Modelling the effect of risk mitigation strategies on cattle exposure to *Salmonella* in pasture after application of organic fertiliser

Elving, Josefine[1*], Nyberg Karin[1], Ann Albihn[1], Ottoson Jakob[1,2]

(1) *Department of Chemistry, Environment and Feed Hygiene, National Veterinary Institute (SVA), SE-789 51 Uppsala, Sweden*

(2) *Swedish University of Agricultural Sciences (SLU), Biomedical Sciences and Veterinary Public Health, Box 7028, 75007 Uppsala, SE*

*Corresponding author: josefine.elving@sva.se

Abstract

Exposure to *Salmonella* in cattle grazing land after application of manure was quantified and the relative effect of three risk mitigation strategies were evaluated; sanitisation treatment, application method and holding time. By a quantitative risk assessment approach the dose calculation was used to estimate *Salmonella* exposure on the first day of grazing. The model predicted that land application of solid manure and manure slurry would greatly benefit from adding a sanitisation treatment before land application. A holding time between land application and grazing gradually reduces the risk of *Salmonella* ingestion to the same level as for treated material, but increases the risk of vector-borne pathogen transmission and run-off.

Introduction

In Sweden relatively few cattle farms are infected by *Salmonella* each year [1]. This can be attributed to the Swedish salmonella control programme, which aims to prevent *Salmonella* in any part of the production chain. In addition, the relatively low number of reported human cases in Sweden (approximately 600 per year), not counting cases of salmonellosis contracted abroad, is also thought to be a result of the good *Salmonella* status among food producing animals. Thus maintaining good *Salmonella* status among domestic animals is an important measure for continued low incidence also in the human population. If manure is not treated to reduce the content of pathogenic organisms prior to land application, there is a risk of pathogen transmission to animals and humans [2]. Introduction of *Salmonella* infections into herds can be both costly and time-consuming for the farmer in terms of *e.g.* lost profits and cleaning and disinfection of animal houses. Due to their dentition grazing cattle will consume soil together with grass as a result of soil attaching to the roots [3]. Thus, if *Salmonella* is present in the soil of a pasture, there is a risk that grazing animals will ingest pathogens [4, 5].

The aims of this study were to evaluate the relative risk of *Salmonella* ingestion by grazing cattle and to compare the effect of some common risk mitigation strategies; holding time, application method and sanitisation treatments. Quantitative microbial risk assessment (QMRA) enables prediction of the consequences of microbial exposure by mathematical modelling rather than actual experiments. Measured data and data from the literature were used in a QMRA approach, with the objective of providing a basis for recommendations on risk mitigation strategies.

Material and Methods

The model was designed to estimate the exposure of grazing cattle to *Salmonella* originating from manure fertilised pasture. Exposure was modelled from the prevalence and reduction of *Salmonella* from pathogen source to pasture, comparing different mitigation strategies (Figure 1). Data from studies conducted in Sweden, or conditions similar to those in Sweden, were preferred to studies conducted elsewhere, since the primary use of the model was to study the risk under Swedish conditions. Studies with data presented showing the variation were preferred to studies presenting data as point estimates. In total, 64 scenarios were analysed.

Hazard characterisation and exposure assessment

The end-point of the QMRA was the exposure dose of viable *Salmonella* bacteria, which was determined on the first day of grazing. It was assumed that equal amounts of organic fertiliser were ingested irrespective of application method.

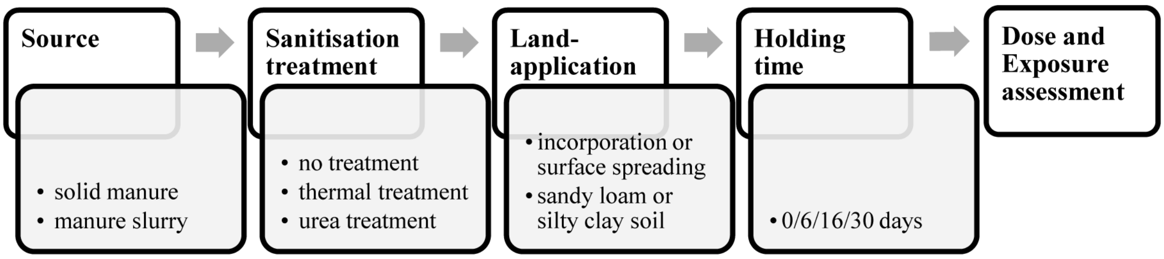

Figure 1. Schematic overview of the model modules

A literature search was conducted to find appropriate values for each of the model parameters. Manure and manure slurry was assumed to be stored for one year, during which filling of the manure pit and addition to the manure heap occurred in a semi-continuous way with material containing the same level of *Salmonella* throughout the whole year. The concentration of *Salmonella* after storage served as input for the treatment module. Spreading of solid manure and manure slurry was calculated in accordance with Swedish regulations (SJVFS 2011:25) and thereby limited to a volume corresponding to maximum 22 kg phosphorus ha^{-1} year^{-1} over a five-year period. The model incorporated two application methods, surface application with and without incorporation into the upper 10 cm of soil, and two soil types, sandy loam and silty clay. Descriptions of distributions and constant parameters for the model are presented in Table 1.

Table 1. Distribution and constant parameters used in the exposure assessment.

Module	Distributions and parameters	Reference(s)
Source		
Shedding of *Salmonella* in cattle (CFU/g)	Uniform: min = 20, max = 50,000	[6]
Within-herd prevalence	Uniform: min = 3.3%, max = 53.1%	[7]
Salmonella prevalence in dairy herds	Beta: α_1 = 40, α_2 = 1029	[8]
Dilution of manure in manure pit	2.8-fold	[9]
Reduction during storage		
- solid manure (log10/year)	Normal: μ = -1.68, σ =0.02	[10]
- slurry (log10/year)	Normal: μ = -1.78, σ = 0.03	
Sanitisation treatment		
Inactivation rate (based on upper 95% confidence interval limit) during:		
- thermal treatment, 50.5°C ($\log_{10}$ CFU/h)	Normal: μ = 0.56, σ = 0.03	Unpublished
- urea treatment, 14°C, 2% urea ($\log_{10}$ CFU/day)	Normal: μ = 0.49, σ = 0.03	[11]
Land-application		
Phosphorus content: solid manure/slurry (kg/ton)	1.5/0.6	[9]
Bulk density of soil (ton/m^3)	1.44	
Dilution ratio soil:fertiliser in solid manure/slurry	98/39-fold	
Holding time		
Reduction after incorporation (log10)	Normal:	
- sandy loam	day 6; μ = 0.74, σ = 0.33	[12]
	day 16; μ = 1.63, σ = 0.57	
	day 30; μ = 2.94, σ = 0.1 4	
- silty clay	day 6; μ = 1.27, σ = 0.54	
	day 16; μ = 2.42, σ = 0.37	
	day 30; μ = 3.59, σ = 0.67	
Reduction ratio surface/incorporated	1.95	[13]
Ingestion dose		
Dietary intake (kg dm/cow day)	13.7	[14]
Moisture content of sandy loam/silty clay soil (%)	8.8/15.6	[12]
Amount soil ingested/kg dm intake (%)	2.25	[3]

Risk modelling and data analysis

The model was constructed using Microsoft Excel with the @Risk add-in software (version 5.7, Palisade Corporation, NY, USA). A Monte Carlo simulation of the model sampling was performed with 10,000 Latin hypercube iterations. The comparison of the different risk mitigation strategies was performed in relative terms, thereby decreasing the influence of variation (variability and uncertainty). To identify the most important predictive factors in the distribution inputs, *i.e.* the parameters that correlated most with the final dose estimate, the Spearman correlation coefficient in @Risk was used.

Results

The estimated mean number of *Salmonella* in untreated but stored solid manure and manure slurry was 5.4×10^3 and 1.6×10^3 kg^{-1}, resulting in a viable *Salmonella* cell soil load of 55 and 40 kg^{-1}, respectively. Sanitisation treatment of solid manure and manure slurry decreased the *Salmonella* load in soil to 1.6×10^{-4} and 1.2×10^{-4} kg^{-1}, respectively. The amount of viable *Salmonella* was further reduced by the inclusion of a holding time between land application and grazing. In terms of pathogen exposure in cattle, land application of untreated materials increased the *Salmonella* dose by 10^5-fold compared with sanitised materials and by at least 10^{11}-fold compared with the scenarios including both a sanitisation treatment and a holding period of 30 days. There was also a difference in the dose estimate between the different materials. Application of untreated manure increased exposure by 1.37-1.43-fold compared with untreated manure slurry. Rank correlation revealed that the predictor with the overall greatest importance for the dose estimates was variation in *Salmonella* reduction during the holding time, when this was included in the scenario, followed by variations in initial density of *Salmonella* in the material and the effect of the sanitisation treatment.

Conclusion and perspectives

The main objective of this study was to compare the effect of mitigation strategies on the risk of *Salmonella* ingestion by grazing cattle. By presenting the relative risk of several scenarios, a model that is easy to communicate to risk managers can be obtained, and it is also possible to decrease the influence of variation (variability and uncertainty).

Since the present study relies on a review of literature data it is important to take into consideration the limitations in available data when interpreting the outcome of the study. Literature reporting levels of *Salmonella* in manure in Sweden is limited. The uniform distributions, used here to reflect the lack of knowledge concerning level of *Salmonella* at source, is usually not a good reflection of the perceived uncertainty of a parameter, since all values within the range have the same probability density, which changes to zero at minimum and maximum values. Additional data would allow the model to be refined, and hence also the estimated loads and doses. The *Salmonella* strains used in studies of thermal and urea treatment are both thought to exhibit higher tolerance to the respective treatment than other strains [11, 15]. Thus, it was assumed that treating the materials in accordance with the recommendations on temperature or urea addition during the time period required for the respective treatment would achieve at least a 5 $\log_{10}$ reduction, irrespective of which *Salmonella* strains are present in the materials. Also worth considering is that the data used in the treatment module do not take into account any risk of recontamination or regrowth in treated material. Furthermore, storage times before land application can vary substantially, as can the level of *Salmonella* in the material added to the manure heap and manure pit over one year.

The Spearman rank correlation analysis revealed that the parameter most influencing the exposure dose was bacterial reduction in soil after land application. Reduction of microorganisms in the soil environment is a complex process and depends on several parameters such as soil type, competing microbiota, moisture and UV-radiation [16]. Hence, the variation in the input parameter is to a large extent due to variability in inactivation rates and additional studies and data sets may not necessarily decrease the variation. This is in contrast to the influence of the source parameter on exposure dose. Due to the large uncertainty in the actual prevalence of *Salmonella* at between-herd and within-herd level, as well as concerning shedding of the pathogen from infected animals, increased knowledge concerning these data points can help in reducing the uncertainty.

It is evident that sanitisation treatment, either heat or urea, greatly reduces the *Salmonella* load to soil. In terms of *Salmonella* reduction, surface spreading of contaminated materials is preferable, since it leads to greater reduction by exposure to UV-light and desiccation. However, incorporation of manure into soil will reduce nitrogen losses and the risk of *Salmonella* transmission in the environment by vector animals and surface run-off to water recipients [17-19]. Risk mitigation strategies applied after land application, such as holding time, will further reduce the risk of *Salmonella* ingestion. At present, fertilisation of grazing land with organic fertilisers is not recommended in Sweden due to a strict regime to prevent disease transmission. Looking into the regulations of *e.g.* the UK, a holding time of 2 months is required between fertilisation and letting animals graze the land [20]. Based on the present approach, the risk of *Salmonella* ingestion would most likely be limited at that point, irrespective of soil type and treatment before land application. When setting restrictions on grazing, the immune status of the animals should also be taken into consideration. For example, prolonged holding periods could be used for young calves that are more susceptible to infection, with the possibility of shorter holding times for animals with good immune status.

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