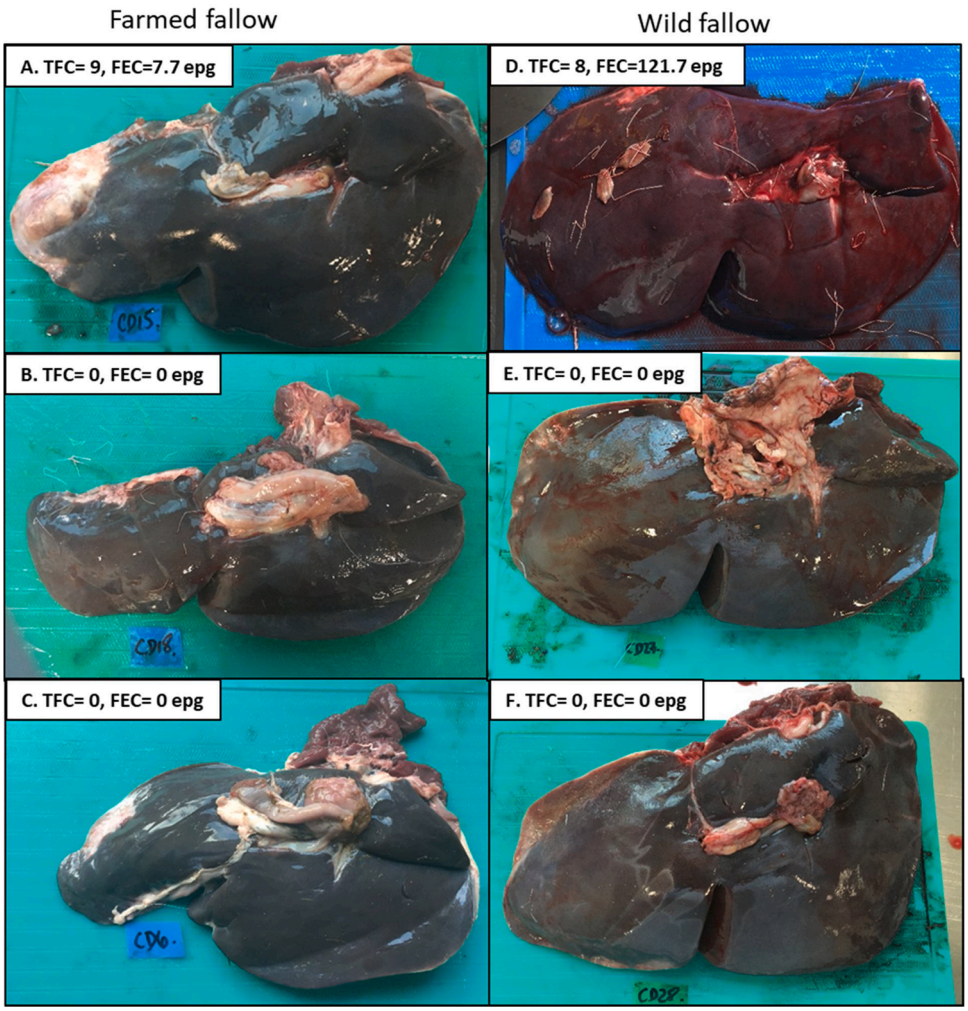

Fig. 2. Visceral surface of deer livers at site A (Figures A - C. farmed deer with atrophy of the left lobe and hypertrophy of right lobe, Figures D - F. wild deer with mild fibrosis of the left lobe).

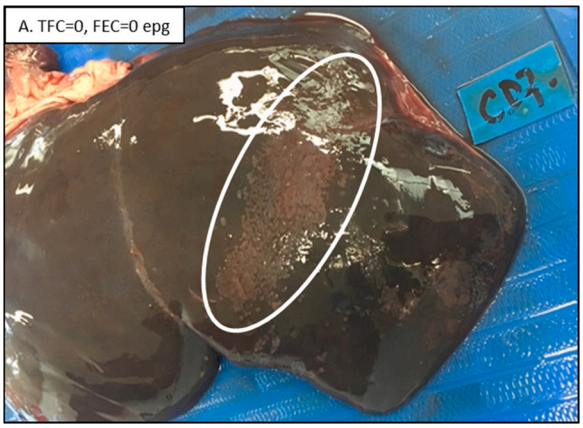

Fig. 3. Plaque deposits (circled) on the Glisson's capsule of the diaphragmatic surface of the left lobe.

on group mean KFI, body condition was significantly different by group (Fig. 6) and between females at site B and between males at site A (Table 5). As deer were euthanased at different time points, over different seasons and were of mixed ages, comparative differences between infected and non-infected deer were not assessed.

At site A, male deer positive for liver fluke and euthanised at the same time point (June 2019) had a lower KFI ($p = 0.106$) than male deer without fluke (Fig. 7).

5. Discussion

The prevalence of liver fluke (active and resolved infections) was significantly higher in farmed deer (84.2 %) than wild deer (site A - 45.0 %, site B - 40 %). Farmed deer maintained at a high stocking rate within a fluky habitat would have a higher risk of exposure especially when food supplies were limited. Wild deer that are free-roaming, have less pressure to graze fluky habitats with an expansive grazing area and vegetation of different heights. The prevalence levels observed in the wild deer are consistent with that of wild fallow deer (53 %) recently surveyed in south eastern NSW (Jenkins et al., 2020). When liver fluke prevalence was compared by sex, female deer had a higher prevalence than males although some caution is warranted in this interpretation as there were only 25 males of the total 79 deer. Borkowski and Pudełko (2007) showed that male fallow deer have a larger home range (9.75 km^2) than females (2.1 km^2) when monitored with radio-collared tracking devices. Having an expansive home range may contribute to lower prevalence levels and frequency grazing fluky habitats but may also account for the lower number of male deer available for comparative analyses in this study. Nevertheless, sample size, season, age and gender immune response may have also influenced fluke prevalence (French et al., 2019).

The frequency of fibrotic lesions on the left lobe, including grey-rust

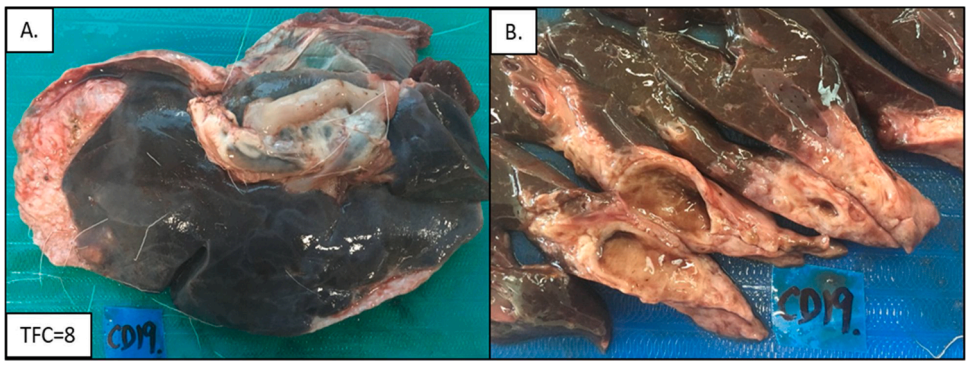

Fig. 4. A. Liver with fibrotic lesions at the perimeter of the left lobe. **B.** Left lobe excised displaying dense fibrosis and capsules which confine fluke.

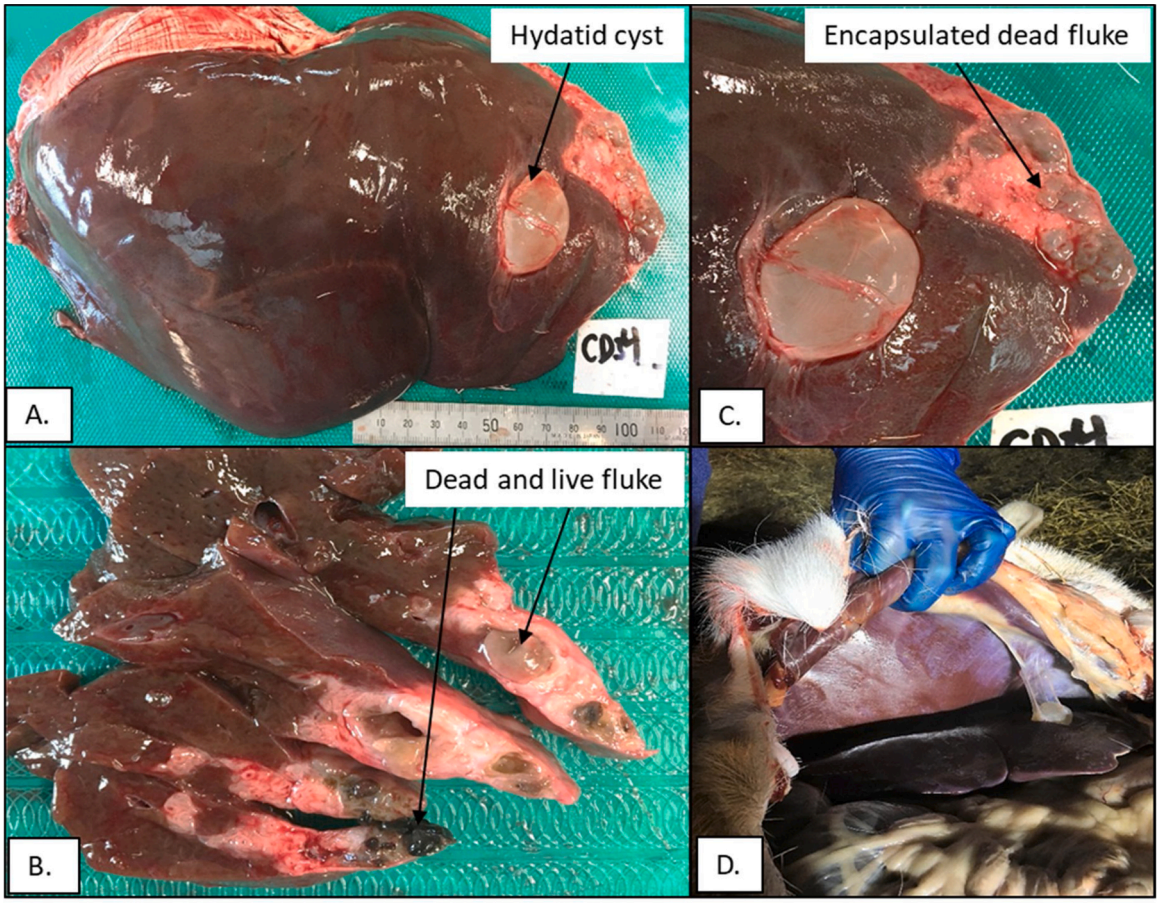

Fig. 5. A. Deer liver infested with liver fluke and hydatid tapeworm cyst **B.** Excised left lobe with peripheral fibrotic capsules encapsulating live and dead (black) fluke. **C.** Fibrotic lesions with encapsulated fluke. **D.** Liver (in situ) with hydatid tapeworm cyst attached to the diaphragm wall by fibrotic adhesions.

coloured plaques on the diaphragmatic surface of the Glisson's capsule, suggest the left lobe is the most direct entry point for immature fluke during the intraperitoneal migration phase. In situ, the left lobe is positioned adjacent to the small intestine providing a direct route of entry. These findings are consistent with earlier reports in white-tailed deer when artificially infected with dose levels ranging from 100−2,500 metacercariae. The left lobe was predominately affected with plaques on the diaphragmatic surface of the Glisson's capsule and clearly visible up to 15 weeks post infection (Presidente et al., 1974). Fluke recovered in this study were predominately confined or trapped within dense thick-walled fibrotic capsules of the left lobe which minimised tissue damage in the right lobe. Similar fibrotic capsules have been reported in moose (*Alces alces*) and white-tailed deer infected with *Fascioloides magna* (Lankester, 1974; Vannatta, 2016).

Fibrotic lesions were denser in the farmed deer with concomitant atrophy of the left lobe. Being confined within a fluky habitat for up to 5 years, the farmed deer were at higher risk of recurrent exposure to liver fluke. The density of these lesions suggests recurrent exposure has increased tissue response over time, forming a physical barrier to migrating fluke. At site A, farmed deer also had a lower FEC despite having a similar fluke burden to the wild deer at this site. The dense fibrotic capsules in farmed deer also appear to restrict the normal flow and release of fluke eggs. Deer have no gall bladder and the release of fluke eggs into the small intestine would normally be unimpeded. Similar observations were reported in wild red deer surveyed in the Scottish Highlands (French et al., 2016). In the liver, fluke confined in

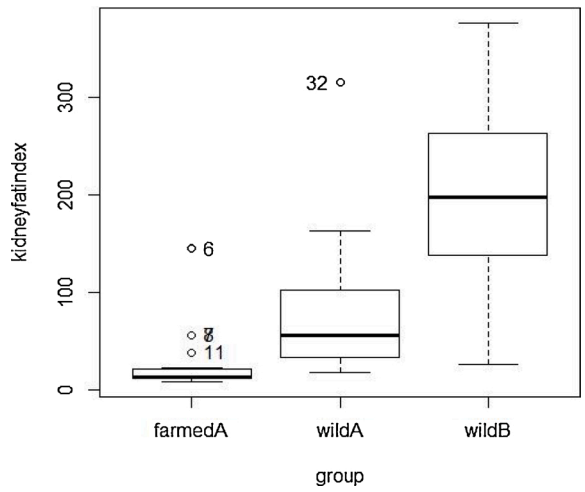

Fig. 6. KFI (%) by group.

"pockets" was thought to restrict egg flow contributing to the low FEC sensitivity observed in the study. Other deer species infested with liver fluke have also been detected with low FECs. Wild fallow deer (n = 66), red deer (n = 4) and sika (*Cervus nippon*) (n = 5) surveyed in Ireland had FECs ≤ 3 epg (O'Toole et al., 2014). These studies highlight that further research is warranted to ascertain how pathomorphological lesions of the liver influence diagnostic testing in deer. The low FECs detected in the fallow deer of this study however may also be attributed to daily fluctuations in egg shedding as faecal samples were collected at different times of the day (Valero et al., 2002). In addition, the duration of infection and immune response may also contribute to lower FECs and also needs to be considered.

Overall, the fallow deer had low fluke burdens including those farmed within a fluky habitat at a high stocking rate and with limited pasture. Reports of naturally acquired fluke burdens in fallow deer have been variable, ranging from 3 to 100 fluke (Vengušt et al., 2003; Jenkins et al., 2020). The fluke burdens of this study were also consistent with wild red deer in the Scottish Highlands which ranged from 1 to 13 fluke (French et al., 2016). The low numbers of fluke trapped within the peripheral fibrotic regions of the liver suggest a strong inherent resistance to fluke infestation. Furthermore, decaying fluke found within the fibrotic capsules provides further evidence of their ability to resist infections. Further investigations however are required to establish what level of infection (trickle or bolus) generates this strong tissue response and how quickly fallow deer can resolve infections. Expanding investigations to encompass other organs may also be warranted as the dense fibrotic lesions seen in the fallow deer may redirect migration and establishment. Migration of immature fluke through the diaphragm to the thoracic cavity of artificially infected white-tailed deer during the intraperitoneal migration phase has been reported (Presidente et al., 1974, 1975).

Liver fluke had low pathogenicity in the fallow deer of this study based on fluke burden and liver pathology. Interestingly, no immature fluke were recovered from livers and all fluke had reached the adult development stage and were shedding eggs with faeces. Fluke typically migrate throughout the liver parenchyma for 6–7 weeks and then enter the bile ducts to mature into adult fluke (Boray, 2017). In this study, adult fluke were predominately confined within fibrotic capsules of the peripheral parenchyma suggesting tissue response in the liver effectively traps fluke before migration to the major bile ducts. Fluke weight and width did not differ between wild and farmed deer infections, however fluke length was significantly longer in the farmed deer. We can only speculate that farmed deer were exposed to a greater fluke challenge. This may have reduced their ability to resolve infections as quickly, and hence, fluke may be older in age attributing to the longer fluke length. Other factors however can also influence fluke size such as immune

Table 5
Mean KFI (%) of farmed and wild deer by group and sex ± standard deviation.

KFI (%)	Farmed A	Wild A	Wild B	p-value
Season sampled	Jul 2019	Mar 2019 - Jun 2020	Sept 2020	
Group	25.8^{a}± 32.2	78.8^{b}± 70.3	188.2^{c}± 89.8	0.001
Female	35.8^{a}± 53.3	94.8^{a}± 78.0	210.4^{b}± 72.0	0.001
Male	21.2^{a}± 17.3	49.0^{b}± 43.6	32.7^{ab}± 8.3	0.0113
Fluke prevalence (active infections) (%)	57.9 (11 of 19)	15.0 (3 of 20)	2.5 (1 of 40)	

[a],[b] Rows with different superscripts are significantly different at $p < 0.05$.

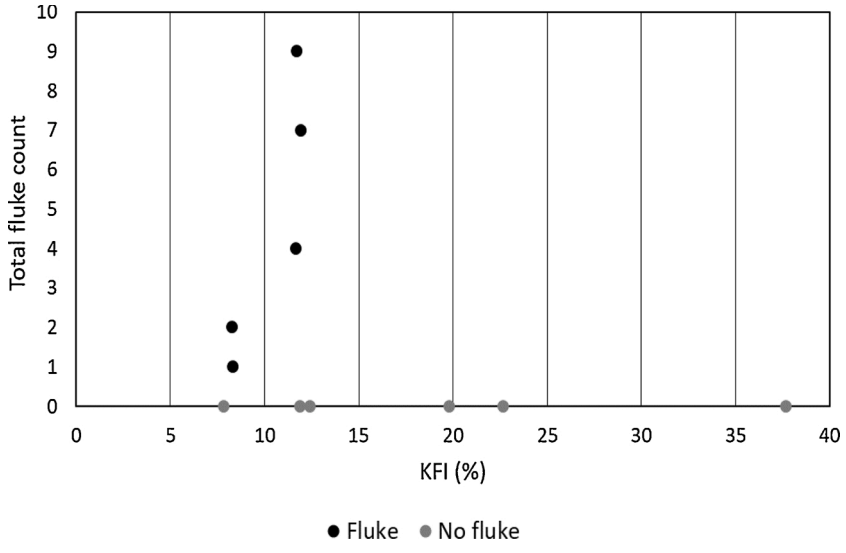

Fig. 7. KFI (%) vs TFC of farmed male deer euthanised in June 2019.

response, host species, number of fluke and crowding effect which need to be considered (Kistner and Koller, 1975; Muro et al., 1997; Valero et al., 2001, 2006).

Despite all deer appearing visually healthy, KFI varied between deer. The low KFI in farmed deer would largely be a result of their restricted grazing area whilst being maintained at a high stocking rate. KFI is acknowledged as a valuable indicator of body condition with fat reserves varying by season and sex (Riney, 1955; Caughley, 1970; Kistner et al., 1980). Deer typically deposit body fat throughout summer and autumn when food is usually abundant and utilise fat reserves throughout the winter months (Kistner et al., 1980). As deer were of mixed age and sampled over different seasons from different habitats, it is difficult to conclude any impact of parasite burden on KFI. Nevertheless, infected male deer harvested at site A had a lower KFI than male deer with no fluke suggesting fluke burden may have influenced KFI. Liver weights were also lower in farmed deer which may also be attributed to the limited diet as a result of the high stocking rate. However we cannot rule out lower liver weights as being a result of recurrent fluke infections. The marked atrophy of the liver and dense fibrotic lesions in 84.2 % of farmed deer supports this notion.

Artificial infections conducted by Kistner and Koller (1975) showed that liver fluke originating from deer are highly infective in sheep. In this study, fallow deer cohabiting with livestock were shedding fluke eggs on pasture, facilitating the liver fluke life cycle in this region. Nevertheless, egg shedding from fallow deer may be at a lower level relative to domestic livestock or other feral species. Furthermore, fallow deer cohabiting the grazing environment may appear clinically healthy with infections unnoticed while domestic livestock would be at a greater risk of developing clinical signs of infection. In order to establish impact of deer on disease transmission and make comparable analyses between studies and deer populations, sampling time is critical. Equally, the inherent ability of fallow deer to resolve infections may present misleading results regarding true fluke burdens.

6. Conclusion

This study has shown that farmed and free-roaming wild fallow deer within this livestock grazing region are a definitive host for liver fluke. There is strong evidence that fallow deer have a level of resistance to liver fluke and are capable of resolving infections. Recurrent exposure to liver fluke, generating dense fibrotic lesions and capsules, may also reduce egg shedding requiring further investigations. Gross pathomorphological lesions in the liver clearly limit tissue damage during the migratory phase of infestation. As deer are widespread throughout this region, future monitoring of prevalence levels during regulated culling programmes could provide further insight to the role deer play as reservoirs for liver fluke and risks to dispersal when cohabiting the livestock grazing environment.

Author statement

Jane Lamb – Conceptualisation, study design, field investigation, sample collection, post mortem assessments, data analysis and writing.

Emma Doyle – Review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge and thank the land holders and their managers along with the NSW Government Local Land Services for providing access to deer for sampling. This work was supported by the Australian Commonwealth Government through the award of the Australian Government Research Training Program Scholarship.

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