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Culling decisions of dairy farmers during a 3-year *Salmonella* control study

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Abstract

Salmonella enterica subsp. *enterica*-serotypes lead to periodically increased morbidity and mortality in cattle herds. The bacteria can also lead to serious infections in humans. Consequently, Denmark has started a surveillance and control programme in 2002. The programme focuses on *Salmonella* Dublin which is the most prevalent and most persistent serotype in the Danish cattle population.

A field study in ten dairy herds with persistent *Salmonella* infections was carried out over three years to gain experience with control procedures including risk assessment, targeted control actions and test-and-cull procedures. From autumn 2003 until end of 2006 quarterly milk quality control samples from all lactating cows and biannual blood samples from all young stock above the age of three months were tested using an indirect antibody ELISA. The most recent and previous test results were used to categorise all animals into risk groups. These risk groups and all individual ELISA-results were communicated to the farmers as colour-coded lists four to six times per year. Farmers were advised to manage the risk of *Salmonella* transmission from cattle with repeatedly high ELISA results (flagged as "red") or cows with at least one recent moderately high ELISA result (flagged as "yellow") on the lists. Risk management included e.g. culling or separation of the cows at calving.

We analysed culling decisions using two models. For heifers a hierarchical multivariable logistic model with herd as random effect evaluated if animals with red and yellow flags had higher probability of being slaughtered or sold before first calving than animals without any risk flags. For adult cows a semi-parametric proportional hazard survival model was used to test the effect of number of red and yellow flags on hazards of culling at different time points and interactions with prevalence in the herd while

accounting for parity, stage of lactation, milk yield, somatic cell count and the hierarchical structure of the data with animals clustered at herd level.

This study illustrates how investigation of culling decisions made by herd managers when they have access to test-status of individual animals and overall apparent prevalence during control of an infection can lead to useful new knowledge. Overall herd managers were more likely to cull cattle with increasing number of yellow and red flags than animals with no flags. However, cattle were more likely to be culled with yellow and red flags during times with low or medium high within-herd seroprevalence than at times with high seroprevalence. These results are valuable knowledge for modelling and planning of control strategies and for making recommendations to farmers about control options.

1. Introduction

Salmonella enterica subsp. *enterica* serovar Dublin (*S*. Dublin) is the most commonly isolated serotype of salmonella in cattle in Denmark (Anonymous, 2010). Infected herds typically experience periodically increased morbidity and mortality among calves and abortions in adult cows (Richardson and Watson, 1971; Wray and Davies, 2000). *S.* Dublin infections in humans are rare in incidence, but invasive leading to a syndrome of sustained bacteraemia with fever, resulting in high case fatality (Helms et al., 2003). Consequently, the Danish cattle industry and the Danish Veterinary and Food Administration started a surveillance and control campaign in cattle herds aimed at reducing *S*. Dublin prevalence to zero (or below detection limits) by end of 2014.

Control of *S*. Dublin in cattle herds is achieved through strict and persistent management procedures aimed at blocking transmission routes within the herd to stop or reduce spread of *S*. Dublin between animals in the herd, or to and from the environment (Wray et al., 1989; Jensen et al., 2004). Furthermore, purchase of replacement stock and contact to other herds need to be restrictive (Vaessen et al., 1998; van Schaik et al., 2002; Nielsen et al., 2007; Jordan et al., 2008). *S*. Dublin appears to have a tendency to produce persistently infected cattle that do not show any clinical signs and thus pose a risk of spread of infection in the herd (Richardson, 1973; Wray et al., 1989; House et al., 1993). It has been suggested that persistently infected animals have persistently high antibody responses to the infection as opposed to temporarily infected cattle, in which the level of antibodies in blood or milk will drop to low levels within two to four months after the time of infection (Spier et al., 1990; House et al., 1993). This provides an opportunity to classify individual cattle into high or low risk animals for differential management or culling decisions on the basis of repeated antibody measurements during control programmes for *S*. Dublin (Smith et al., 1992).

In intervention field studies it is often desirable to extract information about which management procedures were used by the herd managers and relate these to success rates or prevalence reductions (Jensen et al., 2004; Ellis-Iversen et al., 2008; Collins et al., 2010). In addition, drivers of decision making during control of infectious diseases are of interest (Ellis-Iversen et al., 2010). Factors affecting culling decisions can be objectively analysed when there are detailed data available about calving, movement of animals, production and health on individual animal level over an extended period of time. Survival analysis including health disorders as time-dependent variables has been suggested as most appropriate for such analyses (Beaudeau et al., 2000). To our knowledge, the effect of the salmonella status of individual animals on culling in dairy herds has never been studied before, probably because such laboratory-results are not usually available to the farmers and recorded centrally in a database. However, in the Danish *S.* Dublin control program farmers have the opportunity to request individual animal ELISA-testing through the milk recording scheme or by having blood samples collected for testing. The laboratory enters the results in the Danish Cattle Database and all tested animals are assigned a risk group at the time of sampling based on the current and previous up to four samples collected from the same individual.

This study aimed at demonstrating how culling decisions of herd managers in 10 dairy herds during a field study on *S.* Dublin control were affected by access to repeated ELISA-results and *Salmonella* risk classification from individual cattle in the herds. It was hypothesised that herd managers were more likely to cull animals that had had persistently high antibody titres in blood or milk samples than those that did not. Furthermore, investigation of whether the underlying prevalence affected the culling decisions was of interest.

2. Material and Methods

2.1 Selection of herds

A field study was carried out in 10 dairy herds over a period of three years to gain experience with a structured approach to control of *S*. Dublin including risk assessment followed by herd-specific targeted control actions in the herds, and test-and-cull or test-and-manage procedures. The herds were followed intensively through herd visits and frequent testing of all animals. The herds had seroprevalences above 5% among cows at time of inclusion in the study. All 10 herds had high (>25 corrected optical density-values (ODC%)) *Salmonella*-antibody levels in bulk-tank milk measured through the Danish cattle *Salmonella* surveillance programme for one to three years prior to the onset of the study (Nielsen and Ersbøll, 2005; Nielsen and Nielsen, 2011). This strongly indicated that *Salmonella* had been present in the herds for a period and still was present in the herds at the beginning of the study period (Veling et al., 2000; Nielsen, 2003; Warnick et al., 2006). The serotype most likely to be present was *S*. Dublin even though information about relevant serotype was only available for six of the herds (five with only *S*. Dublin isolated and one with dual *S*. Dublin and *S*. Typhimurium infections). All farmers joined the study because they were motivated to actively try to eradicate the infection from their herd.

The demographics of the herds and information of management has been described in detail elsewhere (Nielsen and Nielsen, 2011). In short, herd size went from an average of 97 cows (95%CI: 75-119) at the beginning of the study period to an average of 123 cows (95%CI: 97-150) at the end of the study period. One was a Jersey herd and nine were Danish Holstein breeds. Eight of the herds were conventional, one was organic during the first 1½ year of the study period, and one herd was organic throughout the study period from mid 2003 to end of 2006.

136 *2.2 Sampling of individual cattle*

137 From autumn 2003 until end of 2006 milk recording samples from all lactating
138 cows were collected every three months and blood samples from all young stock above the
139 age of three months and until first calving were collected twice per year. The samples were
140 tested using an indirect ELISA that measured antibodies directed against O-antigens of
141 *Salmonella* serogroup-D. *S*. Dublin is with very few exceptions the only serogroup-D
142 *Salmonella* type isolated in cattle. The test results were used to categorise all animals into
143 risk groups based on current and previous test results, and the risk groups and ELISA-
144 results were communicated to the farmers four to six times per year, usually one month
145 after each new testing round. The test procedures and validity estimates are described in
146 Section 2.3, and the criteria for the risk groups are described in Section 2.4.

147 Farmers were advised to consider culling cows with repeatedly high ELISA results,
148 in particular if they were not able to manage the risk of transmission of bacteria by
149 isolating the high risk cows from young calves during and after calving and from other
150 cows in the calving area. However, farmers were advised to make their choice of control
151 procedures specific to their own herd instead of following general advice, and they were
152 asked to regularly evaluate the progress and adjust their decision-making if necessary.
153 Thus, it was not possible to classify the herds according to a certain set of management
154 procedures.

155

156 *2.3 Serological method*

157 The in-house ELISA used for the blood and milk samples at Eurofins Laboratory
158 (Holstebro, Denmark) has been described in detail elsewhere (Nielsen and Ersbøll, 2004;
159 Nielsen et al., 2004). The ODC% was calculated for each sample as follows:

160

$$ODC\% = \frac{\left(\overline{OD}_{sample} - \overline{OD}_{neg\ ref} \right)}{\left(\overline{OD}_{pos\ ref} - \overline{OD}_{neg\ ref} \right)} * 100\%$$

where $\overline{OD}_{sample}$ is the mean value of two test wells, and $\overline{OD}_{neg\ ref}$ and $\overline{OD}_{pos\ ref}$ are the mean values of four negative and four positive reference wells in the ELISA plates. The scale of ELISA values goes from 0 to approximately 200 ODC% and can be interpreted as a semi-quantitative scale of the concentration of antibodies in the sample. Although the antigen used in the assay was developed to detect antibodies directed against *S.* Dublin, cross-reactions with other serotypes of *Salmonella* are known to occur (Konrad et al., 1994). Under Danish conditions it would mainly be *S.* Typhimurium-serotypes that might cause cross-reactions.

The sensitivity (Se) of single measurements at animal level has been estimated to be approximately 50% and the specificity (Sp) approximately 98% at cut-off 50 ODC% in cattle above 300 days old for the serum test (Nielsen and Ersbøll, 2004). For the milk ELISA, Se was estimated to be approximately 43% and Sp approximately 90% (Nielsen, 2003). The Se is much higher (94%) for actively shedding carriers (Veling et al., 2000). However, the test sensitivity and specificity estimates and the predictive values for these tests are not essential for this study, because conclusions were not drawn about true infection status of the tested animals nor the effect of culling animals classified as high-risk on success or failure of control.

2.4 Risk groups and seroprevalence

The criteria of the serologically determined risk groups were modified from recommendations in previous experimental and field studies (Smith et al., 1989; Spier et al., 1990; House et al., 1993). Heifers and cows were categorised as high risk indicated by

a "red flag" on the result lists provided to the farmers, if they had at least two samples above 80 ODC% with a minimum of 120 days in between, the most recent sample was above 80 ODC% and the average of the last up to four samples was above 80 ODC%. The animals were categorised medium risk indicated by a "yellow flag" if the most recent ELISA and the average of the last up to four samples were above 50 ODC%, but not high enough to be categorised as high risk. Animals with ELISA values below 50 ODC% in the most recent sample did not have any colour indicators on the decision support lists.

Two datasets were created for further analysis, one for heifers (female young stock) and one for adult cows. This split of data was used because milk production data could only be included for lactating cows. In the heifer dataset, the within-herd prevalence of *Salmonella* was calculated as the number of animals with yellow or red flags out of all tested animals in the herd in the relevant sampling round (twice per year). The within-herd prevalence was considered low if <5% (the mean within-herd prevalence) and high if ≥5%. In the cow-dataset, the prevalence was calculated as the number of cows with yellow or red flags out of all tested cows in the herd in the relevant sampling round (four sample rounds per year). Prevalence was categorised as low if <5%, medium if between 5 and 15% and high if >15%.

2.5 Data management

Heifer dataset

The dataset of heifers included animals that had been sampled at least three times and was constructed with one observation per animal indicating herd-id, animal-id, number of red and yellow flags, within-herd seroprevalence at the last sampling date before culling or first calving, and whether or not the heifer was sold or slaughtered before the first calving.

211 *Cow dataset*

212 The adult cow dataset was constructed with one observation per sampling interval. The
213 first interval went from the first ELISA test date to next ELISA test date (or in case the
214 cow was culled before the next sampling round, the last date of the interval was set to be
215 the culling date). The next interval went from the second ELISA test date to the next
216 ELISA date and so forth. Thus, the cows entered the study on the first date they were
217 ELISA tested. Cows were either censored on the last ELISA test date plus 92 days, if they
218 were not culled within this period, or were set to have a failure ("culled" implying sold or
219 sent to slaughter) and left the study on the date of culling. For each interval the relevant
220 *Salmonella* risk group was given. Cumulative numbers of red and yellow flags up to and
221 including the most recent ELISA date was counted for each cow-interval.

222

223 *Confounding variables in cow dataset*

224 Milk yield was recorded 11 times per year through a milk recording scheme at which
225 kilograms of milk, percentage of fat and percentage of protein were determined. Energy
226 corrected milk yield (ECM) was calculated on each milk quality control test date as (kg of
227 milk × (383 × fat% + 242 × protein% + 780.8))/3140 (Nielsen et al., 2009). The following
228 expected confounding variables were constructed for each of these intervals: The mean
229 energy corrected milk yield (mean-ECM) and mean of the natural logarithm to the somatic
230 cell counts (mean-lnSCC) measured in each interval based on all milk recordings
231 performed in that interval; days in milk (DIM) and parity on the first day of the interval.
232 Six two-level predictive models for ECM were constructed for first, second and third
233 and higher parities and for each of the two types of breed groupings in the study herds
234 (large breeds (9 herds) and Jersey (1 herd), respectively. The models predicted the test day
235 ECM including Wilminks correction as DIM * exp (-0.065*DIM) (Silvestre et al., 2006).

236 The mean deviation from the predicted milk yield (in %) according to the models were
237 included in the dataset as a potentially confounding variable (mean-pctECM).
238
239 *2.6 Statistical analysis of heifer data*
240 A two-level hierarchical logistic regression model was used to analyse the data on
241 heifers to account for the clustering of animals in herds. The analysis was performed in
242 STATA® IC/11 (StataCorpLP, College Station, Texas, USA) using a subject specific
243 model (xtmelogit). Outcome in the model was a binary variable indicating whether the
244 heifer was culled before first calving or not. Herd was included in the model as a random
245 effect to account for clustering of animals at herd level. Forward stepwise inclusion of
246 variables was used to assess significance of the main effects and interactions of all
247 explanatory variables. The model was fit using maximum likelihood estimation. The model
248 fit when allowing for random slopes of the herd effect was assessed by comparing log-
249 likelihood to the final model without random slopes.
250
251 *2.7 Statistical analysis of cow data*
252 All the statistical analyses of cows were performed in STATA® IC/11. The time to
253 culling in adult cows was analysed using a semi-parametric survival model (Cox
254 proportional hazards model). Efron's method was used to handle ties in the data (multiple
255 culling events on the same end of study days for cows). The hierarchical structure of the
256 data with animals clustered at herd level was accounted for by including herd as a gamma
257 distributed shared frailty in the proportional hazards model. The estimation of the shared
258 frailty was done using a penalised likelihood function (Dohoo et al., 2009).
259 Initially mean-ECM, mean-lnSCC, DIM and parity were forced into the model due
260 to expected strong confounding effects. The optimal functional form of continuous and

discrete predictors with more than 10 levels was determined by the use of fractional polynomials and evaluation of lowess smoothed graphs of Martingale residuals (Royston and Sauerbrei, 2008). The fractional polynomial form (up to 4 terms) which best fit the data was forced into all consecutive models to control for confounding.

Then a stepwise forward selection procedure was used to test the rest of the explanatory variables including possible two-way interactions between the explanatory variables of interest in the model. All effects were evaluated at a 5% significance level. Inclusion of time-varying variables was used at the end of the modelling procedure where it was evaluated as necessary by assessment of significance levels and differences in log-likelihood between subsets of models.

The assumption of proportional hazards was evaluated graphically for the categorical variable year and by graphical and statistical test evaluation of Schoenfeld residuals for continuous variables included in the final model. These procedures evaluated whether of not there was evidence that some hazard ratios, conditional on the frailty effect (i.e. the effect of a change in the number of flags within a herd), were non-proportional (i.e. changed over time). The assumption of independent censoring was evaluated by sensitivity analysis comparing scenarios with complete positive and negative correlations between censoring and culling. The overall fit of the model was assessed by graphical evaluation of the Cox-Snell residuals (Dohoo et al., 2009). Finally, we checked for outliers by plots of deviance residuals vs. time and influential points by plots of score residuals vs. time.

3. Results

3.1 Results of logistic analysis of culling of heifers

285 The risk group variable was categorised into a three-level flag variable counting the
286 number of yellow and red flags. Only 76 out of the 1491 heifers included in the study had
287 yellow or red flags. Risk flag=0 indicated no yellow or red flags, risk flag=1 indicated one
288 or more yellow flags and risk flag=2 indicated one or more red flags. Within-heifer
289 prevalence was categorised as low if below, and high if above or equal to 5% (the mean
290 heifer prevalence). There were only two heifers with red risk flags when the within-herd
291 prevalence was low. In general there were more animals included in the dataset in 2005
292 and 2006 due to the criteria that the animal had to have been tested at least three times to
293 be included. Table 1 shows the distribution of the categorised prevalence and risk flag
294 variables in culled and non-culled heifers. In the initial univariable cross-tabulations the
295 risk of culling appeared to be significantly higher with increasing risk flag number (χ=33.8,
296 p<0.0001).The results of the final multivariable model are shown in Table 2. Heifers with
297 one or more yellow flags had 2.7 (95%CI: 1.3-5.8) times higher odds of being culled, and
298 heifers with one or more red flags had 11.5 (95%CI: 4.7-28.3) times higher odds of being
299 culled than heifers with no flags. Furthermore, heifers had twice the odds of being culled
300 when prevalence was low as opposed to when prevalence was high (in the table OR for
301 high prevalence=0.5, p=0.009). However, the risk of culling did not change between years.
302 Fig. 1 illustrates the associations between having yellow or red risk flags and the
303 probabilities (shown both as raw proportions in the dataset and model predicted
304 probabilities) that a heifer was culled before the first calving during low and high within-
305 herd prevalence.
306
307 *3.2 Results of survival analysis of culling of adult cows*
308 The distribution of observations in each of the prevalence-flag groups are shown in
309 Table 3. In Fig. 2 the functional form of the continuous confounding variables and log

hazards of culling in the cows are illustrated. A total of 4400 cows were included in the dataset. Some cows were represented in several prevalence-flag groups, because they changed test status or the herd changed seroprevalence as time went by in the study period. The variables included in the final survival model are presented together with parameter estimates, standard errors, hazard ratios and *p*-values in Table 4. The effects of three parameters varied with time: 0 flags and >5 flags in medium prevalence and 0 flags in high prevalence. The time effects gave similar results when modelling the variation over time as linear and log-linear, so for simplicity it was decided to base the results on the linear form. Fig. 3 illustrates the hazard ratios for each flag group relative to the reference group with 0 flags within each prevalence group at the median number of study days for the time-varying prevalence-flag groups. For instance, cows with >5 flags had 2.6 times higher hazard of being culled than cows with no flags during low prevalence periods and this remained constant over the study period. The difference in risk of having >5 flags vs. no flags during medium high prevalence times changed over the study period from no difference (HR=0.1, Table 4) at the beginning of the study period to more than three times the hazard (HR=3.3, Fig. 3) at the medium number of study days for that group. In contrast, cows with >5 flags were not more likely to be culled than cows with no flags during periods with high prevalence in the herd (HR=0.4, Table 4) and this difference in risk did not change significantly over time.

The functional forms of the confounders illustrated in Fig. 2 were evaluated to be reasonable. For instance they showed that the risk of culling increased during the lactation (DIM) and with increasing somatic cell count (lnsccc), and risk of culling decreased with increasing milk yield (ECM) and the more the milk yield exceeded the expected milk yield for each cow (pct-ECM).

The model fit as assessed by plots of Shoenfeld residuals for continuous variables did not raise concerns (data not shown). Neither did plots of the Cox-Snell residuals for the overall fit of the model (data not shown). We did not find influential outliers in the data. The assumption of independent censoring was evaluated to be reasonable by sensitivity analyses of correlations between censoring and culling.

4. Discussion

To our knowledge this is the first study to evaluate the effect of individual animal level *Salmonella*-test status on culling probabilities of heifers and cows in dairy herds that are attempting to control *Salmonella*-infection. The cut-off values used for the classification of the animals were not decided by the authors aiming to be used in the study. They were used by the classification system set up in the Danish Cattle Database. In this study the classifications (yellow and red flags) that were communicated to the farmers during the study period were simply used to analyse how the farmers made decisions based on these results. To our knowledge it is not known how large a proportion of cattle in the red or yellow flag groups are truly infected or infectious. However, one study found that three out of nine animals with repeated antibody measurements that would lead to a red flag in this study carried the infection in internal organs, but none of them shed bacteria in faeces or milk (Lomborg et al., 2007).

There were high hazard ratios for >5 flags in the low prevalence group and 2-5 flags in the medium prevalence group, but not in the high prevalence group. One flag appeared to be protective against culling in the high prevalence group. Overall, there appeared to be decreased hazard ratios for culling in the high prevalence groups. Exceptions to this were medium and high prevalence groups with no flags. Due to the time-varying effect in these groups the hazard ratios went from low to high over the course

of the study. The fact that increasing number of risk flags was associated with increased risk of culling was expected, because in the study farmers were advised to consider culling these animals as part of the control strategy, in particular if they were not able to otherwise manage the risk of *Salmonella*-transmission from the high risk animals by isolation or separation. However, the analyses of the data provided a more nuanced culling pattern, in that farmers were more hesitant to cull animals with risk flags during periods with high within-herd prevalence than during periods with low within-herd prevalence. One explanation for this could be that when the prevalence is high the number of animals with risk flags is higher than when prevalence is low, and it is not feasible to cull too many heifers and cows at the same time in a herd without losing too much of the production capacity and having to purchase replacement heifers. This is important to take into account when evaluating potential control strategies for instance in simulation models. The herds were followed using four annual bulk-tank milk measurements from 2007 to 2010 after the control period ended (data not shown), and in all herds repeated individual ELISA results indicated that the herds were able to stop transmission of *Salmonella* despite the fact that culling was not used consistently in the control period (Nielsen and Nielsen, 2011).

In our survival model, herd was included as a frailty (random effect) and the model fit improved by keeping it in the model. This can be interpreted as overall differences between herds in general culling strategies. Investigating differences among herds in the effects of prevalence-flag groups would have required fitting a model with up to 11 additional variance components (random slopes). The data would not support this expansion of the model.

Survival analysis with implementation of time-varying effects of health conditions has been suggested as the most appropriate method for analysis of farmers' culling decisions (Beaudeau et al., 2000). Parity, mastitis, teat injuries, poor milk yield and to some extend

384 metabolic, reproductive and foot disorders have been shown to be drivers of culling
385 (Beaudeau et al., 2000; Cramer et al., 2009). In this study we took into account parity,
386 lactation stage, somatic cell counts and milk yield, both as absolute yield and as the
387 deviation from the average of the herd mates at the same parity and lactation stage. We
388 were not able to include other disorders due to lack of reliable data for those.

389 Care has to be taken in the interpretation of the results, because as shown in Table 1
390 and Table 3 some flag or prevalence-flag groups had few observations. We have included
391 95% confidence intervals in Figs. 1 and Fig. 3 to illustrate the uncertainties of the
392 estimates. Some of the prevalence-flag groups in Fig. 3, which show culling hazard
393 estimates at medium number of study days for each prevalence-flag group, have reasonable
394 narrow confidence interval and conclusive estimates. For cows there was a protective
395 effect of having one flag in the medium and high prevalence groups. This effect became
396 even more pronounced as number of study days increased (results not shown). The
397 explanation for this could be that during the study farmers became aware that it might be a
398 good idea to wait and see if the next ELISA-measurement would confirm the status of the
399 cow as being a high risk animal, or if it was just a temporary increase in antibodies that
400 caused the first flag. Having 2-5 risk flags was associated with increased risk of culling in
401 the medium and high prevalence groups, but not in the low prevalence group. This group
402 only had 11 culled cows and 66 cows in total across all herds, so it is difficult to say if it is
403 due to poor sample size that we were not able to show an effect. Cows having >5 risk flags
404 had higher risk of culling compared to cows with no flags in the low and medium
405 prevalence groups, but not in the high prevalence group. The high prevalence group only
406 included 30 cows out of which 8 were culled across all 10 herds. Culling of high risk cows
407 has been recommended during the control period to avoid re-infection of the increasingly
408 susceptible herd (Spier et al., 1990; House et al., 1993; Jensen et al., 2004), but if there are

too many of them on the list it might not be financially wise to cull them all at the same time.

In Denmark, all farmers can order single or repeated ELISA measurements for *Salmonella* antibodies on all or selected animals and have easy access to the results either electronically or by letter. This study illustrates behavioural patterns of farmers provided with such decision tools during a control programme. The herds were selected to participate in the study because they had expressed interest in participating either directly or through their local veterinary advisors. Thus, these herds are representative of herds with motivated farmers or herd managers that choose to actively intervene against *Salmonella* through management and testing strategies. Hence, they might not be representative of farmers that are less encouraged to control the infection, but might be more or less forced to for instance through national legislation.

According to a simulation study about optimal control strategies for *Salmonella* in cattle one of the most effective ways to achieve national prevalence reduction is to reduce the time period a herd is infected (Jordan et al., 2008). It is supported by literature to be a rational approach to *Salmonella* control in cattle herds to try to reduce the spread of the infection through separation and hygienic routines instead of initiating a test-and-cull strategy when there is still widespread infection among the animals and environment in the herd (Wray et al., 1989; Wray and Davies, 2000). After this control study ended, the recommendation to only use culling according to repeated ELISA-measurements in the face of low prevalence among young stock became incorporated in the Danish *Salmonella* Dublin control campaign.

5. Conclusion

Using a two-level multivariable logistic analysis model for culling of heifers and a Cox proportional hazards survival model for culling of cows we were able to demonstrate that farmers were more likely to cull animals detected as high risk for *Salmonella* in 10 dairy herds during a 3-year control period. However, the culling risk of cows was strongly influenced by the within-herd seroprevalence in the herd probably due to the fact that too many animals would have to be culled during high-prevalence times if this was not taken into account when making culling decisions. These results are valuable knowledge for modelling of control strategies and for making recommendations to farmers about control options. Furthermore, this study illustrates a statistical method applied to data from a field study to explore how culling decisions of farmers are affected by access to knowledge about the test-status of individual animals during control.

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449 **References**

450

451 Anonymous, 2010. Annual Report on Zoonoses in Denmark 2009. In: Birgitte Helwigh,
452 Anne Louise Krogh (Eds.), Danish Zoonosis Centre, National Food Institute, Technical
453 University of Denmark, pp. 1-56.

454 Beaudeau, F., Seegers, H., Ducrocq, V., Fourichon, C., Bareille, N., 2000. Effect of health
455 disorders on culling in dairy cows: a review and a critical discussion. Ann. Zootech. 49,
456 293-311.

457 Collins, M.T., Eggleston, V., Manning, E.J.B., 2010. Successful control of Johne's disease
458 in nine dairy herds: results of a six-year field trial. J. Dairy Sci. 93, 1638-1643.

459 Cramer, G., Lissemore, K.D., Guard, C.L., Leslie, K.E., Kelton, D.F., 2009. The
460 association between foot lesions and culling risk in Ontario Holstein cows. J. Dairy Sci. 92,
461 2572-2579.

462 Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research. 2nd Edition.
463 *Eds. Margaret McPike*. VER Inc., Charlottetown, Prince Edwards Island, Canada.

464 Ellis-Iversen, J., Cook, A.J., Watson, E., Nielen, M., Larkin, L., Wooldridge, M.,
465 Hogeveen, H., 2010. Perceptions, circumstances and motivators that influence
466 implementation of zoonotic control programs on cattle farms. Prev. Vet. Med. 93, 276-285.

467 Ellis-Iversen, J., Smith, R., Van Winden, S., Paiba, G., Watson, E., Snow, L., Cook, A.,
468 2008. Farm practices to control *E.coli* O157 in young cattle - A randomised controlled
469 trial. Vet. Res. 39.

470 Helms, M., Vastrup, P., Gerner-Smidt, P., Mølbak, K., 2003. Short and long term mortality
471 associated with foodborne bacterial gastrointestinal infections: registry based study. Br.
472 Med. J. 326, 357-361.

473 House, J.K., Smith, B.P., Dilling, G.W., Roden, L.D., 1993. Enzyme-linked
474 immunosorbent assay for serologic detection of *Salmonella dublin* carriers on a large dairy.
475 Am. J. Vet. Res. 54, 1391-1399.

476 Jensen, A.M., Kjeldsen, A.M., Alban, L., 2004. Control of *Salmonella* Dublin in 6 Danish
477 dairy herds. A case study. (Sanering for *Salmonella* Dublin i 6 malkekvægsbesætninger.
478 En case-undersøgelse.). Dan. Veterinærtidsskr. 87, 26-36.

479 Jordan, D., Nielsen, L.R., Warnick, L.D., 2008. Modelling a national programme for the
480 control of foodborne pathogens in livestock: the case of *Salmonella* Dublin in the Danish
481 cattle industry. Epid. Infect. 136, 1521-1536.

482 Konrad, H., Smith, B.P., Dilling, G.W., House, J.K., 1994. Production of *Salmonella*
483 serogroup D (O9)-specific enzyme-linked immunosorbent assay antigen. Am. J. Vet. Res.
484 55, 1647-1651.

485 Lomborg, S., Agerholm, J., Jensen, A., Nielsen, L., 2007. Effects of experimental
486 immunosuppression in cattle with persistently high antibody levels to *Salmonella* Dublin
487 lipopolysaccharide O-antigens. BMC Veterinary Research 3, 17.

488 Nielsen, L.R., 2003. *Salmonella* Dublin in dairy cattle: Use of diagnostic tests for
489 investigation of risk factors and infection dynamics. PhD thesis. The Royal Veterinary and
490 Agricultural University, pp. 1-219. Available at:
491 http://www.iph.life.ku.dk/forskning/Salmonella/Publications.aspx (accessed March 9,
492 2011).

493 Nielsen, L.R., Ersbøll, A.K., 2004. Age stratified validation of an indirect *Salmonella*
494 Dublin serum ELISA for individual diagnosis in cattle. J. Vet. Diagn. Invest. 16, 205-211.

495 Nielsen, L.R., Ersbøll, A.K., 2005. Factors associated with variation in bulk-tank-milk
496 *Salmonella* Dublin ELISA ODC% in dairy herds. Prev. Vet. Med. 68, 165-179.

497 Nielsen, L.R., Nielsen, S.S., 2011. A structured approach to control of *Salmonella* Dublin
498 in 10 Danish dairy herds based on risk scoring and test-and-manage procedures. Food Res.
499 Int. In press. Doi:10.1016/j.foodres.2011.02.027

500 Nielsen, L.R., Toft, N., Ersbøll, A.K., 2004. Evaluation of an indirect serum ELISA and a
501 bacteriological faecal culture test for diagnosis of *Salmonella* serotype Dublin in cattle
502 using latent class models. J. Appl. Microbiol. 96, 311-319.

503 Nielsen, L.R., Warnick, L.D., Greiner, M., 2007. Risk factors for changing test
504 tlassification in the Danish surveillance program for *Salmonella* in dairy herds. J. Dairy
505 Sci. 90, 2815-2825.

506 Nielsen, S.S., Krogh, M.A., Enevoldsen, C., 2009. Time to the occurrence of a decline in
507 milk production in cows with various paratuberculosis antibody profiles. J. Dairy Sci. 92,
508 149-155.

509 Richardson, A., 1973. The transmission of *Salmonella* dublin to calves from adult carrier
510 cows. Vet. Rec. 92, 112-115.

511 Richardson, A., Watson, W.A., 1971. A contribution to the epidemiology of *Salmonella*
512 Dublin infection in cattle. Br. Vet. J. 127, 173-182.

513 Royston, P., Sauerbrei, W., 2008. Multivariable model-building. A pragmatic approach to
514 regression analysis based on fractional polynomials for modelling continuous variables.
515 *Eds. Royston, P. and Sauerbrei, W.* John Wiley & Sons Ltd., Chichester, England.

516 Silvestre, A.M., Petim-Batista, F., Colaço, J., 2006. The accuracy of seven mathematical
517 functions in modeling dairy cattle lactation curves based on test-day records from varying
518 sample schemes. J. Dairy Sci. 89, 1813-1821.

519 Smith, B.P., House, J.K., Dilling, G.W., Roden, L.D., Spier, S.J., 1992. Identification of
520 *Salmonella dublin* carrier cattle. Proceedings of the International symposium *Salmonella*
521 and salmonellosis. Zoopôle, Ploufragan, France., pp. 225-230.

522 Smith, B.P., Oliver, D.G., Singh, P., Dilling, G., Marvin, P.A., Ram, B.P., Jang, L.S.,
523 Sharkov, N., Orsborn, J.S., 1989. Detection of *Salmonella dublin* mammary gland
524 infection in carrier cows, using an ELISA for antibody in milk or serum. Am. J. Vet. Res.
525 50, 1352-1360.

526 Spier, S.J., Smith, B.P., Tyler, J.W., Cullor, J.S., Dilling, G.W., Da Pfaff, L., 1990. Use of
527 ELISA for detection of immunoglobulins G and M that recognize *Salmonella dublin*
528 lipopolysaccharide for prediction of carrier status in cattle. Am. J. Vet. Res. 51, 1900-1904.

529 Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A., Klunder, T., 1998. Risk factors for
530 *Salmonella* Dublin infection on dairy farms. Vet. Quart. 20, 97-99.

van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A., Barkema, H.W., Benedictus, G., 2002. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev. Vet. Med. 54, 279-289.

Veling, J., van Zijderveld, F.G., Zijderveld-van Bemmel, A.M., Barkema, H.W., Schukken, Y.H., 2000. Evaluation of three newly developed enzyme-linked immunosorbent assays and two agglutination tests for detecting *Salmonella enterica* subsp. *enterica* Serovar Dublin infections in dairy cattle. J. Clin. Microbiol. 38, 4402-4407.

Warnick, L.D., Nielsen, L.R., Nielsen, J., Greiner, M., 2006. Simulation model estimates of test accuracy and predictive values for the Danish *Salmonella* surveillance program in dairy herds. Prev. Vet. Med. 77, 284-303.

Wray, C., Davies, R.H., 2000. *Salmonella* infections in cattle. In: *Wray, C., Wray, A. (Eds.)*, *Salmonella* in Domestic Animals. CABI Publishing, New York, New York State, pp. 169-190.

Wray, C., Wadsworth, Q.C., Richards, D.W., Morgan, J.H., 1989. A three-year study of *Salmonella* Dublin infection in a closed dairy herd. Vet. Rec. 124, 532-535.

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Table 1. Distribution of culled and non-culled heifers in different years, within-herd prevalence groups and *Salmonella* risk groups in 10 dairy herds during a three year *Salmonella* control study

Explanatory variables	n	Culled before first calving (%)	Not culled before first calving (%)
Number of risk flags			
Zero flags	1415	145 (10.2%)	1270 (89.8%)
One or more yellow flags	52	10 (19.2%)	42 (80.8%)
One or more red flags	24	11 (45.8%)	13 (54.2%)
Within-herd prevalence groups			
Low prevalence (<5%)	909	119 (13.1%)	790 (86.9%)
High prevalence (≥5%)	582	47 (8.1%)	535 (91.9%)
Year			
2004	141	13 (9.2%)	128 (90.8%)
2005	500	57 (11.4%)	443 (88.6%)
2006	850	96 (11.3%)	754 (88.7%)

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Table 2 Parameter estimates *(β),* standard error (S.E.), odds ratios (OR), 95% confidence interval of OR and significance level (*P*) in the final logistic regression model for probability of culling in heifers in 10 dairy herds during a three year *S.* Dublin intervention study. Risk flags indicate if heifers have been assigned medium (yellow flags) or high (red flags) risk for spreading *Salmonella*.

Explanatory variables	Estimate *(β)*	S.E.	OR	95% CI of OR	*P*
Intercept	-2.10	0.24			-
Risk flags					<0.0001
Zero flags	0		1		
One or more yellow flags	1.00	0.39	2.7	1.3-5.8	
One or more red flags	2.44	0.46	11.5	4.7-28.3	
Prevalence groups					0.009
Low prevalence (<5%)	0		1		
High prevalence (≥5%)	-0.79	0.30	0.5	0.3-0.8	
Random effect of herd					
Variance component estimate	0.38	0.22			

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Table 3 Distribution of cows in twelve *Salmonella* prevalence-risk flag groups in the dataset used for survival analysis of culling of cows during a three year intervention study in 10 dairy herds. Flags are the cumulative number of yellow (medium risk) or red (high risk) flags for each animal in the given time-interval.

Prevalence-flag group	n*	culled	Mean number of days spent in that prevalence-flag group
Low prev, 0 flags	2172	540	309
Low prev, 1 flag	24	3	87
Low prev, 2-5 flags	66	11	116
Low prev, >5 flags	25	7	87
Medium prev, 0 flags	1603	277	241
Medium prev, 1 flag	75	4	100
Medium prev, 2-5 flags	145	27	127
Medium prev, >5 flags	41	19	171
High prev, 0 flags	1090	195	284
High prev, 1 flag	411	34	121
High prev, 2-5 flags	273	56	200
High prev, >5 flags	30	8	206

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557 *n= number of cows represented in each group. Cows can be represented in several

558 different groups over time.

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Table 4 Parameter estimates (β), standard error (S.E.), hazard ratios (HR), 95% confidence intervals for HRs and significance level (*P*) in the final proportional hazards survival model for probability of culling in adult cows in 10 dairy herds during a three year *S.* Dublin intervention study. Risk flags indicate the number of times heifers have been assigned medium or high risk of spreading *Salmonella*.

Predictors	Estimate *(β)*	S.E.	HR	95%CI of HR	*P*
Year					< 0.0001
2004	0	-	1		
2005	-0.74	0.13	0.5	0.4-0.6	
2006	-0.17	0.12	0.8	0.7-1.1	
Prevalence-flag groups					< 0.0001
Low prev, 0 flags	0	-	1		
Low prev, 1 flags	0.28	0.58	1.3	0.4-4.2	
Low prev, 2-5 flags	0.02	0.38	1.0	0.5-2.2	
Low prev, >5 flags	0.94	0.51	2.6	0.9-7.0	
Medium prev, 0 flags	-0.89	0.17	0.4	0.3-0.6	
Medium prev, 1 flags	-1.28	0.59	0.3	0.1-0.9	
Medium prev, 2-5 flags	0.55	0.23	1.7	1.1-2.7	
Medium prev, >5 flags	-2.14	1.11	0.1	0.0-1.0	
High prev, 0 flags	-1.17	0.21	0.3	0.2-0.5	
High prev, 1 flags	-1.63	0.29	0.2	0.1-0.3	
High prev, 2-5 flags	-0.61	0.21	0.5	0.4-0.8	
High prev, >5 flags	-0.91	0.42	0.4	0.2-0.9	
Time effect per 100 days	0.15	0.03	1.2	1.1-1.2	<0.001
Time effect per 100 days	0.40	0.13	1.4	1.2-1.6	0.002
Time effect per 100 days	0.12	0.04	1.1	1.0-1.2	0.005

Effect of continuous confounding variables[b]			
$LnSCC^3$	0.004	0.0003	0.000
PctECM	9.08	5.78	0.116
$PctECM^{0.5}$	-19.51	6.97	0.005
$PctECM^2$	-0.15	1.15	0.898
$(Days\ in\ milk/100)^3$	0.01	0.003	0.001
$1/(Parity^2)$	-0.65	0.14	0.000
LnECM	194.76	24.85	0.000
$LnECM^2$	58.22	7.91	0.000
$ECM^{0.5}$	-576.55	76.18	0.000
$LnECM^{0.5}$	66.59	8.82	0.000
Frailty effect of herd	0.14	0.07	

[a] the time effect per 100 days is the estimate adjusting the main effect of the relevant prevalence-flag group by study days

[b] HR and 95%CIs for HRs not shown for confounding variables

565 **Figures**
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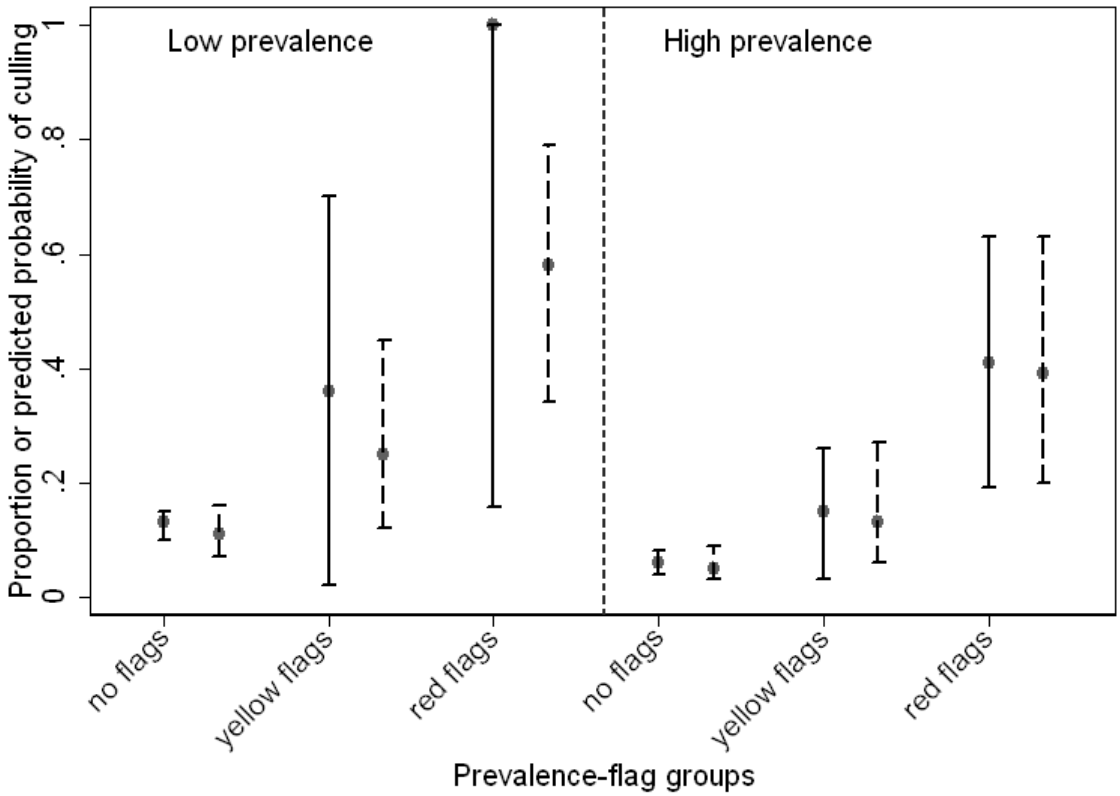

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571 **Fig.1.** Proportions in the raw data (solid lines) and predicted probabilities (dashed lines)
572 with 95% confidence intervals from a logistic analysis of heifers being culled before the
573 first calving in different *Salmonella* risk flag groups under low (<5%) and high (≥5%)
574 within-herd seroprevalences. There were only two heifers with red flags in the low
575 prevalence group and both were culled, thus the exact one-sided 97.5% confidence interval
576 was calculated for this proportion.

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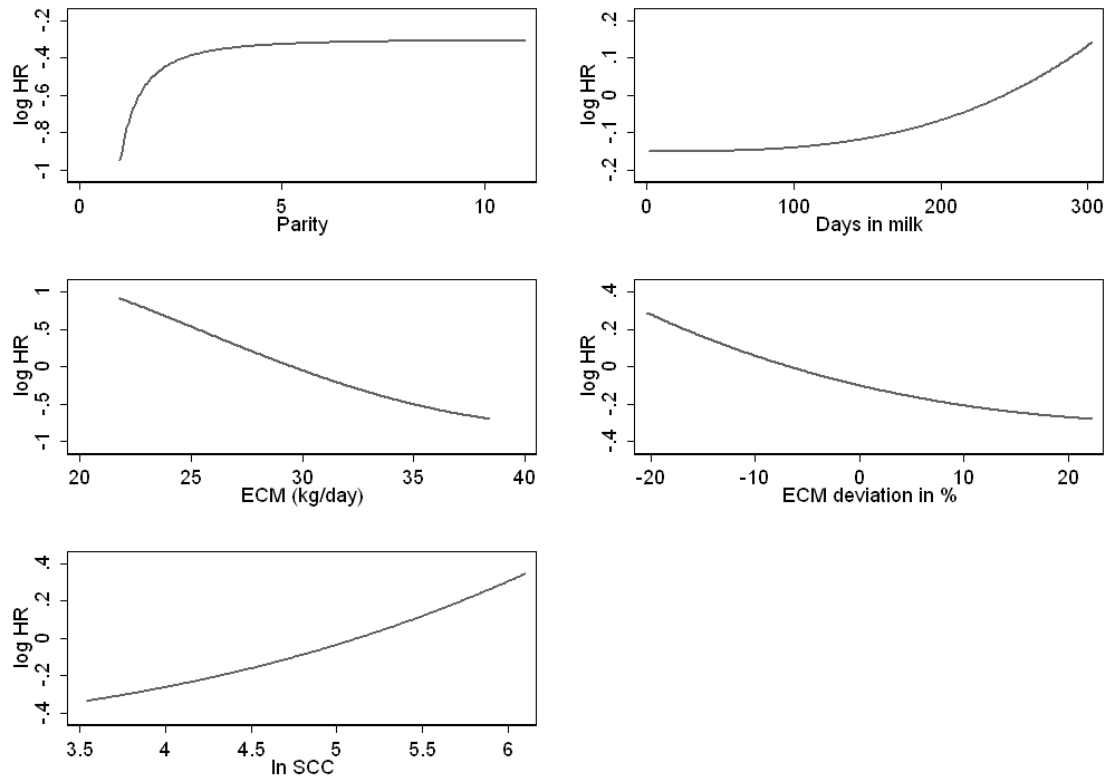

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582 **Fig. 2.** Functional forms of the relationships between continuous confounders and the log
583 hazard ratio (log HR) of culling in adult cows. The confounders were: Parity (1 to 11),
584 number of days from calving (Days in milk), energy corrected milk yield (ECM), deviation
585 in % from the expected energy corrected milk yield adjusted for breed, parity and days in
586 milk (ECM deviation in %) and the logarithm of the somatic cell count in milk (ln SCC).

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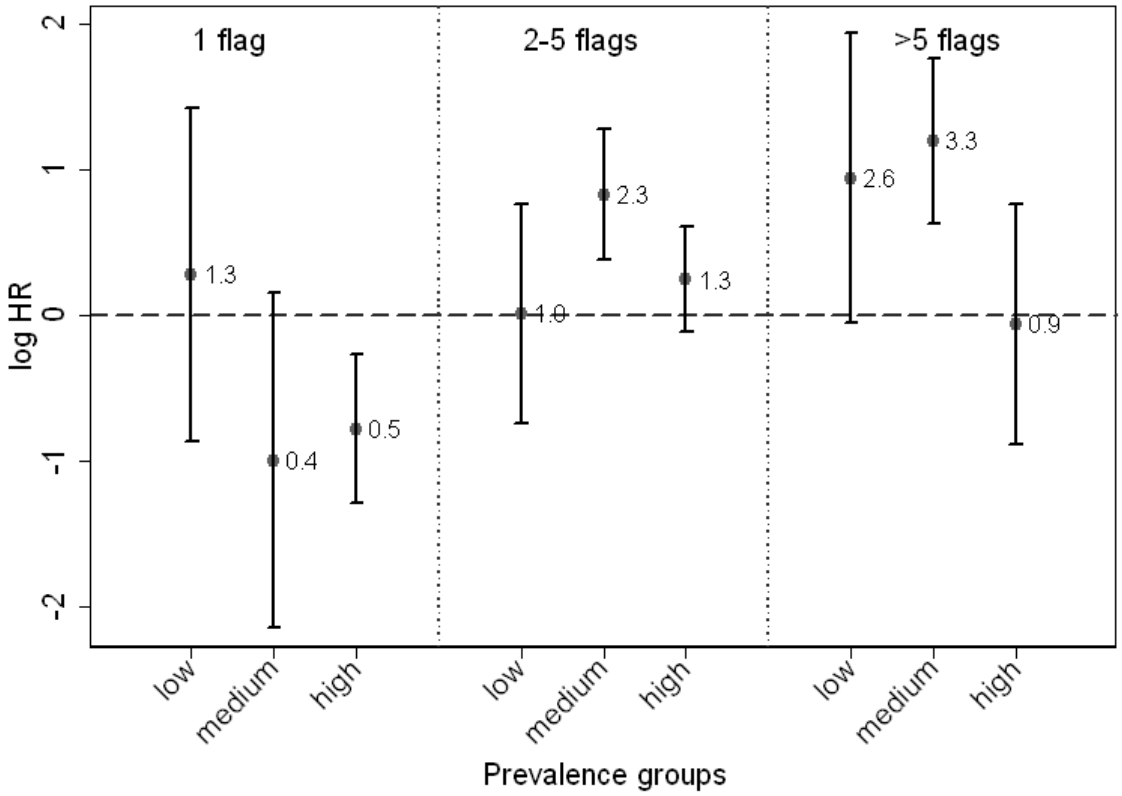

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590 **Fig. 3.** Log hazard (log HR) of culling in all *Salmonella* prevalence-flag groups with 95%
591 confidence intervals at the median number of study days for the time-varying prevalence-
592 flag groups. The numbers next to the dots on each line show the corresponding hazard ratio
593 of the prevalence-flag combination compared to the reference group "0 flags" for each
594 prevalence level.

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