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Published in:
Preventive Veterinary Medicine

DOI:
10.1016/j.prevetmed.2022.105776

Print publication: 01/11/2022

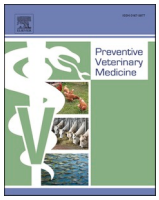
Document Version
Publisher's PDF, also known as Version of record

Preventive Veterinary Medicine 208 (2022) 105776

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Determining the prevalence of antibodies to *Salmonella* Dublin in dairy herds in Great Britain by quarterly bulk tank testing

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ARTICLE INFO

Keywords:
Salmonella Dublin
Prevalence
Dairy herd

ABSTRACT

Salmonella enterica subspecies *enterica* serovar Dublin has been the most common *Salmonella* serovar isolated from cattle in Great Britain for the previous 22 years. It can cause a wide variety of clinical presentations and result in significant welfare and productivity concerns in infected herds. Bulk tank antibody testing undertaken every three or four months forms the basis of eradication and monitoring programmes in Denmark and the Netherlands and has been shown to be a sensitive, specific and cost-effective way of establishing seroprevalence and monitoring infection at a herd level. A prevalence estimate based on quarterly bulk tank testing has not been previously carried out in Great Britain. This study recruited 410 herds across Great Britain, who submitted milk samples on a quarterly basis for screening by an ELISA for *Salmonella* Dublin antibody. Classifying herds according to the Danish eradication scheme classification gave an apparent prevalence of 38% (95% confidence intervals 34–43%) and an estimated true prevalence of 40% (95% confidence intervals 35–45%), taking into account the test sensitivity and specificity. Of the 401 herds which completed the quarterly bulk tank testing, 45% had one or more positive bulk tank results.

1. Introduction

Salmonella enterica subspecies *enterica* serovar Dublin has been the most common *Salmonella* serovar isolated in Great Britain for the previous 22 years, comprising 58% of cattle *Salmonella* isolates from clinical samples in 2020 (Animal and Plant Health Agency (APHA, 2021a). The majority (82%) of *Salmonella* Dublin diagnoses between 2016 and 2020 in which the herd type was known were made in dairy herds, with 13% in beef herds and 5% in calf rearing units (APHA, 2021b).

Confirmed clinical diagnoses of *Salmonella* Dublin made by APHA, Scotland's Rural College (SRUC) and APHA partner providers are recorded through the Veterinary Investigation Diagnosis Analysis (VIDA) system. However, in addition to this there is a need for an accurate prevalence estimate to allow veterinary surgeons and farmers to be aware of the risk of incursion of *Salmonella* Dublin, and the likelihood of the bacterium being present in their herd. The wide range of clinical presentations (Henderson and Mason, 2017) and subclinical production effects (Nielsen et al., 2012) mean that *Salmonella* Dublin is likely to be under-diagnosed and thus relying on reports of clinical disease will under-estimate the impact of *Salmonella* Dublin on the national dairy herd.

An estimate of the current herd level prevalence of *Salmonella* Dublin in Great Britain has not been published since Davison et al. (2005) carried out a study in 449 dairy herds in England and Wales. They identified *Salmonella* Dublin on pooled slurry cultures from 7.5% of dairy herds sampled.

Velasova et al. (2017) carried out single sample pan-*Salmonella* serology on 225 dairy herds in Great Britain and identified that 48% were positive for antibodies to a *Salmonella* species.

A review of the methods of herd level diagnosis for *Salmonella* Dublin was carried out by Veling et al. (2002) and found that in general serological techniques are more sensitive than culture-based techniques. Additionally, Warnick et al. (2006) found the herd level sensitivity of quarterly bulk tank testing to be 95%, based on four samples taken three months apart. For a herd to be classified as negative the average of the previous four results had to be below 25 ODC% and the last of the four samples could not be more than 20 ODC% higher than the average of the previous three.

False negative results can occur in herds where within herd seroprevalence is less than 5%, consistent with bulk tank testing for other

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https://doi.org/10.1016/j.prevetmed.2022.105776
Received 6 April 2022; Received in revised form 30 September 2022; Accepted 4 October 2022
Available online 5 October 2022

diseases. Veling et al. (2001) identified that case herds most often tested negative on a single bulk tank sample when the percentage of seropositive cows was less than 5%, with only 35% of these herds testing positive on bulk tank testing.

Bulk tank antibody titres have been shown to correlate with the bacteriological farm status, the mean yield corrected antibody measurements in individual cows and the number of cows with high antibody titres. The bulk tank antibody titre is strongly correlated with the spread of infection within the adult herd (Wedderkopp et al., 2001; Nielsen and Ersbøll, 2005). False positive results can occur due to infection with other serogroup B and D *Salmonella* serovars. Nielsen (2003) found evidence of cross-reactivity with *Salmonella* Typhimurium infection, whereas the work by Ågren et al. (2016) did not confirm this.

As of 2020, the British dairy herd comprised 1 537 000 milking dairy cows (AHDB, 2022a), across 9306 holdings (AHDB, 2022b). The mean herd size in 2020 was 163 in England, 156 in Wales and 201 in Scotland (AHDB, 2022c). There are currently no voluntary or compulsory control programmes for *Salmonella* Dublin in Great Britain.

This study aims to establish the apparent and true national seroprevalences of *Salmonella* Dublin infection in Great Britain using quarterly bulk tank testing.

2. Materials and methods

This study was carried out between April 2020 and February 2021.

A sample size calculation to identify the number of herds required to estimate the true prevalence was carried out using EpiTools (Ausvet, 2021). The estimated true prevalence was set at 25%, based on the herd seroprevalence of 48% for all *Salmonella* serovars (Velasova et al., 2017), and the observation that 58% of cattle *Salmonella* isolates from clinical samples were *Salmonella* Dublin (APHA, 2021a). This seroprevalence is also similar to that identified in Denmark (Warnick et al., 2006). The test sensitivity and specificity were set at 95% and 99%, respectively (Warnick et al., 2006; Nyman et al., 2013) and the desired precision for the prevalence estimate at 0.05. This gave a sample size of 379. Four hundred and ten herds were recruited to account for farms withdrawing from the study.

Stratified sampling was used to get a representative sample at regional and county level. The farms were assigned geographically according to the distribution of herds within Great Britain (AHDB, personal communication and Scottish Dairy Cattle Association, 2020). For example, if an area had 10% of the British dairy herd present, 41 herds were recruited from that area. This was also applied for the counties within the designated areas.

Herds were recruited either through a single milk purchaser or their veterinary practice. The milk purchaser was asked to supply quarterly milk samples with bronopol preservative in them from a random selection of the herds within a particular county. If the milk purchaser had an insufficient number of clients within that county, veterinary practices within that county were asked to randomly select a number of their dairy clients to achieve the total number of samples required. If a farm had multiple bulk tanks, they were asked to sample only one. Three hundred and seventy three herds were recruited through the milk purchaser, and 37 through veterinary practices. While both the milk purchaser and veterinary practice were asked to select farms for a particular county at random from their database, the method by which this was carried out was not dictated by the authors and therefore the samples may not be truly random.

The samples were supplied to the authors anonymously, having been assigned a number which allowed the quarterly samples to be linked to each other and to the county from which they had been recruited. No herd level information was available and notably no vaccination history.

Milk samples were tested using the PrioCHECK® Salmonella Ab bovine Dublin ELISA (Thermofisher, 2019). This is an indirect ELISA for the detection of antibodies in cattle. The wells were coated with purified lipopolysaccharide isolated from *Salmonella* Dublin. 100 µl ul of milk was added to the wells and incubated at room temperature (18–25 °C) for 1 h. The plates were washed and conjugate (goat anti-bovine IgG with horseradish peroxidase) added to the plate and incubated again for one hour at room temperature. Plates were washed after incubation, a chromogen (tetramethylbenzidine) added and further incubated at room temperature for 15 min to develop. The colour development is stopped with stop solution and the plate is read on a spectrophotometer at 450 nm to obtain the optical density (OD) to calculate the percent positivity.

The cut-off was altered from the kit cut-off of 35 ODC% to 25 ODC% in line with internal validation work and the work carried out by Warnick et al. (2006).

Herds were classified as positive or negative according to the criteria used in the Danish eradication scheme (Warnick et al., 2006). For a herd to be classified as negative the average of the previous four results had to be below 25 ODC% and the last of the four samples could not be more than 20 ODC% higher than the average of the previous three.

In addition to this, the number of herds which had one or more antibody positive test results was recorded.

Prevalence estimates were carried out using EpiTools 'estimated true prevalence with an imperfect test' tool (Ausvet, 2021). The true prevalence estimates were calculated using the method described by Rogan and Gladen (1978), using the formula TP = (AP +Sp-1)/(Se +Sp −1), where TP = true prevalence, AP = apparent prevalence, Se = sensitivity and Sp = specificity. The confidence intervals for apparent prevalence were calculated using the Wilson interval method as described by (Brown et al., 2001). The confidence intervals for the true prevalence estimate were calculated using Blaker's confidence intervals to provide exact two sided confidence intervals which were adjusted for test sensitivity and specificity. Blaker's confidence intervals were calculated as described by Reiczigel et al. (2010).

3. Results

Of the 410 herds recruited, 401 completed testing. There were 181 herds (45%) that had one or more positive bulk tank results, and 153 herds (38%) were positive according to the Danish eradication scheme criteria. The apparent national seroprevalence based on the Danish classification (Warnick et al., 2006) was 38% and the estimate of the true national seroprevalence was 40% (95% confidence interval: 35–45%). Of the 401 herds which completed the quarterly bulk tank testing, 45% of herds had one or more bulk tank samples which were positive for antibodies to *Salmonella* Dublin. Table 1 shows the number and percentage of herds which were positive at each of the four sampling points.

Of the 401 herds, 339 herds (85%) maintained the same status across the four sampling points. Two hundred and twenty of these had four negative results, while 119 had four positive results. The results of the remaining 62 herds are shown in table 2.

3.1. Seroconversions

There were 244 herds which tested negative initially, and 18 of these seroconverted, giving an incidence of 7 newly infected herds per 100

Table 1
The number and percentage of herds which were positive at each of the quarterly sampling points.

Quarter	Number of herds with an antibody positive bulk tank (Percentage)
One (April to May 2020)	158 (39%)
Two (July to August 2020)	128 (32%)
Three (October to November 2020)	148 (37%)
Four (January to February 2021)	151 (38%)

Table 2
The variation in antibody levels in herds with alterations in herd status over the study period.

Variation in status	Number of herds (Percentage of herds)
Fluctuating between positive and negative	25 (6% of all herds)
Apparent recovery	19 (12% of initially positive herds)
Seroconversion	18 (7% of initially negative herds)

herds over the course of the study.

Of these herds, three seroconverted between quarter one and two, six between quarter two and quarter three and nine between quarter three and quarter four.

Fourteen of the eighteen herds would have been classified as positive at the end of the study according to the Danish eradication scheme criteria.

3.2. Herd level recoveries

Nineteen herds had a positive bulk tank in quarter one and a negative bulk tank in quarter four, and so represented apparent herd level recoveries. Of these nineteen herds, fourteen were classified as negative according to the Danish eradication scheme criteria, all of which had only one antibody positive bulk tank result in quarter one followed by three negative bulk tank results. Of the five herds which were still classified as positive at the end of the study, one was bulk tank negative in quarter four only, two were bulk tank negative in quarters three and four and two were negative in quarters two, three and four.

3.3. Herds with fluctuating antibody levels

Twenty-five herds had antibody levels which fluctuated between positive and negative. Of these herds sixteen were classified as positive according to the Danish eradication scheme criteria, while nine were classified as negative.

Five herds only had one antibody positive bulk tank result, one in quarter two and four in quarter three. All these herds were classified as negative.

Ten herds had two positive bulk tank antibody results. Seven of these herds were classified as positive according to the Danish eradication scheme criteria.

Ten herds had three positive bulk tank results. Of these, one was classified as negative, on the basis of bulk tank results of 36, 0, 28 and 31 ODC% for quarters one to four respectively. Of these ten herds with a single bulk tank antibody negative result, nine had a negative bulk tank result in quarter two (July to August).

The seroprevalence of *Salmonella* Dublin was examined regionally as well as nationally. Fig. 1 illustrates the regional prevalences with 95% confidence intervals. Because of similar seroprevalence estimates and their small sample size, the east, south-east and south regions were grouped together for analysis.

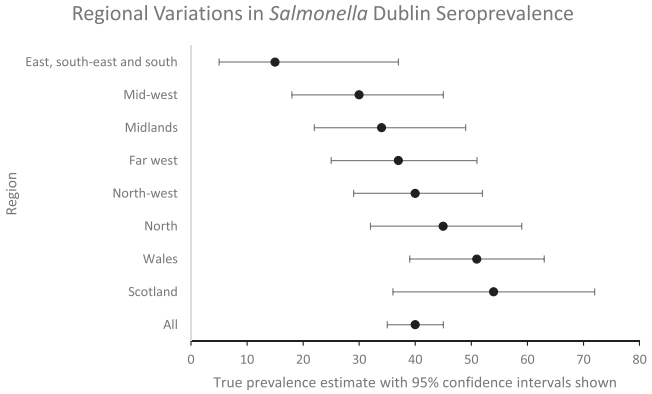

Fig. 1. Regional variations in Salmonella Dublin seroprevalence.

3.4. ODC% values

Figure two shows the frequency of the mean ODC % values for all 401 herds in the study. The majority of the herds which were classified as negative had mean ODC % values between 0% and 5%. Of the 153 herds classified as positive using the Danish eradication scheme criteria, only seven (5%) had mean ODC% values within 10% of the cut-off (Fig. 2).

3.5. Producer recruited herds verses veterinary practice recruited herds

To investigate the variation between the herds selected by the milk purchaser and veterinary practices, the difference in seroprevalence in regions which had farms selected both by the milk purchaser and a veterinary practice were examined. Only four regions had farms selected both by the milk purchaser and a veterinary practice and the number of farms and seroprevalence is shown in Table 3. In three of the four regions, the farms selected by the veterinary practices had an identical or lower seroprevalence than those selected by the milk purchaser, and so any selection bias by veterinary practitioners does not appear to have artificially increased the seroprevalence.

4. Discussion

4.1. Prevalence estimate

This is the first study to estimate the seroprevalence of *Salmonella* Dublin in Great Britain and assign status on the basis of quarterly bulk tank testing. Previous seroprevalence estimates relied either on a single pan-*Salmonella* bulk tank sample or bacteriological techniques. Of the 401 herds tested, 181 (45%) had one or more *Salmonella* Dublin antibody positive bulk tank samples. This is similar to the percentage of positive bulk tank results identified in the pan-*Salmonella* bulk tank survey carried out by Velasova et al. (2017) in Great Britain, and also by O'Doherty et al. (2013) in Ireland, at 48% and 49% respectively. It might be expected that the figure obtained through this study would be lower compared to Velasova et al. (2017), given that other *Salmonella* serovars would not be detected here. However, the quarterly sampling is likely to have increased the sensitivity in this study. This would be consistent with the breakdown of the quarterly results in Table 1, where at each quarter the percentage of herds which were antibody positive ranged from 32% to 39%.

In using the criteria for the Danish eradication scheme to assign herd level status, this study also sought to identify those herds which would be classed as positive or negative at the end of the study period. Thus, while the true prevalence based purely on the number of herds with one or more positive bulk tank results would be 47% (95% confidence

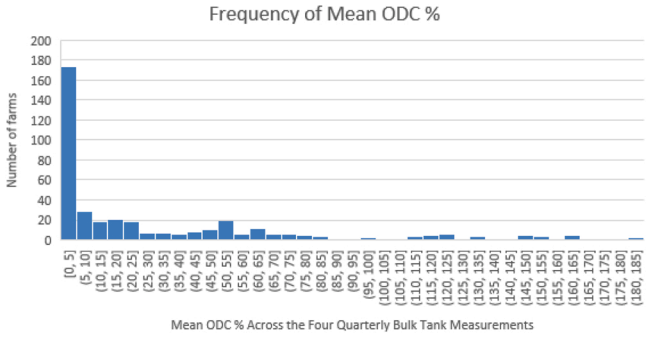

Fig. 2. Frequency of mean ODC% across four quarterly bulk tank measurements for all herds.

Table 3
A comparison between the seroprevalence of milk purchaser selected farms and veterinary practice selected farms for regions where farms were selected by both methods.

Region	Percentage of farms classified as positive – farms selected by milk purchaser (number of herds recruited)	Percentage of farms classified as positive– farms selected by veterinary practice (number of herds)
Dumfries and Galloway	83% (12)	67% (3)
Pembrokeshire	67% (9)	33% (3)
Carmathenshire	67% (9)	78% (9)
South Wales	0% (5)	0% (1)

intervals 42–52%) the true prevalence based on herds which were classified as positive was 40% (95% confidence intervals 35–45%).

Using the Danish eradication scheme classification to assign herd level status, the true prevalence of herd level infection is higher than the Danish prevalence, which was 26% before the control programme began (Nielsen et al., 2007). Vaccination may have artificially elevated the prevalence in British dairy herds. However, Velasova et al. (2017) indicated that the percentage of British herds vaccinating for *Salmonella* Dublin was low, at 5%, and therefore, does not explain the difference between the Danish and British prevalence estimates.

4.2. Herd classification

Because the Danish eradication scheme is well established and evidenced, and the ELISA test kit and sampling protocol used were the same as is used in the scheme, herd level status was assigned as being positive or negative based on the interpretation criteria used as part of this scheme (Warnick et al., 2006). For a herd to be classified as negative the average of the previous four results had to be below 25 ODC% and the last of the four samples could not be more than 20 ODC% higher than the average of the previous three. This gave a true prevalence estimate of 40% (95% confidence intervals 35–45%).

However, while this detected herds which seroconverted between sample points three and four, there were a number of herds which seroconverted between sample points two and three that were still classified as negative under the Danish interpretation criteria. While cross-reactions are possible, it is likely that some of these herds will be genuinely infected, and thus the 40% true prevalence estimate is likely to be a conservative estimate.

The impact of this may be more profound in this survey than in the Danish eradication scheme, due to differences in the risk of incursion of *Salmonella* Dublin into a herd. Incursion of *Salmonella* Dublin may be more common in British dairy herds compared to Danish dairy herds. Initially, in Denmark, negative herds purchasing from level two (not-negative) herds are locked-in to a level two (not-negative) status for a period of between three weeks and three months depending on the stage of the eradication programme (Nielsen, 2009), which dramatically altered purchasing behaviour (Nielsen, 2013a). Currently level two herds cannot sell cattle to level one herds, further reducing the risk of incursion into negative herds (Danish Agricultural and Food Council, 2020). In British dairy herds, bulk tank testing for *Salmonella* Dublin is a relatively recent introduction, herd level status is not commonly known and in the authors' experience purchasing decisions are unlikely to take the *Salmonella* status of the herd of origin into account.

Of the twenty-eight herds which had a positive bulk tank result, but were not classified as positive according to the Danish eradication scheme, fourteen were herds that had an apparent recovery, ten were herds with fluctuating antibody titres and four were herds which seroconverted. Longer term surveillance of these herds, as is the case in the Danish eradication scheme would determine whether these herds had been classified correctly, or whether the categorisation of herds which had seroconverted as negative represented a missed opportunity to detect the incursion of *Salmonella* Dublin.

4.3. Possibility of cross-reactions and false positive results

Although the ELISA used was to detect antibodies to *Salmonella* Dublin, the possibility of cross-reaction with B and other D group *Salmonella* serovars must be considered. Nielsen (2003) found evidence of cross-reactivity with *Salmonella* Typhimurium infection, whereas the work by Ågren et al. (2016) did not confirm this. Herds were determined to have a cross-reaction in Nielsen (2003) where individual sera or bulk tank serology was positive, and where *Salmonella* Dublin was not isolated on culture of faecal samples from a cohort of the herd over a nine month period. These may not, therefore, have represented cross-reactions, but instead a lack of sensitivity of the faecal sampling protocol and a lack of sensitivity of faecal culture in non-clinically affected animals, which has been documented (Nielsen et al., 2004).

Ågren et al. (2016) compared the results of *Salmonella* serotypes isolated from Swedish dairy herds over a four year period, using the PrioCHECK® Salmonella Ab bovine Dublin ELISA and the PrioCHECK® Salmonella Ab bovine ELISA. They identified that, even lowering the positive cut-off ODC% to 20%, no herds that had *Salmonella* serotypes other than *Salmonella* Dublin had a positive bulk tank result using the Dublin ELISA. Ågren et al. (2016) found that, in common with Great Britain (APHA, 2021a), the most common B or D serotype, other than *Salmonella* Dublin was *Salmonella* Typhimurium.

One vet-recruited herd in this study had an outbreak of *Salmonella* Typhimurium in the adult cattle between the first and second sampling points but the bulk tank remained antibody negative.

Of the 153 herds which were classified as positive according to the Danish eradication scheme criteria, only seven herds had ODC% values for all four samples which were within 10% of the cut-off. These may still be genuinely positive herds, given the work by Ågren et al. (2016). They may reflect herds with a lower proportion of seropositive cows.

It is of note that the sensitivity of a bulk tank sample is lower when the percentage of seropositive cows is less than 5% or when infection is confined to youngstock (Veling et al., 2001). Therefore, fluctuating or low titres may not reflect cross-reaction, but instead be an early indicator of infection or reflect herds where the percentage of seropositive cows is less than 5%.

The effect of vaccination on serology is unknown, in particular how long the antibodies will persist. The antibody response in an infected animal is not long-lasting unless there is carrier status or ongoing exposure to the bacterium (Robertsson et al., 1984; Smith et al., 1989, in Nielsen, 2013b). Velasova et al. (2017) excluded vaccinating herds in their study and found that only 5% of 221 herds were vaccinating for *Salmonella* Dublin. Even assuming all vaccinating herds would otherwise be antibody negative, removing 5% of herds classified as positive herds from the study on the assumption their results were vaccinal, would only reduce the true prevalence estimate by 3%. Additionally, in the authors' experience, vaccination against *Salmonella* Dublin is not generally carried out unless a clinical problem has been identified and so it is very unlikely that all vaccinating herds would otherwise be antibody negative.

4.4. Quarterly testing results

The importance of testing quarterly rather than assigning status on a single bulk tank sample in order to increase sensitivity has been established by (Veling et al. 2002; Warnick et al., 2006). In this study, 85% of herds had the same serostatus at every sampling and so would have been correctly assigned a status on a single sample, but 15% could have been incorrectly classified.

It is interesting to note that of the ten herds which had fluctuating antibody titres and only one bulk tank antibody negative result, nine of these had that negative result in quarter two (July to August). In the authors' experience bulk tank antibody titres drop in grazing herds

during the summer, which may be the case here, although the grazing history of these herds was not available. However, this observation combined with the fact that the quarter two sampling had the lowest percentage of positive bulk tanks would indicate that negative herd status should not be assumed based on a single bulk tank status in summer in particular. Regardless of grazing status, the increased incidence of clinical *Salmonella* Dublin infection in the autumn and winter means that this period of time should be surveyed (APHA, 2021a).

The duration of the study limited the ability to assess true changes of serostatus, as herds could not enter the study with a negative status, due to the fact that this requires four quarterly results. Instead, an apparent seroconversion was taken as a herd which was initially antibody negative on bulk tank, before becoming antibody positive and remaining positive for the remainder of the study period. There was also limited time for herds to recover from infection. An apparent recovery was classed as a herd that changed from bulk tank positive to negative, without returning to being antibody positive during the study period.

In this study, eighteen (7%) herds that were negative in the first quarter seroconverted, which is of note for herd biosecurity and the risk of incursion. Fourteen of these herds would have been classified as positive using the Danish eradication scheme classification criteria.

It is encouraging to note that within a nine-month period, there were nineteen herds that were seropositive at the beginning of the study and became seronegative by quarter four. While this is an apparent rather than a true recovery, and future titre fluctuations may occur, thirteen of these herds would have been classified as negative at quarter four. Fifteen of the nineteen herds had three negative results by the end of the study, and therefore there can be a reasonable degree of confidence in the recovery of these herds.

Danish data highlights that herd level recovery is not only possible, but that the average duration of infection in Danish dairy herds during the surveillance and control programmes was two years (Nielsen and Dohoo, 2013).

Veling (2004) noted that in the Netherlands, only 50% of infected herds became endemically infected, and whether this occurred was largely dependent on the hygiene and management in the initial stages of infection. Therefore, as well as clinical vigilance, quarterly bulk tank testing can provide a relatively early indication of infection circulating in the adult herd. This would allow control measures to be put in place similar to those described in Nielsen and Nielsen (2012) and adapted by Henderson and Mason (2017).

4.5. Significance of an antibody positive bulk tank result

Interestingly, Velasova et al. (2017) found that, while 48% of bulk tanks were positive for antibodies to *Salmonella*, only 20% of farmers believed the bacterium to be present on farm, and none believed it to be causing a problem at that time point. Possible reasons for this may be that the infection goes unrecognised clinically or presents only with subclinical production effects, such as a decrease in milk yield (Nielsen et al., 2012; Veling, 2004). Additionally, given that prior infection has been shown to reduce the severity of subsequent infections at an individual animal level (Steinbach et al., 1996) it is possible that the impact of a positive bulk tank result is affected by the degree of immunity within a herd.

One important factor in making an inference about the clinical significance of a positive bulk tank antibody result is whether clinical salmonellosis is recognised as such. While *Salmonella* Dublin has historically been reported as a cause of sudden death and diarrhoea, a review of carcase and clinical pathology submissions to SRUC Veterinary Services highlighted the wide range of clinical presentations, and the fact that only 66% of confirmed *Salmonella* Dublin cases in adult cattle and 53% in calves present with diarrhoea (Henderson and Mason, 2017). Presentations such as poor growth rates and pneumonia in calves and milk drop in adult cattle may not be attributed to salmonellosis, although it is also possible that calves with mild diarrhoea are less likely to be sampled than those with systemic disease.

Additionally, strain virulence, host immunity, host physiology and the degree of exposure will all influence the severity of clinical disease (Wallis et al., 1995; Steinbach et al., 1996; Mattila et al., 1988; Wray and Sojka, 1981). It is known that previous exposure to the bacterium will result in milder clinical signs, therefore the extent of clinical disease and mortality are likely to be lower in herds with endemic infection rather than where the bacterium enters a naïve herd (Steinbach et al., 1996). However, even in endemically infected herds, there is the possibility of differing immune statuses in different groups and a positive bulk tank result should therefore act as a warning against complacency in hygiene and management protocols. Additionally, the introduction of naïve animals to an infected herd, or infected animals to a naïve herd is still likely to have clinical and subclinical consequences (Bazeley, 2006; Nielsen and Dohoo, 2012).

As well as the clinical impact of *Salmonella* Dublin infection within a herd, there are subclinical effects on production. Several studies have examined the correlation between the herd's bulk tank *Salmonella* antibody status and milk yield. Nielsen et al. (2012) found that a hundred cow herd in the first year following a change in the bulk tank status from negative to positive lost 40 000 kg of energy corrected milk yield, relative to the same herd the year before the change in status. The milk yield took more than a year to return to pre-infection levels. Nielsen et al. (2010) also found a correlation between high calf mortality and bulk tank *Salmonella* Dublin antibody status, with bulk tank antibody positive herds having twice the odds of having high calf mortality as bulk tank antibody negative herds. High calf mortality was defined as mortality of greater than 6.5%, as this is the target calf mortality set by the Danish Cattle Federation. This may be confounded by other management factors on farm which contribute to the presence of endemic salmonellosis and simultaneously affect calf health, but the impact of *Salmonella* Dublin on calf health is well-established (Peters, 1985; Bazeley, 2006).

Nielsen et al. (2013) modelled financial losses associated with the introduction of *Salmonella* Dublin into a herd and identified that, while losses were highest in the first year, they continued for ten years, with ongoing annual losses of between €1400 and €34,800.

The likely significance of a positive bulk tank antibody result is also supported by the fact that bulk tank antibody levels have been shown to correlate with the bacteriological farm status, the antibody measurements in individual cows and spread of infection within the adult herd (Nielsen, 2013b). While a degree of immunity may exist within a herd, the herd level prevalence indicates the importance of biosecurity considerations for the seronegative herds.

4.6. Regional seroprevalence

The failure of nine herds to complete the sampling programme may have influenced the regional seroprevalence, as these herds were not distributed evenly geographically. Six of these herds were Scottish. However, based on between one and three bulk tank results, 60% of the herds which dropped out had one or more positive bulk tank results. Given that 45% of herds which completed testing had one or more positive bulk tank results, failure of these herds to complete testing would not have over-estimated the prevalence of herds with positive bulk tank results.

4.7. Herd recruitment

The method of herd recruitment may have introduced a potential bias, as herds were recruited either through a single milk purchaser, or by their veterinary practice. While both the milk purchaser and vet practice were asked to randomly select herds within a particular region from their database of clients, the method by which this was done was not specified. Given that consulting vets will have a knowledge of their client's *Salmonella* status, this may have affected their farm selection.

However, Table 3, which compared the herds selected by milk producers and veterinary practices, did not indicate that the selection of herds by the veterinary surgeons would have led to an increased prevalence.

5. Conclusions

Quarterly bulk tank testing has estimated the prevalence of herd level infection with *Salmonella* Dublin in British dairy herds to be 40% (95% confidence intervals 35–45%). It has provided some information on regional variations within this. It is of note that vaccinated herds were not excluded from this study but the relatively small percentage of herds vaccinating (Velasova et al., 2017) and the overlap between infected and vaccinated herds meant that this is considered unlikely to have dramatically altered the prevalence estimate.

The estimate of true prevalence of *Salmonella* Dublin in the dairy herd was significantly higher than the only previous estimate of 7.5% based on bacteriological cultures. This concurs with the work by Veling et al. (2002) which identifies that culture-based techniques have a much lower herd level sensitivity for the monitoring of infection. Culture based techniques would, however, remain the diagnostic tool of choice in the acute clinical situation, due to the length of time required for seroconversion to occur.

The knowledge of the seroprevalence of Salmonella Dublin enables veterinary surgeons and farmers to be aware of the risk of incursion to the herd, and of the likelihood of *Salmonella* Dublin being present in their herd. Additionally, knowledge of national seroprevalence provides the dairy industry with a figure on which to base decisions around control strategies.

Conflicts of Interest

The prevalence study was funded by MSD Animal Health, who have provided input into the final manuscript but did not have any role in the study design, collection and analysis of data.

Acknowledgements

SRUC VS receives financial support from the Scottish Government for farm animal disease surveillance activities. We would like to extend our thanks to MSD, ARLA and to all the farmers and vets for their involvement. We are grateful to George Caldow for his input into the manuscript.

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