



Original Article

The milk production impacts of liver fluke (*Fasciola hepatica*) infection in dairy cows on the West Coast of the South Island, New ZealandA. Dowling^{a,*}, K.E. Lawrence^b, L. Howe^b, I. Scott^b, W. Pomroy^b^a PGG Wrightson Limited, 1 Robin Mann Place, Christchurch, New Zealand^b School of Veterinary Science, Massey University, Palmerston North, New Zealand

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ABSTRACT

The impact of fasciolosis has been estimated on many occasions and can vary from mild to severe effects on animal productivity. The aim of the current study was to utilise observations on seroconversion of dairy cattle to *Fasciola hepatica* over the course of a lactation and compare their milk production with other cattle who remained serologically negative throughout the same lactation. Four spring-calving dairy herds on the West Coast of the South Island of New Zealand were selected based on prior knowledge of endemic liver fluke infection. Over the four herds, a total of 485 cows were blood sampled twice during one lactation, in early November (spring) and early March (autumn). The *F. hepatica* antibody titre of *F. hepatica* antibody was quantified using a commercial ELISA test (IDEXX Fasciolosis Verification, IDEXX Europe BV, Hoofddorp, The Netherlands). Milk production was estimated by a series of 3–4 herd tests over the lactation where each cow is individually measured during the morning and evening milking on the same day with samples analysed for milk yield, milk fat (MF), protein (MP), lactose (ML) and total milk solids (MS). Energy corrected milk (ECM) was then calculated using the formula: $ECM (kg/d) = 12.55 \times MF (kg/d) + 7.39 \times MP (kg/d) + 0.2595 \times \text{milk yield} (kg/d)$. At the spring sampling, 52 % of cows had antibodies detected which increased to 63 % in autumn. For the sampled cows, those that were in the *negative* IDEXX test category in autumn and spring were categorised as 'uninfected' while those which were *negative* in spring and seroconverted to the *strong positive* category in autumn were categorised as 'infected'. Those in the intermediate categories were ignored. A total of 235 cows were categorised as 'uninfected' at the spring testing, being in the *negative* IDEXX diagnostic category. Of those 235, at the autumn testing, 152 remained in the *negative* diagnostic category and 50 were in the *strong positive* IDEXX diagnostic category and were categorised as 'infected'. A model was fitted which described the lactation curve of each milk component (MF, ML, MP, ECM), a variable describing the infection status of the cow was then tested in the model. The only significant change detected was mean Milk Fat (MF) % being 0.24 MF% points (95 % CI 0.04–0.44 %) lower for 'infected' compared to 'uninfected' cows. Using a value of 6.044/kg MF New Zealand dollars (NZD) this represents an economic loss of NZD 60.2 per 'infected' cow in a West Coast herd. Although small, such an effect will still have an appreciable impact on the economic return to a dairy farmer.

1. Introduction

Fasciolosis, caused by the trematode parasite *Fasciola hepatica* (liver fluke) is a worldwide problem (Vercruyse and Claerebout, 2001; Pritchard et al., 2005; Charlier et al., 2014; Kelley et al., 2020; de Waal and Mehmood, 2021) resulting in economic losses for farmed ruminants. Hepatobiliary pathology is caused by both migration (Dawes (1963); (Wilson et al., 1998) and residence of the flukes (Behm and Sangster, 1999; Charlier et al., 2007; Behm and Sangster, 1999) with lesser

impacts due to secondary factors including blood loss (Dawes, 1964), hypoalbuminemia (Anderson et al., 1977) and enhancing the impacts of contiguous infections (Aitken et al., 1978; Claridge et al., 2012).

Milk production (litres or kilograms) losses due to the presence of liver fluke infection or improved production after treatment have been reported as 3–15 % in Europe (Black and Froyd, 1972; Charlier et al., 2007; Mezo et al., 2011; Charlier et al., 2012a; Howell et al., 2015; Kostenberger et al., 2017; May et al., 2020; Novobilsky et al., 2020; Springer et al., 2021; Takeuchi-Storm et al., 2021) and 16–32 % in the

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central Americas (Arenal et al., 2018; Villa-Mancera and Reynoso-Palomar, 2019). Impacts on the milk constituents (MF, ML, MP) of 0.06 kg/cow/day or 0.06–2.2 % (Charlier et al., 2007; Charlier et al., 2012a; Kostenberger et al., 2017; May et al., 2020) have also been reported. To develop a model to predict the impact of liver fluke infection on cows in Switzerland, Schweizer et al. (2005) determined that infected herds had a 10 % reduction in milk yield (litres/cow/day). Not all studies, however, have been able to determine a statistically significant impact on milk production (Hayward et al., 2021) with impacts at the cow level not always being evident at the herd level. These effects may be lost due to too few cows in the herd having a production limiting infection, even though the prevalence was high.

Studies investigating the impact of liver fluke infection on milk production have either measured the impact of active infection against no infection, or flukicide treatment against no treatment in infected animals, at the individual and herd level. These studies are often conducted in herds which calve all year round, with individual cows entering and leaving the herd throughout the year and are thus at different stages of lactation when the trials are conducted. For example, the milk volume, fat and protein composition of milk changes markedly over the lactation period (Silvestre et al., 2009) which makes measuring the effect of infection or treatment difficult to estimate and since cows will be at different stages of lactation at the time of testing, the impact of liver fluke infection on these parameters, although apparent at the individual cow level may be masked at the herd level. This situation is different in New Zealand, where all cows are at a similar stage in lactation due to seasonal spring calving with a greater likelihood that changes at the cow level will also be apparent at the herd level. Another significant difference in farming systems in New Zealand is the absence of housing animals over the winter period. Due to this difference, cattle can be exposed to metacercariae on pasture for most, if not all year, whereas housing animals removes any further potential of infection and if an anthelmintic treatment is administered at housing, the liver has a period of recovery.

To the authors knowledge there have been no previous studies to quantify the impact of liver fluke infection on production in New Zealand dairy herds. International studies may not be relevant in New Zealand due to significant differences in farm practices; seasonal calving over a two-month period, cessation of lactation in the late autumn/early winter, a pasture-based diet grazed in situ, with no housing for long periods.

The aim of this longitudinal study was to serum sample dairy cows, preferentially selecting those in the first or second lactation, from four herds on two occasions during their lactation, once in November (spring) and again in March (autumn) to investigate the associations between the change of anti-fasciola antibody titre at those two time points with milk production parameters, with the economic cost of any production loss estimated.

2. Materials and methods

2.1. Animal selection and sampling schedule

This was a longitudinal study of the impact on milk production of liver fluke infection of dairy cows from four herds from the West Coast region of the South Island, New Zealand (Latitude -41.369 to -42.690 degrees South, Longitude 172.096 to 171.036 degrees East) between November 2018 (spring) and March 2019 (autumn). The herds used in this study were spring calving dairy cows that supplied milk to Westland Milk Products (WMP), Hokitika, New Zealand, formerly known as Westland Milk Cooperative. These herds were selected based on having a history of endemic liver fluke infection identified by bulk milk ELISA and the farmer's willingness to be part of the study.

Cow diet was pasture grazed in situ supplemented when necessary with pasture silage. Grain or palm kernel expeller meal mix may be fed in the milking shed. Cows in their first lactation were grazed separately

from lactating cows before joining the herd at calving. Anthelmintics specifically targeting liver flukes were used on the farms in non-lactating cows and young stock, although these were not recorded for the purpose of this study. The most recent treatment would be no less than five months prior to the first sampling.

In each herd, up to 170 cows were sampled during the morning milking at the first visit, preferentially selecting those in their first and second lactation with the remainder being older cows, with all being identified by the numbered farm ear tag. For the autumn sampling, cows sampled in spring and still present in the lactating herd were identified and sampled during the morning milking.

2.2. Cow breed categorisation

Cow breeds were classified according to the farmers records as either cross-bred (may have a combination of Jersey, Friesian, Ayrshire or other genetics), Jersey cross (predominantly Jersey but not purebred), Jersey, Friesian cross (predominantly Friesian but not purebred) or Friesian. No attempt was made to determine how each farmer classified the breed of the cows in their herd.

2.3. Serum sampling

At both visits cattle were blood sampled from the coccygeal vein. Samples were transferred to an insulated carrier containing frozen pads to be sent to the laboratory on overnight courier on the day of sampling or stored chilled and transported by car to Massey University on the following day. On arrival at the laboratory the blood samples were centrifuged at 1100 rpm for 15 min (Thermo Scientific, Heraeus Megafuge 40) and the serum was pipetted into labelled 1.5 mL Eppendorf tubes. Paired samples of serum from each animal were stored at -20 °C.

2.4. Serum ELISA testing

The *F. hepatica* specific f2 antigen antibody titre, in the serum samples was quantified using a commercial ELISA test (IDEXX Fasciolosis Verification, IDEXX Europe BV, Hoofddorp, The Netherlands) including positive control (PC) and negative control (NC) samples as per kit instructions. Both the autumn and spring serum samples were thawed and analysed by the same technician 21–27 months after sampling. The colour reaction is read as an optical density (OD) at 450 nm and the sample-to-positive percentage ratio (S/P%) is calculated with this formula:

$$SP\%_{sample} = 100 \times \left(\frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \right)$$

where NC denotes negative control and PC positive control, both supplied with the kit.

Depending on the SP%, the results were categorised where $SP\% \leq 30$ as *negative*, for the presence of *Fasciola hepatica* antibodies, $30 < SP\% \leq 80$ as *mild positive*, $80 < SP\% < 150$, as *positive* and $SP\% \geq 150$ as *strong positive*.

2.5. Milk production data

Farmers were requested to provide access to their herd performance recording data (herd test) collected up to four times for the current lactation, carried out by trained technicians. For this procedure, milk was collected at both a morning and afternoon milking at each sample point and analysed for volume (L), milk fat, protein and lactose (percentage (%)) and weight (kg), milk solids (kg) and somatic cell count. Data was categorised by farm identifier, cow identification number, number of the herd test for that lactation (one to four) and date of the herd test, cow breed, cow age (years), lactation number (1,2, 3+),

IDEXX serum SP% and category and the milk production data from the herd test results. Energy corrected milk (ECM) was calculated using the following formula (Santschi et al., 2011): $ECM (kg/d) = 12.55 \times \text{fat} (kg/d) + 7.39 \times \text{protein} (kg/d) + 0.2595 \times \text{milk yield} (kg/d)$. New Zealand herd test data is recorded as volume and not as weight. The density of milk is estimated at 1.03 kg/L and although using volume instead of weight introduces a small bias, the calculation of ECM was not altered to be consistent with Mason et al. (2012).

2.6. Calculating the economic impact of liver fluke infection on milk production

In the 2017/18 lactation the average herd supplying Westland Milk had 415 cows and produced 4150 L of milk per cow (<https://www.westland.co.nz/people-and-place/our-farmers-suppliers/overview>). The values of NZD 6.75/kg for milk solids, comprising milk protein at 7.6506/kg NZD and milk fat at NZD 6.044/kg were used in calculations of the economic cost of infection. These values were those to dairy farmers in New Zealand during 2022/23.

2.7. Statistics

A model of the lactational change in milk production parameters was developed, describing the lactation curve for each milk component using R (R Core Team (2022)). The data structure itself was hierarchical and had 3 levels of information and potential variability, the 1st level was the repeated herd tests on individual cows, the 2nd level was the individual cows, and the 3rd level was the herds from which the individual cows were selected. Separate statistical models with the following milk production parameters, ECM, milk solids (MS), milk fat % (MF%), milk protein % (MP%), milk lactose % (ML%) as the outcome variable were first created for all cows from all herds using a repeated measures random effects model in R using packages 'lme4' (Bates et al., 2015). The variable days in milk (DIM) was calculated as the number of days from calving to the date of each herd test. The variable was centred to reduce multicollinearity with two polynomial variables also created, DIM2 (squared) and DIM3 (cubed). Lactation was used as a proxy for cow age with cows categorised by lactation as either first, second or third and more. Cow breed was categorised by the farmers description of each cow, and herd test was categorised as 1,2,3 or 4. Since there were only 4 dairy herds, herd was entered as a fixed effect and each cow was given a unique ID "CowHerd" created by concatenating the cow ID and herd e.g. cow 68 from herd B became 68B to avoid any confusion where cows in different herds had the same cow ID. Herd was entered into the model a priori and the other fixed effects were tested and retained in the models if $p < 0.05$ using the likelihood ratio test (LRT). Once a model was fitted which described the lactation curve for each milk production parameter, a variable describing the infection status of the cow, based on the results of the spring and autumn antibody testing, was then tested in the model. This variable was created by first selecting all the cows that tested *negative* at the spring IDEXX ELISA test. Of these cows, those that were still in the *negative* category in autumn were categorised as 'uninfected' while those which had seroconverted and were now in the *strong positive* category were categorised as 'infected'. Cows which were in the intermediate categories were ignored for this comparison. The marginal effects of liver fluke infection on each milk production parameter were estimated by comparing 'infected' to 'uninfected' categories using the 'emmeans' package across all four herds (Lenth, 2023). The model goodness of fit was determined by graphing the residuals and measuring the R^2 Value.

3. Results

3.1. Cow sampling and herd testing

A total of 485 cows were blood sampled from November 12 to 15,

2018 (Spring), and again from March 6–10, 2019 (Autumn), with corresponding herd test data (Table 1). A total of 1694 complete cow data points were analysed, ranging from 102 to 153 (17–26 %) cows from each of the four herds. Using calving dates provided by farmers, the average (and range) of days in milk (DIM) at Spring sampling was 83 (37–160) and Autumn 197 (151–274). Herds B and H performed three herd tests while herds A and I performed four. Of the 485 cows, 266 were first lactation, 125 second and 94 third or more lactation.

3.2. IDEXX category change

Between the Spring and Autumn sampling points there was a decrease in the percentage of cows in the *negative*, *mild positive* and *positive* categories with an increase in the *strong positive* category. The percentage of cows in each diagnostic category was skewed toward either *negative* or *strong positive* at both sampling points (Table 2).

3.3. IDEXX antibody category change and milk testing data

A total of 235 cows were categorised as 'uninfected' at the spring testing, being in the *negative* diagnostic category. Of those 235, at the autumn testing 152 remained in the *negative* diagnostic category and 50 had converted to the *strong positive* diagnostic category. The 152 cows that were *negative/negative* and classified as 'uninfected' and 50 cows that were *negative/strong positive* were categorised as 'infected'. There was a total of 701 herd milk test results for these 235 cows. The separate lactation models showed no effect of infection status on ECM ($p = 0.40$), on MS ($p = 0.28$), ML% ($p = 0.20$), or on MP% ($p = 0.07$). However, there was support for a significant effect of change of infection status on MF% ($p = 0.017$) with the mean MF% being 0.24 MF% points (95 % CI 0.04–0.44 %) lower for 'infected' compared to 'uninfected' cows. Lactation number was not significant in the final MF% model ($p = 0.34$). The conditional and marginal r^2 were 0.733 and 0.453 respectively with residuals normally distributed (Tables 3 and 4).

3.4. Calculating the economic cost of liver fluke infection on milk production

A 0.24 %-point reduction in MF in an 'infected' cow equates to 2.4 g MF/L lower production compared to an 'uninfected' cow. With MF valued at NZD 6.044/kg and the average herd producing 4150 l of milk per cow, this is a loss of NZD 60.2 per cow. In this study 50/235 (21 %) cows were 'uninfected' at the first sampling and 'infected' at the second. If this were representative of the average West Coast herd of 415 cows, the economic cost would be NZD 5246.3 per herd.

Table 1

Dates of herd tests, blood sampling and the number of paired serum samples for each herd.

Herd	Blood sampling dates		Herd test dates				Number of cows with paired samples
A	12/	06/	30/	11/	12/	19/	126
	11/	03/	09/	12/	02/	04/	
	2018	2019	2018	2018	2019	2019	
	13/	08/		19/	03/	02/	
B	11/	03/		12/	03/	05/	104
	2018	2019		2018	2019	2019	
	14/	09/	27/	05/	04/		
	11/	03/	09/	12/	02/		
H	2018	2019	2018	2019	2019		101
	15/	10/	24/	22/	21/	16/	
	11/	03/	09/	11/	01/	04/	
	2018	2019	2018	2018	2019	2019	
I	2018	2019	2018	2018	2019	2019	155

Table 2

The number of cows in each IDEXX serum diagnostic category in Spring and Autumn. For the diagnostic categories, the number in parenthesis indicate the percentage of cows from the Spring category in each category in Autumn (calculated across each row). The Total figures are for all results for Spring and Autumn. Percentages may not equal 100 due to rounding.

	Autumn IDEXX Category				Total
	Negative	Mild Positive	Positive	Strong Positive	
Spring IDEXX Category					
Negative	152 (65 %)	24 (10 %)	9 (4 %)	50 (21 %)	235 (48 %)
Mild Positive	13 (30 %)	5 (12 %)	5 (12 %)	20 (47 %)	43 (9 %)
Positive	5 (11 %)	1 (2 %)	8 (18 %)	30 (68 %)	44 (9 %)
Strong Positive	14 (9 %)	8 (5 %)	8 (5 %)	134 (82 %)	164 (34 %)
Total	184 (38 %)	38 (8 %)	30 (6 %)	234 (48 %)	486

Table 3

Coefficients for final repeated measures model predicting the effect of being *Fasciola hepatica* serum positive on milk fat percent.

Outcome variable	Milk Fat Percent (MF%)		
	Estimates	CI	P
(Intercept)	4.8	4.5–5.1	<0.001
Days In Milk* centre	0.35	0.25–0.46	<0.001
IDEXX fluke test positive	–0.24	–0.44 to –0.04	0.017
Breed [Jersey]	0.80	0.26–1.34	0.004
Breed [Crossbred]	0.26	–0.05–0.56	0.101
Herd [B]	–0.1	–0.33–0.13	0.407
Herd [C]	–0.24	–0.52–0.04	0.088
Herd [D]	–0.18	–0.40–0.04	0.106
Days In Milk centre ²	0.19	0.15–0.23	<0.001
Days In Milk centre ³	0.14	0.08–0.19	<0.001

* Days in Milk is calculated from the farmer recorded calving date and the sampling date for each cow.

Table 4

Number (and percentage) of cows 'Uninfected' and 'Infected' in their first, second or third and subsequent lactation.

	First lactation	Second Lactation	Third or more lactation	total
Negative spring and Negative autumn (Uninfected)	80 (53 %)	38 (25 %)	34 (22 %)	152
Negative spring and Strong Positive autumn (Infected)	21 (42 %)	18 (36 %)	11 (22 %)	50

4. Discussion

Although there was relatively minor change of IDEXX diagnostic category for all cows over a four-month period, cows that were *negative* in the spring and *strong positive* in the autumn ('infected') relative to cows *negative* at both sampling points ('uninfected') had a significant reduction in MF%. An increase in antibody titre subsequent to liver fluke infection can be detected using the IDEXX antibody ELISA 2 weeks post infection (wpi) and reaches a maximum SP% 8 wpi (Reichel, 2002), so it can be assumed that the change from 'uninfected' to 'infected' category is due to liver fluke infection at, or very close to the period between the two sampling points. It is presumed that the impacts of hepatic pathology and immunological activity resulted in a reduction in MF%. Further studies investigating the duration of the impact on MF%, are warranted, since the estimated loss NZD 60.2 was for animals that become infected within the lactation and may underestimate the impact on the subsequent lactation (Charlier et al., 2012b) or lifetime production. It would not be possible for a farmer to visualise such a small drop in MF% for individual cattle. Nevertheless, a drop such as described here, does result in an appreciable decline in monetary return for the farmer. Rainfall was below average during the spring and summer of this study (data not shown) and is likely to have resulted in lower snail numbers over this period (Dowling, 2023). This is likely to have reduced the risk

of cows becoming infected from cercariae released from snails over this period, so the 21 % of cows that seroconverted from 'uninfected' to 'infected' may underestimate infection when rainfall is average, or indeed above average. The financial impact at a herd level may be much greater than the NZD 5246.3 calculated in this study.

For a farmer to determine the financial return of interventions that reduce the risk of cows becoming infected with liver fluke, and therefore impacting milk production, an estimation of the within herd prevalence of infection at time points throughout the lactation is essential. If infection occurs early in lactation, any impact on milk production would be over a longer period and potentially have a greater impact than infection late in lactation. The currently favoured single sampling point of late lactation may be useful to determine anthelmintic treatment options during the non-lactating period but does little to help determine when infection occurred. The authors suggest that bulk milk sampling of the herd more frequently throughout the lactation will aid farmers to determine both when infection occurs and the range of prevalence of infection. This serial monitoring will likely be required each year since weather conditions have a major impact on the fluke life cycle and therefore risk of infection (Rowcliffe and Ollerenshaw, 1960; Boray, 1969; Harris and Charleston, 1976; Andrews et al., 2022).

In overseas studies the greatest impact on milk production is noted between herds that are determined to have a high antibody titre relative to negative titres (Charlier et al., 2007; Charlier et al., 2012b; Kostenberger et al., 2017). Milk production parameters are influenced by many factors with liver fluke infection being one, with greatest impacts in volume noted between negative and the highest positive diagnostic category (Charlier et al., 2007; May et al., 2020; Mezo et al., 2011; Takeuchi-Storm et al., 2021), high producing herds (Howell et al., 2015), or a high herd prevalence of infection (Vercruyse and Claerebout, 2001) to result in a financial cost. Unfortunately, two of the herds in this study only performed three herd tests, weakening the dataset. Repeating the study using a larger number of herds would be valuable to determine if the result seen in this study is repeatable. Antibody titre indicates the magnitude of the current immune response of the cow to the presence of *F. hepatica*. Weaknesses of using antibody based diagnostics include the inability to determine if a positive result indicates current or historic infection, the time of initial infection, whether infection is continuous or sporadic, the number of flukes present, nor will titres indicate any hepatic insufficiency related to previous fluke infections, thus it may underestimate the impact (Charlier et al., 2014). The antibody titre rise may be the result of the presence of only a few fluke, less than the suggested production limiting thresholds of 10 (Charlier et al., 2008) and 30 flukes (Vercruyse and Claerebout, 2001).

Animals that were positive in the spring testing may have been infected from any time since the last effective anthelmintic treatment. While we could not determine the drench history of individual cows in this study, for those treated it would have been at least 5 months prior to sampling. Antibody titre decreases after successful treatment but may still record a positive result at the time of sampling dependant on the initial titre and time since treatment (Charlier et al., 2014). A previous study in the region (Dowling et al., 2025.) using an antibody bulk milk ELISA test, found herds treated with an anthelmintic specifically

targeting liver fluke at the end of lactation (early winter) had no change in the SP% from an autumn and spring sampling. This indicates that on those farms the cows possibly became reinfected after treatment, supporting the possible reinfection of cow in the current study after successful treatment. The life expectancy of flukes is not clearly determined with 75 % of fluke surviving five to 21 months post natural infection, although in cattle with low numbers of flukes present some still remained at 26 months (Ross, 1968) with flukes also surviving at least two years in a Danish study (Takeuchi-Storm et al., 2018). The use of a diagnostic test able to detect current infection, such as antigen detection, could be used in future studies to better determine the current infection state of animals. Faecal egg counts in cattle in the study region were found to be very low (data not published) and to have a very low sensitivity (Dowling et al., 2024) so not considered as suitable for this study.

At the spring sampling 52 % of cows (*mild positive*, *positive* and *strong positive*, Table 2) already had a positive antibody titre, either indicating chronic infection or new infection acquired since the last anthelmintic treatment. Further work is required to determine the survival of metacercariae over the winter on the West Coast to aid farmers to develop control measures to reduce infection. In the only New Zealand study to date which was in the Manawatu region in the lower North Island, metacercariae did not survive winter (Harris and Charleston, 1976), whereas overseas studies in similar climates have demonstrated that metacercariae have survived over winter (Luzon-Pena et al., 1994). If metacercariae can survive the winter period and remain infective, the impact of an anthelmintic treatment at the end of lactation (in late autumn/early winter) is unlikely to reduce the risk of reinfection, so unless this treatment is shown to improve milk production in the following lactation it has questionable value. This study did not investigate hepatic pathology because of prior infection, which may be sufficient to impact milk production regardless of new infection as indicated by Charlier et al. (2012b).

The increase in antibody titre between the two sampling points was less than expected and could be related to lower than average rainfall recorded over the spring and summer during this study. The IDEXX serum test did appear to bias the *negative* and *strong positive* diagnostic categories with relatively few cows in the intervening two categories (Table 2). While it cannot be shown whether this general increase in antibody titre is the result of new infection or progression of current infection and amplification of the immune response, the increase does come at a cost to the host.

It was also interesting to note that 10 % of cows decreased at least one diagnostic category between the two sampling points, possible because of self-cure or those where the IDEXX SP% was near the cut-off values between diagnostic categories. Nevertheless, there were still 50/235 cows that were *negative* in Spring and *strong positive* in Autumn that could be used to calculate the impact of becoming infected with liver fluke compared to cows that remained uninfected.

Age of cow (lactation) was not significant in this study. While the litres (yield) increases until aged five to seven years, percentage of the milk constituents does not change considerably with age of cow (Silvestre, 2009), but is rather impacted by factors such as diet and cow health.

In conclusion, the calculated economic cost of NZD 60.2 in the 'infected' cows, combined with estimated prevalence infection in a herd determined from bulk milk antibody analysis enables farmers to estimate of the impact of infection in their herd to determine if the economic cost warrants intervention.

Ethical approval

This experiment was performed under the approval of Massey University Animal Ethics Committee, Protocol 18/36.

CRedit authorship contribution statement

A. Dowling: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **K.E. Lawrence:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **L. Howe:** Writing – review & editing, Supervision. **I. Scott:** Writing – review & editing, Supervision. **W. Pomroy:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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 article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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References

- Aitken, M.M., Jones, P.W., Hall, G.A., Hughes, D.L., Collis, K.A., 1978. Effects of experimental *Salmonella Dublin* infection in cattle given *Fasciola hepatica* thirteen weeks previously. *J. Comp. Pathol.* 88.
- Anderson, P.H., Berrett, S., Brush, P.J., Hebert, C.N., Parfitt, J.W., Patterson, D.S.P., 1977. Biochemical indicators of liver-injury in calves with experimental fascioliasis. *Vet. Rec.* 100, 43–45.
- Andrews, S.J., Cwiklinski, K., Dalton, J.P., 2022. The discovery of *Fasciola hepatica* and its life cycle. In: Dalton, J.P. (Ed.), *Fasciolosis*, 2 Edn. CABI.
- Arenal, A., Garcia, Y., Quesada, L., Velazquez, D., Sanchez, D., Pena, M., Suarez, A., Diaz, A., Sanchez, Y., Casaert, S., 2018. Risk factors for the presence of *Fasciola hepatica* antibodies in bulk-milk samples and their association with milk production decreases, in Cuban dairy cattle. *BMC Vet. Res.* 14, 8.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Behm, C., Sangster, N., 1999. In: Dalton, J.P. (Ed.), *Pathology, Pathophysiology and Clinical Aspects. Fasciolosis*. CABI, pp. 185–224.
- Black, N.M., Froyd, G., 1972. The possible influence of liver fluke infestation on milk quality. *Vet. Record* 90, 71–72.
- Boray, J.C., 1969. Experimental fascioliasis in Australia. *Adv. Parasitol.* 7, 95–210.
- Charlier, J., Duchateau, L., Claerebout, E., Williams, D., Vercruysse, J., 2007. Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. *Prev. Vet. Med.* 78, 57–66.
- Charlier, J., De Meulemeester, L., Claerebout, E., Williams, D., Vercruysse, J., 2008. Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. *Vet. Parasitol.* 153, 44–51.
- Charlier, J., Van der Voort, M., Hogeveen, H., Vercruysse, J., 2012a. ParaCalc® -A novel tool to evaluate the economic importance of worm infections on the dairy farm. *Vet. Parasitol.* 184, 204–211.
- Charlier, J., Hostens, M., Jacobs, J., Van Ranst, B., Duchateau, L., Vercruysse, J., 2012b. Integrating fasciolosis control in the dry cow management: the effect of closantel treatment on milk production. *Plos One* 7.
- Charlier, J., Vercruysse, J., Morgan, E., van Dijk, J., Williams, D., 2014. Recent advances in the diagnosis, impact on production and prediction of *Fasciola hepatica* in cattle. *Parasitology* 141, 326–335.
- Claridge, J., Diggle, P., McCann, C.M., Mulcahy, G., Flynn, R., McNair, J., Strain, S., Welsh, M., Baylis, M., Williams, D.J.L., 2012. *Fasciola hepatica* is associated with the failure to detect bovine tuberculosis in dairy cattle. *Nat. Commun.* 3.
- Dawes, B., 1963. Migration of juvenile forms of *fasciola hepatica* l. through wall of intestines in mouse, with some observations on food and feeding. *Parasitology* 53, 109–+.
- Dawes, B., 1964. Fasciolosis - the invasive stages of *Fasciola hepatica* in mammalian hosts. *Adv. Parasitol.* 89.
- Dowling, A., 2023. The Prevalence and Production Effects of Liver Fluke (*Fasciola hepatica*) in New Zealand Cattle Including Evaluation of Diagnostic Tests. PhD thesis, Massey University, New Zealand. <https://mro.massey.ac.nz/items/19408d21-e316-4e0e-8de5-84a82b89b617>.
- Dowling, A., Lawrence, K.E., Howe, L., Scott, I., Pomroy, W., 2025. The seroprevalence and spatial distribution of liver fluke infection in a sample of west coast and Canterbury dairy herds. *Parasitology. Reg. Stud.* 58.

- Dowling, A.F., Lawrence, K.E., Pomroy, W., Scott, I., Howe, L., 2024. The use of a Bayesian latent class model to estimate the test characteristics of three liver fluke diagnostic tests under New Zealand field conditions. *Vet. Parasitol.* 332.
- Harris, R., Charleston, W., 1976. The epidemiology of *Fasciola hepatica* infections in sheep on a *Lymnaea columella* habitat in the Manawatu. *New Zeal. Vet. J.* 24, 11–17.
- Hayward, A.D., Skuce, P.J., McNeilly, T.N., 2021. The influence of liver fluke infection on production in sheep and cattle: a meta-analysis. *Int. J. Parasitol.* 51 (11), 913–924.
- Howell, A., Baylis, M., Smith, R., Pinchbeck, G., Williams, D., 2015. Epidemiology and impact of *Fasciola hepatica* exposure in high-yielding dairy herds. *Prev. Vet. Med.* 121, 41–48.
- Kelley, J., Rathinasamy, V., Elliott, T., Rawlin, G., Beddoe, T., Stevenson, M., Spithill, T., 2020. Determination of the prevalence and intensity of *Fasciola hepatica* infection in dairy cattle from six irrigation regions of Victoria, South-Eastern Australia, further identifying significant triclabendazole resistance on three properties. *Vet. Parasitol.* 277, 109019.
- Kostenberger, K., Tichy, A., Bauer, K., Pless, P., Wittek, T., 2017. Associations between fasciolosis and milk production, and the impact of anthelmintic treatment in dairy herds. *Parasitol. Res.* 116, 1981–1987.
- Luzon-Pena, M., Rojovazquez, F.A., Gomezbautista, M., 1994. The overwintering of eggs, intramolluscal stages and metacercariae of *Fasciola hepatica* under the temperatures of a mediterranean area (Madrid, Spain). *Vet. Parasitol.* 55, 143–148.
- Mason, W.A., Pomroy, W.E., Lawrence, K., Scott, I., 2012. The effect of repeated, four-weekly eprinomectin treatment on milk production in pasture-based, seasonally-calving dairy cattle. *Vet. Parasitol.* 189.
- May, K., Bohlsen, E., Konig, S., Strube, C., 2020. *Fasciola hepatica* seroprevalence in northern German dairy herds and associations with milk production parameters and milk ketone bodies. *Vet. Parasitol.* 277, 8.
- Mezo, M., Gonzalez-Warleta, M., Castro-Hermida, J.A., Muino, L., Ubeira, F.M., 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. *Vet. Parasitol.* 180, 237–242.
- Novobilsky, A., Rustas, B.-O., Grandi, G., Hogberg, N., Hoglund, J., 2020. Selective flukicide treatment of non-lactating cows and the corresponding production impact of *Fasciola hepatica* in dairy herds in Sweden. *Vet. Parasitol.* 283, 109180.
- Pritchard, G., Forbes, A., Williams, D., Salimi-Bejestani, M., Daniel, R., 2005. Emergence of fasciolosis in cattle in East Anglia. *Vet. Rec.* 157, 578–582.
- R (R Core Team), 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.Rproject.org/>.
- Reichel, M., 2002. Performance characteristics of an enzyme-linked immunosorbent assay for the detection of liver fluke (*Fasciola hepatica*) infection in sheep and cattle. *Vet. Parasitol.* 107, 65–72.
- Rowcliffe, S.A., Ollerenshaw, C.B., 1960. Observations on the bionomics of the egg of *Fasciola Hepatica*. *Ann. Tropical Med. Parasitol.* 54, 172–181.
- Ross, J.G., 1968. The life span of *Fasciola hepatica* in cattle, pp. 587–589.
- Santschi, D.E., Lefebvre, D.M., Cue, R.L., Girard, C.L., Pellerin, D., 2011. Complete-lactation milk and component yields following a short (35-d) or a conventional (60-d) dry period management strategy in commercial Holstein herds. *J. Dairy Sci.* 94, 2302–2311.
- Schweizer, G., Braun, U., Deplazes, P., Torgerson, P.R., 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. *Vet. Record* 157, 188–193.
- Silvestre, A.M., Santos, V.A., Ginja, M.M., Colaco, J.A., 2009. Lactation curves for milk, fat and protein in dairy cows: A full approach. *Livest. Sci.* 122.
- Springer, A., Jordan, D., Kirse, A., Schneider, B., Campe, A., Knubben-Schweizer, G., Muller, K.E., Hoedemaker, M., Strube, C., 2021. Seroprevalence of major pasture-borne Parasitoses (gastrointestinal nematodes, liver flukes and lungworms) in German dairy cattle herds, association with management factors and impact on production parameters. *Animals* 11.
- Takeuchi-Storm, N., Denwood, M., Petersen, H.H., Enemark, H.L., Stensgaard, A.S., Sengupta, M.E., Beesley, N.J., Hodgkinson, J., Williams, D., Thamsborg, S.M., 2018. Patterns of *Fasciola hepatica* infection in Danish dairy cattle: implications for on-farm control of the parasite based on different diagnostic methods. *Parasit. Vectors* 11.
- Takeuchi-Storm, N., Thamsborg, S.M., Enemark, H.L., Boes, J., Williams, D., Denwood, M.J., 2021. Association between milk yield and milk anti-*Fasciola hepatica* antibody levels, and the utility of bulk tank milk samples for assessing within-herd prevalence on organic dairy farms. *Vet. Parasitol.* 291, 109374.
- Vercruyse, J., Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. *Vet. Parasitol.* 98, 195–214.
- Villa-Mancera, A., Reynoso-Palomar, A., 2019. High prevalence, potential economic impact, and risk factors of *Fasciola hepatica* in dairy herds in tropical, dry and temperate climate regions in Mexico. *Acta Trop.* 193, 169–175.
- de Waal, T., Mehmood, K., 2021. Editorial: trematode infection in ruminants. *Front. Vet. Sci.* 8.
- Wilson, L.R., Good, R.T., Panaccio, M., Wijffels, G.L., Sandeman, R.M., Spithill, T.W., 1998. *Fasciola hepatica*: characterization and cloning of the major cathepsin B protease secreted by newly excysted juvenile liver fluke. *Exp. Parasitol.* 88, 85–94.