INTERPRETIVE SUMMARY

Relative importance of herd-level risk factors for probability of infection with paratuberculosis in Irish dairy herds

McAloon

The objective of this study was to identify the most important herd-level risk factors for Johne's Disease in dairy herds. Analysis of management practices, animal movement and diagnostic test data from 925 Irish dairy herds identified routine use of the calving area for sick or lame cows, and length of time spent in the calving pen as the most important risk factors in Irish dairy herds.

RISK FACTORS FOR PARATUBERCULOSIS

Relative importance of herd-level risk factors for probability of infection with paratuberculosis in Irish dairy herds

Conor G. McAloon*, Michael L. Doherty*, Paul Whyte*, Simon J. More*, Luke O'Grady*, Lorna Citer†, Martin J. Green‡

*Section of Herd Health and Animal Husbandry, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

†Animal Health Ireland, Carrick-on-Shannon, Co. Leitrim, Ireland

‡School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, United Kingdom

ABSTRACT

Control of paratuberculosis is challenging due to the relatively poor performance of diagnostic tests, a prolonged incubation period and protracted environmental survival. Prioritisation of herd-level interventions is not possible because putative risk factors are often not supported by risk factor studies. The objective for this study was to investigate the relative importance of risk factors for an increased probability of herd paratuberculosis infection. Risk assessment data, comprehensive animal purchase history and diagnostic test data were available for 936 Irish dairy herds. Both logistic regression and a Bayesian beta regression on the outcome of a Latent Class Analysis were conducted. Population Attributable Fractions and proportional reduction in variance explained were calculated for each variable in the logistic and Bayesian models respectively. Routine use of the calving area for sick or lame cows was found to be a significant explanatory covariate in both models. Purchasing behaviour for the previous 10 years was not found to be significant. For the logistic model, length of time calves spend in the calving pen (25%), and routine use of

the calving pen for sick or lame animals (14%) had the highest attributable fractions. For the Bayesian model, the overall R-squared was 16%. Dry cow cleanliness (7%) and routine use of the calving area for sick or lame cows (6%) and had the highest proportional reduction in variance explained. These findings provide support for several management practices commonly recommended as part of paratuberculosis control programmes, however a large proportion of the observed variation in probability of infection remained unexplained suggesting other important risks factors may exist.

INTRODUCTION

Bovine paratuberculosis, also called Johne's Disease (**JD**) is characterised by chronic granulomatous enteritis which manifests clinically as a protein-losing enteropathy causing diarrhoea, hypoproteinaemia, emaciation and eventually death (Sweeney et al., 2012). Adverse effects on animal productivity in terms of lower milk yield (McAloon et al., 2016), higher cull rates (Hendrick et al., 2005), reduced value for culled animals (Richardson and More, 2009), possible adverse effects on fertility (Johnson-Ifearulundu et al., 2000) and losses due to continued spread of infection are key drivers in the attempt to control the disease at farm level. In addition, some research exists to suggest that the aetiologic pathogen *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) may pose a zoonotic risk (Chiodini et al., 2012).

Control of JD is difficult due to the relatively poor performance of diagnostic tests (Nielsen and Toft, 2008), a prolonged incubation period (Sweeney et al., 2011) and protracted environmental survival (Whittington et al., 2004). Several simulation studies have concluded that test and cull programmes are unlikely to be effective in isolation and that control of the disease on farm should centre primarily on closing infection routes, ideally in combination with testing and culling (Kudahl et al., 2011; Lu et al., 2010; Robins et al., 2015). However, there is little empirical evidence to support many of the specific interventions introduced at

herd level to reduce the probability of introduction and transmission of disease. Although several risk factor studies have been conducted, results often fail to agree with putative risk factors that inform key aspects of control programmes, making prioritisation of implementable control measures difficult (McAloon et al., 2015).

At least part of the disparity in these studies may be due to misclassification of positive and negative herds. Conventionally, herd level risk factor studies are conducted by attributing an infection status to each herd based on a set number of test reactors. However, such dichotomised approaches may discard important information regarding the likelihood of infection and may be biased in larger herds due to imperfect test specificity.

The use of Bayesian Latent Class methods allows the estimation of a probability of infection for each herd conditional on the test characteristics, number of test positive animals and the total number of animals in the herd (Branscum et al., 2004). In addition, Bayesian methods account for uncertainty associated with model parameters by modelling each parameter as a random variable with an associated probability distribution. Bayesian inference allows direct inference on the parameter of interest, conditional on the observed data and the prior distributions (Messam et al., 2008).

In Ireland, control of non-statutory diseases such as JD is coordinated by Animal Health Ireland (**AHI**) (More et al., 2011). In 2013, a pilot Voluntary Johne's Disease Control Programme was introduced which combined annual testing of all animals over 24 months of age with an on-farm Risk Assessment and Management Plan (**RAMP**) that captured herd management practices relevant to JD. RAMP has been widely adopted across many countries with recognisable control programmes (Geraghty et al., 2014). The Risk Assessment (**RA**) component involves assigning risk scores to different management procedures and areas based on observations and farmer reported practice. In addition, within the Irish system,

animal movement data for the herd is provided for the practitioner to assess bioexclusion risks. The outcome of the RA is used to inform a Management Plan (**MP**) and, in national programmes, may have some bearing on herd categorisation or herd risk score.

A reduction in animal-level test positivity associated with the implementation of management practices has been found in a number of small scale investigations on demonstration or study herds using the RAMP approach (Ferrouillet et al., 2009; Pillars et al., 2011; Espejo et al., 2012) but progress has not been reproduced in larger studies on commercial farms (Sorge et al., 2011). Furthermore, to the authors' knowledge there are no studies available investigating the risk associated with RAMP scores in combination with comprehensive herd purchase history, or modelling herd level infection status on a probabilistic scale.

The objective of this study was to identify and evaluate the relative importance of risk factors for JD probability of infection using diagnostic test results, RA scores and animal movement history for herds enrolled in the national voluntary Johne's Disease Control Programme.

MATERIALS AND METHODS

Dataset

The dataset for the current study was obtained from herds enrolled in the national voluntary Johne's Disease control programme. Enrolled herds were required to have all animals that were 24 months of age and older serologically tested using either serum or milk samples.

Diagnostic testing was conducted in approved government and commercial laboratories using one of 3 commercial ELISA kits approved for use in the AHI programme; Parachek (Prionics, Switzerland), Paratuberculosis Antibody Screening Test (Idexx, USA) and ID Screen (IDVet, Montpellier, France). Producers that elect to test using blood or milk sample were required to test all eligible animals once or twice per year respectively. Test data were stored centrally in the Irish Cattle Breeding Federation (**ICBF**) computer database. Data were

extracted for the period beginning 1st November 2013 and ending 30th December 2014 and included anonymised cow and herd identifiers, test-date, sample-to-positive (S/P) ratio, laboratory interpretation (negative, suspect, positive), sample type (blood or milk), testing laboratory (test kit) and county. Diagnostic test data were available for 1,040 herds.

Given that the time frame for extraction exceeded 12 months, several herds had results from more than one herd screen. To reduce the potential for reverse causality, i.e. the effect of changes in management occurring as a result of a positive diagnosis, the last herd screen occurring before the RAMP was preferentially selected, followed by the soonest herd screen occurring after the RAMP. Test and animal movement data were extracted separately and datasets were aligned using coded herd identifiers. An additional binary variable was created to investigate the effect of having the test before (1) versus after (0) the RAMP. The values for sensitivity (Se) and specificity (Sp) used in the models were appropriate for a single test.

In addition to ongoing testing, enrolled herds were required to have an annual RAMP carried out by a programme approved veterinary practitioner. The RAMP contained questions on the history of the disease on each farm as well as the risk of introduction of infection from sources other than animal movement – e.g. colostrum, slurry contractors etc. The biocontainment component of the RAMP consisted of an additional 28 questions regarding management practices and observations made on the farm at the day of the visit, which were deemed to be relevant to the spread of JD.

In the RA used in the Irish programme, questions were scored using an ordinal scale of 1, 4, 7 and 10. Within the AHI programme, this method was used to reduce the potential for subjectivity that might be associated with the use of a continuous scale, since each specific management practice may be associated with a particular score on the ordinal scale. In addition, the use of 1, 4, 7 and 10 rather than 1, 2, 3 and 4 was used as a means of weighting

134 the risk associated with each management practice. Higher scores were associated with
135 increased risk of transmission. However, for this study, RA scores were modelled as
136 categorical variables, thereby reflecting the risk associated with specific practices rather than
137 the risk scores *per se* and ensuring that the scale used would have no effect on the model
138 outcome. Questions asked as part of the RAMP are shown in Table 1.

139 To assist in assessing bioexclusion, the RAMP was pre-populated with animal movement
140 data for the herd over the preceding 10 years. Movement data included herd size, number of
141 male and female introductions, number of source herds and number of overseas imports for
142 every year from 2005 to 2014. Herd sizes less than 20 in 2014 were dropped from the
143 analysis. Herd size was next summarised across the 10-year period: herds that had a herd size
144 of <105% of herd size in 2005 were categorised as non-growing herds; the remaining herds
145 were then broken into mild, moderate and large growth by categorising the percentage growth
146 into 3 equal quantiles: 5-25%, 26-46% and >46%.

147 Movement and herd size data were then aggregated over two 5-year periods, 2005-2009 and
148 2010-2014. Within each 5-year period, herds were described as "closed" if no purchases had
149 been made, herds where no females were purchased and males were purchased at <5% of the
150 overall herd size were described as "Replacement bulls only", for the remaining herds, the
151 number of female purchases was averaged across the 5-year period and broken into 3 equal
152 quantiles: low, medium and high replacement purchase. Given small number of herds were in
153 the "closed" category for each 5-year period, this category was combined with "Replacement
154 bulls only" for the analysis. An additional binary variable was created to identify herds where
155 males were purchased at greater than 5% of the overall herd size. These herds were
156 considered likely to be purchasing male animals for beef production in addition to the dairy
157 enterprise. Finally, the number of source herds purchased from each year was averaged
158 across each 5-year period.

159 Herds were removed from the dataset when one or more of the 3 components of the scheme:
160 diagnostic test results, herd movement history and RAMP results, were missing or
161 incomplete. The final dataset included data from 925 herds.

162 ***Analytical Models***

163 ***Model 1; Logistic regression analysis.*** The outcome variable was herd infection
164 status (positive or negative) and herds were defined as positive when they had 2 or more
165 cows with positive tests. A cut point of 2 positive cows was used to account for imperfect test
166 specificity; with herd sizes represented in this study, it was less likely that two positive results
167 would both be false positives. In addition, for the purposes of comparison, the final model
168 was reassessed with a cut-point of 1 reactor, the final single reactor model is included as
169 supplementary material (Supplementary Material 1 - Table 1). Data analysis was conducted
170 in R-studio version 1.0.44 (The R Core Team, 2016). Individual explanatory covariates were
171 initially investigated within a univariable logistic regression framework and carried forward
172 for multivariable regression analysis when $P<0.2$. Before addition to the multivariable model,
173 covariates were assessed for correlation. When 2 variables were highly correlated (>0.8), one
174 was selected and brought forward for multivariable analysis based on whichever variable
175 resulted in the model with the lowest Akaike Information Criterion (**AIC**). Variables dropped
176 due to collinearity were replaced into the final model to test for significance. The model was
177 constructed using a forward stepwise elimination and variables with a significance
178 probability $P<0.05$ were retained in the model. Herd size and test medium were forced into
179 the model from the beginning of the multivariate analysis to account for the potential
180 confounding effect of these variables on test sensitivity and specificity. In addition, for the
181 purpose of comparison, the model was reconstructed using the AIC solely as the selection
182 criteria. Finally, the Population Attributable Fraction (**PAF**) was calculated for each variable
183 in the model based on distribution of exposure in cases (Hanley, 2001);

$$PAF = \frac{RR-1}{RR} \times \frac{\text{number of exposed cases}}{\text{overall number of cases}} \quad (1)$$

Adjusted relative risks were calculated from the Odds Ratios of the final model using the method described by Zhang (1998).

Model 2; Bayesian analysis. This analysis was conducted in two stages. First, a probability of infection for each herd was estimated using a Bayesian latent class model. This model had the same structure, and was implemented using the same methods as described in McAloon et al.(2016a). Briefly, the number of test positive animals in a given herd was assumed to follow a binomial distribution with a probability equal to the apparent prevalence and n equal to the number of animals tested. The apparent prevalence (**AP**) was related to the true prevalence (**TP**) and the test sensitivity (**Se**), and specificity (**Sp**) by the formula;

$$AP = TP \times Se + (1 - TP) \times (1 - Sp) \quad (2)$$

TP was modelled as a mixture of a Bernoulli distribution, with a probability equal to the probability of infection for the herd, and a beta distribution equal to the within herd true prevalence.

In the second step, the mean probability of infection for each herd was used as the outcome variable in a Bayesian beta regression model with a logit link (Branscum et al., 2007). The model was built using a forward stepwise analysis and variables were retained in the model when the 95% credible interval did not include zero.

The model had the following structure;

$$\mu_i \sim \text{beta}(a_i, b_i) \quad (3)$$

$$a_i = \psi_i \times \gamma \quad (4)$$

$$b_i = (1- \psi_i) \times \gamma \quad (5)$$

$$\text{logit}(\psi) <- \beta_0 + \beta_1 X_1 \ldots \quad (6)$$

$$\gamma \sim \text{gamma}(G_1, G_2) \quad (7)$$

where μ_i was the probability of infection for the i-th herd which was modelled by a beta distribution. To facilitate incorporation of the covariate information into the regression model, the beta distribution was parameterised in terms of its mean, ψ, and a parameter related to its variance, γ (Bransum et al., 2007). A logit link was used to estimate the regression coefficient, β, for each covariate, X. G_1 and G_2 were the shape and scale parameters for the gamma distribution, γ. Larger values of γ correspond to less heterogeneity in the data. For this analysis, a prior gamma distribution with a low mean and high variance was used (G_1=G_2=0.01).

Diffuse normal distributions (mean = 0, precision = 0.01) were used for the priors of each coefficient in the model. Model outcomes for each covariate were reported as probability of infection by converting the coefficients according to the formula;

$$p = \frac{1}{1+ e^{-(\beta_0 + \sum \beta_j X_j)}} \quad (8)$$

Where p is the probability of infection, β_0 is the intercept and β_j is the coefficient of the j-th covariate, X_j (Dohoo et al., 2010). To fit the model, mean probabilities of infection less than 0.01 (n=8) were rounded to 0.01 and those greater than 0.99 (n=100) to 0.99.

Model fit was assessed using posterior predictive simulations (Gelman et al., 2000). The predictive simulation incorporated within the model was;

$$\text{Pred}\mu_i \sim \text{beta}(a_i, b_i) \quad (9)$$

$\text{Pred}\mu_i$ was monitored for the final 5000 iterations of the overall simulation. Predicted probability of infection was compared to the probability of infection outcome from the latent class model and the mean difference and the mean squared difference was used to compare

models. The proportional reduction in variance explained was calculated for each variable by removing each variable in turn from the full model, re-estimating model parameters and, calculating the difference in R-squared relative to the full model.

The model was implemented in WinBUGS 1.4.1 (Lunn et al., 2000), the first 5,000 iterations were discarded as burn in, by which time convergence had occurred, and 15,000 iterations used for posterior inference. Convergence was assessed by visual assessment of the chain as well as by running multiple chains from dispersed starting values (Christensen et al., 2012). The code for analysis is provided as supplementary material (Supplementary Material 2).

RESULTS

Descriptive Statistics

925 herds were present in the final dataset. Overall median herd size was 80, Using a cut-point of 2 reactors, 265 herds were positive, giving an apparent prevalence of 0.29. RAMP scores are summarised in Figure 1 and animal purchase data for the 10 years prior to diagnostic testing are summarised in Table 2.

Median herd growth from 2005 to 2015 was 25%. From 2005-2009 only 30 herds were classified as closed, with a further 70 herds classified as replacement bull purchases only. Similarly, from 2010-2014, 37 and 109 herds were closed or replacement bull only. From 2005-2009, 30% of herds purchased replacement females at an annual average of more than 7.5% of the total herds size, whilst from 2010-2014, the equivalent figure was 28%. In each 5-year block the mean annual number of source herds was more than 1 for 54% of the herds in 2005-2009 and 38% in 2010-2014.

Model Outcomes

Model 1; Logistic regression.

The outputs from the final multivariable logistic regression model are shown in Table 3. The reference category for each variable has been selected to avoid negative coefficients. Herd size was positively associated with herd positivity with an odds ratio of 1.01 for each additional animal. Herds testing with milk were 1.57 times as likely to test positive as those testing with blood. A large seasonal effect was apparent with herds testing in January 2.1 times as likely to test positive as those tested in May. Herds where pooled colostrum was used for more than 10% of the calves were 2.1 times as likely to be positive compared to those herds where calves were fed colostrum from their own test-negative dam, this category had a PAF of 11.6%. Herds where weaned heifers were grazed near adult animals, but without direct or indirect contact were 1.7 times as likely to test positive, and had a PAF of 10.5%, as those where direct or indirect contact was possible. Herds where the milking cow environment had clearly visible manure contamination were 1.7 times as likely to be defined as positive compared to herds where only trace amounts of manure were visible with a PAF of 7.6%. Herds where the calving area was routinely used for housing sick and lame cows were 2.2 times as likely to be positive than those where the calving area was never used for non-calving cows and had a PAF of 14.2%. Herds where more than 50% of the calves were removed from the dam within 30 minutes of birth were 2.3 times as likely to test positive as those where 90% of the calves were removed within 15 minutes of birth, the PAF of this variable was 24.7%. Finally, herds that experienced small growth (5-25%) were 1.7 times as likely to test positive as those that expanded to a high (>50%) growth in herd size.

Model 2; Bayesian Model. Outputs from the final Bayesian beta-regression model are shown in Table 4. Overall the model explained 16% of the variation, indicating that a considerable amount of the variation in the probability of a herd being positive remained

unexplained. The reported presence of previous clinical or test positive animals was responsible for 22.6% of the overall variance explained (R-squared) and resulted in a mean probability of infection (95% probability interval) of 0.72 (0.66-0.77). A strong seasonal effect was again observed which was responsible for 35% of the overall R-squared, with herds testing in January having a probability of infection of 0.77 (0.69, 0.83). The proportion of the herd tested comprised 3.2% of the overall R-squared and was negatively associated with the probability of infection. The probability of infection dropped by 5% with each additional 10% of the herd tested. Feeding of forages to weaned heifers that had been spread with slurry in the previous season increased the probability of infection by 8% (0-16%). Dry cow cleanliness comprised 7.1% of the overall R-squared and herds where dry cows had no faecal contamination visible had a mean probability of infection of 0.67, compared to 0.60 in herds where faecal contamination was visible on the legs but not extending above the dewclaws. The use of the calving pen for non-calving animals comprised 5.8% of the overall R-squared and herds where the calving pen was routinely used for sick or lame animals had a probability of infection of 0.69 (0.65-0.74).

DISCUSSION

The present study used a combination of frequentist and Bayesian methods to investigate risk factors for positivity and infection probability in Irish dairy herds using data collected as part of the AHI voluntary programme.

In the logistic regression model, the speed with which calves were removed from the calving pen was the most important variable (PAF = 24.7%). Herds in which >90% calves were removed within 15 minutes of birth had the lowest risk of being positive, with herds where calves were still removed within 30 minutes were 2.2 times as likely to be positive. In this case the large PAF is caused by a combination of the relatively large odds ratio, combined

300 with the large proportion of herds within the higher risk category (n=507). The practice of
301 removing the calf immediately from the dam is commonly advocated for the purpose of
302 paratuberculosis control however, despite investigating this risk factor, a number of studies
303 have failed to find this practice associated with an increased risk of positivity (Johnson-
304 Ifearulundu and Kaneene, 1998; Wells and Wagnher, 2000; Nielsen and Toft. 2011).
305 However, Cashman et al. (2008), found an increased risk of culturing MAP from milk filters
306 in herds where a greater proportion of calvings where not supervised. Interestingly, the
307 practice of immediate separation from the dam is also recommended for the control of calf
308 diseases (McGuirk and Collins, 2004), although studies into the benefit of calf removal have
309 been equivocal (Weary and Chua, 2000; Trotz-Williams et al., 2007), McAloon et al., 2016b
310 recently found improved passive transfer in calves removed immediately from the calving
311 pen, compared with those spending more than 30minutes with the dam.

312 The use of the calving pen to house sick or lame animals was the second most important
313 management factor in both the Bayesian and logistic model with a proportional reduction in
314 R-squared of 5.8% and a PAF of 14.2%). Herds that routinely used the calving pen for sick or
315 lame cows had a mean probability of infection of 0.69 (0.65-0.74) and were 2.3 times as
316 likely to be defined as positive compared with herds in which the calving pen was never used
317 for sick or lame cows. The use of the calving pen for sick or lame cows is often discouraged
318 as part of JD control programmes (Sweeney et al., 2012). This is based on the rationale that
319 cows that are subclinically infected with JD are more likely to be susceptible and therefore
320 affected with other diseases but there appears to be little empirical evidence to support this
321 claim. It is however likely that “sick” cows would also include those suffering from
322 symptoms of clinical JD and this practice could facilitate disease transmission. In addition,
323 routine use of the calving pen for sick animals could be an indicator of increased stocking

density and insufficient building space, potentially resulting in increased exposure of calves to infected faecal material.

The source of colostrum was significant in the logistic regression model and had a PAF 11.6%). However, this variable was not significant in the Bayesian model. Herds in which over 10% of calves were fed colostrum from sources other than the dam (Risk Score 10) were 2.1 times as likely to be defined positive compared with herds in which dam-only colostrum was practiced. Nielsen et al. (2008) found that calves fed colostrum from multiple sources were 1.2 times as likely to be positive than those fed dam-only colostrum. However, this finding is not consistent. For example, in a longitudinal study, Pithua et al. (2011) found that calves fed PCR-positive colostrum were not at a significantly greater risk of testing positive as adults compared to those fed PCR-negative colostrum. In contrast, the same author found that calves fed a commercial colostrum replacer were less likely to be identified as positive as adults than those fed conventional colostrum (Pithua et al., 2009). Similarly, Stabel (2008) found that colostrum pasteurisation reduced the incidence of disease in calves as measured by interferon gamma. However, in the long-term, risk of infection for this cohort of calves was not different between groups (Godden et al., 2015).

Dry cow cleanliness was significantly associated with probability of infection in the Bayesian model and was responsible for a reduction in R-squared of 7.1%). The finding that the lowest dry cow contamination score was associated with an increased risk of infection compared to the second lowest score seems counterintuitive. This finding could potentially be explained given the seasonal calving system operated on Irish dairy herds, i.e. the fact that the dry cow pen is not in use for a large majority of the year. However, whenever the month when the RAMP was conducted was forced into the model, the variable remained significant suggesting that the time when the RAMP was conducted was not confounding this variable.

It is worth noting that risk scores of 7 and 10 were associated with increased risk compared to risk score 4 although these associations were not significant.

Similarly, the finding that herds where heifers were housed or grazed near cows but had no direct contact (Q23) were at greater risk of testing positive compared with those where there was direct contact or heifers were exposed via run-off or slurry spreading, is difficult to explain. The susceptibility to infection has been shown to decrease with age (Windsor and Whittington, 2010), however, more recently, Mortier et al. (2013) demonstrated that calves up to the age of 12 months could be infected with both high and low doses of MAP. Despite been identified as the lowest risk category for this model, the large proportion of herds where weaned heifers had direct or indirect contact with adult cows (45%) is a significant concern.

The milking cow environment score had a PAF of 7.6% with herds where manure was clearly visible were 1.7 times as likely to test positive as those where trace amounts of manure was visible. Although infection of adult animals is possible with sufficiently high doses of MAP (Whittington and Windsor, 2010), in this case it is more likely that the finding is indicative of the overall hygiene of the farm, rather than the specific risk to adult animals *per se*.

In the Bayesian model, the feeding of forages that had received slurry from adult animals was significantly associated with the probability of infection, however this variable only comprised 1.3% of the overall variation. Interestingly, a similar finding was observed in a North American study (Obasanjo et al., 1997). In contrast, Kohl et al. (2010) was unable to culture MAP from baled grass silage following inoculation, although samples were positive by PCR. The authors in that study suggested that conserved forages constituted a minor risk for transmission. In a pasture based system where conserved forages are consumed during the housed period, avoiding the use of slurry on harvested grass may difficult to avoid, which is reflected in the high proportion of herds in the higher risk category (95%). In addition, on

372 many farms, avoiding spreading slurry on grass harvested for younger animals would
373 necessitate segregation of conserved forage for different age groups of animals. Furthermore,
374 increased application on adult ground would lead to an increase in potassium content (Soder
375 and Stout, 2003), resulting in an increased Dietary Cation Anion Difference and therefore an
376 increased risk of hypocalcaemia (Goff, 2004).

377 The change in herd size from 2005 to 2014 was only significant in the logistic model with a
378 PAF of 6.7%. In that case, the lowest risk of testing positive was observed in herds that had
379 undergone significant expansion (>50%) over the 10-year period. Anecdotally, herd
380 expansion has been associated with an increased risk of poor heath in general. However, in a
381 previous Irish study, Jago and Berry (2011) found improved reproductive performance in
382 dairy herds undergoing higher levels of expansion suggesting that this finding could be
383 confounded by improved management in general on these farms. In addition, the same study
384 found that the average parity number decreased in herds as the rate of expansion increased.
385 The sensitivity of the ELISA is known to increase with increased age (Nielsen et al., 2013),
386 therefore as the mean age of the herd decreases, the effective herd level sensitivity of the
387 ELISA screen is also likely to have decreased.

388 In the Bayesian model, previous presence of test positive or clinical cases of JD explained the
389 largest proportion of variance explained (41%), however, in the logistic model, this variable
390 had a PAF of 12.6%. The finding is unsurprising and highlights awareness of the herd
391 infection status in many herds. It was decided to couple this variable with whether or not the
392 RAMP had been conducted prior to or after the herd screen in an attempt to remove any
393 possible confounding associated with prior knowledge of the disease in the herd. When the
394 variable was removed from the model, all of the variables remained significant.

Given the imperfect specificity of the test, herd size was included as a variable, largely to account for confounding since larger herds would have an inherently greater risk of having false positive test results. In agreement with this, herd size was found to be significant in the logistic model, whereas in the Bayesian model, herd size was not significant. However, previous studies have documented increased risk of infection status in association with increased herd size. Collins (1994) found that larger herds in Wisconsin were more likely to be defined positive based on serological methods, however this association was not statistically significant. Similarly, Daniels et al., (2002) found that clinical disease was more likely to be present on Scottish farms in the preceding 10 years when herd size was larger. Finally, based on analysis of submitted laboratory samples, Barrett et al. (2011), found a significant association between herd positivity and herd size.

To the authors' knowledge this study represents the first use of herd level outputs from a Bayesian latent class model to fit a beta regression on herd level risk factors. Furthermore, the use of PAF from a classical logistic regression model has not yet been used to investigate the relative importance of risk factors for paratuberculosis. The Bayesian model reduced the risk for misclassification due to imperfect test performance as test Se and Sp were incorporated within the latent class model. On the other hand, the logistic model was based on the binary outcome of assigned herd status facilitated the use of PAF, giving a more intuitive impression of the relative importance of significant risk factors. Both methods are limited by the sampling method in this study. The Irish JD control programme is voluntary and therefore may not be representative of the average Irish dairy farm. In addition, the study utilised a cross sectional design based on a single test, single RA strategy. Although the recommendation from the national programme is to conduct the RA prior to testing, it is possible that testing may have been conducted prior to the completion of the RA, prompting the introduction of management changes and thereby introducing the risk of reverse causality

into the analysis. The authors attempted to reduce this risk by using RA data from the first year of the programme. Given that the management practices identified as significant in this are biologically plausible and largely agree with putative risk factors, it seems unlikely that reverse causality was a significant issue in this analysis.

An unforeseen, outcome of the analysis was the strong seasonal effect that was observed in both models. In each model, January, February and March were associated with a greater risk of positivity. The risk decreased in April, May and June before increasing again in the autumn and winter. Within the Irish system, seasonality has the potential to be confounded by stage of lactation and therefore milk yield. Nielsen and Toft (2012), found that the risk of being test positive on milk ELISA was greatly increased in the first 7 DIM and increased linearly over the course of the lactation after correcting for milk yield which appeared to have a diluting effect. To investigate the current dataset further, we separated the dataset into herds using milk and those using blood. Although the lowest risk month for both datasets was the same, i.e. May, different temporal trends were apparent depending on the test media used. In the milk dataset, the risk steadily increased from March to August with a large peak in September before declining again from September to December. With the serum dataset, the highest risk of test positivity was in January with a decline until May, with a second smaller peak in July. These findings require further investigation to examine whether this trend repeats in subsequent years.

CONCLUSIONS

This study demonstrates the use of PAF and Bayesian beta-regression as a means of investigating the relative importance of herd-level interventions on a national scale for the control of paratuberculosis. The findings of this study suggest that the national control programme should emphasise avoiding the use of the calving pen to house sick and/or lame

cows, reducing the length of time calves spend in the calving pen to less than 15 minutes and reducing the prevalence of pooled colostrum feeding as key interventions to reduce the prevalence of paratuberculosis in Irish dairy herds. It should also be noted however, that a large proportion of the observed variation in probability of infection remained unexplained suggesting other important risks factors may exist.

AKNOWLEDGEMENTS

This study was carried out as part of the ICONMAP multidisciplinary research programme funded by the Irish Department of Agriculture, Food and the Marine. The authors wish to acknowledge the assistance of Animal Health Ireland and the Irish Cattle Breeding Federation in providing data for the study.

REFERENCES

Central Statistics Office (CSO):
http://www.cso.ie/multiquicktables/quickTables.aspx?id=aaa06 accessed 19/12/2016

Barrett, D., J. Mee, P. Mullowney, M. Good, G. McGrath, T. Clegg, S. More, S. 2011. Risk factors associated with Johne's disease test status in dairy herds in Ireland. Vet. Rec. 168, 410.

Branscum, A. J., I. A. Gardner, W. O. Johnson. 2004. Bayesian modeling of animal-and herd-level prevalences. Prev. Vet. Med. 66, 101-112.

Branscum, A.J., W.O. Johnson, M.C. Thurmond. 2007. Bayesian beta regression: applications to household expenditure data and genetic distance between foot-and-mouth disease viruses. Aus. NZ. J. Stat. 49, 287-301.

Chiodini, R.J., W.M. Chamberlin, J. Sarosiek, R.W. McCallum, 2012. Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association? Crit. Rev. Microbiol. 38, 52-93.

Christensen, R., W. Johnson, A. Branscum, T. Hanson, T. 2012. Bayesian Ideas and Data Analysis : An Introduction for Scientists and Statisticians CRC Press, Boca Raton, FL.

Collins, M., D. Sockett, W. Goodger, T. Conrad, C. Thomas, D. Carr, D. 1994. Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. J. Am. Vet. Med. Assoc. 204, 636-636.

Daniels, M., M. Hutchings, D. Allcroft, I. McKendrick, A. Greig. 2002. Risk factors for Johne's disease in Scotland-the results of a survey of farmers. Vet. Rec. 150, 135-138.

Dohoo, I.R., W. Martin, H.E. Stryhn. 2010. Veterinary epidemiologic research. 2nd ed. VER Inc., Prince Edward Island, Canada.

Dorshorst, N.C., M.T. Collins, J.E. Lombard, 2006. Decision analysis model for paratuberculosis control in commercial dairy herds. Prev. Vet. Med. 75, 92-122.

Espejo, L., S. Godden, W. Hartmann, S. Wells. 2012. Reduction in incidence of Johne's disease associated with implementation of a disease control program in Minnesota demonstration herds. J. Dairy Sci. 95, 4141-4152.

Ferrouillet, C., S. Wells, W. Hartmann, S. Godden, J. Carrier. 2009. Decrease of Johne's disease prevalence and incidence in six Minnesota, USA, dairy cattle herds on a long-term management program. Prev. Vet. Med. 88, 128-137.

Garry, F., 2011. Control of Paratuberculosis in Dairy Herds. Vet. Clin. North Am. Food Anim. Pract. 27, 599-607.

Geraghty, T., D.A. Graham, P. Mullowney, S.J. More. 2014. A review of bovine Johne's disease control activities in 6 endemically infected countries. Prev. Vet. Med. 116, 1-11.

Godden, S., S. Wells, M. Donahue, J. Stabel, J. Oakes, S. Sreevatsan, J. Fetrow. 2015. Effect of feeding heat-treated colostrum on risk for infection with Mycobacterium avium ssp. paratuberculosis, milk production, and longevity in Holstein dairy cows. J. Dairy Sci. 98, 5630-5641.

Hanley, J.A., 2001. A heuristic approach to the formulas for population attributable fraction. J. Epidemiol. Community Health, 55, 508-514.

Hendrick, S.H., D.F. Kelton, K.E. Leslie, K.D. Lissemore, M. Archambault, T.F. Duffield. 2005. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. J.Am. Vet. Med. Assoc. 227, 1302-1308.

Johnson-Ifearulundu, Y., J. Kaneene, D. Sprecher, J. Gardiner, J. Lloyd. 2000. The effect of subclinical Mycobacterium paratuberculosis infection on days open in Michigan, USA, dairy cows. Prev. Vet. Med. 46, 171-181.

Kennedy, D.J., G. Benedictus. 2001. Control of Mycobacterium avium subsp. paratuberculosis infection in agricultural species. Rev. Sci. Tech. 20, 151-179.

Kudahl, A.B., S.S. Nielsen, S. Østergaard. 2011. Strategies for time of culling in control of paratuberculosis in dairy herds. J. Dairy Sci. 94, 3824-3834.

Lu, Z., Y.H. Schukken, R.L. Smith, Y.T. Grohn. 2010. Stochastic simulations of a multi-group compartmental model for Johne's disease on US dairy herds with test-based culling intervention. J. Theor. Biol. 264, 1190-1201.

Lunn, D.J., A. Thomas, N. Best, D. Spiegelhalter. 2000. WinBUGS — a Bayesian modelling framework: concepts, structure, and extensibility. Stat. Comput. 10, 325–337.

510 Maher, P., M. Good, M. S.J. More. 2008. Trends in cow numbers and culling rate in the Irish
511 cattle population, 2003 to 2006. Irish Vet. J. 61, 455-463.

512 Marcé, C., P. Ezanno, H. Seegers, D.U. Pfeiffer, C. Fourichon. 2011. Within-herd contact
513 structure and transmission of Mycobacterium avium subspecies paratuberculosis in a
514 persistently infected dairy cattle herd. Prev. Vet. Med. 100, 116-125.

515 McAloon, C.G., P. Whyte, S.J. More, L. O'Grady, M.L. Doherty. 2015. Development of a
516 HACCP-based approach to control paratuberculosis in infected Irish dairy herds. Prev. Vet.
517 Med 120, 152-161.

518 McAloon, C.G., P. Whyte, S.J. More, M.J. Green, L. O'Grady, A. Garcia, M.L. Doherty.
519 2016. The effect of paratuberculosis on milk yield—A systematic review and meta-analysis.
520 J. Dairy Sci. 99, 1449-1460.

521 McAloon, C.G., M.L. Doherty, P. Whyte, L. O'Grady, S.J. More, L.L.M. Messam, M. Good,
522 P. Mullowney, S. Strain, M.J. Green. 2016a. Bayesian estimation of prevalence of
523 paratuberculosis in dairy herds enrolled in a voluntary Johne's Disease Control Programme in
524 Ireland. Prev. Vet. Med. 128, 95-100.

525 Messam, L.L.M., A.J. Branscum, M.T. Collins, I.A. Gardner. 2008. Frequentist and Bayesian
526 approaches to prevalence estimation using examples from Johne's disease. Anim. Health Res.
527 Rev., 9, 1-23

528 More, S., M.L. Doherty, L. Downey, K. McKenzie, C. Devitt, J. O'Flaherty. 2011. Animal
529 Health Ireland: providing national leadership and coordination of non-regulatory animal
530 health issues in Ireland. Rev. Sci. Tech. 30, 715.

Nielsen, S.S., H. Bjerre, N. Toft. 2008. Colostrum and Milk as Risk Factors for Infection with *Mycobacterium avium* subspecies *paratuberculosis* in Dairy Cattle. J. Dairy Sci. 91, 4610-4615.

Pillars, R., D. Grooms, J. Gardiner, J. Kaneene. 2011. Association between risk-assessment scores and individual-cow Johne's disease-test status over time on seven Michigan, USA dairy herds. Prev. Vet. Med. 98, 10-18.

Pithua, P., S.M. Godden, S.J. Wells, M.J. Oakes. 2009. Efficacy of feeding plasma-derived commercial colostrum replacer for the prevention of transmission of Mycobacterium avium subsp paratuberculosis in Holstein calves. J. Am. Vet. Med. Assoc. 234, 1167-1176.

Pithua, P., S.M. Godden, S.J. Wells, J.R. Stabel. 2011. Evaluation of the risk of paratuberculosis in adult cows fed Mycobacterium avium subsp paratuberculosis DNA-positive or-negative colostrum as calves. Am. J. Vet. Res. 72, 1456-1464.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richardson, E., S.J. More. 2009. Direct and indirect effects of Johne's disease on farm and animal productivity in an Irish dairy herd. Irish Vet. J. 62, 526.

Robins, J., S. Bogen, A. Francis, A. Westhoek, A. Kanarek, S. Lenhart, S. Eda, S. 2015. Agent-based model for Johne’s disease dynamics in a dairy herd. Veterinary research, 46, 68.

Sorge, U., K. Lissemore, A. Godkin, J. Jansen, S. Hendrick, S. Wells, D. Kelton. 2011. Changes in management practices and apparent prevalence on Canadian dairy farms participating in a voluntary risk assessment-based Johne's disease control program. J. Dairy Sci. 94, 5227-5237.

553 Stabel, J.R., 2008. Pasteurization of Colostrum Reduces the Incidence of Paratuberculosis in
554 Neonatal Dairy Calves. J. Dairy Sci. 91, 3600-3606.

555 Sweeney, R.W., M.T. Collins, A.P. Koets, S.M. McGuirk, A.J. Roussel. 2012.
556 Paratuberculosis (Johne's disease) in cattle and other susceptible species. J. Vet. Intern. Med.
557 26, 1239-1250.

558 Zhang J, F.Y. Kai. 1998. What's the relative risk?: A method of correcting the odds ratio in
559 cohort studies of common outcomes. J. Am. Med. Assoc. 280:1690-1691.

560

561 **Table 1.** Questions asked as part of on-farm Risk Assessment (**RA**) conducted on 925 dairy

562 herds enrolled in the Irish national Johne's Disease Control Programme

Q1. Have you ever completed a Johne's Disease herd test?
Q2. Has there been any suspect cases of JD on the farm?
Q3. Have you had any confirmed clinical JD or test positive cows in your herd?
Q4. Do you use your own equipment to spread slurry on your farm?
Q5. Do you spread cattle/slurry from other herds on your pasture?
Q6. Do you graze cattle purchased by you for fattening on your pasture?
Q7. Do you graze cattle/cows on commonage or with cattle from other herds
Q8. Do you graze on rented ground?
Q9. Do you use contract rearers or rear calves/heifers under a different herd number?
Q10. Do sheep cograze on this farm?
Q11. Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial
Q12. Are at least 3 litres of colostrum (first milking) consumed within the first 2 hours?
Q13. Are calves fed on low risk whole milk
Q14. How often is non-saleable whole milk (high risk) fed?
Q15. Are calves housed in individual or group pens in the first week?
Q16. Is there exposure to cow manure in the calf housing or grazing area?
Q17. Is there exposure to cow manure by watering or feeding utensils?
Q18. Are calves fed forages that have received slurry from adult animals within the last year?
Q19. Do you feed or have you fed colostrum from other herds?
Q20. When was this last fed?
Q21. Do you feed milk from cows from other herds
Q22. When was this last fed?
Q23. Are weaned heifers exposed to cows or their manure at any time?
Q24. Are maiden heifers exposed to cows or their manure at any time?
Q25. What is the overall hygiene and cleanliness score of weaned heifers
Q26. What is the overall hygiene and cleanliness score of maiden or incalf heifers?
Q27. Are weaned heifers (≥6 months) fed forages that have received slurry from adult animals within the last year?
Q28. Are maiden or incalf heifers (≥6 months) fed forages that have received slurry from adult animals within the last year?
Q29. Dry cow area environment hygiene score
Q30. Milking cow area environment hygiene score
Q31. Dry cow cleanliness
Q32. Milking cow cleanliness
Q33. Single or multiple cows in calving areas?
Q34. Manure build up
Q35. Manure on soiled udders and legs of cows?
Q36. Calving area used for lame or sick cows?
Q37. Calving area used for JD clinical or JD test positive cows?
Q38. Birth of calves in areas other than designated calving area?

Q39. Likelihood of calf nursing cow(s)?
Q40. How fast are newborn dairy calves removed from their mothers?

563

564

565

566 **Table 3.** Summary of herd-level characteristics and animal introduction data for 925 dairy
567 herds enrolled in the Irish national Johne's Disease Control Programme. Definition of
568 categories and proportion of herds defined as positive based on ≥2 animals testing positive.

Variable	Number in category	Percent in category	Number positive	Percent positive
Herd size				
≤60	246	27%	70	17%
61-80	217	23%	66	23%
81-116	232	25%	61	31%
>116	230	25%	101	44%
Test medium				
Blood	588	64%	166	28%
Milk	337	36%	99	29%
Test precedes RAMP[1]				
Yes	493	53%	157	32%
No	432	47%	108	25%
Herd growth 2005-2014				
<5%	232	25%	56	24%
5-25%	219	24%	66	30%
26-46%	218	24%	67	31%
>45%	256	28%	76	30%
Mean annual purchases 2005 – 2009[2]				
Closed/Replacement Bulls Only	101	11%	28	28%
Females at <2% of herd size	266	29%	81	30%
Females at 2-7.5% of herd size	281	30%	72	26%
Females at >7.5% of herd size	277	30%	84	30%
Mean number of herds purchased from 2005 - 2009				
<0.4	240	26%	70	29%
0.4-1.0	180	19%	51	28%
1.0-2.2	234	25%	71	30%
>2.2	271	29%	73	27%
Beef purchases 2005-2009[3]				
Yes	383	41%	107	28%

No	542	59%	158	29%
Mean number of herds purchased from 2010-2014				
Closed/Replacement Bulls Only	146	16%	43	29%
Females at <2% of herd size	316	34%	87	28%
Females at 2-7.5% of herd size	204	22%	57	28%
Females at >7.5% of herd size	259	28%	78	30%
Beef purchases 2010-2014				
Yes	327	35%	95	29%
No	598	65%	170	28%
Mean number of herds purchased from 2010-2014				
<0.4	363	39%	98	27%
0.4-1.0	209	23%	56	27%
1.0-2.2	195	21%	63	32%
>2.2	158	17%	48	30%

[1]RAMP = Risk Assessment and Management Plan

[2]Replacement Bulls Only = herds not introducing females and only introducing males at ≤5% of the overall herd size each year

[3]Beef purchases = herds purchasing males at >5% of the overall herd size each year

574 **Table 4.** Results from multivariable logistic regression model assessing the association
575 between RA questions, animal movement data and the outcome "herd positivity", defined as
576 herds with 2 or more positive animals in the herd

Variable	n[1]	Coefficient	Odds Ratio	95% Confidence Intervals	p	PAF[2]
Herd Size		0.01	1.01	1.01, 1.01	<0.001	
Test Medium						
Milk	337	0.45	1.57	1.57, 1.57	0.021	9.7%
Blood	588	REF				
Test Month						
January	48	2.12	8.33	3.61, 19.24	<0.001	5.5%
February	53	1.69	5.42	2.34, 12.57	<0.001	4.9%
March	69	1.34	3.82	1.77, 8.23	0.001	4.4%
April	114	0.66	1.93	0.96, 3.91	0.064	3.2%
May	160	REF				
June	129	0.38	1.46	0.73, 2.92	0.277	2.0%
July	91	1.53	4.62	2.35, 9.08	<0.001	8.2%
August	67	1.23	3.42	1.59, 7.38	0.002	4.0%
September	53	1.03	2.8	1.21, 6.5	0.016	2.6%
October	69	1.52	4.57	2.17, 9.64	<0.001	5.5%
November	44	1.69	5.42	2.33, 12.61	<0.001	4.2%
December	28	1.19	3.29	1.16, 9.31	0.026	1.5%
Q3. Presence of clinical JD or test positive cows in past[3]						
No and RA conducted after testing	257	REF				
No and RA conducted before testing	348	0.38	1.46	0.91, 2.35	0.114	7.8%
Yes and RA conducted after testing	175	1.03	2.8	1.67, 4.69	<0.001	12.2%
Yes and RA conducted before testing	145	1.21	3.35	1.92, 5.84	<0.001	12.6%
Q11. Are calves fed colostrum from own mother or from known low risk colostrum sources?						
Calves receive colostrum from their own test negative mother	291	REF				
Calves receive colostrum from their own mother (no selection)	278	0.39	1.48	0.94, 2.31	0.088	6.9%
1-10% of calves receive colostrum from source other than dam	166	0.35	1.42	0.85, 2.38	0.190	4.2%

>10% of calves receive colostrum from source other than dam	190	0.74	2.1	1.28, 3.42	0.003	11.6%
Q23. Are weaned heifers exposed to cows or their manure at any time?						
Never housed/grazed with adult animals, no direct contact and no exposure to manure. Not fed uneaten rations from cows and not sharing water troughs	241	0.27	1.31	0.85, 2.02	0.212	4.1%
Housed/grazed near cows but no direct or indirect contact	269	0.54	1.72	1.15, 2.55	0.007	10.5%
Housed/grazed near cows, direct or indirect contact possible	415	REF				
Q30. Milking cow environment hygiene score						
No visible manure contamination of feeding areas or water troughs	188	0.38	1.46	0.95, 2.26	0.084	4.6%
Trace amount of manure visible, feeding areas/water troughs cleaned > 1/week	565	REF				
Manure clearly visible, feeding areas/water troughs cleaned < 1/week	172	0.55	1.73	1.14, 2.63	0.010	7.6%
Q36. Calving area used for lame or sick cows?						
Calving area is never used by non-calving cows	516	REF				
Calving area is used by non-calving cows once in 3 months	125	0.05	1.05	0.63, 1.75	0.841	0.5%
Calving area is used by non-calving cows at least once monthly	75	0.28	1.32	0.73, 2.41	0.357	1.7%
Calving area is used by non-calving cows at least once weekly	209	0.81	2.25	1.48, 3.42	<0.001	14.2%
Q40. How quickly are calves removed from their dam?						
>90% are removed within 15 minutes of birth	97	REF				
>50% are removed within 30 minutes	507	0.84	2.32	1.23, 4.37	0.010	24.7%
10-50% are removed within 30 minutes	236	0.42	1.52	0.76, 3.03	0.237	5.6%

<10% are removed within 30 minutes	85	0.49	1.63	0.72, 3.7	0.239	3.2%
Herd Growth						
Stable (<5%)	256	0.23	1.26	0.78, 2.03	0.350	4.2%
Small Growth (5-25%)	219	0.47	1.6	1, 2.56	0.049	6.7%
Medium (26 - 46%)	218	0.41	1.51	0.95, 2.39	0.079	6.1%
Large (>46%)	232	REF				

577

578 [1]n = number in category

579 [2]PAF = Population Attributable Fraction

580 [3]RA = Risk Assessment

581 **Table 5.** Results from final multivariable Bayesian beta regression model assessing the
582 association between RA questions, animal movement data and the probability of infection as
583 estimated by a Bayesian latent class analysis.

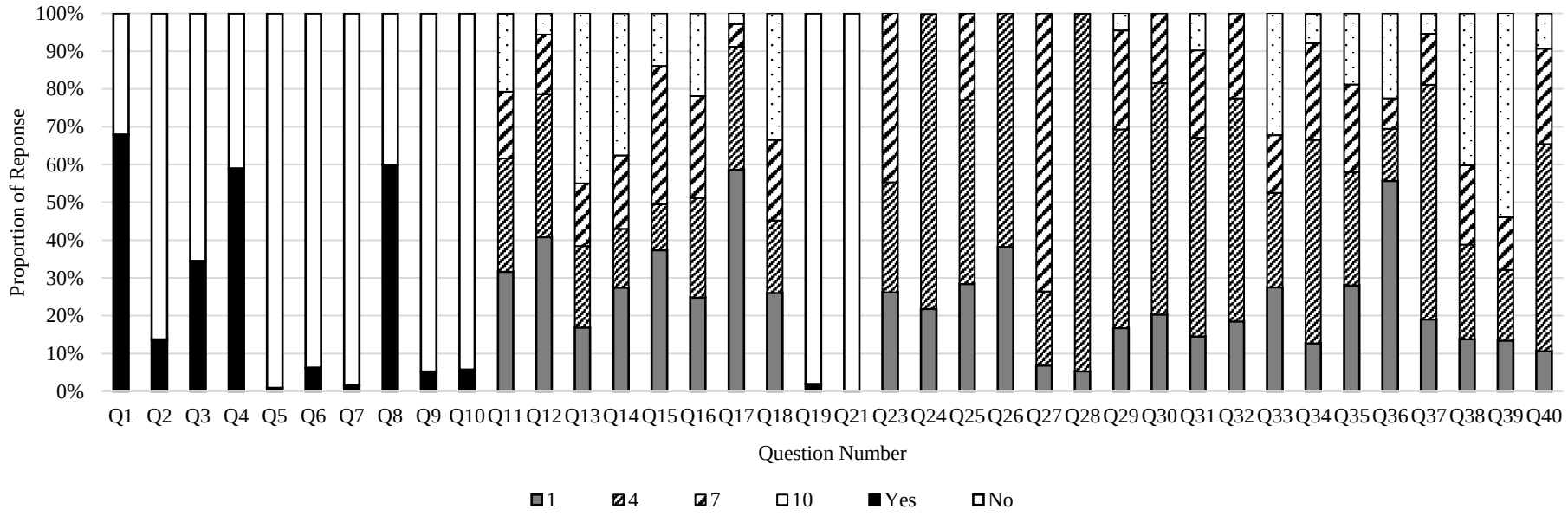
Variable	n[1]	Coefficient	Probability of infection	95% Probability Interval	Proportional reduction in R-squared
Intercept			0.60	0.25, 0.86	
Q3. Presence of clinical JD or test positive cows in past					
No and after testing	257	REF			
No and before testing	348	0.52	0.72	0.66, 0.77	
Yes and after testing	175	0.08	0.62	0.57, 0.67	
Yes and before testing	145	0.44	0.70	0.65, 0.75	
					22.6%
Test Month					
January	48	0.80	0.77	0.69, 0.83	
February	53	0.66	0.74	0.66, 0.81	
March	69	0.31	0.67	0.59, 0.75	
April	114	0.12	0.63	0.55, 0.69	
May	160	REF			
June	129	0.10	0.62	0.55, 0.69	
July	91	0.49	0.71	0.64, 0.77	
August	67	0.37	0.68	0.60, 0.76	
September	53	0.38	0.69	0.60, 0.76	
October	69	0.63	0.74	0.66, 0.80	
November	44	0.87	0.78	0.70, 0.85	
December	28	0.35	0.68	0.56, 0.78	
					35.3%
Proportion of herd tested					
Increase of 10%		-0.22	0.55		
					3.2%
Q28. Are maiden or incalf heifers (≥6 months) fed forages that have received slurry from adult animals within the last year?					
No forages fed to heifers have been spread with slurry in the previous season	50				
Fresh or conserved forages that were spread with slurry in the	875	0.35	0.68	0.6, 0.76	

previous season are fed to heifers					
					1.3%
Q31. Dry cow cleanliness					
No manure visible on hind legs or udder	135	0.28	0.67	0.61, 0.72	
Manure present on hind legs but not above dewclaws	486	0.00	0.60		
Manure present on hind legs but not above hocks, or is present on the udder or teats	213	0.14	0.63	0.59, 0.68	
Manure present above the hocks	91	0.26	0.66	0.60, 0.72	
					7.1%
Q36. Is the Calving Area ever used for lame or sick cows?					
Calving area is never used by non-calving cows		REF			
Calving area is used by non-calving cows once in 3 months		0.13	0.63	0.57, 0.69	
Calving area is used by non-calving cows at least once monthly		0.14	0.63	0.56, 0.7	
Calving area is used by non-calving cows at least once weekly		0.41	0.69	0.65, 0.74	
					5.8%

584

585 [1]n = number in each category

McAloon Figure 1. Stacked bar graph showing distribution of responses to Risk Assessment for 925 dairy herds enrolled in the Irish national Johne's Disease Control Programme. Questions 23-25 and Question 17 are scored to a maximum 7, questions 26 and 28 are scored to a maximum of 4.



McAloon Figure 2. Predicted median probabilities of infection from final Bayesian beta regression model versus data outputs (observed) from Bayesian latent class model. R-squared = 0.16.