Interpretative Summary

Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain

Velasova

There is a need for reliable nationwide information on dairy cattle health in Great Britain (GB). The aim of this study was to estimate the herd-prevalence of selected endemic infectious diseases in the population of GB dairy herds. Our results show that dairy cows across GB are frequently exposed to the studied pathogens and showed some geographical variation. More than half of farms showed evidence of exposure to bovine viral diarrhoea virus (BVDV) (66%), Mycobacterium avium subspecies paratuberculosis (Johnne’s disease) (68%), bovine herpesvirus type 1 (BHV-1) (62%) and Coxiella burnetii (80%). On approximately 50% of farms, antibodies against Leptospira hardjo and Salmonella spp were detected. Further, almost all farms (95%) showed evidence of exposure to Ostertagia ostertagi, 55% farms to Fasciola hepatica and 46% farms to Neospora caninum. Given the burden to efficient production that those pathogens pose, and in some cases their public health implications, the implementation of measures to control and possibly eliminate some of the studied pathogens should be given consideration.

PREVALENCE OF ENDEMIC DISEASES

Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain

Martina Velasova*, Angela Damaso*, Bhagyalakshmi Chengat Prakashbabu*, Jenny Gibbons†, Nick Wheelhouse†, David Longbottom†, Steven Van Winden*, Martin Green§, Javier Guitian*
ABSTRACT

In order to implement appropriate and effective disease control programs at national level, up-to-date and unbiased information on disease frequency is needed. The aim of this study was to estimate the prevalence of selected endemic infectious diseases in the population of dairy herds in Great Britain. Bulk milk tank (BMT) samples from 225 randomly selected dairy farms stratified by region and herd size were tested for antibodies against bovine viral diarrhoea virus (BVDV), bovine herpesvirus type 1 (BHV-1), Mycobacterium avium subspecies paratuberculosis (MAP), Leptospira hardjo (L. hardjo), Salmonella spp., Coxiella burnetii (C. burnetii), Fasciola hepatica (F. hepatica), Neospora caninum (N. caninum) and Ostertagia ostertagi (O. ostertagi). Furthermore, the presence of BVDV, C. burnetii and
Chlamydia-like organisms was determined by polymerase chain reaction (PCR). The apparent herd prevalence was estimated as a weighted proportion of positive farms. For some of the pathogens, the true prevalence was calculated by adjusting for the herd level sensitivity and specificity of bulk milk tests and the expected ability of the diagnostic test to classify as positive or negative farms above or below a minimum proportion of positive animals among those in milk. Amongst unvaccinated farms, the true prevalence of BMT antibodies against BVDV (number of farms, n=102) was estimated at 66% (95% Confidence Interval, CI: 56-77), MAP (n=222) 68% (95% CI: 59-77), BHV-1 (n=118) 62% (95% CI: 52-73), L. hardjo (n=111) 47% (95% CI: 34-60) and Salmonella spp. (n=209) 48% (95% CI: 39-56). The apparent prevalence of BMT antibodies against C. burnetii (n=221) was 80% (95% CI: 75-85), F. hepatica (n=224) 55% (95% CI: 48-62), N. caninum (n=222) 46% (95% CI: 38-54), and O. ostertagi (n=221) 95% (95% CI: 91-98). BVDV (n=225), C. burnetii (n=220) and Chlamydia-like (n=220) antigens were detected on 5% (95% CI: 2-9), 29% (95% CI: 21-36) and 31% (95% CI: 24-38) of farms, respectively. Our results show that dairy cows across GB are frequently exposed to the studied pathogens, which are endemic at high levels with some geographical variations. These prevalence estimates provide a much needed basis to assess whether nationwide control programs for the studied pathogens are justified by their potential economic, environmental and public health implications. Should surveillance and control programs be initiated, the estimates presented here are a baseline against which progress can be assessed.

Keywords: prevalence, endemic infectious disease, dairy cow, bulk milk, ELISA
INTRODUCTION

A number of infectious diseases of dairy cows such as bovine viral diarrhoea (BVD), Johne’s disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), infectious bovine rhinotracheitis (IBR) and liver fluke are generally regarded as being widespread and endemic in the United Kingdom (Carslake et al., 2011; Sekiya et al., 2013). These diseases are known to have a significant impact on dairy production due to their effect on fertility (Fray et al., 2000; Lanyon et al., 2014; Walz et al., 2015), milk production (Charlier et al., 2007; Tiwari et al., 2007; Richardson and More, 2009; McAloon et al., 2016) and subsequently on culling (Wilson et al., 1993; Tiwari et al., 2005; Murphy et al., 2006; Smith et al., 2010).

In Great Britain (GB), in 2005, the total costs of cattle endemic diseases (disease, control and prevention) was estimated to range from £800,000 ($984,675) for Johne’s disease and Pasteurellosis to £39.6 million ($48.7 million) per annum for BVD (Bennett and Ijpelaar, 2005). With the exception of bovine tuberculosis (bTB) in GB and BVD in Scotland, controlling such diseases is voluntary for GB farmers. The need to control endemic infectious disease can however be overlooked by farmers as it can be difficult to associate their presence with visible losses. This is often because clinical signs associated with such diseases on a given animal in an infected herd are absent, mild or non-specific, leading towards a general acceptance of their occurrence on dairy farms in endemic areas (Richardson and More, 2009; Cummings et al., 2010; Carslake et al., 2011; Statham, 2011). In such cases, from the farmers’ perspective, there is often very little, if any, financial incentive to control the disease (Stott et al., 2005). Nevertheless, examples from European countries suggest that the control or elimination of some of these pathogens (e.g. bovine herpesvirus type 1 in Scandinavian countries and Austria, BVDV in Sweden) can be achieved and would be beneficial (Ackermann and Engels, 2006; Lindberg et al., 2006).
When control programs are implemented, it is important, that they are accompanied by continuous monitoring of herd status to assess the effectiveness of the program and progress towards goals. This can be achieved through serological testing at herd level (Lindberg and Alenius, 1999; Houe et al., 2006). Testing of bulk milk samples is a particularly cost-effective strategy and has become part of surveillance and disease control programs for a number of endemic infectious diseases of dairy cattle (Paton et al., 1998; Leonard et al., 2004; Kim et al., 2005; Nielsen and Nielsen, 2012; Booth et al., 2013; Sekiya et al., 2013).

The application of a suitable disease control or elimination program at national or regional level and the monitoring of the progress of that program should be based on knowledge of the baseline frequency and distribution of the disease in the population (Dohoo et al., 1994; Ackermann and Engels, 2006; Humphry et al., 2012; Sayers et al., 2015). Such estimates can allow informed decisions on the justification of a program at national level and provide a baseline against which the impact of the control program can be assessed. With the exception of BVD in Scotland, for which a survey of Scottish dairy farms has recently been carried out to inform the Scottish BVD elimination program (Humphry et al., 2012), presently, in GB, there is a lack of reliable and up-to-date estimates of the prevalence of endemic diseases in the national dairy herd. This is because, for the majority of endemic diseases, there is no active disease surveillance in place. A number of private and public routine recording systems exist; however, at national level, the information they provide is likely to be biased (Velasova et al., 2015).

In addition to these ongoing recording systems, one-off surveys are often carried out (Paton et al., 1998; Pritchard, 1999; Davison et al., 2005; Salimi-Bejestani et al., 2005; Woodbine et al., 2009b; Williams and Winden, 2014), but although useful, their results should be interpreted with caution because of issues such as non-probabilistic selection of
studied farms (Paton et al., 1998; Woodbine et al., 2009b; Williams and Winden, 2014) and failure to adjust prevalence estimates for the study design (Paton et al., 1998) or for test performance (Davison et al., 2005; Woodbine et al., 2009a; Williams and Winden, 2014). Furthermore, one-off studies are only useful for a limited period of time, as the level of endemicity can change as a result of the implementation of control measures and changes in the dairy industry, the more apparent of which are increased herd size, genetic selection and application of new technological innovations (Barkema et al., 2015). It is therefore reasonable to assume that the few available estimated prevalence figures could no longer be accurate.

Accordingly, the aim of this study was to generate new information on the prevalence and distribution of selected major infectious diseases of dairy cows at national level to provide a basis for a future monitoring of disease trends over time and for the implementation of suitable and effective disease control or elimination programs at national level.

**MATERIAL AND METHODS**

*Study Population and Sampling Design*

A nationwide cross-sectional study of commercial dairy herds was conducted in Great Britain from April 2014 to March 2015. The study population was selected by means of stratified random sampling from a sample frame comprising 10,491 dairy farms, representing approximately 95% of the total population of all dairy farms in GB, held by the dairy industry (AHDB Dairy, division of the Agricultural and Horticultural Development Board). The farms were stratified by region (North England, Midlands, South East England, South West England, Scotland and Wales) and herd size (small: < 50 cows, medium: 50 - 149 cows, large: ≥ 150 cows). Equal number of farms within each stratum was selected using simple
random sampling. The total number \((n)\) of farms to study was calculated as follows (EpiCentre, IVABS, Massey University, New Zealand):

\[
\begin{align*}
n &= \frac{\sum_{i=1}^{e} \left(n_i \right)^2 \times p_i \times (1 - p_i) \times w_i}{N^2 \times \frac{AE^2}{Z^2} + \sum_{i=1}^{e} [n_i \times p_i \times (1 - p_i)]}, \\
W_i &= \frac{n_i \times \sqrt{p_i \times (1 - p_i)}}{\sum_{i=1}^{e} [n_i \times \sqrt{p_i \times (1 - p_i)}]}
\end{align*}
\]

Where \(e\) is the number of strata, \(n_i\) is the number of farms in strata \(i\), \(p_i\) is the expected prevalence in strata \(i\) (50% was used as worst-case scenario), \(N\) is the total number of farms in the population, \(AE\) is absolute acceptable error (7%), \(Z\) is a constant corresponding to a 95% confidence interval (1.96) and \(w_i\) is a weighting factor of each stratum. For purpose of sample size calculation, perfect sensitivity and specificity of the diagnostic tests were assumed. A total sample of 200 farms was found to be sufficient to generate the desired estimates and it was decided to aim to recruit 250 farms.

Based on previous experiences of the dairy industry, it was expected that around 20% of farmers contacted would be willing to participate in the study. The selected farmers were contacted by post, receiving information about the project and their participation. Farmers not responding to the initial letter received a reminder. Cattle veterinary practitioners were also informed about the project through the British Cattle Veterinary Association (BCVA) newsletter and by email and were also asked to encourage their clients to participate if they received a letter inviting them. Farmers who agreed to participate were included in the study and the status of their farms with respect to ten different pathogens was assessed. Selection of specific pathogens was based on the results of a workshop run by the Royal Veterinary College in April 2012. In the workshop, the participants were asked to identify and rank cattle health conditions considered important for individual farmers and the dairy industry and for which no reliable and up-to-date nationwide estimates were available (Velasova et al.,
Additionally, five more pathogens for which no up-to-date nationwide estimates were identified and which could be detected using bulk milk samples were included.

**Ascertainment of Disease Status**

Farm level status regarding ten different pathogens was assessed by testing a single or repeated bulk milk tank (BMT) samples for the presence of specific antibodies (Ab) (Table 1). In addition, for three of the pathogens direct detection of the antigen in bulk milk was carried out.

From each farm, a BMT sample of approximately 30 mL was collected and kept refrigerated until arrival at the laboratory. On arrival fresh milk samples were put into refrigerated storage set at the temperature between 1°C – 7°C. To each sample 5 mL of Bronopol preservative was added. Commercially available enzyme linked immunosorbent assays (ELISAs) were performed according to the manufacturers’ instructions. If there was an option for short and long incubation, the long incubation was used. The results were calculated according to the manufacturer’s instructions either as 1) percent positivity calculated as the ratio of the optical density of the sample (ODS) to the mean optical density of the positive control (ODPC) x 100 or as, 2) the percentage inhibition calculated as (1-ODS/ODNC) x 100, where ODNC is the mean optical density of the negative control.

The presence of BVDV antigen was studied by means of a real-time polymerase chain reaction (real-time PCR) protocol (TaqVet® BVDV screening test - LSI, France). The presence of *Coxiella burnetii* (*C. burnetii*) antigen was assessed by means of an in-house real-time PCR protocol developed by Klee et al. (2006). An in-house real-time PCR (16S *Chlamydiales* PCR) according to Lienard et al. (2011) was also used to detect *Chlamydia*-like organisms.
All the analyses were carried out on a single BMT sample with the exception of the detection of antibodies against MAP and *Fasciola hepatica* (*F. hepatica*) and the detection of BVDV antigen, which were carried out on four samples collected at three monthly intervals. This was carried out to increase the detection of positive farms considering the low diagnostic performance of bulk milk ELISA tests for the detection of MAP (van Weering et al., 2007) and *F. hepatica* (Reichel et al., 2005) and higher prevalence of BVDV amongst young stock (Booth et al., 2013).

**Data Collection**

A standardised questionnaire was used to gather information on general farm characteristics (i.e. herd size, production type), vaccination status, the main herd health problems as perceived by the farmer at the time of the visit and the farmer’s knowledge of the disease status of the farm for each disease in question. With respect to the disease status, the farms were divided into five categories: 1) disease definitely present (based on previous laboratory testing or abattoir monitoring), 2) disease present but unsure (no previous laboratory testing was carried out), 3) disease definitely not present (previous laboratory testing was carried out), 4) disease not present but unsure; and 5) unknown disease status. The questionnaire was designed in consultation with two veterinary practitioners and was piloted on four farms prior to use. Questions, which appeared to be unclear to farmers, were rephrased to improve the clarity. Interviews were carried out by seven interviewers (three qualified veterinarians and four final year veterinary students), all of whom were trained to ensure consistency and robustness of the collected data.

**Data Analysis**
All questionnaire data and the results of the laboratory testing were entered into a Microsoft Access 2007® (Microsoft Corp., Redmond, WA, USA) database. The accuracy of information in the database was cross-checked with the questionnaire. All categorical variables were summarised using frequencies and percentages. All continuous variables were checked for deviations from the normal distribution using histograms and the normality test for skewness and kurtosis. They were summarised using frequencies and medians with ranges (minimum and maximum). The descriptive statistics were carried out using Stata 11.2® (StataCorp, Texas, USA) software.

**Prevalence Estimation - Single Disease Testing.** For the estimation of prevalence at herd level, the results of assays were dichotomised as positive/negative based on the cut offs summarised and presented in Table 2. The apparent herd prevalence \((p)\) of individual pathogens at national level was calculated as the total number of positive herds divided by the total number of herds sampled weighted to account for the stratified study design. Sampling weights were calculated for each stratum as: 1/probability of a farm being selected. The probability of a farm being selected was calculated as proportion of farms studied in each stratum from the total number of farms in the stratum using the AHDB Dairy sampling frame described above. The population prevalence \((p)\) can be calculated as follows (EpiCentre, IVABS, Massey University, New Zealand):

\[
p = \frac{\sum_{i=1}^{e}(N_i \times p_i)}{N}
\]

Where \(e\) is the number of strata, \(N_i\) is the number of farms in the population in stratum \(i\), \(p_i\) is the estimated proportion of farms with the condition of interest in stratum \(i\), \(N\) is the total number of farms in the population (AHDB Dairy sampling frame). The weighted proportions and their 95% confidence intervals adjusted for the study design by estimating
the standard error using linearization method with a first order Taylor approximation of the point estimates were obtained in Stata 11.2 software (StataCorp, 2013).

The true herd prevalence was calculated for those conditions for which it was considered biologically meaningful to dichotomise farms as not infected or infected and for which reliable information on the diagnostic test performance was available. The latter information included: a) herd level sensitivity (Se) and specificity (Sp) of the diagnostic tests used; and b) a positive cut off value (as per test manufacturer instruction) to classify herds as positive or negative above or below this threshold. The point estimates and confidence intervals were adjusted for the Se and Sp of the diagnostic tests as described by Rogan and Gladen (1978). Information on herd level Se and Sp of the diagnostic tests as well as the minimum proportion of positive animals for the establishment of herd sensitivity and specificity was obtained either directly from the manufacturers or through available literature and is summarised in Table 2. In the case of the prevalence of Ostertagia ostertagi (O. ostertagi), F. hepatica, Neospora caninum (N. caninum) and C. burnetii only apparent prevalence is presented, as no reliable information on the respective diagnostic tests Se and Sp were obtained. All PCR tests were assumed to have 100% Se and Sp. Because of the inability of the antibody assays that were performed to distinguish between vaccinated and unvaccinated herds, vaccinated herds and herds for which vaccination status was unavailable were removed from the analysis.

Correlations between studied pathogens on unvaccinated farms were assessed by Phi correlation coefficient (φ) calculated as the square root of chi-square divided by n, the total number of observations (Olivier and Bell, 2013). A chi-squared test was performed to assess the association of herd status (positive/negative) with region or herd size. Variations in the prevalence taking into account the effect of both region and herd size (independent variables) were assessed using logistic regressions. The strengths of the associations was measured by
calculating adjusted odds ratios (ORs) and their confidence intervals (CI). Statistical significance of the associations of both independent variables to the farm status was tested using a Wald test at a relaxed significance level \( \alpha = 5\% \).

**Repeated Quarterly Testing.** The apparent and true herd prevalence of antibodies against MAP and *F. hepatica* and the presence of BVDV at each quarterly test were estimated as described above. Only farms that completed all four quarterly tests were included in the analysis. To estimate overall period prevalence, a farm was considered positive if at least one of the samples tested positive in a given quarterly test during the period of 12 months. The true period herd prevalence was then calculated based on a combined \( Se \) and \( Sp \) of the tests in parallel as: 

\[
Se_{combined} = Se \times n - (Se)^n \quad \text{and} \quad Sp_{combined} = Sp^n
\]

where \( n \) = number of tests carried out. Because not all the farms participated in all quarterly tests, the calculations of combined \( Se \) and \( Sp \) were performed based on a minimum of two quarterly tests carried out. The \( Se_{combined} \) and \( Sp_{combined} \) of MAP ELISA test in parallel were calculated as 1.0 and 0.85 respectively. For the BVDV PCR test \( Se_{combined} \) and \( Sp_{combined} \) of one were used.

**Farmers’ perception.** Positive and negative predictive values (PPV and NPV) were calculated as the proportion of farms on which farmers correctly classified the status of the farm with respect to the pathogens under study using the results of the BMT as the gold standard. Farms vaccinating against the studied pathogens or those were farmers did not know the status of the tested pathogens were excluded from the calculations.

**Spatial Analysis**
Choropleth maps showing the distribution of positive herds across the studied regions were generated, by dividing the number of positive herds by the number of herds tested within each region (where possible adjusted for the performance of the diagnostic tests used), using ArcGIS 10 (ESRI Inc., CA, USA, 2010) software. Presence of spatial autocorrelation was tested using the univariate Moran’s I test for global spatial autocorrelation and Queen contiguity (i.e. considering as neighbouring units those that have any point such as boundaries or corners in common). To account for the variation in number of farms tested and the underlying population structure, the prevalence estimates were adjusted towards the overall average by applying the empirical Bayes smoothing (Anselin et al., 2004; Anselin, 2004-2005). Statistical significance of the Moran’s I was tested using Monte Carlo randomisation with 9,999 permutations. The analyses of global spatial autocorrelation were carried out using the GeoDa 1.6.7 software (https://geodacenter.asu.edu).

Areas with significantly higher or lower proportion of BMT positive farms (clusters) were identified using a spatial scanning method, the scan statistics. The testing was performed using Bernoulli probability model by Kulldorff (1997) in SatScan™ version 9.4.2 (www.satscan.org). The maximum cluster size tested was 50% of the population at risk. The geographic information was based on the farm postcode (easting and northing coordinates) corresponding to the farm address registered within the AHDB Dairy database collected as part of the recruitment process. Identified clusters were considered significant at $P < 0.05$, based on Monte Carlo hypothesis testing with 9,999 permutations.

The project was approved by the Ethics and Welfare committee at the Royal Veterinary College (approval number URN 2013 0097H).

RESULTS
**Farm Recruitment**

Of the 1483 selected dairy farms, 553 farms responded (37% response rate); 279 negative and 274 positive answers. Of the 274 farms that agreed to participate, 225 farms were studied (had milk sample tested for some or all of the diseases and completed the questionnaire), representing approximately 2% of the total population of dairy farms in GB. The remaining 49 farms that initially answered positively either went out of milk production, were no longer contactable or no longer interested in the study for various reasons.

**Farm Characteristics**

The median herd size was 133 adult cows and ranged from 14 to 603. Approximately half (117/225) of the farms were mixed dairy farms (dairy farms with other production animals, i.e. beef or sheep) and the majority of the farms (93%, 209/225) were conventional (as opposed to organic) dairy producers. One hundred and sixty-four farms (73%) managed their milking herd as one production group and the remaining farms had two or more groups of high and low yielding cows. The average milk yield per cow per year in 2013 was 7613.2 litres (median=7822.1, range from 3100 to 11679 litres). Information on calving intervals was recorded from 205 farms with median of 406 days (range from 310 to 474 days). On majority of farms cows calved all year round (74%, 165/224). The most common grazing system was grazing in summer and housed in winter (89%, 200/225). On 13 (6%) farms, cows were kept indoor all year round and on remaining farms, cows were kept outdoor all year round. Cubicles were the most common (79%, 164/208) type of housing for milking cows, with 27 (13%) farms housing milking cows in straw yards and the remaining farms using both type of housing. One hundred and seven farms (48%) purchased a new stock during a period of 12 months prior to the farm visit.
**Point Prevalence**

Initial BMT samples were obtained for all farms between July 2014 and March 2015, with 144 farms (64%) tested between July and September 2014. The estimated herd prevalence of the studied pathogens based on the presence of antibodies (on unvaccinated farms) or antigen (all farms) in the initial BMT samples was high with higher proportion of positive farms found amongst medium (50-150 cows) and large (≥150 cows) herds (Table 3). The true prevalence of antibody positive unvaccinated farms ranged from 48% (95% CI: 40-56) to 68% (95% CI: 61-76) for *Salmonella* spp and MAP, respectively. Of the nine BVDV antigen positive farms, seven farms vaccinated against BVDV. Amongst BVDV unvaccinated farms, two farms were both BVDV antigen and antibody positive. Of the 57 *C. burnetii* antigen positive farms, 55 farms had also antibodies detected. The distribution of ELISAs antibody levels expressed as percent positivity or percent inhibition (BVDV) is presented in Figure 1. Of the pathogens tested, amongst unvaccinated farms, a correlation of positive status was found between: (1) BVDV antibody and BHV-1, *Leptospira hardjo* (*L. hardjo*) and *F. hepatica*; (2) BHV-1 and MAP and *L. hardjo*; and (3) *C. burnetii* antibody and antigen positive farms (Table 4). Very low positive correlation was detected between BVDV antibody and antigen.

**Associations of prevalence with region and herd size**

In the univariable analysis, region was significantly associated with testing positive to BVDV antibody (*P* = 0.01), BVDV antigen (*P* = 0.03), *L. hardjo* (*P* < 0.001), MAP (*P* = 0.04), *Salmonella* spp (*P* = 0.001), *C. burnetii* antibody (*P* = 0.01), *Chlamydia*-like organisms (*P* = 0.04) and *F. hepatica* (*P* < 0.001). Herd size was significantly associated with testing
positive to *C. burnetii* antibody ($P < 0.001$), *F. hepatica* ($P = 0.02$) and *O. ostertagi* ($P = 0.05$).

In the multivariable analysis (Table 5), taking into account the effect of herd size, farms located in Wales had significantly higher odds of being BVDV antibody positive compared to the farms in South East England (OR = 14.2, 95% CI: 2.7 – 75.0). The odds of a farm being positive to BHV-1 was increased for farms located in Scotland (OR = 6.2, 95% CI: 1.1-36.2) and Wales (OR = 4.3, 95% CI: 1.3 – 14.0); and of MAP for farms in South West England (OR = 3.3, 95% CI: 1.3 - 8.6). Farms located in the central and northern parts of the country had significantly increased odds of being positive to *L. hardjo* compared to the farms in South East England. Large herds and farms located in South West England (OR = 3.8, 95% CI: 1.4 – 10.3), North England (OR = 4.3, 95% CI: 1.5 – 12.4), Scotland (OR = 6.2, 95% CI: 1.8 – 22.0) and Wales (OR = 7.1, 95% CI: 2.6 – 19.6) had significantly increased odds of being positive to *Salmonella* spp. Both herd size and region were significantly associated with being positive to *C. burnetii*, with higher odds detected for medium and large herds and farms located in South West England, Wales, Midlands and North England (Table 5). Accounting for the effect of region, large herds were less likely to be positive to *F. hepatica* compared to the small herds (OR = 0.2, 95% CI: 0.1 – 0.9). Odds of being positive to *F. hepatica* were significantly increased for all regions but Midlands when compared to South East England. BVDV antigen and *O. ostertagi* were dropped from the multivariable analysis due to omitted observations in some of the categories of region or herd size.

**Repeated Quarterly Testing**

The quarterly testing for the presence of BVDV antigen and antibodies against MAP and *Fasciola hepatica* in BMT samples was carried out between October 2014 and
November 2015. The median interval between the second, third and fourth quarterly test was 90, 92 and 89 days, respectively with a minimum of 19 days and a maximum of 190 days between any two tests carried out. The overall prevalence of BVDV and antibodies against MAP and *F. hepatica* in bulk milk, based on all four tests results, was estimated for 203, 206 and 206 farms respectively. The results of prevalence at each quarterly testing as well as the overall (period) prevalence during the whole study period are presented in Figure 2. The true prevalence of BVDV antigen positive farms was 5%, 11%, 11% and 12%, and of MAP antibody positive farms was 68%, 72%, 83% and 80%, at each quarterly test, respectively. The apparent prevalence of *F. hepatica* antibody positive farms at first, second, third and fourth test was 55%, 60%, 57% and 56% respectively. During the whole study period, the true prevalence of farms testing positive at least once to BVDV antigen or antibodies against MAP was 19% (95% CI: 13 – 26) and 89% (95% CI: 81 – 94), respectively. The apparent period prevalence of *F. hepatica* was 67% (95% CI: 61– 73).

**Farmers’ Knowledge of Disease Status**

Approximately 19% (42/224) of farms were members of one of the accredited herd health schemes and 3% (7/224) of farms were working towards one at the time of the visit. Farmers’ knowledge of the status of their herds with respect to the studied pathogens is summarised in Figure 3. Amongst unvaccinated farms, farmers believed MAP, *F. hepatica* and BVD to be present on 55%, 46% and 30% of farms, respectively. Of the studied pathogens, most frequently reported problems were due to MAP (41% of farms), whereas no problems due to *Salmonella* spp., *C. burnetii* or *O. ostertagi* were reported (Figure 3). Percentage of farms, where farmers correctly believed the disease in question was present that actually tested positive (positive predictive value) was high for *C. burnetii* (100%), *O.*
ostertagi (97%) and BVDV antibody (92%) but very low for BVDV antigen (5%), Table 6. High negative predictive value of the farmers’ perception was estimated for BVDV antigen (96%).

Spatial Distribution

Accounting for the vaccination status, herds that tested positive for the individual pathogens were found in all studied regions. However, the variation in the distribution of the positive herds was marked across the regions (Figure 4 a,b,c) with the lowest estimates found mostly in the South East England. Global spatial autocorrelation of positive unvaccinated farms was detected for BVDV antibodies ($I = 0.23$, $P = 0.02$), F. hepatica ($I = 0.22$, $P = 0.008$) and Salmonella spp. ($I = 0.18$, $P = 0.02$). Spatial autocorrelation of C. burnetii PCR positive farms was also detected ($I = 0.03$, $P = 0.02$). By means of the Scan statistic, both low and high-risk clusters of positive unvaccinated farms were found for F. hepatica, L. hardjo, Salmonella spp., and for BVDV antibodies. Further, one high-risk cluster for BVDV antigen and O. ostertagi, and one low-risk cluster for C. burnetii antibody positive farms were found (Figure 5 a,b). All low-risk clusters were located in the South East England.

DISCUSSION

To inform decisions regarding disease priorities and suitable control programs and to allow for monitoring of disease trends over time, reliable and up-to-date information on disease prevalence is highly desirable. With this in mind, the present study was designed to provide prevalence estimates representative of the national GB dairy herd for a number of infectious diseases assumed to be endemic. Participation in the study was voluntary. However, when compared nationally, although the estimated weighted average herd size of
187 cows was slightly higher than that of 144 cows for the GB dairy herd; the estimated average annual milk yield was comparable with the national estimate of 7,535 litres (DairyCo, 2013). This is suggesting that the data where not noticeably biased in this respect. The use of stratified sampling by region and herd size has further allowed us to produce national prevalence estimates with smaller standard errors compared to a non-stratified study of the same size.

The results confirm that the studied pathogens are spread widely across GB and that, at the time of the study, a large proportion of the dairy herds in GB had previously been exposed to them. The results further suggests that active disease transmission is occurring amongst the dairy cattle population and that available control measures are either not being implemented or not being effective. The estimated prevalence values broadly agree with those reported from other countries, where studied pathogens are considered endemic, suggesting similar pathogen dynamics (Boelaert et al., 2000; Muskens et al., 2000; Nielsen et al., 2000; Alonso-Andicoberry et al., 2001; Otranto et al., 2003; van Schaik et al., 2003; Kampa et al., 2004; Scott et al., 2006; Talafha et al., 2009; Habing et al., 2012; Saa et al., 2012; Lombard et al., 2013; O’Doherty et al., 2013; Agger and Paul, 2014; van Engelen et al., 2014; Sayers et al., 2015; Fernandes et al., 2016), except for higher prevalence estimates of *F. hepatica* and *N. caninum* infections compared to some other countries (Sanderson et al., 2000; Cringoli et al., 2002; O’Doherty et al., 2013; Olsen et al., 2015).

Bulk milk samples were used to assess herd status based on the presence of specific antibodies or antigen. Our prevalence estimates are therefore herd-level estimates and they are subject to misclassification bias as a result of suboptimal sensitivity or specificity of the tests applied at the level of the herd. Ascertainment of the infection status of a herd by means of testing a single milk sample from the bulk tank is well established and has obvious logistical and financial advantages. On the other hand, the use of bulk milk comes with
limitations as the ability to identify infected herds (sensitivity) is compromised, in particular for pathogens which can be present in the herd at low within-herd prevalence level. In this situation, negative results should be interpreted as a herd with less than a minimum proportion of positive animals among those in milk needed for the expected ability of the diagnostic test to classify farm as positive. When possible, we tried to adjust the apparent prevalence obtained for the imperfect performance of the test using available values of herd-level sensitivity and specificity. This information was however not available for some of the studied pathogens such as *C. burnetii, F. hepatica, N. caninum* and *O. ostertagi*. As a result, only estimates of their apparent prevalence are presented. In addition, we assumed all PCR tests to be 100% *Se* and *Sp*, which could have resulted in the misclassification bias. Another limitation is that the antibodies detected in BMT sample may be indicative of historical rather than active or recent infection (Duffell and Harkness, 1985; Lindberg and Alenius, 1999; Booth et al., 2013; Sayers et al., 2015) and that the bulk milk sample does not include the whole herd. Young stock, clinically ill cows and dry cows are excluded from the sample. As a result, for example, the prevalence of BVDV antigen in bulk milk can be underestimated due to premature culling of infected young stock (Bishop et al., 2010). Similarly, the prevalence of MAP can be underestimated due to the susceptibility of cows infected with MAP to secondary conditions (e.g. mastitis or lameness) (Villarino and Jordan, 2005) and the subsequent exclusion of cows treated with antibiotics from milk sampling and testing.

Regional variations in prevalence of some of the studied pathogens have been reported previously (Paton et al., 1998; Leonard et al., 2004; Ryan et al., 2012; Howell et al., 2015; Sayers et al., 2015). Overall, we found lower proportion of the positive farms in the South East of England where cattle density is lower (< 10 dairy cows per 100 hectares of farmed land) compared to the other studied regions (CHAWG, 2012). Other factors, such as herd size, management practices (biosecurity, purchase of new stock), and environmental
conditions (i.e. temperature, type of land) can be used to explain the observed regional differences in the number of positive farms. However, comparison at regional level has to be done with caution, as the present national study was not designed to generate prevalence estimates at regional level.

A relatively high number of studied farms were vaccinated against BVDV, BHV-1 and *L. hardjo*, which indicates farmers’ understanding of a need for disease control measures. Only the results of unvaccinated herds are presented as the diagnostic tests used in this study were unable to differentiate between vaccinated and infected farms. Further, the presence of a correlation between the positive status for BVDV antibodies, BHV-1, *L. hardjo*, MAP and *F. hepatica* is suggesting that there are similar risk factors for infections due to these pathogens, which is in agreement with the previous reports (Paton et al., 1998; Kampa et al., 2004; Williams and Winden, 2014). The high level of antibodies against BVDV and BHV-1 detected on a number of farms is suggestive of the presence of active infection or in case of BVDV, also presence or recent removal of persistently infected (PI) animal(s) (Kampa et al., 2004; Booth et al., 2013). However, the detection of low positive correlation between BVDV antibody and antigen positive farms in this study is indicative of detection of historical infections on a number of farms, as the antibodies can persist in bulk milk up to three to four years in previously infected herds (Lindberg and Alenius, 1999). The observed variation in the level of BHV-1 BTM antibody detected agrees with the previous report of farms being either strongly positive or with very low or no antibody detected (Paton et al., 1998). Furthermore, the estimated prevalence of BHV-1 is almost identical to the values reported from previous surveys indicating the stability of the virus in the population of GB dairy farms (Paton et al., 1998; Williams and Winden, 2014).

The high apparent prevalence of BMT antibodies against *O. ostertagi*, *F. hepatica* and *N. caninum* is not surprising. It has been reported that *O. ostertagi* is present on all farms and
that the majority of type 1 ostertagiosis infections occur during summer months (Sekiya et al., 2013). Higher proportions of *O. ostertagi* and *F. hepatica* BMT antibody positive farms were found in the northern parts of GB. This is most likely due to the effect of environmental factors (i.e. higher rainfall in these regions in 2014 (Office, 2014) as well as differences in grazing practices (i.e. access to pasture and duration of grazing) (Sekiya et al., 2013). In relation to *N. caninum*, seasonal variation in the prevalence has been previously reported (O’Doherty et al., 2013). Due to limited financial resources, only a single testing was carried out which could have resulted in some positive farms being missed, especially on farms tested during early stages of the lactation (O’Doherty et al., 2013). However, in this study, the majority of the herds (74%) were calving all year round. Furthermore, the first testing was carried out between July 2014 and March 2015 with the majority (64%) of the samples tested between July and September 2014 minimising the number of false negative results on farms with seasonal calving.

The prevalence of *Salmonella* spp, and *C. burnetii* in a population of dairy farms in GB was high. However, no farmer reported problems due to these pathogens, indicating that they are mostly subclinical. As a result, without routine screening, infected herds will remain undetected posing a risk for disease transmission, especially in areas with high cattle density. The differences in environmental and climatic conditions (i.e. type of landscape, cattle density, temperature, rainfall, wind) were also reported to play an important role in relation to the regional variations we observed for these pathogens (Davison et al., 2005; Nusinovici et al., 2015). Similarly, diverse ecological niche and a wide hosts range for *Chlamydia*-like organisms have been reported (Taylor-Brown et al., 2015). In addition to their presence in environment, previous studies in GB have also observed the evidence of *Chlamydia*-like organisms in 18% of bovine placenta samples in Scotland (Wheelhouse et al., 2012) and in approx. 10% of bovine samples in England and Wales (Wheelhouse et al., 2015).
The repeated testing for BVDV antigen and antibodies against MAP and *F. hepatica* allowed us to observe trends in antibody levels. The exposure of farms to *F. hepatica* appeared to be stable during the whole follow up period, suggesting the endemicity of the infection on the farms. In relation to MAP infections, changes from positive to negative or negative to positive status were observed on more than half of the farms during the follow up period. The changes could be due to the low diagnostic performance of the MAP ELISA (van Weering et al., 2007), purchase of seropositive animals on open farms or exclusion of dry or seropositive animals from the BMT testing. Changes in BVDV antigen status during the study period could also be due to a purchase or removal of infected animals from the herd or bulk milk sample at the time of the testing or due to a PI heifer entering the milking herd (Booth and Brownlie, 2012). The observed changes in prevalence of BVDV antigen and antibodies against MAP, together with the results of farmers’ perception of disease status highlight the importance and value of repeated testing in correctly identifying infected herds and hence appropriate control measures.

Given the importance of accurate and reliable baseline data for the effective implementation and monitoring of disease control programs, the results of this study are particularly valuable. That is because the results of this study not only provide much needed baseline data for the control of endemic pathogens (for which monitoring is already underway in GB, i.e. BVDV), but also for other pathogens which are not presently being monitored at a national level in GB.

**CONCLUSIONS**

Dairy herds in Great Britain are frequently exposed to a number of endemic pathogens that are prevalent at high levels and exhibit some geographical variations. Given
the burden to efficient production that those pathogens pose, and in some cases their public
health implications, the implementation of measures to control and possibly eliminate some
of these pathogens should be given consideration. Despite some limitations, the prevalence
figures estimated in this study provide a basis for the future monitoring of disease trends over
time and can be used to assess the effectiveness of future disease control programs
implemented at a national level.

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REFERENCES

113:293-302.

Alonso-Andicoberry, C., F. J. Garcia-Pena, J. Pereira-Bueno, E. Costas, and L. M. Ortega-
Mora. 2001. Herd-level risk factors associated with Leptospira spp. seroprevalence in


of spatial data. J. Geogr. Syst. 6:197-218.


Frossling, J., A. Lindberg, and C. Bjorkman. 2006. Evaluation of an iscom ELISA used for
detection of antibodies to *Neospora caninum* in bulk milk. Prev. Vet. Med. 74:120-
129.

level associations with the shedding of *Salmonella* and antimicrobial-resistant


Howell, A., M. Baylis, R. Smith, G. Pinchbeck, and D. Williams. 2015. Epidemiology and

Prevalence of antibodies to bovine viral diarrhoea virus in bulk tank milk and

*BVDV* and BHV-1 infections in dairy herds in Northern and Northeastern Thailand.


Highly sensitive real-time PCR for specific detection and quantification of *Coxiella

26:1481-1496.


http://www.metoffice.gov.uk/climate/uk/summaries/2014/annual/regional-values


StataCorp. 2013. Stata: Release 13. Statistical Software. Stata Press, College Station, TX: StataCorp LP.


Figure 1. Results of antibody titres on unvaccinated farms detected by serological testing of bulk milk samples between July 2014 and March 2015 interpreted as a percent positivity or percent inhibition (for bovine viral diarrhoea virus). The blue vertical line indicates cut offs for negative/positive ELISA results. BVDV = bovine viral diarrhoea virus, MAP = *Mycobacterium avium* subspecies *paratuberculosis*, BHV-1 = bovine herpes virus 1.

Figure 2. a) The estimated point and overall (period) true prevalence of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and bovine viral diarrhoea virus (BVDV) antigen and 95% confidence intervals of estimates tested using the bulk tank milk samples as part of the cross-sectional study (n = 225 dairy farms in Great Britain studied between July 2014 and November 2015). For *Fasciola hepatica*, the estimates and 95% confidence intervals of apparent prevalence are presented. Dynamics of tested pathogens: b) expressed as proportion of old and new positive farms of the total number of positive farms detected at each quarterly test compared to the previous test result of a given pathogen; and c) proportion of farms that tested always positive, changed between positive/negative or always tested negative during the whole study period (July 2014 to November 2015)

Figure 3. Farmers’ perception of the herd disease status at the time of the visit prior to the laboratory testing of bulk milk samples being carried out, accounted for the vaccination status. “Believed as present” represents farms where farmers knew the disease was present based on the results of previous test or based on their perception. “Believed as problem” represents farms where farmers believed that the listed disease was a problem at the time of the visit. “Antibody test positive” represents the true proportion of positive unvaccinated farms based on the detection of antibodies against tested pathogens using single bulk tank
milk samples collected between July 2014 and March 2015 (n = 225 dairy farms in Great Britain). BVDV = bovine viral diarrhoea virus, MAP = Mycobacterium avium subspecies paratuberculosis, BHV-1 = bovine herpes virus 1.

**Figure 4 a,b,c.** Choropleth maps showing the proportion of seropositive unvaccinated farms by region. The results account for sensitivity and specificity of the tests used, except for Coxiella burnetii and studied parasites (n = 225 dairy farms in Great Britain studied as part of the cross-sectional study between July 2014 and March 2015). BVDV = bovine viral diarrhoea virus, MAP = Mycobacterium avium subspecies paratuberculosis, BHV-1 = bovine herpes virus 1.

**Figure 5 a,b.** Location of low (blue colour) and high (red colour) risk clusters of bulk tank milk (BTM) antibody or antigen (bovine viral diarrhoea virus - BVDV) positive unvaccinated farms tested using bulk milk tank samples between July 2014 and March 2015 as part of the cross-sectional study (n = 225 dairy farms in Great Britain). Relative risk (RR) of significant high and low risk clusters (*P < 0.05 and P ≥ 0.01; **P < 0.01).
Velasova Figure 1
Velasova Figure 2.
Velasova Figure 3

[Bar chart showing the percentage of farms with different parasites, with categories such as MAP, BYH, Leptospira hardjo, and others. The chart includes three categories of results: antibody test positive, believed as present, and believed as problem.]
Velasova Figure 4a
Table 1. Pathogens for which farm status was assessed by bulk milk testing as part of the cross-sectional study of 225 dairy farms in Great Britain studied between July 2014 and November 2015 and criteria used to ascertain farm status

<table>
<thead>
<tr>
<th>Pathogen (disease)</th>
<th>Detection(^1) of Ab/Ag</th>
<th>Diagnostic test</th>
<th>Frequency of testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Viral Diarrhoea Virus (BVDV)</td>
<td>Ab</td>
<td>BVDV p80 antibody test, IDEXX Laboratories, USA</td>
<td>Once</td>
</tr>
<tr>
<td>(\text{Ag} )</td>
<td>TaqVet(^\text{®} ) BVDV screening test, LSI, France</td>
<td>Quarterly</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium subspecies (\text{paratuberculosis} ) (Johnes’ disease)</td>
<td>Ab</td>
<td>Paratuberculosis screening test, IDEXX Laboratories, USA</td>
<td>Quarterly</td>
</tr>
<tr>
<td>Leptospira hardjo</td>
<td>Ab</td>
<td>Bovine \textit{Leptospira Hardjo} ELISA test, Linnodee Ltd, Northern Ireland</td>
<td>Once</td>
</tr>
<tr>
<td>Bovine herpesvirus type 1 (Infectious bovine rhinotracheitis (IBR))</td>
<td>Ab</td>
<td>BHV-1 Bulk milk antibody test, IDEXX Laboratories, USA</td>
<td>Once</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Ab</td>
<td>PrioCHECK(^\text{®} ) Salmonella Ab ELISA, Prionics Lelystad B.V, Netherlands</td>
<td>Once</td>
</tr>
<tr>
<td>Coxiella burnetii (Q fever)</td>
<td>Ab</td>
<td>LSI\textit{Vet}™ Ruminant Q Fever serum/milk ELISA, LSI, France</td>
<td>Once</td>
</tr>
<tr>
<td>(\text{Ag} )</td>
<td>In house real-time PCR</td>
<td>Once</td>
<td></td>
</tr>
<tr>
<td>Chlamydia-like organisms</td>
<td>Ag</td>
<td>In house, real-time 16S Chlamydiales PCR</td>
<td>Once</td>
</tr>
<tr>
<td>Neospora caninum</td>
<td>Ab</td>
<td>SVANOVIR(^\text{®} ) \textit{Neospora-} Ab test, Svanova, Sweden</td>
<td>Once</td>
</tr>
<tr>
<td>Fasciola hepatica (Liver fluke)</td>
<td>Ab</td>
<td>Fasciolosis verification antibody test, IDEXX Laboratories, USA</td>
<td>Quarterly</td>
</tr>
<tr>
<td>Ostertagia ostertagi (Parasitic gastroenteritis, PGE)</td>
<td>Ab</td>
<td>SVANOVIR(^\text{®} ) \textit{Ostertagia-} Ab test, Svanova, Sweden</td>
<td>Once</td>
</tr>
</tbody>
</table>

\(^1\)Detection: Ab = antibody, Ag = antigen
Table 2. Information on diagnostic test performance, sensitivity and specificity of commercially available assays used for testing of bulk milk samples as part of the cross-sectional study of dairy farms in Great Britain (n = 225 farms studied between July 2014 and November 2015)

<table>
<thead>
<tr>
<th>Commercial test</th>
<th>Positive cut off</th>
<th>Results calculated as</th>
<th>Within-herd prevalence threshold for a positive cut off†</th>
<th>Herd level Sensitivity</th>
<th>Herd level Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV p80 Ab</td>
<td>&gt;20</td>
<td>% Inhibition</td>
<td>&gt;10%</td>
<td>100</td>
<td>100</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>Paratuberculosis Ab screening test</td>
<td>&gt;12.5</td>
<td>% Positivity</td>
<td>&gt;3%</td>
<td>85</td>
<td>96</td>
<td>(van Weering et al., 2007)</td>
</tr>
<tr>
<td>Linnoddee Leptosira Hardjo ELISA test</td>
<td>&gt;3</td>
<td>% Positivity</td>
<td>Not available</td>
<td>94.1</td>
<td>94.8</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>BHV-1 Ab test</td>
<td>≥25</td>
<td>% Positivity</td>
<td>Not available</td>
<td>100</td>
<td>99.6</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>PrioChECK® Salmonella Ab bovine ELISA</td>
<td>≥35</td>
<td>% Positivity</td>
<td>Not available</td>
<td>99.4</td>
<td>97.9</td>
<td>Manufacturer, (Nyman et al., 2013)</td>
</tr>
<tr>
<td>LSIVet™ Ruminant Q Fever ELISA</td>
<td>&gt;30</td>
<td>% Positivity</td>
<td>&gt;10%</td>
<td>90</td>
<td>-</td>
<td>Manufacturer, (Ryan et al., 2011)</td>
</tr>
<tr>
<td>IDEXX Fasciolis Verification Test</td>
<td>&gt;30</td>
<td>% Positivity</td>
<td>&lt;20%</td>
<td>-</td>
<td>-</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>SVANOVIR® Neospora- iscom Ab test</td>
<td>≥20</td>
<td>% Positivity</td>
<td>&gt;10 - 15%</td>
<td>33.3</td>
<td>97.7</td>
<td>(Frossling et al., 2006)</td>
</tr>
<tr>
<td>SVANOVIR® Ostertagia ostertagi Ab test</td>
<td>&gt;0.5</td>
<td>% Positivity</td>
<td>Not available</td>
<td>-</td>
<td>-</td>
<td>Manufacturer</td>
</tr>
</tbody>
</table>

† The minimum within herd prevalence used for establishment of herd sensitivity and specificity
Table 3. The true herd prevalence estimates for selected pathogens and 95% confidence intervals based on the results of single testing of bulk milk samples on unvaccinated farms, weighted to account for the study design, carried out between July 2014 and March 2015 as part of the cross-sectional study of 225 dairy farms in Great Britain.

<table>
<thead>
<tr>
<th>Pathogen and type of test (antigen or antibody detection in BMT)</th>
<th>Number of vaccinated farms excluded from the analysis*</th>
<th>Number of farms for prevalence estimation</th>
<th>Number of positive farms</th>
<th>ELISA readings (expressed as % positivity/% inhibition) on positive farms</th>
<th>Prevalence % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV (antigen)</td>
<td>0</td>
<td>225</td>
<td>9**</td>
<td>Median (min-max)</td>
<td>5.2 (1.5-8.9)</td>
</tr>
<tr>
<td>BVDV (antibody)</td>
<td>121</td>
<td>102</td>
<td>61</td>
<td>71.7 (21.7-95.6)</td>
<td>66.3 (55.6-77.1)</td>
</tr>
<tr>
<td>MAP (antibody)</td>
<td>2</td>
<td>222</td>
<td>134</td>
<td>21.3 (12.6-83.9)</td>
<td>68.3 (59.3-77.4)</td>
</tr>
<tr>
<td>*Farms for which information on vaccination was missing were also excluded from the analysis of prevalence: BVDV (1 farm); MAP (1 farm); Leptospira hardjo (2 farms); bovine herpes virus-1 (3 farms); Salmonella spp (1 farm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptospira hardjo (antibody)</td>
<td>112</td>
<td>111</td>
<td>46</td>
<td>26.2 (3.4-80.7)</td>
<td>46.9 (33.9-59.8)</td>
</tr>
<tr>
<td>Bovine herpes virus-1 (antibody)</td>
<td>105</td>
<td>118</td>
<td>71</td>
<td>201.0 (26.4-364.2)</td>
<td>62.4 (52.1-72.7)</td>
</tr>
<tr>
<td>Salmonella spp (antibody)</td>
<td>12</td>
<td>209</td>
<td>90</td>
<td>79.4 (35.2-333.1)</td>
<td>47.6 (39.3-55.8)</td>
</tr>
<tr>
<td>Coxiella burnetti (antibody)</td>
<td>NA</td>
<td>221</td>
<td>157</td>
<td>92.8 (30.0-222.2)</td>
<td>79.8 (74.6-84.9) †</td>
</tr>
<tr>
<td>Coxiella burnetti (antigen)</td>
<td>NA</td>
<td>220</td>
<td>57</td>
<td>-</td>
<td>28.6 (21.2-36.1)</td>
</tr>
<tr>
<td>Chlamydia-like organisms (antigen)</td>
<td>NA</td>
<td>220</td>
<td>69</td>
<td>-</td>
<td>31.0 (23.7-38.3)</td>
</tr>
<tr>
<td>*The estimated apparent prevalence figures where no reliable information on herd level sensitivity and specificity of bulk milk ELISA test was available</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciola hepatica (antibody)</td>
<td>NA</td>
<td>224</td>
<td>106</td>
<td>132.5 (30.4-555.1)</td>
<td>55.1 (48.4-61.9) †</td>
</tr>
<tr>
<td>Neospora caninum (antibody)</td>
<td>NA</td>
<td>222</td>
<td>99</td>
<td>34 (20.1-94.6)</td>
<td>45.8 (38.1-53.5) †</td>
</tr>
<tr>
<td>Ostertagia ostertagi (antibody)</td>
<td>NA</td>
<td>221</td>
<td>209</td>
<td>1.07 (0.5-1.7)</td>
<td>94.9 (91.3-98.4) †</td>
</tr>
</tbody>
</table>

†Seven out of nine BVDV PCR positive farms were vaccinated

Pathogen: BVDV = bovine viral diarrhoea virus; MAP = Mycobacterium avium subspecies paratuberculosis; BMT = bulk milk tank; NA = not applicable
Table 4. Correlation between positive status of the tested pathogens measured by $\Phi^1$ - correlation coefficient on unvaccinated farms, studied as part of the cross-sectional study of 225 dairy farms in Great Britain carried out between July 2014 and March 2015

<table>
<thead>
<tr>
<th>Pathogens$^2$</th>
<th>BVDV Ab</th>
<th>BVDV Ag</th>
<th>BHV-1</th>
<th>MAP Ab</th>
<th>MAP Ag</th>
<th>L. hardjo</th>
<th>Salmonella spp.</th>
<th>C. burnetii Ab</th>
<th>C. burnetii Ag</th>
<th>Chlamydia-like</th>
<th>F. hepatica</th>
<th>N. caninum</th>
<th>O. ostertagi</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV Ab</td>
<td>0.11</td>
<td>0.30</td>
<td>0.11</td>
<td>0.41</td>
<td>0.34</td>
<td>0.30</td>
<td>0.34</td>
<td>0.59</td>
<td>0.34</td>
<td>0.33</td>
<td>0.04</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>BVDV Ag</td>
<td>0.10</td>
<td>0.07</td>
<td>0.03</td>
<td>0.08</td>
<td>0.13</td>
<td>0.10</td>
<td>0.06</td>
<td>0.04</td>
<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>BHV-1</td>
<td>0.34</td>
<td>0.59</td>
<td>0.20</td>
<td>0.09</td>
<td>0.02</td>
<td>0.08</td>
<td>0.02</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>MAP</td>
<td>0.16</td>
<td>0.28</td>
<td>0.18</td>
<td>0.20</td>
<td>0.07</td>
<td>0.02</td>
<td>0.07</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>L. hardjo</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
<td>0.08</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
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<td>0.04</td>
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<td>0.28</td>
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<tr>
<td>C. burnetii Ab</td>
<td>0.33</td>
<td>0.06</td>
<td>0.06</td>
<td>0.16</td>
<td>0.04</td>
<td>0.06</td>
<td>0.12</td>
<td>0.06</td>
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<td>0.03</td>
<td>0.13</td>
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<td>0.04</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
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<td>0.04</td>
<td>0.04</td>
<td>0.00</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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<td>0.07</td>
<td>0.15</td>
<td>0.07</td>
<td>0.05</td>
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</tbody>
</table>

$^1$Phi coefficient was calculated as the square root of chi-square divided by n, the total number of observations; medium (0.3) and large (0.5) effect size in bold letters

$^2$Pathogens: BVDV = bovine viral diarrhoea virus, BHV – 1 = bovine herpesvirus type 1, MAP = Mycobacterium avium subspecies paratuberculosis, L. hardjo = Leptospira hardjo, C. burnetii = Coxiella burnetii, F. hepatica = Fasciola hepatica, N. caninum = Neospora caninum, O. ostertagi = Ostertagia ostertagi

Ab = antibody, Ag = antigen
Table 5 Multivariable logistic regression analysis of the associations between the studied pathogens and region and herd size on unvaccinated farms. Data collected as part of the cross-sectional survey of 225 dairy farms in Great Britain conducted between July 2014 and March 2015.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>OR (N=102)</th>
<th>95% CI</th>
<th>OR (N=118)</th>
<th>95% CI</th>
<th>OR (N=222)</th>
<th>95% CI</th>
<th>OR (N=111)</th>
<th>95% CI</th>
<th>OR (N=209)</th>
<th>95% CI</th>
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<tbody>
<tr>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>0.3</td>
<td>0.0-1.6</td>
<td>1.7</td>
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<td>0.5-4.4</td>
<td>0.9</td>
<td>0.2-4.5</td>
<td>2.6</td>
<td>0.8-8.7</td>
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<td></td>
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</tr>
<tr>
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<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
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<td>1.4-10.3**</td>
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<td>Midlands</td>
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<td>0.3-2.2</td>
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<td>4.4-183.3***</td>
<td>6.2</td>
<td>1.8-22.0**</td>
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<td>1.3-14.0*</td>
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<td>0.8-4.7</td>
<td>32.2</td>
<td>6.0-173.9***</td>
<td>7.1</td>
<td>2.6-19.6***</td>
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<table>
<thead>
<tr>
<th>Pathogen</th>
<th>OR (N=221)</th>
<th>95% CI</th>
<th>OR (N=220)</th>
<th>95% CI</th>
<th>OR (N=220)</th>
<th>95% CI</th>
<th>OR (N=224)</th>
<th>95% CI</th>
<th>OR (N=220)</th>
<th>95% CI</th>
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<td>Ref</td>
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<td>Ref</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>2.9</td>
<td>1.0-8.5*</td>
<td>2.9</td>
<td>1.0-8.5*</td>
<td>2.9</td>
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<td>0.4-3.4</td>
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<tr>
<td>≥150</td>
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<td>3.0-30.6***</td>
<td>9.6</td>
<td>3.0-30.6***</td>
<td>4.1</td>
<td>0.8-19.7</td>
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<td>0.1-0.9*</td>
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<td>0.5-4.4</td>
</tr>
<tr>
<td><strong>Region</strong></td>
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<td>Ref</td>
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<tr>
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<td>2.2-18.0**</td>
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<td>2.9-31.6***</td>
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<td>4.2</td>
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<td>Ab Count</td>
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<td>Density</td>
<td>OR (95% CI)</td>
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<td>0.8-9.3</td>
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<td>0.4-4.2</td>
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<td>20.7-1923.0***</td>
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<td>0.2-1.7</td>
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<tr>
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<td>1.5-10.4**</td>
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<td>1.5-10.4**</td>
<td>1.6</td>
<td>0.7-3.9</td>
<td>12.8</td>
<td>3.8-42.5***</td>
<td>1.3</td>
<td>0.5-3.1</td>
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</table>

1BVDV = bovine viral diarrhoea virus; MAP = Mycobacterium avium subspecies paratuberculosis, BHV-1 = bovine herpesvirus type 1, L. hardjo = Leptospira hardjo, C. burnetii = Coxiella burnetii, F. hepatica = Fasciola hepatica, N. caninum = Neospora caninum
Ab = antibody, OR = odds ratio, CI: Confidence interval, *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001
2Region: SE = South East, SW = South West, N = North
Table 6. Farmers’ perception of the disease status expressed as positive and negative predictive values using the bulk milk results as the gold standard. The perception was recorded at the time of the visit prior to the single laboratory testing of the bulk milk samples carried out as part of the cross-sectional study of 225 dairy farms in Great Britain carried out between July 2014 and March 2015.

<table>
<thead>
<tr>
<th>Pathogen1 and type of test (antigen or antibody detection in bulk milk sample)</th>
<th>Farmers’ perception of current disease status2</th>
<th>N*</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV (antigen)</td>
<td>All farms</td>
<td>202</td>
<td>5.0</td>
<td>95.9</td>
</tr>
<tr>
<td>BVDV (antibody)</td>
<td>Unvaccinated</td>
<td>92</td>
<td>92.3</td>
<td>53.0</td>
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<tr>
<td>MAP (antibody)</td>
<td>Unvaccinated</td>
<td>204</td>
<td>70.7</td>
<td>51.0</td>
</tr>
<tr>
<td><em>Leptospira hardjo</em> (antibody)</td>
<td>Unvaccinated</td>
<td>98</td>
<td>75.0</td>
<td>70.5</td>
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<tr>
<td>Bovine herpes virus-1 (antibody)</td>
<td>Unvaccinated</td>
<td>93</td>
<td>75.0</td>
<td>46.6</td>
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<tr>
<td><em>Salmonella</em> spp (antibody)</td>
<td>Unvaccinated</td>
<td>157</td>
<td>68.7</td>
<td>63.2</td>
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<tr>
<td><em>Coxiella burnetii</em> (antibody)</td>
<td>All farms</td>
<td>75</td>
<td>100.0</td>
<td>31.5</td>
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<tr>
<td><em>Coxiella burnetii</em> (antigen)</td>
<td>All farms</td>
<td>75</td>
<td>100.0</td>
<td>80.8</td>
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<tr>
<td><em>Fasciola hepatica</em> (antibody)</td>
<td>All farms</td>
<td>192</td>
<td>64.9</td>
<td>73.5</td>
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<tr>
<td><em>Neospora caninum</em> (antibody)</td>
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<td>165</td>
<td>61.3</td>
<td>63.1</td>
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<tr>
<td><em>Ostertagia ostertagi</em> (antibody)</td>
<td>All farms</td>
<td>166</td>
<td>97.2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

1Pathogens: BVDV = bovine viral diarrhoea virus; MAP = *Mycobacterium avium* subspecies *paratuberculosis*

2Farmers’ perception: PPV = positive predictive value; NPV = negative predictive value

*N* = number of farms. Farms on which the disease status was unknown were excluded from the calculation of the PPV and NPV.