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The use of a Bayesian latent class model to estimate the test characteristics of three liver fluke diagnostic tests under New Zealand field conditions

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ABSTRACT

The liver fluke *Fasciola hepatica* is a trematode parasite of farmed livestock with worldwide distribution, causing chronic production losses and possible death from hepatobiliary damage. The effective management of liver fluke infection requires diagnostic tests which can accurately identify infected animals at both the individual and herd level. However, the accuracy of liver fluke diagnostic tests performed on individual New Zealand cattle is currently unknown. The aim of this study was to use a Bayesian latent class model (LCM) to estimate the test characteristics of three liver fluke diagnostic tests, the coproantigen ELISA, the IDEXX antibody ELISA and the faecal egg count. One hundred and twenty dairy cows each from two dairy farms were blood and faecal sampled in April 2021. The samples were transported to Massey University, Palmerston North, and the three diagnostic tests completed following the respective manufacturer instructions. A Bayesian LCM model, adapted from the original Hui and Walter 2 tests 2 populations model, was built to estimate the test characteristics of the three diagnostic tests in the two dairy herds. The model was implemented in JAGS using Markov chain Monte Carlo sampling. The first 30,000 iterations were discarded as burn-in, and the next 200,000 iterations were used to construct the posterior distributions. Uninformed priors, beta (1,1), were used as the prior distributions for the prevalence estimation and informed beta priors, based on published results, were used as the prior distributions for estimating the sensitivity and specificity of each diagnostic test. Model convergence was confirmed by inspection of trace plots and examination of the results of the Gelman and Rubin test. The results found that the coproantigen ELISA test was the most accurate for diagnosing liver fluke infection in individual animals with a sensitivity = 0.98 (95 % CI 0.95–1.00) and specificity = 0.95 (95 % CI 0.81–1.00) compared to the IDEXX antibody ELISA test, sensitivity = 0.39 (95 % CI 0.32–0.47) and specificity = 0.86 (95 % CI 0.75–0.96) or the FEC, sensitivity = 0.23 (95 % CI 0.17–0.30) and specificity = 0.92 (95 % CI 0.86–0.97). Based on these results clinicians should be encouraged to use the coproantigen ELISA test to diagnose liver fluke infection in individual cattle.

1. Introduction

The liver fluke *Fasciola hepatica* is a trematode parasite of farmed livestock with worldwide distribution causing chronic production losses and possible death from hepatobiliary damage (Charlier et al., 2014). The lifecycle of *Fasciola hepatica* is well described (Charleston, 1997) with the adult fluke resident in the bile ducts of the infected animal and the intermediate host mud snails *P. columella* and *L. tomentosa* found in permanently wet areas. The intensity and geographical extent of liver fluke infection in New Zealand cattle has been surveyed once using slaughterhouse data (Charleston et al., 1990) and is predicted to increase due to climate change (Haydock et al., 2016), with previously naïve areas being more suitable to the establishment and survival of the intermediate host snails. This will result in some regions experiencing liver fluke infection for the first time, becoming another production limiting factor in their farm system. Effective management of liver fluke infection requires diagnostic tests which can accurately identify infected animals and estimate the infection intensity at both the individual and herd level, aspects complicated by other factors including a two-host lifecycle, a migratory phase in the host, a long prepatent period and variable host immune response to the presence of the parasite.

There is an excellent test to determine *F. hepatica* infection on post-

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mortem material, the total fluke count (TFC), where the liver is sliced into thin sections and massaged to remove flukes, with an estimated sensitivity (Se) and specificity (Sp) of 99 % and 98 % respectively (Mazeri et al., 2016). However, the test is only useful for research purposes, being too impractical for use as a diagnostic tool in the field, due to the time taken to systematically examine each liver and the requirement for post-mortem tissue. The resulting diagnosis from TFCs is highly indicative of the state of infection in that animal but the range of infection within a cohort of animals can vary greatly from those with no flukes present to those with many (Dowling, 2023). Therefore, the TFC is most suited to being used as a gold standard against which the test characteristics of a new diagnostic test can be measured.

The requirement for a liver fluke test to have high sensitivity or high specificity is dependent on the purpose of the test and the disease prevalence. In a situation where a disease is to be eradicated, a high Se is required to ensure that very few infected animals are missed, keeping in mind that depending on the Sp, several uninfected animals may be treated or culled unnecessarily, which also has a cost. When the disease prevalence is high, even a test with a moderate Se will still detect many of the infected animals, but this decreases sharply when the disease prevalence is low (Bentley et al., 2012).

Diagnostic assays are almost invariably developed and tested using artificial infections in a naïve population and then compared to an uninfected population, with the results often measured against those from a non-gold standard test. When any imperfect diagnostic test is used as the gold standard for determining the test characteristics of a second test, the results can be biased towards the imperfect gold standard test (Molloy et al., 2005; Munita et al., 2019; Salimi-Bejestani et al., 2007). Furthermore, the host response to an artificial infection can be quite different to that of naturally infected animals and indicates that tests developed on artificial infections should also be evaluated in the field, on animals that are naturally infected with multiple stages of the parasite life cycle. It is also important that when new diagnostic tests are evaluated in the field, they are compared to the results from a true gold standard not an imperfect test.

There currently is no gold standard test for diagnosing liver fluke infection in live animals in the field (Mazeri et al., 2016). Antibody detection ELISA in either milk or serum, and faecal egg counting (FEC) are two commonly used diagnostic tools for determining the presence and intensity of liver fluke infection in farmed livestock, with neither of these tests being sufficiently robust to be considered a gold standard. Furthermore, the test characteristics for these two tests have never been fully assessed on naturally infected cows in New Zealand.

A more recent liver fluke diagnostic development is an antigen ELISA assay which detects the presence of excretory - secretory antigens (ESA) in either serum or faeces of infected people or animals. The ESA requires the presence of flukes or parts of fluke bodies in the hepatic parenchyma (serum) and biliary system (faeces) and a positive result indicates current or very recent infection where not all parts of fluke bodies have been eliminated from the host. Since this test does not rely on the host's immune response but infers infection by detecting antigen, it is not confounded by any factors such as concurrent infection or nutritional stress which could affect the host's antibody response to liver fluke infection. The coproantigen ELISA can detect ESA from as few as 2 flukes (Mezo et al., 2004) and can reliably detect infections of 10 or more flukes from 6 weeks post infection (Charlier et al., 2008).

One of the more popular approaches to estimating the test characteristics of a diagnostic test, in the absence of a gold standard, is through using Bayesian latent class modelling (Joseph et al., 1995; Rapsch et al., 2006; Mazeri et al., 2016) and is based on Bayes' theorem of conditional probability. Bayesian latent class models (LCM) provide robust estimates of disease prevalence and test parameters including Se, Sp, positive predictive value (PPV) and negative predictive value (NPV), aiding a better interpretation of assay results. Furthermore, the use of Bayesian LCM allows for smaller sample sizes and the comparison of multiple independent dichotomous diagnostic tests across different populations, where the only known variable is the apparent disease prevalence (Pouillot et al., 2002; Rapsch et al., 2006; Mazeri et al., 2016). This method of analysis has been used in many studies investigating disease prevalence and test characteristics where there was no gold standard, including estimating the prevalence of liver damage caused by pithomycotoxicosis in cattle (Laven et al., 2021), the prevalence of liver fluke in Swiss cattle (Rapsch et al., 2006) and the prevalence of Strongyloides infection in humans (Joseph et al., 1995). It has also been used for the estimation of diagnostic test characteristics (Horigan et al., 2011; Mazeri et al., 2016). An important requirement for using Bayesian LCM is that the diagnostic tests being compared are independent i.e., they are based on different biological assays, and that the true prevalence of disease in the populations being compared are quite different.

The aim of this study was to estimate the test characteristics of three common liver fluke diagnostic tests, the FEC, the IDEXX ELISA test, and the coproantigen ELISA test using Bayesian LCM, in naturally infected adult cows from two New Zealand dairy farms.

2. Materials and methods

This was a prospective observational study using a convenience sample of cows from two West Coast dairy farms. The West Coast is a region of New Zealand on the west coast of the South Island. The West Coast has very high rainfall and is recognised as having a high prevalence of liver fluke infection (Dowling, 2023)

2.1. Animal selection and sampling

All cattle in this study were adult lactating dairy cows from the West Coast of the South Island with samples collected from two separate herds, A and B. Cows from Herds A and B were convenience sampled in the same week in April (autumn) 2021. At each farm, 150 cows were blood sampled (10 ml) from the caudal tail vein at the morning milking and identified with a coloured marker for faecal sampling the following morning milking, between 6 am and 8 am. On both farms, cows were milked on a rotary platform with a continuous flow of cows entering and exiting, with the first author (AD) standing on a fixed raised platform of 2 m length on the outer circumference of the rotating platform. Cows were selected for sampling at the choice of AD as those that were most easily accessed to total 150, this total being met when approximately 2/3 of cows had been milked. A 70 ml sample of vat milk was also collected at the completion of miking. The following morning, those cows with the raddle mark were faecal sampled during milking until 120 animals were successfully sampled. Blood and faecal samples were identified to the cow using the herd ID tag.

Serum samples were transferred to a refrigerator once a clot had formed and collected serum samples were stored at 4°C with the milk sample. Faecal samples from the first herd were stored in chilly bins with frozen pads changed each day. All samples were transported to the laboratory in chilled containers with serum and faecal samples placed in a chiller at 4°C on arrival and milk stored at −20°C, 14 hours after the second herd was faecal sampled. The following day, 5 g of faeces was weighed into 70 ml plastic containers and stored at 4°C, while the remaining faecal material in the original plastic container was stored at −20°C. On the same day, serum samples were centrifuged at 1100 g for 15 minutes with duplicate samples pipetted into 1.5 ml Eppendorf tubes and stored frozen at −20°C.

2.2. Coproantigen ELISA testing

The coproantigen ELISA (Bio K 201 – Monoscreen AgELISA Fasciola hepatica, Bio-X Diagnostics S.A. Rochefort, Belgium) was conducted as per the kit instructions with the amendment of overnight incubation of faeces with the dilution buffer (Brockwell et al., 2013). After farm collection faecal samples were frozen at −20°C for 16–17 weeks. Faeces were thawed, then 2 g of each were transferred into 10 ml plastic conical

tube with 2 ml of dilution buffer added, inverted several times to mix and to ensure the faecal material was at the base and then stored vertically overnight. The coproantigen ELISA was performed on all samples on the same day for Herd A and B. Animals with an OD(450 nm) value ≥0.014 (1.4 %) were assigned a positive result (Brockwell et al., 2013).

2.3. IDEXX serum and milk antibody ELISA testing

The IDEXX antibody ELISA (IDEXX Fasciolosis Verification, IDEXX Europe BV, Hoofdorp, The Netherlands) was conducted as per kit instructions. Serum samples were refrigerated after collection and centrifuged within one week before being stored at −20 °C until analysis 9 weeks later when they were processed on the same day for Herds A and B. Animals with a calculated SP% ≥30 were assigned a positive result (Munita et al., 2019). The two bulk milk samples were thawed and analysed 16 weeks after collection. Diagnostic categories used as per kit instructions for Herds A and B.

2.4. Faecal egg counts

The FEC were performed using 5 g faeces sieved through a Flukefinder® (Richard Dixon, ID, US) and then filtered using the sedimentation process. Faeces were refrigerated for up to one week before being prepared for counting by filtration and sedimentation. Prepared samples were refrigerated for up to one month before examination under a microscope for counting. A sample containing one or more fluke eggs was assigned a positive result.

2.5. Statistics

The performance of the coproantigen ELISA, the IDEXX antibody ELISA, and the FEC was evaluated with a Bayesian latent class model (LCM). Using this framework, the latent or unknown class is the true infection status of the cow, and in this study was defined as an infection with adult fluke which had persisted long enough for secretory antigens to be excreted, for eggs to be passed or for a detectable immune response to be triggered in the host. The Bayesian LCM model was adapted from the original Hui and Walter 2 tests 2 populations model (Hui and Walter, 1980) to one analysing 3 tests and two populations, following an approach described by Drewe et al. (2010). The Hui and Walter model makes three assumptions; 1) that the prevalence of disease is different between the two populations, 2) that the sensitivity and specificity of each diagnostic test is the same for each population, and 3) that the tests are conditionally independent. Regarding test independence, the following assumptions were made; the FEC relies upon the presence of eggs, the IDEXX antibody ELISA detects antibodies specific to the f2 component of the ESA in serum, and the coproantigen ELISA test detects the presence of fluke specific ESA in faeces, since the biological mechanism of action of each test was interpreted as different these three diagnostic tests were initially considered conditionally independent. However, Mazeri et al. (2016), conducting a similar analysis, considered that the coproantigen ELISA test and the FEC were potentially dependent so a covariance factor between the coproantigen ELISA test and the FEC test was also included in the LCM model.

The structure of the model meant there were 14 degrees of freedom (seven in each population) and 8 parameters to estimate (3 Sensitivities, 3 Specificities and 2 prevalences) which made the model mathematically solvable. The model was implemented in JAGS (Plummer, 2003), a software using MCMC simulations to construct posterior distributions for the analysis of Bayesian hierarchical models. JAGS was run within R (Version 4.2.1) using the runjags package (Denwood, 2016). The first 30,000 iterations were discarded as burn-in and the next 200,000 iterations were used to construct the posterior distributions. The LCM model incorporates prior knowledge by specifying beta (α, β) prior distributions for each diagnostic test's sensitivity, specificity, and the true disease prevalence in each population. These prior distributions may be based on literature or expert opinion, but if neither is available, uninformed, or weak priors are used instead.

The probabilities of all the possible combinations of test outcomes conditional on the unknown disease status were specified using the Se and Sp of each test and the true prevalence (*pi*) of liver fluke infection in each herd (Mazeri et al., 2016). For example, the probability that all three diagnostic tests are positive in Herd A =

$$p1[1] = pi1 * Se1 * Se2 * Se3 + (1 - pi1) * (1 - Sp1) * (1 - Sp2) * (1 - Sp3)$$

Where $p1[1]$ is the probability of all three tests being positive in Herd A, $pi1$ is the true prevalence of liver fluke infection in Herd A, $Se1$, $Se2$ and $Se3$ are the sensitivity of the coproantigen test, the IDEXX ELISA and FEC, respectively and $Sp1$, $Sp2$ and $Sp3$ are the specificity of the coproantigen test, the IDEXX ELISA and FEC, respectively.

Animals could be positive or negative for each of the three diagnostic tests, giving eight possible combinations of test results (2^3). Uninformed uniform, beta (1, 1), priors were used for estimating the prevalence of liver fluke infection in each herd. However, due to the sparsity of the observed data (i.e., zero cell observations for some of the cross-classified results, see Table 3), the ability of the LCM model to estimate the diagnostic tests' parameters was impaired (Meletis et al. 2022). The only solution to this problem is to incorporate external information by informing the prior distribution of the diagnostic tests. Hence, informative priors were introduced for the diagnostic tests, using the following method. From published data for each of these diagnostic tests (Table 1) the mode was calculated using the method of Venter (Venter, 1967) and then a beta distribution was selected with this mode, using the epiR beta buster function from the epiR library (Stevenson and Sergeant, 2024), and which reflected our belief that we were 80 % confident that the diagnostic test sensitivity or specificity was greater than the lowest published value.

Convergence of the Bayesian LCM MCMC was assessed by simultaneously running two chains from different starting values and visual inspection of the trace plots using the R Package coda (Plummer et al., 2006) along with examining the results of the Gelman and Rubin test and the Heidelberger-Welch diagnostic test. Geweke's diagnostic test was used to adjust the burn-in period (Geweke, 1992).

A sensitivity analysis was conducted to examine the influence of the

Table 1
Studies referenced for coproantigen ELISA, IDEXX antibody ELISA, and faecal egg count (FEC) sensitivity and specificity estimates from which the beta prior distributions for the Bayesian LCM were calculated.

Coproantigen ELISA studies	Sensitivity	Specificity
(Kelley et al., 2021)	100 %	not determined
(Palmer et al., 2014)	87 %	99 %
(Charlier et al., 2008) spring	98 %	92 %
(Charlier et al., 2008) autumn	90 %	94 %
(Mazeri et al., 2016)	77 %	99 %
IDEXX antibody ELISA studies	Sensitivity	Specificity
(Hutchinson and Macarthur, 2003)	99 %	95 %
(Charlier et al., 2008)	82 %	80 %
(Charlier et al., 2008)	95 %	88 %
(Rapsch et al., 2006)	92 %	94 %
(Molloy et al., 2005)	98.20 %	98.30 %
FEC studies	Sensitivity	Specificity
(Kelley et al., 2021) 2 g faeces	88 %	not determined
(Boray, 1969)	30 %	not determined
(Rapsch et al., 2006) 10 g faeces	69 %	not determined
(Anderson et al., 1999) 5 g faeces	67 %	100 %
(Charlier et al., 2008) 4 g faeces	42 %	100 %
(Charlier et al., 2008) 10 g faeces	63 %	96 %
(Mazeri et al., 2016) 5 g summer	81 %	99 %
(Mazeri et al., 2016) 5 g winter	77 %	99 %
(Mazeri et al., 2016) 5 g autumn	58 %	99 %

prior information on the posterior estimates of the diagnostic tests, by running the same model with uninformative priors, beta (1, 1), for all the diagnostic tests and for just the prior sensitivities. The full Bayesian LCM model, as a R script, is found in the supplementary material. The published sensitivities and specificities for the three diagnostic tests used in Herds A and B are presented in Table 1 and the priors constructed from these data are shown in Table 2.

3. Results

The diagnostic test results from 109 cows from Herd A and 99 cows from Herd B were used to build the Bayesian LCM model. The bulk milk ELISA SP% from Herds A and B were 166 % and 286 % respectively, indicating ≥50 % of cows infected in each herd. The frequency of each possible combination of the three-liver fluke diagnostic tests results for Herd A and Herd B, are shown in Table 3.

The degree of inter-test agreement for positive and negative test results is shown in Figs. 1 and 2. The data show that it was rare for all three tests to agree on a positive result: in only 9.7 % of cases of at least one positive result were all three tests positive (Fig. 1). In 23.2 % of cases both coproantigen and ELISA were positive, but FEC was negative, and for 48.6 % of the time the coproantigen result was positive when the other two tests were negative (Fig. 1). Only a small percent of ELISA and FEC tests were positive when the coproantigen test was negative, and no cases were ever positive for both ELISA and FEC when the coproantigen test was negative (Fig. 1). A different trend was seen in agreement between negative test results with the highest level of agreement (47.4 %) occurring when ELISA and FEC gave the same (negative) result (Fig. 2). In 12.1 % of cases all three tests gave a negative test and the coproantigen test was never negative when the other two tests returned a positive result (Fig. 2).

The posterior distributions for the covariance between the sensitivity and specificity of the coproantigen test and the sensitivity and specificity of FEC, respectively, included zero, so the results from the Bayesian LCM model with no covariance factor are presented.

The estimates of sensitivity and specificity for the three diagnostic tests determined by MCMC simulation are shown in Table 4. The diagnostics for the model were satisfactory, the trace plots for each of the estimated parameters showed that the MCMC algorithm had converged to a stationary distribution (Fig. 1, supplementary material). In addition, the Gelman and Rubin point estimate was 1, for all the estimated parameters, and the model also passed the Heidelberger-Welch convergence test. Geweke's diagnostic test showed that a burn-in period of 30,000 was required to give a result of less than 2 for all the estimated parameters for both chains. The estimated median prevalence of liver fluke infection in Herd A was high at 0.67 (95 % CI 0.54–0.78) and even higher in Herd B at 0.99 (95 % CI 0.96–1.00).

The results of the sensitivity analysis showed that when uninformed diagnostic sensitivity priors were used, the posterior estimates for the median sensitivity and median specificity of all the diagnostic tests were similar to the results of the informed model. However, when uninformed priors were used for both the sensitivity and specificity of the diagnostic tests, the posterior estimates for median sensitivity of the coproantigen ELISA and for the median specificities of all three diagnostic tests were much lower, with wider 95 % credible intervals.

Table 2
Informed beta distribution priors used for each liver fluke diagnostic test in the Bayesian LCM.

Test	Sensitivity	Specificity
IDEXX antibody ELISA	Beta (10.0,1.3)	Beta (9.2,1.3)
Coproantigen ELISA	Beta (8.0,1.4)	Beta (23.1,1.2)
FEC	Beta (1.5,1.2)	Beta (59.7,1.6)

Table 3
The cross-classified results of three liver fluke diagnostic tests completed on two West Coast dairy farms.

Coproantigen ELISA	IDEXX antibody ELISA	Faecal egg count	Frequency Herd A	Frequency Herd B
Positive	Positive	Positive	10	8
Positive	Negative	Negative	44	46
Negative	Positive	Negative	6	0
Negative	Negative	Positive	7	0
Positive	Positive	Negative	9	34
Positive	Negative	Positive	11	10
Negative	Positive	Positive	0	0
Negative	Negative	Negative	22	1

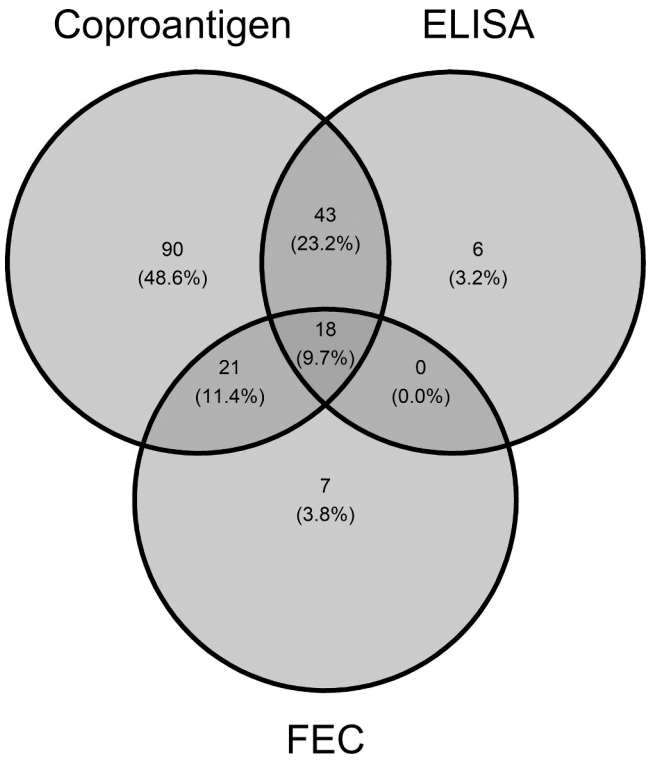

Fig. 1. Percentage agreement between positive test results for three diagnostic tests for liver fluke infection conducted on two West Coast dairy herds (n = 185) where at least one test gave a positive result.

4. Discussion

The aim of this study was to measure the accuracy of three diagnostic tests for liver fluke in New Zealand cattle. A Bayesian LCM was used to estimate the test characteristics of three liver fluke diagnostic tests when there was no gold standard test available, and without knowledge of the true infection status of individual cows, with the aim of identifying the suitability of these diagnostics tests for use in New Zealand cattle by veterinary practitioners. The estimated test characteristics for the coproantigen ELISA were Se = 0.98 (95 % CI 0.95–1.00) and Sp = 0.95 (95 % CI 0.81–1.00) for the IDEXX antibody ELISA were Se = 0.39 (95 % CI 0.32–0.47) and Sp = 0.86 (95 % CI 0.75–0.96) and for the FEC were Se = 0.23 (95 % CI 0.17–0.30) and Sp = 0.92 (95 % CI 0.86–0.97).

Whereas both the sensitivity and specificity estimates for the coproantigen ELISA test and the specificity estimates for the IDEXX ELISA and FEC tests were close to published studies, the sensitivity estimates for the IDEXX ELISA and the FEC were far lower than previously recorded (Table 1). The lower test sensitivity found for these two latter tests means that many false negative diagnoses, i.e., the animal is

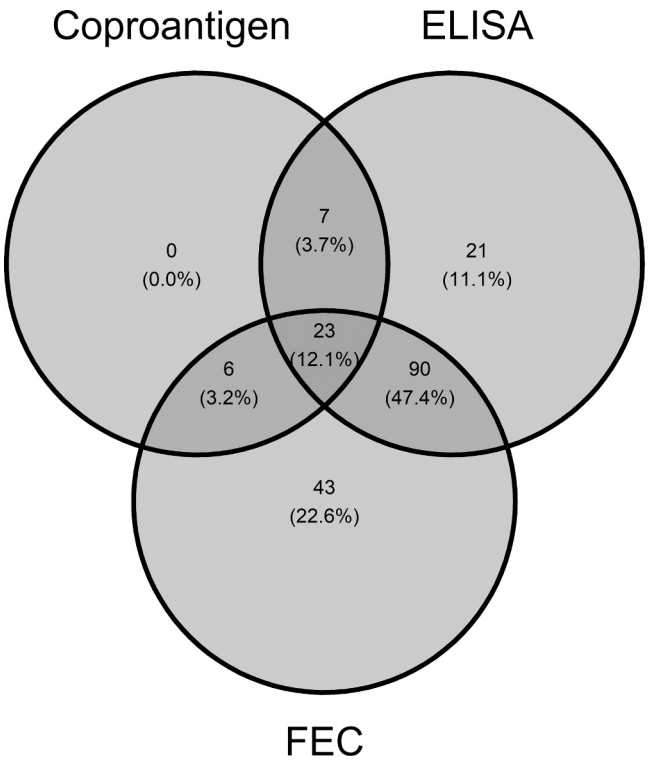

Fig. 2. Percentage agreement between negative test results for three diagnostic tests for liver fluke infection conducted on 2 West Coast dairy herds (n = 190) where at least one test gave a negative result.

Table 4
Results of Bayesian Latent Class Model showing the median and the 95 % Bayesian credible intervals for the true prevalence of liver fluke infection in Herd A and Herd B and for the test properties of the coproantigen ELISA test, the IDEXX antibody ELISA and the faecal egg count (FEC).

	Median	Lower credible interval	Upper credible interval
Infection prevalence Herd A	0.67	0.54	0.78
Infection prevalence Herd B	0.99	0.96	1.00
Se Coproantigen ELISA	0.98	0.95	1.00
Se IDEXX antibody ELISA	0.39	0.32	0.47
Se FEC	0.23	0.17	0.30
Sp Coproantigen ELISA	0.95	0.81	1.00
Sp IDEXX antibody ELISA	0.86	0.75	0.96
Sp FEC	0.92	0.86	0.97

infected, but the diagnostic test is negative, will result when they are used in the field. The poor sensitivity found for the FEC is not surprising when you consider that it is only able to diagnose patent infections whereas the coproantigen ELISA and IDEXX ELISA can diagnose both patent and prepatent infections. Also, it is important to note that the test characteristics estimated for the FEC are specific to the methodology described in this study i.e., a 5 g faecal sample and the use of a Flukefinder® and will be different for other FEC techniques used for diagnosing liver fluke infection.

When undertaking a FEC to diagnose liver fluke infection, it is important to consider the dynamics of egg excretion. Egg output from flukes in cattle peak 18–20 weeks post infection (wpi) before decreasing to very low levels at 38–40 wpi (Bouvry and Rau, 1986; Boulard et al., 1995). This means that false negative results, i.e., no eggs observed but adult flukes are still present in the bile ducts, are more common in long-standing chronic infections or when the fluke burden is low (Kelley et al., 2021). Antibody ELISA assays detect the presence of circulating antibodies produced by the host in response to the presence of the flukes (Walsh et al., 2021). These antibody ELISAs also have inherent problems which can manifest as false negative results whilst the immune response ramps up in response to new infection, and false positive results due to the persistence of antibodies after active infection has been eliminated. The antibody response is greatly influenced by other factors including naivety of the host; the number of metacercariae ingested and whether this is as an experimental infection or a natural infection (Walsh et al., 2021); which stages of the fluke lifecycle are present (Kuerpick et al., 2013; Robinson et al., 2008; Walsh et al., 2021); the immune capacity of the host; and the season of sampling (Charlier et al., 2008). In addition, the time taken for the antibodies to decay cannot be accurately determined in part due to the unknown titre at the time of elimination of the flukes and host variability. This means that antibody ELISA assays cannot always distinguish between historical and current infections and may be less useful for diagnosing actual infection in individual animals.

From the results of this study the coproantigen ELISA is clearly the most accurate assay for both confirming infection or freedom from infection. This assay detects ESA released from adult flukes into the faeces (via the bile) and does not rely on a host humoral response. The high accuracy of the test is very exciting, and means clinicians finally have access to a reliable liver fluke test for individual animals. While the coproantigen ELISA requires expensive, specialist equipment usually found in commercial and research laboratories, the overall cost of running the ELISA is similar to the FEC due to the ability to process multiple samples.

Currently lateral flow tests are being developed to allow rapid animal side testing (Aftab et al., 2024). Although none are commercially available, they do offer the promise of easy and cost-effective diagnosis.

In this study the faecal samples were frozen at −20°C for nearly 17 weeks prior to carrying out the coproantigen ELISA test. Although no literature was found on the effect of freezing on the performance of the test in cattle, faecal samples were frozen for 12 weeks in sheep and 12 months in red deer without impacting the optical density (Flanagan et al., 2011; French et al., 2016). Arguably an even greater impediment to the routine use of the coproantigen ELISA is the potential loss of antigen recognition in samples which are not frozen or preserved (Flanagan et al., 2011), representing a logistical problem particularly in remote areas.

When choosing which liver fluke diagnostic test to use, the veterinarian must first ask themselves what they are trying to accomplish. The wide-ranging test characteristics estimated in this study reinforces the limitations of using some diagnostic tests in an individual at one time point and the benefit of using tests in parallel (Drewe et al., 2010; Sekiya et al., 2013; George et al., 2019). However, despite its poor performance the FEC remains a useful diagnostic tool, especially for rural veterinary clinics with limited access to diagnostic laboratories. If liver fluke infection is suspected the presence of any eggs confirms this. Furthermore, a production limiting infection of 10 or more adult flukes can be inferred by the presence of one or more eggs in a 10 g faecal sample (Mezo et al., 2010). Performing repeated counts from the same faecal sample can further increase the sensitivity (Rapsch et al., 2006) making this a useful, if somewhat laborious, diagnostic test.

The reasonable specificity of the IDEXX ELISA does mean that this test also continues to have a place in the diagnostic toolbox. Since there will be very few false positives, a positive result is likely to indicate recent exposure to infection. However, as described earlier, the issue of antibody persistence after active infection is eliminated, means that the infection could either be historic or current. The test characteristics estimated in this study were for the individual animal but if a similar specificity was found when the IDEXX ELISA is performed on bulk milk, then a positive bulk milk test (BMT) should give good confidence that a herd is currently infected, since a flukicide treatment cannot be given to lactating cows. Unfortunately, if the BMT IDEXX ELISA has a similar low sensitivity to the individual test then a negative result is not as

informative and if there is a high level of suspicion that this herd could have liver fluke then a random sample of cows would need to be tested using the coproantigen ELISA test. The bulk IDEXX ELISA milk test was shown to be highly repeatable for herds sampled in consecutive weeks (Dowling, 2023) so serial sampling throughout the lactation may provide more useful information around the dynamics of infection in the herd. The number of cows to sample for the coproantigen test will depend on what you believe the prevalence of infection in the herd is e.g., if you believe the prevalence of liver fluke in the herd is > 0.3 then 9 faecal samples will be sufficient.

Both herds had a high estimated prevalence of liver fluke in the sampled cows with Herd B having almost 100 % of cows infected. The bulk milk ELISA SP% from Herds A and B were 166 and 286 respectively, indicating $\geq$50 % of cows infected, which supports the prevalence estimates from the LCM model and shows just how common liver fluke is on the West Coast (Dowling, 2023) and why sustainable solutions are needed.

The animals in this study were all sampled at a similar calendar date when infection prevalence in New Zealand is predicted to be at a peak (autumn). By restricting the sampling period to the autumn, this study has removed the potential seasonal bias reported by other researchers (Charlier et al., 2008; Mazeri et al., 2016). In the future it may be necessary to repeat the study in the spring to investigate whether the same seasonal effect is also present in New Zealand. However, it is possible that the seasonal effect seen overseas is an artefact of winter housing and that in New Zealand, where cattle are not housed and continue to ingest metacercariae into late autumn or early winter, a similar effect may not be seen. Another potential limitation of this study is that Herd B had a very high disease prevalence which could have affected the model performance, although all respective diagnostic trace plots showed good convergence. The sensitivity analysis showed that the posterior distribution of the diagnostic tests' specificity is largely a product of the prior assumptions for these specificities. This is a concern and was unavoidable owing to the small data set and the zero cell observations for some of the cross-classified results. However, the reliance of our study on strong priors should be considered when interpreting the results; if the priors assumed for specificity of the tests turn out to be incorrect, then our results will be extremely misleading. Future work should aim to repeat this analysis with a larger and more complete data set.

4.1. Conclusion

In conclusion, the coproantigen ELISA test performed very well under New Zealand field conditions and should be considered the preferred test for diagnosing liver fluke infection in individual live cattle. Future work should aim at repeating the study in spring to investigate if there are seasonal effects and using the coproantigen test to validate the test characteristics of the bulk milk IDEXX ELISA test.

Ethical approval

This experiment was performed under the approval of Massey University Animal Ethics Committee, Protocol 21/06

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110305.

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