Bovine tuberculosis (BTB) is a significant and intractable disease of cattle caused by *Mycobacterium bovis*. In the United Kingdom, despite an aggressive eradication programme, the prevalence of BTB is increasing with an unexplained, exponential rise in cases year on year. Here we show in a study involving 3,026 dairy herds in England and Wales that there is a significant negative association between exposure to the common, ubiquitous helminth parasite, *Fasciola hepatica* and diagnosis of BTB. The magnitude of the single intradermal comparative cervical tuberculin test used to diagnose BTB is reduced in cattle experimentally co-infected with *M. bovis* and *F. hepatica*. We estimate an under-ascertainment rate of about one-third (95% confidence interval 27–38%) among our study farms, in the hypothetical situation of no exposure to *F. hepatica*. This finding may in part explain the continuing spread of BTB and the failure of the current eradication programme in the United Kingdom.
Accurate diagnosis of an infectious disease is vital for its control, eradication and for the treatment of affected individuals. The diagnosis of many diseases requires detection of the host’s immune response to the causative agent. Some pathogens, notably helminth parasites, impair their host’s immune response, increasing susceptibility to infection and also affecting the sensitivity of immunologically based diagnostic tests.

Bovine tuberculosis (BTB), caused by *M. bovis*, is a serious disease of cattle; affected animals show loss of productivity and the economic consequences for countries with endemic BTB are exacerbated by international restrictions placed on bovine exports. *M. bovis* is an important zoonosis; it is estimated to be responsible for ~10% of human tuberculosis (TB) in Africa and historically was a major public health problem in Europe, with ~2,500 deaths occurring annually in the United Kingdom in the 1930s before pasteurization of milk was introduced. BTB has been eradicated in several countries including much of the EU, Australia, Canada and most of the United States but it is still endemic in the United Kingdom, Ireland and New Zealand.

The United Kingdom has had an eradication programme in place since the 1950s, based on diagnosis using the single intradermal comparative cervical tuberculin (SICCT) test and immediate slaughter of positive animals, yet the incidence of BTB has increased exponentially in recent years. The number of new herd incidents in Great Britain in 2009 was 4,525 and government expenditure on the eradication programme was £108.4 million. The number of cattle slaughtered in 2010 was 32,411 (4,703 new herd incidents) and provisional statistics suggest an overall increase in TB incidence rate of 5.8% in 2011 compared with the same period in 2010. By comparison, in 1999 the total cost of controlling BTB was £8.2 million with 1,666 new herd incidents recorded. It has proved difficult to account not only for the increase in new incidents of BTB within endemic areas but also the spread of infection into new areas. Several factors have been identified as contributing to this increase such as cattle movements and reservoirs of infection within wildlife species, notably the Eurasian badger (*Meles meles*). Restrictions are now placed on cattle movements with pre-movement testing introduced in 2001, but despite this, incidence is continuing to increase with spread predominantly local but with a small number of long-range jumps.

The SICCT test, approved for use in the EU and the principal test used in the United Kingdom and Ireland for ante-mortem identification of infected animals, measures a delayed type hypersensitivity response to the tuberculin antigen-purified protein derivative (PPD) and is dependent on functional antigen-specific T-cells and their capacity to secrete interferon-γ (IFN-γ). The diagnostic sensitivity of the SICCT test is estimated to be between 52–100% with a median value of 80% using the standard interpretation of the test. Several factors may account for the poor sensitivity of the SICCT test; one factor that has been considered but not fully evaluated in the field is the effect of concurrent infection with pathogens that may suppress the immune response to *M. bovis*.

*F. hepatica* is a common trematode parasite of livestock worldwide. The prevalence of *F. hepatica* infection has increased substantially between 2000–2009 in the United Kingdom, partly due to changes in the climate that foster the development of the intermediate host and free-living stages of the parasite, partly due to changes in land use, in particular drainage practices, and partly due to the lack of drugs licensed for use in milking cattle and increasing evidence of resistance to triclabendazole, a drug commonly used for the treatment of *F. hepatica* infection. Recent estimates indicate a herd-level prevalence of 70–80% in the UK dairy herd. *F. hepatica* is known to induce an anti-inflammatory state in its host; pro-inflammatory cytokine responses are suppressed, increasing susceptibility to intracellular pathogens normally controlled by pro-inflammatory responses, such as *Bordetella pertussis* and *Salmonella dublin*. In cattle experimentally co-infected with *F. hepatica* and *M. bovis*, Bacille Calmette Guerin (BCG), the SICCT test and PPD-specific IFN-γ responses were negative in 7/9 and 8/9 animals, respectively, whereas four out of five cattle infected with BCG alone were positive by both SICCT and the IFN-γ tests. This trend was true irrespective of whether BCG was given before or after *F. hepatica*. These observations led us to investigate if *F. hepatica* infection in cattle compromises the sensitivity of the SICCT test used in the United Kingdom to diagnose BTB in the field.

We show here that a significant negative association exists between exposure to *F. hepatica* and diagnosis of BTB, and that the magnitude of the SICCT test used to diagnose BTB is reduced in cattle experimentally co-infected with *M. bovis* and *F. hepatica*. These results indicate that in the presence of *F. hepatica*, the SICCT test used to diagnose BTB is compromised; we estimate that in our study farms, in the hypothetical situation of no exposure to *F. hepatica*, there is an under-ascertainment rate of about one-third (95% confidence interval (CI) = 27–38%). This finding may in part explain the continuing spread of BTB and the failure of the current eradication programme in the United Kingdom.

**Results**

**Prevalence of BTB and *F. hepatica* in England and Wales.** Data on the prevalence of BTB and exposure to *F. hepatica* in 3,026 dairy herds in England and Wales were collected and the national smoothed prevalence for each pathogen calculated (Fig. 1a and b). Smoothened BTB prevalence in 2004–2007 was highest in the south and west of England and Wales (Fig. 1a). Three large clusters are apparent: northern Cornwall and Devon, the south Midlands and the southwest of Wales. A fourth, smaller and less-intense cluster is visible north of the Midlands, centred on Derbyshire. This map of BTB prevalence presents a similar picture to previous maps of BTB distribution in Great Britain calculated from all beef and dairy farms rather than, as here, a sample of dairy farms that were originally tested for exposure to *F. hepatica* using a bulk tank milk test.

The smoothed *F. hepatica* percentage positivity (pp) value was greatest in the west of Britain (Fig. 1b). Four major clusters are the northwest of England (Cumbria), an adjoining cluster stretching south through Lancashire to the north Midlands, the northwest of Wales and a small part of southwest Wales. Some parts of Cornwall and Devon in the far southwest have moderate *F. hepatica* pp values.

Comparison of Fig. 1a and b shows that there is almost no overlap in the west of England and Wales in the distributions of the intense clusters of BTB prevalence and *F. hepatica* pp value. In the northwest of England and the northwest of Wales, *F. hepatica* pp values are high and BTB is of very low prevalence. In south Wales, the cluster of high *F. hepatica* pp values lies approximately between two clusters of high BTB prevalence. In the Midlands, a large area of high BTB prevalence is of low *F. hepatica* pp value. In Cornwall and Devon, and in the north Midlands, both BTB prevalence and *F. hepatica* pp values are at moderate levels. In contrast to the situation in the west of England and Wales, both BTB prevalence and *F. hepatica* pp values are low in most of the east of England.

**Multivariable logistic regression models for BTB.** One hypothesis for the strong negative spatial association between BTB and *F. hepatica* is that the two infections do not directly interact, but that a mutually exclusive set of environmental conditions favour BTB and *F. hepatica*, respectively. To test this hypothesis, we developed a model for BTB, based on a previously published model that successfully predicts BTB presence or absence using a combination of environmental and farm-level variables, including animal movements between farms. If BTB presence or absence is not affected by *F. hepatica*, then smoothed square-root-transformed *F. hepatica*...
Tables 1 and 2 | Multivariable logistic regression model of BTB occurrence excluding smoothed square root of *F. hepatica* pp value.

| Predictor | Coefficient | SE    | P      | OR   | L 95% CI   | U 95% CI   |
|-----------|-------------|-------|--------|------|------------|------------|
| Constant  | −30.045     | 3.577 | <0.001 | 1.0  | 1.83       | 4.21       |
| F. hepatica_PP | −0.109     | 0.016 | <0.001 | 0.91 | 0.89       | 0.94       |
| NN_BT | 1.020      | 0.213 | <0.001 | 2.77 | 1.83       | 1.65       |
| HerdSize | 0.182      | 0.021 | <0.001 | 1.20 | 1.15       | 1.25       |
| NDVI     | 0.009      | 0.002 | <0.001 | 1.01 | 1.01       | 1.01       |
| Sqrt MOff | 3.595     | 0.147 | <0.001 | 3.86 | 2.90       | 5.14       |
| MeanT    | 1.350      | 0.335 | <0.001 | 0.09 | 0.05       | 0.17       |
| VPD      | −2.443     | 0.158 | <0.001 | 1.17 | 1.10       | 1.25       |

Abbreviations: F. hepatica, percentage positive ELISA value; NN_BT, nearest neighbour’s smoothed BTB probability; HerdSize, square root of herd size; NDVI, normalized difference vegetation index; Sqrt MOff, root of movements off farm; Mean T, mean temperature (°C); VPD, Vapour Pressure Deficit is a measure of lack of moisture equilibrium between an object and the atmosphere, the higher the VPD, the more rapid the rate of desiccation; TVar, temperature variance, indicates the magnitude of the seasonality, that is, larger values indicate larger differences between winter and summer extremes; OR, odds ratio; L/U 95% CI, lower and upper 95% confidence intervals of OR. Log-likelihood = −1016.374.

Table 2 | Multivariable logistic regression model of BTB occurrence including smoothed square root of *F. hepatica* pp value.

| Predictor | Coefficient | SE    | P      | OR   | L 95% CI   | U 95% CI   |
|-----------|-------------|-------|--------|------|------------|------------|
| Constant  | −33.561     | 3.861 | <0.001 | 0.61 | 0.56       | 0.67       |
| F. hepatica_PP | 0.046     | 0.046 | <0.001 | 1.59 | 1.03       | 2.47       |
| NN_BT | 0.467      | 0.223 | 0.036 | 1.18 | 1.13       | 1.23       |
| HerdSize | 0.163      | 0.022 | <0.001 | 1.18 | 1.13       | 1.23       |
| NDVI     | 0.015      | 0.002 | <0.001 | 1.18 | 1.13       | 1.23       |
| Sqrt MOff | 0.089     | 0.017 | <0.001 | 0.91 | 0.88       | 0.95       |
| MeanT    | 1.196      | 0.154 | <0.001 | 3.31 | 2.45       | 4.47       |
| VPD      | −2.344     | 0.352 | <0.001 | 0.10 | 0.05       | 0.19       |
| TVar     | 0.071      | 0.036 | 0.048 | 1.07 | 1.00       | 1.15       |

Abbreviations: F. hepatica, percentage positive ELISA value; NN_BT, nearest neighbour’s smoothed BTB probability; HerdSize, square root of herd size; NDVI, normalized difference vegetation index; Sqrt MOff, root of movements off farm; Mean T, mean temperature (°C); VPD, Vapour Pressure Deficit is a measure of lack of moisture equilibrium between an object and the atmosphere, the higher the VPD, the more rapid the rate of desiccation; TVar, temperature variance, indicates the magnitude of the seasonality, that is, larger values indicate larger differences between winter and summer extremes; OR, odds ratio; L/U 95% CI, lower and upper 95% confidence intervals of OR. Log-likelihood = −953.26.

Figure 1 | Smoothed distribution of BTB and *F. hepatica*. (a) Smoothed log-odds of an individual dairy animal having been detected as BTB-positive in 2004–2007. BTB data for 3,026 farms also tested for exposure to *F. hepatica* were obtained from the UK Department for Environment, Food and Rural Affairs (DEFRA) VETnET database. A data cleaning process was undertaken to ensure only records pertaining to actual livestock tests were analysed; (b) Smoothed square root of the pp value for *F. hepatica*. To determine the prevalence of exposure to *F. hepatica*, bulk milk samples were obtained from the same 3,026 dairy farms in England and Wales and tested using an antibody detection ELISA. Samples were obtained from three major milk-testing companies in England and Wales between November 2006 and January 2007.

pp value (our variable, *F. hepatica_PP*) should not explain any additional variance in a statistical model for BTB driven by environmental, farm and movement variables. We therefore developed a multivariable logistic regression model for the occurrence of BTB using a sample of 1,821 farms, with model validation undertaken on the remaining subset, using proxies of the most important published variables6,7, without and with *F. hepatica* (Tables 1 and 2). In the best model excluding *F. hepatica*, proxies of six variables found to be significant in previous studies6,7 were highly significant (all *P* < 0.001), as was herd size (*P* < 0.001). The risk of BTB being present on a farm increases with higher average temperature and more variable temperature and increases slightly with the normalized difference vegetation index (NDVI), but decreases with higher vapour pressure deficit (VPD). The risk increases when the nearest neighbouring farm has a higher probability of being BTB-positive and for larger herd sizes, and the risk decreases with larger numbers of movements off the farm.
Importantly, when we add Fasciola_PP to the model, all the variables remain significant (with negligible change in coefficients and odds ratios) but Fasciola_PP has an additional, significant, negative effect on the odds of BTB being present on a farm (OR = 0.61; 95% CI = 0.56–0.67, P < 0.001; Table 1). The model that includes Fasciola_PP fits the data significantly better than the model without that variable (difference in deviance = 126.22, P < 0.001). We therefore conclude that F. hepatica is an additional environmental risk factor for BTB and is negatively associated with the odds of BTB being diagnosed on a farm.

The effect of Fasciola_PP on the odds of detection of BTB, after the inclusion of environmental and farm-level variables, is robust at smaller spatial scales than in England and Wales, including both countries separately, southern England and southwest Wales.

Under-ascertainment rate of BTB in the absence of F. hepatica. To assess the impact of F. hepatica on BTB detection in England and Wales, we estimated the probability of farms having BTB given their environmental and farm-level characteristics, in the presence and (hypothetical) absence of F. hepatica. The results indicate that, in the absence of F. hepatica, the probability of farms being diagnosed with BTB would increase substantially in several parts of England and Wales (Fig. 2). The most marked increases are in the southwest, Wales, the northern Midlands (Derbyshire), and Lancashire and Cumbria in the northwest. Using our model, we estimate that in the hypothetical absence of F. hepatica, the detected prevalence of BTB in our study farms would increase from 35.5 to 53%, a difference of 17.5% (95% CI, 14.5–20.4%). This equates to an under-ascertainment rate in our study farms of about one-third (0.175/0.53; 95% CI = 27–38%).

Experimental infection of calves with M. bovis and F. hepatica. The negative association between the presence of F. hepatica and detection of BTB could be explained by F. hepatica impairing the SICCT test used to diagnose BTB as there is evidence to suggest that F. hepatica infections reduced bystander IFN-γ responses and compromised SICCT tests in calves infected with BCG, an avirulent strain of BTB\(^{12}\). To address this, six calves were experimentally infected with 150 metacercariae of F. hepatica, 4 weeks later they were challenged with a target dose of 5×10⁵ colony-forming units (CFU) of a virulent strain of M. bovis; six calves were infected with M. bovis alone. SICCT tests were conducted 10 and 21 weeks after M. bovis infection (Table 3). The data were analysed using a linear mixed effects model fitted by maximum likelihood with week and co-infection as fixed effects and cow as a random effect. SICCT responses of co-infected cattle were significantly less than animals infected with M. bovis alone (\(P = 0.011\)), and were significantly less at week-21 post infection compared with week-10 post infection (\(P = 0.0013\)). The effect of co-infection did not change between 10 and 21 weeks post infection (\(P = 0.235\)). These results show that cattle co-infected with F. hepatica react less strongly to the SICCT test than those infected with M. bovis alone over a significant period of time.

**Discussion**

Here, we show a strong negative spatial association between diagnosis of BTB and exposure to F. hepatica using 3,026 dairy herds in England and Wales. When we included smoothed square-root-transformed F. hepatica pp value into a previously published model of BTB, which successfully predicted BTB presence or absence using a combination of environmental and farm level variables, including animal movements between farms\(^6\), the model fitted the data significantly better than the model without the presence of F. hepatica, confirming that the negative spatial association between BTB and F. hepatica was not associated with a mutually exclusive set of environmental conditions favouring BTB\(^{5,7}\) and F. hepatica\(^{10}\), respectively. This leads to the conclusion that F. hepatica is an additional environmental risk factor for BTB and, importantly, is negatively associated with the odds of BTB being diagnosed on a farm. These data suggest that in the presence of F. hepatica infection, the SICCT test is less effective, a conclusion that we corroborated by an experiment.
in which calves were infected with *M. bovis* alone or in combination with *F. hepatica*. The calves co-infected with *F. hepatica* reacted less strongly to the SICCT test than those infected with *M. bovis* alone when tested on two occasions at week 10 and week 21 after *M. bovis* infection. Previous studies have shown that cattle infected with *F. hepatica* and *M. bovis* BCG had significantly reduced skin thickness following standard SICCT tests, and this was associated with impaired IFN-γ responses and enhanced IL4, IL10 and transforming growth factor (TGF)-β secretion. Concors with murine models in which *F. hepatica* or specific *F. hepatica* antigens have been shown to modulate dendritic cell and macrophage function, leading to the induction of regulatory T-cell populations expressing TGF-β and IL10, which in turn inhibit bystander and auto-antigen-specific Th1 and Th17 responses and release of inflammatory mediators such as IFN-γ and IL12. Similarly in humans, helminth infections suppress T-cell and IFN-γ responses to PPD and reduce the efficacy of BCG vaccination. These data are consistent with the conclusion from our findings that in the presence of *F. hepatica* infection, the efficacy of the SICCT test used to diagnose BTB is compromised.

Although the magnitude of the response to the SICCT test in the co-infected calves was significantly less than that in calves infected with *M. bovis* alone, all six co-infected animals had responses that would be considered positive under field conditions (comparative increase in skin thickness of more than 4 mm), nevertheless the mean difference in skin thickness was 42% less than in those infected with *M. bovis* alone. It was not feasible to replicate under experimental conditions the situation in the field where animals are likely to have higher *F. hepatica* burdens and we have not yet explored the interaction between intensity and relative timing of each infection and the impact each may have on the SICCT. But it is plausible that a *F. hepatica* infection has the effect of pushing weak SICCT positives into a negative classification in the field where interpretation of the test is not always clear cut.

An alternative explanation for both our epidemiological analysis and the results from the experimental infections is that *F. hepatica* reduces the susceptibility of cattle to BTB. In a study in which 200 in-contact and 200 cattle classified as BTB-infected by SICCT (that is, reactors) were investigated in depth, a consistent negative association was found between the SICCT and the PPD-specific IFN-γ responses to PPD and reduced the efficacy of BCG vaccination. These data are consistent with the conclusion from our findings that in the presence of *F. hepatica* infection, the efficacy of the SICCT test used to diagnose BTB is compromised.

Methods

**F. hepatica** data. Bulk milk samples from 3,026 dairy farms in the England and Wales 13 were tested using an antibody detection enzyme-linked immunosorbent assay (ELISA) 27. The test result was expressed as the pp value, which is the ratio of the optical density reading for the test sample and the optical density reading for a positive control sample. A cutoff pp value of 27 was used to define a positive herd; at this cutoff the diagnostic sensitivity of the test is 96% (95% CI = 89–100%) and the diagnostic specificity is 80% (95% CI = 66–94%).

**BTB data.** BTB data was obtained for each of the 3,026 farms. Two sources of uncertainty are acknowledged in the BTB data. First, SICCT test sensitivity and specificity are imperfect, leading to false-positive and false-negative results. Positive test results are normally confirmed if typical TB lesions are found at post mortem examination or if positive culture for *M. bovis* is obtained. Owing to the low sensitivity of laboratory culture and the frequent absence of visible lesions, the number of animals reacting to the skin test was used to denote farms with BTB breakdowns, rather than the number of confirmed cases of BTB. Second, BTB testing occurs at different frequencies according to parish prevalence of BTB. Testing intervals range from every 1 to every 4 years respectively; but are also conducted when necessary for pre-movement testing, providing the farm has not been tested in the last 60 days; testing is repeated 60 days later if a reactor animal is detected. Once a herd is free from reactors it must test negative to two subsequent whole-herd tests, 60 days apart, to be considered ‘Officially TB free’. We analysed the cumulative incidence of BTB in the 3,026 farms from 1 January 2004 to 31 December 2007; this 4-year period was chosen to, first, ensure that all farms had been tested for BTB at least once and, second, include the period when the same farms were tested for *F. hepatica*.

Smoothing of *F. hepatica* pp value and BTB data. Smoothing is a statistical process that aims to capture important patterns in data while reducing noise. We used model-based geostatistics 28 to smooth the two sets of disease data. First, the 3,026 farms were randomly allocated to 1,984 to be used for model development (designated as analysis locations), while the remaining 1,042 (hold-out locations) were reserved for model validation. The underlying statistical model is that $Y_i = X_i + E_i$, where $Y_i$ is the observed value of a response variable at location $x_i$, $X_i$ is an unobserved spatially varying surface that is modelled as the realisation of a spatial stochastic process and $E_i$ is an independent random perturbation with variance $\sigma^2$ that accounts for sampling variation and/or micro-scale spatial variation in the immediate vicinity of $x_i$ (‘immediate’ meaning, roughly, less than the smallest distance between any two data locations). The properties of the stochastic process $X(\cdot)$ are encapsulated in its mean, its variance, $\sigma^2$ and the covariance, $\sigma^2(\rho)$ between values of $X(\cdot)$ at a pair of locations a distance $\rho$ apart, where $\rho$ is a parameter representing the rate at which the spatial correlation decays with increasing distance. For *F. hepatica*, we used the response variable square-root-transformed pp value, while for BTB we used the log-odds of prevalence.

The theoretical variogram of $Y$ is the function $v(\rho) = \tau^2 + \sigma^2(1 - \rho/\sigma)$.$\phi$. We specified the parametric model $\rho(\phi) = \exp(-\rho/\phi)$ by inspecting the sample variogram of the data, estimated the model parameters, $\mu, \tau, \sigma^2, \phi$ and $\sigma^2$ by the method of maximum likelihood and used the fitted model to compute the minimum mean square error predictor of $X(\cdot)$ at each of the 1,984 data locations, at each of the 1,042 hold-out locations and on a regular grid spanning England and Wales at 5×5 km resolution so as to generate a national map of the predicted spatial variation in $X(\cdot)$. The variograms for BTB and *F. hepatica* pp value are shown in Fig. 3.

The analysis was conducted using the R open-source software environment (www.r-project.org).

**Covariate data.** Covariate data were obtained for published descriptions of exemplary variables for BTB or close proxies of it. We derived environmental variables from both meteorological and satellite-derived data. Meteorological variables, including minimum and maximum monthly temperatures, and vapour pressure (VP) were obtained for the period 2001–2005, from the UK’s Meteorological Office as raster maps with a 5×5 km resolution. For each variable, data for each farm location were extracted from the raster layer using ArcGIS v 9.3 (ArcMap version 9.3, ESRI, USA). In brief, the raster layer was fitted to the base map containing point data on farm location, using the Georeferencing tool bar. The raster layer

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was then stretched to fit the underlying base map closely in ~50 positions around the coastline of the base map. The transformation option was highlighted from the georeferencing menu, and a first-order transformation was decided as most acceptable. The root mean square of the transformation should be less than or equal to the pixel size of the data set when checked. Point data pertaining to each test was added to the base map and values of the overlying raster layer were extracted to the attributes table for each newly created layer contained in a ‘variable point’ column that contained the newly extracted raster data as point data. Microsoft Access 2007 was then used to assimilate a table with each farm location and each raster layer value for that farm. The average temperature for each farm was then calculated from the maximum and minimum temperatures extracted. This method produces smoother values with fewer high outliers.

VPD was calculated by VPD = SVP − VP, where SVP = saturation vapour deficit (mb), VP = vapour pressure (mb) and \( \log_a \left( \frac{SVP}{500/((2305/T)-(500/((2305/((100000/T)))}} \) \right) \), where \( T \) = mean monthly temperature (K).

**Figure 3** | Empirical variograms for incidence of BTB, *F. hepatica* infection and the residuals from the fitted model. (a) Log-odds of the herd-level cumulative incidence of BTB; (b) square-root of the pp value; and (c) the residuals from the fitted model; the grey lines are empirical variograms computed from 99 independent permutations of the residuals over the locations. These provide an informal tolerance envelope for the empirical variogram when the residuals are spatially independent. A formal Monte Carlo test rejects the spatial independence hypothesis \( (P = 0.01) \) but the absence of a clear rising trend in the empirical variogram, in contrast to the behaviour of the other two variograms, suggests that the residual spatial correlation is relatively weak. The \( x \) axis, \( u \), denotes distance in kilometres; the \( y \) axis, \( V(u) \), denotes the estimated value of the variogram at distance \( u \).

**Table 4** | Complete list of variables available for multivariable logistic regression analysis.

| Variable      | Definition                                                                 | Source         |
|---------------|---------------------------------------------------------------------------|---------------|
| BTBID         | Identification number for each premises                                    | Assigned       |
| pp value      | Anti-*F. hepatica* antibody ELISA percentage-positivity value              | Ref. 14       |
| Sqrtpp        | Square-root transformation of pp value                                     | Calculated     |
| Fasciola_pp   | Smoothed extracted value of sqrtpp from map                               | Calculated     |
| BTBPosneg     | Whether a farm has tested positive for a BTB test during 2004–2007        | VETNET, DEFRA |
| NN_BTB        | Log-odds of BTB prevalence of nearest neighbouring farm                   | Calculated     |
| Total reactors, total confirmed | The total number of reactor animals found between 2004–2007. The total number of animals confirmed as BTB positive on PME or culture 2004–2007 | VETNET, DEFRA |
| SqrtHerd      | Herd Size, square-root transformed for this model                         | VETNET, DEFRA |
| NUTS2, NUTS1, AHOID, PCArea | The NUTS2 classification of the premises, the NUTS1 classification of the premises, the Animal Health Office classification of the premises, the postcode area of the premises | VETNET, DEFRA |
| NDVI, EVI     | Normalized difference vegetation index, enhanced vegetation index         | MODIS         |
| SqrtMOff, Sqrt(On+1) | Square-root transformation of cattle movements off the premises, Square-root transformation of one plus cattle movements onto the premises | RADAR, DEFRA |
| MaxT, MinT, RainyDays, RH, VP | The maximum temperature recorded on the farm (C), the minimum temperature recorded on the farm (C), the mean number of rainy days (rainfall >1mm) per year, mean relative humidity on the farm, mean vapour pressure on the farm | MetOffice |
| MeanT         | The mean temperature on the farm calculated as a mean of maximum and minimum | Calculated     |
| VPD           | Vapour pressure deficit                                                   | Calculated     |
| TVar          | Temperature variance, calculated from MODIS Fourier curves                 | Calculated     |
| EnglWales     | Location of a farm in either the England or Wales                          | Assigned       |
2004–2007 were obtained from DEFRA (RAPID) and Detection of Animal-related Risks). Finally, we estimated the log-odds of prevalence of BTB of the nearest neighbour, from our analysis farms, using the Hawth’s Analysis Tools extension (version 3.27, www.spatialalegory.com/htools/download.php) in ArcMap.

Data for herd size and movements on and off premises were square root transformed to normalise their distributions.

**Multivariable logistic regression model.** A BTB-positive farm was defined as a premises having at least one animal reacting positively to the BTB SICCT test in the 4-year period. The association between BTB and the explanatory variables was explored using binary logistic regression analysis (Minitab 16). Significance was determined at the 5% level.

The variables (Table 4) were initially available and tested in the logistic regression model. Variables were removed individually by backward elimination, beginning with the variable with the least significant P-value. This approach allowed any changes towards significance to be monitored with every variable removed. The final model contained only variables that were statistically significant (P < 0.05) and where the confidence limits around the adjusted odds ratio did not include 1. Stepwise regression and forward regression models produced the same final model.

Initially, 3,026 farms were included in the *F. hepatica* ELISA dataset. Four pairs of farms had different county-parish-holding (CPH) numbers but shared the same co-ordinates. Data were missing from 224 farms due to incomplete coverage by raster layers. This left a total sample size of 2,794 farms for regression analysis. These were randomly divided into two sets: 1,821 analysis farms, for model development, and 973 hold-out farms, for model validation.

A varioram of the residuals of the logistic regression model (Fig. 3) indicated some remaining but weak, unexplained spatial correlation, decaying from ~0.2 to zero over the range 0–30 km.

**Experimental infections.** All procedures were conducted under the Animals (Scientific Procedures) Act, 1986 and animals were housed in a containment level 3 facility for the duration of the experiment. Twelve castrated male Friesian calves aged between 17 and 21 weeks of age were used; six were infected with a single oral dose of 150 *F. hepatica* metacercariae of the Cullompton isolate (Ridgeway Research Ltd, UK). Four weeks later, all 12 calves were infected with *M. bovis* (iso- 

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**Author contributions**

J.C.: model development, data processing. P.D.: statistical analysis and smoothing methodology. C.M.Mc.: collection of milk samples and testing for antibodies to *F. hepatica*. G.M., R.E., J.M., S.S.: M.W.: experimental infection of calves with *M. bovis* and *F. hepatica*, immunological analysis, SICCT determination, post mortem v...
examinations and bacterial analysis. M.B., D.J.L.W.: conception of project, project design. The manuscript was written by J.C., M.B. and D.J.L.W. but all authors contributed.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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