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Management practices as risk factors for the presence of bulk milk antibodies to *Salmonella*, *Neospora caninum* and *Leptospira interrogans* serovar *hardjo* in Irish dairy herds.

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Abstract

A survey of management practices in 309 Irish dairy herds was used to identify risk factors for the presence of antibodies to *Salmonella*, *Neospora caninum* and *Leptospira interrogans* serovar *hardjo* in extensively managed unvaccinated dairy herds. A previous study documented a herd-level seroprevalence in bulk milk of 49%, 19% and 86% for *Salmonella*, *Neospora caninum* and *Leptospira interrogans* serovar *hardjo*, respectively in the unvaccinated proportion of these 309 herds in 2009. Association analyses in the present study were carried out using multiple logistic regression models. Herds where cattle were purchased or introduced had a greater likelihood of being positive to *Leptospira interrogans* serovar *hardjo* ($P<0.01$) and *Salmonella* ($P<0.01$). Larger herds had a greater likelihood of recording a positive bulk milk antibody result to *Leptospira interrogans* serovar *hardjo* ($P<0.05$). Herds that practiced year round calving were more likely to be positive to *Neospora caninum* ($P<0.05$) compared to herds with a spring-calving season, with no difference in risk between herds that practiced split calving compared to herds that practiced spring calving. No association was found between presence of dogs on farms and prevalence of *Neospora caninum* possibly due to limited access of dogs to infected materials including afterbirths. The information from this study will assist in the design of suitable control programmes for the diseases under investigation in pasture-based livestock systems.

Introduction

An investigation of the temporal trends in bulk milk antibody levels to *Salmonella*, *Neospora caninum* (*N. caninum*) and *Leptospira interrogans* serovar *hardjo* (*L. hardjo*) in Irish dairy herds was completed in 2009 (O' Doherty et al., 2013). This study documented herd-level seroprevalences of 49%, 19% and 86% for *Salmonella, N. caninum,* and *L. hardjo,* respectively, with no association found to exist between the prevalence of these pathogens with one another. The clinical manifestations of these infectious agents, which include abortion, poor calf health and mortality, have been shown in international cattle populations to have an adverse effect on the economic performance of dairy herds (Bennett, 1993; Visser et al., 1997; Chi et al., 2002). Control of these pathogens at farm level is therefore important to dairy farmers.

Previous dairy herd studies have identified herd size, purchase of animals and calving season as risk factors for testing positive for *Salmonella* (Evans and Davies, 1996; Vaessen et al., 1998; Carrique-Mas et al., 2010), *N. caninum* (Bjorkman, et al., 1996; Ould-Amrouche et al., 1999; Schares et al., 2004; Dubey et al., 2007) and *L. hardjo* (Van Schaik et al., 2002; Leonard et al., 2004; Ryan et al., 2012).

Risk factors specific for presence of and exposure to *Salmonella* in dairy herds include geographical region, the prevalence of *Salmonella* positive herds in the surrounding geographical region, importation of farm manure, concurrent infection with liver fluke, use of calving facilities to house sick animals and access of birds or rodents to animal feed supplies (Evans and Davies, 1996; Vaessen et al., 1998; Wedderkoop et al., 2001; Carrique-Mas et al., 2010).

N. caninum specific risk factors include the presence of farm dogs, access to pond water, the presence of older animals in herds and rearing home-bred replacements (Bartels et al., 1999; Ould-Amrouche et al., 1999; Schares et al., 2004; Frossling et al., 2005).

Additional risk factors specific to *L. hardjo* include geographical region, co-grazing with infected animals, access to contaminated water sources, and natural-mating (Leonard et al., 2004; Ryan et al., 2012).

Irish dairying is based on an extensive, pasture-based system of livestock production, operating at a stocking rate of, on average, 1.85 livestock units per hectare (LU/Ha) (Dillon, 2011). Such systems involve calving cows to coincide with the period of maximum grass growth i.e. springtime (Dillon et al., 1995), with cows being fed grazed grass outdoors for up to 235 days of lactation (Drennan et al., 2005). Few studies have documented pathogen exposure risk factors for such extensive production systems (Leonard et al., 2004). In addition, the majority of previous studies examined risk factors for the presence of the actual pathogen (Evans and Davies, 1996; Vaessen et al., 1998; Bartels et al., 1999; Carrique-Mas et al., 2010) with limited studies on identification of risk factors associated with bulk milk seropositivity (Wedderkoop et al., 2001; Schares et al., 2004; Leonard et al., 2004).

As bulk milk analysis is becoming an important diagnostic tool at farm level for the purposes of herd health planning (McElroy 2012), identifying risk factors associated with bulk milk seropositivity would prove beneficial. Identification of such risk factors would also assist with promotion of biosecurity implementation (Villarroel et al., 2007) on Irish dairy farms which, at present, is sub-optimal (Sayers et al., 2012). The objective of this study therefore was to establish the association between general management and biosecurity-related practices, and bulk milk tank antibody status in unvaccinated herds in order to identify risk factors for exposure to *Salmonella*, *N. caninum* and *L. hardjo* in an extensive grazing production system.

Materials and Methods

Herd selection

Selection of herds for use in this study has been previously described in detail by O' Doherty et al. (2013). Briefly, 312 herds which were members of HerdPlus®, a breeding information decision support tool for farmers co-ordinated by the Irish Cattle Breeding Federation (ICBF), volunteered to participate in the study. Herds were randomly selected within strata of herd size (31 to 65 cows, 66 to 99 cows, and >99 cows) and geographical location in Ireland (county; n=26). The number of herds selected per strata was weighted by the number of herds within-stratum in the entire Irish population.

Survey design

A survey questionnaire comprising 57 questions (Appendix 1) was compiled from multiple sources including a web-based herd health management tool[1], a parasite and grazing management questionnaire (Charlier et al., 2005), and a comprehensive review of literature. Questions broadly related to livestock management, farm visitors, equipment, hygiene and disinfection and bioexclusion measures such as not purchasing animals, quarantine procedures, access to dogs and to water courses, and wildlife control measures. An additional set of questions was used to obtain data on vaccination protocols employed on each farm for *Salmonella* and *L. hardjo*. Of the 57 variables (survey questions), 30 had multiple classes, 27 had binary (yes/no) responses and all were closed-ended. A selection of variables is included in Table 1. The questionnaire was piloted by farm managers on seven Teagasc (Irish Agriculture and Food Development Authority) research farms which led to minor adjustment

[1]www.myhealthyherd.com

of the questionnaire. A consultation with researchers at the Animal and Grassland, Research and Innovation Centre, Teagasc, Moorepark also took place to review the final questionnaire before circulation to participating farmers.

Survey administration

Survey packs were mailed to each of the 312 study participants in December 2009. Survey packs contained a cover letter, the questionnaire, and a self-addressed envelope. A reminder letter was issued to non-respondents in March 2010 followed by a reminder telephone call in May 2010. Surveys were not received from three participants and these herds were removed from further analysis.

Ancillary information

In addition, to the self-declared variables collected from the farmer questionnaire, data on a further nine additional risk factors were sourced from the ICBF database. These included the number of dairy cows in 2009, variation in dairy cow numbers between 2006 and 2009, percentage of first lactation animals in the herd in 2009, percentage of home born dairy cows in the herd in 2009, percentage of Holstein-Friesian animals in the herd in 2009, the presence of a natural-mating bull on the farm and whether the natural-mating bull was purchased or home-born. The geographic location of each study herd was also considered as a risk factor. Location of study herds was divided into seven geographical regions according to Irish Central Statistics Office (CSO, 2007) survey procedures. These seven regions were subsequently combined into three logical regions based on dairy herd distribution in Ireland. Calving season was also examined as a risk factor for bulk milk seropositivity. Calving season in 2009 was split into three categories; spring-calving (i.e. ≥85% of the herd calved between January and March), split-calving (i.e. ≤ 85% of the herd calved between January and March with remaining cows calved between August and December), and herds that did not meet the criteria for inclusion as spring-calving or split-calving were classified as year-round calving. Of the nine additional variables, six had multiple classes and three were binary (Table 2).

Survey validation

The internal consistency of the survey was determined by evaluating the consistency of responses to questions that were repeated throughout the survey using a standardised Chronbach's coefficient alpha. Results of the Chronbach's coefficient alpha were interpreted using a guide by George and Mallery (2008). A total of three questions were repeated in varying formats. Repeated questions related to importation of slurry from other farms, feeding of feedstuffs to farm animals that had been in contact with other animals (i.e. wildlife), and access of farm animals to watercourses that have passed through other farms.

Herd classification

Bulk milk samples were collected from each study herd at four time points in 2009 (March, June, August and November) and were tested for antibodies against *Salmonella*, *N. caninum* and *L. hardjo* using commercially available Enzyme Linked Immunosorbant Assay (ELISA) kits. The sensitivity (Se) of the *Salmonella*, *N. caninum* and the *L. hardjo* ELISAs was 63.2%, 99% and 96.4%, respectively. The specificity (Sp) of each ELISA was 99.7%, 96% and 96.7%, respectively. Herds were classified as vaccinated or unvaccinated for *Salmonella* and *L. hardjo* based on farmer-declared survey data. There is no vaccine for *N. caninum* currently licensed in the Republic of Ireland. The ELISA test result and vaccination status of each herd were combined to determine the antibody status (test negative *vs*. test positive) of study herds. ELISA tests, used for detection of antibodies against *Salmonella* and

L. hardjo in the present study, did not differentiate between vaccinated and exposed herds; therefore vaccinated herds were excluded from further analysis. Unvaccinated herds were classified as negative for exposure to the respective pathogen if the herd recorded a negative bulk milk antibody reading at all of the four sampling time points in 2009 (O' Doherty et al., 2013). Participants were not aware of the disease status of their herds when completing the survey.

Statistical analysis

Univariate logistic regression analysis using PROC GENMOD (SAS Version 9.1, USA) was firstly used to determine the association between each of the 66 risk factors (i.e. survey results and ancillary herd information) and bulk milk antibody status in unvaccinated herds. In all analyses the likelihood of a positive antibody status was modelled. A binomial error distribution of the data was assumed in all models and a logit link function was used. In the case of *Salmonella* and *N. caninum*, independent variables were dichotomised when logical to do so (Tables 1 and 2). Variables with a never, occasionally (i.e. an event that occurs on rare occasions) and frequently (an event that occurs on a regular basis) response profile were dichotomised to no *vs*. yes responses. In addition, variables on housing and turnout of cows and calves were dichotomised to 'early' versus 'late' housing and 'early' versus 'late' turnout. Variables regarding heifer and calf-rearing location were dichotomised to 'reared on the home farm' (i.e. the farm where all the activities associated with the dairy enterprise take place) or 'reared on an outside farm' (i.e. parcel(s) of land located away from the home farm on which animals are grazed and winter feed supplies are harvested) (Table 1). Multiple responses were retained for certain variables e.g. calving season and region, as these provided biologically important information. Due to the small number (n=10) of unvaccinated herds that tested negative for exposure to *L. hardjo*, all independent variables were dichotomised for the purpose of identifying risk factors for *L. hardjo*.

Where an association ($P \leq 0.15$) between independent and dependent variables in the univariate analyses was identified, that independent variable was subsequently considered for inclusion in a multivariable analysis. All non-significant ($P>0.05$) variables were removed in a backwards elimination until only significant ($P<0.05$) variables remained in the model. In a subsequent forward step each of the non-significant variables were re-introduced into the model individually and if associated ($P<0.05$) with the dependent variable, they were retained in the final model. Variables that exhibited a low number of responses and for which odds ratios (OR) could not be calculated were removed from the analyses. All two way interactions between significant variables were quantified.

The predicted probability of a positive ELISA result was calculated from the multivariable model using:

$$P = (1 + e^{-(\alpha+\beta \mathbf{x})})^{-1}$$

where α is the intercept of the model, β is the predicted regression coefficient(s), and $\mathbf{X}$ is the design matrix for the variables in the model. The OR and their associated 95% confidence intervals (CI) were also calculated using contrast statements.

Results

Survey validation and response rate

A 99% response rate (309 herds) to the delivered questionnaire was achieved. These herds have previously been shown to geographically represent the Irish national dairy farm population (O' Doherty et al., 2013). The mean number of non-responders to individual questions was five [range zero (i.e. all respondents provided a response to a particular question) to 36 (i.e. thirty-six respondents did not supply a response to a particular question)].

The Chronbach coefficient alpha analysis yielded values of 0.79, 0.71 and 0.76 for responses to the same question indicating acceptable internal consistency of responses supplied in the survey.

Descriptive data and herd level antibody status

The mean herd size of the study population was 101 cows; 27% of herds had less than 65 cows, 34% had between 65 and 99 cows and 39% of herds had in excess of 99 cows. Of the study herds, 87% were spring-calving, 11% were split-calving and 2% were year-round calving. One third of respondents were located in the South West of the Republic of Ireland, 40% were located in the midlands and 26% were located in the North of the Republic of Ireland. Cattle were not purchased by 18% of herds, with 24% of survey respondents operating a quarantine facility for cattle entering the farm. Of the study population, only 21% reported that visitors to their farm were never allowed to enter the livestock areas without being dressed in appropriate protective clothing and without having their boots disinfected. Three variables on sharing grazing and buildings with cattle from other farms, regularly inspecting farm boundaries and rapidly disposing of dead animals exhibited a low response rate within categories and OR values could not be calculated for these variables; 99% of respondents reported that their cattle never shared grazing or buildings with cattle from other farms, 1 % reported occasional sharing of grazing and buildings with other cattle, with no respondents reporting frequent sharing of grazing and buildings with cattle from other farms. A total of 298 (96%) respondents reported that they regularly inspected farm boundaries. Of the study population only 1% reported that they did not rapidly dispose of dead animals. As reported by O’ Doherty et al. (2013) 76% (n=235) of the study herds vaccinated for *L. hardjo* while 49% (n=151) vaccinated for *Salmonella*. A total of 158 unvaccinated herds were included in the analysis for *Salmonella* and 74 unvaccinated herds were included in the analysis for *L. hardjo*. Of the 158 herds that did not vaccinate for *Salmonella*, 78 (49%) recorded a test positive result (O' Doherty et al., 2013). Of the 74 herds that did not vaccinate for *L. hardjo*, 64 (86%) recorded a test positive result (O' Doherty et al., 2013). A total of 60 herds (19%) recorded a test positive result for *N. caninum* (O' Doherty et al., 2013).

Test positive vs. test negative *N. caninum* herds

Univariate analyses highlighted several general management and biosecurity-related risk factors associated (P<0.15) with an increased likelihood of being bulk milk test positive to *N. caninum*. General management factors included earlier housing of cows (P=0.03), year round calving of cows (P=0.01), heifers grazing on cow's pasture (P=0.005) and grazing different age groups of cattle together (P=0.10). Biosecurity-related factors included the lack of secure farm boundaries (P=0.05), non-provision of adequate quarantine facilities for newly purchased animals (P=0.15), maintenance of clean and secure feed areas (P=0.13) and not testing newly purchased female animals for exposure to *N. caninum* (P=0.14). A comprehensive description of the associations between these both biosecurity-related and general management factors with *N. caninum* antibody status from the multivariable model are summarised in Table 3. A greater likelihood of being positive for exposure to *N. caninum* was observed in herds that practiced year-round calving and in herds that housed cows earlier (Table 3). The multivariable analysis also indicated a greater likelihood of being bulk milk positive to *N. caninum* in herds where secure farm boundaries were not present (Table 3). No significant two-way interactions were found

Test positive vs. test negative unvaccinated *Salmonella* herds

Univariate analyses highlighted biosecurity-related risk factors associated (P<0.15) with an increased likelihood of being test positive to *Salmonella*. These factors included frequent access to water courses that passed through other farms (P=0.05), herds having greater than three neighbouring farms containing cattle directly bordering the farm (P=0.10), herds that occasionally used agricultural contractors and did not insist that their equipment was clean and disinfected (P=0.15), herds that maintained a clean and secure feed area (P=0.11) and herds that maintained clean housing and yards (P=0.09). General management related risk factors associated (P<0.15) with an increased likelihood of testing positive to *Salmonella* in unvaccinated herds included larger herd size (P=0.10), herds that had a greater than 10% reduction in herd size between 2006 and 2009 (P=0.15) and herds that contained less than 70% home born animals (P=0.07).

The associations between both general management and biosecurity-related factors with *Salmonella* antibody status in unvaccinated herds in the multivariable model are summarised in Table 4. A greater likelihood of having a positive bulk milk reading for *Salmonella* was detected in herds that were located in the southern region of the Republic of Ireland, herds that contained less than 70% home born cows, herds that had frequent access to watercourses that passed through other farms, and herds where heifers co-grazed with cows (Table 4).No significant two-way interactions were found.

Test positive vs. test negative unvaccinated *L. hardjo* herds

Biosecurity related risk factors associated (P<0.15) with recording a positive bulk milk reading to *L. hardjo* in the univariate analysis included movement of cattle onto and off the farm (P=0.02), use of agricultural contractors without insisting that their equipment was clean and disinfected (P=0.10), and not minimising the numbers of visitors to the farm (P=0.15). General management related risk factors associated (P<0.15) with recording a positive bulk milk reading to *L. hardjo* included greater percentage of first lactation animals (P=0.13), rearing of calves on out farms (P=0.13), housing of calves later in the year (P=0.06), herds with higher numbers of dairy cows in 2009 (P=0.05), and herds that grazed calves on cows pasture (P=0.03). Results from the multivariable model (Table 5) indicate a greater probability of recording a positive bulk milk reading to *L. hardjo* was detected in herds that moved cattle onto and off the farm and in herds where calves grazed cows pasture. Herds containing higher numbers of cows and herds where oral drenching equipment was regularly cleaned had a higher probability of being antibody positive to *L. hardjo.* No significant two-way interactions were found.

Discussion

The objective of this study was to determine which farm practices, both general management and biosecurity-related, were risk factors for positive bulk milk results for *Salmonella*, *N. caninum* and *L. hardjo* in unvaccinated Irish dairy herds. As respondents were geographically representative of the national population of dairy farmers (O' Doherty et al., 2013), and an acceptable measure of survey internal consistency was achieved, this study provides risk information appropriate to pasture-based livestock dairy systems. The results from this study will assist in the design of suitable national control measures to reduce the presence of *Salmonella*, *N. caninum* and *L. hardjo* in Irish dairy herds and similar livestock systems internationally.

N. caninum

This study highlighted a greater likelihood of being bulk milk positive to *N. caninum* in herds where cows calve throughout the entire year compared to farms operating more compact calving systems. Herds that operate a year-round calving system have an opportunity to "recycle" non-pregnant cows throughout the year to minimise culling rates and extend the lactation of cows (Patton, 2012). This makes the opportunity to identify sub-fertile animals more difficult, potentially leading to retention of cows that would normally be culled from a spring-calving herd due to poorer fertility performance. As split-calving herds also operate discrete calving-seasons (albeit at two different periods), identification of sub-fertile cows is eased compared to year-round systems which may explain why a split-calving has not been identified as a risk factor. Alternatively year-round calving may result in more prolonged exposure of definitive hosts (i.e. canines) to placentas and afterbirths thereby perpetuating the lifecycle of *N. caninum*. Prolonged exposure to *N. caninum* infected material may be integral to its persistence within a herd, as this study also highlighted that herds that were housed earlier for the winter period were twice as likely to record a *N. caninum* positive bulk milk result. This earlier housing may inadvertently prolong exposure of bovine incidental hosts to oocyst contaminated feedstuffs/concentrates while indoors.

An unexpected finding was a greater likelihood (P=0.05) of being positive for exposure to *N. caninum* on farms with non-secure farm boundaries. Secure farm boundaries play a vital role in preventing disease spread between animals on neighbouring farms. There is no evidence, however, to suggest that direct cow to cow transmission of *N. caninum* exists (Dubey et al., 2007) unlike the more infectious viral diseases such as Bovine Viral Diarrhoea (BVD) and Infectious Bovine Rhinotracheitis (IBR) where direct animal contact is a highly efficient method of disease spread (Houe, 1999). The presence of non-secure boundaries may be indicative of poor overall farm management which may contribute to increased exposure to *N. caninum*. However, as *N. caninum* is a relatively newly identified pathogen which was only described first in 1988 (Dubey et al., 2007), the role of an, as yet, undetermined transmission method cannot be ruled out.

In the current study no association (P=0.51) was found between access of dogs and a bulk milk positive reading to *N. caninum*, which was unexpected. The presence of dogs, a definitive host for *N. caninum*, has previously been identified as a major risk factor for *N. caninum* in other populations (Bartels et al., 1999; Schares et al., 2004). Even though 82% of respondents in the present study reported that dogs had access to cow feeding and calving areas, prolonged exposure of dogs to potentially infected placentas and afterbirths would be limited as the majority of study herds calved cows in spring over a very short period of time. In addition, it should also be noted that a bovine brucellosis national eradication programme has operated in Ireland since 1965 (Hayes et al., 2009). Correct disposal of placentas and calving related materials (e.g. gloves etc.) was widely promoted as was the role of afterbirths in the spread of infectious disease. This will have contributed to many Irish farmers routinely adopting practices to adequately dispose of these materials, thereby preventing infection of farm dogs. Calving of cows indoors is common in spring-calving herds which again may increase the efficiency of placental disposal and minimise exposure of dogs to infected material. Finally, as the majority of cows in spring-calving systems are maintained on pasture, and the level of concentrates in the diet is limited, the potential for exposure of cows to faecal-contaminated feed is limited. Alternatively, even though dogs may have been present on study herds, it is possible that the dogs were not infected with *N. caninum*. It is possible, nonetheless, that this study may have lacked the statistical power to fully evaluate the role of the dog in the spread of *N. caninum* on study farms. Further investigation of this finding in extensive dairy systems is warranted.

In a pasture-based system, the tight calving season and resultant culling policies would appear to be protective against *N. caninum*. The relationship between culling policy and herd status for *N. caninum* deserves further investigation to definitively highlight its usefulness as a routine control method.

Salmonella

Carrique-Mas et al. (2010) reported a higher prevalence and incidence of *Salmonella* in areas of England with greater densities of dairy cattle. The greatest density of dairy cattle occurs in southern regions of the Republic of Ireland (Lesschen et al., 2011). It was expected, therefore, that a greater probability of being positive to *Salmonella* would occur in that region. The results generated did indeed highlight this trend with both multivariable models indicating a greater likelihood of being bulk milk positive to *Salmonella* in the southern region. A recent review of farm biosecurity has highlighted maintenance of a closed herd (not purchasing animals) as an important component of a farm biosecurity plan (Mee et al., 2012). In the present study, however, only 18% of respondents did not introduce new purchased cattle onto their farms. Similarly, a nationwide study of biosecurity on Irish dairy farms (n=450) found that only 12% of Irish dairy farmers operated a closed herd policy (Sayers, et al., 2012). In the current study of unvaccinated herds, those that purchased cows i.e. contained fewer than 70% home-born cows were 3.7 times more likely (Table 4) to record a positive bulk milk result to *Salmonella*. This is in agreement with Vaessen et al. (1998) and Evans and Davies (1996) who found that introduction of cattle is an important risk factor in the spread of *Salmonella*. A novel finding of this study showed that in herds where heifers grazed cow's pasture, they were over twice as likely to be bulk milk positive to *Salmonella*. As far back as 1975, faecal contamination of pasture was highlighted as an efficient method of transmitting *Salmonella* (Williams, 1975). In predominantly pasture-based dairy systems, therefore, grazing of paddocks by management groups of differing ages (e.g. cows and heifers) may be an important mode of *Salmonella* transmission. A study on biosecurity and risk management practices on dairy replacement rearing units in the USA by Maunsell and Donovan (2008) identified the minimisation of direct and indirect contact between different age groups of cattle as a practice to prevent new infections occurring in young stock. Grazing of heifers on cows pasture in such livestock systems should therefore be avoided to prevent new infections occurring in young stock.

Salmonella spp. have been previously isolated from rivers and streams, and water from a stream contaminated with *Salmonella* was linked to an outbreak of the disease in cattle in the United Kingdom (Williams, 1975). Results from the current study support this finding, with herds that had frequent access to watercourses that passed through other farms being more likely to record a positive bulk milk antibody reading to *Salmonella*. However, it was also highlighted that those herds with occasional access to such watercourses were less likely to be bulk milk positive than those herds with no access. These results suggest that further investigations are necessary to fully understand the role of watercourses in the transmission of *Salmonella* in pasture-based systems of dairy production.

L. hardjo

A limitation of the current study was the small number of herds that tested negative for *L. hardjo*, which reduced the statistical power for detection of significant risk factors for the presence of this pathogen. Leonard et al. (2004) found a higher prevalence of *L. hardjo* in larger herds in a study of 347 unvaccinated Irish dairy herds. As contact with urine from infected animals is an efficient method of spread of *L. hardjo* (Levett, 2001), greater contact between susceptible animals and urine from potentially infected animals is more likely in larger herds. The results from the current study support this finding with larger herds being likely to record a positive bulk milk result to *L. hardjo*. Additionally, the probability of having at least one positive animal was higher in larger herds and this combined with the high Se of the diagnostic test, resulted in larger herds recording a positive test result for exposure to *L. hardjo*.

A study by van Schaik et al. (2002) showed that direct contact between cattle through animal movement on and off the home farm e.g. allowing cattle to return to the farm when not sold at market, should be avoided to avoid introduction of infectious diseases including *L. hardjo*. Results from the current study support this finding, with herds where cattle were reintroduced into the herd after returning from marts and shows or from temporary grazing had an increased likelihood of being bulk milk positive to *L. hardjo*. Cattle that are moved off the farm and returned again can potentially become infected with *L. hardjo* through contact with other animals or through access to contaminated pastures or water sources. The findings in the present study are also in agreement with Noremark et al., (2011) who reported that markets are potential sources of infectious disease in Sweden. Similar to *Salmonella*, grazing of contaminated pasture by young-stock was a risk factor for herds being bulk milk positive to *L. hardjo* and again highlights that direct and indirect contact between different age groups of cattle should be minimised to prevent new infections occurring in young-stock. Grazing of calves on cows' pasture should therefore be avoided to prevent possible transmission of *L. hardjo* in dairy herds operating pasture-based systems. An unexpected finding of this study was a greater likelihood of testing positive for antibodies to *L. hardjo* in herds where oral drenching equipment was regularly cleaned. As cleaning of oral drenching equipment is indicative of good management practice, a lower likelihood of testing positive to *L. hardjo* on these farms was expected. One possible reason for the greater likelihood of testing positive for exposure to *L. hardjo* is that the cleaning of oral drenching equipment was carried out in response to the presence of *L. hardjo* or other infectious diseases in the herd.

The use of bulk milk tank testing in the current study identified similar risk factors to those found in previous studies. The majority of risk factors in previous studies were identified using individual animal testing to define herd status. This study, therefore, highlights the usefulness of bulk milk tank testing as a less expensive approach of classifying herd-level disease status and undertaking risk factor identification. Such studies are often prohibitively expensive where individual animal testing is used as the method of herd classification. In the present study bulk milk tank samples were used to classify herds as negative or positive for exposure to the pathogens under investigation and risk factors associated with exposure were also identified. However, the results of the present study need to be interpreted with caution due to uncertainty in the events prior to the study occurring e.g. was the risk factor present prior to introduction of the disease and therefore it cannot be stated for definite that the risk factors identified were the causative factor for the presence of the particular pathogen.

Conclusions

This study provides useful information on general management and biosecurity measures which can be used to reduce the risk of exposure to *Salmonella*, *N. caninum* and *L. hardjo* in herds operating pasture-based livestock systems. Incorporation of these findings into the design of control programmes should facilitate more effective disease management and appropriate application of resources. This study highlights an overlap in risk factors between pasture-based production systems and more intensive systems, although factors specific to pasture-based systems have also been identified. This study also highlighted a possible protective effect against *N. caninum* in compact seasonal pasture-based calving systems.

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Table 1. Description of selected variables

Table 2: Description of the 9 variables derived from ancillary data

Variable	Original response	N	Binary response
Do you buy or introduce cattle (including bulls) onto the farm	Never Occasionally Frequently	54 230 15	Never *vs*. occasionally and frequently
Cattle entering the farm (either newly purchased or returning from mart, show etc.) undergo adequate quarantine i.e. isolation for at least 30 days at a distance of at least 3 meters with no mixing of dung and urine	Never Occasionally Frequently	212 42 26	Never *vs*. occasionally and frequently
Are your farm boundaries secure and do not allow any contact between your cattle and others	Totally secure Almost secure Not secure	153 143 8	Totally secure *vs*. almost secure and not secure
Do you move cattle onto and off the farm including to shows or temporary grazing	Never Occasionally Frequently	190 103 14	Never *vs*. occasionally and frequently
Do heifers graze cows pasture	No Yes	156 144	No *vs*. Yes
Do you regularly clean oral drenching equipment	Yes No	265 42	No *vs*. Yes
Do calves graze cows pasture	No Yes	147 153	No *vs*. Yes
Are visitors allowed to enter the animal areas of the farm or have contact with the cattle without wearing appropriate protective clothing	Never Occasionally Frequently	65 206 37	Never *vs*. occasionally and frequently
What month were cows housed	September/October November December	80 166 44	September/October *vs*. November and December
What month were cows turned out to pasture	January February March/April	33 220 45	January and February *vs*. March/April
What month were calves housed	September/October November December	48 161 64	September/October *vs*. November and December
What month were calves turned out to pasture	January February March/April	38 127 123	January and February *vs*. March/April
Where were calves reared	Home farm Out farm Contract reared	147 145 4	Home farm vs. out farm and contract reared
Where were heifers reared	Home farm Out farm Contract reared	111 178 6	Home farm vs. out farm and contract reared

Change in herd size in the previous three years	>10% decrease	28	>10 decrease to <10% increase *vs.* ≥10% increase
	<10 decrease to <10% increase	81	
	≥10% increase	195	
Percentage of first lactation animals in the herd in 2009	<15%	30	<25% *vs.* ≥25%
	>15% to <25%	139	
	>25% to <35%	106	
	≥35%	34	
Proportion of Holstein Friesian cows in the herd	<50 %	7	
	≥50%	302	
Was a natural mating bull present on the farm	No	87	
	Yes	222	
Was the natural mating bull purchased	No	52	
	Yes	257	
What percentage of the cows were home born	<70%	71	<70% and >70% to <90% *vs.* ≥90%
	>70% to <90%	103	
	≥90%	134	
Calving Season	Entire spring calving	269	Entire spring calving vs. split calving and year round calving
	Split calving	33	
	Year round calving	7	
Herd size in 2009	<65 cows	85	< 65 cows and 66 to 99 cows vs. > 99 cows
	66 to 99 cows	104	
	>99 cows	120	
Region of Ireland	South west	105	South west and midlands vs. North
	Midlands	124	
	North	80	

Table 3. Predicted probabilities (PP), odds ratios (OR) and associated 95% confidence intervals (95% CI) for management practices associated with presence *vs*. absence of antibodies to *N. caninum* in 309 Irish dairy herds in 2009 in the multivariable analysis

Risk Factor	PP	Contrast	OR	95% CI	P value	Model P value
Calving season						
Year round	0.65	Year-round *vs*. Split	7.67	1.08, 54.26	0.04	
Split	0.19	Year-round *vs*. Spring	7.96	1.43, 44.15	0.02	0.04
Spring *	0.19	Split *vs*. Spring	1.04	0.37, 2.93	0.95	
When are cows housed						
Sept/Oct	0.33	Sept/Oct *vs*. Nov/Dec	2.16	1.15, 4.06	0.02	0.02
Nov/Dec *	0.19					
Are your boundaries secure						
Yes *	0.11	Yes *vs*. no	0.54	0.29, 1.00	0.05	0.05
No	0.19					

*=referent category for calculation of predicted probabilities

Table 4. Predicted probabilities (PP), odds ratios (OR) and associated 95% confidence intervals (95% CI) for management practices associated with presence *vs*. absence of antibodies to *Salmonella* in 158 unvaccinated Irish dairy herds in 2009 in the multivariable analysis

Risk Factor	PP	Contrast	OR	95% CI	P value	Model P value
Region of Ireland						
North	0.35	North *vs*. midlands	0.42	0.18, 0.99	0.05	
Midlands	0.57	North *vs*. southwest	0.37	0.15, 0.91	0.03	0.05
Southwest *	0.60	Midlands *vs*. southwest	0.88	0.38, 2.04	0.76	
Do cattle have access to watercourses that have passed through other farms						
Occasionally	0.49	Occasionally *vs*. frequently	0.12	0.03, 0.50	0.004	
Frequently	0.89	Occasionally *vs*. never	0.63	0.29, 1.38	0.25	0.006
Never *	0.60	Frequently *vs*. never	5.30	1.34, 20.97	0.02	
Do heifers graze cows pasture						
No *	0.40	No *vs*. yes	0.44	0.22, 0.89	0.02	0.02
yes	0.60					
What percentage of the dairy cows were home born						
<70%	0.85	<70% *vs*. >70 <90%	3.69	1.41, 9.64	0.008	
>70 <90%	0.61	<70% *vs*. ≥90%	3.91	1.48, 10.29	0.006	0.008
≥90% *	0.60	>70 <90% *vs*. ≥90%	1.06	0.48, 2.35	0.89	

*= Referent category for calculation of predicted probabilities

Table 5. Predicted probabilities (PP), odds ratios (OR) and associated 95% confidence intervals (95% CI) for management practices associated with presence *vs*. absence of antibodies to *L. hardjo* in 74 unvaccinated Irish dairy herds in 2009 in the multivariable analysis

Risk Factor	PP	Contrast	OR	95% CI	P value	Model P value
Do calves graze cows pasture						
Yes *	0.99					
No	0.98	Yes *vs*. no	13.69	1.21, 154.54	0.03	0.03
Do you move cattle onto and off the farm including to shows or temporary grazing						
Yes *	0.99					
No	0.98	Yes *vs*. no	15.15	1.35, 170.27	0.03	0.03
Herd size in 2009						
<99 cows	0.45	<99 cows *vs*. > 99 cows	0.02	0.0005, 0.62	0.03	0.03
> 99 cows *	0.98					
Do you regularly clean oral drenching equipment						
Yes *	0.98					
No	0.48	No *vs*. yes	0.02	0.0005, 0.74	0.03	0.03

*=Referent category for calculation of predicted probabilities