



A questionnaire study of associations between potential risk factors and salmonella status in Swedish dairy herds



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ARTICLE INFO

Article history:

Received 14 November 2016

Received in revised form 22 March 2017

Accepted 6 May 2017

Keywords:

Dairy

Cattle

Salmonella

Risk factor

Questionnaire

Additive bayesian network

ABSTRACT

In this study associations between potential risk factors and salmonella status in Swedish dairy herds were investigated. A case-control study design was used, including existing as well as new cases. Herds were assigned a salmonella status on the basis of antibody analysis of bulk milk samples. Information on potential risk factors was collected from registry data and from farmers via a questionnaire. Univariable and multivariable logistic regression analyses were used to investigate associations between salmonella status and potential risk factors. In addition, multivariate analysis with Additive Bayesian Network (ABN) modelling was performed to improve understanding of the complex relationship between all the variables. Because of the difficulty in identifying associations between potential risk factors and infections with low prevalence and a large regional variation, exposure of potential risk factors in the high-prevalence region (Öland) were compared to exposure in other regions in Sweden.

In total 483 of 996 (48%) farmers responded to the questionnaire, 69 herds had test-positive bulk milk samples. The strongest association with salmonella status was 'presence of salmonella test-positive herds <5 km' (OR 4.3, 95% CI 2.0–9.4). Associations with salmonella status were also seen between 'feeding calves residue milk only' (OR 2.4, 95% CI 1.2–4.6), 'certified organic herds' (OR 2.5, 95% CI 1.2–4.9) and 'frequently seeing signs of rodents' (OR 0.4, 95% CI 0.13–0.97). The ABN model showed associations between Öland and four of the variables: salmonella status, presence of test-positive herds <5 km, shared pastures and providing protective clothing for visitors. The latter is probably a reflection of increased disease awareness in Öland. The ABN model showed associations between herd size and housing as well as several management procedures. This provides an explanation why herd size frequently has been identified as a risk factor for salmonella by other studies.

The study confirms the importance of local transmission routes for salmonella, but does not identify specific components in this local spread. Therefore, it supports the use of a broad biosecurity approach in the prevention of salmonella. In Öland, some potential risk factors are more common than in other parts of Sweden. Theoretically these could contribute to the spread of salmonella, but this was not confirmed in the present study. The study also highlights the difficulty in identifying associations between potential risk factors and infections with low prevalence and large regional variation.

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Abbreviations: NUTS, nomenclature des Unités Territoriales Statistiques; ABN, additive bayesian network; DAG, directed acyclic graph; SCC, somatic cell count; OR, odds ratio; CI, confidence interval; ODC%, percent optical density corrected.

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1. Introduction

Salmonella is an important zoonotic pathogen and therefore a target for surveillance and control in many countries. The Swedish salmonella control program has been in place since the 1960ies, it includes all serotypes and all animal species in the food chain from feed to table (Swedish Board of Agriculture, 2004). The program has resulted in very low prevalence of salmonella in poultry and pig production (National Veterinary Institute, 2014; EFSA, 2015),

and a recent study confirms that it is also low in dairy herds (Ågren et al., 2016). Swedish authorities have concluded that there is no need for further reduction of salmonella in Swedish animal production, as the number of human cases caused by Swedish animal products is very low (National Board of Health and Welfare, 2016; Wahlström et al., 2011). However, the importance of keeping the program cost efficient is emphasized in the same document, and the cost efficiency of the current control in cattle has been questioned.

To ensure that surveillance and control is efficient, knowledge about relevant risk factors is needed. A large number of studies have been performed concerning risk factors for salmonella in cattle herds (Nielsen, 2013; Belluco et al., 2015). However, many of the studies used registry data and most studies were performed in regions with considerably higher prevalence than in Sweden. Also, many of them are limited to a single serotype, mostly the cattle-adapted serotype *S. Dublin*, whereas the Swedish program encompasses all serotypes.

In Sweden, *S. Dublin* occurs primarily on an island in the south east (Öland). In this region, the prevalence of bulk milk positive dairy herds is 15%, as compared to 0–1.2% in all other counties (Ågren et al., 2016). Historical data indicate that *S. Dublin* has been endemic in this region for several decades (Lewerin et al., 2011). This raises questions whether there are management routines or herd conditions in this region that contribute to persistence and spread of *S. Dublin*.

The purpose of this study was to investigate associations between potential risk factors and salmonella infection in Swedish dairy herds, a low prevalence population with a high-prevalence region. Also, exposure to potential risk factors in the high-prevalence region (Öland) were compared to exposure in other regions in Sweden. The results of the study should provide a knowledge basis for government decisions on modifications of the control program, as well as advice to Swedish dairy farmers on how to reduce the risks for introduction and persistence of salmonella infection in their herds.

2. Materials and methods

In this study a case-control design was used, including existing as well as new cases. As described in detail below, herds were assigned a salmonella status on the basis of antibody analysis of bulk milk samples. Information on potential risk factors was collected from registry data and questionnaires distributed to farmers. Data were analysed by logistic regression and Additive Bayesian Network (ABN) modelling (Lewis et al., 2011; Lewis and Ward, 2013). All statistical analyses were performed in Stata Version 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP), except for the ABN modelling that was performed in R (R Development Core Team 2013; abn-package, 2014).

2.1. Study design and sample size

In April 2013, a national bulk milk screening was performed and the results were used for assigning salmonella status to herds in this study (Ågren et al., 2016). Individual test results and salmonella status were not disclosed to the farmers. All samples had been analysed with Prionics PrioCHECK[®] salmonella Ab bovine Dublin ELISA (including *S. Dublin* O-antigens 1, 9 and 12; hereafter referred to as the Dublin ELISA) and Prionics PrioCHECK[®] salmonella Ab bovine ELISA (including *S. Dublin* O-antigens 1, 9, 12 and *S. Typhimurium* O-antigens 1, 4, 5, 12; hereafter referred to as the Bovine ELISA) (Thermo Fischer Scientific, Massachusetts, US). Herds with a percent optical density corrected value (ODC%) ≥ 20 in at least one of the tests ($n = 141$) were considered likely to be infected with

salmonella and were classified as salmonella positive. The infection status of herds with a test result of 15–20 ODC% ($n = 60$) was considered ambiguous. Herds with a bulk milk result ≤ 5 ODC% in both tests ($n = 4016$) were considered likely not to be infected with salmonella and classified as negative. Questionnaires were sent to the owners of the 141 test-positive herds, all 60 herds with an ambiguous result, and 700 randomly selected herds in the negative group. In addition, questionnaires were sent to all test-negative herds in Öland to compare differences in routines in herds in Öland with those in the rest of Sweden. In other words, in addition to the randomly selected test-negative herds in Öland, the remaining 95 test-negative herds also received a questionnaire. Since the sensitivity is low for identifying infected herds with a single bulk milk sample (Veling et al., 2002; Warnick et al., 2006), the questionnaire asked farmers if they accepted collection of a second bulk milk sample six months after the screening, as repeated testing improves sensitivity (Warnick et al., 2006). Herds assigned a positive salmonella status based on the follow-up samples were moved to the test-positive group, if not already in this group. Herds previously assigned a positive salmonella status based on the screening were kept in the test-positive group, even if the follow-up samples were test-negative. Herds with ambiguous test-results were excluded before analyses.

2.2. Questionnaire

A questionnaire containing 57 closed questions, but with room for comments, was sent to the farmers ($n = 996$). The farmers were not informed of the salmonella status of their herd. The answers could be anonymous or supplied with the farm identifier. Therefore all questionnaires were coded, so that anonymous answers could be entered in the correct category, albeit not individually identifiable. The questions addressed general information about the farm and aspects of external as well as internal biosecurity. Previously reported risk factors for salmonella in cattle herds were covered by the questions. A pilot version of the questionnaire was tested on three dairy farmers and five veterinarians working with cattle consultations, research, and epidemiology. Their feedback was used to improve the questionnaire. The full questionnaire, translated into English, is provided as a supplementary file (Supplement 1). To increase the response rate, information about the study was published in a dairy journal (Växa Sverige, 2017) six weeks before sending out the questionnaires. In addition, all selected dairy farmers with a known e-mail address received an e-mail five days prior to the questionnaire, informing them about the study and questionnaire to come. To facilitate data collection, an e-mail with a link to the electronic questionnaire in Qestback (Qestback, 2017) was also sent to all selected farmers with a known e-mail address.

2.3. Data entry

The paper questionnaire replies were manually transferred to the online Qestback database by the first author and one additional person. All numerical data in all questionnaires were double checked by the first author. Data were extracted from Qestback and imported into Stata for data cleaning and analyses. Farms that had received both an electronic and a paper questionnaire were checked for duplicate responses. This was done by searching for duplicates of farm identifiers and, in the case of anonymous responders, by comparison of general information about the herds such as location and herd size.

2.4. Data management

The R package abn, only accepts variables with binary, Gaussian and Poisson distributions (Lewis and McCormick, 2012; abn-

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