Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12577

Prevalence of liver fluke infection in Irish horses and assessment of a serological test for diagnosis of equine fasciolosis

A. QUIGLEY*, M. SEKIYA, S. EGAN, A. WOLFE, C. NEGREDO and G. MULCAHY

School of Veterinary Medicine, University College Dublin, Ireland.

*Correspondence email: aoife.quigley@ucd.ie; Received: 16.7.15; Accepted: 27.3.16

Summary

Background: There is little information on the prevalence of *Fasciola hepatica* infection in the horse population in Ireland or the potential impact of fluke infection on animal health.
Objectives: To investigate *F. hepatica* infection in the Irish horse population and to assess the diagnostic potential of an indirect enzyme-linked immunosorbent assay (ELISA) based on the *F. hepatica* recombinant cathepsin L1 (CL1) antigen.
Study design: Cross-sectional abattoir survey of horses for liver fluke status.
Methods: Animals (n = 200) were examined at an abattoir between May 2013 and April 2014. Horses were graded *ante mortem* for body condition score. Blood and faeces were collected and livers were examined *post mortem* by gross morphology. A cohort (n = 35) of livers were also examined histologically. Haematology and blood biochemistry, including serum liver enzyme activities, were measured and faeces were sedimented for egg counts. Serum was assayed by indirect ELISA using a recombinant CL1.
Results: The prevalence of liver fluke infection was 9.5%. There was no correlation between liver fluke status and time of year, breed classification, age group, sex, body condition score, *ante mortem* assessment, strongyle infection status, serum liver enzyme activities or CL1 concentration. A comparison of the CL1 ELISA in horse sera compared with a reference standard diagnosis showed high specificity of 95.6% (95% confidence interval [CI] 91.5–98.0%), but low sensitivity of 42.1% (95% CI 20.2–66.5%).
Main limitations: This study is limited by its nature as an abattoir study, the relatively small number of animals examined (n = 200), and the absence of a known negative group of horses.
Conclusions: Blood biomarkers are not good indicators of liver fluke infection and the CL1 ELISA is not a sensitive tool for diagnosis of fluke infection in the horse. The prevalence of *F. hepatica* in horses indicates that further research is required to assess the potential impact of liver fluke on equine liver health.

Keywords: horse; *Fasciola hepatica*; liver fluke; ELISA

Introduction

Fasciola hepatica is a common trematode parasite of livestock in Ireland, causing significant economic losses and affecting animal welfare. The parasite has long been known to infect horses [1], but its impact on the health of infected horses is poorly understood. The few reports on the prevalence of liver fluke in horses come from regions where the parasite is endemic in ruminants [2,3]. *F. hepatica* infection in horses may be under-reported as there is currently no rapid and reliable method of diagnosis. Definitive diagnosis relies on faecal egg counts (FECs), which have low diagnostic sensitivity, detecting only 60–70% of patent infections, or *post mortem* identification [1,4,5].

Liver fluke is sometimes reported incidentally at *post mortem* examination in horses. However, there are also anecdotal reports of horses that exhibit weight loss and diarrhoea and fail to respond to routine anthelmintics but appear to respond to flukicide treatment. There is a lack of evidence in these cases and more research in the area is required.

The objectives of this study were to assess the prevalence of *F. hepatica* in Irish horses and to assess the utility of an indirect enzyme-linked immunosorbent assay (ELISA) based on the *F. hepatica* recombinant cathepsin L1 (CL1) antigen for diagnosis of infected animals. Haematological, biochemical and faecal analyses were carried out in 200 animals. The definitive diagnosis of fluke infection was based on either positive identification of fluke in the liver or on FEC. In ruminant sera, individual and bulk milk, the CL1 ELISA has very high sensitivity and specificity. However, little is known about the humoral immune response of horses to *F. hepatica* or the pathogenesis and pathology of fasciolosis in horses.

Materials and methods

Experiment design

Ante mortem data, *post mortem* tissues, faeces and blood samples were collected from 200 horses slaughtered at an abattoir in Thomastown, County Kilkenny between May 2013 and April 2014.

The horses were chosen at random from those presented for slaughter throughout the testing period and included a variety of breeds, ages and sexes (Supplementary Item 1).

Ante mortem examination and score

Each horse was examined *ante mortem* and age, breed, body condition and signs of disease were recorded. The horses were categorised into 4 groups: *Group 1* included horses in recent work (shod or recently clipped); *Group 2* included horses with findings of neutral or no clinical significance (i.e. fresh skin abrasions, serous nasal discharge, etc.); *Group 3* included unkempt horses in which findings were consistent with a chronic underlying condition (i.e. rainscald, ringworm, pot belly, etc.), and *Group 4* included horses with signs of current disease (i.e. mucopurulent nasal discharge, diarrhoea).

Post mortem examination

Each liver was examined visually and by palpation. Livers were sliced at approximately 1 cm intervals through all lobes and the cross-sections were examined for the presence of adult fluke, thickening of bile ducts and alterations in bile colour, consistency or volume. Livers were graded from 1 to 6 (Table 1). *Grade 1* indicated that no pathology was seen. *Grade 2* indicated findings of mild, nonspecific liver changes. *Grades 3*, *4* and *5* indicated, respectively, mild, moderate and marked changes in bile duct

TABLE 1: Grading of livers at *post mortem* examination in an abattoir survey of fluke status in 200 Irish horses

Grade	Pathology seen	Horses, n
1	None	34
2	Mild, nonspecific liver changes	50
3	Mild bile duct thickening/calcification/increased bile viscosity	71
4	Moderate bile duct thickening/calcification/increased bile viscosity	21
5	Marked bile duct thickening/calcification/increased bile viscosity	8
6	Fluke found in liver	16

thickening, calcification and increased bile viscosity. Finally, *grade 6* indicated findings of fluke in the liver.

Two tissue samples containing a section of bile duct and adjacent liver parenchyma (1 cm^3 each) were taken from both the left and right lobes of each liver and preserved in 10% formalin.

Animals were classed as positive if adult fluke were seen at *post mortem* examination, eggs were found during faecal sedimentation, or adults or juveniles were seen on histology. In the absence of adults, juveniles or eggs, livers were classed as negative for liver fluke regardless of other morphological changes.

Histological examination

Liver samples were examined from 35 animals ranging in age from 4 to 23 years, and including 15 females and 20 males. These livers were divided into 3 categories: positive (adult fluke found on *post mortem* examination or fluke eggs found on sedimentation); suspect (showing gross *post mortem* changes consistent with fluke infection, but no fluke found *post mortem*, and negative on FEC), and negative (showing no *ante mortem*, *post mortem*, haematological, biochemical or faecal evidence of fluke infection). All livers that showed positive findings for fluke on gross examination *post mortem* were included in the subset for histopathological examination.

Tissue samples were fixed in 10% neutral buffered formalin for at least 48 h, embedded in paraffin wax, cut into 4 μm sections and stained with haematoxylin and eosin (H&E).

Histopathological changes in the bile ducts were characterised according to 3 criteria: 1) amount of peri-ductular fibrosis; 2) amount of peri-ductular cellular infiltrates, and 3) hyperplastic changes in the biliary epithelium. Each of the 3 criteria were graded on a scale of 0–3, where 0 = no changes, 1 = mild changes, 2 = moderate changes and 3 = marked changes. Thus each liver sample was scored for each of the 3 criteria to generate a cumulative score where 0 (no changes) represented the lowest possible score and 9 represented the highest possible score. Overall, scores of 1–4 were deemed to represent mild changes, scores of 5–7 were considered to indicate moderate changes, and scores of 8 or 9 were deemed to indicate marked changes.

Blood sampling

All horses were processed in the manner regulated at the abattoir. Blood samples were collected at the commencement of the exsanguination process to reduce stress to the horse using Vacutainers with no anticoagulant. Vacutainers were centrifuged at 5000 ***g*** for 10 min and supernatant was aliquoted and stored at -80°C until assay. Complete blood counts were analysed using a Siemens ADVIA 2120 haematology analyser[a]. Biochemical markers (total protein, albumin, globulin, γ-glutamyltrasferase [GGT] and glutamate dehydrogenase [GLDH]) were measured using a Randox RX Imola analyser[b]. A cohort of samples (n = 25), which included all positive animals, were also tested for bile acid levels using the Randox analyser[b].

Faecal sampling

Faecal samples were taken from the rectum *post mortem* and FECs for nematodes were determined using the McMaster technique [4]. *F. hepatica* eggs were quantified by sedimentation [4] using 3 g of faeces per animal. Faecal coproantigen testing was carried out on a number of samples (n = 42). These samples were frozen at -20°C within 24 h of collection and stored until assay. The samples were tested using a Bio-X-Bovine *Fasciola hepatica* Antigen ELISA[c] according to the manufacturer's instructions.

Indirect serum ELISA for horses

The 200 serum samples were run in duplicate on an in-house ELISA based on a recombinant version of the major *F. hepatica* excretory secretion protein (E/S), CL1. Plates were coated with recombinant mutant CL1 [6] at a concentration of 1 μg/ml in 50 mmol/l carbonate coating buffer and incubated for 1 h at 37°C. Columns were alternately coated with antigen or with buffer only to provide a background control. Plates were washed 3 times with phosphate-buffered saline with Tween 20 (PBST) (this was repeated after each incubation step). Plates were blocked with 5% milk powder[d] in PBST at 100 μl per well, and incubated for 1 h at 37°C. Sera were diluted 1:100 in 2% milk powder in PBST, and added in quantities of 100 μl per well, in duplicate, and incubated for 1 h at 37°C. Conjugate anti-horse immunoglobulin Gt (IgGt)[e] was diluted 1:20,000 in 2% milk powder in PBST, added at 100 μl per well, and incubated for 1 h at 37°C. 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate[d] was added, at 100 μl per well, and plates were allowed to develop for 10 min and then stopped with 1 mol/l H_2SO_4, at 100 μl per well. Plates were read on a Dynamica LEDetect plate reader[f] and corrected optical densities (ODs) were calculated by subtracting the background OD from the reading of the antigen-coated well. The cut-off value for the single endpoint test was determined to be 0.15 at OD 450 nm. A panel of reference standard positive sera from horses positive for faecal eggs (n = 6) and reference standard negative sera from horses negative for faecal eggs and historically free of liver fluke (n = 28) was used to develop the cut-off, which was the mean $\pm$2 s.d. of the negative sera. Cut-of values were verified using horse sera generously gifted by Professor Diana Williams, University of Liverpool.

Data analysis

Statistical analysis was performed to: 1) determine the associations between fluke infection status and various environmental and subjective health indicators, and 2) assess the effects of parasitic infection on various blood haematological and biochemical variables.

Preliminary steps established the stability of the variance for each of the continuous variables; where normality was not identified, a transformation step was applied. In this case, a square root transformation was applied to the variables white blood cell (WBC) count, neutrophil count, GGT and GLDH, a sqrt transformation was applied to Hct and a log (FEC + 1) transformation was applied to strongyle FECs.

A Chi-square goodness-of-fit (Pearson's) test was applied to test the individual relationships between fluke infection status and the independent categorical variables of date, age, sex, body condition, breed, *ante mortem* status and strongyle infection status. Where a putative relationship was identified (based on $P \leq 0.15$), a linear regression was modelled with that independent variable to describe the association.

To assess and describe the combined effects of independent variables on each of the blood haematological and biochemical measurements, manual backward elimination stepwise regression procedures were utilised. In total, 10 models were developed for blood parameters Hct, WBC count, neutrophil count, eosinophil count, GGT, GLDH, total protein, albumin, globulin and A/G ratio. Assessment of residual normality was determined using standardised normal probability plots.

Data management and graphical representations were completed using Microsoft Excel Version MSO 365 (16.0.6729.1019)[g]. All statistical analysis was performed using Stata/SE Version 12.1[h]. P values of ≤ 0.05 were considered to indicate statistical significance.

Results

Prevalence of liver fluke

In total, 9.5% (19/200) of the horses examined were positive for *F. hepatica* infection. Of these, 12 were positive for adult fluke on *post mortem* examination. Four were positive for both adult fluke on *post mortem*

examination and FEC, 2 were positive on FEC only, and one juvenile showed fluke on histological examination.

Ante mortem examination and score

The average age of the 200 horses examined was 11 years and the average body condition score was 2.4/5. The average age and body condition score of the cohort of horses positive for *F. hepatica* were 10.6 years and 2.3/5, respectively. No sex or breed predilection was noted; 7.6% of females and 10.4% of males were positive for fluke and infection was spread fairly evenly across the various breeds represented (7.7% of sport horses, 10.6% of Thoroughbreds, 11.1% of ponies, 12.5% of Cobs).

Of the 19 positive horses, 15 were categorised as belonging to *Groups 2* (neutral) or *3* (unkempt). None showed evidence of recent work and only 4 showed signs of current disease, all of which were nonspecific clinical signs (scour and mucopurulent nasal discharge).

Post mortem examination

In the 19 positive horses, liver examination revealed *grade 6* changes (adult fluke found) on gross examination in 16 cases. One of the remaining 3 positive livers showed *grade 5* changes and histopathological examination revealed the presence of a juvenile fluke. The final 2 positive horses showed no gross changes of the liver, but were positive for fluke eggs on faecal sedimentation. A further 5 horses had *grade 5* changes, which correlate to pathological findings that could indicate liver damage attributable to fluke (Fig 1 and Table 1).

Bile varied in colour from cream to brown, and mineralisation was noted in the walls of some bile ducts. A number of the livers were enlarged, with rounded edges, and texture ranged from normal to rubbery to firm. A few of the livers were dark-coloured and displayed gritty parasitic tracts throughout the hepatic parenchyma. The number of fluke harvested from positive livers ranged from 3 to 30.

Histological examination

In the 18 livers positive for the presence of adult liver fluke at gross examination or on FEC, the mean histological score was 6.5/9 (Fig 2). In 6 livers judged to be negative for adult liver fluke but showing changes suspicious for the presence of liver fluke, the mean histological score was 7.4/9. This group included the one liver in which a juvenile fluke was seen on histological examination. Finally, 11 livers that were judged to be negative for gross changes consistent with liver fluke had a mean histological score of 2.2/9.

Blood biomarkers

Fluke status was not significantly associated with any of the blood parameters measured. Eosinophils, total protein, globulin and A/G ratio were not affected by the presence of fluke, but all were significantly associated with strongyle burden.

Bile acids were analysed in a total of 25 horses, including 17 *F. hepatica*-positive animals. All samples were found to be within normal limits. There was no significant difference between the positive and negative horse samples.

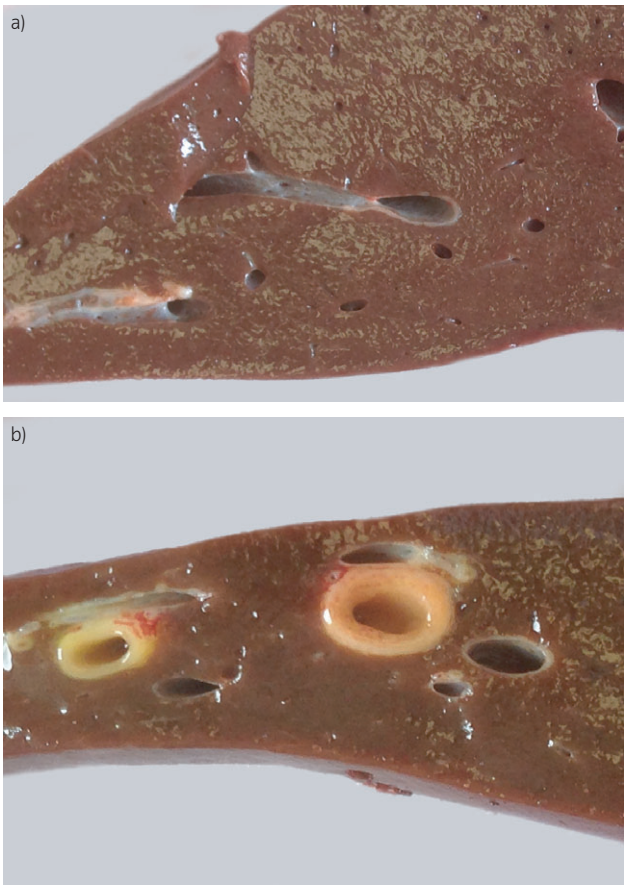

Fig 1: *Post mortem* gross examination of liver. Image a) shows normal liver. Image b) shows liver with significantly thickened bile ducts (*grade 5*).

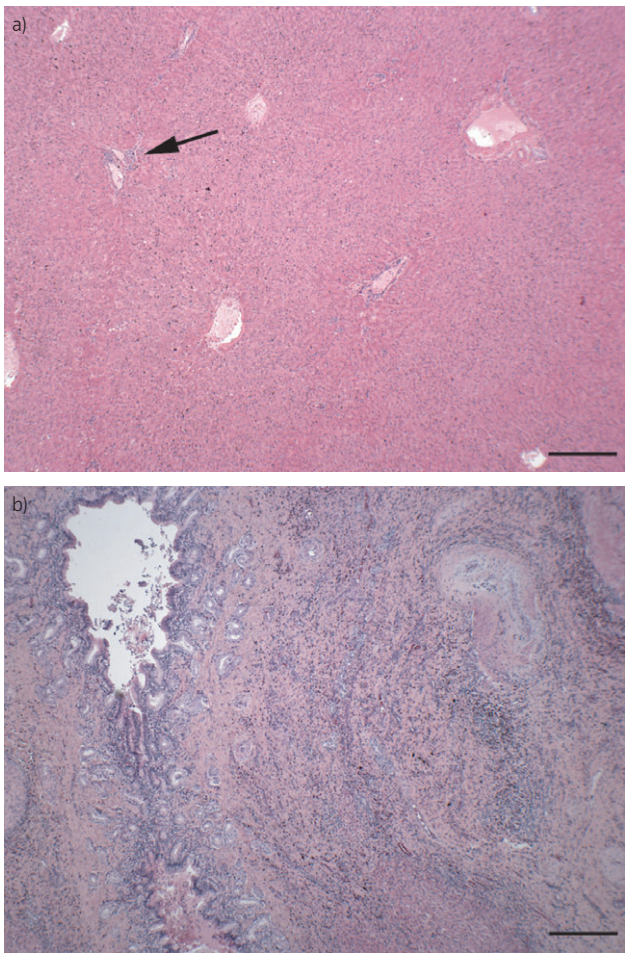

Fig 2: Histological liver examination. Image a) shows a section of normal liver with a normal portal triad area including bile duct (arrow). Image b) shows a section of liver with severe changes (*grade 9*), including a hyperplastic biliary epithelium with peri-ductular fibrosis and cellular infiltrates (arrow). (Haematoxylin and eosin stain; original magnification ×40.)

Faecal sampling

Six horses were positive for *F. hepatica* eggs on faecal sedimentation. Four of these horses were also found to have adult liver fluke on gross *post mortem* examination.

All of the 42 horses examined for liver fluke antigen using the faecal coproantigen test were found to be negative. This cohort included 6 animals found to be positive for fluke in gross *post mortem* examination, 2 of which were also positive for *F. hepatica* eggs on faecal sedimentation.

Recombinant CL1 ELISA

The cut-off value for the single endpoint recombinant CL1 ELISA was determined to be 0.15 at OD 450 nm. The 200 horse sera were run in duplicate on the ELISA. The comparison of OD values with fluke status (*post mortem* findings or FEC) led to sensitivity and specificity of 42.1% (95% confidence interval [CI] 20.2–66.5%) and 95.6% (95% CI 91.5–98.0%), respectively (Supplementary Item 2).

Fluke status

Fluke status was not associated with any of the categorical variables tested (Supplementary Item 3). However, strongyle infection resulted in increases in eosinophil count, total protein, globulin and A/G ratio (Supplementary Item 4). No associations were seen between strongyles and age, sex, body condition, breed or *ante mortem* score. There were significant associations between blood parameters and body condition and *ante mortem* examination score (Supplementary Item 4). An increase in body condition score was associated with increases in all blood parameters except WBC and neutrophil counts, both of which were decreased in these animals. Animals with higher *ante mortem* scores (the more diseased animals) had decreased serum albumin concentrations, whereas those with lower *ante mortem* scores (the healthier animals) had increased protein and globulin concentrations (Supplementary Item 4). The standardised normal probability plot indicated satisfactory agreement with normality in all cases.

Discussion

This is the first abattoir survey of liver fluke in Irish horses. The prevalence of liver fluke infection was 9.5% during the period of May 2013 to April 2014. The horses sampled represent a relatively balanced range of breed, age and sex; however, the nature of the study and the availability of horses at the abattoir preclude any interpretation of the statistics as definitive and representative. Reports on the prevalence of *F. hepatica* in horses vary widely according to geographical region and the sensitivity of the diagnostic assay. In northwest Spain, where liver fluke is endemic, 60% of horses tested positive on an indirect ELISA based on the recombinant *F. hepatica* 2.9-kDa antigen, although FECs were negative in all animals [3]. In the same region, prevalences of fasciolosis in ruminants ranged from 28 to 65% as determined by FECs and necropsy [7], whereas seroprevalences in ruminants ranged between 65 and 92% [7,8]. In the Black Sea region of Turkey, seroprevalence was found to be 18% using an indirect ELISA with *F. hepatica* E/S as the capture antigen, whereas the coprological prevalence was 11.5% [2,9]. In the Brandenburg region of Germany, a coprological study in horses found no evidence of liver fluke infection, and another study conducted in the same region found a relatively low prevalence of 7% in cattle [10,11]. In Ireland, recent data from bulk milk surveys indicate the prevalence of liver fluke in cattle to be 82% [12], and a 2006 abattoir study recorded the prevalence of liver fluke in cattle to be 65% [13]. Taken together, these data suggest there is abundant habitat for liver fluke in many regions, but this is not reflected in a high prevalence of liver fluke in horses. Although there are potentially many reasons for differences in prevalences of liver fluke between ruminants and horses, we suspect that in Ireland, where liver fluke is pervasive, the exposure of Irish horses to the parasite may be much higher than the 9.5% prevalence in horses reported here.

The higher prevalence of infection in cattle than in horses suggests that horses are more resistant to *F. hepatica* infection than ruminants [14,15]. Indeed, the lack of a gallbladder in the horse may account for a reduction in egg shedding in comparison with ruminants. However, in the present study, the finding of mature fluke in equine livers, the observation of *F. hepatica* eggs in faecal samples, and the fact that a homogenised fluke harvested from one horse yielded large numbers of eggs indicate that fluke can reach maturity and become patent in the horse.

There was no statistically significant correlation between liver fluke status and any of the environmental or *ante mortem* parameters examined. This may be attributable to our use of a relatively small number of animals in this study (n = 200). In addition, changes associated with liver fluke status may be undetected as infection with *F. hepatica* was not synchronous or controlled. Finally, no true known negative, or naïve, group of horses was included in this study.

Liver fluke status was not significantly associated with any of the blood parameters examined. Previous studies of infected horses showed that GLDH increased from 1 to 2 months post infection and peaked again at 4, 7 and 8 months post infection. GGT increased from 3 months post infection, peaked at 4 months and remained low thereafter [16]. In this study, the time of infection of the animals is unknown, and the lack of elevation of GGT and GLDH indicates that these biomarkers are of limited use in diagnosing liver fluke infection. This is not surprising because normal results on serological tests have been shown to be poor indicators of the absence of liver disease [17]. However, it should be noted that as this was an abattoir study, this group of horses does not represent a classical 'diseased' group, and as such could not be deemed to be showing any clinical signs specifically attributable to liver dysfunction.

The results of this study indicate that nematodes rather than trematodes appear to have a greater impact on blood results, and that eosinophil and globulin concentrations are seen to increase in animals with significant strongyle burdens (Supplementary Item 4).

Liver damage in horses is often cumulative; biochemical markers of liver damage are not seen in the blood until a minimum of 50% of the liver is damaged, and the horse will usually not show signs associated with liver failure until over 80% of the liver is damaged [18]. GGT is considered to be the most useful diagnostic enzyme for liver disease, as although sorbitol dehydrogenase (SDH) is more sensitive, it is difficult to measure. GGT levels increase with hepatobiliary disease and cholestasis, but may not be significantly increased in cases of chronic, nonactive fibrosis [18]. Bile acids are considered an excellent screen for liver insufficiency in the horse and have been shown to increase 6-fold in horses with ligated bile ducts [19]. We observed bile duct fibrosis and calcification in the livers of the fluke-positive horses, which appeared to cause blockage of some smaller bile ductules; however, no increases in either GGT activity or bile acids were seen. It is possible that the wide diameter of the larger bile ducts and the fact that bile is continuously secreted into the duodenum in the horse preclude any true blockage of the bile and therefore elevations commonly associated with cholestasis are not seen.

We were unable to determine the duration of infection in the current study or to verify whether any of the 'negative' cohort had been previously exposed to liver fluke. The liver and bile duct tissues have a limited number of potential responses to insult and hence it was impossible to categorically determine whether changes seen histologically in the 'negative' animals were caused by parasite migration or toxicoses, or represented age-related changes. As histological examination of liver tissue did not provide a definitive diagnosis of whether the changes seen reflected the presence of fluke, rather than other causes, the use of biopsies to evaluate the presence of *Fasciola* sp. infection in the horse is not recommended. As well as being unable to establish whether the changes seen reflected *Fasciola* sp. infection, the multifocal and scattered nature of such pathological changes indicates that biopsies will be very insensitive as indicators of fluke-related liver damage. Liver parenchyma immediately adjacent to areas of suspected liver fluke damage was frequently remarkably normal in appearance. It should also be noted that not all livers were examined histologically, and although one liver was deemed positive for fluke on the finding of a juvenile fluke on histology, it is possible that the prevalence of juvenile fluke, and therefore of positive horses, is higher than reported here.

The changes seen in this study on *post mortem* examination are consistent with those seen in chronic infections in cattle and manifest as dilated, thickened bile ducts and enlarged livers [20]. However, further studies using experimental infection are required to assess the degree of

damage caused by fluke in the horse and the timeline from infection to visible liver pathology.

Assessment of the CL1 ELISA as a diagnostic tool for the detection of *F. hepatica* infection in the horse showed that the ELISA has high specificity but low sensitivity. This was surprising because our earlier findings showed the CL1 ELISA to have both high sensitivity and specificity in ruminant serum and milk samples [21]. However, sensitivity and specificity are dependent on the population in which the assay is evaluated and are not fixed properties of the test itself [22], which may account for some of the differences we report. The liver fluke 2.9-kDa antigen ELISA described by Arias and co-workers [3] appears to have high sensitivity and specificity when applied to horse sera, which may be attributable to the more potent antibody response raised against this small antigen. We found the coproantigen ELISA was also not suitable for use as a diagnostic in horses, which is in agreement with the results of a study conducted in Western Australia [23]. These findings raise questions about the differences in the humoral immune response to liver fluke infection between horses and ruminants and will be further investigated.

It is difficult to assess the impacts of fluke-associated liver damage on equine health, performance and welfare. These infections are largely subclinical in nature and therefore go undiagnosed and untreated. The liver is involved in many vital functions, including metabolism, nutrient absorption and storage, and bile excretion. The liver and bile duct fibrosis caused as a result of fluke infection in the animals examined in this study is irreversible and therefore it is likely that horses previously affected with fluke that encounter another insult to the liver (ragwort, fatty liver, etc.) are in a higher risk category than those without pre-existing fibrosis. This fibrosis in an organ responsible for metabolism and protein synthesis is likely to contribute to cumulative liver insufficiency, which, in turn, may impact on performance and indeed welfare.

Conclusions

The finding of a 9.5% prevalence of liver fluke in horses during the period of this study indicates that *F. hepatica* may be a relevant factor in equine health and welfare in Ireland. Environmental and *ante mortem* factors showed no statistical association with fluke infection in this study. During this study, blood biomarkers, GLDH, GGT and bile acid concentration were not useful for diagnosis of infection, and the CL1 ELISA was not a sensitive diagnostic assay for fluke infection. Further research is required to assess the haematological, biochemical and immunological response of the naïve horse to fluke infection.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight was not required; the study was performed on material collected at an abattoir.

Sources of funding

This research was supported by seed funding from University College Dublin.

Acknowledgements

The authors would like to acknowledge the assistance of the laboratory staff at University College Dublin, the veterinary inspectors and staff at B&F Meats Ltd (Thomastown, Ireland), and Gearoid Sayers of Epsilon Ltd (Cork, Ireland) for statistical assistance. We also gratefully acknowledge the gift of sera from Professor Diana Williams, University of Liverpool (Liverpool, UK). We gratefully acknowledge Professor John Dalton of Queens University, Belfast for the gift of the recombinant CL1.

Authorship

A. Quigley, M. Sekiya and G. Mulcahy were responsible for the conception and design of the study, data analysis and the preparation of the manuscript. C. Negredo, S. Egan and A. Quigley were involved in the acquisition and analysis of data. S. Egan and A. Wolfe were involved in the analysis and interpretation of data. All authors approved the final version of the manuscript.

Manufacturers' addresses

[a]Siemens Healthcare GmbH, Erlangens, Germany.
[b]Randox Laboratories Ltd, London, UK.
[c]Bio-X Diagnostics Sprl, Jemelle, Belgium.
[d]Sigma-Aldrich Ireland Ltd, Arklow, Ireland.
[e]AbD Serotec Ltd, Oxford, UK.
[f]Dynamica Treuhand GmbH, Uster, Switzerland.
[g]Microsoft Corp., Redmond, Washington, USA.
[h]StataCorp LP, College Station, Texas, USA.

References

1. Owen, J.M.M. (1977) Liver fluke infection in horses and ponies. *Equine Vet. J.* **9**, 29-31.
2. Acici, M., Bolukbas, C.S., Gurler, A.T., Umur, S. and Buyuktanir, O. (2013) Seroprevalence of fasciolosis in equines of the Black Sea region, Turkey. *J. Equine Vet. Sci.* **33**, 62-66.
3. Arias, M.S., Piñeiro, P., Hillyer, G.V., Francisco, I., Cazapal-Monteiro, C.F., Suárez, J.L., Morrondo, P., Sánchez-Andrade, R. and Paz-Silva, A. (2012) Enzyme-linked immunosorbent assays for the detection of equine antibodies specific to a recombinant *Fasciola hepatica* surface antigen in an endemic area. *Parasitol. Res.* **110**, 1001-1007.
4. Taylor, M.A., Coop, R.L. and Wall, R.L. (2007) *Veterinary Parasitology*, 3rd edn., Blackwell Publishing Ltd, Oxford.
5. Rapsch, C., Schweizer, G., Grimm, F., Kohler, L., Bauer, C., Deplazes, P., Braun, U. and Torgerson, P.R. (2006) Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. *Int. J. Parasitol.* **36**, 1153-1158.
6. Collins, P.R., Stack, C.M., O'Neill, S.M., Doyle, S., Ryan, T., Brennan, G.P., Mousley, A., Stewart, M., Maule, A.G., Dalton, J.P. and Donnelly, S. (2004) Cathepsin L1, the major protease involved in liver fluke (*Fasciola hepatica*) virulence: propetide cleavage sites and autoactivation of the zymogen secreted from gastrodermal cells. *J. Biol. Chem.* **279**, 17038-17046.
7. Sanchez Andrade, R., Paz-Silva, A., Suarez, J., Panadero, R., Pedreira, J., Lopez, J., Diez-Banos, C. and Morrondo, P. (2002) Influence of age and breed on natural bovine fasciolosis in an endemic area (Galicia, NW Spain). *Vet. Res. Commun.* **26**, 361-370.
8. Arias, M., Morrondo, P., Hillyer, G., Sanchezandrade, R., Suarez, J., Lomba, C., Pedreira, J., Diaz, P., Diezbanos, P. and Pazsilva, A. (2007) Immunodiagnosis of current fasciolosis in sheep naturally exposed to *Fasciola hepatica* by using a 2.9 kDa recombinant protein. *Vet. Parasitol.* **146**, 46-49.
9. Umur, S. and Açıcı, M. (2009) A survey on helminth infections of equines in the central Black Sea region, Turkey. *Turkish J. Vet. Anim. Sci.* **33**, 373-378.
10. Hinney, B., Wirtherle, N.C., Kyule, M., Miethe, N., Zessin, K.-H. and Clausen, P.-H. (2011) Prevalence of helminths in horses in the state of Brandenburg, Germany. *Parasitol. Res.* **108**, 1083-1091.
11. Kuerpick, B., Schnieder, T. and Strube, C. (2012) Seasonal pattern of *Fasciola hepatica* antibodies in dairy herds in northern Germany. *Parasitol. Res.* **111**, 1085-1092.
12. Selemetas, N., Ducheyne, E., Phelan, P., O'Kiely, P., Hendrickx, G. and De Waal, T. (2015) Spatial analysis and risk mapping of *Fasciola hepatica* infection in dairy herds in Ireland. *Geospat. Health* **9**, 281-291.
13. Murphy, T.M., Fahy, K.N., Mcauliffe, A., Forbes, A.B. and Clegg, T.A. (2006) A study of helminth parasites in culled cows from Ireland. *Prev. Vet. Med.* **76**, 1-10.
14. Boray, J.C. (1969) Experimental fascioliasis in Australia. *Adv. Parasitol.* **7**, 96-204.
15. Nansen, P., Andersen, S. and Hesselholt, M. (1975) Experimental infection of the horse with *Fasciola hepatica*. *Exp. Parasitol.* **37**, 15-19.

16. Soulé, C., Boulard, C., Levieux, D., Barnouin, J. and Plateau, E. (1989) Experimental equine fascioliasis: evolution of serologic, enzymatic and parasitic parameters. *Ann. Rech. Vet.* **20**, 295-307.
17. Durham, A.E., Smith, K.C. and Newton, J.R. (2003) An evaluation of diagnostic data in comparison to the results of liver biopsies in mature horses. *Equine Vet. J.* **35**, 554-559.
18. Barton, M.H. (2010) Disorders of the liver. In: *Equine Internal Medicine*, 3rd edn., Eds: S.M. Reed, W.M. Bayly and D.C. Sellon, Saunders, St Louis. pp 939-975.
19. Hoffman, W., Baker, G., Rieser, S. and Dorner, J.L. (1987) Alterations in selected biochemical constituents in equids after induced hepatic disease. *Am. J. Vet. Res.* **48**, 1343-1347.
20. Affroze, M., Begum, N., Islam, M.S., Rony, S.A., Islam, M.A. and Mondal, M.M.H. (2013) Risk factors and gross pathology of bovine liver fluke infection at Netrokona District, Bangladesh. *J. Anim. Sci. Adv.* **3**, 83-90.
21. Golden, O., Flynn, R.J., Read, C., Sekiya, M., Donnelly, S.M., Stack, C., Dalton, J.P. and Mulcahy, G. (2010) Protection of cattle against a natural infection of *Fasciola hepatica* by vaccination with recombinant cathepsin L1 (rFhCL1). *Vaccine* **28**, 5551-5557.
22. Leeflang, M.M.G. and Bossuyt, P. (2005) Test accuracy is likely to vary depending on the population it is used in. *Vet. Parasitol.* **134**, 189.
23. Palmer, D.G., Lyon, J., Palmer, M.A. and 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.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Signalment of horses included in an abbatoir survey of fluke status in Irish horses.

Supplementary Item 2: CL1 ELISA results in horses with and without evidence of fluke infection in an abbatoir survey of 200 Irish horses.

Supplementary Item 3: Chi square analysis of environmental and ante-mortem variables with fluke status in an abbatoir survey of 200 Irish horses.

Supplementary Item 4: Significant associations between blood parameters and body condition score, ante mortem score and strongyle status in an abbatoir survey of 200 Irish horses.

.