

Liver fluke (*Fasciola hepatica*) co-infection with bovine tuberculosis in cattle: A prospective herd-level assessment of herd bTB risk in dairy enterprises

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Abstract

Co-infection of tuberculosis (TB) and helminths is recognized as a significant problem in regions where such pathogens are endemic and chronic cases exist. Co-infection can modulate the immune system leading to interference with diagnostic tests, increased pathological impacts and pathogen persistence. However, research has found that such interactions between pathogens can be context and species specific. Recent studies have suggested that liver fluke, *Fasciola hepatica*, infection may impact on immunological responses and diagnostics for bovine tuberculosis (bTB; caused by *Mycobacterium bovis*) in cattle. Where evidence of such interaction exists, there would be an onus on policy makers to adjust eradication programs to minimize impacts. We assessed the association between herd-level bTB breakdown risk and seasonal variation in liver fluke exposure based on 5,753 bulk tank milk (BTM) samples from 1,494 dairy herds across Northern Ireland. BTM was tested by an IDEXX antibody specific enzyme-linked immunosorbent assay (ELISA) using the 'f2' antigen as a detection agent. The ELISA determined the result based on a sample to (known) positive ratio (S/P%) from which binary status and categories of exposure were derived. Associations were tested using multivariable random effects models. Models predicting bTB risk were not improved with the inclusion of liver fluke exposure levels. Variations in modelling liver fluke exposure (S/P%, binary, categories of exposure) and bTB risk (skin test breakdowns, post-mortem confirmed breakdowns, breakdown size and lag effects) also failed to support associations (neither positive nor negative) between the pathogens at herd-level. These results, along with previously published animal-level data from Northern Ireland, suggest that the nexus between bTB and *F. hepatica* may have small size effects at the population-level. However, our results also highlight the high prevalence of *F. hepatica* in cattle in our study population, and therefore we cannot fully discount the potential hypothesis of population-level depression of immune response to *M. bovis* due to co-infection.

KEYWORDS

bulk milk sampling, concurrent infection, endemic disease, *Fasciola*, *Mycobacterium*

1 | INTRODUCTION

Co-infection is a known modulator of host immune response to infection in a number of systems (Graham, 2008; Mabbott, 2018). Such immunological modulation has significant effects on hosts, including exacerbating the impact of pathological progression and virulence of pathogens, the cost of which can ultimately be increased mortality risk (Ezenwa & Jolles, 2011). Tuberculosis co-infection with helminth parasites have been widely recognized as a case in point (Ezenwa & Jolles, 2011; du Plessis & Walzl, 2014; Rafi, Ribeiro-Rodrigues, Ellner, & Salgame, 2012).

There has been some empirical and theoretical progress in understanding the generalities of mechanisms impacting the outcome of co-infection (Graham, 2008). However, the patterns are often complex, being both context specific and species-specific (for host and parasite; du Plessis & Walzl, 2014; Ezenwa & Jolles, 2011; Rafi et al., 2012). Indeed, understanding co-infection dynamic effects on host susceptibility and infectivity is still an evolving area of research (Salgame, Yap, & Gause, 2013).

For the specific case of bovine tuberculosis (bTB) caused by *Mycobacterium bovis*, research has demonstrated that the diagnosis and pathological progression of infection could be modulated by co-infection with *Fasciola hepatica* (liver fluke), a common helminth parasite of cattle. Experimental infection studies indicate that co-infection in this system can lead to reduced immunological response during bTB diagnostic skin tests (based on reaction the tuberculin Purified Protein Derivative [PPD]), potentially reducing the efficacy of bTB control programs (Flynn, Mannion, Golden, Hacariz, & Mulcahy, 2007; Flynn et al., 2009). Recent experimental research has indicated that co-infected animals may harbour lower *M. bovis* burdens, suggesting a potential role for therapeutic effects of co-infection or the drive towards a latent stage of bTB infection (Garza-Cuartero et al., 2016). There has been some evidence presented that suggest that such interaction can have an impact at the herd level also. A negative association was found between bTB herd risk and liver fluke using a geospatial model of English and Welsh dairy herds based on bulk milk sample testing (Claridge et al., 2012), which was interpreted to indicate underascertainment of bTB disclosure in areas with co-infection.

In Northern Ireland, despite ongoing and costly eradication efforts, bTB herd-level incidence has risen in recent years to approximately 9% (DAERA, 2017). The herd-level prevalence for fluke, as estimated using abattoir surveillance data, exceeds 65% (Byrne et al., 2016), with levels of fluke exposure in dairy herds based on antibody detection being >90% (Byrne, Graham, McConville, et al., 2018). Both bTB and liver fluke are considered priority diseases in Northern Ireland, with considerable economic impacts. The bTB eradication programme costs over £37 million in 2017 (DAERA, 2017), and Northern Irish farmers bear a burden of £50 million related to production losses caused by fluke infection, and via costs of fluke control (Cooper, McMahon, Fairweather, & Elliott, 2015). Any association between bTB and *F. hepatica* infestation is therefore important in understanding the epidemiology of both diseases and guiding eradication efforts (Allen, Skuce, & Byrne, 2018).

To better understand the impact of co-infection between bTB and *F. hepatica* in Northern Irish cattle, a prospective longitudinal study was designed, comprising of an approximate 50% sample of active milking dairy herds in Northern Ireland (NI), to assess herd-level associations of *F. hepatica* with yearly bTB status. To estimate seasonal variation in liver fluke risk, bulk milk sampling from a statutory scheme was related to whole herd bTB test results. It was anticipated that this study would clarify the population-level association between *F. hepatica* and bTB in NI, and complement retrospective animal-level analyses from the same population (Byrne et al., 2019). Specifically, we wanted to test the hypothesis that there was a negative association between liver fluke infection risk and bTB breakdown risk at the herd level (*sensu* Claridge et al., 2012).

2 | METHODS

2.1 | Prospective bulk milk survey

Bulk tank milk (BTM) samples were selected from active, milk-producing dairy herds in NI, utilizing data submitted as part of a bulk milk surveillance scheme from 2016 (Diagnostic Surveillance and Investigation Branch [DSIB], AFBI). The bulk milk survey has been detailed elsewhere (Byrne, Graham, McConville, et al., 2018), however, the process is broadly outlined here. The design was a prospective longitudinal cohort study, with four cross-sectional cohort observations, aimed to sample approximately 50% of active dairy herds in NI periodically over 1 year (census data suggested a total 2,694 dairy herds were active during 2016). Given the resources available, we attempted to maximize the number of herds included within the study, while allowing for multiple samples per herd to capture seasonal variation in exposure (Byrne, Graham, McConville, et al., 2018). Herd selection was based on selected dairies that were representative of the whole target population in Northern Ireland. This was established by undertaking a representativeness study on data gathered during 2015. Selected herds from dairies were tested for their representativeness with respect to herd size (a potential risk factor and metric of production intensity), geographic spread (across all 10 Divisional Veterinary Office [DVO] areas) and bTB risk. Overall, no substantial differences in either herd size (modelled using a negative binomial; $p > 0.1$) or bTB risk (logistic model; $p > 0.1$) were detected between selected herds, relative to non-selected active herds across dairies (Byrne, Graham, McConville, et al., 2018). The herds were also widely geographically distributed, with all DVOs represented in the dataset (minimum 120 herds per DVO). This representativeness study suggested that the herd selection was not biased with regards key parameters of interest.

Samples were tested on four different occasions throughout 2016, representing each season: spring (March), summer (June), autumn (September) and winter (December). Each bulk milk was sampled using the ISO17025 accredited Enzyme-linked Immunosorbent Assay (ELISA) test at the DSIB, AFBI. The test kit employed was the IDEXX *F. hepatica* antibody ELISA kit that uses the 'f2' antigen to

TABLE 1 Interpretation of results from liver fluke ELISA for bulk tank milk samples (from manufacturers recommendations)

S/P%	Infestation level	Within-herd prev.
≤30%	0	Negative
>30 to ≤80%	+	<20% (low infestation)
>80% to <150%	++	20%–50% (medium infestation)
≥150%	+++	>50% (strong infestation)

detect *F. hepatica* antibodies. Optical Density (OD) values (absorbance) were obtained, and the presence or absence of antibody to *F. hepatica* was determined by the sample to positive (S/P%) ratio; based on a known positive sample provided in the IDEXX kit, and calculated using Equation 1.

$$\%S/P = \frac{100 \times \text{corrected OD value of the sample}}{\text{mean corrected OD value of POS control}} \quad (1)$$

Corrected OD values were calculated by subtracting the OD value of the corresponding wells coated with the negative control antigen from the positive control antigen well, with both having the sample added, in order to correct for non-specific antigen-antibody binding.

The prevalence of infestation in the herd can be correlated with these S/P% values (Reichel, Vanhoff, & Baxter, 2005) and were divided into groups of percentage infestation (within-herd prevalence; Table 1). Results were interpreted in line with research, manufacturer's recommendations and in-house validation (AFBI SOP, 2016; Charlier, Meyns, Soenen, & Vercruyse, 2013; Reichel, 2002; Reichel et al., 2005). Both binary (positive/negative) and categorical (negative, low, moderate, high; Table 1) levels of within-herd infection were modelled during the study. Furthermore, the raw S/P% values were modelled without categorization as a continuous (linear) predictor. The kit has been demonstrated to exhibit high sensitivity (95%–99%) and specificity (95%–100%) across studies (Molloy, Anderson, Fletcher, Landmann, & Knight, 2005; Reichel et al., 2005; Hutchinson and Macarthur, 2003 in Höglund et al., 2010). This test has very similar performance to other ELISA based tests used to assess fluke prevalence and seasonal variation thereof, allowing for cross-comparison (e.g. Ildana Biotech Kit, Dublin, Ireland, with a reported SE/SP of 98%; Bloemhoff et al., 2015).

2.2 | Bovine tuberculosis data

Anonymized bTB herd-level data were extracted from the Animal and Public Health Information System (APHIS) database (Houston, 2001). All NI herds undergo annual bTB testing, using the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. Different types of testing regime occur depending on the epidemiological context and herd-history (including annual whole herd tests, backward tracing tests, forward tracing tests, contiguous [neighbouring] herd testing). Where chronic breakdowns occur, ancillary use of severe

interpretation SICCT can occur, which can result in increased disclosure of reactors.

The SICCT test exhibits very high specificity (>99.9%; Goodchild, Downs, Upton, Wood, & Rua-Domenech, 2015; Lahuerta-Marin et al., 2018; Nuñez-García et al., 2018), and moderate animal-level sensitivity (50%–80%; De la Rua-Domenech et al., 2006; Lahuerta-Marin et al., 2018; Nuñez-García et al., 2018) under standard interpretation. However, herd-level sensitivity improves depending on true prevalence and number of animals tested (Cameron & Baldock, 1998; Martin, Shoukri, & Thorburn, 1992); for example, a herd of 100 animals with 3% bTB prevalence tested by a diagnostic that has 65% animal-level sensitivity, the herd-level sensitivity reaches 88% (calculated from Cameron & Baldock, 1998). All animals (both SICCT reactors and non-reactors) were assessed for the presence of visible bTB lesions at the abattoir as part of a passive disease surveillance program within NI. Additionally, laboratory confirmation of bTB lesions detected for *M. bovis* occurred over study period (Roring, Hughes, Skuce, & Neill, 2000). For the purposes of this study, a Lesion at Routine Slaughter (LRS) animal was an animal that disclosed as ante-mortem test-negative but was found to have bTB lesions at slaughter. Also for this study, a herd was considered to have experienced a confirmed bTB breakdown when either SICCT test reactors were disclosed with post-mortem evidence of bTB (by the presence of visible lesions and/or laboratory confirmation) or when a herd was found with an LRS animal (see 'Modelling Approach' for bTB metrics used).

2.3 | Modelling approach

Multivariable random effects (RE) modelling was used throughout, to control for the non-independence of multiple observations per farm (i.e. a herd random effect). A series of models were developed to assess whether there was an association between bTB disclosure within herds, their liver fluke status and levels of fluke infection. A base model was initially built to control for a-priori variables known to impact on bTB at the herd level on the island of Ireland (Byrne, White, McGrath, James, & Martin, 2014). Our aim was to develop a parsimonious base model that explained variation in bTB, and then add the fluke exposure data based on the BTM ELISA results. Following this, models were assessed to determine whether liver fluke variables explained additional variation in the outcome (following a similar approach to Claridge et al., 2012). The factors chosen for the base model have consistently been important predictors of herd-level risk in studies from the island of Ireland (Byrne et al., 2014; Denny & Wilesmith, 1999; Griffin et al., 2005) and elsewhere (Broughan et al., 2016; Humblet, Boschirol, & Saegerman, 2009; Skuce, Allen, & McDowell, 2012), and are potential confounders which should be controlled for in our model, while keeping the model as parsimonious as possible. The variables offered to the baseline models included herd size (log transformed), local cattle bTB prevalence (clustered within DVO areas) and metrics of bTB history. We generated a number of variables to capture bTB history,

including the confirmation status of the herd during the previous calendar year, breakdown status measured as the disclosure of one or more skin test reactors, LRS disclosed during the previous calendar year, or the total number of reactors disclosed during the previous calendar year. However, these metrics were highly correlated, and therefore we only included one bTB history metrics in each of our respective models to avoid multicollinearity problems. We included the parameter that improved the fit of the model most. The season during which the BTM sample was taken, and the proportion of the herd which was made up of dairy cattle breeds (this latter variable could help to identify herds with mixed [dairy and non-dairy] enterprises) were also offered to the model. To control for any underlying spatial heterogeneity unaccounted for within the model (Milne et al., 2019), the DVO that the herd was located within was included in multivariable models (spatial variables [DVO; 10 categories]).

Correlations, cross-tabulations and χ^2 tests were used to assess potential associations between independent variables. To avoid multicollinearity, predictors that were strongly associated were not entered into the same model. Instead, univariable random effects logit models were used to select which variables expressed the strongest association with the outcome, and models with lowest Akaike information criterion (AIC) retained for further model building of the base model. During the model building process, parsimonious models were selected through backwards selection, followed by re-entering removed variables, and then comparing competing models using AIC.

In final candidate models, fit (calibration) was assessed using a Hosmer–Lemeshow (LR) test applied to logit models without random effects (HL tests cannot be performed with random effects models). Final candidate random effects model discriminatory ability was assessed by calculating the Area Under the Curve (AUC). The convention that an AUC ≥ 0.7 suggested an adequate predictive model to discriminate binary outcomes was followed (Hosmer & Lemeshow, 2005). A likelihood ratio test was used to compare REs component of the models, against a non-cluster adjusted model, where the test assessed whether the alpha parameter was equal to zero.

During this study, all testing data available for each herd were used to assign bTB statuses to herds. As the animal-level sensitivity of the statutory test (SICCT) is moderate (Lahuerta-Marin et al., 2018; De la Rua-Domenech et al., 2006), we modelled bTB outcome in five ways. Firstly, we modelled herd risk as whether a herd had a confirmed breakdown, defined as a breakdown with one or more lesion and/or laboratory confirmed animals culled during the calendar year of 2016 (binary outcome modelled using logit distribution). Secondly, as only approximately 50% of SICCT reactor animals were confirmed as part of this process, we also modelled bTB risk as a herd with a SICCT test positive breakdown (logit model). These herds disclosed at least one skin test (SICCT) positive animal during 2016. Thirdly, the number of animals disclosed through skin testing during the year as a proxy measure of breakdown size was modelled as a count using a Poisson distribution, again applying a RE for herd. This was also followed-up by fitting zero-truncated Poisson (ZTP) models to counts of reactors

in only herds which disclosed reactors in 2016. These ZTP models employed the cluster variance-adjusted (sandwich estimator) to deal with multiple observations per herd. As some infections within herds may be missed during *ante-mortem* testing, we also modelled the probability of a herd having a LRS status in 2016 (that is, one or more LRS animals were found during the calendar year). Finally, we also explored potential for temporal lag effects with models being developed for 2015 and 2017.

Liver fluke exposure was also modelled in a three ways. In the first scenario, the raw S/P% values were included as a continuous linear predictor. In the second scenario, herds were categorized as positive or negative to *F. hepatica* (i.e. a binary outcome termed 'exposure status'). In the third scenario, a categorical predictor was generated using the continuous *F. hepatica* S/P% values, by categorizing herds into one of four *F. hepatica* infection level classes (negative, low, moderate and high, as recommended by manufacturers, termed 'categorical within-herd prevalence'). The impact of liver fluke exposure on the fit of the model was assessed by comparing the change in AIC, Bayesian Information Criterion (BIC), the change in log-likelihood tested using a likelihood ratio (deviance) nested test, and the change in discriminatory ability (measured using the AUC).

3 | RESULTS

3.1 | Summary results

There were 5,758 BTM samples taken from 1,494 herds within the dataset for liver fluke antibody testing. The median herd size of these farms was 165 animals (mean: 202; IQR: 104–257). 91.43% (1,366 herds) were sampled on four occasions over 2016; with 128 herds with three or less samples taken (8.57%). Five samples (5/5,758; 0.087%) failed to yield a test result (therefore $n = 5,753$).

On average, 1,438 (range: 1,421–1,449) herds were sampled each season, with the highest liver fluke infection risk being observed in winter and the lowest in summer. Overall, 401/5753 (6.97%) of samples were considered negative for liver fluke antibodies with a S/P% < 30%. 38.03% of samples were classed in the highest exposure (within-herd prevalence) category (2,188/5753; see Table S1).

There were 85 herds with standard skin test reactors (breakdowns) in 2015 (5.69%; 85/1,494 herds), 101 herds in 2016 (6.76%; 101/1,494) and 151 in 2017 (10.11%; 151/1,494 herds). In 2015, the proportion of herds which disclosed one or more confirmed animals within this cohort was 3.82% (57/1,494 herds), 2016 it was 4.42% (66/1,494 herds) and in 2017, 6.76% (101/1,494 herds). Median number of reactors disclosed in 2015 was 3 (mean: 4.73; IQR: 1–5; Max: 30), in 2016 was 2 (mean: 3.84; IQR: 1–4; Max: 32), and in 2017 was 2 (mean: 4.43; IQR: 1–5; Max: 29). One or more LRS animals were disclosed in 52 herds without standard reactors during a breakdown in 2015, 76 during 2016 and 54 during 2017.

Univariable sample-level models suggested (see Supporting Information for detail) limited evidence in support of relationships between bTB confirmed infection herd status and BTM liver fluke status (Fisher's exact χ^2 $p = 0.532$), categorical within-herd

prevalence (Fisher's exact $\chi^2 p = 0.138$), and the liver fluke S/P% result (two-sided t -test; $p = 0.340$; Figure 1). Similarly, there was little evidence for an association between herd breakdown status based on the presence of standard reactors and BTM liver fluke status (Fisher's exact $\chi^2 p = 0.150$) nor S/P% (two-sided t test; $p = 0.253$). There was some evidence of an association between herd breakdown status and BTM liver fluke categorical within-herd prevalence (Fisher's exact $\chi^2 p = 0.020$).

Unadjusted count models suggested that there was limited evidence for an association between herd reactor counts and BTM exposure status (unadjusted Poisson model: Incidence rate ratio 1.23; $p = 0.104$). There was variation in reactor counts between liver fluke within-herd prevalence categories, with a higher count of reactors in low fluke risk herds relative to fluke negative herds (unadjusted Poisson model: IRR 1.44; $p = 0.007$), and a negative relationship with increasing S/P% antibody levels (IRR: 0.999; $p = 0.039$).

There was little evidence of a relationship between the probability of LRS with liver fluke status (Fisher's exact: $p = 0.484$), nor with S/P% value (univariable logit model: $p = 0.246$). There was an association between LRS and liver fluke infection categories (Fisher's exact: $p = 0.005$); there was higher risk of LRS disclosure in herds with low and moderate level of liver fluke exposure relative to negative or high levels of liver fluke exposure.

3.2 | Multivariable models

3.2.1 | *Fasciola hepatica* association with confirmed bTB infection risk

There was co-linearity between the variables capturing historic (2015) bTB risk, with the preferred predictor used in model building

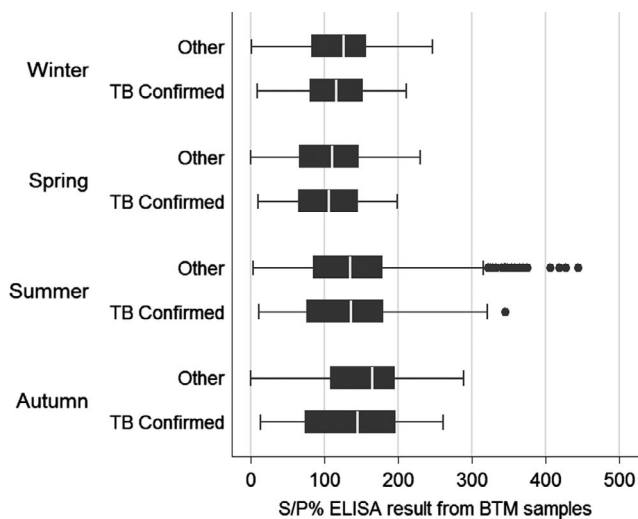


FIGURE 1 Boxplot depicting variation in S/P% values for liver fluke exposure measured by antibody ELISA using bulk tank milk (BTM) from dairy herds in Northern Ireland across (a) seasons and (b) amongst herds which had confirmed bTB infection, or did not, during 2016. There were no significant differences amongst bTB status, but there was significant seasonal variation in risk

being whether a herd had a breakdown in 2015. The most supported (lowest AIC) baseline random effect logit model for predicting 2016 herd bTB confirmation status only contained two independent variables—historic (2015) breakdown status and herd size. However, we also retained the DVO spatial predictor as a fixed effect, to control for any underlying spatial heterogeneity not controlled for in our model (e.g. Ballymena had lower confirmed bTB risk than any other DVO area in 2016; $p < 0.02$). The logit baseline model did not exhibit evidence of poor fit (HL test: $p = 0.893$), and exhibited fair discriminatory ability (AUC: 0.69; 95%CI: 0.66–0.73).

Adding the continuous *F. hepatica* S/P% antibody reading from the BTM samples as opposed to the herd prevalence grouping interpretations (see Table 1), did improve the random effect model's ability to discriminate bTB confirmation status marginally, however, there was considerable overlap between 95%CI (AUC prior to S/P% addition: 0.69 [95%CI: 0.66–0.73]; after addition: 0.71 [95%CI: 0.68–0.75]). Furthermore, the AIC (+54) and BIC (+61) increased with the addition of the variable, with such increases in units considered 'significantly' worse model (following Burnham & Anderson, 2004; Raftery, 1995; Table 2). The adjusted OR for liver fluke S/P% was 1.00 (95%CI: 0.99–1.01), with a p -value of 0.730, indicating little support for an association between liver fluke exposure as measured as a continuous linear variable. The log-likelihood for the model including liver fluke S/P% value was -294.66 , without the S/P% value variable the log-likelihood was -268.55 ; likelihood ratio (deviance) test (LR $\chi^2(df:1) = -52.22$; $p = 1.000$) indicated no improvement to the model with the inclusion of S/P% value.

Similar results were found when the liver fluke exposure was measured as a binary outcome (OR: 1.259; 95%CI: 0.128–12.367; $p = 0.843$; Supporting Information S5) or when categorized into within-herd prevalence classes (Negative versus low: OR: 1.257 [95%CI: 0.150–10.512]; Negative versus moderate: OR: 1.392 [95%CI: 0.178–10.889]; Negative versus high: OR: 1.397 [95%CI: 0.165–11.802]; $p = 0.990$; Supporting Information S6).

3.2.2 | *Fasciola hepatica* association with bTB skin test reactors herd risk

Unsurprisingly, a similar base model was selected for predicting breakdowns based on the disclosure of SICCT test reactors within herds in 2016 (as there was a strong association between SICCT breakdowns and confirmed breakdowns within herds; Fisher's exact: $p < 0.001$). The base model exhibited an AUC 0.70 (95%CI: 0.66–0.74), the non-clustered model did not suggest a lack of fit to the bTB data (HL test: $p = 0.427$). *F. hepatica* S/P% value was not strongly associated with skin test reactor risk (OR: 1.001; 95%CI: 0.995–1.007; $p = 0.655$). The addition of the *F. hepatica* S/P% value did not improve the model in terms of BIC (+5.645), but the AIC metric suggested models were equivocal (AIC -0.975). The AUC for the model including S/P% was 0.70 (95%CI: 0.67–0.74), and the likelihood ratio test did not support the model with the inclusion of the additional variable ($p = 0.085$). The final model is presented in Table S7.

Predictor	OR (95%CI)	SE	χ^2	DF	p
<i>F. hepatica</i> S/P% value	1.001 (0.993–1.010)	0.005	0.12	1	0.730
DVO district			15.31	9	<0.083
Reactor status 2015					
Breakdown herd versus non-breakdown herd	51.072 (11.869–219.770)	38.027	27.90	1	<0.001
Log (herd size)	6.890 (3.072–15.452)	2.839	21.93	1	<0.001

TABLE 2 Final logistic random effect model predicting confirmation status of herds for *Mycobacterium bovis* in 2016, assessing the relationship between *Fasciola hepatica* exposures modelled as a continuous S/P% value

Liver fluke infection status (liver fluke status measured as a binary variable) and the categorical classes of infection were also 'non-significantly' related to bTB breakdown risk in 2016 ($p = 0.342$ and $p = 0.810$, respectively); the point estimate from the binary model was OR 1.990 (95%CI: 0.482–8.215). The likelihood ratio test were $p = 0.121$ and $p = 0.951$, respectively. Model AIC (binary: +0.406; categorical: +5.689) or BIC (binary: +6.213; categorical: +25.548) values were not improved when fluke variables were added to respective models. Final models are presented in Tables S8–S9.

3.2.3 | *Fasciola hepatica* association with LRS risk

When modelling the risk of lesions at routine slaughter (LRS), we excluded any herds that disclosed standard reactors, therefore reducing the dataset marginally ($n = 5,145$). The base model had better discriminatory power than other models with an AUC of 0.74 (95%CI: 0.71–0.76).

No liver fluke variables were strongly associated with LRS risk in 2016 when added to baseline models (*F. hepatica* S/P% value: OR: 1.000 [95%CI: 0.993–1.006]; $p = 0.967$; herd infection status: OR: 1.082 [95%CI: 0.273–4.291]; $p = 0.911$; liver fluke infection class level: χ^2 (DF: 3) = 0.97; $p = 0.810$). Nor were any model improved with the inclusion of *F. hepatica* S/P% value (Δ AIC: +2.554; Δ BIC + 3.992), herd binary infection status (Δ AIC: +3.136; Δ BIC + 3.410) or liver fluke infection class level (Δ AIC: +22.936; Δ BIC + 42.573). None of the models improved the discriminatory performance of the base model, with 95%CI of the estimated AUC overlapping with the baseline model. Final models are presented in Tables S10–S12.

3.2.4 | *Fasciola hepatica* association with bTB breakdown size

Random effects Poisson count models were fitted to the number of reactors disclosed in 2016, while controlling for base model confounders. There was limited evidence that *F. hepatica* S/P% value ($p = 0.846$), herd infection status ($p = 0.874$) and liver fluke infection class level ($p = 0.998$) were associated with reactor counts per herd, while controlling for DVO district, herd size and bTB history, respectively in separate models. Both AIC and BIC increased when each respective liver fluke variable was added to the model.

Restricting the dataset to only herds which experienced a breakdown during 2016, there remained no evidence that the inclusion of

F. hepatica S/P% value ($p = 0.692$), herd infection status ($p = 0.849$) and liver fluke within-herd prevalence class ($p = 0.982$) improved a random effects Poisson model (all increased AIC values), while controlling for baseline confounders ($n = 394$). These findings were also supported when reactor counts were modelled using a zero-truncated negative binomial model (*F. hepatica* S/P% value: $p = 0.137$; herd infection status: $p = 0.451$; liver fluke infection class level: $p = 0.246$).

4 | DISCUSSION

During this study we did not find support for the hypothesis that there was a negative association between the levels of within-herd infection prevalence of liver fluke, based on bulk milk ELISA testing for *F. hepatica* antibodies, and herd-level bovine tuberculosis risk. We also did not find evidence of an association between liver fluke infection and lag historic bTB herd status in 2015 or 'future' effects in 2017 (see Supporting Information S16–S17). Point estimates from almost all models used to investigate co-infection association in our dataset were positive in direction, which conflicts with the initial hypothesis based on previous herd-level findings. The confidence intervals around these point estimates also straddled zero (or 1 when reporting odds ratios from logistic models).

These results concur with the overall findings of recent animal-level analyses of concurrent infection from Northern Ireland (Byrne et al., 2017; Byrne, Graham, Brown, et al., 2018; Byrne et al., 2019), but do not appear to be consistent with other recent herd-level epidemiological models from Great Britain (Claridge et al., 2012). Furthermore, our results do not appear to be consistent with animal experiments, whereby co-infection has reduced the immunological reaction during bTB testing, such that it could impact on the diagnosis of the pathogen (Claridge et al., 2012; Flynn et al., 2007). However, a recent review of the literature on the impact of fluke infection on *M. bovis* has highlighted variable results of co-infection studies between the two pathogens (Howell, 2017). Howell (2017) undertook a systematic review of liver fluke-bTB studies and found that while a number of studies demonstrated reductions in measures of bTB diagnosis (skin test response, IFN-g response, lesions or confirmation), generally the size effects were small and/or 'non-significant'. Furthermore, the research suggested that there was the potential for a number of biases, for example incomplete outcome data reporting, confirmation bias and selective reporting (e.g. results were over interpreted or claims were made that were not supported by the results; Howell, 2017).

An animal-level case-control study from Northern Ireland for animals that were exposed to *M. bovis* failed to establish an association between bTB visible lesion (VL) presence and abattoir reported liver fluke status (either current infection or historic infection based on scaring of the liver; Byrne et al., 2017). However, for animals in the same cohort with bTB-VLs, there was an association between maximum lesion size classes (categorized broadly as small, medium or large lesions) and liver fluke status (Byrne, Graham, Brown, et al., 2018), with fluke positive animals disclosing smaller lesions than animals without evidence of fluke infection. Using a larger surveillance dataset (~130,000 animals), Byrne et al. (2019) did not report an association with a number of metrics of bTB-SICCT results, presence of lesions, laboratory confirmation—and fluke status. However, there was a negative association found between the disclosure of lesions at routine slaughter (essentially, these are animals with bTB-VL lesions found at abattoir that have not been disclosed during ante-mortem testing) and fluke status. Broughan et al. (2009) reported that there was a decreased risk of bTB confirmation in bTB reactors and exposed non-reactors using an antibody ELISA to assess cattle liver fluke exposure status ($n = 400$). It was interpreted to mean that fluke co-infection could drive the false positive rate, however, given the potential for fluke to impact on the pathogenesis of bTB (e.g. by being associated with smaller size or counts of lesions, Byrne, Graham, Brown, et al., 2018 or reduced bacterial load Garza-Cuartero et al., 2016), it is possible that, if anything, liver fluke infection could reduce the bTB-VL confirmation rates of truly infected cattle. Other research from Northern Ireland on the impact of liver fluke status on SICCT tuberculin reaction sizes uncovered a negative association at univariable level, but this association was confounded by age-class (Byrne et al., 2019). Furthermore, the size effect was very small (<1 mm), indicating low clinical or practical impact, concurring with the conclusions of Howell (2017). Other experimental work found significant decreases in reaction to tuberculin when animals were co-infected with *F. hepatica*, however, the size effect was not great enough to elicit a change in interpretation of the skin test result in these experiments (Claridge et al., 2012). The differences between field and experimental findings are difficult to explain, but factors like the dose, timing and route given to experimental animals relative to natural exposure conditions may be important (Pollock, Rodgers, Welsh, & McNair, 2006).

Several factors may have contributed to the (lack of) effects found in the present study. Firstly, the strong *F. hepatica*-TB interaction effects may be only apparent under certain situations. For example, when a host experiences chronic untreated parasitic infection that severely challenges host immuno-competency. This situation is uncommon in farming practice in the UK and Ireland, where clinical bTB or liver fluke infection resulting in severe morbidity or mortality is rare (All Island Animal Disease Surveillance Report, 2015). With regards bTB, only 10 per 1.5 million animals slaughtered in the early 2000s in the Republic of Ireland had disclosed with generalized bTB (O'Keefe, 2006). All animals are bTB tested during annual herd tests (Abernethy et al., 2006), and despite the moderate sensitivity of the SICCT test, the repeated

nature of testing means many infected animals will be culled before major pathological progression occurs. Similarly, liver fluke is treated in Northern Ireland, and predictive models have been developed to inform farmers as to when to treat to maximize impact (Goodall, Menzies, & Taylor, 1993; McIlroy, Goodall, Stewart, Taylor, & McCracken, 1990; Ross, 1970). In developing countries where coordinated control schemes using ante-mortem testing may be limited, more severe pathological progression due to infection may occur. This may impact the nexus between fascioliasis and bovine tuberculosis infection (Kelly et al., 2018; Munyeme et al., 2012). Wild populations may also be governed by differing ecological rules than that of managed populations (Ezenwa & Jolles, 2015; Graham, 2008), as managed populations have a very strong evolutionary pressure on the pathogen due to test and cull (as is the case of bTB in Northern Ireland), and so too on detected infected hosts (Allen et al., 2018). For example, Ezenwa and Jolles (2015) found that anthelmintic treatment of free-ranging buffalo in South Africa resulted in increased animal survival, which then led to increases in bTB risk for the population. This was despite evidence to suggest at the individual-level clearing of helminth infection could improve immuno-competency.

Recent analyses looking at the interaction between helminths and bacterial disease highlight how idiosyncratic the outcomes of co-infections can be, depending on the host species and pathogen (du Plessis & Walzl, 2014; Rafi et al., 2012). Furthermore, the timing of exposure and infection by each pathogen can have an impact (Flynn et al., 2007) on the ultimate effect of co-infection (negative for the host [facilitation]; positive for the host [competition]; or neutral [non-interactive]).

4.1 | Limitations

One of the limitations of this study was that only dairy herds were appropriate for recruitment, as this work relied on bulk milk samples to assess levels of fluke infection within herds. Furthermore, only milking animals could contribute to the herd-level assessment of risk (for liver fluke), which means there are some within-herd biases introduced in terms of herd representativeness. Whilst non-dairy animals were excluded, previous research suggested that if an impact of co-infection was to occur, it may be more likely to occur in dairy than non-dairy herds (Broughan et al., 2009; DEFRA, 2005). We used all herd testing data available to assign bTB herd status. Where herd's experienced prolonged or recurrent problems clearing infection, additional removal of severe interpretation reactors may have occurred. The use of higher sensitivity testing may have increased the probability of disclosing truly bTB infected herds, which may have introduced some small positive bias into our classification for such herds. However, we used both ante- and post-mortem data, and multiple metrics of bTB status, in an attempt to mitigate some of the risk of biased disclosure.

A second limitation is that bulk milk testing gives an overall level of infection across the (milking) herd, but this may mask substantial inter-individual risk variation. However, we have undertaken additional analyses at the animal level to try to verify the conclusions

of this work (Byrne et al., 2017; Byrne, Graham, Brown, et al., 2018; Byrne et al., 2019). Taking both pieces of work (retrospective animal- and prospective herd-level analyses) together would tend to support the hypothesis of limited observed association between bTB and *F. hepatica* infection in Northern Ireland.

An additional limitation of this work is the absence of information to assess the impact of recent flukicide application to cattle. The timing of flukicides could potentially impact the immunological dynamics within hosts, such that the impact of co-infection may be diminished (Broughan et al., 2009). However, the literature on anthelmintics effects on tuberculosis control (e.g. by BCG vaccination) in humans has seen variable results (Abate et al., 2015; Salgame et al., 2013).

With regards to the base model used during this study, an a-priori decision to not include animal movement (trade) as a co-variable was made, in an attempt to retain parsimonious models. However, this omission could be criticized as some work highlights the importance of 'buying in' of infection (Brown, Marshall, Mitchell, & Byrne, 2018; Skuce et al., 2012). We explored this risk by assessing the relationship between herd confirmation or breakdown risk, and the inward movement of cattle into herds (measured in three ways) in 2015 (mean: 9.87; median: 2 animals/herd) and 2016 (mean: 9.97; median: 2 animals/herd), respectively (Table S18). At univariable level, inward movements were inconsistently associated with bTB risk (associated in 2/12 models; however, sign difference between the two models where evidence suggested an association; Table S18). Furthermore, exploring multivariable random effects models with the inclusion of a binary predictor for inward movement during 2016 did not improve model fit, nor had it any influence on the association with liver fluke exposure (e.g. Table S19). These results support our initial decision.

4.2 | Impact

These findings have impact for both policy makers and practitioners invested in the management bovine tuberculosis in endemic regions, which rely upon the accurate diagnosis of infected individuals (Allen et al., 2018; De la Rua-Domenech et al., 2006; Schiller et al., 2010). Co-infection management has been proposed as an important policy approach when moving towards eradication of notifiable pathogens (e.g. Claridge et al., 2012). Management of co-infection may have clear animal welfare and production benefits. However, in the case of bTB and liver fluke in Northern Ireland, our work suggests that there is little epidemiological evidence to support the hypothesis that changes in liver fluke prevalence will have large impacts on the management of bTB. However, a word of caution on the generalizability of our results: Ireland has very high liver fluke burdens relative to other countries across Europe and elsewhere (Byrne, Graham, McConville, et al., 2018; Byrne et al., 2016; Ducheyne et al., 2015; Selemetas et al., 2015). For example, 83% of Irish dairy herds have been shown to be exposed to liver fluke (Selemetas et al., 2015), 61%–65% of herds in Northern Ireland have liver fluke infected animals disclosed at slaughter (Byrne et al., 2016) and 38% of dairy

herds in Northern Ireland have high levels (>50%) of within-herd infection (Byrne, Graham, McConville, et al., 2018; present study). It may be that the potential for synergistic/antagonistic interactions between pathogens could arise when levels of infection are lower or more variable. Furthermore, there are potential differences in the impact of co-infection in the face of variations in anthelmintic use (and the parasitic resistance to such products; Kelley et al., 2016) in comparison with herds in the present study. Finally, because of the widespread nature of fluke infection, we cannot discount fully the possibility that there is a population-wide immunological depression in the national herd, potentially impacting both the tuberculin in the comparative test (avian PPD and bovine PPD), relative to other countries with lower fluke infection levels. We would strongly advocate, in the spirit of countering the claimed 'reproducibility crisis' in scientific reporting (Baker, 2016), for additional studies to assess whether our results can be replicated or refuted for other comparable populations.

5 | CONCLUSION

We have found little herd-level support for an association between levels of within-herd liver fluke prevalence, measured by antibody ELISA tests of bulk milk samples, and bTB risk measured using ante- and post-mortem diagnostics. This suggests at the population level, liver fluke co-infection may have limited measurable impact on bTB dynamics, or the size effects of such interactions are small relative to the variation across our dataset. While management of both pathogens is prudent from animal welfare and production (economic) perspectives, this research suggests that the management of liver fluke will have little measurable impact on bTB dynamics. However, further work to assess the impact of anthelmintic treatment, and the timing of such, is warranted to ensure any (even minor) animal-level potential benefits are maximized if bTB eradication is to be achieved.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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