

**Final grant report form: Norman Hayward Fund**

<b>PROJECT/STUDY TITLE:</b>	THE IMPACT OF LIVER FLUKE ON THE WELFARE OF HORSES
<b>PRINCIPAL APPLICANT(S)</b>	Prof Diana Williams
<b>GRANT AWARDED (DATE):</b>	19 <sup>th</sup> July 2016

**Lay summary of project outcomes, achievements and potential impact on animal welfare:**  
Max 300 words

A serological test to detect exposure to liver fluke was used to test 224 UK horses killed in an abattoir during 2017; 10% were found to have antibodies for liver fluke. Flukes isolated from the livers of infected horses were subjected to DNA analysis and found to be from the same population as flukes infecting sheep and cattle, indicating that horses are at risk if they share grazing with fluke-infected ruminants.

A case control study of 269 horses from across Great Britain found that horses with liver fluke are about 6 times more likely to have suspected liver disease than uninfected horses. A cross section of ages and breeds were included, ranging from pet ponies to race- and competition-horses.

Information was obtained from a small number of infected horses; clinical signs were typically non-specific and included inappetence, lethargy and dullness. There were no consistent blood test abnormalities, with about half of the horses exhibiting raised liver enzymes, and none had eosinophilia or anaemia. Sixty-four% of infected horses had a history of co-grazing with ruminants indicating that this should be considered as an important risk factor.

These results indicate that liver fluke is a cause of liver disease and should be considered as a differential diagnosis in horses exhibiting clinical signs or blood test abnormalities consistent with liver disease, particularly where horses have grazed with ruminants. Fluke infected horses have been found across the UK.

Direct communication was made with over 80 veterinary surgeons and 120 horse owners during the study, leading to many interesting discussions about clinical histories and risk factors associated with liver fluke in horses. In response to requests for information and treatment advice, a leaflet was produced for distribution to veterinary surgeons. A veterinary CPD event was organized to share knowledge.

Information from this study is of direct benefit to animal welfare because it confirms that liver fluke is an important parasite of horses and is responsible for a proportion of cases of liver disease of unknown cause. This supports the need for diagnostic testing and treatment in horses deemed to be at risk. Further dissemination of results is planned, to include an academic journal and article in the veterinary press.

**Detailed progress against original objectives:** List outcomes against original objectives. Discuss what has been achieved, including any statistical analysis completed as part of the project.

1. An abattoir study to establish the prevalence of infection in horses at slaughter, to determine what proportion of fluke reach maturity and lay eggs and to molecularly define these fluke populations to establish if they are similar to those infecting sheep and cattle in the UK.

Over the course of the year, nine abattoir visits were made and a total of 315 horses were examined of which 224 horses were from England and Wales. For horses originating from Britain, sero-prevalence was estimated to be 10.2% (95% CI 5.3%-17.1%) by ELISA, and 1.8% of horses had adult flukes in the liver.

At the abattoir, it was not practical to further dissect livers to establish if immature fluke were present in each horse and serological results were only obtained once the serum was taken back to the laboratory and processed. This meant it was not possible to estimate what proportion of

fluke reach maturity. Molecular analysis of 123 flukes from nine horses, including four animals from this study, was performed. Based on analysis of 15 microsatellite loci amplified by PCR and analysed by capillary sequencing compared with results from 1579 flukes from sheep and cattle, we showed that 96% of alleles were shared between fluke isolated from all three hosts.  $FST = 0.025$  indicating that genes are able to flow freely between fluke infecting sheep, cattle and horses, hence there is no evidence that a separate fluke population exists specific to horses.

2. A case control study will be conducted, where cases are patients with liver disease and controls will be horses receiving veterinary care for other reasons and from which a blood sample has been taken.

109 cases (horses with suspected liver disease – clinical signs and/or blood test results consistent with liver disease) and 160 controls (horses without liver disease – GGT and GLDH within normal limits) were included in the study. Signalment data and geographical origin were compared between the cases and controls. The locations of origin were different for the two groups but all other parameters including age, breed, sex etc, were similar. The analysis showed that horses with liver fluke were significantly more likely to have suspected liver disease (OR 6.4, 95% CI 1-6-25.7), confirming our hypothesis that liver fluke is a potential cause of unexplained liver disease.

3. A logistic regression analysis of clinical data to identify clinical correlates of infection.

Insufficient data were collected from fluke positive horses, hence a full logistical regression analysis was not possible. However descriptive statistics were used and the results showed that (i) reported clinical signs were typically non-specific and included inappetence, lethargy and dullness; (ii) there were no consistent haematological or biochemical abnormalities, with about half the positive horses exhibiting raised liver enzymes, and none having either eosinophilia or anaemia.

4. A knowledge exchange programme to inform Veterinary Surgeons and horse owners about control of fluke infection.

A Facebook page and a Twitter account were set up to raise awareness of the study, to aid in recruiting patients and to feedback information on prevalence, diagnosis and treatment of fasciolosis in horses. A leaflet was prepared and sent to all the participating practices (~140 practices), Norbrook's representatives also distributed copies to practices they visited. A CPD event for vets was held at the University of Liverpool and the results of the project presented at conferences, namely the World Association for the Advancement of Veterinary Parasitology (Kuala Lumpur, September 2017) and the British Society for Parasitology (Aberystwyth, April 2018).

Further details of the results from the project are in the attached draft manuscript.

**Were there any challenges or barriers/modifications to the project?** Explain the nature of and reasons for any changes in project focus, scope, delivery, schedule or evaluation.

Due to restrictions at the abattoir, it was only possible to examine the surface of the livers unless these had already been condemned. This probably contributed to low numbers of liver fluke being found in the livers.

Too few positive cases meant we were unable to complete the logistic regression analysis but descriptive statistics were used instead.

Anecdotally, vets in practices in liver fluke endemic areas, especially Lancashire, South Wales, said they were familiar with and were routinely treating horses with suspected fluke infections.

**Provide details of knowledge transfer activities to date and any future plans/actions.**

<p>Publicity and awareness of the study led to an increased awareness of liver fluke in horses led to many interactions with vets and horse owners during the study and we received numerous requests for advice and information. Leaflets with basic advice and treatment options for liver fluke were produced. A CPD talk for vets and presentations at two parasitology conferences (WAAVP and BSP) were made. Twitter and Facebook were used for public engagement during the study.</p>
<p><b>Provide details of any original peer-reviewed research papers, book chapters and books/monographs that have resulted directly from your work supported by this grant.</b></p>
<p>A manuscript is in preparation for publication.</p>
<p><b>Have any other funding bodies been involved in supporting the development of the work supported by this grant?</b></p>
<p>A small grant (£400) from Norbrook was obtained to support a 5<sup>th</sup> Year BVSc student to complete a full literature review on different treatment options for horses infected with liver fluke. This information was used in the leaflet sent to participating veterinary practices and also put on line through the Facebook and Twitter feeds.</p>
<p><b>Briefly tell us about the staff who received a salary or stipend from this grant (including yourself) - Name, job title, full or part time</b></p>
<p>One post-doctoral research assistant was employed for 12 months to undertake the project. Dr Alison Howell is a veterinary surgeon who completed her PhD in 2016. Through undertaking the study she has become familiar with contacting and working with horse owners and veterinarians. She has learnt new statistical skills including new mapping methods. She was also trained in molecular techniques including using a panel of DNA microsatellite markers, capillary sequencing and subsequent genetic population analysis.</p>
<p><b>How has the grant contributed to the professional development of the staff named above (including yourself)? Max 250 words</b></p>
<p>Through conducting this project, all the staff involved have increased their knowledge of equine fasciolosis, developed collaborations and links with other professionals in the industry and found an increasing awareness and interest in fluke infections in horses.</p> <p>We have been contacted and are now working with practices, racing yards and leisure horse owners, indicating a greater awareness of liver fluke infections in horses.</p>
<p><b>Have the results been published? If yes please state when:</b></p>
<p>A manuscript is in preparation, we hope to submit it for publication either to the Veterinary Record or to Equine Veterinary Journal in the summer.</p>