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Seroprevalence of *Mycoplasma bovis* in bulk milk samples in Irish dairy herds and risk factors associated with herd seropositive status

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ABSTRACT

Mycoplasma bovis is a serious disease of cattle worldwide; mastitis, pneumonia, and arthritis are particularly important clinical presentations in dairy herds. *Mycoplasma bovis* was first identified in Ireland in 1994, and the reporting of *Mycoplasma*-associated disease has substantially increased over the last 5 years. Despite the presumed endemic nature of *M. bovis* in Ireland, there is a paucity of data on the prevalence of infection, and the effect of this disease on the dairy industry. The aim of this observational study was to estimate apparent herd prevalence for *M. bovis* in Irish dairy herds using routinely collected bulk milk surveillance samples and to assess risk factors for herd seropositivity. In autumn 2018, 1,500 herds out of the 16,858 herds that submitted bulk tank milk (BTM) samples to the Department of Agriculture testing laboratory for routine surveillance were randomly selected for further testing. A final data set of 1,313 sampled herds with a BTM ELISA result were used for the analysis. Testing was conducted using an indirect ELISA kit (ID Screen *Mycoplasma bovis*). Herd-level risk factors were used as explanatory variables to determine potential risk factors associated with positive herd status (reflecting past or current exposure to *M. bovis*). A total of 588 of the 1,313 BTM samples were positive to *M. bovis*, providing an apparent herd prevalence of 0.45 (95% CI: 0.42, 0.47) in Irish dairy herds in autumn 2018. Multivariable analysis was conducted using logistic regression. The final model identified herd size, the number of neighboring farms, in-degree and county as statistically significant risk factors for herd BTM seropositivity to *M. bovis*. The results suggest a high apparent herd prevalence of seropositivity to *M. bovis*, and evidence that *M. bovis* infection is now endemic in the Irish dairy sector. In addition, risk factors identified are closely aligned to what we would expect of an infectious disease. Awareness raising and education about this important disease is warranted given the widespread nature of exposure and likely infection in Irish herds. Further work on the validation of diagnostic tests for herd-level diagnosis should be undertaken as a matter of priority.

Key words: *Mycoplasma bovis*, prevalence, risk factor, epidemiology

INTRODUCTION

Bovine mycoplasmosis, caused by *Mycoplasma bovis*, has been recognized as a major emerging disease of cattle worldwide (Nicholas, 2011). It was first identified in the United States in the early 1960s and has since spread to most countries in the world (Nicholas and Ayling, 2003). Infection may result in severe pain and therefore has consequential welfare implications for affected cattle, as well as production losses for the farm (Nicholas and Ayling, 2003). Infected cattle can present with several clinical manifestations, the most common of which in dairy herds are mastitis, pneumonia, and arthritis. In general, treatment and control of disease caused by *M. bovis*, particularly mastitis is frequently unrewarding (Maunsell et al., 2011; Lysnyansky and Ayling, 2016). Recent European data has shown a rapid increase in the development of antimicrobial resistance in *M. bovis* across the continent (Klein et al., 2019), further complicating treatment and necessitating the need for robust national and farm-level measures for control and prevention.

As with many infectious diseases, it is important to investigate the prevalence of the disease in a population to understand its impact. Previous prevalence studies

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have focused on either determining serological evidence of exposure to *M. bovis*, using ELISA testing, or detection of the pathogen, using tests such as PCR. A recent Belgian study reported an estimated herd-level true prevalence of 24.8%, based on ELISA testing of bulk tank milk (**BTM**) samples (Gille et al., 2018). In Denmark, herd-level apparent prevalences of 7 and 2% were reported using BTM samples, based on ELISA and PCR respectively, using the manufacturers' recommended cut-offs (Nielsen et al., 2015). The most recent countries to report infection with *M. bovis*, with this considered to reflect recent introduction rather than endemic states, are Finland in 2012 (Haapala et al., 2018) and New Zealand in 2017 (Jordan et al., 2021). Despite the serious impact of *M. bovis*-associated disease, it has been reported that it has not yet been sufficiently addressed by international control authorities (Calcutt et al., 2018).

Mycoplasma bovis was first identified and confirmed in Ireland in 1994 from a pneumonic calf imported from France (Doherty et al., 1994). *Mycoplasma bovis* was subsequently documented as a cause of arthritis (1996), mastitis (1998), and abortion (1999) in Irish dairy herds (Byrne et al., 2001). Since then, there has been a substantial increase in reporting of *Mycoplasma*-associated disease in Ireland, particularly cases of arthritis and mastitis in dairy herds over the last 5 years. *Mycoplasma bovis* is now among the 5 most common pathogens detected at necropsy in bovine respiratory disease cases reported by the Department of Agriculture Food and the Marine (**DAFM**; Veterinary Laboratory Service, 2019). Explosive outbreaks of *M. bovis* occur sporadically, often with devastating consequences for the farms involved, and these outbreaks are regularly investigated by the authors. Despite the presumed endemic nature of *M. bovis* in Ireland, there is a paucity of data on the prevalence of infection, and the impact of this disease on the dairy industry. In Ireland, 90% of dairy herds adhere to a compact spring calving system of production, which is predominantly pasture based (Ramsbottom et al., 2015); this may affect the dynamics of within-herd transmission.

Methodological advances now make it possible to test for the presence of the pathogen, or alternatively exposure to the pathogen at herd level through a variety of tests. Bulk tank milk serological screening, as well as methods used to detect the presence of the pathogen, are widely employed in screening for biosecurity purposes and in national disease surveillance (Johnson et al., 2014; Ryan et al., 2018). The use of bulk tank screening by ELISA for serological evidence of *M. bovis* infection has been used in other studies (Petersen et al., 2016; Parker et al., 2017). However, no national prevalence estimates or research specific to *M. bovis* has been undertaken in Ireland to date. Serological tests enable a rapid and cost-effective means to establish presence of infection in a herd (Andersson et al., 2019), where exposure is deemed a proxy measure for infection. Estimates of between-herd prevalence of *M. bovis* infection are not widely available with the exception of a few studies (Nielsen et al., 2015; Vähänikkilä et al., 2019). In addition to the collection of prevalence data, risk factor information is also essential to inform any control strategies. Risk factors for between-herd spread of *M. bovis* infection largely relate to herd size and those associated with biosecurity practices. These include increasing herd size, herd expansion, and purchase of cattle, (McCluskey, 2003; Lysnyansky et al., 2016) with the potential to inadvertently introduce infected carrier animals (Punyapornwithaya et al., 2010). More recently, presence of a breeding bull (Gille et al., 2018) was identified as a risk factor for the presence of *M. bovis* within a herd. In addition, the use of contaminated semen for AI (Haapala et al., 2018) has been reported as a potential risk factor for the introduction of the pathogen.

Given the background of *M. bovis* and its impact as a pathogen, we aim to adopt these methodological advances in herd screening to address the knowledge gap relating to *M. bovis* in Ireland. The aim of this observational study was to estimate apparent herd prevalence for *M. bovis* in Irish dairy herds using routinely collected bulk milk surveillance samples and to assess risk factors for herd seropositivity status.

MATERIALS AND METHODS

Sample Collection and Testing

Bulk Tank Milk Sample: Collection and Sampling. The Blood Testing Laboratory of the Veterinary Laboratory Service of the DAFM acquires BTM samples from all Irish dairy herds in the spring and autumn of each year, primarily for surveillance of targeted bovine diseases. Simple random sampling was undertaken, drawing from all herds (approximately 17,500) that submitted milk samples to the Blood Testing Laboratory in 2015. A total of 1,500 herds were identified, and the milk samples from these herds have been tested twice a year since this time for several infectious diseases, including bovine infectious diarrhea (**BVD**) and infectious bovine rhinotracheitis. Figure 1 presents the percentage of all dairy herds per country that were sampled. After the removal of herds with missing or insufficient samples, 1,327 sampled herds were tested for *M. bovis* in the current study. After removing herds

without herd identifier information or herds with fewer than 10 females over 2 yr old, a total of 1,313 sampled herds remained in the data set for analysis.

ELISA Testing and Interpretation. Testing was conducted using ID Screen *Mycoplasma bovis* indirect ELISA kit (ID-Vet) according to the manufacturer's instructions, using the short incubation protocol. Bulk milk samples with a sample-to-positive percentage ≥20% were considered positive. The coefficients of variation for intra-assay and interassay variability provided by the manufacturer were 3 and 5.4%, respectively.

Risk Factors. Herd-level risk factors were used as explanatory variables to determine potential risk factors associated with exposure to *M. bovis* and positive herd status. Herd-level risk factors were constructed from herd-level variables extracted from the DAFM Animal Identification and Movement computer system and the Irish Cattle Breeding Federation. Data available for analysis included annual herd size from 2012 to 2018 inclusive (based on the number of females over 2 yr old, hereafter referred to as herd size) on August 1 each year, the number of births per month from August 2017 through July 2018, the number of males over 2 yr old (to identify if a breeding male may have been present) on August 1, 2018, the annual number of movements into the herd by year (July 31 to August 1 in the following year), the number of herds from which movements came (identified as in-degree), if there were any imports from other country from 2012 to 2018, the number of contiguous herds (number of neighbors), the number of land parcels per farm (both at August 1, 2018), county, and province. Land parcels are noncontiguous areas of land under common ownership. Therefore, a farm with 2 land parcels will be composed of 2 areas of land that are separated from each other. Counties with fewer than 10 tested herds were combined with neighboring counties. This applied to Dublin, which was combined with Wicklow, and to Leitrim and Roscommon, which were combined with neighboring Sligo.

In addition, new variables were constructed for calving season using the number of births in each month from August 1, 2017, to July 31, 2018. Herds were categorized as spring calving if >95% of births in 2018 occurred in the months January through June 2018, otherwise they were classed as non-spring-calving herds. Categorical variables were constructed from continuous variables if there was a nonlinear relationship with the outcome. The first related to herd expansion. Herd size based on the number of females over 2 yr old from 2012 to 2018 was used to assess if herds were expanding or not relative to the year 2012. Herd size was standardized against herd size in 2012 and herds were classified as expanding if the herd size had increased in 2018 by >5%, as stable if the herd size remained within 0 to 5% or as contracting if the percentage expansion was <0%, each compared with herd size in 2012. The 0- to 5-percentage-unit interval used to define herds as stable was chosen based on the distribution of the percentage expansion. These figures corresponded approximately to the 20th and 65th percentiles of the distribution. The mean number of introductions to the herd per year was calculated and a categorical variable created based on the quantile of number of introductions for the years 2014 through 2018. The variable in-degree, which included movements from 2014 to 2018 inclusive, was subdivided into quantiles, either zero herds from which animals were introduced, 0 to 1 herd, 1 to 2 herds or >2 herds. Binary categorical variables were also created for the presence of a breeding bull (if there was a male over 2 yr old present in 2018 or not) and if any imports occurred.

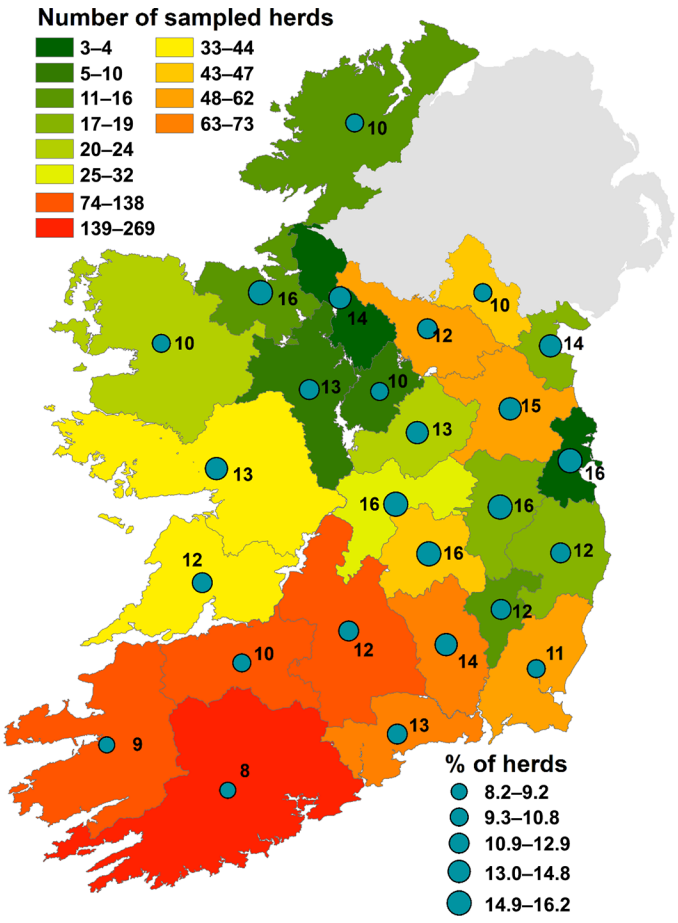

Figure 1. Map showing number of sampled herds from each county (choropleth) in Ireland and the percentage of all dairy herds in each county that were sampled (graduated circles and numeric values).

Data Analyses. An estimate for apparent herd-level point prevalence was calculated from the results and risk factors analyzed. Multivariable analysis was conducted using logistic regression modeling with BTM herd positivity as the outcome variable of interest with herd-level risk factors tested as explanatory variables.

Each variable was first assessed to determine if it was normally distributed by visually appraising histograms of the variable. Variables that were not normally distributed were log-transformed. In addition, the relationship between each continuous variable and the binary outcome was visually assessed using locally estimated scatterplot smoothing (Cleveland and Devlin, 1988). Variables with a nonlinear relationship with the logit of the outcome were categorized in quintiles. Each available variable was then tested individually for significance as an explanatory variable for herd BTM-positive status in a univariate analysis. Variables with $P < 0.2$ in the univariable analysis were brought forward to the multivariable analysis. The multivariable models were constructed using a forward stepwise approach; variables were then added to the multivariable model in order of their univariable P-value, with variables with the lowest P-value added first. Variables with $P < 0.05$ were retained in the multivariable model, including both categorical and continuous variables. After the addition of each new variable, P-values for the remaining variables in the model were recalculated. Variables with a $P > 0.05$ were removed from the model before further variables were added. For related variables such as province and county, the decision over which variable to include was made based on which variable resulted in the best overall model fit as assessed by the Akaike information criterion (**AIC**). As the inclusion of county resulted in a lower AIC, this was selected as the variable for location to be included in the final model. Similarly, upon the addition of each new variable in the model, the correlation between the variable to be added and the existing variables in the model was calculated. Where variables were strongly correlated ($r > 0.8$), only one of the correlated variables was selected for inclusion. The decision over which variable to use was based on the variable resulting in the best model fit as assessed by the AIC. Given the strengths and weaknesses of different model building approaches, we also repeated the model building steps using a backward elimination method and checked for consistency between the final models. Finally, the assumption of linearity between continuous variables in the final model, and the logit of the outcome variable was assessed using Box-Tidwell tests.

Data analysis and visual presentation of results were performed in R (2017; https://r-project.org) using the 'dplyr' (Wickham et al., 2015) and 'ggplot2' (Wickham and Wickham, 2007) packages. Results are presented as odds ratio and 95% confidence intervals are also reported. The STROBE guidelines on reporting for observational studies were consulted during the writing of this manuscript (von Elm et al., 2008).

RESULTS

A total of 588 of the 1,313 BTM samples were positive to *M. bovis*, providing an apparent herd prevalence of 0.45 (95% CI 0.42, 0.47) in Irish dairy herds in autumn 2018. Tables 1, 2, and 3 and Figure 2 include descriptive statistics about each variable included in the analysis.

Eleven variables, as listed in Table 1, were statistically significant at the univariable level and added to the multivariable analysis in order of significance. Descriptive tables of the variables used in the analysis are included in Tables 2 and 3 and in Figure 2.

The forward stepwise and backward elimination approaches each returned the same 4 variables in the final model. However, use of a correlation matrix identified that the number of land parcels and the number of neighbors was correlated. Both variables when included separately resulted in an identical measure of model fit. Therefore, the number of neighbors was retained and the number of land parcels removed. Results of the final multivariable model are presented in Table 4. Variables including herd size (based on the number of females over 2 yr old in 2018), the number of neighboring farms, the number of herds purchased from (in-degree) and the categorical variable representing quantiles of the mean number of herd introductions (between 2014 and 2018 inclusive) were all found to be significant ($P < 0.05$) in the initial phase of model building. For every unit increase in ln herd size, there was 2.65 times increased odds of having a BTM-positive result for *M. bovis* ($P <$

Table 1. List of statistically significant variables at the univariable level that were used to build the multivariable model

Variable
Log herd size (number of females over 2 yr old, August 2018)
Log number of neighbors, August 2018
Number of land parcels, August 2018
In-degree (number of farms purchased from) 2014–2018
Mean number introductions 2014–2018
Growth category 2012–2018
Presence of a male over 2 years old Aug 2018
Imports 2012–2018
Spring-calving-only herds (defined as 95% births occurred January to June 2018)
County
Province

Table 2. Summary of categorical variables included in the univariable analysis for association with herd bulk milk seropositivity to *Mycoplasma bovis*

Variable and Category	No. of herds per category
In-degree (no. of herds purchased from), 2014–2018	
0	407
1	358
2	199
>2	349
Mean no. of animal introductions, 2014–2018	
0	110
0–0.60	174
0.61–2.20	251
2.21–6.20	268
6.21–16.70	247
>16.70	263
Growth category, 2012–2018[1]	
Contracting	268
Stable	562
Expanding	483
Presence of male over 2 yr old, 2018	
No	343
Yes	970
Herds with imports, 2012–2018	
No	1,294
Yes	19
Spring-calving-only herds (95% of births occurred January–June 2018)	
No	458
Yes	855

[1]Growth categories: contracting = herds with percentage expansion <0%; stable = herds with percentage expansion between 0 and 5%; expanding = herds with percentage expansion >5%.

0.001), in other words, each 25% increase in herd size resulted in 1.24 times greater odds of having a BTM-positive result for *M. bovis*. For herds buying from multiple sources (in-degree category >2), there was 2.34 times greater odds of having a BTM result positive for *M. bovis* ($P < 0.001$). For each unit increase in the number of neighbors, there was 1.33 times greater odds of being a positive herd ($P = 0.029$). Other variables such as number of imports, number of land parcels, and the category that described herd growth between 2012 and 2018 were found to be not statistically significant when added to the multivariable model. Presence of a male over 2 yr old in 2018, as well as whether the herd was spring calving or not were also not found to be significantly associated with herd BTM seropositivity to *M. bovis* in the final multivariable analysis. When geography for each herd, as denoted by county, was added to the model, the category of mean number of introductions to the herd became insignificant and was removed. County was associated with the odds of having a herd-positive BTM result for *M. bovis*; County Clare had the lowest odds for herds being positive for *M. bovis* and was used as the referent county. Relative to County Clare, herds in County Monaghan had the highest odds of having BTM-positive herds (13.36; $P < 0.001$), followed by counties Galway and Meath.

The final model identified herd size, the number of neighboring farms, in-degree, and county as statistically significant risk factors for herd BTM seropositivity to *M. bovis*.

DISCUSSION

The aim of our study was to estimate herd-level *M. bovis* prevalence in Ireland, and to identify herd-level risk factors associated with BTM seropositivity for *M. bovis* as a proxy for exposure to *M. bovis* in Irish dairy herds. This work was conducted using routinely recorded data. There is high apparent herd prevalence among dairy herds in Ireland, with evidence from 45% of tested herds of at least past exposure to *M. bovis*. Further, risk factors associated with herd-level seropositivity included increasing herd size, the buying in behavior (reflected as an increased risk associated with those herds who bought from more than 2 sources), the number of contiguous neighboring farms and region (as identified by county).

Prevalence

This study provides considerable clarity with respect to the current epidemiological status of *M. bovis* infection in the Irish dairy herd. Considerable time has passed since the first report of *M. bovis* in Ireland by Doherty et al. (1994), and this study is the first to estimate apparent herd prevalence for *M. bovis* exposure in Irish dairy herds. The results highlight the high apparent herd prevalence, with evidence of exposure present on 45% of sampled dairy herds. Of equal importance, the geographical distribution is widespread, with exposed herds present in all Irish counties. Collectively, these findings (high apparent herd prevalence, widespread geographical distribution) and the extended period of pathogen presence in Ireland collectively suggest that *M. bovis* is now an endemic infection in Ireland. This is an important finding, with implications for the Irish dairy industry as outlined subsequently.

The apparent herd prevalence in this study is substantially higher than recent reports from Belgium with an estimated true herd prevalence of 24.8% (Gille et al., 2018), and Denmark with an apparent herd prevalence of 7% (Nielsen et al., 2015). We accept that these country comparisons should be interpreted with care, given differing criteria for herd recruitment and the use of different diagnostic test kits. Of relevance, a recent study using bovine serum reported large differences in

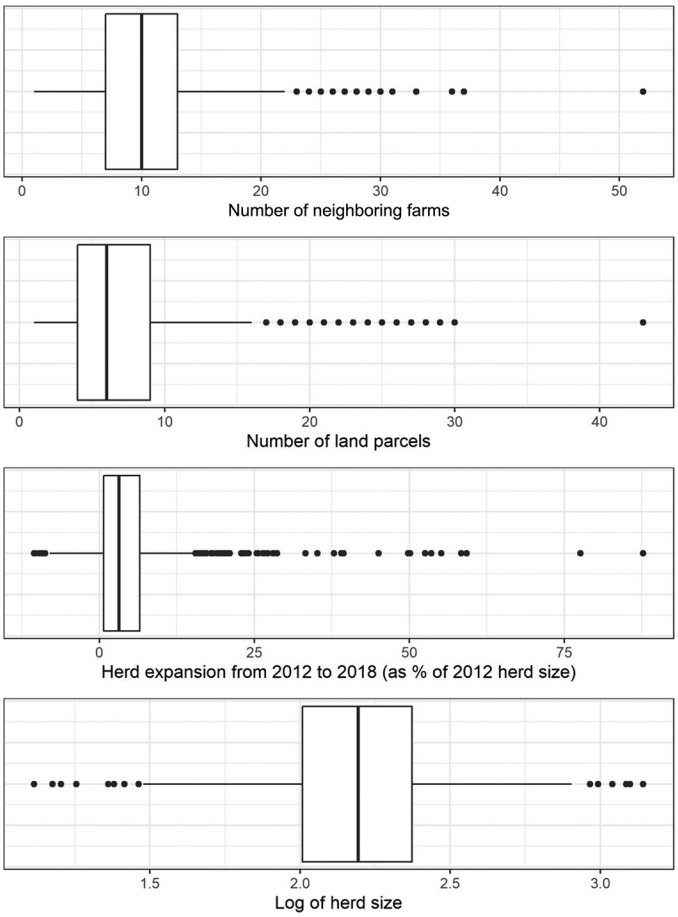

Figure 2. Box plots of each continuous variable used in the univariable analysis, i.e., association with herd bulk milk seropositivity to *Mycoplasma bovis*. For each variable, the box represents the 25th, 50th, and 75th percentiles; whiskers are 1.5 times the interquartile range (above and below 25th and 75th percentiles); and dots are values outside of the whisker range. All variables relate to herd characteristics on August 1, 2018. Number of neighbors is the number of unique farms with land bordering the herd of interest; number of land parcels is the number of separate, noncontiguous land areas owned by the herdowner; percentage expansion is the percentage increase in herd size between 2012 and 2018; Log(HerdSize) refers to the log of the number of females over 2 yr in 2018.

performances between commonly used *M. bovis* ELISA test kits (Andersson et al., 2019).

Risk Factors

The identified risk factors for herd exposure are similar to those reported elsewhere. They are also consistent with those factors that would be expected to increase herd risk in a country with endemic infection.

Larger herds are at increased exposure risk, a result that is consistent across a range of international studies. International studies have identified larger herds at higher risk of being infected with *M. bovis* (Fox et al., 2003; McCluskey, 2003). McCluskey (2003) reported large herds, which were classified as herds with over 500 cows, being up to 66 times more likely to have a BTM culture positive for *M. bovis*. Fox et al. (2003) found that volume of milk production, which was thought to be a function of herd size, was correlated with the risk of being culture positive for *M. bovis*. A more recent study of *M. bovis* in Japan identified larger herds, or corporation-type farms as more likely to be positive for *M. bovis* based on PCR testing (Murai and Higuchi, 2019). Similar findings were observed in Israel with increasing herd size and an increase in *Mycoplasma* mastitis (Lysnyansky et al., 2016).

Increased herd size is recognized as a risk factor for many infectious diseases (Sayers et al., 2015), reflecting an increased likelihood of introducing stock from other farms and also increased opportunity for transmission and maintenance of infection (Lysnyansky et al., 2016). Similarly, larger herds have a greater number susceptible (and potentially more infectious) animals during an outbreak with the potential to perpetuate the transmission of infectious diseases such as *M. bovis*. We speculate that the national picture could have been influenced by the abolition of milk quota in 2015 and the large-scale expansion that occurred in Ireland subsequently, leading to a substantial increase in average herd size. Anecdotally, outbreaks of *M. bovis*–related

Table 3. Summary of results of herd-level bulk milk testing using the ID-Vet indirect ELISA to *Mycoplasma bovis* antibody, per county in Ireland; samples were collected in autumn 2018

County	No. (%) of herds testing		Total no. of herds
	Negative	Positive	
Clare	36 (83.7)	7 (16.3)	43
Carlow	6 (42.9)	8 (57.1)	14
Cavan	33 (62.3)	20 (37.7	53
Cork	178 (66.7)	89 (33.3)	267
Donegal	6 (37.5)	10 (62.5)	16
Dublin/Wicklow	11 (50.0)	11 (50.0)	22
Galway	12 (28.6)	30 (71.4)	42
Kerry	89 (74.2)	31 (25.8)	120
Kildare	6 (33.3)	12 (66.7)	18
Kilkenny	27 (37.5)	45 (62.5)	72
Laois	20 (42.6)	27 (57.4)	47
Leitrim/Roscommon/Sligo	18 (72.0)	7 (28.0)	25
Limerick	66 (62.9)	39 (37.1)	105
Longford	5 (10)	5 (10)	10
Louth	5 (31.3)	11 (68.8)	16
Mayo	14 (60.9)	9 (39.1)	23
Meath	16 (28.6)	40 (71.4)	56
Monaghan	14 (30.4)	32 (69.6)	46
Offaly	10 (31.3)	22 (68.8)	32
Tipperary	65 (47.8)	71 (52.2)	136
Waterford	47 (71.2)	19 (28.8)	66
Westmeath	12 (50.0)	12 (50.0)	24
Wexford	29 (48.3)	31 (51.7)	60

disease in Ireland are much more common in the last 5 to 6 years. We note, however, that herd expansion was not retained as a statistically significant variable in the final model.

Buying-in behavior was also identified as a risk factor for herd exposure. The introduction of carrier cattle without clinical signs is thought to be the primary means of introduction of *M. bovis* to farms (Maunsell et al., 2011). However, the subsequent development of clinical disease is less well characterized. Some farms will have cows that develop severe clinical disease shortly after introduction of a carrier animal as a result of shedding and transmission of *M. bovis*. In other herds, transmission appears to be delayed, reflecting a later shedding event. Haapala et al. (2018) has outlined the potential means of introducing *M. bovis* to herds, either through buying in, cattle movements, imports or introduction via fomites, or germplasm. Animal movements and purchase of animals has been shown to be associated with introduction and transmission of *M. bovis* into herds (Amram et al., 2013; Aebi et al., 2015). The importance of biosecurity practices is also implicit in our study results. In particular, the odds of infection with *M. bovis* were much higher in herds buying from multiple sources, compared with those buying from just one other herd or not buying/closed herds. Animal movement is a key feature of cattle production in Ireland (McGrath et al., 2018). Due to the data available, it was possible to consider biosecurity with some granularity, focusing not just on whether a herd is open or closed but also specific buying in behavior. In our study, the number of introductions was not retained in the final model, and the type of buying behavior appears to be more important than absolute number of introductions, reflected by the results that herds buying from more than one source were more likely to be seropositive. The biosecurity risk associated with *M. bovis* infection increases with an increase in the number of source herds from which animals are purchased.

Contiguity was also identified as important with respect to herd exposure to *M. bovis*. As reflected previously, the introduction of any infectious disease to a dairy herd is potentially related to inadequate biosecurity practices, such as whether the herd has contact with cattle from other farms or whether the herd is buying in which will bring the inherent risk of intro-

Table 4. Results of final multivariable model of association of herd risk factors and bulk tank milk seropositivity for *Mycoplasma bovis*

Variable and Category	Estimate	SE	Odds ratio (95% CI)	*P*-value
Log(herd size) (no. of females >2 yr old, August 1, 2018)	0.97	0.13	2.64 (2.07, 3.39)	<0.001
Log(no. of neighbors)	0.29	0.13	1.34 (1.03, 1.73)	0.029
In-degree (no. of herds purchased from)				
0 (closed herd)	Referent			
Bought from 1 herd	0.13	0.17	1.14 (0.82, 1.58)	0.428
Bought from 2 herds	0.24	0.2	1.27 (0.87, 1.87)	0.219
Bought from >2 herds	0.85	0.17	2.34 (1.69, 3.26)	<0.001
Herd location (county)				
Clare	Referent			<0.001
Carlow	1.69	0.71	5.42 (1.36, 21.7)	0.017
Cavan	1.19	0.52	3.29 (1.18, 9.13)	0.023
Cork	0.71	0.45	2.03 (0.85, 4.9)	0.111
Donegal	2.18	0.69	8.85 (2.26, 34.42)	0.002
Dublin/Wicklow	1.4	0.62	4.06 (1.21, 13.57)	0.023
Galway	2.54	0.55	12.68 (4.27, 37.44)	<0.001
Kerry	0.52	0.48	1.68 (0.66, 4.3)	0.271
Kildare	2.25	0.67	9.49 (2.56, 34.85)	0.001
Kilkenny	1.69	0.5	5.42 (2.05, 14.28)	0.001
Laois	1.71	0.52	5.53 (1.99, 15.41)	0.001
Leitrim/Roscommon/Sligo	0.79	0.64	2.2 (0.63, 7.65)	0.215
Limerick	0.88	0.48	2.41 (0.95, 6.12)	0.064
Longford	1.96	0.81	7.1 (1.46, 34.46)	0.015
Louth	2.2	0.71	9.03 (2.26, 35.9)	0.002
Mayo	1.26	0.62	3.53 (1.04, 11.93)	0.044
Meath	2.35	0.53	10.49 (3.74, 29.62)	<0.001
Monaghan	2.59	0.55	13.33 (4.57, 39.03)	<0.001
Offaly	2.21	0.58	9.12 (2.92, 28.6)	<0.001
Tipperary	1.55	0.46	4.71 (1.91, 11.62)	0.001
Waterford	0.31	0.51	1.36 (0.5, 3.75)	0.545
Westmeath	1.49	0.61	4.44 (1.35, 14.57)	0.014
Wexford	1.5	0.5	4.48 (1.66, 11.99)	0.003

duction of diseases. In the present study, the number of neighbors was identified as a statistically significant risk factor for herd BTM seropositive status. This is an interesting finding and may reflect the biosecurity threat posed by neighboring farms, noting that this was recently identified as a plausible mode of transmission of bovine viral diarrhea in a recent Irish study (Guelbenzu-Gonzalo et al., 2021). We accept that herd contiguity may be confounded by herd size, as larger herds could have more neighbors. However, the Irish farming landscape is highly fragmented. A farm may have multiple land parcels, each with a series of neighboring farms. In the absence of detailed information on whether there is nose-to-nose contact or the possibility for stock mixing, it is difficult to make inferences about the exact implications that the number of neighbors has for the risk of introduction of *M. bovis*. As with many infectious diseases, the role of fomites and indirect transmission sources between herds such as shared equipment or visitors is difficult to quantify but should not be ruled out. These indirect transmission pathways were studied in detail in Irish herds in a recent study by Guelbenzu-Gonzalo et al. (2021) on bovine viral diarrhea virus and highlight the biosecurity challenges facing most Irish herds.

Finally, county was also identified as a risk factor for *M. bovis* herd exposure. The association between county and herd seropositivity status is an interesting finding, with County Clare being the county with the lowest odds of having herd BTM seropositivity to *M. bovis*. Relative to County Clare, County Monaghan had the highest odds of a having herds positive for *M. bovis* on BTM serology. Other counties with a statistically significant higher risk of *M. bovis* exposure included counties Galway and Meath. This finding is difficult to interpret in terms of whether there is any plausible reason why herds in these counties may have higher prevalence of *M. bovis*, although it is not uncommon that farming systems may vary by county, such as arable or livestock enterprises, or differing cattle densities and herd types.

Limitations and Future Directions

For several reasons, the study results need to be interpreted with care. There is an absence of robust data in the peer reviewed literature on the performance of the ID-Vet ELISA as a herd-level test for BTM analysis for detection of *M. bovis* antibodies, and consequently it is difficult to extrapolate from apparent to true herd prevalence. In a recent Danish study, a correlation between individual animal blood and milk results was identified for the ID-Vet ELISA. However, this study did raise specificity concerns and outlined the need for further validation work if this test were to be used as a BTM screening tool (Petersen et al., 2020). More broadly, the use of BTM ELISA as a herd screening tool is limited and should be interpreted with care due to the many influences on ELISA results. For example, factors such as seasonality and stage of lactation could potentially affect antibody response (Parker et al., 2017; McAloon et al., 2020). McAloon et al. (2020) found an effect of stage of lactation and yield on serological response to Johne's disease in an Irish seasonal system; however, even when correcting for these factors, there was little effect, and categories of infection status did not really change. A further limitation is that our samples provide an estimate of prevalence at a single point in time. Petersen et al. (2020) discussed the much higher sensitivity of the ID-Vet ELISA relative to other kits. In addition, this study also suggested that the ID-Vet ELISA may detect antibodies for a longer time due to increased sensitivity, potentially providing exposure estimates for a substantial period in the past. It is a limitation of this study that we are reporting evidence of past exposure and do not have information on the number of recent active infections. In addition to work validating the ID-Vet ELISA as a diagnostic test for herd-level screening, further work is necessary to better understand the epidemiology of *M. bovis* in Irish dairy herds. Of particular concern are anecdotal reports from field veterinarians of ongoing disease problems on many farms, sometimes with high mortality. A proposed approach to validate the use of this assay as a herd-level screening test would be to use a Bayesian latent class analysis approach to evaluate test sensitivity and specificity given the absence of a gold standard test for determining herd-level infection.

The results suggest a high apparent herd prevalence of seropositivity to *M. bovis*, and evidence of endemicity of *M. bovis* infection, in the Irish dairy sector. This would concur with the previous clinical experience of the authors, given the frequency with which *M. bovis* has been diagnosed in herd outbreaks and clinical scenarios in Ireland. The risk factors are biologically plausible, and consistent with what would be expected for a disease such as *M. bovis*. For example, buying in behavior (such as sourcing from multiple herds) and larger herds each provide increased opportunity for introduction and establishment of herd infection. Mycoplasmosis is a biosecurity challenge, and particularly so in the fragmented Irish farming environment where cattle movements are common. In recent years, considerable progress has been made to increase awareness of farm biosecurity, particularly in association with the national BVD eradication program (Graham et al.,

2021). Awareness raising of *M. bovis* is needed, including the key role of farm biosecurity in effective control. Further work on the validation of diagnostic tests for herd-level diagnosis should be undertaken as a matter of priority.

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