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

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Key Words:	prevalence, endemic infectious disease, dairy cow, bulk milk

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1 ***Interpretative Summary***

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

4 **Velasova**

5 In a nationwide study, the herd-level prevalence of selected endemic infectious diseases was
6 estimated using bulk milk in 225 randomly selected Great Britain dairy herds. More than half
7 of the herds showed evidence of exposure to bovine viral diarrhoea virus, *Mycobacterium*
8 *avium* subspecies *paratuberculosis*, bovine herpesvirus type 1, and *Coxiella burnetii*.
9 Approximately 50% of the herds had antibodies against *Leptospira hardjo* and *Salmonella*
10 spp detected. Further, bulk milk of almost all herds had antibodies to *Ostertagia ostertagi*,
11 55% to *Fasciola hepatica* and 46% to *Neospora caninum*. Control and possibly elimination
12 of some of the studied pathogens should be given consideration.

13

14 PREVALENCE OF ENDEMIC DISEASES

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

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33

34 **ABSTRACT**

35 In order to implement appropriate and effective disease control programs at national level,
36 up-to-date and unbiased information on disease frequency is needed. The aim of this study
37 was to estimate the prevalence of selected endemic infectious diseases in the population of
38 dairy herds in Great Britain. Bulk milk tank (BMT) samples from 225 randomly selected
39 dairy farms stratified by region and herd size were tested for antibodies against bovine viral
40 diarrhoea virus (BVDV), bovine herpesvirus type 1 (BHV-1), *Mycobacterium avium*
41 subspecies *paratuberculosis* (MAP), *Leptospira hardjo* , *Salmonella* spp., *Coxiella burnetii* ,
42 *Fasciola hepatica* , *Neospora caninum* , and *Ostertagia ostertagi* . Furthermore, the presence
43 of BVDV, *C. burnetii* and *Chlamydia*-like organisms was determined by polymerase chain
44 reaction (PCR). The apparent herd prevalence was estimated as a weighted proportion of
45 positive herds. The true prevalence was calculated when a test was used with known test
46 characteristics for the cut-off value used. Amongst unvaccinated herds, the true prevalence of

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BMT antibodies against BVDV was estimated at 66% (95% Confidence Interval, CI: 56-77%), MAP 68% (95% CI: 59-77%), BHV-1 62% (95% CI: 52-73%), *L. hardjo* 47% (95% CI: 34-60%) and *Salmonella* spp. 48% (95% CI: 39-56%). The apparent prevalence of BMT antibodies against *C. burnetii* was 80% (95% CI: 75-85%), *F. hepatica* 55% (95% CI: 48-62%), *N. caninum* 46% (95% CI: 38-54%), and *O. ostertagi* 95% (95% CI: 91-98%). BVDV, *C. burnetii* and *Chlamydia*-like antigens were detected in 5% (95% CI: 2-9%), 29% (95% CI: 21-36%) and 31% (95% CI: 24-38%) of herds, 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

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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 effects on fertility (Fray et al., 2000; Lanyon et al., 2014; Walz et al., 2015), milk production (Tiwari et al., 2007; McAloon et al., 2016) and subsequently on culling (Murphy et al., 2006; Smith et al., 2010).

In Great Britain (GB), in 2005, the total costs of dairy and beef cattle endemic infectious diseases (disease, control and prevention) was estimated to be as high as £10 million ($12.4 million) for Johne's disease and £61.1 million ($75.7 million) per annum for BVD (Bennett and Ijpelaar, 2005). However, due to a lack of reliable prevalence data at national level, these figures are likely to underestimate the true situation. 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 to~~wards~~ a general acceptance of their occurrence on dairy farms in endemic areas (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 (BHV-1) in Scandinavian countries and Austria, BVDV in Sweden) can be achieved and would be beneficial (Ackermann and Engels, 2006; Lindberg et al., 2006).

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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 the 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 (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 (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).

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In addition to these ongoing recording systems, one-off surveys are often carried out (Davison et al., 2005; Salimi-Bejestani et al., 2005; Woodbine et al., 2009b), 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~~prevalence 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 important ~~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.

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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 registered farms were stratified by six regions (North England, Midlands, South East England, South West England, Scotland and Wales) ~~and~~ and then within each region by herd size ~~-~~(small: < 50 cows, medium: 50 - 149 cows, large: ≥ 150 cows) creating 18 strata. The herd size was based on the total number of lactating and dry cows. An ~~E~~equal number of

137 farms within each stratum was selected using simple random sampling. The total number (*n*)
138 of farms to study was calculated using ProMesa software v.1.62 (http://www.promesa.co.nz/
139)~~as follows~~according to the formula:

140 $$n = \frac{\sum_{i=1}^{e}\left[\frac{(n_i)^2 \times p_i \times (1-p_i)}{w_i}\right]}{N^2 \times \frac{AE^2}{Z^2} + \sum_{i=1}^{e}[n_i \times p_i \times (1-p_i)]}, \qquad w_i = \frac{n_i \times \sqrt{p_i \times (1-p_i)}}{\sum_{i=1}^{e}[n_i \times \sqrt{p_i \times (1-p_i)}]}$$

141 Where e is the number of strata, n_i is the number of farms in stratum~~a~~ ~~i~~*I* (i.e. large farms in
142 Scotland), p_i is the expected prevalence in stratum~~a~~ *i* (50% was used as worst-case scenario),
143 N is the total number of farms in the population (10,491), AE is absolute acceptable error
144 (error of 14% to achieve 7% precision for the assumed 50% prevalence), 1.96 is the critical Z
145 score value for a 95% confidence interval and w_i is a weighting factor of each stratum. ~~-~~For
146 purpose of sample size calculation, perfect sensitivity and specificity of the diagnostic tests
147 were assumed. A total sample of 200 farms was found to be sufficient to generate the desired
148 estimates and it was decided to aim to recruit 250 farms (approximately 40 farms from each
149 region with approximately equal number of farms within each herd size category).

150 Based on previous experiences of the dairy industry, it was expected that around 20%
151 of farmers contacted would be willing to participate in the study. The selected farmers were
152 contacted by post, receiving information about the project and their participation. Farmers not
153 responding to the initial letter received a reminder. Cattle veterinary practitioners were also
154 informed about the project through the British Cattle Veterinary Association (BCVA)
155 newsletter and by email and were also asked to encourage their clients to participate if they
156 received a letter inviting them. Farmers who agreed to participate were included in the study
157 and the status of their farms with respect to ten different pathogens was assessed. Selection of
158 specific pathogens was based on the results of a workshop run by the Royal Veterinary
159 College in April 2012. In the workshop, the participants were asked to identify and rank

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160 cattle health conditions considered important for individual farmers and the dairy industry
161 and for which no reliable and up-to-date nationwide estimates were available (Velasova et al.,
162 2015). Additionally, five more pathogens for which no up-to-date nationwide estimates were
163 identified and which could be detected using bulk milk samples were included.

164

165 ***Ascertainment of Disease Status***

166 Farm level status with regard to BVDV, MAP, BHV-1, *Salmonella* spp., *Leptospira*
167 *hardjo*, *Coxiella burnetii*, *Fasciola hepatica*, *Neospora caninum*, and *Ostertagia ostertagi*
168 was assessed by testing a single or repeated bulk milk tank (BMT) samples for the presence
169 of specific antibodies (Ab) (Table 1). In addition, for three of the pathogens (BVDV, *C.*
170 *burnetii* and *Chlamydia*-like organisms) direct detection of the antigen in bulk milk was
171 carried out.

172 From each farm, a BMT sample of approximately 30 mL was collected and kept
173 refrigerated until arrival at the laboratory. On arrival fresh milk samples were put into
174 refrigerated storage set at a temperature between 1°C – 7°C. To each sample 5 mL of
175 Bronopol preservative was added. Commercially available enzyme linked immunosorbent
176 assays (ELISAs) described in Table 1 were performed according to the manufacturers'
177 instructions. If there was an option for short and long incubation, the long incubation was
178 used. The results were calculated according to the manufacturer's instructions either as 1)
179 percent positivity calculated as the ratio of the optical density of the sample (OD_S) to the
180 mean optical density of the positive control (OD_{PC}) x 100 or as, 2) the percentage inhibition
181 calculated as (1-OD_S/OD_{NC}) x 100, where OD_{NC} is the mean optical density of the negative
182 control.

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183 The presence of BVDV antigen was studied by means of a real-time polymerase chain
184 reaction (real-time PCR) protocol (TaqVet® BVDV screening test - LSI, France). The
185 presence of *C. burnetii* antigen was assessed by means of an in-house real-time PCR protocol
186 developed by Klee et al. (2006). An in-house real-time PCR (16S *Chlamydiales* PCR)
187 according to Lienard et al. (2011) was also used to detect *Chlamydia*-like organisms.

188 All the analyses were carried out on a single BMT sample with the exception of the
189 detection of antibodies against MAP and *F. hepatica* and the detection of BVDV antigen,
190 which were carried out on four samples collected at three monthly intervals. This was carried
191 out to increase the detection of positive farms considering the low sensitivitydiagnostic
192 performance of bulk milk ELISA tests for the detection of MAP (van Weering et al., 2007)
193 and *F. hepatica* (Reichel et al., 2005) and higher prevalence of BVDV amongst young stock
194 (Booth et al., 2013).

195

196 ***Data Collection***

197 A standardised questionnaire was used to gather information on general farm
198 characteristics (i.e. herd size, production type), vaccination status, the main herd health
199 problems as perceived by the farmer at the time of the visit and the farmer's knowledge of the
200 disease status of the farm for each disease in question. With respect to the farmer's perception
201 of their farm's disease status, the farms were divided into five categories: 1) disease
202 definitely present (based on previous laboratory testing or abattoir monitoring), 2) disease
203 present but unsure (no previous laboratory testing was carried out), 3) disease definitely not
204 present (previous laboratory testing was carried out), 4) disease not present but unsure; and 5)
205 unknown disease status. The questionnaire was designed in consultation with two veterinary
206 practitioners and was piloted on four farms prior to use. Questions, which appeared to be

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207 unclear to farmers, were rephrased to improve the clarity. Interviews were carried out by
208 seven interviewers (three qualified veterinarians and four final year veterinary students), all
209 of whom were trained to ensure consistency and robustness of the collected data.
210

211 ***Data Analysis***

212 All questionnaire data and the results of the laboratory testing were entered into a
213 Microsoft Access 2007® (Microsoft Corp., Redmond, WA, USA) database. The accuracy of
214 information in the database was cross-checked with the questionnaire. All categorical
215 variables were summarised using frequencies and percentages. All continuous variables were
216 checked for deviations from the normal distribution using histograms and the normality test
217 for skewness and kurtosis. They were summarised using frequencies and medians with ranges
218 (minimum and maximum). The descriptive statistics were carried outcalculated using Stata
219 11.2® (StataCorp, Texas, USA) software.
220

221 ***Prevalence Estimation - Single Disease Testing.*** For the estimation of prevalence at
222 herd level, the results of assays were dichotomised as positive/negative based on the cut offs
223 summarised and presented in Table 2. The apparent herd prevalence (*p*) of individual
224 pathogens at the national level was calculated as the total number of positive herds divided by
225 the total number of herds sampled weighted to account for the stratified study design.
226 Sampling weights were calculated using the AHDB Dairy sampling frame described above
227 (where the dairy cattle population was stratified by six regions and within each region further
228 by three herd size categories). fFor each stratum *i* (i.e. small farms in Wales) the sampling
229 weight was calculated as: 1/probability of a farm being selected. The probability of a farm
230 being selected in stratum *i* was calculated as a number proportion of farms studied fromin

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~~each~~ stratum *i*~~from~~ divided by the ~~total~~ number of registered farms in th~~e~~ate stratum. The weighted population prevalence (*p*) and 95% confidence intervals were calculated using survey package in Stata 11.2 according to formulae described in Stata manual (StataCorp, 2013). The 95% confidence intervals were adjusted by estimating the standard error using linearization method with a first order Taylor approximation of the point estimates (StataCorp, 2013).~~.~~

The true herd prevalence was calculated for those conditions for which it was considered biologically meaningful to dichotomise herds as not infected or infected and for which reliable information on the diagnostic test characteristic for the cut-off were available. The latter information included: a) herd level sensitivity (*Se*) and specificity (*Sp*) of the diagnostic tests used; and b) a 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 *O. ostertagi, F. hepatica, 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 in unvaccinated herds 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

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256 the association of herd status (positive/negative) with region or herd size. Variations in the
257 prevalence taking into account the effect of both region and herd size (independent variables)
258 were assessed using logistic regression, and strength of the associations was measured by
259 calculating adjusted odds ratios (OR~~s~~) and their confidence intervals (CI). Statistical
260 significance of the associations of both independent variables with the herd status was tested
261 using a Wald test ~~at a relaxed significance level~~with alpha = 5%.
262

263 ***Repeated Quarterly Testing.*** The apparent and true herd prevalence of antibodies
264 against MAP and *F. hepatica* and the presence of BVDV at each quarterly test were
265 estimated as described above. Only farms that completed all four quarterly tests were
266 included in the analysis. To estimate overall period prevalence, a herd was considered
267 positive if at least one of the samples tested positive in a given quarterly test during the
268 period of 12 months. The true period herd prevalence was then calculated based on a
269 combined *Se* and *Sp* of the tests in parallel as: $Se_{combined} = Se$ × ~~x~~ n - $(Se)^n$ and $Sp_{combined} = Sp^n$,
270 where n= number of tests carried out. The $Se_{combined}$ and $Sp_{combined}$ of MAP ELISA test in
271 parallel were calculated as 1.0 and 0.85 respectively. For the BVDV PCR test $Se_{combined}$ and
272 $Sp_{combined}$ of one were used.

273 ***Farmers' perception.*** Positive and negative predictive values (PPV and NPV) were
274 calculated as the proportion of farms on which farmers correctly classified the status of the
275 herd with respect to the pathogens under study using the results of the BMT as the gold
276 standard. Herds vaccinated against the studied pathogens or those were farmers did not know
277 the status of the tested pathogens were excluded from the calculations.
278

279 ***Spatial Analysis***

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280 Choropleth maps showing the distribution of positive herds across the studied regions
281 were generated by dividing the number of positive herds by the number of herds tested within
282 each region (where possible adjusted for the performance of the diagnostic tests used), using
283 ArcGIS 10 (ESRI Inc., CA, USA, 2010) software. Presence of spatial autocorrelation was
284 tested using the univariate Moran's *I* test for global spatial autocorrelation and Queen
285 contiguity (i.e. considering as neighbouring units those that have any point such as
286 boundaries or corners in common). To account for the variation in number of farms tested
287 and the underlying population structure, the prevalence estimates were adjusted towards the
288 overall average by applying the empirical Bayes smoothing (Anselin et al., 2004; Anselin,
289 2004-2005). Statistical significance of the Moran's *I* was tested using Monte Carlo
290 randomisation with 9,999 permutations. The analyses of global spatial autocorrelation were
291 carried out using the GeoDa 1.6.7 software (https://geodacenter.asu.edu).

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

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

302

303 ## RESULTS

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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 litres (median=7822, 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 the 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 (i.e., freestalls) 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~~new cattle during a period of 12 months prior to the farm visit.

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328

329 ***Point Prevalence***

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

346

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

348 In the univariable analysis, herd-level prevalence differed among regions for BVDV
349 antibody ($P = 0.01$), BVDV antigen ($P = 0.03$), *L. hardjo* ($P < 0.001$), MAP ($P = 0.04$),
350 *Salmonella* spp ($P = 0.001$), *C. burnetii* antibody ($P = 0.01$), *Chlamydia*-like organisms ($P =$
351 0.04) and *F. hepatica* ($P < 0.001$). Differences in herd-level prevalence by herd size were

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352 also observed for *C. burnetii* antibody ($P < 0.001$), *F. hepatica* ($P = 0.02$) and *O. ostertagi* (P
353 $= 0.05$).

354 Accounting for the effect of herd size, regional variations in herd-level prevalence
355 observed in Figure 4a-c remained apparent for most of the studied pathogens (Table 5). ~~Herds
356 located~~Herds located in Wales and Scotland had higher odds of being positive to BVDV
357 antibody, BHV-1, *L. hardjo*, *Salmonella* spp. and *F. hepatica*. ~~Whereas h~~Herds located in
358 South West England had the highest odds of being positive to MAP and *C. burnetii* compared
359 to the herds in South East England. Accounting for the effect of region, large herds (≥ 150
360 cows) had increased odds of being positive to *Salmonella* spp and *C. burnetii* (Table 5)
361 compared to the small herds (<50 cows). BVDV antigen and *O. ostertagi* could not be
362 included in the multivariable analysis due to omitted observations in some of the categories
363 of region or herd size.

364

365 ***Repeated Quarterly Testing***

366 The quarterly testing for the presence of BVDV antigen and antibodies against MAP
367 and *F. hepatica* in BMT samples was carried out between October 2014 and November 2015.
368 The median interval between the second, third and fourth quarterly test was 90, 92 and 89
369 days, respectively with a minimum of 19 days and a maximum of 190 days between any two
370 tests carried out. The overall prevalence of BVDV and antibodies against MAP and *F.
371 hepatica* in bulk milk, based on all four tests results, was estimated for 203, 206, and 206
372 farms, respectively. The results of prevalence at each quarterly testing as well as the overall
373 (period) prevalence during the whole study period are presented in Figure 2. The true
374 prevalence of BVDV antigen positive herds was 5, 11, 11 and 12%, and of MAP antibody
375 positive farms was 68, 72, 83 and 80%, at each quarterly test, respectively. The apparent

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376 prevalence of *F. hepatica* antibody positive herds at first, second, third and fourth test was
377 55, 60, 57 and 56% respectively. During the whole study period, the true prevalence of herds
378 testing positive at least once to BVDV antigen or antibodies against MAP was 19% (95% CI:
379 13 – 26%) and 89% (95% CI: 81 – 94%), respectively. The apparent period prevalence of *F.*
380 *hepatica* was 67% (95% CI: 61– 73%).

381

382 ***Farmers' Knowledge of Disease Status***

383 Approximately 19% (42/224) of farms were members of one of the accredited herd
384 health schemes and 3% (7/224) of farms were working towards one at the time of the visit.
385 Farmers' knowledge of the status of their herds with respect to the studied pathogens is
386 summarised in Figure 3. Amongst unvaccinated herds, farmers believed MAP, *F. hepatica*
387 and BVDV to be present on 55, 46 and 30% of farms, respectively. Of the studied pathogens,
388 most frequently reported problems were due to MAP (41% of farms), whereas no problems
389 due to *Salmonella* spp., *C. burnetii* or *O. ostertagi* were reported (Figure 3). The percentage
390 of herds where farmers correctly believed the disease in question was present that actually
391 tested positive (positive predictive value) was high for *C. burnetii* (100%), although more
392 than 50% of the farmers did not know the status*, O. ostertagi* (97%) and BVDV antibody
393 (92%) but very low for BVDV antigen (5%), Table 6. High negative predictive value of the
394 farmers' perception was estimated for BVDV antigen (96%).

395

396 ***Spatial Distribution***

397 Accounting for the vaccination status, herds that tested positive for the individual
398 pathogens were found in all studied regions. However, the variation in the distribution of the
399 positive herds was marked across the regions (Figure 4 a,b,c) with the lowest estimates found

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400 mostly in the South East England. Global spatial autocorrelation of positive unvaccinated
401 herds was detected for BVDV antibodies (I = 0.23, P = 0.02), *F. hepatica* (I = 0.22, P =
402 0.008) and *Salmonella* spp. (I = 0.18, P = 0.02). Spatial autocorrelation of *C. burnetii* PCR
403 positive herds was also detected (I = 0.03, P = 0.02). By means of the Scan statistic, both low
404 and high-risk clusters of positive unvaccinated herds were found for *F. hepatica, L. hardjo,*
405 *Salmonella* spp., and for BVDV antibodies. Further, one high-risk cluster for BVDV antigen
406 and *O. ostertagi,* and one low-risk cluster for *C. burnetii* antibody positive herds were found
407 (Figure 5 a,b). All low-risk clusters were located in the South East England.

408

409 **DISCUSSION**

410 To inform decisions regarding disease priorities and suitable control programs and to
411 allow for monitoring of disease trends over time, reliable and up-to-date information on
412 disease prevalence is highly desirable. With this in mind, the present study was designed to
413 provide prevalence estimates representative of the national GB dairy herd for a number of
414 non-statutory infectious diseases assumed to be endemic. Bovine tuberculosis although
415 identified as important during the workshop was not included in the study due to the fact that
416 existing mandatory surveillance provides reliable information on its occurrence at the
417 national level (Velasova et al., 2015). Participation in the study was voluntary. However,
418 when compared nationally, although the estimated weighted average herd size of 187 cows
419 was slightly higher than that of 144 cows for the GB dairy herd; the estimated average annual
420 milk yield was comparable with the national estimate of 7,535 litres (DairyCo, 2013). This is
421 suggesting that the data where not noticeably biased in this respect. The use of stratified
422 sampling by region and herd size has further allowed us to produce national prevalence
423 estimates with smaller standard errors compared to a non-stratified study of the same size.

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424 The results of high prevalence and wide geographic distribution confirm that the
425 studied pathogens are spread widely across GB and that, at the time of the study, a large
426 proportion of the dairy herds in GB had previously been exposed to them. The resultsThe
427 high prevalence levels further suggest that active disease transmission is occurring amongst
428 the dairy cattle population and that available control measures are either not being
429 implemented or not being effective. The estimated prevalence values of most of the studied
430 pathogens broadly agree with those reported from other countries, where thesestudied
431 pathogens are considered endemic, suggesting similar pathogen dynamics (BVDV and BHV-
432 1 (Kampa et al., 2004; Sayers et al., 2015; Fernandes et al., 2016), MAP (Muskens et al.,
433 2000; van Schaik et al., 2003), *C. burnetii* (van Engelen et al., 2014), *Salmonella* spp., and *L.*
434 *hardjo* (Habing et al., 2012; O' Doherty et al., 2013). , except for hHigher prevalence
435 estimates of *F. hepatica* (Cringoli et al., 2002; Olsen et al., 2015) and *N. caninum* infections
436 were estimated in this study compared to some other countries (Sanderson et al., 2000; O'
437 Doherty et al., 2013).

438 Bulk milk samples were used to assess herd status based on the presence of specific
439 antibodies or antigen. Our prevalence estimates are therefore herd-level estimates and they
440 are subject to misclassification bias as a result of imperfectsuboptimal sensitivity or
441 specificity of the tests applied at the level of the herd. Ascertainment of the infection status of
442 a herd by means of testing a single milk sample from the bulk tank is well established and has
443 obvious logistical and financial advantages. On the other hand, the use of bulk milk comes
444 with limitations as the ability to identify infected herds (sensitivity) is compromised, in
445 particular for pathogens which can be present in the herd at low level. In this situation,
446 negative results should be interpreted as a herd with less than a minimum proportion of
447 positive animals among those in milk needed for the expected ability of the diagnostic test to
448 classify herd as positive. When possible, we tried to adjust the apparent prevalence obtained

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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 have 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 (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. Furthermore, exclusion of the vaccinated herds from the study population resulted in lower precision of the prevalence estimates, such that the smallest number of the studied unvaccinated herds (102 herds) was sufficient to estimate 50% prevalence (the worst-case scenario) with 10% precision and 95% confidence.

Regional variations in prevalence of some of the studied pathogens have been reported previously (Ryan et al., 2012; Howell et al., 2015; Sayers et al., 2015). Overall, we found a lower proportion of positive herds 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

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473 herds. However, comparison at regional level has to be done with caution, as the present
474 national study was not designed to generate prevalence estimates at regional level.

475 A relatively high number of studied farms were vaccinated against BVDV, BHV-1
476 and *L. hardjo*, which indicates farmers' understanding of a need for disease control measures.
477 Only the results of unvaccinated herds are presented as the diagnostic tests used in this study
478 were unable to differentiate between vaccinated and infected herds. The presence of a
479 correlation between the positive status for BVDV antibodies, BHV-1, *L. hardjo,* MAP and *F.*
480 *hepatica* suggests that there are similar risk factors for infections due to these pathogens,
481 which is in agreement with the previous reports (Paton et al., 1998; Kampa et al., 2004;
482 Williams and Winden, 2014). The high level of antibodies against BVDV and BHV-1
483 detected in a number of herds is suggestive of the presence of active infection or in case of
484 BVDV, also presence or recent removal of persistently infected (PI) animal(s) (Kampa et al.,
485 2004; Booth et al., 2013). However, the detection of low positive correlation between BVDV
486 antibody and antigen positive herds in this study is indicative of detection of historical
487 infections on a number of farms, as the antibodies can persist in bulk milk up to three to four
488 years in previously infected herds (Lindberg and Alenius, 1999). The observed variation in
489 the level of BHV-1 BMT antibody detected agrees with the previous report of herds being
490 either strongly positive or with very low or no antibody detected (Paton et al., 1998).
491 Furthermore, the estimated prevalence of BHV-1 is almost identical to the values reported
492 from previous surveys indicating the stability of the virus in the population of GB dairy herds
493 (Paton et al., 1998; Williams and Winden, 2014).

494 The high apparent prevalence of BMT antibodies against *O. ostertagi, F. hepatica* and
495 *N. caninum* is not surprising. It has been reported that *O. ostertagi* is present in all herds and
496 that the majority of type 1 ostertagiosis infections occur during summer months (Sekiya et al.,
497 2013). Higher proportions of *O. ostertagi* and *F. hepatica* BMT antibody positive herds were

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498 found in the northern parts of GB. This is most likely due to the effect of environmental
499 factors (i.e. higher rainfall in these regions in 2014 (MetOffice, 2014) as well as differences
500 in grazing practices (i.e. access to pasture and duration of grazing) (Sekiya et al., 2013). In
501 relation to *N. caninum*, seasonal variation in the prevalence has been previously reported (O'
502 Doherty et al., 2013). Due to limited financial resources, in this study, only a single testing
503 was carried out which could have resulted in some positive herds being missed, especially on
504 farms tested during early stages of the lactation (O' Doherty et al., 2013). However, in this
505 study, the majority of the herds (74%) were calving all year round. Furthermore, the first
506 testing was carried out between July 2014 and March 2015 with the majority (64%) of the
507 samples tested between July and September 2014 minimising the number of false-negative
508 results on farms with seasonal calving.

509 The prevalence of *Salmonella* spp, and *C. burnetii* in a population of dairy herds in
510 GB was high. However, no farmer reported problems due to these pathogens, indicating that
511 they are mostly subclinical or unrecognised. It further suggests that the importance of a
512 pathogen or disease and willingness to act on depend not just on prevalence but also on
513 attributable economic impacts. As a result, without routine screening, infected herds will
514 remain undetected posing a risk for disease transmission, especially in areas with high cattle
515 density. The differences in environmental and climatic conditions (i.e. type of landscape,
516 cattle density, temperature, rainfall, wind) were also reported to play an important role in
517 relation to the regional variations we observed for these pathogens (Davison et al., 2005;
518 Nusinovici et al., 2015). Similarly, diverse ecological niches and a wide hosts range for
519 *Chlamydia*-like organisms have been reported (Taylor-Brown et al., 2015). In addition to
520 their presence in environment, previous studies in GB have also observed the evidence of
521 *Chlamydia*-like organisms in 18% of bovine placenta samples in Scotland (Wheelhouse et al.,

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522 2012) and in approx. 10% of bovine samples in England and Wales (Wheelhouse et al.,
523 2015).

524 The repeated testing for BVDV antigen and antibodies against MAP and *F. hepatica*
525 allowed us to observe trends in antibody levels. The exposure of herds to *F. hepatica*
526 appeared to be stable during the whole follow up period, suggesting the endemicity of the
527 infection on the farms. In relation to MAP infections, changes from positive to negative or
528 negative to positive status were observed in more than half of the herds during the follow up
529 period. The changes could be due to the low ~~diagnostic performance~~sensitivity of the MAP
530 ELISA (van Weering et al., 2007), purchase of seropositive animals on open farms or
531 exclusion of dry or seropositive animals from the BMT testing. Changes in BVDV antigen
532 status during the study period could also be due to a purchase or removal of infected animals
533 from the herd or bulk milk sample at the time of the testing or due to a PI heifer entering the
534 milking herd (Booth and Brownlie, 2012). The observed changes in prevalence of BVDV
535 antigen and antibodies against MAP, together with the results of farmers' perception of
536 disease status highlight the importance and value of repeated testing in correctly identifying
537 infected herds and hence appropriate control measures.

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

544

545 **CONCLUSIONS**

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

554

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

770

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

781

782 **Figure 3.** Farmers' perception of the herd disease status at the time of the visit prior to the
783 laboratory testing of bulk milk samples being carried out, accounted for the vaccination
784 status. "Believed as present" represents farms where farmers knew the disease was present
785 based on the results of previous test or based on their perception. "Believed as problem"
786 represents farms where farmers believed that the listed disease was a problem at the time of
787 the visit. "Antibody test positive" represents the true proportion of positive unvaccinated
788 farms based on the detection of antibodies against tested pathogens using single bulk tank

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789 milk samples collected between July 2014 and March 2015 (n = 225 dairy farms in Great
790 Britain). BVDV = bovine viral diarrhoea virus, MAP = *Mycobacterium avium* subspecies
791 *paratuberculosis*, BHV-1 = bovine herpes virus 1.

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

798

799 **Figure 5 a,b.** Location of low (blue colour) and high (red colour) risk clusters of bulk milk
800 tank (BMT) antibody or antigen (bovine viral diarrhoea virus - BVDV) positive unvaccinated
801 herds tested using bulk milk tank samples between July 2014 and March 2015 as part of the
802 cross-sectional study (n = 225 dairy farms in Great Britain). Relative risk (RR) of significant
803 high and low risk clusters (*$P < 0.05$ and $P \geq 0.01$; **$P < 0.01$).

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1 **Table 1.** Pathogens for which farm status was assessed by bulk milk testing as part of the
2 cross-sectional study of 225 dairy farms in Great Britain studied between July 2014 and
3 November 2015 and criteria used to ascertain farm status

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

4 [1]Detection: Ab = antibody, Ag = antigen
5

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6 **Table 2.** Information on diagnostic test performance, sensitivity and specificity of commercially available assays used for testing of bulk milk
7 samples as part of the cross-sectional study of dairy farms in Great Britain (n = 225 farms studied between July 2014 and November 2015)

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

8 † The minimum within herd prevalence used for establishment of herd sensitivity and specificity

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

Pathogen[1] and type of test (antigen or antibody detection in bulk milk)	Number of vaccinated farms excluded from the analysis*	Number of farms for prevalence estimation	Total number of positive farms	Number (%) of positive farms by herd size			ELISA readings (expressed as % positivity/% inhibition) on positive farms Median (min-max)	True prevalence % (95% CI)
				Small (<50 cows)	Medium (50-149 cows)	Large (≥150 cows)		
BVDV (antigen)	0	225	9**	0 (0)	3 (33)	6 (67)	-	5 (1-9)
BVDV (antibody)	121	102	61	10 (16)	33 (54)	18 (30)	72 (22-96)	66 (56-77)
MAP (antibody)	2	222	134	10 (8)	70 (52)	54 (40)	21 (13-84)	68.3 (59-77)
Leptospira hardjo (antibody)	112	111	46	4 (9)	29 (63)	13 (28)	26 (3-81)	46.9 (34-60)
Bovine herpes virus-1 (antibody)	105	118	71	8 (11)	46 (65)	17 (24)	201 (26-364)	62.4 (52-73)
Salmonella spp (antibody)	12	209	90	4 (4)	45 (50)	41 (46)	79 (35-333)	47.6 (39-56)
Coxiella burnetii (antibody)	NA	221	157	8 (5)	71 (45)	78 (50)	93 (30-222)	79.8 (75-85) †
Coxiella burnetii (antigen)	NA	220	57	3 (5)	23 (40)	31 (55)	-	28.6 (21-36)
Chlamydia-like organisms (antigen)	NA	220	69	2 (3)	33 (48)	34 (49)	-	31.0 (24-38)
Fasciola hepatica (antibody)	NA	224	106	12 (11)	58 (55)	36 (34)	132 (30-555)	55.1 (48-62) †
Neospora caninum (antibody)	NA	222	99	7 (7)	46 (46)	46 (47)	34 (20-95)	45.8 (38-54) †
Ostertagia ostertagi (antibody)	NA	221	209	18 (9)	108 (51)	83 (40)	1. (0.5-2)	94.9 (91-98) †

12 [1]Pathogen: BVDV = bovine viral diarrhoea virus; MAP = *Mycobacterium avium* subspecies *paratuberculosis*; BMT = bulk milk tank; NA = not applicable
13 *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);
14 bovine herpes virus-1 (3 farms); *Salmonella spp* (1 farm)

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15 **Seven out of nine BVDV PCR positive farms were vaccinated

16 †The estimated apparent prevalence figures where no reliable information on herd level sensitivity and specificity of bulk milk ELISA test was available

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Table 4. Correlation between positive status of the tested pathogens measured by *Phi* - 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. Values in bold indicate moderate (*Phi* = 0.30-0.39) to strong positive relationship (*Phi* = 0.40-0.69).

[1]Pathogens	BVDV Ag	BHV-1	MAP	*L. hardjo*	*Salmonella* spp.	*C. burnetii* Ab	*C. burnetii* Ag	*Chlamydia-like*	*F. hepatica*	*N. caninum*	*O. ostertagi*
BVDV Ab	0.11	**0.30**	0.11	**0.41**	0.17	0.10	0.09	0.04	**0.34**	0.21	0.21
BVDV Ag		0.10	0.07	0.03	0.08	0.13	0.03	0.04	0.01	0.04	0.05
BHV-1			**0.34**	**0.59**	0.20	0.06	0.04	0.08	0.17	0.17	0.17
MAP				0.16	0.09	0.02	0.08	0.10	0.07	0.18	0.05
L. hardjo					0.28	0.18	0.17	0.00	0.28	0.10	0.12
Salmonella spp.						0.07	0.02	0.04	0.16	0.12	0.01
C. burnetii Ab							**0.33**	0.06	0.06	0.13	0.02
C. burnetii Ag								0.08	0.04	0.01	0.00
Chlamydia-like									0.03	0.05	0.07
F. hepatica										0.04	0.15
N. caninum											0.05

[1]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

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Table 5. Multivariable logistic regression analysis of the associations between the studied pathogens[1] and region[2] 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.

		BVDV Ab (n=102)		BHV-1 (n=118)		MAP (n=222)		*L. hardjo* (n=111)		*Salmonella* spp (n=209)	
		OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Herd size	<150	1	-	1	-	1	-	1	-	1	-
	≥150	0.6	0.2-1.5	0.9	0.4-2.2	0.7	0.4-1.3	1.0	0.4-2.8	1.9	1.0-3.4*
Region	SE England	1	-	1	-	1	-	1	-	1	-
	SW England	2.2	0.6-8.0	2.4	0.7-7.9	3.2	1.3-8.3*	6.3	0.9-41.4	3.6	1.3-9.6*
	Midlands	1.8	0.5-6.2	3.4	0.9-12.2	0.9	0.4-2.3	9.0	1.6-49.9*	1.5	0.5-4.7
	N England	4.0	1.0-16.1*	2.1	0.6-7.7	1.1	0.4-2.7	6.3	1.0-38.1*	4.0	1.4-11.5**
	Scotland	1.9	0.3-10.3	6.5	1.1-38.1*	0.8	0.3-2.2	28.3	4.4-182.3***	6.7	1.9-23.6**
	Wales	14.2	2.7-74.5**	4.2	1.3-13.5*	1.9	0.8-4.6	32.2	6.0-173.9***	6.9	2.5-19.1***
		C. burnetii Ab (n=221)		*C. burnetii* Ag (n=220)		*Chlamydia*-like (n=220)		*F. hepatica* (n=224)		*N. caninum* (n=220)	
		OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Herd size	<150	1	-	1	-	1	-	1	-	1	-
	≥150	3.8	1.9-7.8***	2.1	1.1-3.9*	1.6	0.9-2.9	0.6	0.3-1.1	1.3	0.7-2.3
Region	SE England	1	-	1	-	1	-	1	-	1	-
	SW England	5.5	1.9-15.4**	1.0	0.4-2.8	0.7	0.3-1.8	9.9	3.0-32.7***	1.4	0.6-3.4
	Midlands	3.9	1.4-11.1*	1.3	0.5-3.8	0.6	0.2-1.7	2.1	0.5-8.1	1.3	0.5-3.2
	N England	3.9	1.4-10.7**	1.5	0.5-4.3	0.3	0.1-1.0	28.4	7.9-102.1***	1.4	0.6-3.6
	Scotland	3.0	0.9-10.0	1.3	0.4-4.8	1.4	0.5-4.4	183.5	19.1-1760.4***	0.5	0.1-1.7
	Wales	3.8	1.4-10.0**	1.3	0.5-3.6	1.6	0.6-3.8	12.6	3.8-41.6***	1.3	0.5-3.0

[1]BVDV = bovine viral diarrhoea virus; MAP = *Mycobacterium avium* subspecies *paratuberculosis,* BHV-1 = bovine herpesvirus type 1, *Leptospira hardjo, Coxiella burnetii, Fasciola hepatica, Neospora caninum*
Ab = antibody, OR = odds ratio, CI: Confidence interval

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[2]Region: SE = South East, SW = South West, N = North
*0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001

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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.

Pathogen[1] and type of test (antigen or antibody detection in bulk milk sample)	Farmers' perception of current disease status[2]			
		n~~N~~*	PPV %	NPV %
BVDV (antigen)	All farms	202	5.0	95.9
BVDV (antibody)	Unvaccinated	92	92.3	53.0
MAP (antibody)	Unvaccinated	204	70.7	51.0
Leptospira hardjo (antibody)	Unvaccinated	98	75.0	70.5
Bovine herpes virus-1 (antibody)	Unvaccinated	93	75.0	46.6
Salmonella spp (antibody)	Unvaccinated	157	68.7	63.2
Coxiella burnetii (antibody)	All farms	75	100.0	31.5
Coxiella burnetii (antigen)	All farms	75	100.0	80.8
Fasciola hepatica (antibody)	All farms	192	64.9	73.5
Neospora caninum (antibody)	All farms	165	61.3	63.1
Ostertagia ostertagi (antibody)	All farms	166	97.2	7.5

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

[2]Farmers' perception: PPV = positive predictive value; NPV = negative predictive value

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

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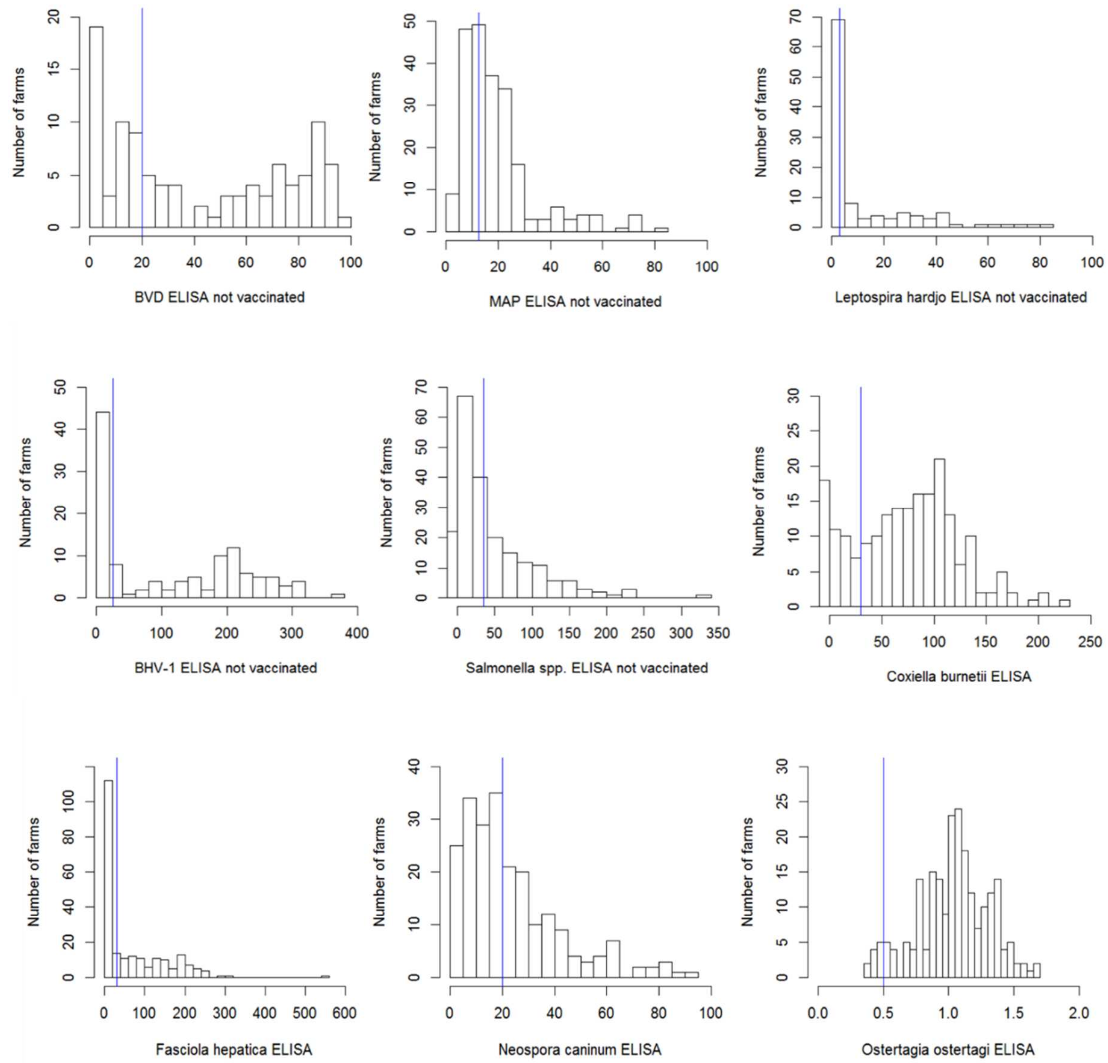
Velasova Figure 1



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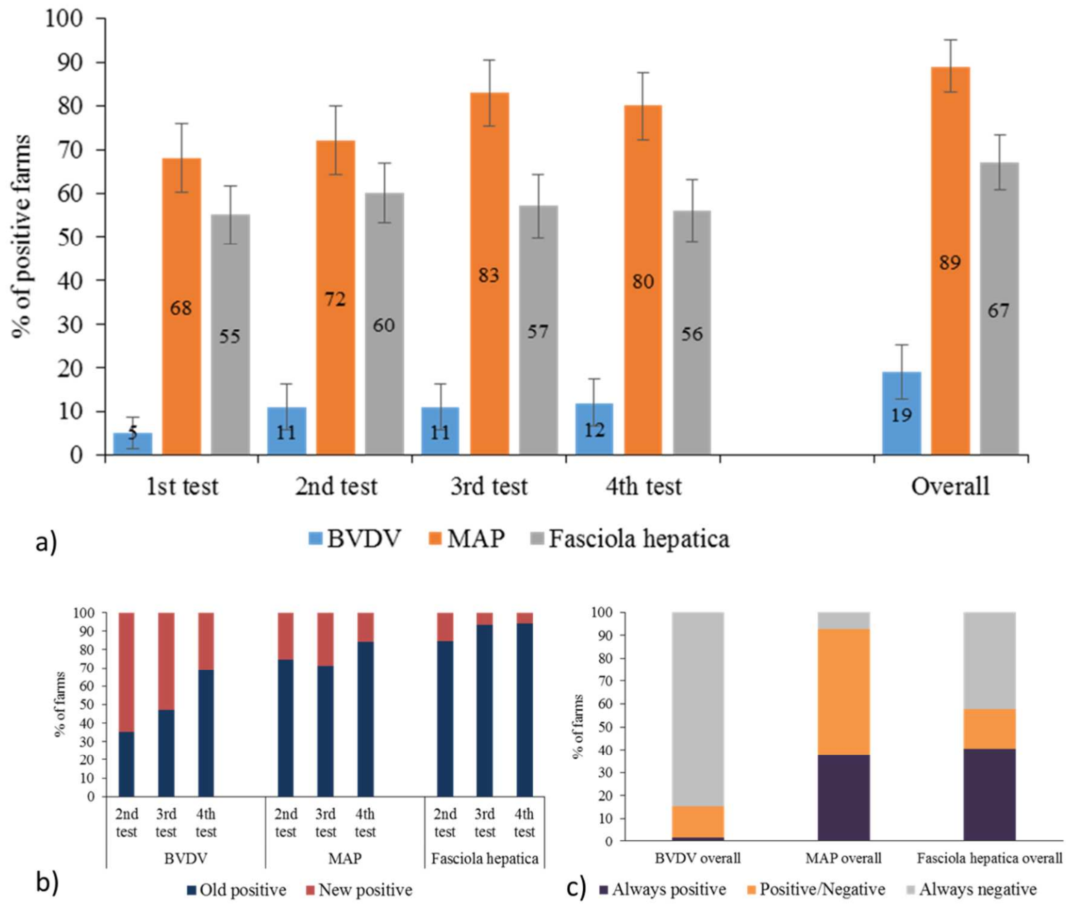

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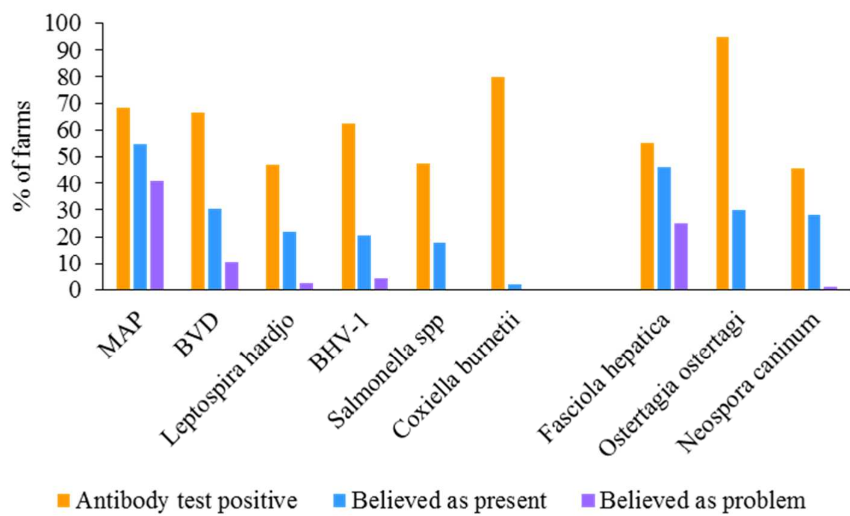

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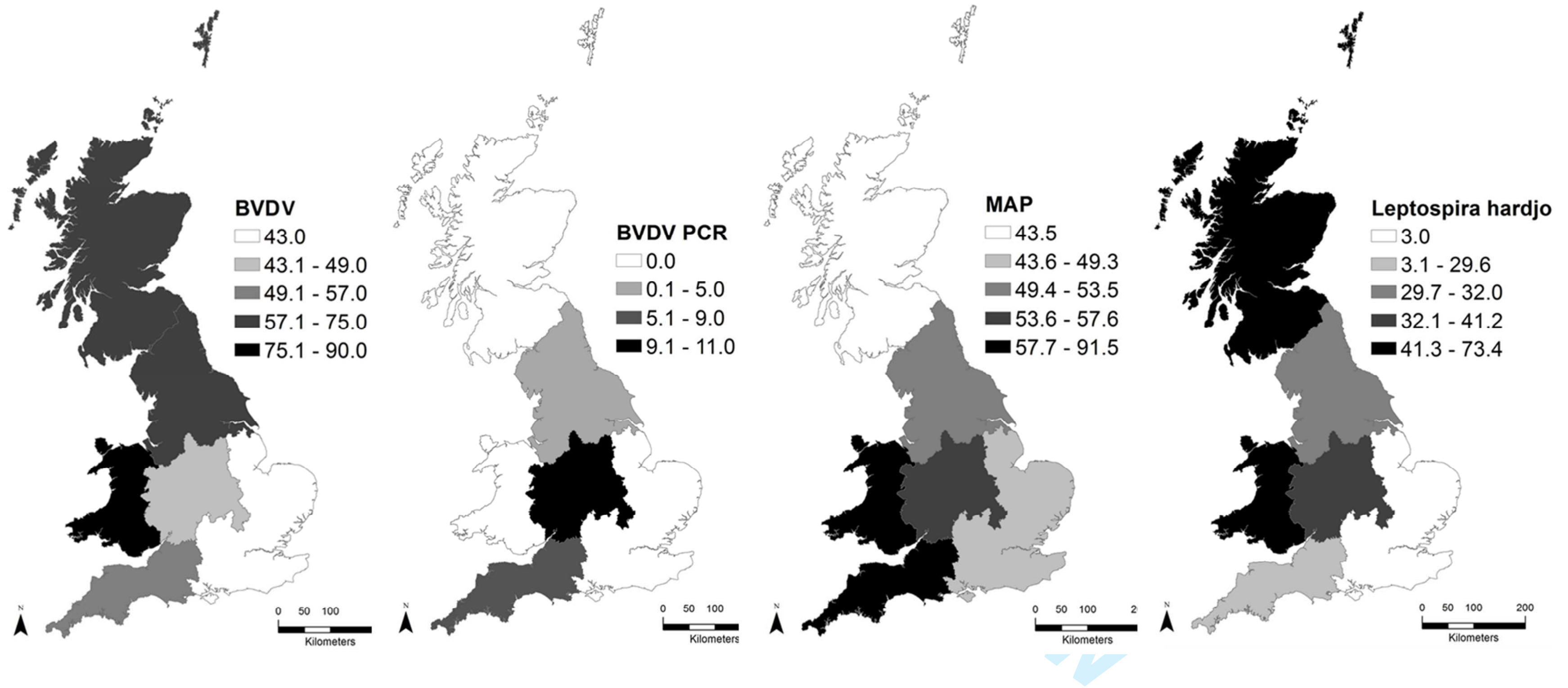
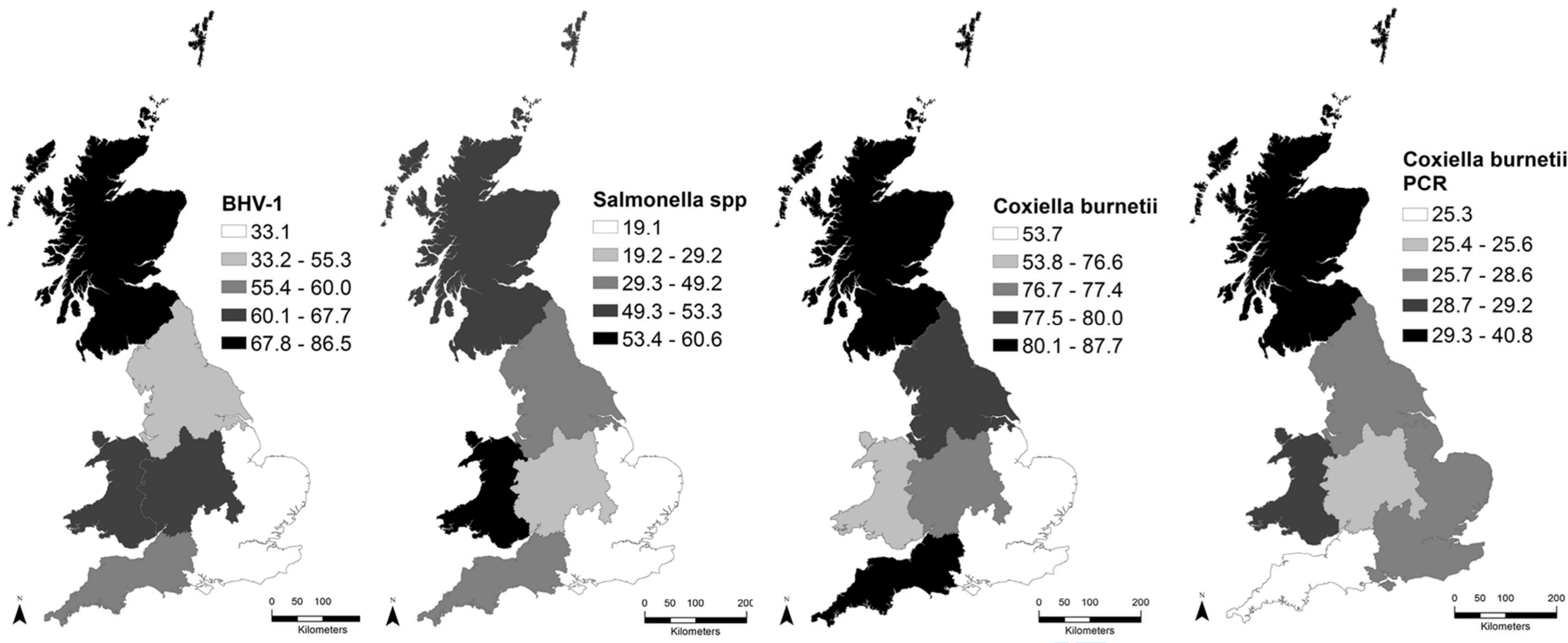
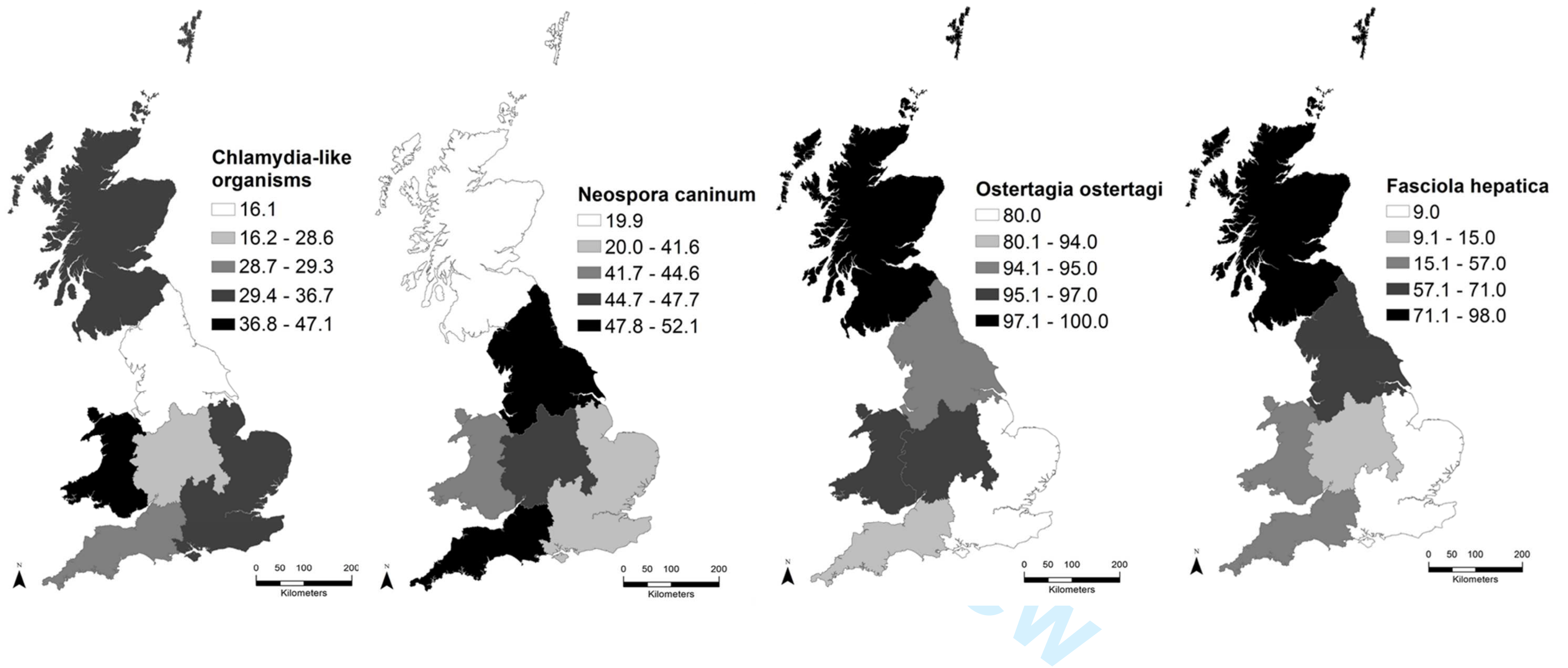
Velasova Figure 4a



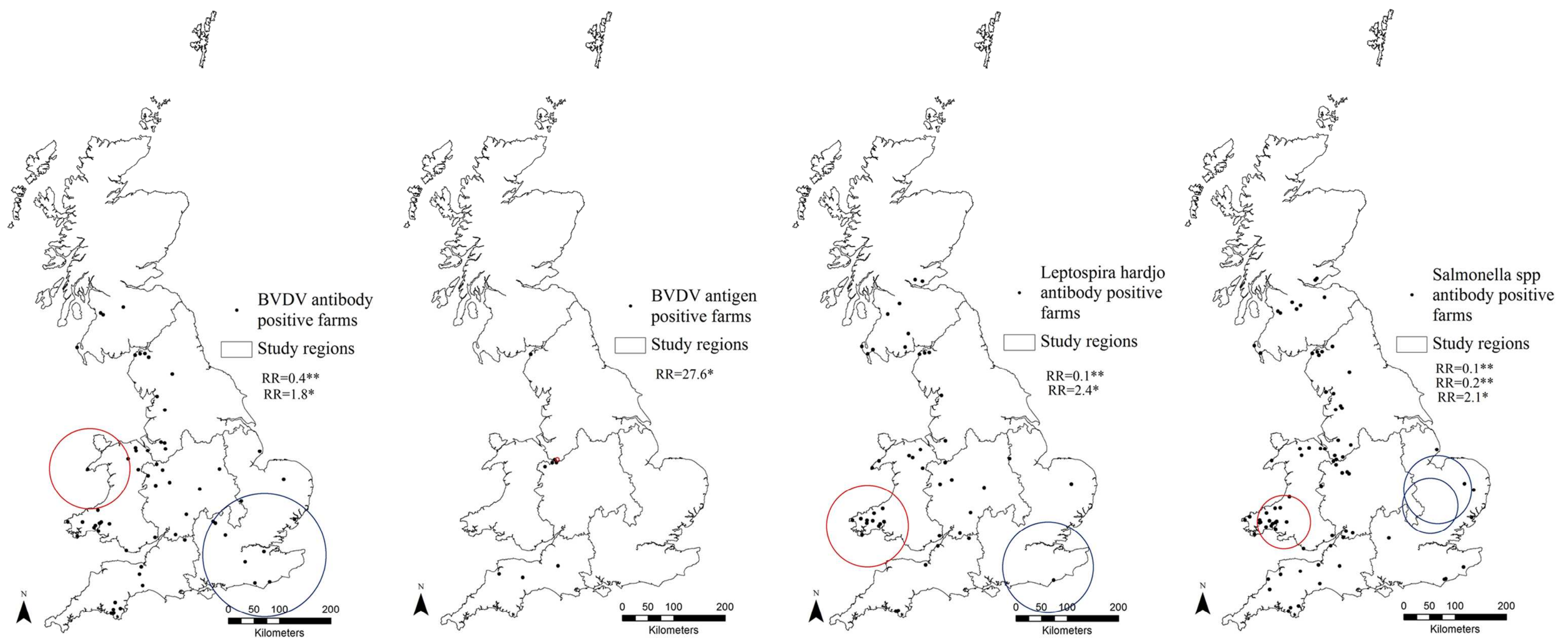
Velasova Figure 4b



Velasova Figure 4c



Velasova Figure 5a



Velasova Figure 5b