Transmission of *Salmonella* in dairy herds quantified in the endemic situation

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**Abstract** – *Salmonella* is a cause of concern in the cattle industry, because it is a zoonosis causing severe invasive infections in humans and because it causes economic and welfare losses in infected herds. In general, cattle in the Netherlands are infected with two types; *Salmonella* Dublin and *Salmonella* Typhimurium. Both types cause clinical signs but *S.* Dublin outbreaks are more prevalent and clinical signs are more severe than *S.* Typhimurium outbreaks. Our knowledge of the transmission of *Salmonella* within herds is still limited, while this is an essential component for modelling the success of intervention strategies to control *Salmonella*. The aim of our study was to estimate the basic reproduction ratio (*R*₀), the number of secondary cases produced from each primary case in a totally susceptible population, for *S.* Dublin and *S.* Typhimurium in dairy herds. Serological data were obtained from eight farms with a clinical outbreak of *Salmonella*, two with an outbreak of *S.* Dublin and 6 of *S.* Typhimurium. *R*₀ was estimated from the serological data of the herds that were in an endemic state of the infection. *R*₀ across herds was estimated to be 2.5 (95% CI 1.7–9.8) and 1.3 (95% CI 1.1–1.7) for *S.* Dublin and *S.* Typhimurium, respectively. The between herd variation was significant and fairly large. The results of the sensitivity analysis showed that the *R*₀ estimate was not sensitive for changes in the latent, infectious or seropositive periods. The *R*₀ estimates indicated that the infection would not spread very extensively in susceptible populations under management systems similar to the ones in the study herds.

*Salmonella* Dublin / *Salmonella* Typhimurium / transmission / dairy cattle

**1. INTRODUCTION**

*Salmonella* is a cause of concern in the cattle industry, because it is a zoonosis causing severe invasive infections in humans and because it causes economic and welfare losses in infected herds. In general, cattle in the Netherlands are infected with two types; *Salmonella* Dublin and *Salmonella* Typhimurium. Both types cause clinical symptoms but *S.* Dublin outbreaks are more prevalent and clinical signs are more severe than *S.* Typhimurium outbreaks. In the Netherlands, 8–9% of the dairy herds are infected with *Salmonella* and about 1% of the herds experience a clinical outbreak each year.

Several studies show that there are differences in the transmission dynamics and epidemic behaviour of *Salmonella* serotypes at the herd level. There is evidence that clinical episodes and shedding of *Salmonella* recur periodically, at intervals of several months, before disappearing

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from the farm environment\textsuperscript{1} [2, 3, 9]. Xiao et al. [24] and Bergevoet et al.\textsuperscript{2} developed mathematical and simulation models to understand such dynamics and to simulate control programs, but these models lack reliable parameter estimates for the description of Salmonella dynamics within herds. Quantification of the transmission potential of Salmonella by means of the basic reproduction ratio ($R_0$) can help to improve such modelling studies. $R_0$, defined as the average number of secondary infections per primary infected in a susceptible population, is a threshold parameter measuring the invasibility of a pathogen (invasion is only possible if $R_0 > 1$), but it also determines its prevalence in a population where it is endemic.

To our knowledge, the only study that quantified $R_0$ for $S.$ Dublin is a study in Danish dairy calves in which the $R_0$ ranged from 1.1 to 2.5 between herds, but with fairly wide confidence intervals. Data were too limited to show possible significant differences in the parameters between the study herds [14]. Xiao et al. [24] made an assumption on the transmissibility of Salmonella of 0.006 new infections per animal per day, which is equivalent to $R_0 = 5.4$ in an average Dutch herd ($= 0.006 \times 10$ days length of infectious period $\times 90$ animals in an average Dutch herd). The aim of our study was to estimate $R_0$ for $S.$ Dublin and $S.$ Typhimurium from data of Dutch dairy herds with endemic Salmonella infections.

2. MATERIALS AND METHODS

2.1. Farms

Data were obtained from eight farms with a clinical outbreak of salmonellosis. The outbreak was confirmed by bacteriological culture of faecal samples or dead animals, which were submitted to the laboratory of the Animal Health Service (AHS, Deventer) in the Netherlands for diagnostic reasons, except for one farm (farm L). Isolated Salmonella serotypes were: Salmonella Dublin (two farms), Salmonella Typhimurium (4 farms) and Salmonella serogroup B (farm C). On farm L the diagnosis was made by detection of antibodies for Salmonella serogroup B in bulk tank milk. More information about the involved serotype was not available for the two herds with a Salmonella serogroup B infection. Both farms C and L were included in the $S.$ Typhimurium group because in the Netherlands, a Salmonella serogroup B infection in dairy herds is usually caused by $S.$ Typhimurium\textsuperscript{3}. The analyses were also carried out without herds C and L to determine whether these herds influenced the $R_0$ estimates for $S.$ Typhimurium.

All farmers stated that, prior to the first Salmonella isolation; there was no history of salmonellosis in the herd. This statement was confirmed by the veterinary practitioner of the farm, and a review of laboratory records of the AHS of the last three years before the outbreak. A questionnaire was administered to obtain records of the clinical signs that were observed in the clinical period and recorded by the farmers in different age-groups (young stock and/or adult cattle) just as reported in an


\textsuperscript{3} Veling J., Personal communication.
earlier study [19]. None of the farms vaccinated against salmonellosis. The farms were spread over The Netherlands with no concentration of farms in a special region. More information about the history of the farms and the cumulative proportion of cows with clinical signs is presented in Table I.

The number of cattle per farm varied from 90 to 202 animals with a median of 114 animals. Percentage animals with clinical signs varied from 8 to 29.5% with a mean and median percentage of respectively, 17.9 and 16.3%. The clinical period ranged from 9 to 377 days. All farms had a free-stall housing system with separate housing for the young calves. All farmers raised their own heifers on-farm. Animals were identified individually by ear tags.

### 2.2. Laboratory procedures

Active carriers were detected by faecal culture. Ten grams of individual faecal samples was inoculated into 100 mL of brilliant-green selenite enrichment broth (Oxoid Ltd, Basingstoke, Hampshire, UK), and 1 g into 100 mL of Rappaport-Vassiliadis broth (Oxoid Ltd). Both enrichment broths were incubated for 18–24 h at 37 °C. Subsequently, 10 μL of both solutions were plated on brilliant-green agar (Oxoid Ltd) that was incubated for 18–24 h at 37 °C. *Salmonella*-suspect colonies were forwarded to the European *Salmonella* Reference Laboratory (RIVM, Bilthoven, the Netherlands) for determination of serotypes of *Salmonella* [7].

Serum samples from the *S*. *Dublin* infected farms were tested using an indirect ELISA with serovar Dublin lipopolysaccharide antigen (LPS ELISA), as described before [18]. Samples were considered positive if the sample showed a relative optical density of ≥ 50% (cut-off) in comparison with a positive reference sample. Serum samples from the *S*. *Typhimurium* and *Salmonella* group B infected farms were tested with a similar LPS ELISA with serovar Typhimurium LPS antigen. The sensitivity of the serum ELISA was expected to be > 80% and the specificity 99.3%.

### 2.3. Sampling

The basic reproduction ratio, $R_0$, the number of secondary cases produced by each primary case in a totally susceptible
population, was estimated with the serological data of the herds. In a herd, all cattle were sampled at each sampling. The two \textit{S}. Dublin herds were sampled within three weeks after the onset of clinical signs and were tested first with biweekly and later with monthly intervals; in total 6 and 11 whole herd samplings were obtained. In the dataset, only data were included that were obtained in the endemic period, which was indicated by a (more or less) constant prevalence. Figure 1 shows that the prevalence was constant for both herds with a \textit{S}. Dublin outbreak after the fourth visit so only the data from the fifth visit and later were kept for the analysis.

Three of the six \textit{S}. Typhimurium herds were sampled only once and three herds were sampled 3 times (2 herds) or 5 times (1 herd). Two herds (with one and three samples) were sampled within a month after the outbreak and 4 herds were sampled at least 4 months after the outbreak; all were in an endemic situation.

2.4. Model

\textit{Salmonella} in cattle is an infection with no life-long immunity. After infection follows a short latent period of about 1–2 days in which an animal is infected but not yet infectious and an average infectious period of 10 days, during which the bacterium can be transmitted to other animals [15]. The infectious period can vary widely, as about 5\% of all animals become carriers [10, 11], whereas most are infectious for only a few days. After the infectious period the animals become seropositive, but this seropositivity only lasts for on average 90 days, after which they are again susceptible for infection [14, 15].

The dynamics of infections in populations can be described by means of compartmental models [1]. For the \textit{Salmonella} dynamics just described, this means that the population of size \( N \) can be divided in four compartments: susceptible animals (denoted by \( S \)), latently infected animals (\( L \)), infectious animals (\( I \)), and seropositive animals (recovered, \( R \)). The change in the numbers of animals in the four compartments is described by the following differential equations:

\[
\begin{align*}
\frac{dS}{dt} &= \frac{R}{dS} - \beta \frac{(S I)}{N} \\
\frac{dL}{dt} &= \beta \frac{(S I)}{N} - \frac{L}{dL} \\
\frac{dI}{dt} &= \frac{L}{dL} - \frac{I}{dI} \\
\frac{dR}{dt} &= \frac{I}{dI} - \frac{R}{dS}
\end{align*}
\]

in which \( dS = 90, dL = 2, \) and \( dI = 10 \), are the average durations of the seropositive, latent, and infectious periods respectively. The parameter \( \beta \) is the transmission parameter, defined as the average number of secondary infections per primary infected animal per day, in a susceptible population assuming homogeneous mixing of animals. By definition, \( R_0 = \beta d_I \). In the model we assume a constant population size and neglect replacement of animals, because the infection dynamics takes place on a much faster time scale than replacement (20–30\% per year).

In an endemic situation, the average number of animals in each class does not change in time, so all differential equations can be set equal to 0. By observing \( R \), the number of seropositive animals, the following relation can be derived:

\[
R_0 = \left( \frac{1}{1 - \left( 1 + \frac{dL + dI}{dS} \right) \times \frac{R}{N}} \right)
\]
Table II. Results of the log-linear regression with a random herd effect and the transmission coefficient of two endemic S. Dublin herds.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>(R_0) 2.5%</th>
<th>(R_0) 97.5%</th>
<th>(CL) 2.5%</th>
<th>(CL) 97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–0.6</td>
<td>0.2</td>
<td>0.2</td>
<td>1.7</td>
<td>1.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Within-herd</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>correlation</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

which can be used to estimate \(R_0\) from the fraction of seropositive animals \((R/N)\) in the endemic situation (1).

The estimations were carried out with a generalized log-linear regression model in STATA/SE8.2 (Statacorp, College Station, Texas, USA) (xtgee \(rtot\), family(poisson) link(log) exposure(n) corr(exchangeable) robust). The dependent variable was the number of seropositives at a certain time \((rtot)\) and the offset was the log of the total herd size \((n)\) at that time multiplied by the constant factor \(1 + \frac{dL + dI}{dS}\), which amounted \(1 + ((2 + 10)/90) = 1.13\). To correct for the repeated observations in a herd an equal-correlation structure was used to model the within-herd correlation. The robust estimator of variance was used to obtain valid standard errors even if the correlations within group are not as hypothesized by the specified correlation structure.

Because of uncertainty about the durations of the latent, infectious, and seropositive period, a sensitivity analysis was carried out to consider the influence on \(R_0\). The periods were changed separately and all at the same time. In addition, the fixed values were replaced by normal distributions in @Risk 3.5 (Pallisade Inc., Newfield, NY, USA) for each period in which the 90% confidence intervals were ± 10% change of the period.

3. RESULTS

The estimates of an intercept-only log-linear regression model for two herds with endemic S. Dublin are presented in Table II including \(R_0\) with confidence limits.

Table III. Results of the log-linear regression with a random herd effect and the transmission coefficient of six endemic S. Typhimurium herds.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>(R_0) 2.5%</th>
<th>(R_0) 97.5%</th>
<th>(CL) 2.5%</th>
<th>(CL) 97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–1.7</td>
<td>0.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Within-herd</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>correlation</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Figure 2. Prevalence of S. Typhimurium at each visit in six Dutch dairy herds.

The overall \(R_0\) for S. Dublin across herds was estimated to be 2.5 (95% CI 1.7–9.8). The within-herd variation was very small (repeated observations on a herd were very similar) but the between herd variation was significant and fairly large (0.45 (95% CI 0.30–0.66)), which is confirmed by the observed S. Dublin prevalence of 41% and 64% in the two herds, respectively and 48% across herds (Fig. 1).

In Table III the results of the intercept-only model for the six herds with S. Typhimurium are presented.

The across herd \(R_0\) for S. Typhimurium is 1.3 (95% CI 1.1–1.7) and the between herd variation was significant (0.72 (95% CI 0.59–0.89)). The S. Typhimurium prevalence across herds varied considerably from 6% to 44% in the six herds (Fig. 2). There is a tendency of \(R_0\) to be lower for S. Typhimurium than for S. Dublin. When the two herds with serotype B (herds C and L) were excluded...
Table IV. Results of the sensitivity analysis for the latent, infectious and seropositive periods for the $R_0$ point estimates for endemic $S$. Dublin herds.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$R_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Default estimate</td>
<td>2.5</td>
</tr>
<tr>
<td>Latent period from 2 to 1 day</td>
<td>2.4</td>
</tr>
<tr>
<td>Infectious period from 10 to 8 days</td>
<td>2.4</td>
</tr>
<tr>
<td>Seropositive period from 90 to 100 days</td>
<td>2.4</td>
</tr>
<tr>
<td>All periods changed at the same time</td>
<td>2.3</td>
</tr>
<tr>
<td>All periods with a 90%-distribution around the default values (90% confidence interval)</td>
<td>2.5 (2.4–2.6)</td>
</tr>
</tbody>
</table>

$R_0$ decreased somewhat to 1.1 (95% CI 1.1–1.2).

The results of the sensitivity analysis show that the $R_0$ estimate is not sensitive for changes in the latent, infectious or seropositive periods (Tab. IV).

4. DISCUSSION

In our study we assumed an endemic equilibrium and Figures 1 and 2 show that this seemed reasonable. The estimates for the basic reproduction ratio for $S$. Dublin and $S$. Typhimurium were fairly low with 2.5 (95% CI 1.7–9.8) and 1.3 (95% CI 1.1–1.7), respectively, but there is a large variation between herds for both $Salmonella$ species. Excluding two herds in which serotype B was found but $S$. Typhimurium was not confirmed (herds C and L) decreased the $R_0$ for $S$. Typhimurium to 1.1 (95% CI 1.1–1.2). The $R_0$ estimates for $S$. Dublin from our study were in accordance with those obtained in young dairy calves [14]. $R_0$ was somewhat smaller than most $R_0$ values calculated in Xiao et al. [24] but theirs were based on an unknown transmission parameter.

The higher value of $R_0$ for $S$. Dublin relates to the smaller probability of minor outbreaks, with only few infected animals and thus often unnoticed, and to larger outbreaks and more likely persistence. Indeed, studies confirm that $S$. Dublin outbreaks show more clinical signs and mortality than $S$. Typhimurium outbreaks [19], and result in persistence more often [6,23]. The long persistence of both types in our study may be due to selection bias, given that farmers contacted the AHS about clinical signs and thus these herds may have been the severe clinical cases. The estimated proportions of infectious animals in the endemic equilibrium are 6% for $S$. Dublin and 2% for $S$. Typhimurium, which are figures also found in other studies [10, 11]. For example, Hoorfar et al. [10] found that approximately 3% of the animals remained seropositive up to 17 months after outbreaks of $S$. Dublin and $S$. Typhimurium, which is an indication that those animals are carriers. About half of these animals in herds with a $S$. Dublin outbreak were also culture positive at slaughter. None of the seropositive animals in $S$. Typhimurium herds were culture positive at slaughter. Thus, the proportion of infectious animals (the carriers) in the study of Hoorfar et al. [10] was between 1.5 and 3% for $S$. Dublin and between 0 and 3% for $S$. Typhimurium. In our study herds only a few active carriers were detected (Tab. I). However, the definition of an active carrier in our study was very conservative being a culture positive sample in 3 consecutive samplings with at least 2-weeks intervals.

These low prevalences indicate an important role for carriers in keeping the infection endemic or re-infections through survival of *Salmonella* in the environment [23].

The $R_0$ estimates were not sensitive for changes in the latent, infectious and seropositive periods. Increased latent and infectious periods would slightly increase $R_0$ and an increased seropositive period would slightly decrease $R_0$. Few studies were available to aid in defining the infectious periods and recovery rates, one study was based on clinical experiments, but confirmed the time of infectiousness in our study [15].

It must be expected that the individual variation of infectiousness is large, with most animals shedding for only 1 or 2 days, and some (intermittently) for years or even for life [4, 16, 21, 23]. This will not affect the $R_0$ estimate, which makes only use of the average of 10 days, but it does affect persistence, since carriers make persistence in a herd more likely. Otherwise, stochastic variations would more often result in quick extinction at such low prevalences. Stochastic simulation models can cope with individual differences between animals but these complex models need detailed data on animal-level. For *Salmonella* control, the high variability implies that a culling strategy to decrease the average infectious period (and thus $R_0$) should aim at the carriers. In addition, hygienic measures can reduce the risk of transmission from the environment [5, 20, 23].

The results were obtained from a small number of herds, 2 with *S*. Dublin and 6 with *S*. Typhimurium and the variation between herds was large. The mean percentage of clinical signs in the study herds was more than two times higher than that in an earlier study with *S*. Typhimurium [19]. Nielsen et al. [12] describe that herds with the highest risk of carrier development were herds with clinical disease outbreaks. Thus, the results from our study may not be applicable for all dairy farms with a *Salmonella* outbreak but are more likely to be valid for dairy herds that experience an outbreak with severe clinical signs in which carriers develop that will shed *Salmonella* for longer periods.

Even though the herds in the study experienced clinical outbreaks there were clear differences in the final prevalences in the herds. The differences between herds may be a result of the number of carriers in the herd. In general, both active and passive shedders are distinguished, active shedders being animals that continuously shed *Salmonella* and passive shedders that are transiently infected and intermittently shed the bacteria [12, 21]. Shedders are hard to detect because a low sensitivity of the test procedures [8, 11, 13]. Very few active carriers were detected in the study herds and those herds did not have a higher prevalence (Tab. I). Thus, some carriers may have been missed. In addition, the variation in prevalence may be a result of the resistance of the animals in a herd. Earlier studies found that herd management and co-infections with liver-fluke and BVDV are risk factors for severe clinical outbreaks [5, 17, 22].

An assumption in our model was that there is random contact between the animals in a herd (homogeneous mixing), which in reality may not be completely true. Calves and heifers are separated from the cows in different degrees; in different pens in the same barn or in separate barns, which may influence the transmission. The herds in our study had a representative management for Dutch dairy herds in that calves were housed in the barn with the cows and the adult cows are kept in one group. The only exception was *S*. Dublin herd Z, in which a group of pregnant heifers was brought in from a distant field between the 9th and 10th visit, causing a slight increase in the prevalence at the 10th visit.
$R_0$ predicts how many animals in a herd will be infectious and seropositive in the endemic equilibrium [1]. Based on our estimates of 2.5 and 1.3 for S. Dublin and S. Typhimurium respectively, the infection will not be highly prevalent in dairy herds under management systems typical for the Netherlands and many other countries.

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REFERENCES


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