Application of causal inference and mathematical modelling to control enzootic diseases in Japanese dairy farms

Yuri Fujimoto

Contents

Abbreviations

AI: artificial insemination

AMAA: agriculture mutual aid association

BLV: bovine leukemia virus

CI: confidence interval

EBL: enzootic bovine leukosis

FACN: Federation of Agricultural Cooperatives in Nemuro

NLBC: National Livestock Breeding Center

NMMCC: Nemuro *Mycoplasma* Mastitis Control Committee

OR: odds ratio

PL: persistent lymphocytosis

RGU: Rakuno Gakuen University

General introduction

Livestock infectious diseases in Hokkaido, Japan

Livestock infectious diseases are critical problems for livestock farmers due to the economic losses they cause. Farmers in Japan recently suffered from outbreaks of several livestock diseases. There were two outbreaks of foot-and-mouth in 2000 [72] and 2010 [58]. The outbreak of classical swine fever started from September 2018 [67] and the last farm case was reported in March 2020 as of June 2020. Highly pathogenic avian influenza was confirmed in Japan in 2004 [60] and outbreaks in poultry farms have been was confirmed in almost every winter since then. These diseases are in the list of monitored infectious diseases and national disease control programs with compensation at culling for the control are in place. However, diseases with milder symptoms are relatively neglected and there are no such national financial supports even a disease is in the list of notifiable diseases. Thus, to control such non-highly pathogenic diseases, livestock workers must concentrate their resources to an effective control method. Moreover, such endemic diseases are bearing huge economic losses every day, and development of tools facilitating voluntary disease control would stabilize livestock production greatly.

Hokkaido was the largest dairy area in Japan. In Hokkaido, 60.1% of dairy cows in the country are kept and 54.4% of milk is produced [47, 50]. Economic impact of Hokkaido in Japanese dairy industry is also high: the amount of production from dairy cattle including its milk was 502.6 billion yen in 2018 and it consists 53.8% of the national total amount [48]. Thus, disease in dairy cattle is the major concern in the livestock industry in Hokkaido. While Japan or Hokkaido does not offer official financial aid to control infectious diseases which is not monitored, local agricultural cooperatives and Self Prevention Promotion Associations at municipal levels provide financial aids to test or vaccinate several livestock diseases.

Epidemiology

Epidemiology is defined as the study of disease in populations and of factors that determine its occurrence [77]. Causal inference is a basic part of epidemiology because the discipline is based on the idea that “causes” (exposures) and “outcomes” (health events) are part of a complex web of relationships [8]. Though epidemiological studies rarely include laboratory experiments, knowledge from laboratory studies is necessary to conduct an epidemiological study to list factors in causation. Thus, epidemiology can connect results of laboratory works of a health issue to measure effects of potential causes on an outcome in a population. There are four types of epidemiological investigations: descriptive, analytical, theoretical and experimental epidemiology [77]. In this thesis, two infectious diseases of dairy cattle were investigated; one is by descriptive and analytical way and the other is by theoretical way.

A causal web is a way of conceptualizing how multiple factors combine to cause disease [8]. A causal-web diagram guides analysis and interpretation of data. In Chapter 1, causal webs were used to make a hypothesis and interpret the result.

Mathematical modelling is an approach which attempts to explain and predict patterns of disease occurrence and what is likely to happen if various alternative control strategies are adopted [77]. Models are classified into deterministic ones which fix the values of input parameters and stochastic ones which describe processes or events subject to random variation [77]. In Chapter 2, an individual-based stochastic model was constructed.

Thesis layout

In Chapter 1, herd-level and cow-level risk factors of *Mycoplasma* mastitis of an outbreak was analyzed by applying causal inference and univariable and multivariable analyses. *Mycoplasma* mastitis of cows is caused by bacteria in *Mycoplasma* spp. and refractory to antibiotic therapy. Economic loss is caused by the disease due to decreased milk production and decreased milk quality by increased somatic cell counts. There was an outbreak of *Mycoplasma* mastitis in Nemuro, Hokkaido, Japan from 2014 to 2015. Risk factors associated with the outbreak were investigated by drawing a causal diagram and using univariable and multivariable analyses.

In Chapter 2, an individual-based simulation model of spread of bovine leukemia virus (BLV) in a dairy herd was constructed. An individual-based model was a type of a model simulating an infectious disease. In the model, individuals are put into various groups by age, geographic location and so on, and then transmission between individuals is modeled as random events according to predefined random rules [6]. In the study, an individual-based model of BLV spread in a dairy farm was confirmed, parameters were estimated and change of prevalence was estimated using data from actual dairy herds.

In the general discussion, results of the previous chapters and further prospects are discussed. This thesis investigated risk factors by causal inference in Chapter 1 and made a simulation model in Chapter 2. Importance of the two epidemiological methods and potential of integrating these methods were discussed.

Chapter 1. A case-control study of herd- and cow-level risk factors associated with an outbreak of *Mycoplasma* mastitis in Nemuro, Japan

1.1 Introduction

Bovine mastitis caused by *Mycoplasma* spp. is a highly contagious disease and a major problem in the global dairy industry [59]. The economic impact of *Mycoplasma* mastitis is high because the disease is considered untreatable with antibiotics, and thus, culling of infected cows is commonly recommended for within-farm control. Several *Mycoplasma* species have been linked to bovine mastitis, with *Mycoplasma bovis* being the most important [17]. In addition to mastitis, *Mycoplasma* spp. also cause a variety of other diseases, including pneumonia, otitis media, and arthritis [42].

The primary route of *Mycoplasma* infection is udder-to-udder spread by milking equipment, hands, or teat dipping [41]. Calves can be infected by ingestion of contaminated colostrum or waste milk and through aerosols [13, 42]. Intra-uterine or intra-mammary transmission of *Mycoplasma* from a dam to a calf has also been reported [15, 65]. Contaminated semen is also a route of *Mycoplasma* infection [19]. Several risk factors for *Mycoplasma* mastitis have been reported as well. Commonly identified risk factors include large herd size and introduction of cattle [17]. Potential reasons larger herds have a higher risk of contracting mastitis are that they tend to have more introduced cattle and a higher chance of a rare infection event [14]. In addition, a higher frequency of *Mycoplasma* infection in winter has been reported [41].

Hokkaido is the largest dairy production area in Japan, producing more than half of the cow milk in the country [49]. Hokkaido is the northernmost prefecture in Japan and typically covered in deep snow in winter. *Mycoplasma* mastitis is a major dairy issue in Japan, with an estimated herd-level prevalence of 3.8% in the Tokachi area of Hokkaido [49, 76]. An increase in the occurrence of *Mycoplasma* mastitis cases was noted from 2014 to 2015 in the Nemuro area (the eastern part of Hokkaido) by the Nemuro *Mycoplasma* Mastitis Control Committee (NMMCC), which consists of local agricultural cooperatives, the Federation of

Agricultural Cooperatives in Nemuro (FACN), the Hokkaido Dairy Milk Recording and Testing Association, veterinarians in the Hokkaido Higashi agriculture mutual aid association (AMAA) and Nemuro Prefectural Livestock Hygiene Service Center, agriculture extension officers in the prefectural agriculture extension office, and the prefectural livestock research institute. Three to six times per year, the committee conducts PCR-based bulk tank milk screening tests [23] for *Mycoplasma* spp. for all of the member dairy farms of the agricultural cooperatives. Although possible risk factors have been reported in the literature, little is known regarding the relative importance of these factors in the Nemuro area.

In the present case-control study, we conducted separate analyses of both cow- and herd-level risk factors for *Mycoplasma* mastitis by (1) comparing infected and non-infected farms, and (2) investigating the records of cattle movement, milk testing, and clinical services regarding infected and non-infected cows at the infected farms.

1.2 Materials and methods

1.2.1 Study design

Case-control analyses at the farm and cow levels for bovine *Mycoplasma* mastitis were conducted using a structured questionnaire and investigation of the records of cattle movement, milk testing, and clinical services, following a participatory appraisal of potential risk factors for *Mycoplasma* mastitis by NMMCC members in March 2015. Four hypotheses regarding factors associated with *Mycoplasma* mastitis were discussed at the appraisal: (1) poor hygiene management facilitates infection of the udder; (2) heifers are infected with *Mycoplasma* at a communal ranch and carry the infection to the farm; (3) cows with a higher milk yield may be more easily infected; and (4) *Mycoplasma* may be harbored for a long period in cows affected by pneumonia, arthritis, or otitis media, until the onset of *Mycoplasma* mastitis (Figure 1).

The study was conducted in the Nemuro region, which is located on a large plain. The Nemuro region is an important dairy production area in eastern Hokkaido, with an average temperature of 6.3°C and typically heavy snow in winter. Some farms in this area send their heifers to communal ranches for grazing during summer. About 99% of Japanese dairy herds use artificial insemination or embryo transfer and use of bulls for breeding is rare.

In the farm-level analysis, case farms were defined as FACN member dairy farms in eastern Hokkaido with at least one cow diagnosed with *Mycoplasma* mastitis during the regular bulk milk screening test followed by a PCR test or with a clinical mastitis diagnosis by an AMAA veterinarian during the period between April 2014 and July 2015. Approximately 96% of the dairy farms in the area belong to the FACN, and the screening results are thus representative of the dairy farm population of the Nemuro region. In cases in which a bulk milk sample tests positive, milk samples from all lactating cows are sent by an AMAA veterinarian to a private company, Nihon Dobutsu Tokusyu Shindan, for PCR-based determination of *Mycoplasma* spp. and *Mycoplasma* species identification. Almost all milk samples from clinical mastitis cases tentatively identified as *Mycoplasma* mastitis are also sent by the AMAA veterinarian to the same private company.

Control farms were defined as follows. First, the case farms were categorized as small-, medium-, or large-scale according to the number of adult cows (<101, 101-200, or >201, respectively). Second, the number of small-, medium-, and large-scale case farms in the Agricultural Cooperative catchment areas within the Nemuro region that were covered by the NMMCC was determined. Third, twice the number of case farms in each respective size and catchment area category at which *Mycoplasma* mastitis had never been reported were randomly selected from a list of farms belonging to the FACN. Surveys using structured questionnaires and an investigation of cattle movement data were conducted among the selected case and control farms for the farm-level risk factor analysis. The first questionnaire asked about experience with *Mycoplasma* infection within 2 years in order to verify the eligibility of case and control farms.

The cow-level analysis was conducted only among case farms where the owners agreed to allow access to production and veterinary clinical records. Case cows were defined as those with a confirmed diagnosis of *Mycoplasma* mastitis based on the diagnostic results from the AMAA returned by the above-mentioned private company between April 2014 and July 2015, regarding both confirmation procedures following bulk milk screening and clinical services. In the clinical records of the AMAA, causal pathogens of mastitis are not recorded. Moreover, although AMAA veterinarians send samples to the company for *Mycoplasma* infection diagnosis for pneumonia, arthritis, and otitis media cases as well, the history of the diagnosed cows is not recorded in the diagnostic results returned by the company. Therefore, the *Mycoplasma* mastitis cows were defined as cows at case farms listed as *Mycoplasma* positive in the records returned by the private company, confirmed as lactating based on FACN records at the time of the tests, and without AMAA clinical records of pneumonia, arthritis, or otitis media in the 2 months before the tests. In Japan, all cattle are registered in the Individual

Identification Information System of Cattle of the National Livestock Breeding Center (NLBC). Cow identifications were matched in the records of the AMAA (clinical records and diagnoses returned by the private company) and FACN using the 10-digit cattle identification number. Control cows at the case farms were matched at a 1:3 ratio with case cows (three non-infected cows per infected cow). The matching criteria were presence at the same farm, parity, and days in milking after calving (difference of less than 30 days). Lists of cows by identification number at the case farms as of July 2016 were provided by the FACN. Case cows that were not matched with non-infected cows were excluded from the study. The cow-level risk factor analysis evaluated cattle movement, milk production, reproduction, and disease history factors using digitized records from different sources.

The study results are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology statement checklist for veterinary medicine (the STROBE-Vet statement) in accordance with recommendation of O'Connor, et al. [62].

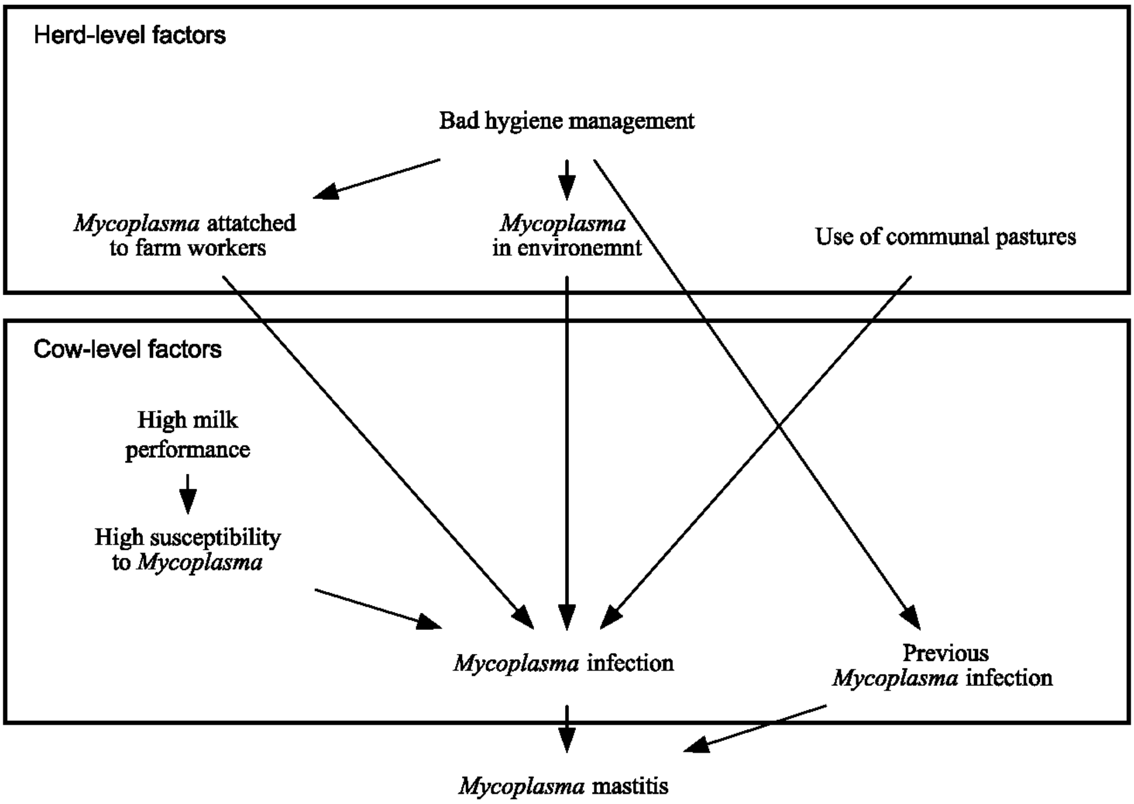

Figure 1. Putative causal web showing hypotheses of the study regarding the relationship between *Mycoplasma* mastitis and poor hygiene management, use of communal ranches, high milk production, and previous *Mycoplasma* infection.

1.2.2 Questionnaire surveys

Two questionnaire surveys were conducted for the farm-level analysis. The first questionnaire focused on hygiene management and introduction of infected cows and collected information relating to the farm, introduction and use of communal ranches for heifers, and hygiene management during the period between April 2014 and July 2015 (Table 1). The designed questionnaire was reviewed by a veterinary epidemiologist and the academic committee of the AMAA and pretested among a few dairy farmers in another region. Feedback was used to improve the questionnaire by adding and rephrasing questions and choices and improving design. The field survey was conducted between December 2015 and February 2016 via face-to-face interviews by staff members of Japan Agricultural Cooperatives. The questionnaire was explained to staff members at the meeting of the NMMCC in August 2015, prior to the field survey. The filled questionnaires were sent to Rakuno Gakuen University (RGU), where the responses were digitized.

The second survey was conducted among the respondents to the first survey in order to clarify the influence of regional *Mycoplasma* mastitis outbreaks in changing behaviors associated with hygiene management (Table 1). The designed questionnaire was reviewed by a veterinary epidemiologist, and face-to-face interviews were conducted in the same manner as with the first survey. The second questionnaire included several questions that were also included in the first questionnaire, but two answer columns were provided for case farms to indicate practices pre- and post-outbreak in order to minimize recall bias. The two questionnaires were written and conducted in the Japanese language and consisted primarily of closed or semi-closed questions.

Table 1. List of collected data and associated content

Category	Content
Herd-level analysis	
Questionnaires	
Farm information	Type of farm (2); number of workers; year the farm was opened; year the farm owner started milking; number of cows (5)
Experience with *Mycoplasma* infection	Number of cows infected with *Mycoplasma* in the outbreak; previous experience with *Mycoplasma* infection (2); knowledge of frequent occurrence of *Mycoplasma* mastitis in the neighborhood; changed hygiene management after the outbreak
Knowledge about *Mycoplasma*	Have ever heard the name; know that *Mycoplasma* causes diseases in calves; know that *Mycoplasma* can be transmitted from a dam to a calf by human hands; considered the possibility of *Mycoplasma* infection in a case of clinical mastitis when no *Mycoplasma* was isolated
Disease prevention	Vaccination against respiratory diseases (3); prevention of wild animal intrusion (3); disinfection of vehicles; management of the sanitation control zone (3); use of disinfectant foot baths (4); use of hydrated lime powder at farm entrance; hygiene management control of vehicles (3)
Milking hygiene	Available milking equipment (3); teat wiping (5); pre-dipping; post-dipping; use of a cart; use of a strip cup; actively called a veterinarian when abnormality was found (2); disinfection of milking equipment (3); order of milking (2); practice mastitis testing (3); disinfection of milking unit after the first calving of heifers
Calf handling	Timing when a calf is separated from its dam; period keeping a calf and dam together; method of feeding colostrum (4); method of feeding milk (4); period of feeding milk to a calf (8); same worker takes care of calves and milking cows; timing of taking care of calves; change gloves and cloths (2)
Communal pastures	Experience of using a communal pasture; type of cows sent to a communal pasture; owner of the communal pasture; type of communal pasture
Introduction	Experience with introduction; type of introduced cows; frequency of introduction (4); number of introduced cows (4); source of introduction (2); mastitis testing of introduced cows (2); quarantine of introduced cows (2); health check of introduced cows

Table 1. (continued)

Category	Content
Barns	Type of housing (4); volume of bedding (3); type of bedding (3); use of hydrated lime powder; frequency of changing bedding (6); frequency of removing manure (3); regular disinfection of barns (3); frequency of barn disinfection (3); type of disinfectant used for barn disinfection (3); type of water supply equipment (3); frequency of cleaning water supply equipment (3); use of machinery ventilation (3)
Hygiene management in winter of 2015	Remember the condition of hygiene management in the winter; change in frequency of ventilation in the winter (4); change in frequency of removing manure in the winter (4); change in frequency of changing bedding in the winter (4)
Movement records	Number of cows in a herd; experience with movement (2); proportion of moved cows (2); experience with introduction (3); proportion of introduced cows (3); experience with using communal pastures; proportion of cows sent to communal pastures; having cows that had been at a livestock market; proportion of cows that had been at a livestock market; number of movements (4); age at movements (4)
Cattle-level analysis	
Movement records	Experience with movement (2); number of movements; age at movements (3); experience with introduction; source of introduction; experience with having been in a livestock market; experience with having been in a communal pasture
Dairy herd test records	Milk yield (4); adjusted milk yield (2); expected milk yield for the next 12 months; fat concentration (3); non-fat milk solids concentration (3); protein concentration (3); milk urea nitrogen concentration; somatic cell count in milk; linear score (2); days in milking; pregnancy status; calving interval; number and timing of artificial insemination (3); details of the last delivery (3); body weight; cow age; amount of concentrates fed
Clinical records	Disease histories: pneumonia (2); peracute mastitis (2); acute mastitis (2); chronic mastitis (2); subclinical mastitis (2); mastitis in dry period (2); mastitis in heifers (2); otitis media; arthritis; *Mycoplasma* infection of other types (3)

Numbers in parentheses indicate the number of questions related to that content. Absence of a number in parentheses indicates that there was only one question for that content item.

1.2.3 Data collection for the farm-level analysis

To enhance the quality of quantitative data regarding movement of cattle for introduction to the farms, including the return of heifers from short-term stays at communal ranches, cattle movement records were obtained from the Search Service of the NLBC Individual Identification Information System of Cattle, collating the identification numbers of cows owned by the case and control farms as of July 2016 provided by the FACN. For the farm-level analysis, movement records dated prior to April 2014 were used. The places where cows had been located were divided into four categories (farm, market, communal ranch, and other) based on place names, interviews with an AMAA veterinarian, and web search results.

1.2.4 Data collection for the cow-level analysis

The cow-level analysis focused on the potential spread of mastitis due to introduction of infected cows, higher susceptibility of cows with a higher milk yield, and disease caused by *Mycoplasma* harbored in the body since a previous infection. Therefore, three types of records were collected: movement, dairy herd testing, and veterinary clinical records.

Based on the expert opinions of the authors, infection of udders with *Mycoplasma* was assumed to have occurred 2 months before the onset or detection of *Mycoplasma* spp. in the diagnostic tests. Therefore, for the case cows, NLBC movement records and AMAA clinical diagnostic records for the associated disease categories involving *Mycoplasma* infection (Table 1) earlier than 2 months prior to the *Mycoplasma* mastitis diagnosis were collected. The milk production and reproduction records (Table 1) for the month that was 2 months prior to the *Mycoplasma* mastitis diagnosis were collected from the FACN. For the control cows, movement, clinical, milk production, and reproduction records for the same months used for the matched case cows were collected. For statistical analysis, days in milking were categorized into four groups: <80, 80-159, 160-240, and >240. Farms were not blinded to the analyst during the study.

1.2.5 Statistical analysis

1.2.5.1 Descriptive epidemiology

The dates of occurrence used for descriptive epidemiology were collected via the first questionnaire. Farms which did not remember the incident dates were removed from the analysis. Information regarding isolated species was based on the results of the laboratory tests. If no laboratory test results were obtained, species designated in the first questionnaire were used. Data regarding monthly amount of snowfall during the study period were obtained from the database of the Japan Meteorological Agency in order to examine the effect of snowfall on *Mycoplasma* mastitis incidence. Temporal associations between *Mycoplasma* mastitis occurrence and snowfall were analyzed using the Spearman correlation test [74].

1.2.5.2 Risk factor analysis

Univariable analyses of herd-level *Mycoplasma* mastitis occurrence were conducted for the items in the two questionnaire survey results and movement records. For the questions asked in both surveys, if the responses in the second survey for pre- and post-outbreak were contradictory, answers for pre-outbreak were used for the analyses; otherwise, answers in the first survey were used. Variables were excluded from the analyses when fewer than half of the farms responded to the items or if the responses were logically invalid (e.g., age of calves sent to a communal ranch was excluded if the farm did not use communal ranches). Categorical questions allowing multiple responses were treated as binomial variables for each choice. New categories were created when more than four farms provided the same content in answers to the free descriptive questions. Some questions were grouped into one on the basis of context.

Categorical variables were examined using Fisher's exact test when more than 20% of a contingency table had an expected value of less than 5; otherwise, the variables were examined using the Pearson's chi-squared test. For binomial variables, the odds ratio (OR) and 95% confidence interval (CI) were calculated. Numerical variables were examined using the Wilcoxon rank sum test.

Herd-level multivariable analyses were conducted for variables exhibiting a *p*-value of less than 0.2 in the univariable analyses. Several variables collected had the same meaning, and in such cases, only the variable that most represented the intended context was selected for the analysis. Variables that could not be included in a causal web were removed. As a large number of variables exhibited a *p*-value of less than 0.2, they were divided into groups based on meaning. Multivariable sub-models were prepared for every group of variables and analyzed using a generalized linear model with binomial error structure and a logit link function, with variables in the group serving as explanatory variables and *Mycoplasma* infection status serving as the response variable using only herds for which complete information regarding the variables was available. Herd size was forced into every model to control for confounding. No interaction terms were included in the models. Final models were selected by both-side stepwise regression according to Akaike's information criterion. Variables exhibiting very large standard errors were removed from the resulting models. The variables selected in the sub-models were integrated into one model, and further model selection was conducted in the same manner. Step-by-step simplification of the integrated model was conducted by comparing models with and without the variable for which the *p*-value was the highest using the likelihood ratio chi-squared test; if the *p*-value was ≥ 0.05, the variable was removed from the model. This step was repeated until the *p*-values for all explanatory variables were <0.05.

Cow-level univariable analyses were conducted for the items in the dairy herd test records, clinical records, and movement records. Variables in which less than half of the records were valid were removed from the analysis. Invalid records included variables such as data for "age of the first movement" for a cow that had never been moved from the home farm.

Categorical variables were examined using Fisher's exact test when more than 20% of a contingency table had an expected value of less than five; otherwise, the variables were examined using the Pearson's chi-squared test. For binomial variables, the OR and 95% CI were calculated. Numerical variables were examined using the Wilcoxon rank sum test. Unconditional tests, not conditional ones, were used to screen the variables with an assumption that variables related with the outcome should have a p-value <0.2 even by unconditional tests.

Cow-level multivariable analyses were conducted for variables exhibiting a *p*-value of less than 0.2 in the univariable analyses. Similar to the herd-level analysis, the most representative variable was selected when multiple variables had the same meaning, and non-related variables in a causal web were excluded from the analysis. No interaction terms were included in the models. Conditional logistic regression with binomial error structure and a logit link function was conducted using these variables as explanatory variables, tuples of an infected cow and non-infected cows as strata, and *Mycoplasma* infection status as the response variable only for cows with complete information regarding the variables. Variables with a very large standard error were removed from the resulting model.

A theoretical causal web was drawn based on the results of the multivariable analyses to illustrate possible relationships between variables. Data were input using Microsoft Excel 2010 and Microsoft Access 2010. All statistical analyses, including random sampling, were performed using R, version 3.5.2 [69]. In addition to those mentioned above, the following R packages were used in the study: dplyr [89], glue [22], foreign [68], lubridate [18], readr [88], readxl [87], and stringr [86] for data handling; DiagrammeR [24], ggplot2 [85], and ggpubr [28] to create graphs; broom [71] and vcd [44] for general statistical analyses.

1.2.6 Ethical approval

This study was conducted at the request of the NMMCC, and ethical concerns regarding access to production and veterinary clinical information were considered by the NMMCC, FACN, and AMAA. AMAA veterinary clinical data were provided to the NMMCC upon written consent from dairy farmers, based on the "Minutes of provision of *Mycoplasma* mastitis investigation data" between the NMMCC and AMAA, which took effect on September 6, 2015. Dairy herd test records were provided to RGU based on the "Minutes of protection of the information assets associated with the collaborative research on the investigation into the cause of *Mycoplasma* mastitis in Nemuro region" between the FACN and RGU, which took effect on December 5, 2016. Consent forms regarding the questionnaire surveys, data collection, and analysis were explained to the farmers in face-to-face interviews with the help of AMAA veterinarians and Japan Agricultural Cooperatives staff. Information was collected and analyzed only for farms that provided signed consent.

1.3 Results

1.3.1 Response rates and data availability

In the herd-level analysis, all 40 infected farms (which belonged to the FACN during the study period) and 73 non-infected farms were selected. In the first questionnaire survey, 37 of the 40 infected farms (92.5%) and 70 of the 73 non-infected farms (95.9%) responded and agreed to participate in the study. In the second questionnaire survey, which was conducted for the participants of the first survey, 25 of the 37 infected farms (67.6%) and 47 of the 70 non-infected farms (67.1%) responded. Movement records for the herd-level analysis were available for 37 infected farms and 67 non-infected farms. The movement records of the remaining 3 non-infected farms could not be obtained.

For the cow-level analysis, 18 infected farms agreed to the use of their cow records in the study. After matching infected and non-infected cows, clinical records, dairy herd test records, and movement records of 42 infected and 107 non-infected cows at 6 infected farms were used for the cow-level analysis. In the 6 farms, 1-19 infected cows (median: 5) were selected out of 118-400 cows (median: 202) in the farms. Of 18 infected farms that agreed to the use of their cow records, 12 farms were removed for the following reasons: no enrollment in the dairy herd testing program (1 farm), non-identification of infected cows (7 farms), no records available for 2 months before the infection occurred (3 farms), and an infected cow being in a dry period 2 months before the infection occurred (1 farm).

1.3.2 Descriptive epidemiology

The mean and median number of infected cows per infected farm were 5.9 and 3.5, respectively (n = 28 farms). Figure 2 shows the temporal relationship between the occurrence of *Mycoplasma* mastitis by species (n = 31 farms) and snowfall. The most frequently isolated species was *M. bovis* (71.0%, 22/31 farms), followed by *M. californicum* (12.9%, four farms), and *M. bovigenitalium* and *M. canadence* (3.2%, one farm each). There was no significant relationship between *Mycoplasma* occurrence and snowfall (Spearman's rank correlation coefficient: 0.214, $p = 0.214$).

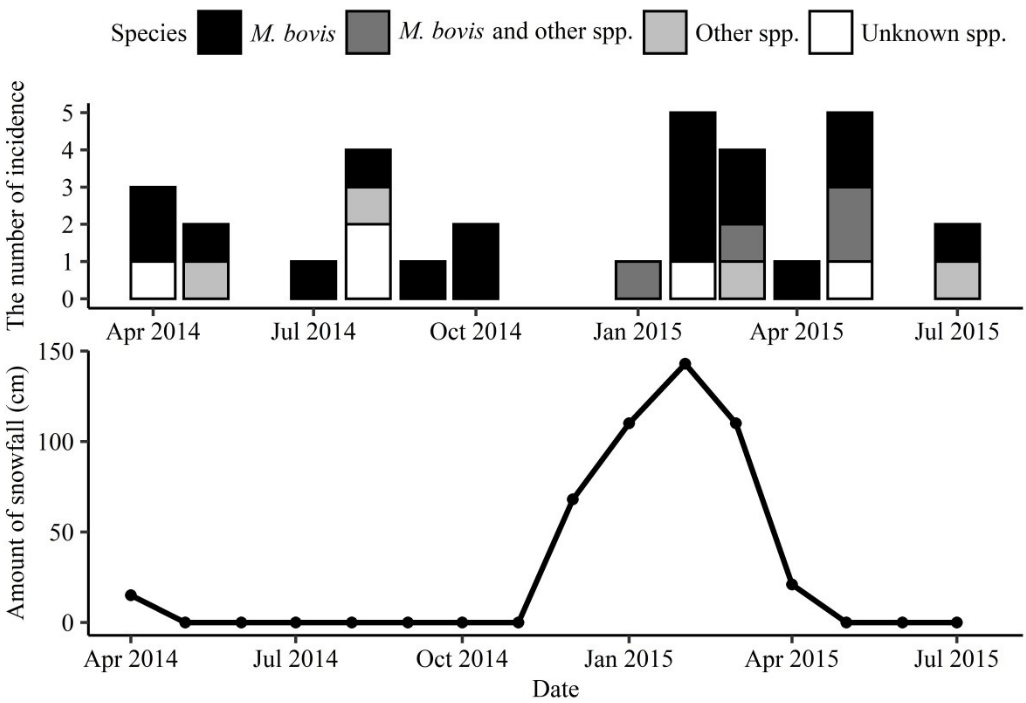

Figure 2. Isolated *Mycoplasma* species and the amount of snowfall during the outbreak. y-axes represent the number of farms at which *Mycoplasma* mastitis was detected in that month based on dates of incident obtained by the first questionnaire (upper) and the total amount of snowfall in Nemuro area in that month (lower).

1.3.3 Herd-level risk factor analysis

In the herd-level univariable analyses, 138 variables from the first questionnaire survey and 18 variables from the second survey for which the answered rate was over 50%, and 23 variables from the movement records for which more than 50% of all farms contained valid values, were analyzed. Table 2 shows the results for variables exhibiting a *p*-value of less than 0.05 in the herd-level univariable analyses using the responses from 37 infected and 70 non-infected dairy farms. In 25 infected farms which answered the second survey and 20 questions for which answer columns were divided to pre- and post-outbreak situation, 8 farms answered differently between pre- and post-outbreak for at least one question (min: 1, median: 2.5, max: 6) and 10 questions were answered differently by at least one farm. The most different questions were “actively call veterinarians when an abnormality was found by PL test” and “disinfect milking equipment before milk a next cow” to which five farms answered differently pre- and post-outbreak.

Variables that exhibited a *p*-value of less than 0.2 in the univariable analyses were selected and grouped into multivariable sub-models based on meaning (Supplemental Table S1). The final herd-level multivariable model included one risk factor: history of introduction of cows, and three preventive factors: tie stall barn for milking cows, consciously wipe teat openings before milking, and use a paper towel after a cloth towel to wipe teats (Table 3).

Table 2. Herd-level univariable risk factor analysis results for *Mycoplasma* mastitis occurrence ($p < 0.05$)

Variable	Infected (%)	Non-infected (%)	Odds ratio (95% CI)	*p*-value
1. Questionnaires				
Mean number of cows				
Milking cows	90.0 (n = 37)	68.5 (n = 70)		0.036
Calves	20.0 (n = 37)	10.0 (n = 70)		0.031
Housing for milking cows				
Tie stall	12/37 (32.4%)	46/70 (65.7%)	0.3 (0.1–0.6)	0.002
Free stall	24/37 (64.9%)	27/70 (38.6%)	2.9 (1.3–6.7)	0.017
Free barn	1/37 (2.7%)	0/70 (0.0%)	5.8 (0.2–145.8)	0.346
Rangeland	2/37 (5.4%)	5/70 (7.1%)	0.7 (0.1–4.0)	1.000
Mycoplasma *infection in milking*				
Consciously wipe teat openings before milking	30/37 (81.1%)	67/70 (95.7%)	0.2 (0.0–0.8)	0.030
Disinfect milking equipment before milk the next cow	16/37 (43.2%)	6/70 (8.6%)	8.1 (2.8–23.5)	0.000
Mycoplasma *transmission from calves to a cow*				
Know that *Mycoplasma* transmits from a calf to a mother cow by human hands	30/37 (81.1%)	37/67 (55.2%)	3.5 (1.3–9.0)	0.015
Use machinery ventilation in the calf barn	14/32 (43.8%)	12/62 (19.4%)	3.2 (1.3–8.3)	0.024
Timing when a calf is separated from its mother cow after a delivery				
Immediately after the delivery	16/23 (69.6%)	17/47 (36.2%)	4.0 (1.4–11.7)	0.018
When realized the delivery finished	8/23 (34.8%)	28/47 (59.6%)	0.4 (0.1–1.0)	0.090
Keep them together for a while	1/23 (4.3%)	4/47 (8.5%)	0.5 (0.1–4.6)	1.000

Table 2. (continued)

Variable	Infected (%)	Non-infected (%)	Odds ratio (95% CI)	*p*-value
Introduction of* Mycoplasma *to a farm				
Occurrence of *Mycoplasma* mastitis within two years in group farms	4/28 (14.3%)	0/55 (0.0%)	20.4 (1.1–393.5)	0.011
Use communal pastures				0.034
Using more than several years	8/37 (21.6%)	32/69 (46.4%)		
Started to use in this year	2/37 (5.4%)	1/69 (1.4%)		
Have been used before	2/37 (5.4%)	5/69 (7.2%)		
Never used	25/37 (67.6%)	31/69 (44.9%)		
Raised awareness by the occurrence of* Mycoplasma *mastitis				
Changed hygiene management after *Mycoplasma* mastitis in the farm or in the neighborhood	17/37 (45.9%)	14/66 (21.2%)	3.2 (1.3–7.6)	0.016
Considered the possibility of *Mycoplasma* infection in a case of clinical mastitis with no bacteria isolated	28/37 (75.7%)	35/67 (52.2%)	2.8 (1.2–6.9)	0.033
Conduct mastitis test for *Mycoplasma* after the first calving of a home-bred heifer	5/37 (13.5%)	1/69 (1.4%)	10.6 (1.2–94.7)	0.019
2. Movement record				
The proportion of moved cows from their home farms	3.7% (n = 37)	25.5% (n = 67)		0.031
The proportion of cows which have been sent to communal pastures	1.2% (n = 37)	10.8% (n = 67)		0.006
Mean number of movements for all cows	0.4 (n = 37)	0.8 (n = 67)		0.020

CI: confidence interval

Table 3. Final herd-level multivariable models for the risk factors for *Mycoplasma* mastitis occurrence for 35 infected and 62 non-infected farms

Variable	Odds ratio	95% CI	*p*-value
(Intercept)	-	-	0.074
Herd size	1.00	1.00–1.00	0.909
Housing for milking cows: tie stall	0.20	0.07–0.60	0.004
Consciously wipe teat openings before milking	0.15	0.02–0.76	0.030
Use a paper towel after a cloth towel to wipe teats	0.31	0.09–-0.92	0.045
Have ever introduced cows	3.43	1.14–10.86	0.030

CI: confidence interval.

1.3.4 Cow-level risk factor analysis

In the cow-level univariable risk factor analyses, 6 variables from the movement records and 32 variables from the productivity records (for which there were valid value for more than 50% of the cows), and all variables in the clinical records were analyzed. In the cow-level univariable risk factor analyses, two factors had *p*-values lower than 0.05: history of being at a livestock market and causal pathogen–unidentified acute mastitis (Table 4). The final cow-level multivariable model included three risk factors: history of being at a livestock market, causal pathogen–unidentified acute mastitis, and higher milk yield at the test day (Table 5). The history of causal pathogen–unidentified acute mastitis exhibited a marginal *p*-value, but the final model with the factor had the lowest AIC. Figure 3 A and B summarizes the postulated causality of *Mycoplasma* mastitis inferred at the herd and cow levels, respectively, for the discussion hereafter.

Table 4. Cow-level univariable risk factor analysis results for *Mycoplasma* mastitis occurrence ($p < 0.05$)

Variable	Infected (%)	Non-infected (%)	Odds ratio (95% CI)	*p*-value
Movement record				
Have ever been at livestock markets	5/35 (14.3%)	3/95 (3.2%)	8.9 (1.0–78.3)	0.019
Dairy herd test record				
Milk yield at the test day (kg)	34.0 (n = 42)	30.2 (n = 107)		0.015
Clinical record				
History of causal pathogen unidentified acute mastitis	13/42 (31.0%)	15/107 (14.0%)	2.8 (1.1–7.4)	0.033

CI: confidence interval

Table 5. Final cow-level multivariable model for risk factors for *Mycoplasma* mastitis occurrence for 35 infected and 95 non-infected cows in 6 farms

Variable	Odds ratio	95% CI	*p*-value
Have ever been at livestock markets	10.80	1.12–104.38	0.040
Milk yield at the test day (kg)	1.09	1.02–1.18	0.014
History of causal pathogen unidentified acute mastitis	3.14	0.86–11.41	0.082

CI: confidence interval

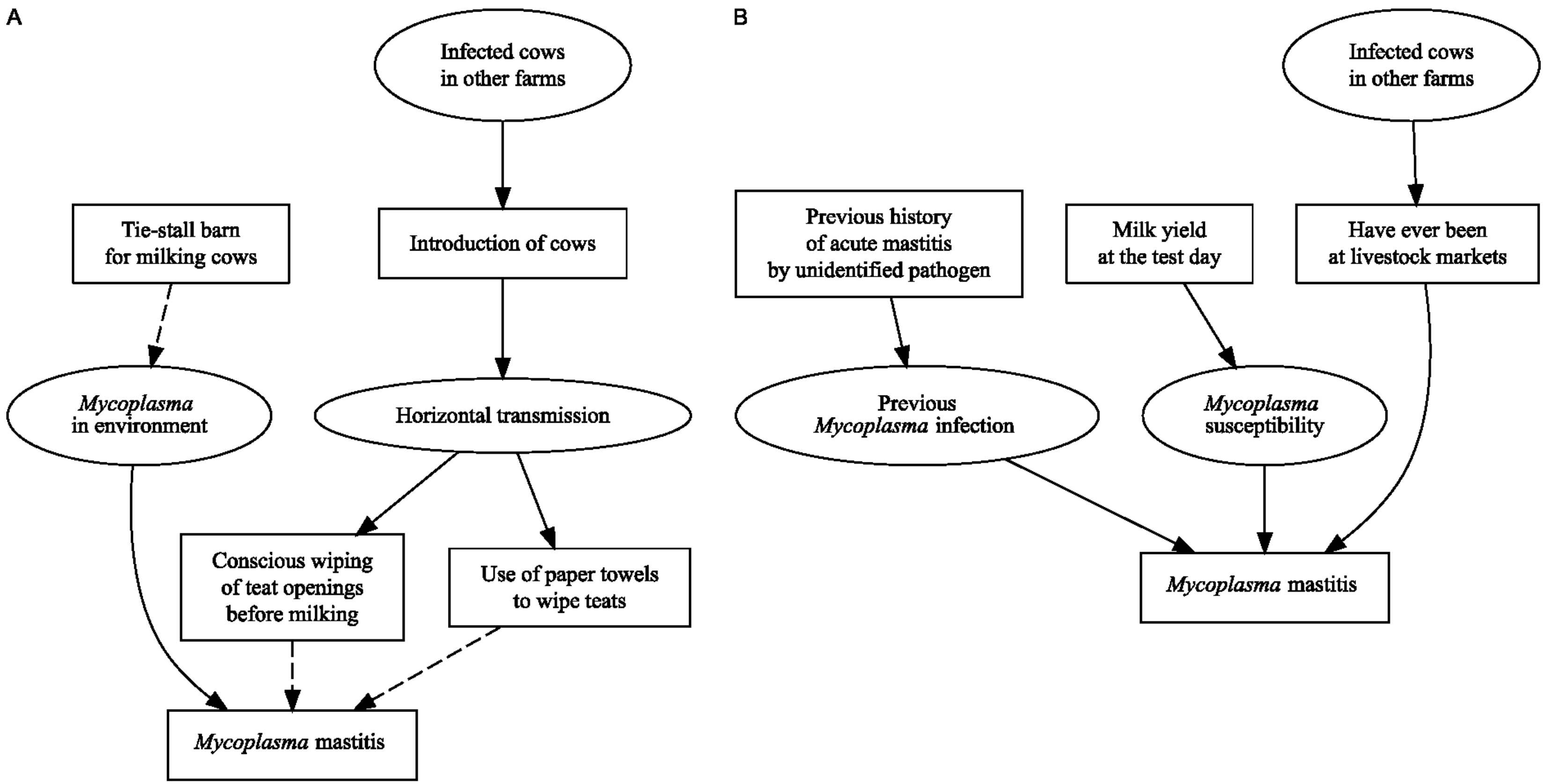

Figure 3. Theoretical causal web indicating risk factors associated with the outbreak of *Mycoplasma* based on the results of the (A) herd-level and (B) cow-level multivariable analyses. Rectangles indicate variables in the final multivariable models, and ellipses indicate hypothetical variables. Solid line indicates facilitating effect, and dashed line indicates preventive effect.

1.4 Discussion

In this study, risk factors for *Mycoplasma* mastitis were investigated at the farm and cow levels, and to the best of the authors' knowledge, this is the first study to have examined an outbreak of *Mycoplasma* mastitis at both levels simultaneously. The multi-level study utilized a variety of data sets: movement records, dairy herd test records, and clinical records, in addition to two questionnaire surveys.

As shown in Figure 3A, at the herd level, introduction of cows poses the risk of introducing *Mycoplasma*-infected animals. Even at the cow level, a history of presence at a livestock market was found to be a risk factor, suggesting the possibility of introducing and/or transmitting *Mycoplasma* from infected cows from other farms (Figure 3B). Cattle introduction is a well-known risk factor for *Mycoplasma* infection [14]. A questionnaire study conducted in Tokachi, which is also located on Hokkaido Island, also identified a history of purchasing cattle as a herd-level risk factor [57]. Interpreting the results related to cattle introduction in the present study was somewhat complicated, however. The dataset consisting of questionnaire results and movement records included information on cattle introduction as a binary response, the number of cows introduced, and the calculated proportion of introduced cows at a given farm (Tables S1 and S2). Among these variables, only history of cattle introduction as a binary response in the first questionnaire was identified as a risk factor. Similarly, although a history of presence at a livestock market in the cow-level analysis was a risk factor, the herd-level factors having cows introduced from livestock markets, the number of cows introduced, and the proportion of cows introduced from livestock markets did not remain risk factors. One possible explanation is low test sensitivity due to the small sample size. In addition, although the *p*-values were comparatively high, the ORs suggested these variables were potential risk factors (Table S1). Another possible explanation is that the risk of introducing *Mycoplasma*-infected cows may not be constant enough to be measured as a proportion or the number of introduced cattle at a farm but instead depends on the history of cattle introduction.

Cows cannot freely move around within a tie stall barn (Figure 3A). However, cows can freely move around in a free stall barn or a free barn, and *Mycoplasma* can enter teat openings soon after milking from bedding that has been contaminated by infected cows. Although they were not included in the final model in the present study, the use of free stall barns or free barns for milking cows were associated with ORs >1, suggesting that they are potential risk factors (these variables were multiple choice and not mutually exclusive, Table S2). Murai and Higuchi [57] also reported a higher prevalence of *M. bovis* in Tokachi, Hokkaido, in herds kept in loose housing than in herds held in tie-stall housing. In addition, Vahanikkila, et al. [79] reported that *M. bovis* commonly circulates for more than 1.5 years in loose-housing barns and that free-stall housing is a risk factor for *Mycoplasma* mastitis. Raaperi, et al. [70] reported an association between higher prevalence of bovine respiratory diseases and loose housing of cows and suggested that there is a greater probability of pathogen transmission in loose-housing barns due to direct contact between cows and frequent regrouping of cows. Employing tie-stall housing may prevent direct contact between infected and non-infected cows and thus serves as a preventive factor for *Mycoplasma* mastitis.

Infection with *Mycoplasma* from other cows within a farm can be prevented by consciously wiping of teat openings before milking or using paper towels after cloth towels to wipe the teats (Figure 3A). There is a general consensus that proper milking hygiene practices are critical for controlling the spread of *Mycoplasma* mastitis [17].

Cows with higher peak milk volume at 2 months before the laboratory testing day had a higher risk of *Mycoplasma* mastitis (Figure 3B). Several herd-level studies have also reported higher milk production at *Mycoplasma*-infected farms than non-infected farms [3, 11, 14]. Aebi, et al. [3] suggested that this is because cows at high-production farms are more likely to have a negative energy balance, which renders them more susceptible to infectious diseases.

During a participatory appraisal of the potential causes of *Mycoplasma* mastitis, it was suggested that *Mycoplasma* harbored in the respiratory tract of calves can remain in the animal and be transferred to the mammary glands via the blood stream, given that *Mycoplasma* can also cause pneumonia, otitis media, and arthritis [42]. In this study, more than a quarter of *Mycoplasma* mastitis cases were associated with a previous case of acute mastitis with the causal pathogen being unidentified, and this was determined to be a risk factor. This suggests that *Mycoplasma* can survive in the body of a cow even after farmers and veterinarians have judged that the animal has recovered from mastitis. However, transmission of *Mycoplasma* that has survived in the body of a cow from the time it was a calf may not be a significant cause of *Mycoplasma* mastitis.

No apparent increase in *Mycoplasma* infection in winter was observed in this study. However, several studies [16, 26, 29] have reported increases in *Mycoplasma*-related diseases in winter. In the winter of 2014, the highest snowfall in 9 years was recorded in Nemuro, and this was suspected as playing a role in the outbreak. However, neither an apparent increase in the number of *Mycoplasma* mastitis cases in winter nor an apparent relationship with the amount of snowfall was observed in the descriptive epidemiology study. In addition, because the ORs for poor hygiene management in cattle barns due to heavy snow were <1, worsening barn hygiene in the winter was assumed to have had little or no relation to the outbreak.

One of the limitations in the present study is the small sample size, particularly in the cow-level analysis, which involved only 6 farms. In addition, more than 2 years had passed between the mastitis outbreak and the time we asked for consent to obtain clinical records of the farms, and not all of the farms belonged to the veterinary association from which clinical records were obtained. Another limitation is ambiguity in case definition in cow-level analysis. Because a list of *Mycoplasma* mastitis cows was not obtained, case cows were defined as lactating cows that was diagnosed as *Mcyoplasma* positive and not diagnosed as pneumonia, arthritis, or otitis media in the previous two months. Thus the cow-level results must be carefully treated. The third limitation is that the studied cows included only Holsteins, which consists of more than 99% of dairy cattle in Japan. However, the consistency between the results of the present study and those of previous studies of *Mycoplasma* suggests that the results can be generalized to a limited degree not only to the whole Japan but also to other countries and other breeds.

1.5 Summary

The objective of this case-control study was to determine the herd- and cow-level risk factors associated with an outbreak of *Mycoplasma* bovine mastitis in the winter of 2014-2015 in Nemuro, Hokkaido, Japan. Two questionnaire surveys were sent to all 40 *Mycoplasma*-infected farms in the area and 73 non-infected farms for the farm-level analysis. Infected cows were matched to twice the number of non-infected cows in the same herds by parity and days after calving. Movement records, dairy herd test records, and clinical records of infected cows and matched non-infected cows were collected for the cow-level analysis. Risk factors for *Mycoplasma* infection were explored by multivariable analyses at both levels. In the herd-level analysis, tie stall housing for milking cows (OR = 0.20, 95% CI: 0.07-0.60, p = 0.004), consciously wiping of teat openings before milking (OR = 0.15, 95% CI: 0.02-0.76, p = 0.030), and use of paper towels to wipe teats (OR = 0.31, 95% CI: 0.09-0.92, p = 0.045) were identified as preventive factors, whereas introduction of cattle (OR = 3.43, 95% CI: 1.14-10.86, p = 0.030) was identified as a risk factor. In the cow-level analysis, a history of presence in livestock markets (OR = 10.80, 95% CI: 1.12-104.38, p = 0.040), higher milk yield 2 months prior to *Mycoplasma* infection (OR = 1.09, 95% CI: 1.02-1.18, p = 0.014), and previous diagnosis of acute mastitis without isolation of the causal pathogen (OR = 3.14, 95% CI: 0.86-11.41, p = 0.082) were identified as risk factors. These results highlight the importance of proper milking hygiene control and quarantine of introduced cattle to prevent *Mycoplasma* infection.

Chapter 2. Construction of a computational simulation model for the spread of bovine leukemia virus in Japanese dairy herd

2.1 Introduction

Enzootic bovine leukosis (EBL) is a disease of cattle caused by bovine leukemia virus (BLV) [45]. BLV is a member of the genus *Deltaretrovirus* in the family *Retroviridae*. No vaccine against BLV is available [90]. EBL is listed by the World Organization for Animal Health as a disease of importance in international trade. In Japan, BLV-infected cows with lymphosarcoma or their products cannot be sold for consumption. This is intended to ensure that livestock products are obtained from healthy animals for consumers rather than to prevent human infection with BLV[63].

BLV infections are usually subclinical. Persistent lymphocytosis (PL) is observed in 30–70% of infected cows, and 2–3% of infected cows develop malignant tumors (lymphosarcomas). BLV preferentially infects B cells in the peripheral blood [51]. The susceptibility of cows to BLV is determined in part by genetic factors, one of which is the bovine major histocompatibility complex, or BoLA system. DRB2 alleles in BoLA genes are closely related to the resistance and susceptibility of cows to BLV-induced PL [81].

Blood-sucking insects, primarily tabanid flies (*Tabanus* spp.) and stable flies (*Stomoxys calcitrans*), are considered the main vectors for BLV infection. In a nationwide survey in Japan, Kobayashi, et al. [32] identified the presence of blood-sucking insects in summer as a risk factor associated with higher within-herd seroprevalence. Other major transmission routes of BLV include colostrum milk from infected dams [12], dehorning [37], use of contaminated needles [9, 37], rectal palpation [33], and *in utero* infection [80].

The prevalence of BLV varies by country. In some countries, such as Australia, New Zealand [83], and 18 countries of the EU, BLV has been eradicated, and these countries have been granted BLV-free status by the OIE [2, 10, 61, 82]. In Japan, by contrast, 78% of dairy farms and 69% of beef farms are infected with BLV [32]. In the US, the herd-level prevalence of BLV is >90% [36]. In Argentina, the herd- and animal-level prevalence is 90.9% and 77.4%, respectively [66].

In Japan, a BLV guideline was published by the Ministry of Agriculture, Forestry, and Fisheries in 2015 [46]. However, no official BLV control program was instituted, and no financial aid was provided to farmers. Thus, to control BLV at the farm level, it is important for farmers to determine the appropriate countermeasures based on their available resources. The objective of the present study was to construct a computational simulation model of BLV spread in a dairy herd which can be a supportive tool for veterinarians and farmers in well-informed decision-making process in choosing a BLV-control strategy.

2.2 Materials and methods

2.2.1 Data and farms

Data from four dairy farms in Hokkaido, Japan, were used in the study. BLV testing was administered to all cows (farms A, C, and D) or to pregnant heifers and delivered cows (farm B) at the farms once or twice each year for 3 to 8 years (Table 6). Samples of cow blood were collected, tested using nested-PCR, BLV-CoCoMo-qPCR, and/or ELISA, and peripheral blood lymphocytes were counted. The European Community's leukosis key was used to judge whether an infected cow had PL. The following data from the first test day were used as the cow data, as described later: cow ID, birth date, sex, stage, infection status, and area where the cow was kept.

The four farms were located in the middle to eastern part of Hokkaido. Farms B and C used communal pastures; farm B sent all their calves to a communal pasture, and farm C occasionally used a communal pasture. Regarding BLV countermeasures, personnel at all four farms changed rectal palpation gloves every time, did not feed raw colostrum milk to newborn calves, and controlled insects. Farms A and B actively culled BLV-infected cows.

Table 6. Farm information

	Farm A	Farm B	Farm C	Farm D
BLV test				
Period	Spring 2012- Autumn 2019	Autumn 2012- Autumn 2019	Spring 2013- Spring 2019	Spring 2017- Autumn 2019
Test frequency (times/year)	1-2	1-2	1-2	2
Target	All cows	Pregnant heifers and delivered cows	All cows	All cows
Number of conducted tests	12	11	13	6
Herd size	138	174 (pregnant heifers+ delivered cows)	263	95
Barns				
Calf	Calf house	Hatch	Hatch	Pen
Heifer	Tie-stall, free-stall	Communal pasture	Free-stall, communal pasture	Rangeland (with dry cows)
Milking cow	Free-stall	Free-stall	Tie-stall, free-stall	Tie-stall
Dry cow	Free-stall	Free-stall	Free-stall	Rangeland (with heifers)
BLV countermeasures	Change rectal palpation gloves every time Feed pasteurized colostrum Control insects Cull infected cows	Change rectal palpation gloves every time Feed pasteurized colostrum Control insects Cull infected cows	Change rectal palpation gloves every time Feed pasteurized colostrum Control insects Do not cull infected cows actively	Change rectal palpation gloves every time Feed artificial colostrum Control insects Do not cull infected cows actively

2.2.2 Model structure

The model was individual-based, which simulates monthly changes in the status of each cow. The model inputs consisted of farm-specific simulation settings and input data (Tables 7 and 8). Farm-specific simulation settings included simulation length and information regarding BLV countermeasures conducted at each farm. Input data were composed of three parts: cow data, area data, and movement data. Cow data included information pertaining to each cow, such as ID, age, sex, and infection status. Area data included information regarding different areas of the farm. Movement data included information regarding when a cow was moved to a different area on the farm. Not all of the variables were necessary in the input data; missing variables were estimated based on other variables. The model calculates monthly changes in the variables listed in Table 9. Figure 4 shows the framework of the model illustrating BLV transmission routes and change of cow and insect infection status considered in the model. The main body of the model consisted of 11 parts (Figure 5): (1) increase month index in a simulation by one; (2) increase age by one; (3) calculate the number of artificial inseminations (AIs) to be conducted, their success and failure, and infections due to AI; (4) change in cow stage (calf/heifer/milking/dry); (5) check which infected cows are detected; (6) add newborn calves and calculate vertical infection and infection via colostrum milk; (7) calculate changes in infection status of infected cows; (8) assign chambers to cows that are roaming in a tie-stall; (9) identify which cows are dead, slaughtered, or culled; (10) move cows meeting the condition to different areas; and (11) remove dead, slaughtered, or culled cows from the herd. Chances of infection considered in the model are listed in Table 10.

The model output was one table for each simulation, which includes the calculated cow status for each month. From the output, the change in monthly prevalence and monthly number of cows infected via each respective infection route can be calculated and visualized as a graph.

The simulation model was constructed using R software, version 3.6.3 [69]. The model was compiled as a package and released on GitHub, where it can be downloaded freely (https://github.com/fmsan51/blvibmjp).

Table 7. Simulation-specific simulation parameters and their default values

Parameter	Value	Possible values
Simulation length (months)	60	-
Number of simulations	1	-
Proportion of female cows among newborn calves	Table 6	-
Proportion of female calves to be replacements		-
Proportion of slaughter in cause of death (slaughter/ slaughter + death at farm)		-
Probability of detecting a cow in heat		-
Calving interval (days)		-
Age of the first delivery (months)		-
Open period (days)		-
Milking period (days)		-
Age at the first service (days)		-
Probability of infection at a communal pasture		-
Capacity of herd	Between 0.9 and 1.1 times the initial herd size	-
Conduct insect control?	No	Yes (insects decrease to 50%)/No/ Proportion to which insects decrease
Change gloves for rectal palpation every time?	Yes	Yes/No
Feed raw colostrum milk to a newborn calf?	No	Yes/No
Cull infected cows?	No	All infected cows/ PL or EBL cows only/ No
Cull one infected cow for every *n*th non-replacement female calves	1	-
BLV test frequency (times/year)	0	-
BLV testing method	No default value	Immunodiffusion test/ ELISA/ Passive hemagglutination reaction test/ Nested PCR/ Real-time PCR/ Sensitivity and specificity of a test

Table 8. Input of the model

Variable	Whether a variable is necessary
Cow data	
Cow ID	No
Age	Either one of age and birth date is necessary
Birth date	
Sex	No
Whether a cow is a replacement or not	No
Stage (Calf/Heifer/Milking cow/Dry cow)	No
Parity	No
Date of last delivery	No
Date a cow got pregnant (if a cow is pregnant)	No
Date a cow was dried (if a cow is in a dry period)	No
Whether a cow needs pregnancy checking	No
Number of AIs conducted after the last delivery	No
Infection status (Non-infected/Asymptomatic/PL/EBL)	No
Date a cow was infected (if a cow is asymptomatic or with PL or EBL)	No
Date of onset of PL (if a cow is with PL or EBL)	No
Date of onset of EBL (if a cow is with EBL)	No
Name of area where a cow was kept	No
Months in which a cow was kept in the current area	No
Location of chamber where a cow was kept (if a cow is kept in a tie-stall barn)	No
Whether a cow is isolated or not (if a cow is kept in a tie-stall barn)	No
Area data	
Name	Yes
Type (Hatch/Free-stall/Outside/Tie-stall/ Communal pasture)	Yes
Capacity	Necessary when type of a barn is hatch or tie-stall
Movement data	
Name of the current area	Yes
Condition in which a cow moves to the next area	Yes
Next area to which a cow will move	Yes
Priority of next area if there was more than one next area	No

Table 9. Cow status calculated in the model

Variable
Cow profile
Cow ID
Age
Birth date
Sex
Stage (Calf/Heifer/Milking cow/Dry cow)
Infection
Infection status (Non-infected/Asymptomatic/PL/EBL)
Date a cow was infected (if a cow is asymptomatic or with PL or EBL)
Date of onset of PL (if a cow is with PL or EBL)
Date of onset of EBL (if a cow is with EBL)
Whether an infected cow is detected
Expected date of onset of PL (if a cow is asymptomatic)
Expected date of onset of EBL (if a cow is asymptomatic or with PL)
Cause of infection (if a cow is infected)
Genetic susceptibility which determines whether a cow will show PL if a cow gets infected
Genetic susceptibility which determines if a cow will show EBL if a cow gets PL
Reproduction
Whether a cow is a replacement or not
Parity
Date of the last delivery
Date a cow got pregnant (if a cow is pregnant)
Date a cow was dried (if a cow is in a dry period)
Whether a cow needs pregnancy checking
Number of AIs conducted after the last delivery
Day of the next heat
Day of the last detected heat
Longevity
Date of removal (if a cow is dead or slaughtered)
Expected date of removal (if a cow is alive)
Cause of removal
Whether a cow is still alive
Area
Name of area where a cow was kept
Months in which a cow was kept in the current area
Location of chamber where cow was kept (if a cow is kept in a tie-stall barn)
Whether a cow was isolated or not (if a cow is kept in a tie-stall barn)
Other
Index month in simulation (from 0 [before a simulation starts] to simulation length)

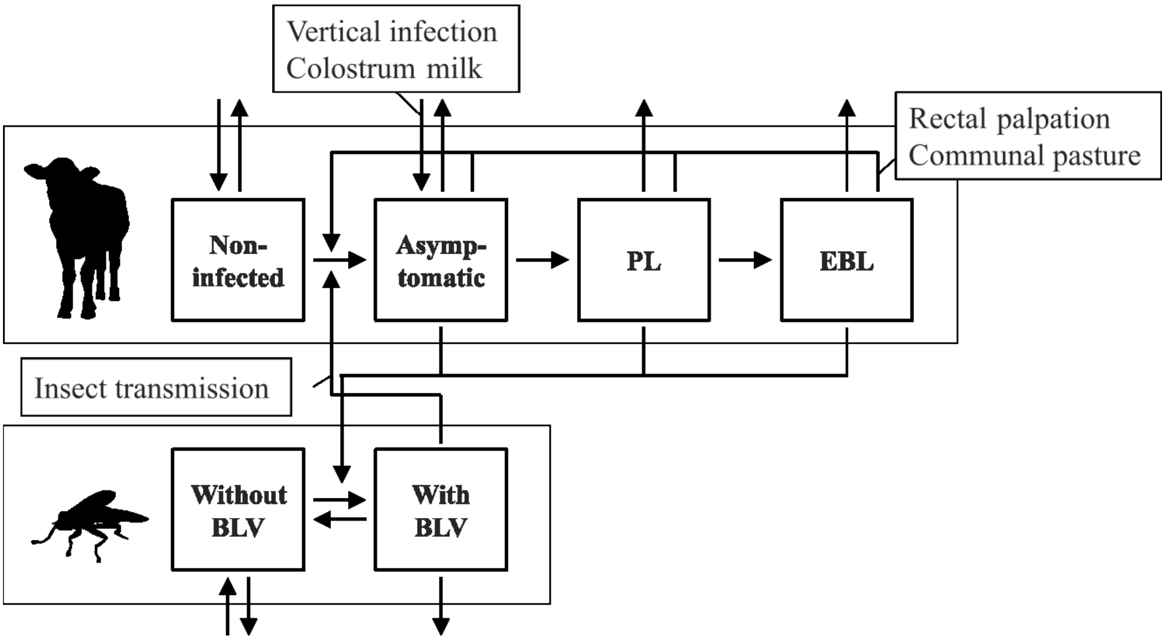

Figure 4. BLV transmission routes and change of infection status of cows and insects considered in the model. The rectangles indicate BLV transmission routes. The squares indicate status of cows and insects. The arrows connecting squares indicate change of infection status and allows connecting squares and allows indicates effect of infected animals in change of infection status. The arrows entering or leaving a square indicate increase or decrease of animals of that status.

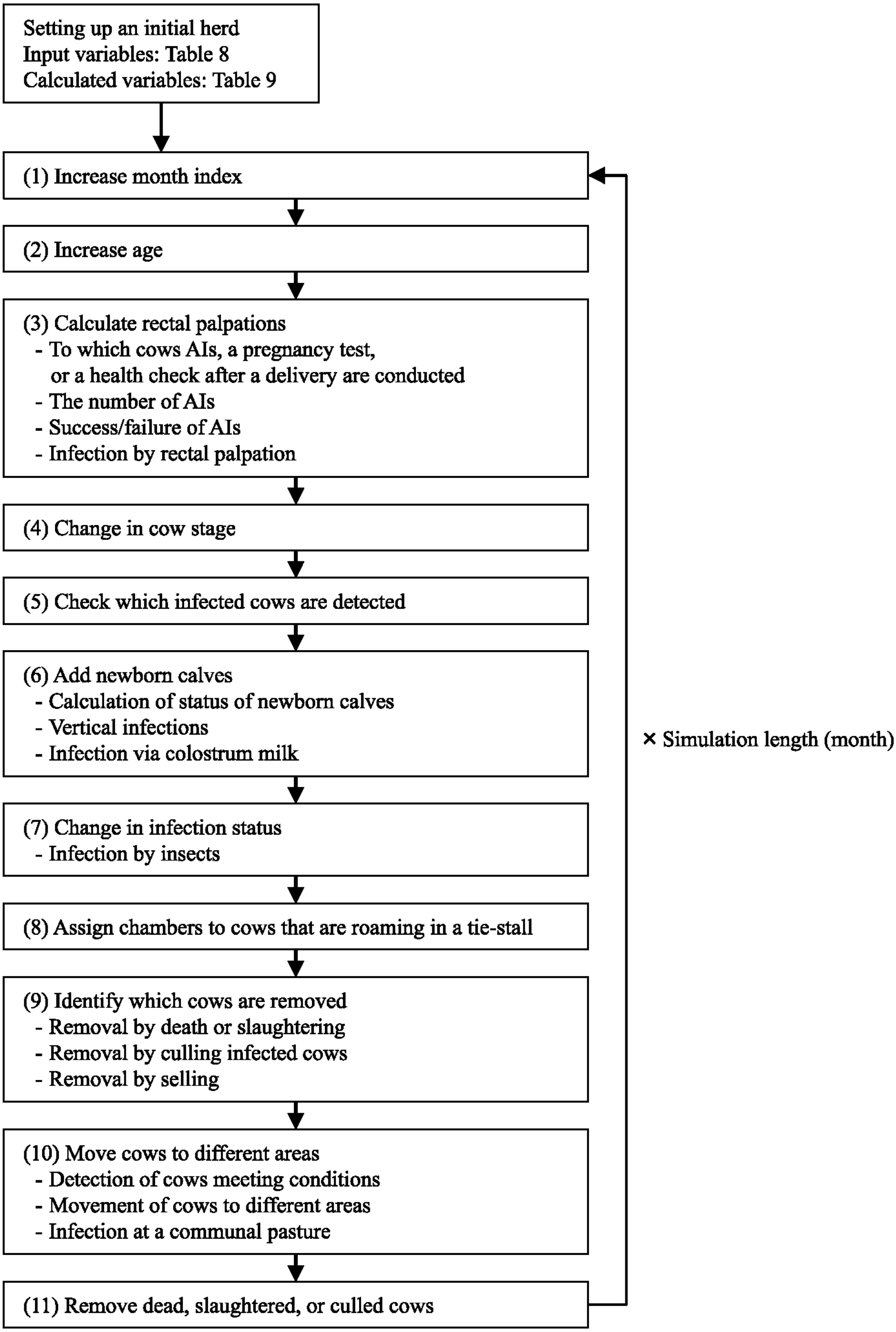

Figure 5. Structure of the constructed simulation model.

Table 10. Infection events, timing, and causes

Infection events	Timing	Cause
Vertical infection	When a calf is born	Vertical
Infection via colostrum milk from an infected dam	When a calf is born	Colostrum milk
Infection via contaminated rectal palpation gloves	When AI is conducted	Rectal palpation
Infection in a communal pasture	When a cow comes back from a communal pasture	Communal pasture
Infection in a barn	When a cow is kept in the same area with an infected cow	Insects

2.2.3 Parameterization

Default parameters used in the model were derived from reports in Japan as far as possible (Table 11). Several parameters in the model were re-estimated by sensitivity analysis as described later. The period from infection to onset of PL was represented as a period until an infected cow develops EBL, because no detailed studies of the period from infection to onset of PL were found. However, a study of the period from infection to onset of EBL [78] had a high level of evidence based on a nationwide survey in Japan. The period between when a cow develops EBL that is not found by a farmer then dies on the farm was assumed to be 2 months based on a general consensus that a cow with lymphosarcoma dies weeks or months after clinical onset [90]. The probability of infection at a communal pasture was randomly drawn from a uniform distribution from 0 to 1 in each run of the model because reported probabilities of seroconversion in communal pastures are highly variable, ranging from 0% to 97.4% [30, 34, 75, 84, 91]. Parameters related to reproduction, such as the probability of detecting a cow in heat or the age of first service, were calculated based on annual reports of dairy testing by the Livestock Improvement Association of Japan [39]. Parameters were randomly drawn from uniform distributions for which the lower and upper limits were equal to the minimum and maximum values of data for Hokkaido obtained from annual reports from 2011 to 2015. Parameters related to death and slaughter were calculated based on reported deaths of female Holsteins in Hokkaido. The age of death at the farm was represented by a mixed exponential and gamma distribution, and age of slaughter was represented by a gamma distribution. Parameters for the distributions were estimated using the number of deaths and slaughters of female Holstein cows at each month in age in Hokkaido each year from 2011 to 2015. The parameters used in a simulation were randomly drawn from uniform distributions, as previously described.

Table 11. List of parameters used in the model and their default values

Parameters	Default value	Source
Probability that an infected cow develops PL	0.3	[90]
Probability that an infected cow develops EBL	0.014	[78]
Months until an infected cow develops EBL	Weibull(3.3, 7.8)	[78]
Proportion of period from infection to onset of PL within period from infection to onset of EBL	0.3	Authors' assumption
Probability that an EBL cow will be detected	Normal(0.397, 0.02)	[78]
Months until an EBL cow dies on a farm	2	Authors' assumption
Test sensitivity and specificity		
Immunodiffusion test	Sensitivity: 0.981, Specificity: 0.967	[54]
ELISA	[Sensitivity: Normal(0.994, 0.005), Specificity: Normal(0.985, 0.010); Normal(0.994, 0.005), Normal(0.984, 0.010); Normal(0.976, 0.011), Normal(0.970, 0.018); Normal(0.893, 0.018), Normal(0.849, 0.033)]	[56]
Passive hemagglutination reaction test	[Sensitivity: 1.000, Specificity: 0.385; 0.909, 0.984]	[1, 7]
Nested PCR	[Sensitivity: Normal(0.928, 0.014), Specificity: Normal(0.767, 0.034); Normal(0.929, 0.015), Normal(0.770, 0.036); Normal(0.916, 0.017), Normal(0.755, 0.039)]	[56]
Real-time PCR	[Sensitivity: 0.800, Specificity: 1.000; 0.933, 1.000; 1.000, 1.000]	[21, 53, 73]
Risk of infection by a stable fly compared with a tabanid	0.038	Calculated from [5]

Table 11. (continued)

Parameters	Default value	Source
Hazard ratio of having an infected neighbor cow in a tie-stall barn	Exponential(Normal(2.52, 0.73))	[31]
Relative risk of infection at a free-stall barn compared with a tie-stall barn	Normal(1.19, 0.097)	[31]
Probability of infection via a contaminated glove for rectal palpation	$1 - (1 - \mathrm{Beta}(3, 1))^{0.25}$	Calculated from [33]
Probability of vertical infection		
From an asymptomatic dam	0.095	[43]
From a PL or EBL dam	0.483	
Probability of infection via contaminated colostrum milk	0.059	[27]
Probability of infection at a communal pasture	Uniform(0, 1)	
Probability of detecting a cow in heat	Uniform(0.59, 0.60)	Calculated from [39]
Heat cycle (days)		
Heifer	Normal(20.5, 1.0)	[40]
Delivered cow	Normal(20.7, 1.1)	
Age at the first service (days)	Uniform(427, 435)	Calculated from [39]
Period from delivery to the first service (days)	Uniform(88, 89)	Calculated from [39]
Probability of success of the first service after a delivery	Uniform(0.32, 0.35)	Calculated from [39]
Average number of AIs conducted	Uniform(2.3, 2.4)	Calculated from [39]

Table 11. (continued)

Parameters	Default value	Source
Sex ratio		
Male	-	Calculated from [39]
Female	Uniform(0.483, 0.503)	
Twins (male and female)	-	
Twins (male and male)	Uniform(0.262, 0.278)	
Twins (female and female)	Uniform(0.255, 0.261)	
Probability that newborns are twins	Uniform(0.029, 0.032)	Calculated from [39]
Probability of stillbirth or abortion		
Parity: 1	Uniform(0.0834, 0.0170)	Calculated from [39]
Parity: 2	Uniform(0.0476, 0.0563)	
Parity: 3	Uniform(0.0487, 0.0572)	
Parity: 4	Uniform(0.0526, 0.0604)	
Parity: ≥5	Uniform(0.0582, 0.0620)	
Proportion of female calves to be replacements		
Number of delivered cows in a herd: <30	0.952	[4]
Number of delivered cows in a herd: <50	0.821	
Number of delivered cows in a herd: <80	0.853	
Number of delivered cows in a herd: <100	0.964	
Number of delivered cows in a herd: ≥100	0.933	
Calving interval (days)	Uniform(427, 432)	Calculated from [39]
Age at the first delivery (months)	Uniform(24.8, 25.2)	
Open period (days)	Uniform(154, 160)	
Milking period (days)	Uniform(363, 366)	

Table 11. (continued)

Parameters	Default value	Source
Proportion of slaughter in cause of death (slaughter/slaughter + death at farm)	Uniform(0.437, 0.448)	Calculated based on reported death of female Holsteins in Hokkaido
Age of death at farm (months)	$\alpha \times$Exponential(β)+(1-α)$\times$Gamma(γ, δ) (α=Uniform(0.172, 0.192); β=Uniform(0.559, 0.695); γ=Uniform(3.943, 4.118); δ=Uniform(0.063, 0.066))	
Age at slaughter (months)	Gamma(α, β) (α=Uniform(4.919, 5.208); β=Uniform(0.068, 0.073))	

[]: One value from a list is selected in each simulation.

2.2.4 Monthly probability of insect transmission of BLV infection

The monthly probability of BLV infection transmitted by insects was calculated based on the number of tabanid flies and stable flies counted in a test barn. Five sticky traps were placed in the test barn of the Animal Research Center of Hokkaido Research Organization from July to November in 2017. The traps were checked once every 6 to 8 days, and the number of trapped tabanid flies and stable flies was determined. The number of tabanid files and stable flies in month m ($N_{tabanid,m}$ and $N_{stable,m}$) was calculated from the data under the assumptions that stable flies appear beginning May 1 and tabanid flies appear beginning June 1. These assumptions were based on reports that tabanid flies appeared in mid-July at a plain in Hokkaido [20], and flies appeared in early May in a dairy herd in Hokkaido [52].

The default value of relative infection risk by a stable fly (RR_{stable}) compared with a tabanid fly was 1/26, based on a report by Buxton, et al. [5] that BLV infection of sheep can occur following exposure to the mouth parts of 25 stable flies or 1 horse fly. The probability of BLV transmission by insects in month i ($P_{ins,i}$) was calculated using the following equation:

$$P_{ins,i} = \left(N_{stable,i} \times RR_{stable} + N_{tabanid,i} \times (1 - RR_{stable})\right) \times c_{ins} ,$$

where c_{ins} represents a coefficient modifying P_{ins} in the equation

$$\sum_{i=1}^{12}\left(1 - P_{ins,i}\right) = 1 - (1 - (1 - \lambda)^2) \times S,$$

where λ is the probability of infection in a 6-month period (4/83), as derived from Tsutsui, et al. [78], and S is the proportion of infections in summer and autumn in a year (13/14) [31].

2.2.5 Sensitivity analysis

A sensitivity analysis was conducted to determine the probability of infection at a communal pasture and evaluate parameters related to transmission of infection by insects, which is considered the major means of BLV infection in Japan. The probability of infection at a communal pasture was calculated using data from farm B, which sent its all heifers to a communal pasture. Parameters related to transmission of infection by insects were evaluated using data from all four farms, except for calculation of the hazard ratio of having an infected cow in a neighboring tie-stall barn for farm B, which did not have a tie-stall barn. Table 12 shows the list of parameters evaluated in the sensitivity analysis and candidate values.

The model simulation was conducted 100 times for each candidate value for each parameter. The simulation results were evaluated based on the χ^2 value calculated using the following formula:

$$\chi^2 = \sum_{m=1}^{M} \frac{\left(I_m - I_{est,m}\right)^2}{I_{est,m}},$$

where M denotes simulation length (Table 13); I_m denotes the actual number of infected cows in month m; and $I_{est,m}$ denotes the number of infected cows estimated by the model in month m. The I_m value for months in which testing was not conducted was estimated from available biannual data using the spline() function in R.

For each candidate value of each parameter, the mean χ^2 value for 100 simulations was calculated for the four farms, and the value with the lowest mean χ^2 value was selected. For the parameter 'insect_pressure', all four values were adopted. For the other parameters, the most frequently selected value for each parameter was adopted. If the highest frequency was shared by more than one value, the median of the values was adopted.

Table 12. Parameters and candidate values for the sensitivity analysis

Parameter	Candidates
Probability of infection at a communal pasture	0, 0.2, 0.4, 0.6, 0.8, 1
Risk of infection transmission by a stable fly compared with a tabanid (RR_{stable}, risk_stable)	0.096, 0.529, 0.746, 0.962, 3.121, 5.281, 9.6
Coefficient of the probability of infection via insects each month (insect_pressure)	0.1, 0.325, 0.55, 0.775, 1, 5.5, 10
Relative risk of infection at a free-stall barn compared with a tie-stall barn (free_pressure)	0.119, 0.387, 0.654, 0.992, 1.19, 6.545, 11.9
Hazard ratio of having an infected neighbor cow in a tie-stall barn (hr_having_infected_neighbor)	1, 1.38, 1.76, 2.14, 2.52, 13.86, 25.2

Table 13. Simulation parameters changed from default values

Parameter	Farm			
	A	B	C	D
Simulation length (months)	90	85	70	30
Probability of infection at a communal pasture	-	0.2	0.2	-
Conduct insect control?	Yes	Yes	Yes	Yes
Cull infected cows?	All infected cows	All infected cows	No (default)	No (default)
Cull one infected cow for every *n*th non-replacement female calves	4	4	-	-
BLV test frequency (times/year)	1	1	-	-
BLV testing method	ELISA	ELISA	-	-

-: The parameter was not used in the simulation.

2.2.6 Integrated simulation

Using the parameters optimized in the sensitivity analysis, 100 simulations were conducted for each farm.

2.2.7 Test scenario comparison

Simulations were conducted with several scenarios using different BLV test sensitivities (0.6/0.8/1) and test frequencies (once a year/twice a year/three times a year). Test specificity was fixed as 1. The culling policy was to cull all infected cows (starting with PL cows and then moving to asymptomatic infected cows), and the culling frequency (cull one infected cow for every *n*th non-replacement female calves) was set as 1. Simulation length was 120 months. Other parameters used in the simulations were the same as those optimized in the sensitivity analysis. The simulation was repeated 100 times for each farm.

2.3 Results

2.3.1 Monthly probability of insect transmission of BLV infection

Table 14 shows the results of tabanid fly and stable fly counts in the test barn, and Table 15 shows the estimated number of insects in each month and probability of transmission of BLV infection by insects.

Table 14. Number of tabanid flies and stable flies counted among the test herd

Period	Number of insects	
	Tabanid fly	Stable fly
July 9-10	2	179
10-11	6	
11-19	4	271
19-26	12	105
July 26-August 1	5	157
August 1-9	2	143
9-16	2	123
16-24	1	366
24-30	0	507
August 30-September 5	0	1189
September 5-13	0	3956
13-20	0	3168
20-27	0	364
September 27-October 4	0	894
October 4-11	0	1327
11-18	0	773
18-25	0	111
25-31	0	64
October 31-November 7	0	0
November 7-15	0	0
15-23	0	0

Table 15. Estimated number of tabanid flies and stable flies and probability of infection transmission by insects

Month	Number of insects		Infection probability
	Tabanid fly	Stable fly	
January	0	0	0
February	0	0	0
March	0	0	0
April	0	0	0
May	0	606	0.0028
June	23	1,740	0.0107
July	61	1,620	0.0146
August	8	1,151	0.0063
September	0	8,787	0.0406
October	0	3,026	0.0140
November	0	27	0.0001
December	0	0	0

2.3.2 Sensitivity analysis

Figure 6 shows the results of the sensitivity analysis of the probability of infection at a communal pasture. A probability of 0.2 was adopted with the lowest mean χ^2 value.

Figures 7 and 8 show the results of the sensitivity analysis of four parameters: risk_stable, free_pressure, insect_pressure, and hr_having_infected_neighbor. The adopted values are shown in Table 16. The adopted values for risk_stable and free_pressure were the same as the original values. The adopted values for insect_pressure and hr_having_infected_neighbor were lower than the original values.

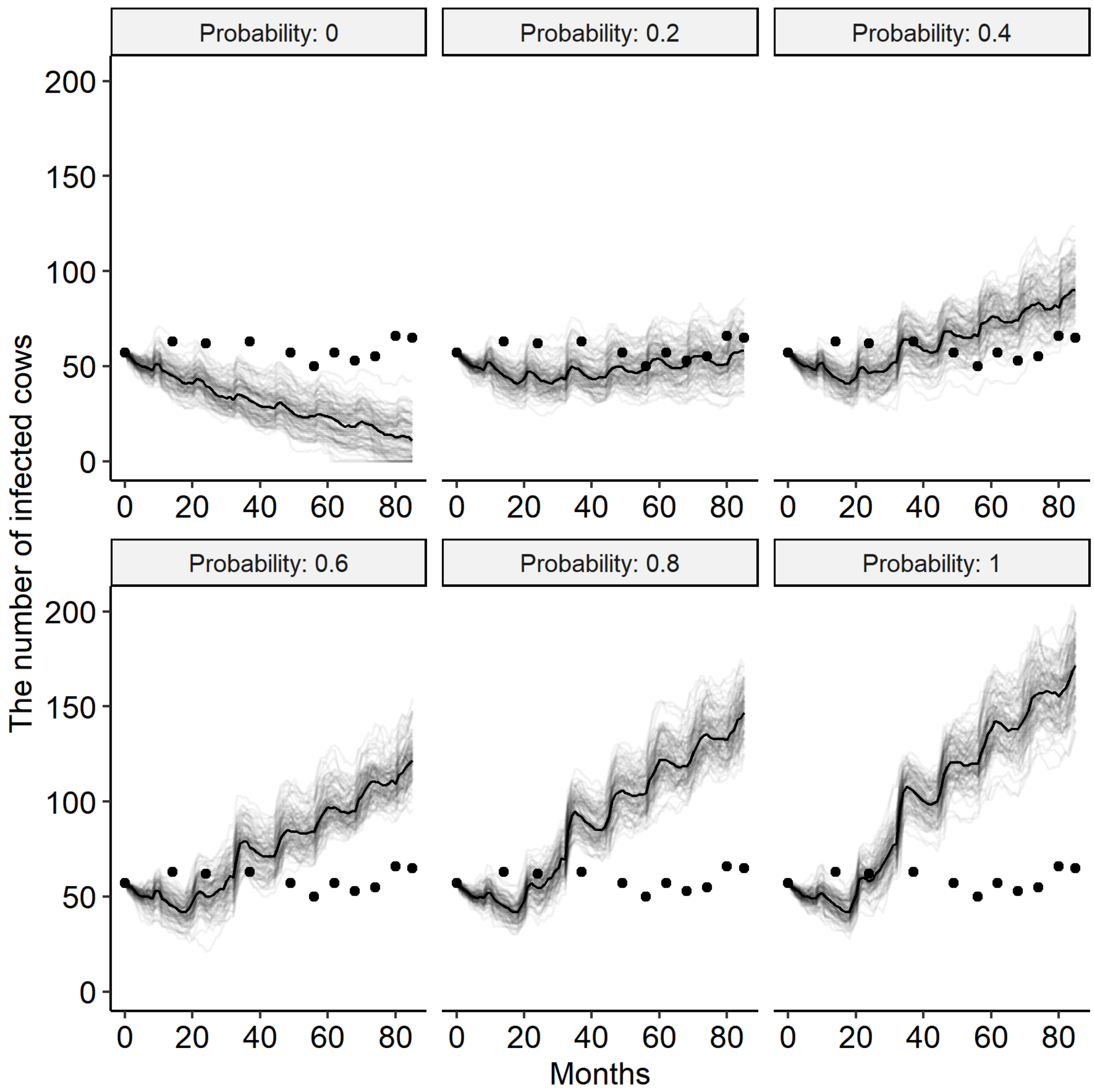

Figure 6. Results of the sensitivity analysis regarding the probability of infection at a communal pasture. The x-axis indicates months in the simulation, and the y-axis indicates the number of infected cows. Points indicate the actual number of infected cows. Gray lines indicate individual simulation results, and black lines indicate the median of the results.

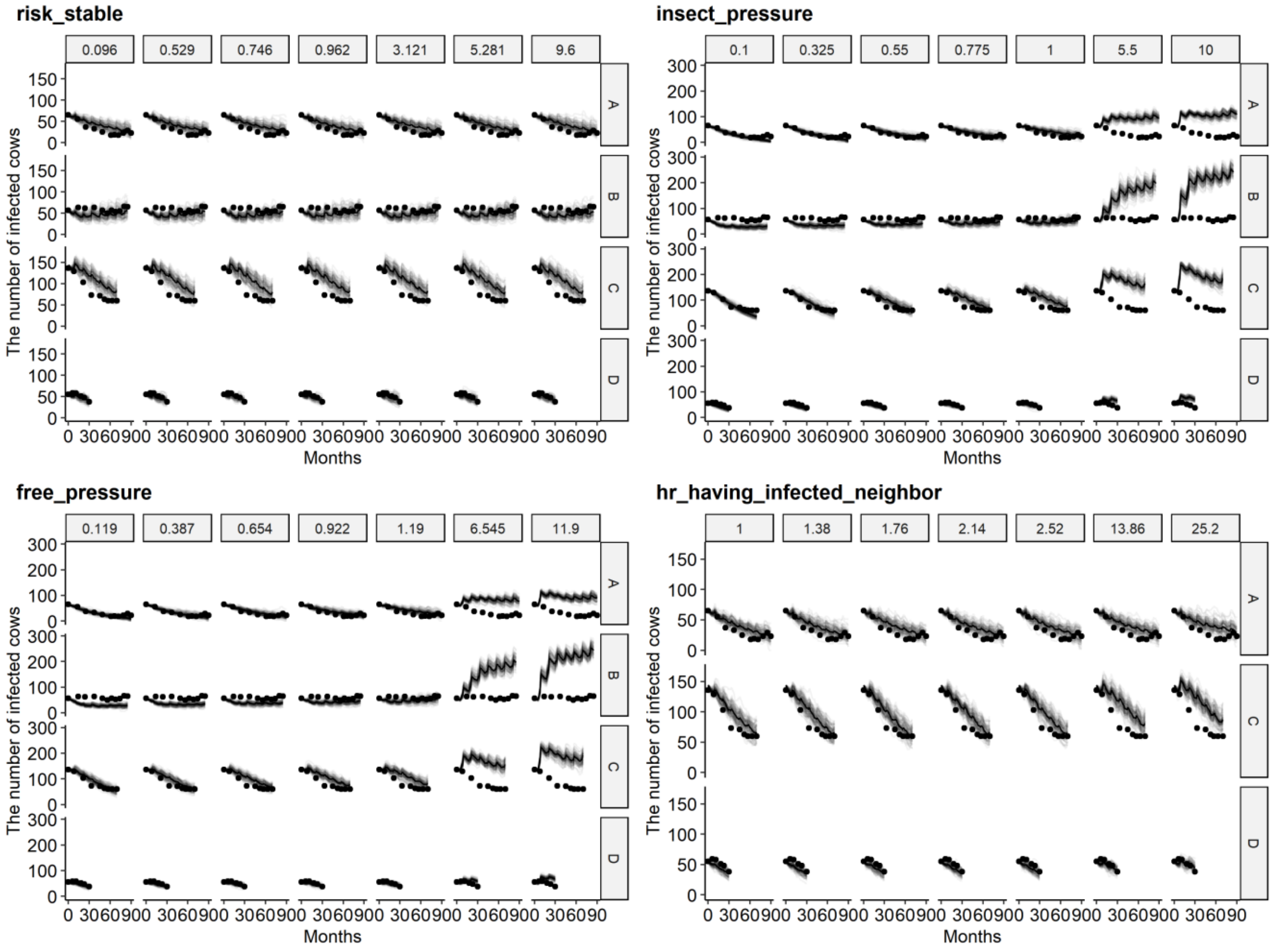

Figure 7. Results of the sensitivity analysis of parameters related to the probability of infection transmission by insects. The x-axis indicates months in the simulation, and the y-axis indicates the number of infected cows. Points indicate the actual number of infected cows. Gray lines indicate individual simulation results, and black lines indicate the median of the results.

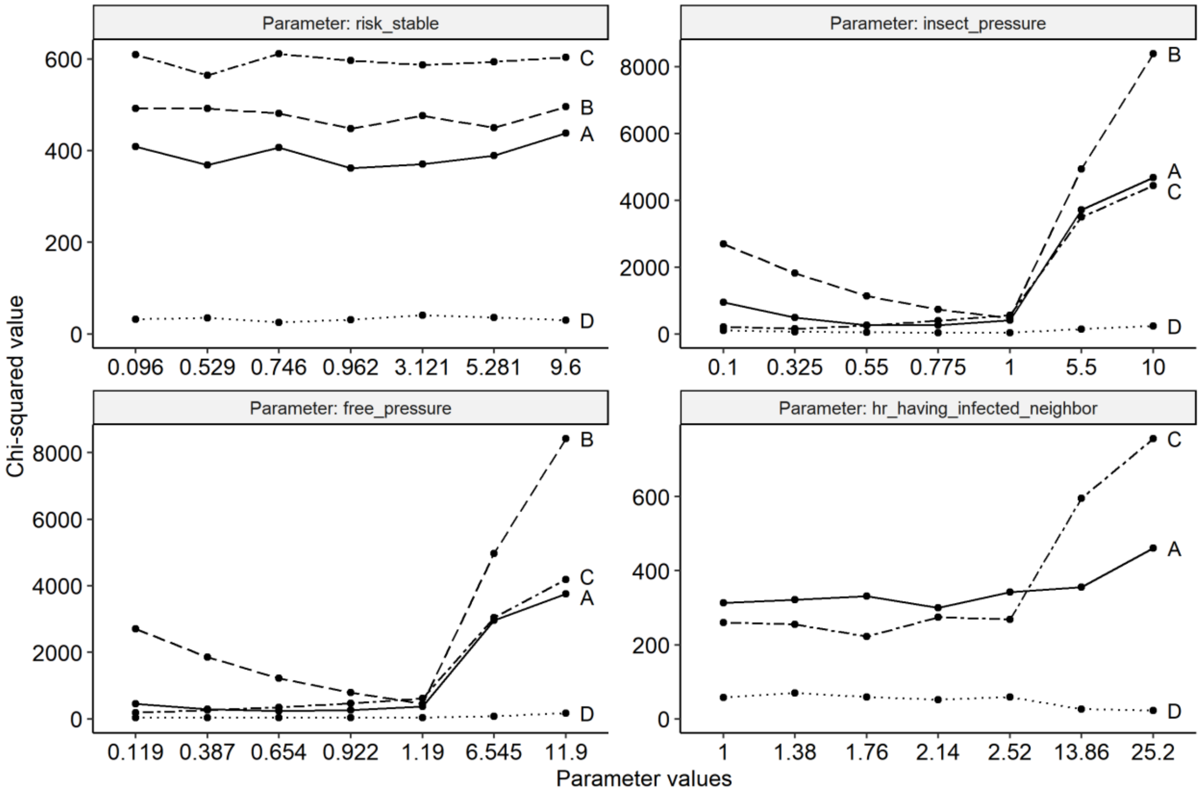

Figure 8. Mean χ^2 values of results of the sensitivity analysis of parameters related to the probability of infection transmission by insects. The x-axis indicates the χ^2 value, and the y-axis indicates values of the parameters. The alphabets next to the lines indicate farm names.

Table 16. Results of parameter optimization in the sensitivity analysis

Parameter	Farm	Selected candidate	Adopted value
Probability of infection at a communal pasture	B		0.2
Coefficient of the probability of infection transmission by insects each month (insect_pressure) (default: 1)	A	0.55	0.55
	B	1	1
	C	0.325	0.325
	D	1	1
Risk of infection transmission by a stable fly compared with a tabanid (RR_{stable}, risk_stable) (default: 0.962)	A	0.962	0.962
	B	0.962	
	C	0.529	
	D	0.746	
Relative risk of infection at a free-stall barn compared with a tie-stall barn (free_pressure) (default: 1.19)	A	0.654	1.19
	B	1.19	
	C	0.119	
	D	1.19	
Hazard ratio of having an infected neighbor cow in a tie-stall barn (hr_having_infected_neighbor) (default: 2.52)	A	2.14	2.14
	C	1.76	
	D	25.2	

2.3.3 Integrated simulation

The number of infected cows estimated by the model with values optimized in the sensitivity analysis and the actual number of infected cows are shown in Figure 9. The number of cows infected via each infection route is shown in Figure 10.

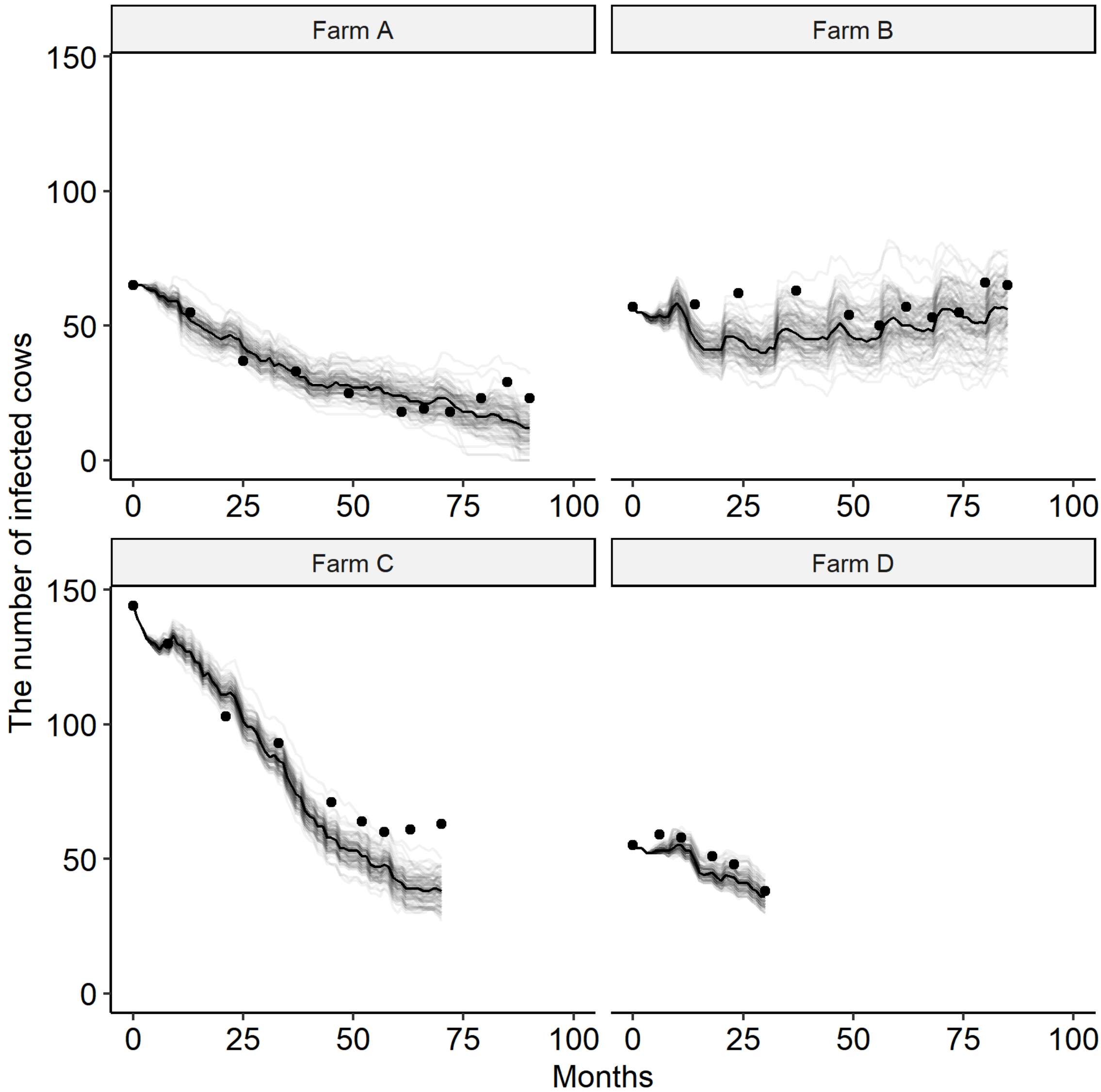

Figure 9. Simulation results with the optimized parameters. The x-axis indicates months in the simulation, and the y-axis indicates the number of infected cows. Points indicate the actual number of infected cows. Gray lines indicate individual simulation results, and black lines indicate the median of the results.

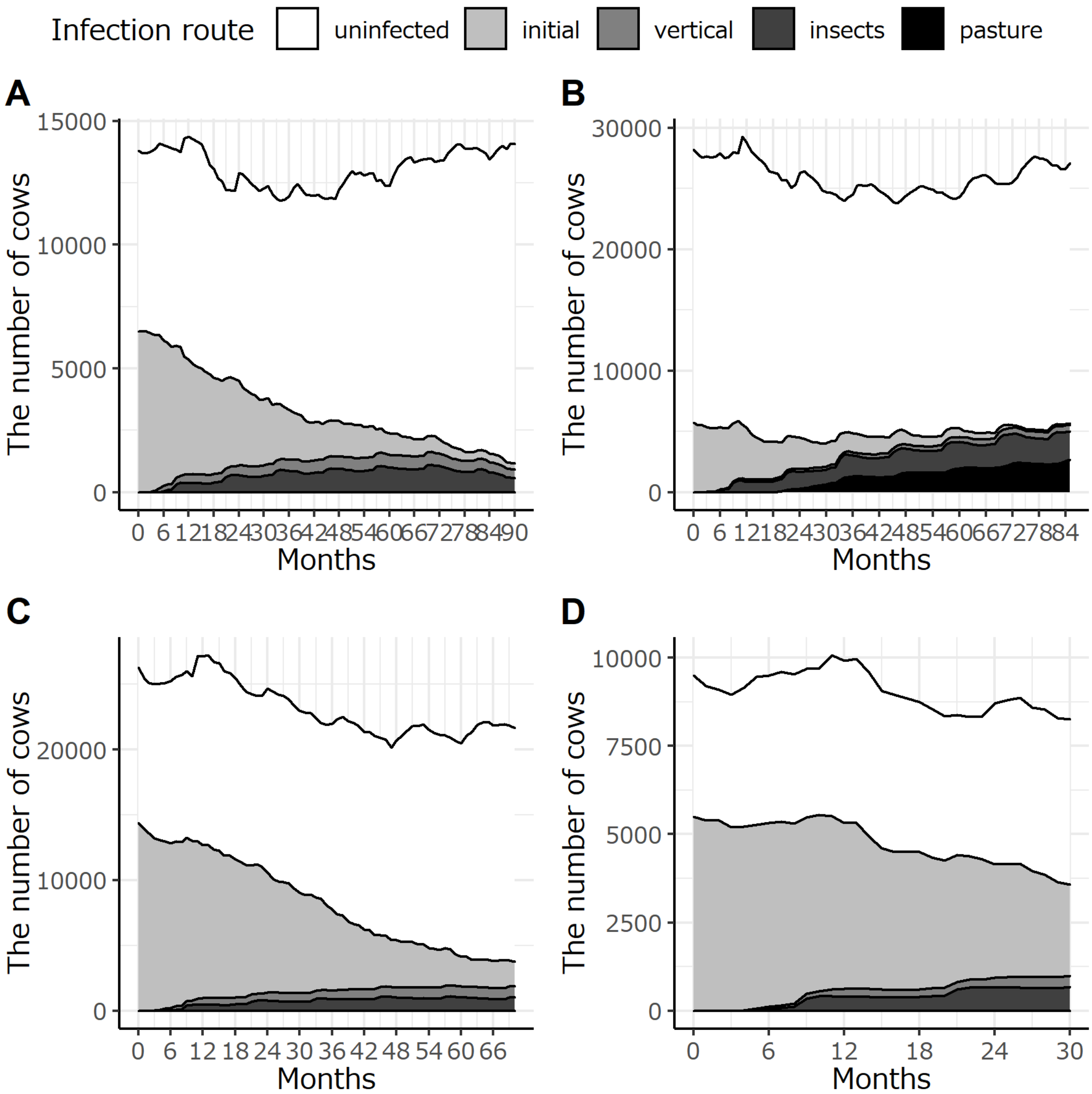

Figure 10. Number of infected cows by infection route in simulation results using optimized parameters. The x-axis indicates months in the simulation, and the y-axis indicates the number of infected cows. “initial” indicates cows infected at the start of the simulation.

2.3.4 Scenario comparison

The results of simulations using different test sensitivities and test frequencies are shown in Figure 11. Test sensitivities and test frequencies did not affect the period needed to eradicate BLV in a herd. BLV was eradicated or nearly eradicated in 10 years in all the farms except for farm B.

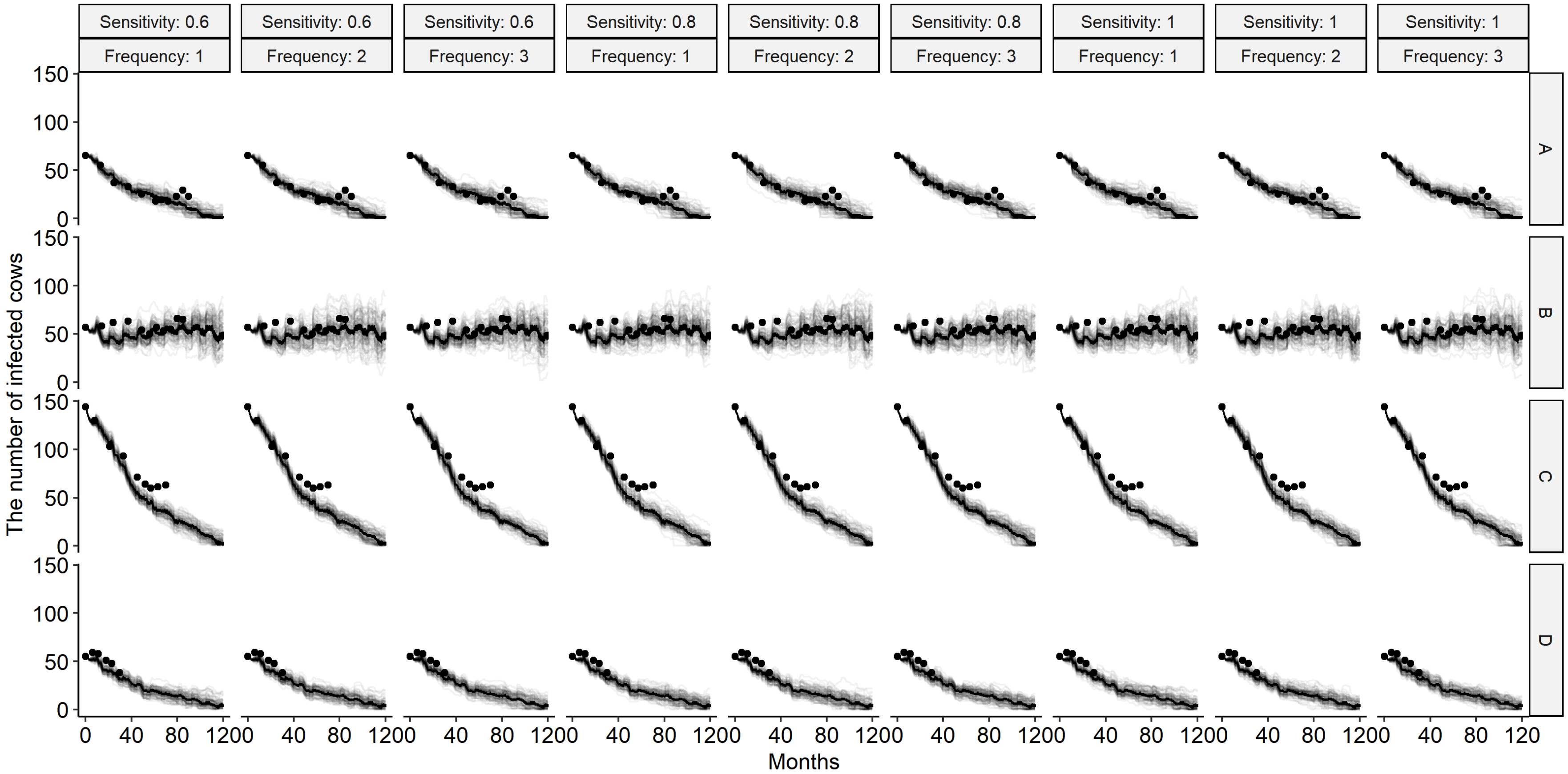

Figure 11. Simulation results under different BLV test strategies. The x-axis indicates months in the simulation, and the y-axis indicates the number of infected cows. Points indicate the actual number of infected cows. Gray lines indicate individual simulation results, and black lines indicate the median of the results.

2.4 Discussion

An individual-based simulation model of BLV infection was constructed in this study. The parameters were optimized using a sensitivity analysis. The number of infected cows was estimated from data from four dairy farms and compared with the actual number of infected cows. The constructed model was released on the Internet.

The estimated probability of infection transmission by insects had two peaks, in July and September, and was zero from December to April (Table 15). This change in the probability of infection was represented as a cyclical increase and decrease in infected cows in the simulation results. It is well known that seroconversion increases in the summer in Japan, when numbers of blood-sucking insects increase. In a study that traced seroconversion at dairy farms, 13 or 14 observed seroconversions occurred between summer and autumn [31]

Figure 6 illustrates the probability of infection at a communal pasture on BLV prevalence on a farm for farm B, which sends all of its calves to a communal pasture. The results suggest that seroconversion at communal pasture impacts BLV prevalence on the farm. Sending heifers to a communal pasture is a risk factor for BLV in Japan [31]. As described in the Materials and Methods section, the reported probability of seroconversion varies widely, from 0% to nearly 100% [34, 75, 84]. Thus, reducing the frequency of seroconversion in a communal pasture is considered an effective way to control BLV and can be easily achieved with the help of the pasture owner and local officials. Ohkatsu, et al. [64] reported a case in which they reduced the probability of seroconversion at a communal pasture used by 75% of local farmers. The seroconversion rate was reduced from 51% in 2016 to 5.6% in 2017 with the help of the owner (an agricultural cooperative) and workers, a Livestock Hygiene Service Center, local veterinarians, a center of the Japan Agricultural Development and Extension Association, and other cooperatives.

In parameter optimization in the sensitivity analysis, farm-specific values were selected for insect_pressure, whereas other parameters were kept constant among the farms. This was because the effects of insect_pressure and controlling insects cannot be separated. All of the farms control insects, but there was no information available as to what extent insect abundance decreased at each farm. The effect of controlling insects thus cannot be separated from the effect of insect_pressure, which is the coefficient of the probability of infection transmission by insects. In addition, the intensity of efforts to control insects differs between farms. Thus, farm-specific values were selected for insect_pressure in the sensitivity analysis.

The optimized values for risk_stable and free_pressure were identical to the original values. Although the adopted value for free_pressure was 1.19, selected candidates of this parameter for farms A and C were <1. This indicates the necessity for further investigation as to whether keeping a cow in a free-stall barn is a risk factor for BLV infection.

Test sensitivities and test frequencies did not affect the period needed to eradicate BLV in a herd. This was because that in all the scenarios the number of culled cows had reached to the limit, which is a number of non-replacement females in newborns. Though BLV was eradicated or nearly eradicated in farm A, C, and D, the number of infected cows reached a plateau in Farm B, where the most infection was occurred in a communal pasture. This also emphasizes the necessity of BLV control in communal pastures.

The model constructed in the study was individual based. Two simulation models of BLV infection were previously reported. One is a compartmental model used to calculate the basic reproduction number, R_0, which indicates the expected number of secondary cases per primary case [6] and evaluate BLV control strategies in Argentina [55], and the other is used to economically evaluate control strategies in Canada, which cannot be classified as a compartment model or individual-based model, but assumes homogenous herds [35]. One advantage of the individual-based model constructed in this study is that it better reflects the real world. A compartmental model assumes a homogenous population, which is very unlikely in a real herd. For example, it is known that bovine leukocyte antigen genes affect the sensitivity of a cow to BLV [38]. Such genetic susceptibility cannot be taken into account in a compartmental model, whereas an individual-based model can account for genetic susceptibility, as the model includes the variables "genetic susceptibility which determines whether a cow shows PL if the cow gets infected" and "genetic susceptibility which determines whether a cow shows EBL if the cow gets PL" (Table 9). The other advantage of an individual-based model is that it is more useful for simulating infections on an actual farm. Using the constructed model, a farmer can simulate the change in BLV prevalence using the age and BLV infection status of the farm's cows as input data.

The constructed model can also be used to calculate R_0. R_0 can be used to evaluate the effectiveness of strategies to control an infectious disease; $R_0 < 1$ indicates that the number of infected individuals in the population will decrease. BLV control strategies can be evaluated by calculating R_0 values in simulations under different BLV control scenarios.

One of the limitations of the model is that infection via several routes was not considered; gauge dehorning, infection via contaminated needles, and introduction of infected cows were not considered in the current model. However, infection resulting from dehorning is considered negligible in Japan, as no significant relationship was observed between dehorning and BLV seroprevalence in a nationwide survey in Japan [32]. Infection via needles also cannot be considered in the current model, as no appropriate reference for the parameter was found. Introduction of infected cows was not considered because the farms rarely introduce cows, and no appropriate reference values were found regarding the prevalence of BLV in introduced cows. Another limitation is that parameters were optimized one by one in the sensitivity analysis. Because the sensitivity analysis was conducted to select the best value from candidates, values not included in the candidates were not selected. In addition, because each parameter was optimized one by one, the results of optimizations when more than one parameter is modified were not evaluated. A final limitation is that the effect of not changing rectal palpation gloves at each procedure was not evaluated, as gloves are changed at each procedure at all of the farms examined.

This model should be useful in comparing the effects of several BLV disease control options a-priori. The functions for such evaluations are ready in the model, and finding effective and also economically favorable solutions is the way forward.

2.5 Summary

In this study, an individual based simulation model of BLV was constructed. BLV is the causative agent of EBL and about 80% of dairy farms and 70% of beef farms are infected with BLV.

The model simulates monthly changes in status of each cow such as age, parity, and infection status. The model input was composed farm-specific simulation settings (*e.g.* BLV control measures conducted in a farm) and data composed of three parts: cow data, area data and movement data. The model output was a table which includes the calculated cow status in each month. Change of BLV prevalence in a herd and the number of infected cows by each infection routes could be calculated from the output. Default parameters of the model were derived from reports in Japan as far as possible. Parameters were optimized by sensitivity analysis. Data from four dairy farms in Hokkaido, Japan was used for parameter estimation and simulation. The constructed model was released on the Internet.

The simulated BLV prevalence showed cyclic increase and decrease reflecting change of monthly probability of insect transmission of BLV infection calculated based on the number of bloodsucking insects counted at a test herd.

Probability of seroconversion at a communal pasture largely affected the within-herd prevalence in a farm which sent all their heifers to a communal pasture. This emphasized the importance of BLV control in communal pastures. BLV test sensitivities and test frequencies did not affect the period to eradicate BLV in a herd.

The constructed model should be a useful tool for veterinarians and farmers in decision-making process in choosing a BLV-control strategy.

General discussion

Highlights of the thesis

In this thesis, two infectious diseases of dairy cattle: *Mycoplasma* mastitis and bovine leukemia were epidemiologically investigated.

In Chapter 1, herd-level and cow-level risk factors associated with the outbreak of *Mycoplasma* mastitis in Nemuro area was revealed. The study used a variety of data sets: two questionnaire surveys, movement records, dairy herd test records, and clinical records. The identified risk factors and preventive factors were in accordance with previous studies or general consensus about *Mycoplasma* mastitis. Two of herd-level preventive factors, consciously wiping of teat openings before milking and use of paper towels to wipe teats, emphasized the importance of performing milking hygiene practice to prevent a disease. In the study, variables to collect and analyze were determined based on the putative causal web. This suggested the importance of drawing a causal web and this study demonstrated how to identify risk factors among candidate variables. Because the causal web was drawn based on previous studies and general consensus of the disease, it can be argued that identifying risk factors of a disease using a causal web is a way to summarize results of studies of a disease.

In Chapter 2, an individual-based simulation model of BLV infection in a dairy herd was constructed. The model was already released online and freely available. Parameters were estimated by sensitivity analysis and the change of prevalence was simulated using data from four dairy farms. The simulation result with the optimized parameters indicated that the model predicted the change of BLV prevalence in the farms well. The estimated probability of infection by insects reflected the number of insects counted at the test barn. The importance of controlling BLV in communal pastures was suggested by comparison of simulation results with different probability of seroconversion at a communal pasture. Difference in the values of BLV test sensitivity and frequency did not affect the period to eradicate BLV, and actually BLV was nearly eradicated in three farms except for one farm which send all their heifers to a communal pasture. The model constructed can simulate change of BLV prevalence in a herd using a real farm data and compare different BLV control strategies a-priori.

Integration of the studies

The thesis composed of two studies: one is the risk factor analysis and another is the modelling of an infectious disease. To construct the BLV model, many parameters were used and the parameters were obtained from previous studies. In addition, transmission routes and cow status calculated in the model were determined consulting previous studies. Building of an infectious disease model needs results of experimental studies and studies of risk factors. Experimental studies explore factors related with a disease. Risk factor analysis identifies which factor is actually related with the disease and assesses size of the effect. In model building process, events that should be considered and parameter values were determined by consulting results of experimental studies and risk factor analysis. In the thesis, the risk factor analysis, whose result can be used to build a simulation model of the disease, and the modelling of an infectious disease were conducted.

Further perspectives of the simulation model

The constructed model can be used to compare different BLV control strategies. Because the model output includes information about infection status, milking status, and whether a cow is culled, slaughtered or sold, volume of production of a herd while a simulation can be calculated from the output. Then cost-effective ness BLV control strategies can be calculated. This helps not only farmers and local livestock workers, but also a government to decide which BLV control strategy they choose and what kind of financial aid should be offered.

In scenario comparison of different culling frequencies and test sensitivities, test sensitivities and test frequencies did not affect the period until eradication of BLV in herds. However, because in the scenarios infected cows, which were adults in many cases, were replaced with newborns, proportion of milking cows decreased in the simulated scenarios. In reality, it is likely that farmers introduce a pregnant heifer or a milking cow to replace an infected cow. Additional comparison of scenarios are needed which uses an introduced cow as a replacement of an infected cow instead of a calf and with different culling frequency.

The model can be improved by adding infection events neglected in the study and by improving parameters that cannot be optimized. Several infection events, for example, gauge dehorning and introduction of cows were neglected in the model. In addition, not all the parameters related with control of BLV were optimized. Especially to optimize parameters related with infection pressure by insects, probability of infection by rectal palpation by using a contaminated glove, and probability of infection by feeding raw colostrum milk, data of a farm which does not conduct BLV countermeasures is needed.

The model can be used to other infectious diseases than BLV infection. While the parameters related with infection were specialized to BLV infection, the structure of the model itself does not contain BLV-specific events. By changing parameters related with infection such as a probability of infection by each infection route and adding events that were not related with BLV infection, the model can be applied to other infectious disease. For example, with result of risk factor analysis of *Mycoplasma* mastitis in Chapter 1 and studies by others, a model of *Mycoplasma* mastitis in a dairy herd can be build.

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Appendices

Supplementary Table S1. Descriptive statistics for variables examined in the study (mean, [n, missing, SD, median, min, max] or n/total [proportion, missing])

Variable	Infected	Non-infected
Herd-level		
Questionnaires		
Farm information		
Type of business		
Family-run	30/37 (81.1%, 0)	58/70 (82.9%, 0)
Cooperative	7/37 (18.9%, 0)	12/70 (17.1%, 0)
Type of farming		
Dairy only	34/36 (94.4%, 1)	62/66 (93.9%, 4)
Mixed	2/36 (5.6%, 1)	4/66 (6.1%, 4)
(If beef cows were kept,) keep beef cows in the same farm	2/2 (100.0%, 35)	3/3 (100.0%, 67)
The number of workers	4.2 (37, 0, 2.7, 3.0, 2.0, 16.0)	3.5 (70, 0, 1.3, 3.0, 1.0, 7.0)
The year the farm was opened	1956.0 (36, 1, 21.7, 1956.0, 1920.0, 2014.0)	1953.6 (65, 5, 19.4, 1950.0, 1924.0, 2007.0)
The year the farm owner started farming	1987.6 (37, 0, 14.0, 1990.0, 1960.0, 2014.0)	1989.4 (68, 2, 13.6, 1990.0, 1943.0, 2014.0)
The number of cows		
Milking cows	109.8 (37, 0, 78.0, 90.0, 20.0, 414.0)	83.6 (70, 0, 45.7, 68.5, 20.0, 228.0)
Dry cows	15.3 (37, 0, 10.6, 14.0, 0.0, 56.0)	13.6 (70, 0, 8.3, 10.0, 2.0, 40.0)
Heifers	66.8 (37, 0, 56.8, 60.0, 6.0, 330.0)	53.3 (70, 0, 47.8, 40.0, 0.0, 250.0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Calves	20.8 (37, 0, 15.6, 20.0, 0.0, 60.0)	15.3 (70, 0, 15.0, 10.0, 0.0, 100.0)
Total	212.6 (37, 0, 149.4, 185.0, 31.0, 860.0)	165.8 (70, 0, 104.1, 126.5, 37.0, 525.0)
Experience of Mycoplasma *infection*		
The number of cows infected by *Mycoplasma* in the outbreak	5.9 (28, 9, 5.9, 3.5, 1.0, 27.0)	- (0, 70, -, -, -, -)
Have experienced *Mycoplasma* mastitis within two years in group farms	4/28 (14.3%, 9)	0/55 (0.0%, 15)
(If yes,) the number of cows infected	3.3 (3, 34, 3.2, 2.0, 1.0, 7.0)	- (0, 70, -, -, -, -)
Knew that frequent occurrence of *Mycoplasma* mastitis in neighborhood recently	24/37 (64.9%, 0)	44/66 (66.7%, 4)
Changed hygiene management after *Mycoplasma* infection in the farm or in the neighborhood	17/37 (45.9%, 0)	14/66 (21.2%, 4)
Knowledge about Mycoplasma		
Have ever heard the name of a bacterium, *Mycoplasma*	37/37 (100.0%, 0)	67/70 (95.7%, 0)
Know that *Mycoplasma* also causes diseases to calves not only mastitis to adult cows	32/37 (86.5%, 0)	52/67 (77.6%, 3)
Know that *Mycoplasma* transmit from a calf to a dam by human hands	30/37 (81.1%, 0)	37/67 (55.2%, 3)
Considered the possibility of *Mycoplasma* infection in a case of clinical mastitis when no bacteria was isolated	28/37 (75.7%, 0)	35/67 (52.2%, 3)
Disease prevention		
Practice of vaccination against respiratory diseases for milking cows		
Yes	8/36 (22.2%, 1)	14/69 (20.3%, 1)
No	22/36 (61.1%, 1)	49/69 (71.0%, 1)
Not sure	6/36 (16.7%, 1)	6/69 (8.7%, 1)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Practice of vaccination against respiratory diseases for heifers		
Yes	23/36 (63.9%, 1)	40/69 (58.0%, 1)
No	10/36 (27.8%, 1)	25/69 (36.2%, 1)
Not sure	3/36 (8.3%, 1)	4/69 (5.8%, 1)
Practice of vaccination against respiratory diseases for calves		
Yes	20/35 (57.1%, 2)	42/69 (60.9%, 1)
No	12/35 (34.3%, 2)	24/69 (34.8%, 1)
Not sure	3/35 (8.6%, 2)	3/69 (4.3%, 1)
Prevention of intrusion of wild animals to the milking cow barn	20/37 (54.1%, 0)	36/69 (52.2%, 1)
Prevention of intrusion of wild animals to the heifer barn	12/34 (35.3%, 3)	18/64 (28.1%, 6)
Prevention of intrusion of wild animals to the calf barn	13/31 (41.9%, 6)	23/60 (38.3%, 10)
Presence of a power sprayer to disinfect vehicles which enter the farm	2/37 (5.4%, 0)	4/70 (5.7%, 0)
Set and apparently divide the sanitation control zone	31/37 (83.8%, 0)	56/70 (80.0%, 0)
Wear dedicated clothes in the sanitation control zone	12/37 (32.4%, 0)	24/70 (34.3%, 0)
Park vehicles of farm workers outside the sanitation control zone	10/37 (27.0%, 0)	28/69 (40.6%, 1)
Set disinfectant foot baths at barns		
At all barns	16/37 (43.2%, 0)	34/69 (49.3%, 1)
One in the whole sanitation control zone	17/37 (45.9%, 0)	30/69 (43.5%, 1)
No foot baths	4/37 (10.8%, 0)	5/69 (7.2%, 1)
Remove dirt on boots before step into disinfectants foot baths	28/33 (84.8%, 4)	50/64 (78.1%, 6)
Frequency of changing disinfectants in foot baths		
More than daily	4/33 (12.1%, 4)	2/63 (3.2%, 7)
Daily	5/33 (15.2%, 4)	13/63 (20.6%, 7)
More than weekly	21/33 (63.6%, 4)	30/63 (47.6%, 7)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Weekly	3/33 (9.1%, 4)	15/63 (23.8%, 7)
Less frequently	0/33 (0.0%, 4)	3/63 (4.8%, 7)
Type of disinfectant used (description question)		
Chlorine disinfectant	26/28 (92.9%, 9)	44/50 (88.0%, 20)
Invert soap	1/28 (3.6%, 9)	6/50 (12.0%, 20)
Hydrated lime	0/28 (0.0%, 9)	2/50 (4.0%, 20)
Scatter hydrated lime powder at farm entrance	27/36 (75.0%, 1)	45/68 (66.2%, 2)
Conducted hygiene control measures to vehicles of farm workers (multiple answers allowed)		
Rinse the vehicle before disinfection	3/34 (8.8%, 3)	4/66 (6.1%, 4)
Disinfect the whole vehicle	0/34 (0.0%, 3)	2/66 (3.0%, 4)
Disinfect the wheel wells	3/34 (8.8%, 3)	8/66 (12.1%, 4)
Disinfect the driver seat floor mat	2/34 (5.9%, 3)	2/65 (3.1%, 5)
Disinfect the bed of the vehicle	1/34 (2.9%, 3)	2/66 (3.0%, 4)
The farm owner set guideline of disinfection of vehicles other than farm workers' ones		
(If yes,) conducted hygiene control measures to vehicles other than farm workers' ones (multiple answers allowed)	1/32 (3.1%, 5)	3/62 (4.8%, 8)
Rinse the vehicle before disinfection	0/1 (0.0%, 36)	0/3 (0.0%, 67)
Disinfect the whole vehicle	0/1 (0.0%, 36)	0/3 (0.0%, 67)
Disinfect the wheel wells	1/1 (100.0%, 36)	3/3 (100.0%, 67)
Disinfect the driver seat floor mat	0/1 (0.0%, 36)	1/3 (33.3%, 67)
Disinfect the bed of the vehicle	0/1 (0.0%, 36)	0/3 (0.0%, 67)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Milking hygiene		
Use a milking parlor	21/22 (95.5%, 15)	20/23 (87.0%, 47)
Existence of a backflush system in the milking system	1/22 (4.5%, 15)	0/23 (0.0%, 47)
Use milking robots	3/22 (13.6%, 15)	1/23 (4.3%, 47)
Use towels to wipe teats		
Yes	34/37 (91.9%, 0)	64/70 (91.4%, 0)
No	0/37 (0.0%, 0)	0/70 (0.0%, 0)
Paper towels only	3/37 (8.1%, 0)	6/70 (8.6%, 0)
Use one towel per cow	27/34 (79.4%, 3)	52/64 (81.2%, 6)
Dip a towel to disinfectant	28/34 (82.4%, 3)	49/64 (76.6%, 6)
Consciously wipe teat openings	30/37 (81.1%, 0)	67/70 (95.7%, 0)
Use a paper towel after a cloth towel to wipe teats	6/37 (16.2%, 0)	24/69 (34.8%, 1)
Do pre-dipping	19/37 (51.4%, 0)	29/70 (41.4%, 0)
Do post-dipping	36/37 (97.3%, 0)	67/70 (95.7%, 0)
Use a cart to convey milking equipment	16/36 (44.4%, 1)	45/69 (65.2%, 1)
Use a strip cup	16/37 (43.2%, 0)	38/70 (54.3%, 0)
Actively call veterinarians when an abnormality was found by a strip cup	15/35 (42.9%, 2)	27/60 (45.0%, 10)
Actively call veterinarians when an abnormality was found by PL test	26/37 (70.3%, 0)	42/70 (60.0%, 0)
Use adequately disinfected milking equipment	36/37 (97.3%, 0)	66/70 (94.3%, 0)
Disinfect milking equipment after milking	33/37 (89.2%, 0)	59/70 (84.3%, 0)
Disinfect milking equipment before milk a next cow	16/37 (43.2%, 0)	6/70 (8.6%, 0)
Milk cows with high somatic cell count last	7/37 (18.9%, 0)	10/68 (14.7%, 2)
Milk mastitis cows last	16/37 (43.2%, 0)	22/68 (32.4%, 2)
Conduct mastitis test by *Mycoplasma* after the first calving of a home-bred heifer	5/37 (13.5%, 0)	1/69 (1.4%, 1)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Conduct mastitis test by pathogen other than *Mycoplasma* after the first calving of a home-bred heifer	8/37 (21.6%, 0)	18/70 (25.7%, 0)
Conduct a self-imposed test of *Mycoplasma* with bulk tank milk	16/36 (44.4%, 1)	24/70 (34.3%, 0)
Disinfect milking units until the result of *Mycoplasma* test was available after the first calving	6/37 (16.2%, 0)	3/69 (4.3%, 1)
Calf handling		
Timing when a calf is separated from its dam after a delivery		
Immediately after the delivery	16/23 (69.6%, 2)	17/47 (36.2%, 0)
When realized the delivery finished	8/23 (34.8%, 2)	28/47 (59.6%, 0)
Keep them together for a while	1/23 (4.3%, 2)	4/47 (8.5%, 0)
Other	0/23 (0.0%, 2)	0/47 (0.0%, 0)
Period to keep a calf and a dam together (days)	2.0 (1, 24, -, 2.0, 2.0, 2.0)	2.6 (4, 43, 3.0, 1.5, 0.5, 7.0)
Way to feed colostrum		
Direct from the dam	1/25 (4.0%, 0)	1/46 (2.2%, 1)
By a feeding tool	21/25 (84.0%, 0)	39/46 (84.8%, 1)
Feed frozen colostrum	7/25 (28.0%, 0)	10/46 (21.7%, 1)
Feed artificial colostrum	8/25 (32.0%, 0)	14/46 (30.4%, 1)
Way to feed milk to calves		
By a dam	2/25 (8.0%, 0)	0/45 (0.0%, 2)
(If yes,) the day start milking	1.0 (2, 23, 0.0, 1.0, 1.0, 1.0)	- (0, 47, -, -, -, -)
The day end milking	1.5 (2, 23, 0.71, 1.5, 1.0, 2.0)	- (0, 47, -, -, -, -)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
By a milking bucket	1/25 (4.0%, 0)	10/45 (22.2%, 2)
(If yes,) the day start milking	1.0 (1, 24, -, 1.0, 1.0, 1.0)	2.3 (10, 37, 6.2, 0.0, 0.0, 20.0)
The day end milking	40.0 (1, 24, -, 40.0, 40.0, 40.0)	34.9 (10, 37, 23.6, 37.5, 3.0, 60.0)
By a milking bin	24/25 (96.0%, 0)	35/45 (77.8%, 2)
(If yes,) the day start milking	0.54 (24, 1, 0.51, 1.0, 0.0, 1.0)	1.2 (35, 12, 1.8, 1.0, 0.0, 7.0)
The day end milking	25.6 (24, 1, 30.2, 9.0, 3.0, 93.0)	17.3 (35, 12, 20.3, 7.0, 1.0, 60.0)
By a bucket	6/25 (24.0%, 0)	14/45 (31.1%, 2)
(If yes,) the day start milking	11.5 (6, 19, 10.5, 9.0, 1.0, 31.0)	9.7 (14, 33, 6.5, 8.0, 4.0, 29.0)
The day end milking	65.0 (6, 19, 12.2, 60.0, 60.0, 90.0)	70.4 (13, 34, 21.1, 60.0, 45.0, 120.0)
Same worker takes care of calves and milking cows	17/25 (68.0%, 0)	33/46 (71.7%, 1)
(If yes,) timing of taking care of calves		
Before milking	8/17 (47.1%, 8)	17/34 (50.0%, 13)
After milking	8/17 (47.1%, 8)	8/34 (23.5%, 13)
Not decided	1/17 (5.9%, 8)	9/34 (26.5%, 13)
Change gloves between taking care of calves and taking care of milking cows		
Yes	12/21 (57.1%, 4)	17/36 (47.2%, 11)
No	6/21 (28.6%, 4)	11/36 (30.6%, 11)
No gloves used	3/21 (14.3%, 4)	8/36 (22.2%, 11)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Change cloths between taking care of calves and taking care of milking cows	2/20 (10.0%, 5)	2/36 (5.6%, 11)
Communal pastures		
Use communal pastures		
Using more than several years	8/37 (21.6%, 0)	32/69 (46.4%, 1)
Started to use in this year	2/37 (5.4%, 0)	1/69 (1.4%, 1)
Have been used before	2/37 (5.4%, 0)	5/69 (7.2%, 1)
Never used	25/37 (67.6%, 0)	31/69 (44.9%, 1)
For farms which have ever used communal pastures,		
Type of cows been send to the communal pasture (multiple answers allowed)		
Heifers	12/12 (100.0%, 25)	33/38 (86.8%, 32)
Dry cows	0/12 (0.0%, 25)	0/38 (0.0%, 32)
Other	2/12 (16.7%, 25)	4/38 (10.5%, 32)
Owner of the communal pasture		
A public organization	2/11 (18.2%, 26)	11/29 (37.9%, 41)
A neighbor farmer	2/11 (18.2%, 26)	3/29 (10.3%, 41)
An agricultural cooperative	5/11 (45.5%, 26)	10/29 (34.5%, 41)
Other	2/11 (18.2%, 26)	5/29 (17.2%, 41)
Introduction		
Have ever introduced cows	13/37 (35.1%, 0)	14/68 (20.6%, 2)
Introduce non-pregnant heifers	4/37 (10.8%, 0)	2/68 (2.9%, 2)
Frequency of introduction of non-pregnant heifers		
Every year	1/37 (2.7%, 0)	1/68 (1.5%, 2)
Once in two years	0/37 (0.0%, 0)	1/68 (1.5%, 2)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Once in five years	3/37 (8.1%, 0)	0/68 (0.0%, 2)
Never	33/37 (89.2%, 0)	66/68 (97.1%, 2)
The number of introduced non-pregnant heifers at the latest introduction	10.5 (2, 35, 13.4, 10.5, 1.0, 20.0)	150.0 (1, 69, -, 150.0, 150.0, 150.0)
Introduce pregnant heifers	5/37 (13.5%, 0)	10/68 (14.7%, 2)
Frequency of introduction of pregnant heifers		
Every year	4/37 (10.8%, 0)	4/67 (6.0%, 3)
Once in two years	0/37 (0.0%, 0)	5/67 (7.5%, 3)
Once in five years	1/37 (2.7%, 0)	0/67 (0.0%, 3)
Never	32/37 (86.5%, 0)	58/67 (86.6%, 3)
The number of introduced pregnant heifers at the latest introduction	11.2 (5, 32, 9.5, 5.0, 3.0, 23.0)	12.2 (6, 64, 18.7, 3.5, 3.0, 50.0)
Introduce delivered cows	8/37 (21.6%, 0)	10/68 (14.7%, 2)
Frequency of introduction of delivered cows		
Every year	2/33 (6.1%, 4)	2/67 (3.0%, 3)
Once in two years	1/33 (3.0%, 4)	4/67 (6.0%, 3)
Once in five years	1/33 (3.0%, 4)	3/67 (4.5%, 3)
Never	29/33 (87.9%, 4)	58/67 (86.6%, 3)
The number of introduced delivered cows at the latest introduction	6.0 (8, 29, 3.6, 5.0, 1.0, 10.0)	5.4 (5, 65, 3.0, 4.0, 3.0, 10.0)
Introduce other cows	0/37 (0.0%, 0)	0/65 (0.0%, 5)
Frequency of introduction of other cows		
Every year	0/36 (100.0%, 1)	0/65 (100.0%, 5)
Once in two years	0/36 (100.0%, 1)	0/65 (100.0%, 5)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Once in five years	0/36 (100.0%, 1)	0/65 (100.0%, 5)
Never	36/36 (100.0%, 1)	65/65 (100.0%, 5)
The number of introduced other cows at the latest introduction	30.0 (1, 36, -, 30.0, 30.0, 30.0)	- (0, 70, -, -, -, -)
For farms which have ever used introduced cows,		
Source of introduction (multiple answers allowed)		
An agricultural cooperative	12/14 (85.7%, 23)	10/13 (76.9%, 57)
A farm of an acquaintance	2/14 (14.3%, 23)	1/13 (7.7%, 57)
A group farm	0/14 (0.0%, 23)	0/13 (0.0%, 57)
A livestock dealer	4/14 (28.6%, 23)	1/13 (7.7%, 57)
Other	1/14 (7.1%, 23)	1/13 (7.7%, 57)
Most frequently introduced source		
An agricultural cooperative	10/14 (71.4%, 23)	10/13 (76.9%, 57)
A farm of an acquaintance	0/14 (0.0%, 23)	1/13 (7.7%, 57)
A group farm	0/14 (0.0%, 23)	0/13 (0.0%, 57)
A livestock dealer	3/14 (21.4%, 23)	1/13 (7.7%, 57)
Other	1/14 (7.1%, 23)	1/13 (7.7%, 57)
Mastitis test by *Mycoplasma* to cows introduced from a livestock market	2/14 (14.3%, 23)	1/13 (7.7%, 57)
Mastitis test by pathogen other than *Mycoplasma* to cows introduced from a livestock market	2/14 (14.3%, 23)	2/13 (15.4%, 57)
Quarantine of introduced cows		
Have a barn only for introduced cows	1/13 (7.7%, 24)	0/13 (0.0%, 57)
Have a barn not only for introduced cows	3/13 (23.1%, 24)	3/13 (23.1%, 57)
No quarantine	9/13 (69.2%, 24)	10/13 (76.9%, 57)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Quarantine period		
One day	0/3 (0.0%, 34)	0/2 (0.0%, 68)
Less than a week	3/3 (100.0%, 34)	2/2 (100.0%, 68)
A week or more	0/3 (0.0%, 34)	0/2 (0.0%, 68)
Farm workers check health condition of introduced cows	10/20 (50.0%, 5)	17/32 (53.1%, 15)
Barns		
Housing for milking cows		
Tie stall	12/37 (32.4%, 0)	46/70 (65.7%, 0)
Free stall	24/37 (64.9%, 0)	27/70 (38.6%, 0)
Free barn	1/37 (2.7%, 0)	0/70 (0.0%, 0)
Rangeland	2/37 (5.4%, 0)	5/70 (7.1%, 0)
Other	1/37 (2.7%, 0)	0/70 (0.0%, 0)
Housing for dry cows		
Tie stall	8/35 (22.9%, 2)	21/67 (31.3%, 3)
Free stall	16/35 (45.7%, 2)	23/67 (34.3%, 3)
Free barn	11/35 (31.4%, 2)	15/67 (22.4%, 3)
Rangeland	6/35 (17.1%, 2)	11/67 (16.4%, 3)
Other	1/35 (2.9%, 2)	4/67 (6.0%, 3)
Housing for heifers		
Tie stall	4/36 (11.1%, 1)	15/68 (22.1%, 2)
Free stall	5/36 (13.9%, 1)	5/68 (7.4%, 2)
Free barn	24/36 (66.7%, 1)	36/68 (52.9%, 2)
Rangeland	10/36 (27.8%, 1)	23/68 (33.8%, 2)
Other	5/36 (13.9%, 1)	6/68 (8.8%, 2)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Housing for calves		
One calf per pen	11/35 (31.4%, 2)	25/68 (36.8%, 2)
Several calves per pen	9/35 (25.7%, 2)	23/68 (33.8%, 2)
Hatch	13/35 (37.1%, 2)	24/68 (35.3%, 2)
Free barn	7/35 (20.0%, 2)	5/68 (7.4%, 2)
Rangeland	2/35 (5.7%, 2)	4/68 (5.9%, 2)
Other	3/35 (8.6%, 2)	2/68 (2.9%, 2)
Volume of bedding in the milking cow barn		
Enough bedding	25/37 (67.6%, 0)	36/70 (51.4%, 0)
The floor can be seen through bedding	10/37 (27.0%, 0)	27/70 (38.6%, 0)
No bedding	2/37 (5.4%, 0)	7/70 (10.0%, 0)
Volume of bedding in the heifer barn		
Enough bedding	17/32 (53.1%, 5)	27/69 (39.1%, 1)
The floor can be seen through bedding	9/32 (28.1%, 5)	27/69 (39.1%, 1)
No bedding	6/32 (18.8%, 5)	15/69 (21.7%, 1)
Volume of bedding in the calf barn		
Enough bedding	30/33 (90.9%, 4)	57/67 (85.1%, 3)
The floor can be seen through bedding	3/33 (9.1%, 4)	10/67 (14.9%, 3)
No bedding	0/33 (0.0%, 4)	0/67 (0.0%, 3)
Bedding in the milking cow barn (multiple answers allowed)		
Mattress	16/37 (43.2%, 0)	28/70 (40.0%, 0)
Sand	1/37 (2.7%, 0)	1/70 (1.4%, 0)
Paper	1/37 (2.7%, 0)	0/70 (0.0%, 0)
Sawdust	13/37 (35.1%, 0)	17/70 (24.3%, 0)
Compost	0/37 (0.0%, 0)	1/70 (1.4%, 0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Straw	14/37 (37.8%, 0)	35/70 (50.0%, 0)
Pasture grass	3/37 (8.1%, 0)	2/70 (2.9%, 0)
Chaff	3/37 (8.1%, 0)	8/70 (11.4%, 0)
Other	4/37 (10.8%, 0)	11/70 (15.7%, 0)
Bedding in the heifer barn (multiple answers allowed)		
Mattress	5/32 (15.6%, 5)	5/60 (8.3%, 10)
Sand	1/32 (3.1%, 5)	2/60 (3.3%, 10)
Paper	1/32 (3.1%, 5)	1/60 (1.7%, 10)
Sawdust	8/32 (25.0%, 5)	11/60 (18.3%, 10)
Compost	0/33 (0.0%, 4)	1/67 (1.5%, 3)
Straw	30/33 (90.9%, 4)	60/67 (89.6%, 3)
Pasture grass	1/33 (3.0%, 4)	5/67 (7.5%, 3)
Chaff	0/33 (0.0%, 4)	1/67 (1.5%, 3)
Other	1/33 (3.0%, 4)	0/67 (0.0%, 3)
Scatter hydrated lime powder on bedding in the milking cow barn	3/37 (8.1%, 0)	8/70 (11.4%, 0)
(If yes,) frequency of use		
Daily or more	7/19 (36.8%, 18)	12/23 (52.2%, 47)
Weekly or more	7/19 (36.8%, 18)	3/23 (13.0%, 47)
Monthly or more	4/19 (21.1%, 18)	5/23 (21.7%, 47)
Yearly or more	0/19 (0.0%, 18)	1/23 (4.3%, 47)
Less frequently	1/19 (5.3%, 18)	2/23 (8.7%, 47)
Frequency of changing bedding in the milking barn in summer		
Daily or more	16/32 (50.0%, 5)	38/64 (59.4%, 6)
Weekly or more	10/32 (31.2%, 5)	18/64 (28.1%, 6)
Monthly or more	4/32 (12.5%, 5)	5/64 (7.8%, 6)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Less frequently	2/32 (6.2%, 5)	3/64 (4.7%, 6)
Frequency of changing bedding in the milking barn in winter		
Daily or more	16/32 (50.0%, 5)	41/65 (63.1%, 5)
Weekly or more	11/32 (34.4%, 5)	17/65 (26.2%, 5)
Monthly or more	5/32 (15.6%, 5)	5/65 (7.7%, 5)
Less frequently	0/32 (0.0%, 5)	2/65 (3.1%, 5)
Frequency of changing bedding in the heifer barn in summer		
Daily or more	7/28 (25.0%, 9)	20/58 (34.5%, 12)
Weekly or more	15/28 (53.6%, 9)	22/58 (37.9%, 12)
Monthly or more	4/28 (14.3%, 9)	10/58 (17.2%, 12)
Less frequently	2/28 (7.1%, 9)	6/58 (10.3%, 12)
Frequency of changing bedding in the heifer barn in winter		
Daily or more	7/28 (25.0%, 9)	20/58 (34.5%, 12)
Weekly or more	15/28 (53.6%, 9)	22/58 (37.9%, 12)
Monthly or more	4/28 (14.3%, 9)	10/58 (17.2%, 12)
Less frequently	2/28 (7.1%, 9)	6/58 (10.3%, 12)
Frequency of changing bedding in the calf barn in summer		
Daily or more	7/29 (24.1%, 8)	21/66 (31.8%, 4)
Weekly or more	19/29 (65.5%, 8)	35/66 (53.0%, 4)
Monthly or more	3/29 (10.3%, 8)	9/66 (13.6%, 4)
Less frequently	0/29 (0.0%, 8)	1/66 (1.5%, 4)
Frequency of changing bedding in the calf barn in winter		
Daily or more	7/29 (24.1%, 8)	21/66 (31.8%, 4)
Weekly or more	19/29 (65.5%, 8)	35/66 (53.0%, 4)
Monthly or more	3/29 (10.3%, 8)	8/66 (12.1%, 4)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Less frequently	0/29 (0.0%, 8)	2/66 (3.0%, 4)
Frequency of removing manure in the milking cow barn (_ times per day)	3.2 (27, 10, 2.2, 2.0, 1.0, 10.0)	4.0 (47, 23, 3.9, 3.0, 1.0, 24.0)
Frequency of removing manure in the heifer barn		
Daily or more	17/30 (56.7%, 7)	34/58 (58.6%, 12)
Weekly or more	10/30 (33.3%, 7)	15/58 (25.9%, 12)
Monthly or more	3/30 (10.0%, 7)	6/58 (10.3%, 12)
Less frequently	0/30 (0.0%, 7)	3/58 (5.2%, 12)
Frequency of removing manure in the calf barn		
Daily or more	8/30 (26.7%, 7)	23/65 (35.4%, 5)
Weekly or more	19/30 (63.3%, 7)	33/65 (50.8%, 5)
Monthly or more	3/30 (10.0%, 7)	9/65 (13.8%, 5)
Regular disinfection in the milking cow barn	15/35 (42.9%, 2)	33/68 (48.5%, 2)
(If yes,) frequency of disinfection		
Weekly or more	2/12 (16.7%, 25)	1/27 (3.7%, 43)
Monthly or more	5/12 (41.7%, 25)	14/27 (51.9%, 43)
Yearly or more	5/12 (41.7%, 25)	12/27 (44.4%, 43)
Less frequently	0/12 (0.0%, 25)	0/27 (0.0%, 43)
Type of disinfectant used (description question)		
Outsourced to an agricultural cooperative	3/12 (25.0%, 25)	12/23 (52.2%, 47)
Chlorine disinfectant	3/12 (25.0%, 25)	3/23 (13.0%, 47)
Invert soap	3/12 (25.0%, 25)	6/23 (26.1%, 47)
Hydrated lime	2/12 (16.7%, 25)	2/23 (8.7%, 47)
Regular disinfection in the heifer barn	13/33 (39.4%, 4)	20/63 (31.7%, 7)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
(If yes,) frequency of disinfection		
Weekly or more	2/11 (18.2%, 26)	0/18 (0.0%, 52)
Monthly or more	3/11 (27.3%, 26)	10/18 (55.6%, 52)
Yearly or more	5/11 (45.5%, 26)	8/18 (44.4%, 52)
Less frequently	1/11 (9.1%, 26)	0/18 (0.0%, 52)
Type of disinfectant used (description question)		
Outsourced to an agricultural cooperative	0/6 (0.0%, 31)	2/11 (18.2%, 59)
Chlorine disinfectant	3/6 (50.0%, 31)	0/11 (0.0%, 59)
Invert soap	1/6 (16.7%, 31)	3/11 (27.3%, 59)
Hydrated lime	3/6 (50.0%, 31)	4/11 (36.4%, 59)
Regular disinfection in the calf barn	20/29 (69.0%, 8)	31/61 (50.8%, 9)
(If yes,) frequency of disinfection		
Weekly or more	5/17 (29.4%, 20)	8/27 (29.6%, 43)
Monthly or more	8/17 (47.1%, 20)	8/27 (29.6%, 43)
Yearly or more	1/17 (5.9%, 20)	10/27 (37.0%, 43)
Less frequently	3/17 (17.6%, 20)	1/27 (3.7%, 43)
Type of disinfectant used (description question)		
Outsourced to an agricultural cooperative	0/10 (0.0%, 27)	1/20 (5.0%, 50)
Chlorine disinfectant	1/10 (10.0%, 27)	1/20 (5.0%, 50)
Invert soap	2/10 (20.0%, 27)	1/20 (5.0%, 50)
Hydrated lime	5/10 (50.0%, 27)	12/20 (60.0%, 50)
Water supply equipment in the milking cow barn		
Water tank	6/7 (85.7%, 30)	6/16 (37.5%, 54)
Water cup	1/7 (14.3%, 30)	12/16 (75.0%, 54)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Frequency of cleaning the water supply equipment in the milking cow barn		
Daily or more	4/27 (14.8%, 10)	4/55 (7.3%, 15)
Weekly or more	14/27 (51.9%, 10)	22/55 (40.0%, 15)
Monthly or more	3/27 (11.1%, 10)	14/55 (25.5%, 15)
Less frequently	3/27 (11.1%, 10)	10/55 (18.2%, 15)
When it get dirty	3/27 (11.1%, 10)	5/55 (9.1%, 15)
Water supply equipment in the heifer barn		
Water tank	3/4 (75.0%, 33)	8/13 (61.5%, 57)
Water cup	2/4 (50.0%, 33)	5/13 (38.5%, 57)
Frequency of cleaning the water supply equipment in the heifer barn		
Daily or more	2/32 (6.2%, 5)	7/68 (10.3%, 2)
Weekly or more	13/32 (40.6%, 5)	20/68 (29.4%, 2)
Monthly or more	6/32 (18.8%, 5)	17/68 (25.0%, 2)
Less frequently	5/32 (15.6%, 5)	16/68 (23.5%, 2)
When it get dirty	6/32 (18.8%, 5)	8/68 (11.8%, 2)
Water supply equipment in the calf barn		
Water tank	0/9 (0.0%, 28)	5/24 (20.8%, 46)
Water cup	4/9 (44.4%, 28)	4/24 (16.7%, 46)
Bucket	4/9 (44.4%, 28)	8/24 (33.3%, 46)
No equipment	1/9 (11.1%, 28)	7/24 (29.2%, 46)
Frequency of cleaning the water supply equipment in the calf barn		
Daily or more	10/29 (34.5%, 8)	20/56 (35.7%, 14)
Weekly or more	9/29 (31.0%, 8)	14/56 (25.0%, 14)
Monthly or more	3/29 (10.3%, 8)	8/56 (14.3%, 14)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Less frequently	4/29 (13.8%, 8)	9/56 (16.1%, 14)
When it get dirty	3/29 (10.3%, 8)	5/56 (8.9%, 14)
Type of ventilation in the milking cow barn		
Windows and doors only	12/37 (32.4%, 0)	26/70 (37.1%, 0)
Fans	25/37 (67.6%, 0)	34/70 (48.6%, 0)
Open barn	9/37 (24.3%, 0)	16/70 (22.9%, 0)
Tunnel ventilation	7/37 (18.9%, 0)	13/70 (18.6%, 0)
Type of ventilation in the heifer barn		
Windows and doors only	20/35 (57.1%, 2)	39/66 (59.1%, 4)
Fans	6/35 (17.1%, 2)	8/66 (12.1%, 4)
Open barn	14/35 (40.0%, 2)	24/66 (36.4%, 4)
Tunnel ventilation	2/35 (5.7%, 2)	2/66 (3.0%, 4)
Type of ventilation in the calf barn		
Windows and doors only	19/32 (59.4%, 5)	37/62 (59.7%, 8)
Fans	13/32 (40.6%, 5)	10/62 (16.1%, 8)
Open barn	8/32 (25.0%, 5)	21/62 (33.9%, 8)
Tunnel ventilation	2/32 (6.2%, 5)	2/62 (3.2%, 8)
Hygiene management during heavy snow		
Remember the condition of hygiene management in the farm from the end of February to early March in 2015 when heavy snow fell	21/37 (56.8%, 0)	37/70 (52.9%, 0)
Volume of ventilation in the period in the milking cow barn		
Could not be done in some period	2/21 (9.5%, 16)	3/37 (8.1%, 33)
Decreased	2/21 (9.5%, 16)	10/37 (27.0%, 33)
Same as usual years	14/21 (66.7%, 16)	17/37 (45.9%, 33)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Volume of ventilation in the period in the heifer barn		
Could not be done in some period	2/21 (9.5%, 16)	4/37 (10.8%, 33)
Decreased	1/21 (4.8%, 16)	9/37 (24.3%, 33)
Same as usual years	11/21 (52.4%, 16)	13/37 (35.1%, 33)
Volume of ventilation in the period in the calf barn		
Could not be done in some period	2/21 (9.5%, 16)	4/37 (10.8%, 33)
Decreased	2/21 (9.5%, 16)	7/37 (18.9%, 33)
Same as usual years	12/21 (57.1%, 16)	14/37 (37.8%, 33)
Volume of ventilation in the period in other barns		
Could not be done in some period	0/21 (0.0%, 16)	1/37 (2.7%, 33)
Decreased	0/21 (0.0%, 16)	1/37 (2.7%, 33)
Same as usual years	8/21 (38.1%, 16)	4/37 (10.8%, 33)
Frequency of removing manure in the period in the milking cow barn		
Could not be done in some period	4/21 (19.0%, 16)	8/37 (21.6%, 33)
Decreased	2/21 (9.5%, 16)	3/37 (8.1%, 33)
Same as usual years	14/21 (66.7%, 16)	20/37 (54.1%, 33)
Frequency of removing manure in the period in the heifer barn		
Could not be done in some period	6/21 (28.6%, 16)	16/37 (43.2%, 33)
Decreased	3/21 (14.3%, 16)	9/37 (24.3%, 33)
Same as usual years	11/21 (52.4%, 16)	9/37 (24.3%, 33)
Frequency of removing manure in the period in the calf barn		
Could not be done in some period	4/21 (19.0%, 16)	11/37 (29.7%, 33)
Decreased	3/21 (14.3%, 16)	5/37 (13.5%, 33)
Same as usual years	12/21 (57.1%, 16)	10/37 (27.0%, 33)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Frequency of removing manure in the period in other barns		
Could not be done in some period	1/21 (4.8%, 16)	2/37 (5.4%, 33)
Decreased	1/21 (4.8%, 16)	3/37 (8.1%, 33)
Same as usual years	8/21 (38.1%, 16)	3/37 (8.1%, 33)
Frequency of changing bedding in the period in the milking cow barn		
Could not be done in some period	2/21 (9.5%, 16)	5/37 (13.5%, 33)
Decreased	1/21 (4.8%, 16)	3/37 (8.1%, 33)
Same as usual years	12/21 (57.1%, 16)	19/37 (51.4%, 33)
Frequency of changing bedding in the period in the heifer barn		
Could not be done in some period	2/21 (9.5%, 16)	12/37 (32.4%, 33)
Decreased	1/21 (4.8%, 16)	8/37 (21.6%, 33)
Same as usual years	11/21 (52.4%, 16)	9/37 (24.3%, 33)
Frequency of changing bedding in the period in the calf barn		
Could not be done in some period	2/21 (9.5%, 16)	8/37 (21.6%, 33)
Decreased	3/21 (14.3%, 16)	5/37 (13.5%, 33)
Same as usual years	11/21 (52.4%, 16)	10/37 (27.0%, 33)
Frequency of changing bedding in the period in other barns		
Could not be done in some period	0/21 (0.0%, 16)	3/37 (8.1%, 33)
Decreased	0/21 (0.0%, 16)	1/37 (2.7%, 33)
Same as usual years	8/21 (38.1%, 16)	3/37 (8.1%, 33)
Movement record		
Total number of cows belonged the farm in the period	115.9 (37, 0, 87.1, 88.0, 6.0, 442.0)	93.0 (67, 0, 52.4, 75.0, 25.0, 227.0)
Have ever moved cows from their home farms	27/37 (73.0%, 0)	52/67 (77.6%, 0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
The number of moved cows from their home farms	16.3 (37, 0, 27.4, 4.0, 0.0, 110.0)	29.3 (67, 0, 36.2, 18.0, 0.0, 164.0)
Have ever moved calves from their home farms	24/37 (64.9%, 0)	46/67 (68.7%, 0)
The number of moved calves from their home farms	10.2 (37, 0, 22.0, 1.0, 0.0, 100.0)	16.0 (67, 0, 27.6, 4.0, 0.0, 164.0)
Have ever introduced cows	17/36 (47.2%, 1)	28/64 (43.8%, 3)
The number of introduced cows	2.1 (32, 5, 4.1, 0.0, 0.0, 16.0)	4.3 (60, 7, 9.4, 0.0, 0.0, 44.0)
Have ever introduced cows from livestock markets	12/32 (37.5%, 5)	21/60 (35.0%, 7)
The number of introduced cows from livestock markets	1.3 (32, 5, 2.6, 0.0, 0.0, 12.0)	2.8 (59, 8, 7.4, 0.0, 0.0, 43.0)
Have ever introduced cows from other farms	9/32 (28.1%, 5)	16/60 (26.7%, 7)
The number of introduced cows from other farms	0.84 (32, 5, 2.5, 0.0, 0.0, 14.0)	0.88 (59, 8, 2.7, 0.0, 0.0, 17.0)
Have ever used communal pastures	22/37 (59.5%, 0)	49/67 (73.1%, 0)
The number of cows which have been sent to communal pastures	10.3 (37, 0, 20.6, 1.0, 0.0, 76.0)	25.1 (67, 0, 35.0, 8.0, 0.0, 164.0)
Have cows which had ever been at livestock markets	15/37 (40.5%, 0)	24/67 (35.8%, 0)
The number of cows which have ever been at livestock markets	1.4 (37, 0, 2.6, 0.0, 0.0, 12.0)	3.6 (67, 0, 8.9, 0.0, 0.0, 44.0)
Mean number of movements for all cows	0.36 (37, 0, 0.54, 0.12, 0.0, 2.0)	0.75 (67, 0, 0.85, 0.53, 0.0, 3.9)
Mean number of movements for moved cows	2.4 (27, 10, 1.0, 2.1, 1.0, 6.0)	2.3 (52, 15, 0.58, 2.1, 1.4, 3.9)
Median number of movements for all cows	0.24 (37, 0, 0.64, 0.0, 0.0, 2.0)	0.54 (67, 0, 0.93, 0.0, 0.0, 4.0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Median number of movements for moved cows	2.2 (27, 10, 1.0, 2.0, 1.0, 6.0)	2.2 (52, 15, 0.63, 2.0, 1.0, 4.0)
Mean age of the first movement (day)	404.1 (27, 10, 386.6, 295.0, 2.0, 1418.8)	411.1 (52, 15, 273.2, 364.9, 2.2, 1313.2)
Median age of the first movement (day)	395.1 (27, 10, 412.5, 335.0, 0.0, 1623.5)	408.1 (52, 15, 290.5, 380.0, 2.0, 1500.0)
Mean age of the last movement (day)	668.3 (27, 10, 405.0, 587.6, 6.0, 1783.2)	593.4 (52, 15, 272.0, 560.2, 157.1, 1663.9)
Median age of the last movement (day)	664.1 (27, 10, 423.9, 625.0, 2.0, 1835.0)	594.3 (52, 15, 264.5, 589.8, 2.0, 1590.0)
Cow-level		
Movement record		
Have ever moved from its home farm	12/44 (27.3%, 0)	59/107 (55.1%, 0)
Have ever moved when it was a calf	0/44 (0.0%, 0)	5/107 (4.7%, 0)
The number of movements	0.55 (44, 1.0, 0.0, 0.0, 4.0)	1.3 (107, 1.8, 1.0, 0.0, 12.0)
Age of the first movement (day)	948.5 (12, 569.5, 759.0, 451.0, 2021.0)	1501.7 (59, 854.8, 1580.0, 9.0, 2969.0)
Age of the last movement (day)	1017.1 (12, 513.3, 784.0, 612.0, 2021.0)	1969.2 (59, 652.5, 2088.0, 375.0, 3142.0)
Mean age of movements (day)	984.8 (12, 538.9, 760.2, 531.5, 2021.0)	1715.2 (59, 685.2, 1650.0, 192.0, 2969.7)
Have introduced	12/44 (27.3%, 0)	22/97 (22.7%, 10)
Source of introduction		
Livestock markets	3/6 (50.0%, 38)	1/9 (11.1%, 98)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Farms	3/6 (50.0%, 38)	8/9 (88.9%, 98)
Have ever been at livestock markets	5/37 (13.5%, 7)	13/95 (13.7%, 12)
Have been sent to a communal pasture	6/40 (15.0%, 4)	16/100 (16.0%, 7)
Dairy herd test record		
Milk yield		
Milk yield at the test day (kg)	35.7 (39, 9.1, 35.2, 14.2, 53.5)	28.5 (563, 9.2, 28.6, 0.0, 57.5)
Milk yield for 305 days (kg)	10203.8 (39, 1719.4, 9959.0, 6314.0, 13993.0)	9537.9 (563, 2078.6, 9564.0, 0.0, 16761.0)
Milk yield in the lactation (kg)	4406.4 (39, 2959.5, 3918.0, 319.0, 10489.0)	6188.7 (563, 3999.5, 6076.0, 0.0, 23278.0)
Peak daily milk yield in the lactation (kg)	41.7 (39, 8.4, 42.2, 25.5, 58.7)	38.4 (563, 10.1, 39.0, 0.0, 65.2)
Adjusted daily milk yield (Solid corrected milk yield adjusted to a cow which is in the second parity, delivered on April and whose days in milking is 150) (kg)	29.1 (39, 9.4, 30.2, 0.0, 44.3)	24.9 (563, 13.2, 27.5, 0.0, 54.8)
Adjusted 305 days milk yield (305 days milk yield adjusted to a cow which is 72 months old, delivered on April, and is milked twice per day) (kg)	10957.0 (39, 1439.2, 11130.0, 7025.0, 14030.0)	10665.7 (563, 2359.5, 10795.0, 0.0, 17351.0)
Expected daily milk yield for next 12 months (kg)	34.0 (39, 12.6, 33.0, 0.0, 54.8)	24.8 (563, 15.4, 28.6, 0.0, 57.3)
Milk components		
Fat concentration at the test day (%)	4.1 (39, 0.84, 4.0, 2.7, 6.0)	
Average fat concentration for 305 days (%)	4.1 (39, 0.38, 4.1, 3.2, 4.9)	4.0 (563, 0.57, 4.0, 0.0, 5.2)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Average fat concentration in the lactation (%)	4.2 (39, 0.72, 4.1, 2.9, 6.4)	4.0 (563, 0.83, 4.0, 0.0, 8.3)
Non-fat milk solids (SNF) concentration at the test day (%)	8.7 (39, 0.35, 8.8, 7.9, 9.6)	8.7 (563, 1.1, 8.8, 0.0, 10.1)
Average SNF concentration for 305 days (%)	8.8 (39, 0.23, 8.8, 8.2, 9.2)	8.7 (563, 0.92, 8.7, 0.0, 9.5)
Average SNF concentration in the lactation (%)	8.7 (39, 0.32, 8.8, 7.9, 9.4)	8.6 (563, 1.1, 8.7, 0.0, 9.8)
Protein concentration at the test day (%)	3.2 (39, 0.35, 3.2, 2.8, 4.3)	3.3 (563, 0.53, 3.3, 0.0, 4.6)
Average protein concentration for 305 days (%)	3.3 (39, 0.21, 3.2, 2.9, 3.9)	3.2 (563, 0.37, 3.2, 0.0, 3.9)
Average protein concentration in the lactation (%)	3.2 (39, 0.34, 3.2, 2.6, 4.0)	3.2 (563, 0.46, 3.2, 0.0, 4.4)
Mulk urea nitrogen concentration at the test day (mg/dl)	11.8 (39, 2.0, 11.8, 7.6, 15.5)	12.4 (563, 2.6, 12.5, 0.0, 30.8)
Somatic cell count		
Somatic cell count (×1,000)	115.9 (39, 237.9, 44.0, 9.0, 1455.0)	184.5 (563, 642.4, 49.0, 0.0, 10458.0)
Linear score	2.2 (39, 1.6, 2.0, 0.0, 7.0)	2.3 (563, 1.8, 2.0, 0.0, 9.0)
The number of months with linear score ≥5 in the lactation	0.46 (39, 1.4, 0.0, 0.0, 8.0)	0.71 (563, 1.6, 0.0, 0.0, 12.0)
Delivery		
Parity	2.9 (39, 1.4, 3.0, 1.0, 6.0)	2.7 (563, 1.7, 2.0, 1.0, 10.0)
Days in milking	122.6 (39, 83.4, 107.0, 9.0, 322.0)	191.2 (563, 123.2, 188.0, 0.0, 766.0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Pregnancy status		
Before the first service	13/39 (33.3%, 0)	150/563 (26.6%, 0)
Artificial insemination (AI) was conducted	15/39 (38.5%, 0)	324/563 (57.5%, 0)
Failed to conceive	11/39 (28.2%, 0)	87/563 (15.5%, 0)
Not designated for AI	0/39 (0.0%, 0)	2/563 (0.4%, 0)
Calving interval (day)	436.7 (32, 127.7, 384.5, 315.0, 760.0)	436.5 (383, 103.0, 405.0, 306.0, 913.0)
The number of AI conducted	1.1 (39, 1.1, 1.0, 0.0, 5.0)	1.6 (563, 1.7, 1.0, 0.0, 13.0)
Period from the last AI (day)	62.1 (26, 54.9, 43.0, 0.0, 202.0)	91.7 (411, 66.3, 84.0, 0.0, 338.0)
Period from the last delivery to the first AI (day)	87.2 (26, 69.0, 76.5, 2.0, 244.0)	148.3 (412, 103.0, 141.5, 0.0, 697.0)
Age at the last delivery (month)	51.6 (39, 18.4, 50.0, 21.0, 89.0)	48.2 (563, 22.6, 46.0, 21.0, 139.0)
Difficulty of the last delivery (1 (easy)–5 (difficult))	1.4 (39, 0.74, 1.0, 1.0, 3.0)	1.5 (563, 0.79, 1.0, 1.0, 5.0)
Type of last delivery		
Singleton	37/39 (94.9%, 0)	503/563 (89.3%, 0)
Multiplets	0/39 (0.0%, 0)	40/563 (7.1%, 0)
Stillbirth or abortion	2/39 (5.1%, 0)	20/563 (3.6%, 0)
Cow profile		
Body weight (kg)	645.5 (2, 78.5, 645.5, 590.0, 701.0)	614.1 (11, 73.8, 590.0, 529.0, 720.0)
Age (month)	55.7 (39, 19.4, 55.0, 23.0, 99.0)	54.5 (563, 23.0, 52.0, 21.0, 144.0)

Supplementary Table S1. (continued)

Variable	Infected	Non-infected
Concentrates fed (kg)	12.4 (39, 2.2, 13.5, 10.0, 15.0)	12.6 (563, 2.0, 13.5, 0.0, 15.0)
Clinical record		
Respiratory disease–pneumonia–*Mycoplasma*	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Respiratory disease–pneumonia–bacteria	5/58 (8.6%, 0)	14/1742 (0.8%, 0)
Disease of udder and teat–peracute mastitis–other bacteria	1/58 (1.7%, 0)	3/1742 (0.2%, 0)
Disease of udder and teat–peracute mastitis–other microbe	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–acute mastitis–other bacteria	13/58 (22.4%, 0)	15/1742 (0.9%, 0)
Disease of udder and teat–acute mastitis–other microbe	5/58 (8.6%, 0)	8/1742 (0.5%, 0)
Disease of udder and teat–chronic mastitis–other bacteria	1/58 (1.7%, 0)	4/1742 (0.2%, 0)
Disease of udder and teat–chronic mastitis–other microbe	0/58 (0.0%, 0)	3/1742 (0.2%, 0)
Disease of udder and teat–subclinical mastitis–other bacteria	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–subclinical mastitis–other microbe	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–mastitis in dry period–other bacteria	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–mastitis in dry period–other microbe	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–mastitis in heifer–other bacteria	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of udder and teat–mastitis in heifer–other microbe	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of sensory organ–otitis media	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease of limb–arthritis–infectious	0/58 (0.0%, 0)	4/1742 (0.2%, 0)
Disease by bacteria or fungus–bovine *Mycoplasma* mastitis	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease by bacteria or fungus–other *Mycoplasma* infection–arthritis	0/58 (0.0%, 0)	0/1742 (0.0%, 0)
Disease by bacteria or fungus–other *Mycoplasma* infection–other	0/58 (0.0%, 0)	0/1742 (0.0%, 0)

Supplementary Table S2. List of herd-level variables and results of the univariable analysis

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Questionnaires								
Farm information								
Type of business					0.9	(0.3–2.5)	1.000	No
Family-run	30/37	(81.1%)	58/70	(82.9%)				
Cooperative	7/37	(18.9%)	12/70	(17.1%)				
Type of farming					1.1	(0.2–6.3)	1.000	No
Dairy only	34/36	(94.4%)	62/66	(93.9%)				
Mixed	2/36	(5.6%)	4/66	(6.1%)				
(If beef cows were kept,) keep beef cows in the same farm	2/2	(100.0%)	3/3	(100.0%)			-	No
The number of workers	3.0	(n = 37)	3.0	(n = 70)			0.365	No
The year the farm was opened	1956.0	(n = 36)	1950.0	(n = 65)			0.480	No
The year the farm owner started farming	1990.0	(n = 37)	1990.0	(n = 68)			0.428	No
The number of cows								
Milking cows	90.0	(n = 37)	68.5	(n = 70)			0.036	No
Dry cows	14.0	(n = 37)	10.0	(n = 70)			0.316	No
Heifers	60.0	(n = 37)	40.0	(n = 70)			0.096	No
Calves	20.0	(n = 37)	10.0	(n = 70)			0.031	No
Total	185.0	(n = 37)	34.5	(n = 70)			0.052	Yes
Experience of Mycoplasma *infection*								
The number of cows infected by *Mycoplasma* in the outbreak	5.9	(Mean; n = 28)	N/A				-	No
Have experienced *Mycoplasma* mastitis within two years in group farms	4/28	(14.3%)	0/55	(0.0%)	20.4	(1.1–393.5)	0.011	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
(If yes,) the number of cows infected	3.3	(Mean; n = 3)	N/A				-	No
Knew that frequent occurrence of *Mycoplasma* mastitis in neighborhood recently	24/37	(64.9%)	44/66	(66.7%)	0.9	(0.4–2.2)	1.000	No
Changed hygiene management after *Mycoplasma* infection in the farm or in the neighborhood	17/37	(45.9%)	14/66	(21.2%)	3.2	(1.3–7.6)	0.016	No
Knowledge about Mycoplasma								
Have ever heard the name of a bacterium, *Mycoplasma*	37/37	(100.0%)	67/70	(95.7%)	3.9	(0.2–77.3)	0.550	No
Know that *Mycoplasma* also causes diseases to calves not only mastitis to adult cows	32/37	(86.5%)	52/67	(77.6%)	1.8	(0.6–5.6)	0.401	No
Know that *Mycoplasma* transmit from a calf to a dam by human hands	30/37	(81.1%)	37/67	(55.2%)	3.5	(1.3–9.0)	0.015	No
Considered the possibility of *Mycoplasma* infection in a case of clinical mastitis when no bacteria was isolated	28/37	(75.7%)	35/67	(52.2%)	2.8	(1.2–6.9)	0.033	No
Disease prevention								
Practice of vaccination against respiratory diseases for milking cows							0.400	No
Yes	8/36	(22.2%)	14/69	(20.3%)				
No	22/36	(61.1%)	49/69	(71.0%)				
Not sure	6/36	(16.7%)	6/69	(8.7%)				

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Practice of vaccination against respiratory diseases for heifers							0.674	No
Yes	23/36	(63.9%)	40/69	(58.0%)				
No	10/36	(27.8%)	25/69	(36.2%)				
Not sure	3/36	(8.3%)	4/69	(5.8%)				
Practice of vaccination against respiratory diseases for calves							0.703	No
Yes	20/35	(57.1%)	42/69	(60.9%)				
No	12/35	(34.3%)	24/69	(34.8%)				
Not sure	3/35	(8.6%)	3/69	(4.3%)				
Prevention of intrusion of wild animals to the milking cow barn	20/37	(54.1%)	36/69	(52.2%)	1.1	(0.5–2.4)	1.000	No
Prevention of intrusion of wild animals to the heifer barn	12/34	(35.3%)	18/64	(28.1%)	1.4	(0.6–3.4)	0.615	No
Prevention of intrusion of wild animals to the calf barn	13/31	(41.9%)	23/60	(38.3%)	1.2	(0.5–2.8)	0.915	No
Presence of a power sprayer to disinfect vehicles which enter the farm	2/37	(5.4%)	4/70	(5.7%)	0.9	(0.2–5.4)	1.000	No
Set and apparently divide the sanitation control zone	31/37	(83.8%)	56/70	(80.0%)	1.3	(0.5–3.7)	0.828	No
Wear dedicated clothes in the sanitation control zone	12/37	(32.4%)	24/70	(34.3%)	0.9	(0.4–2.1)	1.000	No
Park vehicles of farm workers outside the sanitation control zone	10/37	(27.0%)	28/69	(40.6%)	0.5	(0.2–1.3)	0.240	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Set disinfectant foot baths at barns							0.718	No
At all barns	16/37	(43.2%)	34/69	(49.3%)				
One in the whole sanitation control zone	17/37	(45.9%)	30/69	(43.5%)				
No foot baths	4/37	(10.8%)	5/69	(7.2%)				
Remove dirt on boots before step into disinfectants foot baths	28/33	(84.8%)	50/64	(78.1%)	1.6	(0.5–4.8)	0.603	No
Frequency of changing disinfectants in foot baths							0.091	No
More than daily	4/33	(12.1%)	2/63	(3.2%)				
Daily	5/33	(15.2%)	13/63	(20.6%)				
More than weekly	21/33	(63.6%)	30/63	(47.6%)				
Weekly	3/33	(9.1%)	15/63	(23.8%)				
Less frequently	0/33	(0.0%)	3/63	(4.8%)				
Type of disinfectant used (description question)								
Chlorine disinfectant	26/28	(92.9%)	44/50	(88.0%)	1.8	(0.3–9.4)	0.704	No
Invert soap	1/28	(3.6%)	6/50	(12.0%)	0.3	(0.0–2.4)	0.411	No
Hydrated lime	0/28	(0.0%)	2/50	(4.0%)	0.3	(0.1–7.3)	0.534	No
Scatter hydrated lime powder at farm entrance	27/36	(75.0%)	45/68	(66.2%)	1.5	(0.6–3.8)	0.481	No
Conducted hygiene control measures to vehicles of farm workers (multiple answers allowed)								
Rinse the vehicle before disinfection	3/34	(8.8%)	4/66	(6.1%)	1.5	(0.3–7.1)	0.687	No
Disinfect the whole vehicle	0/34	(0.0%)	2/66	(3.0%)	0.4	(0.0–8.0)	0.547	No
Disinfect the wheel wells	3/34	(8.8%)	8/66	(12.1%)	0.7	(0.2–2.8)	0.745	No
Disinfect the driver seat floor mat	2/34	(5.9%)	2/65	(3.1%)	2.0	(0.3–	0.605	No
Disinfect the bed of the vehicle	1/34	(2.9%)	2/66	(3.0%)	1.0	(0.1–11.1)	1.000	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
The farm owner set guideline of disinfection of vehicles other than farm workers' ones	1/32	(3.1%)	3/62	(4.8%)	0.6	(0.1–6.4)	1.000	No
(If yes,) conducted hygiene control measures to vehicles other than farm workers' ones (multiple answers allowed)								
Rinse the vehicle before disinfection	0/1	(0.0%)	0/3	(0.0%)			-	No
Disinfect the whole vehicle	0/1	(0.0%)	0/3	(0.0%)			-	No
Disinfect the wheel wells	1/1	(100.0%)	3/3	(100.0%)			-	No
Disinfect the driver seat floor mat	0/1	(0.0%)	1/3	(33.3%)			-	No
Disinfect the bed of the vehicle	1/1	(100.0%)	3/3	(100.0%)			-	No
Milking hygiene								
Use a milking parlor	21/22	(95.5%)	20/23	(87.0%)			-	No
Existence of a backflush system in the milking system	1/22	(4.5%)	0/23	(0.0%)			-	No
Use a milking robots	3/22	(13.6%)	1/23	(4.3%)			-	No
Use towels to wipe teats							1.000	Yes
Yes	34/37	(91.9%)	64/70	(91.4%)				
No	0/37	(0.0%)	0/70	(0.0%)				
Paper towels only	3/37	(8.1%)	6/70	(8.6%)				
Use one towel per cow	27/34	(79.4%)	52/64	(81.2%)	0.9	(0.3–2.5)	1.000	No
Dip a towel to disinfectant	28/34	(82.4%)	49/64	(76.6%)	1.4	(0.5–4.1)	0.684	No
Consciously wipe teat openings	30/37	(81.1%)	67/70	(95.7%)	0.2	(0.0–0.8)	0.030	Yes
Use a paper towel after a cloth towel to wipe teats	6/37	(16.2%)	24/69	(34.8%)	0.4	(0.1–1.0)	0.072	Yes
Do pre-dipping	19/37	(51.4%)	29/70	(41.4%)	1.5	(0.7–3.3)	0.437	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Do post-dipping	36/37	(97.3%)	67/70	(95.7%)	1.6	(0.2–16.1)	1.000	No
Use a cart to convey milking equipment	16/36	(44.4%)	45/69	(65.2%)	0.4	(0.2–1.0)	0.066	No
Use a strip cup	16/37	(43.2%)	38/70	(54.3%)	0.6	(0.3–1.4)	0.377	No
Actively call veterinarians when an abnormality was found by a strip cup	15/35	(42.9%)	27/60	(45.0%)	0.9	(0.4–2.1)	1.000	No
Actively call veterinarians when an abnormality was found by PL test	26/37	(70.3%)	42/70	(60.0%)	1.6	(0.7–3.7)	0.402	No
Use adequately disinfected milking equipment	36/37	(97.3%)	66/70	(94.3%)	2.2	(0.2–20.3)	0.657	No
Disinfect milking equipment after milking	33/37	(89.2%)	59/70	(84.3%)	1.5	(0.5–5.2)	0.688	No
Disinfect milking equipment before milk a next cow	16/37	(43.2%)	6/70	(8.6%)	8.1	(2.8–23.5)	0.000	No
Milk cows with high somatic cell count last	7/37	(18.9%)	10/68	(14.7%)	1.4	(0.5–3.9)	0.778	No
Milk mastitis cows last	16/37	(43.2%)	22/68	(32.4%)	1.6	(0.7–3.6)	0.370	No
Conduct mastitis test by *Mycoplasma* after the first calving of a home-bred heifer	5/37	(13.5%)	1/69	(1.4%)	10.6	(1.2–94.7)	0.019	No
Conduct mastitis test by pathogen other than *Mycoplasma* after the first calving of a home-bred heifer	8/37	(21.6%)	18/70	(25.7%)	0.8	(0.3–2.1)	0.816	No
Conduct a self-imposed test of *Mycoplasma* with bulk tank milk	16/36	(44.4%)	24/70	(34.3%)	1.5	(0.7–3.5)	0.418	No
Disinfect milking units until the result of *Mycoplasma* test was available after the first calving	6/37	(16.2%)	3/69	(4.3%)	4.3	(1.0–18.2)	0.063	No
Calf handling								
Timing when a calf is separated from its dam after a delivery								
Immediately after the delivery	16/23	(69.6%)	17/47	(36.2%)	4.0	(1.4–11.7)	0.018	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
When realized the delivery finished	8/23	(34.8%)	28/47	(59.6%)	0.4	(0.1–1.0)	0.090	No
Keep them together for a while	1/23	(4.3%)	4/47	(8.5%)	0.5	(0.1–4.6)	1.000	No
Other	0/23	(0.0%)	0/47	(0.0%)			-	No
Period to keep a calf and a dam together (days)	2.0	(n = 1)	1.5	(n = 4)			-	No
Way to feed colostrum								
Direct from the dam	1/25	(4.0%)	1/46	(2.2%)	1.9	(0.1–31.3)	1.000	No
By a feeding tool	21/25	(84.0%)	39/46	(84.8%)	0.9	(0.2–3.6)	1.000	No
Feed frozen colostrum	7/25	(28.0%)	10/46	(21.7%)	1.4	(0.5–4.3)	0.765	No
Feed artificial colostrum	8/25	(32.0%)	14/46	(30.4%)	1.1	(0.4–3.1)	1.000	No
Way to feed milk to calves								
By a dam	2/25	(8.0%)	0/45	(0.0%)	9.7	(0.4–210.0)	0.124	Yes
(If yes,) the day start milking	1.0	(n = 2)	N/A				-	No
The day end milking	1.5	(n = 2)	N/A				-	No
By a milking bucket	1/25	(4.0%)	10/45	(22.2%)	0.1	(0.0–1.2)	0.083	Yes
(If yes,) the day start milking	1.0	(n = 1)	0.0	(n = 10)			-	No
The day end milking	40.0	(n = 1)	37.5	(n = 10)			-	No
By a milking bin	24/25	(96.0%)	35/45	(77.8%)	6.9	(0.8–57.1)	0.083	Yes
(If yes,) the day start milking	1.0	(n = 24)	1.0	(n = 35)			-	No
The day end milking	9.0	(n = 24)	7.0	(n = 35)			-	No
By a bucket	6/25	(24.0%)	14/45	(31.1%)	0.7	(0.2–2.1)	0.723	No
(If yes,) the day start milking	9.0	(n = 6)	8.0	(n = 14)			-	No
The day end milking	60.0	(n = 6)	60.0	(n = 13)			-	No
Same worker takes care of calves and milking cows	17/25	(68.0%)	33/46	(71.7%)	0.8	(0.3–2.4)	0.954	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
(If yes,) timing of taking care of calves							0.118	No
Before milking	8/17	(47.1%)	17/34	(50.0%)				
After milking	8/17	(47.1%)	8/34	(23.5%)				
Not decided	1/17	(5.9%)	9/34	(26.5%)				
Change gloves between taking care of calves and taking care of milking cows							0.768	No
Yes	12/21	(57.1%)	17/36	(47.2%)				
No	6/21	(28.6%)	11/36	(30.6%)				
No gloves used	3/21	(14.3%)	8/36	(22.2%)				
Change cloths between taking care of calves and taking care of milking cows	2/20	(10.0%)	2/36	(5.6%)	1.9	(0.2–14.5)	0.611	No
Communal pastures								
Use communal pastures							0.034	No
Using more than several years	8/37	(21.6%)	32/69	(46.4%)				
Started to use in this year	2/37	(5.4%)	1/69	(1.4%)				
Have been used before	2/37	(5.4%)	5/69	(7.2%)				
Never used	25/37	(67.6%)	31/69	(44.9%)				
For farms which have ever used communal pastures,								
Type of cows been send to the communal pasture (multiple answers allowed)								
Heifers	12/12	(100.0%)	33/38	(86.8%)			-	No
Dry cows	0/12	(0.0%)	0/38	(0.0%)			-	No
Other	2/12	(16.7%)	4/38	(10.5%)			-	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Owner of the communal pasture							-	No
A public organization	2/11	(18.2%)	11/29	(37.9%)				
A neighbor farmer	2/11	(18.2%)	3/29	(10.3%)				
An agricultural cooperative	5/11	(45.5%)	10/29	(34.5%)				
Other	2/11	(18.2%)	5/29	(17.2%)				
Introduction								
Have ever introduced cows	13/37	(35.1%)	14/68	(20.6%)	2.1	(0.9–5.1)	0.163	Yes
Type of introduced cows (multiple answers allowed)								
Introduce non-pregnant heifers	4/37	(10.8%)	2/68	(2.9%)	4.0	(0.7–23.0)	0.181	Yes
Frequency of introduction of non-pregnant heifers							0.063	No
Every year	1/37	(2.7%)	1/68	(1.5%)				
Once in two years	0/37	(0.0%)	1/68	(1.5%)				
Once in five years	3/37	(8.1%)	0/68	(0.0%)				
Never	33/37	(89.2%)	66/68	(97.1%)				
The number of introduced non-pregnant heifers at the latest introduction	10.5	(n = 2)	150.0	(n = 1)			-	No
Introduce pregnant heifers	5/37	(13.5%)	10/68	(14.7%)	0.9	(0.3–2.9)	1.000	No
Frequency of introduction of pregnant heifers							0.114	No
Every year	4/37	(10.8%)	4/67	(6.0%)				
Once in two years	0/37	(0.0%)	5/67	(7.5%)				
Once in five years	1/37	(2.7%)	0/67	(0.0%)				
Never	32/37	(86.5%)	58/67	(86.6%)				

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
The number of introduced pregnant heifers at the latest introduction	5.0	(n = 5)	3.5	(n = 6)			-	No
Introduce delivered cows	8/37	(21.6%)	10/68	(14.7%)	1.6	(0.6–4.5)	0.531	No
Frequency of introduction of delivered cows							0.892	No
Every year	2/33	(6.1%)	2/67	(3.0%)				
Once in two years	1/33	(3.0%)	4/67	(6.0%)				
Once in five years	1/33	(3.0%)	3/67	(4.5%)				
Never	29/33	(87.9%)	58/67	(86.6%)				
The number of introduced delivered at the latest introduction	5.0	(n = 8)	4.0	(n = 5)			-	No
Introduce other cows	0/37	(0%)	0/65	(0%)	1.7	(0.0–89.8)	1.000	No
Frequency of introduction of other cows							1.000	No
Every year	0/36	(0.0%)	0/65	(0.0%)				
Once in two years	0/36	(0.0%)	0/65	(0.0%)				
Once in five years	0/36	(0.0%)	0/65	(0.0%)				
Never	36/36	(100.0%)	65/65	(100.0%)				
The number of introduced other cows at the latest introduction	30.0	(n = 1)	N/A				-	No
For farms which have ever used introduced cows,								
Source of introduction (multiple answers allowed)								
An agricultural cooperative	12/14	(85.7%)	10/13	(76.9%)			-	No
A farm of an acquaintance	2/14	(14.3%)	1/13	(7.7%)			-	No
A group farm	0/14	(0.0%)	0/13	(0.0%)			-	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
A livestock dealer	4/14	(28.6%)	1/13	(7.7%)			-	No
Other	1/14	(7.1%)	1/13	(7.7%)			-	No
Most frequently introduced source								
An agricultural cooperative	10/14	(71.4%)	10/13	(76.9%)			-	No
A farm of an acquaintance	0/14	(0.0%)	1/13	(7.7%)			-	No
A group farm	0/14	(0.0%)	0/13	(0.0%)			-	No
A livestock dealer	3/14	(21.4%)	1/13	(7.7%)			-	No
Other	1/14	(7.1%)	1/13	(7.7%)			-	No
Mastitis test by *Mycoplasma* to cows introduced from a livestock market	2/14	(14.3%)	1/13	(7.7%)			-	No
Mastitis test by pathogen other than *Mycoplasma* to cows introduced from a livestock market	2/14	(14.3%)	2/13	(15.4%)			-	No
Quarantine of introduced cows							-	No
Have a barn only for introduced cows	1/13	(7.7%)	0/13	(0.0%)				
Have a barn not only for introduced cows	3/13	(23.1%)	3/13	(23.1%)				
No quarantine	9/13	(69.2%)	10/13	(76.9%)				
Quarantine period							-	No
One day	0/3	(0.0%)	0/2	(0.0%)				
Less than a week	3/3	(100.0%)	2/2	(100.0%)				
A week or more	0/3	(0.0%)	0/2	(0.0%)				
Farm workers check health condition of introduced cows	10/20	(50.0%)	17/32	(53.1%)	0.9	(0.3–2.7)	1.000	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Barns								
Housing for milking cows								
Tie stall	12/37	(32.4%)	46/70	(65.7%)	0.3	(0.1–0.6)	0.002	Yes
Free stall	24/37	(64.9%)	27/70	(38.6%)	2.9	(1.3–6.7)	0.017	No
Free barn	1/37	(2.7%)	0/70	(0.0%)	5.8	(0.2–145.8)	0.346	No
Rangeland	2/37	(5.4%)	5/70	(7.1%)	0.7	(0.1–4.0)	1.000	No
Other	1/37	(2.7%)	0/70	(0.0%)	5.8	(0.2–145.8)	0.346	No
Housing for dry cows								
Tie stall	8/35	(22.9%)	21/67	(31.3%)	0.6	(0.3–1.7)	0.502	No
Free stall	16/35	(45.7%)	23/67	(34.3%)	1.6	(0.7–3.7)	0.363	No
Free barn	11/35	(31.4%)	15/67	(22.4%)	1.6	(0.6–4.0)	0.450	No
Rangeland	6/35	(17.1%)	11/67	(16.4%)	1.1	(0.4–3.1)	1.000	No
Other	1/35	(2.9%)	4/67	(6.0%)	0.5	(0.0–4.3)	0.658	No
Housing for heifers								
Tie stall	4/36	(11.1%)	15/68	(22.1%)	0.4	(0.1–1.4)	0.268	No
Free stall	5/36	(13.9%)	5/68	(7.4%)	2.0	(0.5–7.5)	0.309	No
Free barn	24/36	(66.7%)	36/68	(52.9%)	1.8	(0.8–4.1)	0.255	No
Rangeland	10/36	(27.8%)	23/68	(33.8%)	0.8	(0.3–1.8)	0.683	No
Other	5/36	(13.9%)	6/68	(8.8%)	1.7	(0.5–5.9)	0.507	No
Housing for calves								
One calf per pen	11/35	(31.4%)	25/68	(36.8%)	0.8	(0.3–1.9)	0.749	No
Several calves per pen	9/35	(25.7%)	23/68	(33.8%)	0.7	(0.3–1.7)	0.537	No
Hatch	13/35	(37.1%)	24/68	(35.3%)	1.1	(0.5–2.5)	1.000	No
Free barn	7/35	(20.0%)	5/68	(7.4%)	3.1	(0.9–10.8)	0.101	Yes

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Rangeland	2/35	(5.7%)	4/68	(5.9%)	1.0	(0.2–5.6)	1.000	No
Other	3/35	(8.6%)	2/68	(2.9%)	3.1	(0.5–19.4)	0.334	No
Volume of bedding in the milking cow barn							0.303	No
Enough bedding	25/37	(67.6%)	36/70	(51.4%)				
The floor can be seen through bedding	10/37	(27.0%)	27/70	(38.6%)				
No bedding	2/37	(5.4%)	7/70	(10.0%)				
Volume of bedding in the heifer barn							0.403	No
Enough bedding	17/32	(53.1%)	27/69	(39.1%)				
The floor can be seen through bedding	9/32	(28.1%)	27/69	(39.1%)				
No bedding	6/32	(18.8%)	15/69	(21.7%)				
Volume of bedding in the calf barn							0.536	No
Enough bedding	30/33	(90.9%)	57/67	(85.1%)				
The floor can be seen through bedding	3/33	(9.1%)	10/67	(14.9%)				
No bedding	0/33	(0.0%)	0./67	(0.0%)				
Bedding in the milking cow barn (multiple answers allowed)								
Mattress	16/37	(43.2%)	28/70	(40.0%)	1.1	(0.5–2.6)	0.906	No
Sand	1/37	(2.7%)	1/70	(1.4%)	1.9	(0.1–31.5)	1.000	No
Paper	1/37	(2.7%)	0/70	(0.0%)	5.8	(0.2–145.8)	0.346	No
Sawdust	13/37	(35.1%)	17/70	(24.3%)	1.7	(0.7–4.0)	0.336	No
Compost	0/37	(0.0%)	1/70	(1.4%)	0.6	(0.0–15.5)	1.000	No
Straw	14/37	(37.8%)	35/70	(50.0%)	0.6	(0.3–1.4)	0.319	No
Pasture grass	3/37	(8.1%)	2/70	(2.9%)	3.0	(0.5–18.8)	0.338	No
Chaff	3/37	(8.1%)	8/70	(11.4%)	0.7	(0.2–2.7)	0.744	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Other	4/37	(10.8%)	11/70	(15.7%)	0.7	(0.2–2.2)	0.688	No
Bedding in the heifer barn (multiple answers allowed)								
Mattress	5/32	(15.6%)	5/60	(8.3%)	2.0	(0.5–7.6)	0.309	No
Sand	1/32	(3.1%)	2/60	(3.3%)	0.9	(0.1–10.7)	1.000	No
Paper	1/32	(3.1%)	1/60	(1.7%)	1.9	(0.1–31.5)	1.000	No
Sawdust	8/32	(25.0%)	11/60	(18.3%)	1.5	(0.5–4.2)	0.630	No
Compost	0/32	(0.0%)	2/60	(3.3%)	0.4	(0.0–7.7)	0.541	No
Straw	21/32	(65.6%)	42/60	(70.0%)	0.8	(0.3–2.0)	0.846	No
Pasture grass	3/32	(9.4%)	4/60	(6.7%)	1.4	(0.3–6.9)	0.691	No
Chaff	0/32	(0.0%)	2/60	(3.3%)	0.4	(0.0–7.7)	0.541	No
Other	2/32	(6.2%)	2/60	(3.3%)	1.9	(0.3–14.4)	0.608	No
Bedding in the calf barn (multiple answers allowed)								
Mattress	1/33	(3.0%)	2/67	(3.0%)	1.0	(0.1–11.6)	1.000	No
Sand	0/33	(0.0%)	0/67	(0.0%)	2.0	(0.0-103.8)	1.000	No
Paper	0/33	(0.0%)	0/67	(0.0%)	2.0	(0.0-103.8)	1.000	No
Sawdust	5/33	(15.2%)	7/67	(10.4%)	1.5	(0.4–5.2)	0.524	No
Compost	0/33	(0.0%)	1/67	(1.5%)	0.7	(0.0–16.7)	1.000	No
Straw	30/33	(90.9%)	60/67	(89.6%)	1.2	(0.3–4.8)	1.000	No
Pasture grass	1/33	(3.0%)	5/67	(7.5%)	0.4	(0.0–3.5)	0.661	No
Chaff	0/33	(0.0%)	1/67	(1.5%)	0.7	(0.0–16.7)	1.000	No
Other	1/33	(3.0%)	0/67	(0.0%)	6.2	(0.2–157.2)	0.330	No
Scatter hydrated lime powder on bedding in the milking cow barn	20/37	(54.1%)	29/70	(41.4%)	1.7	(0.7–3.7)	0.297	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Scatter hydrated lime powder on bedding in the milking cow barn	20/37	(54.1%)	29/70	(41.4%)	1.7	(0.7–3.7)	0.297	No
(If yes,) frequency of use							-	No
Daily or more	7/19	(36.8%)	12/23	(52.2%)				
Weekly or more	7/19	(36.8%)	3/23	(13.0%)				
Monthly or more	4/19	(21.1%)	5/23	(21.7%)				
Yearly or more	0/19	(0.0%)	1/23	(4.3%)				
Less frequently	1/19	(5.3%)	2/23	(8.7%)				
Frequency of changing bedding in the milking barn in summer							0.708	No
Daily or more	16/32	(50.0%)	38/64	(59.4%)				
Weekly or more	10/32	(31.2%)	18/64	(28.1%)				
Monthly or more	4/32	(12.5%)	5/64	(7.8%)				
Less frequently	2/32	(6.2%)	3/64	(4.7%)				
Frequency of changing bedding in the milking barn in winter							0.370	No
Daily or more	16/32	(50.0%)	41/65	(63.1%)				
Weekly or more	11/32	(34.4%)	17/65	(26.2%)				
Monthly or more	5/32	(15.6%)	5/65	(7.7%)				
Less frequently	0/32	(0.0%)	2/65	(3.1%)				

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Frequency of changing bedding in the heifer barn in summer							0.633	No
Daily or more	7/28	(25.0%)	20/58	(34.5%)				
Weekly or more	15/28	(53.6%)	22/58	(37.9%)				
Monthly or more	4/28	(14.3%)	10/58	(17.2%)				
Less frequently	2/28	(7.1%)	6/58	(10.3%)				
Frequency of changing bedding in the heifer barn in winter							0.633	No
Daily or more	7/28	(25.0%)	20/58	(34.5%)				
Weekly or more	15/28	(53.6%)	22/58	(37.9%)				
Monthly or more	4/28	(14.3%)	10/58	(17.2%)				
Less frequently	2/28	(7.1%)	6/58	(10.3%)				
Frequency of changing bedding in the calf barn in summer							0.763	No
Daily or more	7/29	(24.1%)	21/66	(31.8%)				
Weekly or more	19/29	(65.5%)	35/66	(53.0%)				
Monthly or more	3/29	(10.3%)	9/66	(13.6%)				
Less frequently	0/29	(0.0%)	1/66	(1.5%)				
Frequency of changing bedding in the calf barn in winter							0.698	No
Daily or more	7/29	(24.1%)	21/66	(31.8%)				
Weekly or more	19/29	(65.5%)	35/66	(53.0%)				
Monthly or more	3/29	(10.3%)	8/66	(12.1%)				
Less frequently	0/29	(0.0%)	2/66	(3.0%)				

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Frequency of removing manure in the milking cow barn (_ times per day)	2.0	(n = 27)	3.0	(n = 47)			0.386	No
Frequency of removing manure in the heifer barn							0.765	No
Daily or more	17/30	(56.7%)	34/58	(58.6%)				
Weekly or more	10/30	(33.3%)	15/58	(25.9%)				
Monthly or more	3/30	(10.0%)	6/58	(10.3%)				
Less frequently	0/30	(0.0%)	3/58	(5.2%)				
Frequency of removing manure in the calf barn							0.577	No
Daily or more	8/30	(26.7%)	23/65	(35.4%)				
Weekly or more	19/30	(63.3%)	33/65	(50.8%)				
Monthly or more	3/30	(10.0%)	9/65	(13.8%)				
Regular disinfection in the milking cow barn	15/35	(42.9%)	33/68	(48.5%)	0.8	(0.3–1.8)	0.735	No
(If yes,) frequency of disinfection							-	No
Weekly or more	2/12	(16.7%)	1/27	(3.7%)				
Monthly or more	5/12	(41.7%)	14/27	(51.9%)				
Yearly or more	5/12	(41.7%)	12/27	(44.4%)				
Less frequently	0/12	(0.0%)	0/27	(0.0%)				
Type of disinfectant used (description question)								
Outsourced to an agricultural cooperative	3/12	(25.0%)	12/23	(52.2%)			-	No
Chlorine disinfectant	3/12	(25.0%)	3/23	(13.0%)			-	No
Invert soap	3/12	(25.0%)	6/23	(26.1%)			-	No
Hydrated lime	2/12	(16.7%)	2/23	(8.7%)			-	No
Regular disinfection in the heifer barn	13/33	(39.4%)	20/63	(31.7%)	1.4	(0.6–3.4)	0.601	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
(If yes,) frequency of disinfection							-	No
Weekly or more	2/11	(18.2%)	0/18	(0.0%)				
Monthly or more	3/11	(27.3%)	10/18	(55.6%)				
Yearly or more	5/11	(45.5%)	8/18	(44.4%)				
Less frequently	1/11	(9.1%)	0/18	(0.0%)				
Type of disinfectant used (description question)								
Outsourced to an agricultural cooperative	0/6	(0.0%)	2/11	(18.2%)			-	No
Chlorine disinfectant	3/6	(50.0%)	0/11	(0.0%)			-	No
Invert soap	1/6	(16.7%)	3/11	(27.3%)			-	No
Hydrated lime	3/6	(50.0%)	4/11	(36.4%)			-	No
Regular disinfection in the calf barn	20/29	(69.0%)	31/61	(50.8%)	2.2	(0.8–5.5)	0.163	No
(If yes,) frequency of disinfection							-	No
Weekly or more	5/17	(29.4%)	8/27	(29.6%)				
Monthly or more	8/17	(47.1%)	8/27	(29.6%)				
Yearly or more	1/17	(5.9%)	10/27	(37.0%)				
Less frequently	3/17	(17.6%)	1/27	(3.7%)				
Type of disinfectant used (a description type question)								
Outsourced to an agricultural cooperative	0/10	(0.0%)	1/20	(5.0%)			-	No
Chlorine disinfectant	1/10	(10.0%)	1/20	(5.0%)			-	No
Invert soap	2/10	(20.0%)	1/20	(5.0%)			-	No
Hydrated lime	5/10	(50.0%)	12/20	(60.0%)			-	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Water supply equipment in the milking cow barn								
Water tank	6/7	(85.7%)	6/16	(37.5%)			-	No
Water cup	1/7	(14.3%)	12/16	(75.0%)			-	No
Frequency of cleaning the water supply equipment in the milking cow barn							0.393	No
Daily or more	4/27	(14.8%)	4/55	(7.3%)				
Weekly or more	14/27	(51.9%)	22/55	(40.0%)				
Monthly or more	3/27	(11.1%)	14/55	(25.5%)				
Less frequently	3/27	(11.1%)	10/55	(18.2%)				
When it get dirty	3/27	(11.1%)	5/55	(9.1%)				
Water supply equipment in the heifer barn								
Water tank	3/4	(75.0%)	8/13	(61.5%)			-	No
Water cup	2/4	(50.0%)	5/13	(38.5%)			-	No
Frequency of cleaning the water supply equipment in the heifer barn							0.594	No
Daily or more	2/32	(6.2%)	7/68	(10.3%)				
Weekly or more	13/32	(40.6%)	20/68	(29.4%)				
Monthly or more	6/32	(18.8%)	17/68	(25.0%)				
Less frequently	5/32	(15.6%)	16/68	(23.5%)				
When it get dirty	6/32	(18.8%)	8/68	(11.8%)				

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Water supply equipment in the calf barn							-	No
Water tank	0/9	(0.0%)	5/24	(20.8%)				
Water cup	4/9	(44.4%)	4/24	(16.7%)				
Bucket	4/9	(44.4%)	8/24	(33.3%)				
No equipment	1/9	(11.1%)	7/24	(29.2%)				
Frequency of cleaning the water supply equipment in the calf barn							0.968	No
Daily or more	10/29	(34.5%)	20/56	(35.7%)				
Weekly or more	9/29	(31.0%)	14//56	(25.0%)				
Monthly or more	3/29	(10.3%)	8/56	(14.3%)				
Less frequently	4/29	(13.8%)	9/56	(16.1%)				
When it get dirty	3/29	(10.3%)	5/56	(8.9%)				
Use machinery ventilation in the milking cow barn	30/37	(81.1%)	45/70	(64.3%)	2.4	(0.9–6.2)	0.113	Yes
Use machinery ventilation in the heifer barn	8/35	(22.9%)	10/66	(15.2%)	1.7	(0.6–4.7)	0.490	No
Use machinery ventilation in the calf barn	14/32	(43.8%)	12/62	(19.4%)	3.2	(1.3–8.3)	0.024	Yes
Hygiene management during heavy snow								
Remember the condition of hygiene management in the farm from the end of February to early March in 2015 when heavy snow fell	21/37	(56.8%)	37/70	(52.9%)	1.2	(0.5–2.6)	0.856	No
Volume of ventilation was decreased in the period compared with usual winter in the milking cow barn	3/17	(17.6%)	12/29	(41.4%)			-	No
Volume of ventilation was decreased in the period compared with usual winter in the heifer barn	3/14	(21.4%)	12/25	(48.0%)			-	No
Volume of ventilation was decreased in the period compared with usual winter in the calf barn	3/15	(20.0%)	9/23	(39.1%)			-	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Volume of ventilation was decreased in the period compared with usual winter in other barns	0/8	(0.0%)	2/6	(33.3%)			-	No
Frequency of removing manure was decreased in the period compared with usual winter in the milking cow barn	5/18	(27.8%)	10/30	(33.3%)			-	No
Frequency of removing manure was decreased in the period compared with usual winter in the heifer barn	7/18	(38.9%)	23/32	(71.9%)			-	No
Frequency of removing manure was decreased in the period compared with usual winter in the calf barn	6/18	(33.3%)	15/25	(60.0%)			-	No
Frequency of removing manure was decreased in the period compared with usual winter in other barns	1/9	(11.1%)	5/8	(62.5%)			-	No
Frequency of changing bedding was decreased in the period compared with usual winter in the milking cow barn	3/15	(20.0%)	7/26	(26.9%)			-	No
Frequency of changing bedding was decreased in the period compared with usual winter in the heifer barn	3/14	(21.4%)	19/28	(67.9%)			-	No
Frequency of changing bedding was decreased in the period compared with usual winter in the calf barn	4/15	(26.7%)	12/22	(54.5%)			-	No
Frequency of changing bedding was decreased in the period compared with usual winter in other barns	0/8	(0.0%)	4/7	(57.1%)			-	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Movement record								
Total number of cows belonged the farm in the period	88.0	(n = 37)	75.0	(n = 67)			0.163	No
Have ever moved cows from their home farms	27/37	(73.0%)	52/67	(77.6%)	0.8	(0.3–2.0)	0.772	No
The proportion of moved cows from their home farms	0.04	(n = 37)	0.25	(n = 67)			0.031	No
Have ever moved calves from their home farms	24/37	(64.9%)	46/67	(68.7%)	0.8	(0.4–2.0)	0.860	No
The proportion of moved calves from their home farms	0.02	(n = 37)	0.06	(n = 67)			0.133	No
Have ever introduced cows	17/36	(47.2%)	28/64	(43.8%)	1.2	(0.5–2.6)	0.900	No
The proportion of introduced cows	0.00	(n = 32)	0.00	(n = 60)			0.698	No
Have ever introduced cows from livestock markets	12/32	(37.5%)	21/60	(35.0%)	1.1	(0.5–2.7)	0.992	No
The proportion of introduced cows from livestock markets	0.00	(n = 32)	0.00	(n = 59)			0.891	No
Have ever introduced cows from other farms	9/32	(28.1%)	16/60	(26.7%)	1.1	(0.4–2.8)	1.000	No
The proportion of introduced cows from other farms	0.00	(n = 32)	0.00	(n = 59)			0.868	No
Have ever used communal pastures	22/37	(59.5%)	49/67	(73.1%)	0.5	(0.2–1.3)	0.225	No
The proportion of cows which have been sent to communal pastures	0.01	(n = 37)	0.11	(n = 67)			0.006	No
Have cows which had ever been at livestock markets	15/37	(40.5%)	24/67	(35.8%)	1.2	(0.5–2.8)	0.791	No
The proportion of cows which have ever been at livestock markets	0.00	(n = 37)	0.00	(n = 67)			0.916	No
Mean number of movements for all cows	0.4	(n = 37)	0.8	(n = 67)			0.020	No
Mean number of movements for moved cows	2.4	(n = 27)	2.3	(n = 52)			0.950	No

Supplementary Table S2. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds ratio	(95% CI)	p-value	Offered
Median number of movements for all cows	0.2	(n = 37)	0.5	(n = 67)			0.071	No
Median number of movements for moved cows	2.2	(n = 27)	2.2	(n = 52)			0.597	No
Mean age of the first movement (day)	295.0	(n = 27)	364.9	(n = 52)			0.324	No
Median age of the first movement (day)	335.0	(n = 27)	380.0	(n = 52)			0.271	No
Mean age of the last movement (day)	587.6	(n = 27)	560.2	(n = 52)			0.675	No
Median age of the last movement (day)	625.0	(n = 27)	598.8	(n = 52)			0.698	No

‘Offered’ column indicates whether the variable was offered to multivariable analysis.

- in the p-value column means that these variables were not analyzed due to lack of sample size.

N/A means there was no data.

Supplementary Table S3. List of cow-level variables and results of the univariable analysis

Variable	Infected	(%)	Non-infected	(%)	Odds Ratio	(95% CI)	p-value	Offered
Movement record								
Have ever moved from its home farm	11/42	(26.2%)	25/107	(23.4%)	1.6	(0.4–6.7)	0.516	No
Have ever moved when it was a calf	0/24	(0.0%)	5/107	(4.7%)			0.998	No
The number of movements	0.0	(n = 42)	0.0	(n = 107)			0.540	No
Age of the first movement (day)	676.0	(n=11)	559.0	(n = 25)			0.203	No
Age of the last movement (day)	726.0	(n = 11)	719.0	(n = 25)			0.252	No
Mean age of movements (day)	678.3	(n = 11)	639.0	(n = 25)			0.223	No
Have introduced	11/42	(26.2%)	22/97	(22.7%)	1.5	(0.5–4.7)	0.517	No
Source of introduction					3.2	(0.3–36.6)	0.325	No
Livestock markets	3/5	(60.0%)	1/9	(11.1%)				
Farms	2/5	(40.0%)	8/9	(88.9%)				
Have ever been at livestock markets	5/35	(14.3%)	3/95	(3.2%)	3.0	(0.3–33.5)	0.019	Yes
Have been sent to a communal pasture	6/38	(15.8%)	15/101	(14.9%)	8.9	(1.0–78.3)	0.347	No
Dairy herd test record								
Milk yield								
Milk yield at the test day (kg)	34.0	(n = 42)	30.2	(n = 107)			0.015	Yes
Milk yield for 305 days (kg)	9875.0	(n = 42)	9564.0	(n = 107)			0.082	No
Milk yield in the lactation (kg)	3362.0	(n = 42)	2828.0	(n = 107)			0.479	No
Peak daily milk yield in the lactation (kg)	40.0	(n = 42)	39.5	(n = 107)			0.090	No
Adjusted daily milk yield (Solid corrected milk yield adjusted to a cow which is in the second parity, delivered on April and whose days in milking is 150) (kg)	28.1	(n = 42)	27.6	(n = 107)			0.192	No

Supplementary Table S3. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds Ratio	(95% CI)	p-value	Offered
Adjusted 305 days milk yield (305 days milk yield adjusted to a cow which is 72 months old, delivered on April, and is milked twice per day) (kg)	10943.5	(n = 42)	10733.0	(n = 107)			0.095	No
Expected daily milk yield for next 12 months (kg)	32.4	(n = 42)	32.2	(n = 107)			0.054	No
Milk components								
Fat concentration at the test day (%)	3.9	(n = 42)	4.1	(n = 107)			0.590	No
Average fat concentration for 305 days (%)	4.0	(n = 42)	4.1	(n = 107)			0.305	No
Average fat concentration in the lactation (%)	4.1	(n = 42)	4.1	(n = 107)			0.369	No
Non-fat milk solids (SNF) concentration at the test day (%)	8.8	(n = 42)	8.7	(n = 107)			0.241	Yes
Average SNF concentration for 305 days (%)	8.8	(n = 42)	8.8	(n = 107)			0.275	No
Average SNF concentration in the lactation (%)	8.8	(n = 42)	8.8	(n = 107)			0.233	No
Protein concentration at the test day (%)	3.2	(n = 42)	3.2	(n = 107)			0.453	No
Average protein concentration for 305 days (%)	3.2	(n = 42)	3.3	(n = 107)			0.267	No
Average protein concentration in the lactation (%)	3.2	(n = 42)	3.2	(n = 107)			0.293	No
Milk urea nitrogen concentration at the test day (mg/dl)	12.0	(n = 42)	11.6	(n = 107)			0.064	Yes
Somatic cell count								
Somatic cell count (×1,000)	46.0	(n = 42)	43.0	(n = 107)			0.823	No
Linear score	2.0	(n = 42)	2.0	(n = 107)			0.718	No
The number of months with linear score ≥5 in the lactation	0.0	(n = 42)	0.0	(n = 107)			0.674	No

Supplementary Table S3. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds Ratio	(95% CI)	p-value	Offered
Delivery								
Days in milking							-	No
< 80	19/42	(45.2%)	50/107	(46.7%)				
80–159	11/42	(26.2%)	28/107	(26.2%)				
160–239	9/42	(21.4%)	20/107	(18.7%)				
≥ 240	3/42	(7.1%)	9/107	(8.4%)				
Pregnancy status								Yes
Before the first service	17/42	(40.5%)	51/107	(47.7%)			-	
Artificial insemination (AI) was conducted	15/42	(35.7%)	26/107	(24.3%)			0.122	
Failed to conceive	10/42	(23.8%)	30/107	(28.0%)			0.680	
Calving interval (day)	368.5	(n = 30)	397.5	(n = 74)			0.463	No
The number of AI conducted	1.0	(n = 42)	1.0	(n = 107)			0.888	No
Period from the last AI (day)	40.0	(n = 25)	39.5	(n = 56)			0.997	No
Period from the last delivery to the first AI (day)	82.0	(n = 25)	95.5	(n = 56)			0.311	No
Age at the last delivery (month)	48.0	(n = 42)	44.0	(n = 107)			0.737	No
Difficulty of the last delivery (1 (easy)–5 (difficult))	1.0	(n = 42)	1.0	(n = 107)			0.473	No
Type of last delivery								No
Singleton	40/42	(95.2%)	90/107	(84.1%)			-	
Multiplets	2/42	(4.8%)	6/107	(10.3%)			0.686	
Stillbirth or abortion	0/42	(0.0%)	11/407	(10.3%)			1.000	
Cow profile								
Body weight (kg)	590.0	(n = 3)	569.0	(n = 7)			-	No
Age (month)	51.5	(n = 42)	50.0	(n = 107)			0.782	No
Concentrates fed (kg)	13.2	(n = 42)	11.1	(n = 107)			0.087	Yes

Supplementary Table S3. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds Ratio	(95% CI)	p-value	Offered
Clinical record								
Respiratory disease–pneumonia–*Mycoplasma*	0/42	(0.0%)	0/107	(0.0%)			-	No
Respiratory disease–pneumonia–bacteria	5/42	(11.9%)	14/107	(13.1%)	1.0	(0.2–4.1)	1.000	No
Disease of udder and teat–peracute mastitis–other bacteria	1/42	(2.4%)	3/107	(2.8%)	0.5	(0.0–4.5)	0.503	No
Disease of udder and teat–peracute mastitis–other microbe	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of udder and teat–acute mastitis–other bacteria	13/42	(31.0%)	15/107	(14.0%)	2.8	(1.1–7.4)	0.037	Yes
Disease of udder and teat–acute mastitis–other microbe	5/42	(11.9%)	8/107	(7.5%)	1.6	(0.5–5.4)	0.454	No
Disease of udder and teat–chronic mastitis–other bacteria	1/42	(2.4%)	4/107	(3.7%)	0.5	(0.0–5.0)	0.548	No
Disease of udder and teat–chronic mastitis–other microbe	0/42	(0.0%)	3/107	(2.8%)			0.998	No
Disease of udder and teat–subclinical mastitis–other bacteria	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of udder and teat–subclinical mastitis–other microbe	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of udder and teat–mastitis in dry period–other bacteria	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of udder and teat–mastitis in dry period–other microbe	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of udder and teat–mastitis in heifer–other bacteria	0/42	(0.0%)	0/107	(0.0%)			-	No

Supplementary Table S3. (continued)

Variable	Infected	(%)	Non-infected	(%)	Odds Ratio	(95% CI)	p-value	Offered
Disease of udder and teat–mastitis in heifer–other microbe	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of sensory organ–otitis media	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease of limb–arthritis–infectious	0/42	(0.0%)	4/107	(3.7%)			0.998	No
Disease by bacteria or fungus–bovine *Mycoplasma* mastitis	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease by bacteria or fungus–other *Mycoplasma* infection–arthritis	0/42	(0.0%)	0/107	(0.0%)			-	No
Disease by bacteria or fungus–other *Mycoplasma* infection–other	0/42	(0.0%)	0/107	(0.0%)			-	No

‘Offered’ column indicates whether the variable was offered to multivariable analysis.

- in the p-value column means that these variables were not analyzed.

Supplementary Material S4. Questionnaire used for the first survey.

Questionnaire for an epidemiological survey of *Mycoplasma* mastitis in Nemuro region

Nemuro *Mycoplasma* Mastitis Control Committee
Rakuno Gakuen University
November 12, 2015

Please

There are many cases of *Mycoplasma* mastitis in Nemuro and neighboring region in this year. We are investigating the cause by survey to both case farms and randomly selected non-case farms. Our aim is to reveal the way how to prevent the disease by comparing case farms and non-case farms. We would appreciate if you could join the survey.

Please give your signature if you could join the survey. The result will be analyzed at Rakuno Gakuen University with a state in which the respondent can't be identified. Please rest assured that we certainly protect your personal information. About answers of the survey, we will never disclose any information by which a person or a company could be identified, so please rest assured to answer questions.

This page will be stored at the Nemuro *Mycoplasma* Mastitis Control Committee. The following pages will be handed to a person to enter the data.

Your signature:____________________

Date: Year Month Day

Interviewer:____________________ (Name of a worker of a agricultural cooperative)

ID for management (It will be entered at the committee and managed):__________________

1. Please tell us about your knowledge and experience about *Mycoplasma*. ID for management:________

(1) Have you ever head the name of a bacterium, *Mycoplasma*?

Yes [ ] No [ ]

Note: If you answered "yes" to the previous question, please answer the questions in this box.

- Do you know that *Mycoplasma* cause not only mastitis but also disease for calves in their nose, lung, or joints? Yes [ ] No [ ]
- Do you knows that *Mycoplasma* transmit from a calf to a dam by human hands? Yes [ ] No [ ]
- Have you ever considered possibility of *Mycoplasma* infection in a case of clinical mastitis when no bacteria were isolated? Yes [ ] No [ ]

( )

(2) Have you experienced *Mycoplasma* mastitis within two years in the farm? Yes [ ] No [ ]

Note: If you answered "yes," please answer the questions in the following parentheses.

(When:_________Species:______________The number of cows:______Others:______________)

(3) Have you experienced *Mycoplasma* mastitis within two years in group farms (farms which are owned by the same person)? Yes [ ] No [ ]

Note: If you answered "yes," please answer the questions in the following parentheses.

(When:_________Species:______________The number of cows:______Others:______________)

(4) Do you know frequent occurrence of *Mycoplasma* mastitis in neighborhood recently?

Yes [ ] No [ ]

( )

(5) Have you changed hygiene management after *Mycoplasma* infection in the farm or in the neighborhood? Yes [ ] No [ ]

Note: If you answered "yes," please tell us detail of the change.

( )

Note: If you have ever found *Mycoplasma* mastitis by clinical symptom, please answer the questions in this box.

(6) Please tell us clinical symptom you found

[ ] You found the mastitis by clinical symptom in this case.

( )

[ ] You found the mastitis by clinical symptom within two years.

( )

2. Please tell us about your farm.

(1) Type of business

- Family-run [ ] Cooperative [ ]

( )

2. (Continued) ID for management:________

- Dairy only [ ] Mixed (e.g. beef farming, field farming) [ ]
  Note: If you answered "mixed," please tell us the detail. ( )
  Note: If you keep beef cows, are you keeping them in the same site with dairy cows?
  Yes [ ] No [ ]
  ( )
- The number of workers (including the owner) __________
  ( )

(2) Experience about dairy business

- When was the farm opened? Year __________
  ( )
- When did the current owner start to work at the farm? Year __________
  ( )

3. Please tell us about your milking cows.

(1) Please tell us the number of cows.

- Milking cows (Milking cows except for cows in dry period) __________
- Dry cows __________

(2) Please tell us type of housing.

- Milking cows
  Tied [ ] Free stall [ ] Free barn [ ] Rangeland [ ] Others [ ]
  (Detail)
- Do you keep dry cows together with milking cows? Yes [ ] No [ ]
  Note: If you answered "no," please answer the next question.
- Please tell us the type of housing for dry cows.
  Tied [ ] Free stall [ ] Free barn [ ] Rangeland [ ] Others [ ]
  (Detail)

Note: If you keep cows in a free-stall barn, please answer the questions in the box about equipment in the free stall barn.

- Milking parlor Have [ ] Do not have [ ]
- Backflush system in milking system Have [ ] Do not have [ ]
- Milking robot Have [ ] Do not have [ ]

(3) Do you practice vaccination against respiratory disease in milking cows?

Yes [ ] No [ ] Not sure [ ]
( )

(4) Is there enough bedding in bed for milking cows?

No bedding [ ] Have bedding over the bed, but floor can be seen through it [ ]
Enough bedding over the bed [ ]
( )

(5) What type of bedding used in the milking cow barn? (Please check all that used in the milking cow barn.)

Mattress [ ] Sand [ ] Paper [ ] Sawdust [ ] Compost [ ] Straw [ ]
Other [ ]
( )

3. (Continued) ID for management:________

(6) Do you use hydrated lime on the bedding in the milking cow barn? Yes [ ] No [ ]

( )

Note: If you answered "yes," please answer the next question.

How frequently do you scatter hydrated lime powder?

_____ times/day [ ] _____ times/week [ ] _____ times/month [ ]

Less frequently [ ]

( )

(7) Please tell us frequency of changing the bedding in the milking cow barn.

- In summer or year-round

  ______times/day [ ] ______times/week [ ] ______times/month [ ]

  Less frequently [ ]

- In winter

  ______times/day [ ] ______times/week [ ] ______times/month [ ]

  Less frequently [ ]

( )

(8) Please tell us the frequency of removing manure in the milking cow barn.

As same as the frequency of changing the bedding [ ]

Different from the frequency of changing the bedding [ ]

Note: If you answered "different," please answer the frequency in below

_____ times/day [ ] _____ times/week [ ] _____ times/month [ ]

Less frequently [ ]

( )

(9) Do you regularly disinfect the milking cow barn? Yes [ ] No [ ]

Note: If you answered "yes," please answer the questions in this box.

- Please tell us the frequency of the disinfection.

  _____times/week [ ] _____times/month [ ] _____times/year [ ]

  Less frequently [ ]

- Please tell us the detail of the disinfection.

  ( )

(10) How frequently do you clean water tanks or water cups in the milking cow barn?

- Water tank / Water cup (Circle one of them)

  ______times/day [ ] ______times/week [ ] _____times/month [ ]

  Less frequently [ ]

(Detail)

(11) Which is applicable about situation of ventilation of the milking cow barn?

Opening and closing of windows and doors only [ ] Fans [ ] Open barn [ ]

Tunnel ventilation [ ]

( )

(12) Do you take preventive measures against intrusion of wild animals in the milking cow barn?

Yes [ ] No [ ]

(Detail)

4. Please tell us about milking procedure conducted in your farm.

(1) Please tell us about milking procedures.

- Do you use towels to wipe teats? Yes [ ] No [ ] Only paper towels are used [ ]

( )

Note: If you answered "yes," please answer the questions in this box.

- Do you prepare one towel per cow? Yes [ ] No [ ]

  ( )

- Do you dip towels to disinfectant? Yes [ ] No [ ]

  ( )

4. (Continued) ID for management:________

- Do you especially consciously wipe teat openings? Yes [ ] No [ ]
( )
- Do you wipe teats by paper towels after using a cloth towel? Yes [ ] No [ ]
( )
- Do you do pre-dipping? Yes [ ] No [ ]
( )
- Do you do post-dipping? Yes [ ] No [ ]
( )

(2) Please tell us about milking equipment.

- Do you use a cart to convey milking equipment? Yes [ ] No [ ]
( )
- Do you use a strip cup? Yes [ ] No [ ]
( )
- Do you actively call veterinarians when you find an abnormality by the strip cup? Yes [ ] No [ ]
( )
- Do you actively call veterinarians when you find an abnormality by PL test? Yes [ ] No [ ]
( )
- Do you use adequately disinfected milking equipment? Yes [ ] No [ ]
( )
- Do you disinfect milking equipment after milking? Yes [ ] No [ ]
( )
- Do you disinfect milking equipment before milk a next cow? Yes [ ] No [ ]
( )

(3) Please tell us about handling of milking cows with an abnormality.

- Do you milk a cow with high somatic cell count last? Yes [ ] No [ ]
( )
- Do you milk mastitis cows last? Yes [ ] No [ ]
( )
- Do you conduct mastitis test by pathogen other than *Mycoplasma* after the first calving of a home-bread heifer? Yes [ ] No [ ]
( )
- Do you conduct mastitis test by *Mycoplasma* after the first calving of a home-bread heifer? Yes [ ] No [ ]
( )
- Do you conduct a self-imposed test of *Mycoplasma* with bulk tank milk? Yes [ ] No [ ]
( )
- Do you disinfect milking units after the first calving of a cow until the result of *Mycoplasma* test is available? Yes [ ] No [ ]
( )

5. Please tell us about management of your heifers (≧ 4 month old). ID for management:________

(1) Please tell us the number of heifers. ___________

( )

(2) Please tell us type of housing.

Tied [ ] Free barn [ ] Rangeland [ ] Others [ ]

(Detail)

- Do you keep heifers together with milking cows? Yes [ ] No [ ]

Note: If you keep heifers and milking cows in different barns, please answer the questions in this box.

(3) Do you regularly disinfect the heifer barn? Yes [ ] No [ ]

Note: If you conduct disinfection of the barn, please answer the questions in this box.

- Please tell us the frequency of the disinfection.

  _____ times/week [ ] _____ times/month [ ]

  _____ times/year [ ] Less frequently [ ]

- Please tell us the detail of the disinfection.

  ( )

(4) Which is applicable about situation of ventilation of the heifer barn?

Opening and closing of windows and doors only [ ] Fans [ ] Open barn [ ]

Tunnel ventilation [ ]

( )

(5) Do you take preventive measures against intrusion of wild animals in the heifer barn?

Yes [ ] No [ ]

(Detail)

(6) Do you practice vaccination against respiratory disease in heifers?

Yes [ ] No [ ] Not sure [ ]

( )

(7) Is there enough bedding in bed for heifers?

No bedding [ ] Have bedding over the bed, but floor can be seen through it [ ]

Enough bedding over the bed [ ]

( )

(8) What type of bedding used in the heifer barn? (Please check all that used in the heifer barn.)

Mattress [ ] Sand [ ] Paper [ ] Sawdust [ ] Compost [ ] Straw [ ]

Other [ ]

( )

(9) Please tell us frequency of changing the bedding in the heifer barn.

- In summer or year-round

  ______ times/day [ ] ______ times/week [ ] ______ times/month [ ]

  Less frequently [ ]

- In winter

  ______ times/day [ ] ______ times/week [ ] ______ times/month [ ]

  Less frequently [ ]

( )

(10) Please tell us the frequency of removing manure in the heifer barn.

As same as the frequency of changing the bedding [ ]

Different from the frequency of changing the bedding [ ]

Note: If you answered "different," please answer the frequency in below

____ times/day [ ] _____ times/week [ ] _____ times/month [ ]

Less frequently [ ]

( )

(11) How frequently do you clean water tanks or water cups in the heifer barn?

- Water tank / Water cup (Circle one of them)

  ______ times/day [ ] ______ times/week [ ] _____ times/month [ ]

  Less frequently [ ]

(Detail)

6. Please tell us about management of your calves (≦ 4 month old). ID for management:________

(1) Please tell us the number of calves. ___________

( )

(2) Please tell us type of housing.

One calf per pen [ ] Several calves per pen [ ] Hatch [ ] Free barn [ ]
Rangeland [ ] Other [ ]
(Detail)

- Do you keep calves together with milking cows? Yes [ ] No [ ]

Note: If you keep calves and milking cows in different barns, please answer the questions in this box.

(3) Do you regularly disinfect the calf barn? Yes [ ] No [ ]

Note: If you conduct disinfection of the barn, please answer the questions in this box.

- Please tell us the frequency of the disinfection.
  _____ times/week [ ] _____ times/month [ ]
  _____ times/year [ ] Less frequently [ ]
- Please tell us the detail of the disinfection.
  ( )

(4) Which is applicable about situation of ventilation of the calf barn?

Opening and closing of windows and doors only [ ] Fans [ ] Open barn [ ]
Tunnel ventilation [ ]
( )

(5) Do you take preventive measures against intrusion of wild animals in the calf barn?

Yes [ ] No [ ]
(Detail)

(6) Do you practice vaccination against respiratory disease in calves?

Yes [ ] No [ ] Not sure [ ]
(Detail)

(7) Is there enough bedding in bed for calves?

No bedding [ ] Have bedding over the bed, but floor can be seen through it [ ]
Enough bedding over the bed [ ]
( )

(8) What type of bedding used in the calf barn? (Please check all that used in the calf barn.)

Mattress [ ] Sand [ ] Paper [ ] Sawdust [ ] Compost [ ] Straw [ ]
Other [ ]
( )

(9) Please tell us frequency of changing the bedding in the calf barn.

- In summer or year-round
  ______ times/day [ ] ______ times/week [ ] ______ times/month [ ]
  Less frequently [ ]
- In winter
  ______ times/day [ ] ______ times/week [ ] ______ times/month [ ]
  Less frequently [ ]

( )

(10) Please tell us the frequency of removing manure in the calf barn.

As same as the frequency of changing the bedding [ ]
Different from the frequency of changing the bedding [ ]
Note: If you answered "different," please answer the frequency in below
_____ times/day [ ] _____ times/week [ ] _____ times/month [ ]
Less frequently [ ]
( )

(11) How frequently do you clean water tanks or water cups in the calf barn?

- Water tank / Water cup (Circle one of them)
  ______ times/day [ ] ______ times/week [ ] _____ times/month [ ]
  Less frequently [ ]

(Detail)

7. Please tell us about use of communal pastures. ID for management:________

(1) **Do you use communal pastures?**

Use from several years ago [ ] Have started to use from this year [ ]

Have used before (two to five years ago) [ ] Never used [ ]

( )

Note: If you answered other than "never used" communal pastures, please answer the following questions.

(2) **Please tell us the owner of the communal pasture you use.**

A public organization such as city [ ] A neighborhood farmer [ ]

Other (______________________) [ ]

(3) **Please tell us type of cows you send or have sent to the communal pasture. (Check all that apply)**

Heifers [ ] Dry cows [ ] Others [ ]

[ ]

8. Please tell us about introduction of cows.

(1) **Please answer farm situation about introduction and write the number of introduced cows in the most recent year which you introduced cows.**

Non-pregnant heifers [ ] : Every year [ ] Once in two years [ ]
Once in five years [ ] Never [ ]
The number of cows _________

Pregnant heifers [ ] : Every year [ ] Once in two years [ ]
Once in five years [ ] Never [ ]
The number of cows _________

Delivered cows [ ] : Every year [ ] Once in two years [ ]
Once in five years [ ] Never [ ]
The number of cows _________

Other [ ] : (__________) Every year [ ] Once in two years [ ]
Once in five years [ ] Never [ ]
The number of cows _________

[ ]

Note: If you introduce cows, please answer the following questions.

(2) **Which is source of the introduction? (Multiple answers allowed)**

Agricultural cooperative [ ] Farm of an acquaintance [ ] Group farm [ ]

Livestock dealer [ ] Other (_____) [ ]

( )

(3) **Which is the most frequently introduced source? (Check one from which most frequently introduced)**

Agricultural cooperative [ ] Farm of an acquaintance [ ] Group farm [ ]

Livestock dealer [ ] Other (_____) [ ]

( )

(4) **Do you conduct mastitis test by *Mycoplasma* to cows introduced from a livestock market?**

Yes [ ] No [ ]

( )

(5) **Do you do mastitis test by pathogen other than *Mycoplasma* to cows introduced from a livestock market?**

Yes [ ] No [ ]

( )

(6) **Do you have quarantine facility for introduced cows?**

Have a barn only for introduced cows [ ] Have a barn not only for introduced cows [ ]

No quarantine [ ]

Note: If you do quarantine, how long do you quarantine and observe introduced cows?

One day [ ] Less than a week [ ] A week or more [ ]

( )

9. Please tell us about hygiene management in your farm. ID for management:________

(1) Do you have a power sprayer to disinfect vehicles which enter the farm? Yes [ ] No [ ]
( )

(2) Do you set and apparently divide a sanitation control zone? Yes [ ] No [ ]
( )

(3) Do workers wear dedicated clothes in the sanitation control zone? Yes [ ] No [ ]
( )

(4) Do you and workers park vehicles outside of the sanitation control zone when coming to work?
Yes [ ] No [ ]
( )

(5) Which hygiene control measure you always conduct when vehicles enter the sanitation control zone?

- Please tell us about disinfection of vehicles of workers including the owner.

	Rinse the vehicle before disinfection	Disinfect the whole vehicle	Disinfect the wheel wells	Disinfect the drive sear floor mat	Disinfect the bed of the vehicle
Yes	[ ]	[ ]	[ ]	[ ]	[ ]
No	[ ]	[ ]	[ ]	[ ]	[ ]

- Please tell us about disinfection of vehicles other than workers' ones.

Does the farm owner set guideline of disinfection of vehicles other than farm workers' ones?
Yes [ ] No [ ]

Note: If you answered "yes," please answer the next question.
Please tell us about disinfection conducted on vehicles other than farm workers' ones.

	Rinse the vehicle before disinfection	Disinfect the whole vehicle	Disinfect the wheel wells	Disinfect the drive sear floor mat	Disinfect the bed of the vehicle
Yes	[ ]	[ ]	[ ]	[ ]	[ ]
No	[ ]	[ ]	[ ]	[ ]	[ ]

( )

(6) Do you set and use disinfectant foot baths at barn?
At all barns [ ] One foot bath in the whole sanitation control zone [ ] No foot baths [ ]
( )

(7) Do you remove dirt on boots before step into the disinfectant foot baths? Yes [ ] No [ ]
( )

(8) How frequently do you change disinfectants in the foot baths?
Several times per day [ ] Once per day [ ] Once in several days [ ]
Once per week [ ]
( )

(9) What type of disinfectant do you use?
(Type: Brand name:)

(10) Do you scatter hydrated lime powder at farm entrance? Yes [ ] No [ ]
( )

10. Please tell us about farm situation from late February to early March in this year. ID for management:________

(1) A record snowfall was observed from late February to early March in this year. Do you remember how farm operation changed in this period? Yes [ ] No [ ]

Note: If you answered "yes," please answer the following questions in the following about changes due to heavy snow.

(Please check all that apply.)

(2) Milking cow barn(________________)

	Ventilation	Frequency of removing manure	Frequency of changing bedding
Had a period in which it was not conducted	[ ]	[ ]	[ ]
Volume or frequency decreased than usual years	[ ]	[ ]	[ ]
As same as usual years	[ ]	[ ]	[ ]

( )

(3) Heifer barn(________________)

	Ventilation	Frequency of removing manure	Frequency of changing bedding
Had a period in which it was not conducted	[ ]	[ ]	[ ]
Volume or frequency decreased than usual years	[ ]	[ ]	[ ]
As same as usual years	[ ]	[ ]	[ ]

( )

(4) Calf barn(________________)

	Ventilation	Frequency of removing manure	Frequency of changing bedding
Had a period in which it was not conducted	[ ]	[ ]	[ ]
Volume or frequency decreased than usual years	[ ]	[ ]	[ ]
As same as usual years	[ ]	[ ]	[ ]

( )

(5) Other barn(________________)

	Ventilation	Frequency of removing manure	Frequency of changing bedding
Had a period in which it was not conducted	[ ]	[ ]	[ ]
Volume or frequency decreased than usual years	[ ]	[ ]	[ ]
As same as usual years	[ ]	[ ]	[ ]

( )

(6) Other barn(________________)

	Ventilation	Frequency of removing manure	Frequency of changing bedding
Had a period in which it was not conducted	[ ]	[ ]	[ ]
Volume or frequency decreased than usual years	[ ]	[ ]	[ ]
As same as usual years	[ ]	[ ]	[ ]

( )

10. (Continued) ID for management:________

(5) Please tell us when you have other differences compared with usual years.

( )

Note: Finally, if you have any question or request, please freely write.

Thank you very much for your cooperation.
We will try hard to reveal the cause of the *Mycoplasma* mastitis outbreak with your answers.

Nemuro *Mycoplasma* Mastitis Control Committee
Rakuno Gakuen University

根室地区マイコプラズマ乳房炎疫学調査質問票

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学
2015 年 11 月 12 日

お願い

本年度、根室地区および周辺地区では、マイコプラズマ乳房炎が多発しています。原因究明のため、発生農場と、無作為に選ばれた非発生農場の両方を調査しています。発生農場と非発生農場を比較することにより、今後どのように効果的に本病を防いでいけばよいか明らかにしたいので、是非本調査にご協力ください。

この調査にご協力していただける方は、ご署名をお願いします。調査結果は、個人が特定できない状態で酪農学園大学にて解析されます。個人情報は必ず保護されますのでご安心ください。また回答結果についても、個人や事業所が特定できるような情報の公開は行いませんので、御安心してお答えください。

本ページは根室地区マイコプラズマ乳房炎協議会に保存されます。データの入力者には、次ページ以降が手渡されます。

ご署名：＿＿＿＿＿＿＿＿＿＿

年月日：　　　年　　月　　日

質問者：＿＿＿＿＿＿＿＿＿＿　（農協職員名）

管理用ＩＤ（協議会で入力し、管理されます）：＿＿＿＿＿＿＿＿＿

1. マイコプラズマの知識・経験に関して教えてください。　　　　　　管理用 ID：________

（1）マイコプラズマという菌は聞いたことがありますか？　　　はい　□　　いいえ　□

※この枠内は「はい」と回答した方のみ続けて回答してください。

- マイコプラズマは乳房炎だけでなく子牛の鼻や肺、関節にも病気を起こすことを知っていますか？
  はい　□　　いいえ　□
- マイコプラズマは人の手を介して子牛から親牛に移ることを知っていますか？
  はい　□　　いいえ　□
- 菌が出ない臨床型乳房炎の場合にマイコプラズマ乳房炎が頭をよぎったことがありますか？
  はい　□　　いいえ　□

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（2）自身の農場で過去二年以内にマイコプラズマ乳房炎の発生がありましたか？

はい　□　　いいえ　□

※「はい」と回答した方は次の括弧内についても回答してください。

（いつ：________菌種：____________頭数：______その他特記：_________）

（3）系列の農場（経営者が同じ農場）で過去二年以内にマイコプラズマ乳房炎の発生がありましたか？

はい　□　　いいえ　□

※「はい」と回答した方は次の括弧内についても回答してください。

（いつ：________菌種：____________頭数：______その他特記：_________）

（4）最近地域でマイコプラズマ乳房炎が多いことは知っていましたか？

はい　□　　いいえ　□

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（5）マイコプラズマの自農場または地域での発生を受けて衛生対策の内容を変更しましたか？

はい　□　　いいえ　□

※「はい」回答した方は変更の内容を教えてください。

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

※この枠内は発生農場（過去の発生を含む）で臨床から発見した方のみ回答してください。

（6）発見した際の臨床症状を教えてください。

□　今回の発生で臨床症状から発見した

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

□　過去二年以内の発生で臨床症状から発見した

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

2. あなたの農場について教えてください。

（1）経営形態

- 家族経営　□　　法人経営　□

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

2. の続き　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　管理用 ID：________

- 酪農専業　☐　　複合（肉牛・畑作など）　☐
  - ※「複合」とお答えの場合、他の業務は何ですか？（　　　　　　　　　　　　）
  - ※肉用牛を飼養の場合、同じ敷地内で肉用牛を飼養されていますか？
  - はい☐　いいえ☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

- 従事者数（農場主を含め）　　__________名

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（2）酪農業のご経験について

- 農業開設は何年ですか？　　　　　　　　　　　西暦 __________年

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

- 現在の農場主が現牧場に就農したのは何年ですか？　　西暦 __________年

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

3. 搾乳牛について教えてください。

（1）牛の頭数を教えてください。

- 搾乳中の牛（乾乳期の牛を除く搾乳牛）　　　__________ 頭
- 乾乳中の牛　　　　　　　　　　　　　　　　__________ 頭

（2）飼養形態について教えてください。

- 搾乳中の牛
  - 繋ぎ飼い　☐　　フリーストール　☐　　フリーバーン　☐　　放牧場　☐
  - その他　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

- 乾乳中の牛は搾乳中の牛と一緒に飼養していますか。　　はい　☐　　いいえ　☐

※「いいえ」と回答した方のみ次の質問に答えてください。

- 乾乳中の牛の飼養形態を教えてください。
  - 繋ぎ飼い　☐　　フリーストール　☐　　フリーバーン　☐　　放牧場　☐
  - その他　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

※フリーストールで飼養している農場のみ枠内のフリーストール内施設についてお答えください。

● 搾乳パーラー	有　☐	無　☐
● ミルキングシステムのバックフラッシュ機能	有　☐	無　☐
● 搾乳ロボット	有　☐	無　☐

（3）搾乳牛に呼吸器ワクチンプログラムを実施していますか？

はい　☐　　いいえ　☐　分からない　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（4）搾乳牛のベッドに敷料が十分敷かれていますか？

敷料を敷いていない　☐　　全体に敷いているが床のコンクリートが見える　☐

全体に十分に敷いている　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（5）搾乳牛牛舎の敷料は何ですか？（搾乳牛牛舎に使用しているものすべて回答して下さい。）

パスチャーマット　☐　　砂　☐　　紙　☐　　おがくず　☐　　戻し堆肥　☐

敷き藁　☐　　その他　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（6）搾乳牛の寝床に消石灰を使用していますか？　はい　□　いいえ　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

※「はい」と回答した方に質問です。

消石灰はどれくらいの頻度で散布を実施していますか？

毎日______回　□　週______回　□　月______回　□

それより少ない　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

（7）搾乳牛牛舎の敷料の交換頻度を教えてください。

- 夏・通年　毎日______回　□　週______回　□　月______回　□
  それより少ない　□
- 冬　毎日______回　□　週______回　□　月______回　□
  それより少ない　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

（8）搾乳牛牛舎の除糞回数を教えてください。

敷料の交換頻度と同じ　□

敷料の交換頻度と異なる　□

※「異なる」と回答した方は下の頻度に回答してください。

毎日______回　□　週______回　□　月______回　□　それより少ない　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

（9）搾乳牛牛舎の消毒は定期的に行っていますか？　はい　□　いいえ　□

※この枠内は「はい」と答えた方のみ質問に回答してください。

- 消毒の頻度を教えてください。
  週______回　□　月______回　□　年______回　□
  それより少ない　□
- 消毒の内容を具体的に教えてください。

（　　　　　　　　　　　　　　　　　　　　　　　　）

（１０）搾乳牛牛舎の水槽やウォーターカップの掃除はどれくらい頻繁に行いますか？

- 水槽・ウォーターカップ　（いずれか一方に○）
  毎日______回　□　週________回　□　月________回　□
  それより少ない　□

（具体的に　　　　　　　　　　　　　　　　　　　　　）

（１１）搾乳牛牛舎の換気状況に関して該当するものはどれですか？

窓及びドアの開閉のみ　□　扇風機　□　開放　□　トンネル換気　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

（１２）搾乳牛牛舎では鳥獣侵入防止対策は行っていますか？　はい　□　いいえ　□

（具体的に　　　　　　　　　　　　　　　　　　　　　）

4. 農場で実施している搾乳方法に関して教えてください。

（1）搾乳の手順に関して教えてください。

- 清拭タオルは使用していますか？
  はい　□　いいえ　□　ペーパータオルのみ使用している　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

※「はい」と回答された方はこの枠内の質問に回答して下さい。

- 清拭タオルは一頭に一枚準備されていますか？　はい　□　いいえ　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

- 清拭タオルは消毒液につけていますか？　はい　□　いいえ　□

（　　　　　　　　　　　　　　　　　　　　　　　　）

- 乳頭口は特に意識して清拭しますか？ はい □ いいえ □
  （ ）
- 清拭タオルで拭いた後、ペーパータオルで乳頭を拭き取りますか？
  はい □ いいえ □
  （ ）
- プレディッピングをしていますか？ はい □ いいえ □
  （ ）
- ポストディッピングをしていますか？ はい □ いいえ □
  （ ）

（2）搾乳器具に関して教えてください。

- 搾乳ワゴンは使用していますか？ はい □ いいえ □
  （ ）
- ストリップカップは使用していますか？ はい □ いいえ □
  （ ）
- ストリップカップで異常があった場合、積極的に獣医師に連絡しますか？
  はい □ いいえ □
  （ ）
- PL で異常があった場合、積極的に獣医師に連絡しますか？ はい □ いいえ □
  （ ）
- 十分に消毒した搾乳器具を使用していますか？ はい □ いいえ □
  （ ）
- 搾乳終了後に搾乳器具の消毒は実施しますか？ はい □ いいえ □
  （ ）
- 隣の牛に移る時に搾乳器具の消毒は実施しますか はい □ いいえ □
  （ ）

（3）異常のある搾乳牛に対する対応に関して教えてください。

- 体細胞数の高い牛は最後に絞りますか？ はい □ いいえ □
  （ ）
- 乳房炎の牛は最後に搾乳しますか？ はい □ いいえ □
  （ ）
- 自家産初産は分娩後にマイコプラズマ以外の菌種の乳房炎検査はしますか？
  はい □ いいえ □
  （ ）
- 自家産初産は分娩後にマイコプラズマの乳房炎検査はしますか？
  はい □ いいえ □
  （ ）
- バルク乳でマイコプラズマ自主検査の実施は行っていますか？
  はい □ いいえ □
  （ ）
- 初妊牛については、マイコプラズマ検査の結果が出るまでユニット消毒を行っていますか？ はい □ いいえ □
  （ ）

5.育成牛（生後４か月以上の牛）の管理に関して教えてください。　　　管理用 ID：________

（1）育成牛の頭数を教えてください。　　　　　________ 頭
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（2）飼養形態について教えてください。
繋ぎ飼い　☐　　フリーバーン　☐　　放牧場　☐　　その他　　☐
（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）
- 育成牛は搾乳牛と一緒の牛舎で飼養していますか。　　　はい　☐　　いいえ　☐

※この枠内は育成牛と搾乳牛を別々の牛舎で飼育している方のみ回答して下さい。

（3）育成牛牛舎の消毒は定期的に行っていますか？　　　はい　☐　　いいえ　☐

※この枠内は牛舎内の消毒を実施している方のみ質問に回答してください。
- 消毒の頻度を教えてください。
週______回　☐　　月______回　☐　　年______回　☐
それより少ない　☐
- 消毒の内容を具体的に教えてください。
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（4）育成牛牛舎の換気状況に関して該当するものはどれですか？
窓及びドアの開閉のみ　☐　　扇風機　☐　　開放　☐　　トンネル換気　☐
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（5）育成牛牛舎では鳥獣侵入防止対策は行っていますか？　はい　☐　　いいえ　☐
（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（6）育成牛に呼吸器ワクチンプログラムを実施していますか？
はい　☐　　いいえ　☐　　分からない　☐
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（7）育成牛のベッドに敷料が十分敷かれていますか？
敷料を敷いていない　☐　　全体に敷いているが床のコンクリートが見える　☐
全体に十分に敷いている　☐
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

(8)育成牛牛舎の敷料は何ですか？(育成牛牛舎に使用しているものすべて回答して下さい。)
パスチャーマット　☐　　砂　☐　　紙　☐　　おがくず　☐　　戻し堆肥　☐
敷き藁　☐　　その他　☐（　　　　　　　　　　　　　　　　　　　　　）

（9）育成牛牛舎の敷料の交換頻度を教えてください。
- 夏・通年　毎日______回　☐　　週______回　☐　　月______回　☐
それより少ない　☐
- 冬　　　　毎日______回　☐　　週______回　☐　　月______回　☐
それより少ない　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（１０）育成牛牛舎の除糞回数を教えてください。
敷料の交換頻度と同じ　　☐
敷料の交換頻度と異なる　☐
※「異なる」と回答した方は下の頻度に回答してください。
毎日______回　☐　　週______回　☐　　月______回　☐　　それより少ない　☐
（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（１１）育成牛牛舎の水槽やウォーターカップの掃除はどれくらい頻繁に行いますか？
- 水槽・ウォーターカップ　(いずれか一方に〇)
毎日______回　☐　　週______回　☐　　月______回　☐　　それより少ない　☐
（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

6. 子牛（生後４か月以内）の管理に関して教えてください。　　　　管理用 ID：________

（１）子牛の頭数を教えてください。　　　　__________ 頭

（　　　　　　　　　　　）

（２）飼養形態について教えてください。

ペン内に一頭飼育　☐　　ペン内に複数飼育　☐　　ハッチ　☐

フリーバーン　☐　　放牧場　☐　　その他　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　）

● 子牛は搾乳牛と一緒の牛舎で飼養していますか。　　　　はい　☐　　いいえ　☐

※この枠内は子牛と搾乳牛を別々の牛舎で飼育している方のみ回答して下さい。

（３）子牛牛舎の消毒は定期的に行っていますか？　　　　はい　☐　　いいえ　☐

※この枠内は牛舎内の消毒を実施している方のみ質問に回答してください。

● 消毒の頻度を教えてください。

週______回　☐　　月______回　☐　　年______回　☐

それより少ない　☐

● 消毒の内容を具体的に教えてください。

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（４）子牛牛舎の換気状況に関して該当するものはどれですか？

窓及びドアの開閉のみ　☐　　扇風機　☐　　開放　☐　　トンネル換気　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（５）子牛牛舎では鳥獣侵入防止対策は行っていますか？　　はい　☐　　いいえ　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　）

（６）子牛に呼吸器ワクチンプログラムを実施していますか？

はい　☐　　いいえ　☐　　分からない　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（７）子牛のベッドに敷料が十分敷かれていますか？

敷料を敷いていない　☐　　全体に敷いているが床のコンクリートが見える　☐

全体に十分に敷いている　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（８）子牛牛舎の敷料は何ですか？（子牛牛舎に使用しているものすべて回答して下さい。）

パスチャーマット　☐　　砂　☐　　紙　☐　　おがくず　☐　　戻し堆肥　☐

敷き藁　☐　　その他　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（９）子牛牛舎の敷料の交換頻度を教えてください。

● 夏・通年　毎日______回　☐　　週______回　☐　　月______回　☐

それより少ない　☐

● 冬　　　　毎日______回　☐　　週______回　☐　　月______回　☐

それより少ない　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（１０）子牛牛舎の除糞回数を教えてください。

敷料の交換頻度と同じ　☐

敷料の交換頻度と異なる　☐

※「異なる」と回答した方は下の頻度について回答してください。

毎日______回　☐　　週______回　☐　　月______回　☐　　それより少ない　☐

（　　　　　　　　　　　　　　　　　　　　　　　　　　　）

（１１）子牛牛舎の水槽やウォーターカップの掃除はどれくらい頻繁に行いますか？

● 水槽・ウォーターカップ　（いずれか一方に○）

毎日______回　☐　　週______回　☐　　月______回　☐　　それより少ない　☐

（具体的に　　　　　　　　　　　　　　　　　　　　　　　　　　　）

7. 牧野の利用に関して教えてください。　　　　　　　　　　　　　　　　管理用 ID：________

（1）集団牧野を利用していますか？

数年前から利用している　☐　　今年から利用し始めた　　☐

過去（2-5 年前）に利用していた　☐　　利用したことがない　　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

※以下は集団牧野を「利用したことがない」と回答した方以外は回答して下さい。

（2）預託先の牧野を経営している団体を教えてください。

市などの公共機関　☐　　近隣の農場主　☐

その他（____________________）　☐

（3）牧野に預託している・預託していた牧を教えてください。（あてはまるもの全てに回答）

育成牛　☐　　乾乳牛　☐　　その他　☐

[　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　]

8. 導入牛に関して教えてください。

（1）導入状況について回答した後に、最近導入を実施した年度における導入頭数を記入してください。

育成牛　☐：　毎年　☐　　二年に一度　☐　　五年に一度　☐　　しない　☐

　　　　　　　________ 頭

初妊牛　☐：　毎年　☐　　二年に一度　☐　　五年に一度　☐　　しない　☐

　　　　　　　________ 頭

経産牛　☐：　毎年　☐　　二年に一度　☐　　五年に一度　☐　　しない　☐

　　　　　　　________ 頭

その他　☐：　毎年　☐　　二年に一度　☐　　五年に一度　☐　　しない　☐

（________）　________ 頭

[　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　]

※以下は導入を行っている農場のみ回答してください。

（2）導入元はどこですか？（複数回答）

JA　☐　　知り合いの農場　☐　　系列農場　☐　　家畜商　☐

その他（__________）　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

（3）特に導入が多い導入元はどこですか？（もっとも頻度の多いものを一つ選択）

JA　☐　　知り合いの農場　☐　　系列農場　☐　　家畜商　☐

その他（__________）　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

（4）市場導入牛にマイコプラズマ乳房炎検査はしますか？　　　　はい　☐　　いいえ　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

（5）市場導入牛にマイコプラズマ乳房炎以外の菌種の乳房炎検査はしますか？

はい　☐　　いいえ　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

（6）導入牛の隔離施設はありますか？

導入牛専用の牛舎がある　☐　　隔離はしているが専用ではない　☐

隔離はしていない　☐

※隔離を行っている方に質問です。導入牛の隔離観察は何日ほどしますか？

一日　☐　　一週間未満　☐　　一週間以上　☐

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

9. あなたの農場の衛生対策について教えてください。　管理用 ID : ________

（1）農場に入場する車両の消毒に用いる動力噴霧器を設置していますか？

はい □　いいえ □

(　　　　)

（2）衛生管理区域を設定し、分かるように区別していますか？

はい □　いいえ □

(　　　　)

（3）衛生管理区域内では、区域内のみで用いる専用の衣服を着用していますか？

はい □　いいえ □

(　　　　)

（4）あなたと従業員の出勤時に、車両は衛生管理区域の外に駐車していますか？

はい □　いいえ □

(　　　　)

（5）衛生管理区域に車両が侵入する場合、必ず実施している項目はどれですか？

- 農場主を含む作業従事者の車両消毒について教えてください。

	消毒前の水洗い	両車全体の消毒	タイヤハウスの消毒	運転席マットの消毒	荷台消毒
はい	□	□	□	□	□
いいえ	□	□	□	□	□

- 作業従事者以外の車両消毒について教えてください。

作業従事者以外の車両消毒は農場主が基準を決めていますか？　はい □　いいえ □

※「はい」と回答した方に質問です。

作業従事者以外の車両に実施している車両消毒について回答して下さい。

	消毒前の水洗い	両車全体の消毒	タイヤハウスの消毒	運転席マットの消毒	荷台消毒
はい	□	□	□	□	□
いいえ	□	□	□	□	□

(　　　　)

（6）各牛舎に踏込消毒槽を設置し、使用していますか？

各牛舎に設置 □　衛生管理区域に一か所のみ設置 □　設置していない □

(　　　　)

（7）踏込消毒槽に踏み込む前に、長靴の汚れを落としていますか？

はい □　いいえ □

(　　　　)

（8）踏込消毒槽の消毒液はどれくらい頻繁に交換しますか？

一日数回 □　一日一回 □　数日に一回 □　一週間に一回 □

(　　　　)

（9）使用している消毒液はどのようなタイプですか？

(系統 :　　　　商品名 :　　　　)

（１０）石灰帯を設置していますか？　はい □　いいえ □

(　　　　)

10. 今年の 2 月末から 3 月上旬にかけての農場の状況について教えてください。　管理用 ID：________

（1）今年の2月末から3月上旬にかけて根室管内において記録的な積雪が観測されましたが、当時の農作業の内容の変化を覚えていますか。 はい □ いいえ □

※（1）で「はい」と回答された方は以下の積雪・大雪による変化について回答して下さい。（あてはまるものすべてに回答して下さい。）

（2）搾乳牛舎（______________）

	換気状況	除糞の頻度	敷料の交換頻度
実施できなかった期間があった	□	□	□
換気量や実施の頻度が例年よりも減少していた	□	□	□
例年と変化はなかった	□	□	□

（　　　　　　　　　　）

（3）育成牛舎（______________）

	換気状況	除糞の頻度	敷料の交換頻度
実施できなかった期間があった	□	□	□
換気量や実施の頻度が例年よりも減少していた	□	□	□
例年と変化はなかった	□	□	□

（　　　　　　　　　　）

（4）子牛牛舎（______________）

	換気状況	除糞の頻度	敷料の交換頻度
実施できなかった期間があった	□	□	□
換気量や実施の頻度が例年よりも減少していた	□	□	□
例年と変化はなかった	□	□	□

（　　　　　　　　　　）

（5）その他の牛舎（______________）

	換気状況	除糞の頻度	敷料の交換頻度
実施できなかった期間があった	□	□	□
換気量や実施の頻度が例年よりも減少していた	□	□	□
例年と変化はなかった	□	□	□

（　　　　　　　　　　）

（6）その他の牛舎（______________）

	換気状況	除糞の頻度	敷料の交換頻度
実施できなかった期間があった	□	□	□
換気量や実施の頻度が例年よりも減少していた	□	□	□
例年と変化はなかった	□	□	□

（　　　　　　　　　　）

10.の続き　　　　　　　　　　　　　　　　　　　　　　　　　管理用 ID : ________

（５）その他で例年と異なった点があれば教えてください。

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

※最後に何か質問や要望があれば、ご自由にお書きください。

ご協力大変ありがとうございました。
回答していただきましたものを参考に
マイコプラズマ乳房炎発生の原因解明に努力いたします。

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学

Supplementary Material S5. Questionnaire used for the second survey of case farms.

Questionnaire for a resurvey of *Mycoplasma* mastitis in Nemuro region (for a case farm)

Nemuro *Mycoplasma* Mastitis Control Committee
Rakuno Gakuen University
July 20, 2016

<u>Please</u>

We appreciate for your help for our previous questionnaire survey to prevent *Mycoplasma* mastitis. Currently, questionnaires are under analysis in Rakuno Gakuen University and with the result, it was assumed that more investigation is necessary. So, we decided to ask respondents of the previous survey to cooperate in an additional questionnaire survey.

We hope to reveal the cause and countermeasure to the disease and contribute to prevent an outbreak of the disease in Nemuro region. I realize you are very busy, but we would appreciate if you cooperate with us.

Name of a farm:____________________

Date of answer: Year Month Day

Interviewer:____________________ (Name of a worker of an agricultural cooperative)

ID for management (It will be entered at the committee and managed):__________________

Note: **<u>Before answering</u> ...** At items as “before the mastitis in your farm” or “previous,” please answer **hygiene management behavior before the *Mycoplasma* mastitis outbreak from 2014 to 2015** in conducted in your farm. Thank you.

1. Questions about daily routine

- Please tell us the timing when a calf is separated from its dam after a delivery and direct contact of them are limited.

(Before the mastitis in your farm)

- You monitor a delivery of a cow and a calf is separated immediate after the delivery. [ ]
- You do not monitor a delivery and a calf is separated when you realize that the delivery is finished. [ ]
- You keep a calf and a dam for a while and then isolate them. [ ]
- Other (______________________________) [ ]

・If there is a period when you keep a dam and a calf together, please tell us the length of the period.

___________days

(Now)

- You monitor a delivery of a cow and a calf is separated immediate after the delivery. [ ]
- You do not monitor a delivery and a calf is separated when you realize that the delivery is finished. [ ]
- You keep a calf and a dam for a while and then isolate them. [ ]
- Other (______________________________) [ ]

・If there is a period when you keep a dam and a calf together, please tell us the length of the period.

___________days

- Please tell us the way you feed colostrum.

(Previous)

- Feed directly from teats of a dam. [ ]
- A farm worker milks colostrum of a dam and feeds a calf by a feeding tool. [ ]
- Feed thawed frozen colostrum. [ ]
- Feed artificial colostrum. [ ]

(Now)

- Feed directly from teats of a dam. [ ]
- A farm worker milks colostrum of a dam and feeds a calf by a feeding tool. [ ]
- Feed thawed frozen colostrum. [ ]
- Feed artificial colostrum. [ ]

- Please check the all apply to the way you feed calves and tell us the period you use them.

	By a dam	By a milking bucket	By a bin	By a bucket
Previous	[ ] (___days old—___days old)	[ ] (___days old—___days old)	[ ] (___days old—___days old)	[ ] (___days old—___days old)
Now	[ ] (___days old—___days old)	[ ] (___days old—___days old)	[ ] (___days old—___days old)	[ ] (___days old—___days old)

(2) Does the same worker handle calves and milking cows?

	Yes	No
Previous	[ ]	[ ]
Now	[ ]	[ ]

・If you answered “yes,” please answer the following questions.

-When does the worker take care of calves, before or after milking?

	Before milking	After milking	Not decided
Previous	[ ]	[ ]	[ ]
Now	[ ]	[ ]	[ ]

-Does the worker change clothes between taking care of calves and taking care of milking cows?

	Yes	No
Previous	[ ]	[ ]
Now	[ ]	[ ]

		Yes	No	No gloves used
-Does the worker change gloves between taking care of calves and taking care of milking cows?	Previous	[ ]	[ ]	[ ]
	Now	[ ]	[ ]	[ ]

2. Questions about way of milking

(1) Please tell us about milking procedures.

		Yes	No	Only paper towel are used
● Do you use towels to wipe teats?	Before the mastitis in your farm	[ ]	[ ]	[ ]
	Now	[ ]	[ ]	[ ]

Note: If you answered "yes" to at least one of the previous question, please answer the questions in this box.

		Yes	No
Do you use one towel per one cow?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
Do you dip towels to disinfectant?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
Do you wipe teats by paper towels after using a cloth towel?	Previous	[ ]	[ ]
	Now	[ ]	[ ]

		Yes	No
● Do you especially consciously wipe teat openings?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you do pre-dipping?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you do post-dipping?	Previous	[ ]	[ ]
	Now	[ ]	[ ]

(2) Please tell us about milking equipment.

		Yes	No
● Do you use a cart to convey milking equipment?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you use a strip cup?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you actively call veterinarians when you find an abnormality by the strip cup?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you actively call veterinarians when you find an abnormality by PL test?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you use adequately disinfected milking equipment?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you disinfect milking equipment after milking?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you disinfect milking equipment before milk a next cow?	Previous	[ ]	[ ]
	Now	[ ]	[ ]

(3) Please tell us about handling of milking cows with an abnormality.

		Yes	No
● Do you milk a cow with high somatic cell count last?	Previous	[ ]	[ ]
	Now	[ ]	[ ]

● Do you milk mastitis cows last?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you conduct mastitis test by pathogen other than *Mycoplasma* after the first calving of a home-bread heifer?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you conduct mastitis test by *Mycoplasma* after the first calving of a home-bread heifer?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you conduct a self-imposed test of *Mycoplasma* with bulk tank milk?	Previous	[ ]	[ ]
	Now	[ ]	[ ]
● Do you disinfect milking units after the first calving of a cow until the result of *Mycoplasma* test is available?	Previous	[ ]	[ ]
	Now	[ ]	[ ]

3. Questions about use of communal pastures

(1) Have you ever used communal pastures? Yes [ ] No [ ]

・If you answered "yes," please answer the following questions.

-If you have ever used communal pastures before the *Mycoplasma* mastitis outbreak, please tell us the most recent year you used them **before the outbreak**. Year ___________

-Did you use communal pastures **after the outbreak** of *Mycoplasma* mastitis?

Yes [ ] No, but will use them if necessary [ ] No and will never use tem [ ]

(2) Please **select all** the type of cows which you send to the communal pastures and circle the most frequent one.

Calves [ ] Non-pregnant heifers [ ] Dry cows [ ] Other ___________ [ ]

(3) Please **select all** the type of cows which come back from the communal pastures and circle the most frequent one.

Non-pregnant heifers [ ] Pregnant heifers [ ] Other ___________ [ ]

(4) Please **select all** the owners of the communal pastures you use and circle the most frequent one.

An agricultural cooperative [ ] A neighborhood farm [ ]

A public organization such as city [ ] Other _________ [ ]

(5) Please tell us the type of communal pastures you use.

Rangeland [ ] Paddock [ ] Free barn [ ] Tied stall [ ] Other _________ [ ]

4. Questions about cow introduction

If you have ever introduced cows, please answer the following question.		Yes	No
● When you introduce cows, do you check health condition of introduced cows by yourself?	Before the mastitis in your farm	[ ]	[ ]
	Now	[ ]	[ ]

Note: Finally, if you have any question of request, please freely write.

Thank you very much for your cooperation.

We will try hard to reveal the cause of the *Mycoplasma* mastitis outbreak with your answers.

Nemuro *Mycoplasma* Mastitis Control Committee
Rakuno Gakuen University

根室地区マイコプラズマ乳房炎再調査質問票（症例農場用）

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学
2016 年 7 月 20 日

お願い

前回のマイコプラズマ乳房炎対策質問票調査ではご協力いただきありがとうございました。現在、酪農学園大学にて質問票の解析が行われており、結果よりさらなる調査が必要であることが推察されたため、前回調査にご協力いただいた皆様に追加の質問票調査へのご協力をお願いすることとなりました。

本病の発生原因と対策を詳細に究明し、根室管内での本病発生予防に貢献したいと考えておりますので、ご多忙中とは存じますが、ご協力の程、よろしくお願いいたします。

農場名：____________________

回答年月日：　　　年　　月　　日

質問者：____________________　（農協職員名）

管理用ＩＤ（協議会で入力し、管理されます）：__________________

※<u>ご回答いただく前に</u>…質問票内の「自農場でのマイコプラズマ乳房炎発生前」や「発生前」という項目はあなたの農場における**2014年から2015年にかけてのマイコプラズマ乳房炎発生以前の実施内容**を回答していただく項目です。よろしくお願いいたします。

1. 日々の作業内容に関する質問

● 分娩後に子牛を親牛から隔離し、親牛と直接の接触がなくなるタイミングを教えてください。

（自農場でのマイコプラズマ乳房炎発生前）
- 分娩を監視しており、分娩の直後に子牛との隔離を行う。 ☐
- 監視はしておらず、分娩の後に気が付いた段階で隔離を行う。 ☐
- 一定期間、一緒に飼養した後に隔離を行う。 ☐
- その他（ ______________________ ）☐

・親牛と子牛を一緒に飼養する期間がある方はその飼養日数を教えてください。
__________日間

（現在）
- 分娩を監視しており、分娩の直後に子牛との隔離を行う。 ☐
- 監視はしておらず、分娩の後に気が付いた段階で隔離を行う。 ☐
- 一定期間、一緒に飼養した後に隔離を行う。 ☐
- その他（ ______________________ ）☐

・親牛と子牛を一緒に飼養する期間がある方はその飼養日数を教えてください。
__________日間

● 初乳の哺乳方法を教えてください。

（発生前）
- 親牛の乳房から直接哺乳させる。 ☐
- 飼養者が親牛の初乳を搾乳し、哺乳器を用いて哺乳する。 ☐
- 凍結初乳を解凍して哺乳する。 ☐
- 人工初乳製品を哺乳する。 ☐

（現在）
- 親牛の乳房から直接哺乳させる。 ☐
- 飼養者が親牛の初乳を搾乳し、哺乳器を用いて哺乳する。 ☐
- 凍結初乳を解凍して哺乳する。 ☐
- 人工初乳製品を哺乳する。 ☐

● 子牛への哺乳方法に当てはまるものをすべて回答し、その哺乳方法を実施する期間を教えてください。

	自然哺乳	哺乳バケツ	哺乳ビン	バケツ
発生前	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)
現在	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)	☐ (____日齢～ ____日齢)

（2）子牛を扱う作業者と搾乳牛を扱う作業者は同じですか？

	はい	いいえ
発生前	☐	☐
現在	☐	☐

・「はい」と回答した方に質問です。
- 子牛の世話は搾乳の前ですか後ですか。

	搾乳の前	搾乳の後	決めていない
発生前	☐	☐	☐
現在	☐	☐	☐

- 子牛と搾乳牛の世話の間に作業着は交換しますか？

	はい	いいえ
発生前	☐	☐
現在	☐	☐

		はい	いいえ	手袋は使わない
- 子牛を世話と搾乳牛の世話で手袋は交換していますか？	発生前	□	□	□
	現在	□	□	□

2. 搾乳方法に関する質問

（1）搾乳の手順に関して教えてください。

		はい	いいえ	ペーパータオルのみ使用
● 清拭タオルは使用していますか？	自農場での発生前	□	□	□
	現在	□	□	□

※上の質問でどちらか一つでも「はい」と回答された方はこの枠内の質問にも回答して下さい。

		はい	いいえ
清拭タオルは一頭に一枚ずつ使用していますか？	発生前	□	□
	現在	□	□
清拭タオルは消毒液につけていますか？	発生前	□	□
	現在	□	□
清拭タオルで拭いた後、ペーパータオルで乳頭を拭き取りますか？	発生前	□	□
	現在	□	□

		はい	いいえ
● 乳頭口は特に意識して清拭しますか？	発生前	□	□
	現在	□	□
● プレディッピングをしていますか？	発生前	□	□
	現在	□	□
● ポストディッピングをしていますか？	発生前	□	□
	現在	□	□

（2）搾乳器具に関して教えてください。

		はい	いいえ
● 搾乳ワゴンは使用していますか？	発生前	□	□
	現在	□	□
● ストリップカップは使用していますか？	発生前	□	□
	現在	□	□
● ストリップカップで異常があった場合、積極的に獣医師に連絡しますか？	発生前	□	□
	現在	□	□
● PL で異常があった場合、積極的に獣医師に連絡しますか？	発生前	□	□
	現在	□	□
● 十分に消毒した搾乳器具を使用していますか？	発生前	□	□
	現在	□	□
● 搾乳終了後に搾乳器具の消毒は実施しますか？	発生前	□	□
	現在	□	□
● 隣の牛に移る時に搾乳器具の消毒は実施しますか？	発生前	□	□
	現在	□	□

（3）異常のある搾乳牛に対する対応に関して教えてください。

		はい	いいえ
● 体細胞数の高い牛は最後に絞りますか？	発生前	□	□
	現在	□	□

● 乳房炎の牛は最後に搾乳しますか？	発生前	□	□
	現在	□	□
● 自家産初産は分娩後にマイコプラズマ以外の菌種の乳房炎検査はしますか？	発生前	□	□
	現在	□	□
● 自家産初産は分娩後にマイコプラズマの乳房炎検査はしますか？	発生前	□	□
	現在	□	□
● バルク乳でマイコプラズマ自主検査は行っていますか？	発生前	□	□
	現在	□	□
● 初妊牛については、マイコプラズマ検査の結果が出るまでユニット消毒を行っていますか？	発生前	□	□
	現在	□	□

3. 預託に関する質問

（1）預託を実施したことがありますか。　はい □　いいえ □

・「はい」と回答した方に質問です。

- マイコプラズマ乳房炎発生以前に預託をしたことがある方は<u>発生以前で</u>一番最近預託を実施した年を教えてください。 ＿＿＿＿＿年
- マイコプラズマ乳房炎**発生以降**、預託を実施していますか？

実施している □　実施していないが、必要であれば実施する □

実施しておらず、今後も実施しない □

（2）預託する牛の育成ステージに当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

子牛 □　育成牛 □　乾乳期牛 □　その他 ＿＿＿＿＿ □

（3）預託先から戻ってくる牛の育成ステージに当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

育成牛 □　初妊牛 □　その他 ＿＿＿＿＿ □

（4）預託先団体として当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

JA □　近隣の農場 □　市などの公共機関 □　その他 ＿＿＿＿ □

（5）預託先の飼養形態を教えてください。

牧野 □　パドック □　フリーバーン □　繋ぎ牛舎 □

その他 ＿＿＿＿ □

4. 導入に関する質問

過去に導入経験がある方は以下の質問にも回答してください。		はい	いいえ
● 導入の際にはご自身で導入牛の健康状態の確認を実施していますか。	自農場での発生前	□	□
	現在	□	□

※最後に何か質問や要望があれば、ご自由にお書きください。

ご協力大変ありがとうございました。
回答していただきましたものを参考にマイコプラズマ乳房炎発生の原因解明に努力いたします。

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学

Supplementary Material S6. Questionnaire used for the second survey of control farms.

Questionnaire for a resurvey of *Mycoplasma* mastitis in Nemuro region (for a control farm)

Nemuro *Mycoplasma* Mastitis Control Committee
Rakuno Gakuen University
July 20, 2016

Please

We appreciate for your help for our previous questionnaire survey to prevent *Mycoplasma* mastitis. Currently, questionnaires are under analysis in Rakuno Gakuen University and with the result, it was assumed that more investigation is necessary. So, we decided to ask respondents of the previous survey to cooperate in an additional questionnaire survey.

We hope to reveal the cause and countermeasure to the disease and contribute to prevent an outbreak of the disease in Nemuro region. I realize you are very busy, but we would appreciate if you cooperate with us.

Name of a farm:____________________

Date of answer: Year Month Day

Interviewer:____________________ (Name of a worker of an agricultural cooperative)

ID for management (It will be entered at the committee and managed):__________________

1. Questions about daily routine

(1) Please tell us about a dam and a calf after a delivery.

- Please tell us the timing when a calf is separated from its dam after a delivery and direct contact of them are limited.
  - You monitor a delivery of a cow and a calf is separated immediate after the delivery. [ ]
  - You do not monitor a delivery and a calf is separated when you realize that the delivery is finished. [ ]
  - You keep a calf and a dam for a while and then isolate them. [ ]
  - Other ( ) [ ]
  - ・If there is a period when you keep a dam and a calf together, please tell us the length of the period. ________days
- Please tell us the way you feed colostrum.
  - Feed directly from teats of a dam. [ ]
  - A farm worker milks colostrum of a dam and feeds a calf by a feeding tool. [ ]
  - Feed thawed frozen colostrum. [ ]
  - Feed artificial colostrum. [ ]
- Please check the all apply to the way you feed calves and tell us the period you use them.

By a dam [ ]	By a milking bucket [ ]	By a bin [ ]	By a bucket [ ]
(___days old– ___days old)	(___days old– ___days old)	(___days old– ___days old)	(___days old– ___days old)

(2) Does the same worker handle calves and milking cows? Yes [ ] No [ ]

・If you answered "yes," please answer the following questions.

-When does the worker take care of calves, before or after milking?

Before milking [ ] After milking [ ] Not decided [ ]

-Does the worker change gloves between taking care of calves and taking care of milking cows?

Yes [ ] No [ ] No gloves used [ ]

-Does the worker change clothes between taking care of calves and taking care of milking cows?

Yes [ ] No [ ]

2. Questions about use of communal pastures

(1) Have you ever used communal pastures? Yes [ ] No [ ]

・If you answered "yes," please answer the following question.

-Please tell us the most recent year you used them. Year ________

(2) Please **select all** the type of cows which you send to the communal pastures and <u>circle the most frequent one</u>.

Calves [ ] Non-pregnant heifers [ ] Dry cows [ ] Other __________ [ ]

(3) Please **select all** the type of cows which come back from the communal pastures and <u>circle the most frequent one</u>.

Non-pregnant heifers [ ] Pregnant heifers [ ] Other __________ [ ]

(4) Please **select all** the owners of the communal pastures you use and <u>circle the most frequent one</u>.

An agricultural cooperative [ ] A neighborhood farm [ ]

A public organization such as city [ ] Other [ ]

(5) Please tell us the type of communal pastures you use.

Rangeland [ ] Paddock [ ] Free barn [ ] Tied stall [ ] Other __________ [ ]

3. Questions about cow introduction

If you have ever introduced cows, please answer the following question.

- When you introduce cows, do you check health condition of introduced cows by yourself?

Yes [ ] No [ ]

Note: Finally, if you have any question of request, please freely write.

Thank you very much for your cooperation.
We will try hard to reveal the cause of the Mycoplasma mastitis outbreak with your answers.

Nemuro Mycoplasma Mastitis Control Committee
Rakuno Gakuen University

根室地区マイコプラズマ乳房炎再調査質問票（対照農場用）

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学
2016年7月20日

お願い

前回のマイコプラズマ乳房炎対策質問票調査ではご協力いただきありがとうございました。現在、酪農学園大学にて質問票の解析が行われており、結果よりさらなる調査が必要であることが推察されたため、前回調査にご協力いただいた皆様に追加の質問票調査へのご協力をお願いすることとなりました。

本病の発生原因と対策を詳細に究明し、根室管内での本病発生予防に貢献したいと考えておりますので、ご多忙中とは存じますが、ご協力の程、よろしくお願いいたします。

農場名：____________________

回答年月日：　　　年　　月　　日

質問者：____________________　（農協職員名）

管理用ＩＤ（協議会で入力し、管理されます）：__________________

1. 日々の作業内容に関する質問

（1）分娩後の親牛と子牛に関して教えてください。

- 分娩後に子牛を親牛から隔離し、親牛と直接の接触がなくなるタイミングを教えてください。
  - 分娩を監視しており、分娩の直後に子牛との隔離を行う。 □
  - 監視はしておらず、分娩の後に気が付いた段階で隔離を行う。 □
  - 一定期間、一緒に飼養した後に隔離を行う。 □
  - その他（　　　　　　　　　　　　　　　　　　　　　　） □
  - ・親牛と子牛を一緒に飼養することがある方はその飼養日数を教えてください。
    ＿＿＿＿＿日間
- 初乳の哺乳方法を教えてください。
  - 親牛の乳房から直接哺乳させる。 □
  - 飼養者が親牛の初乳を搾乳し、哺乳器を用いて哺乳する。 □
  - 凍結初乳を解凍して哺乳する。 □
  - 人工初乳製品を哺乳する。 □
- 初乳以降の哺乳方法に当てはまるものをすべて回答し、その哺乳方法を実施する期間を教えてください。

自然哺乳 □	哺乳バケツ □	哺乳ビン □	バケツ □
(＿＿日齢～ ＿＿日齢)	(＿＿日齢～ ＿＿日齢)	(＿＿日齢～ ＿＿日齢)	(＿＿日齢～ ＿＿日齢)

（2）子牛を扱う作業者と搾乳牛を扱う作業者は同じですか？ はい □ いいえ □

・「はい」と回答した方に質問です。

- 子牛の世話は搾乳の前ですか後ですか。
  搾乳の前 □ 搾乳の後 □ 決めていない □
- 子牛を世話と搾乳牛の世話で手袋は交換していますか？
  はい □ いいえ □ 手袋を使用していない □
- 子牛と搾乳牛の世話の間に作業着は交換しますか？ はい □ いいえ □

2. 預託に関する質問

（1）預託を実施したことがありますか。 はい □ いいえ □

・「はい」と回答した方に質問です。

- 一番最近預託を実施した年を教えてください。 ＿＿＿＿年

（2）預託する牛の育成ステージに当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

子牛 □　育成牛 □　乾乳期牛 □　その他 ＿＿＿＿＿ □

（3）預託先から戻ってくる牛の育成ステージに当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

育成牛 □　初妊牛 □　その他 ＿＿＿＿＿ □

（4）預託先団体として当てはまるものを**すべて選び**、その中で<u>最も頻度の多いものには○</u>をつけてください。

JA □　近隣の農場 □　市などの公共機関 □　その他 ＿＿＿＿ □

（5）預託先の飼養形態を教えてください。

牧野 □　パドック □　フリーバーン □　繋ぎ牛舎 □

その他 ＿＿＿＿ □

3. 導入に関する質問

過去に導入経験がある方は以下の質問にも回答してください。

- 導入の際にはご自身で導入牛の健康状態の確認を実施していますか。
  はい □ いいえ □

※最後に何か質問や要望があれば、ご自由にお書きください。

ご協力大変ありがとうございました。
回答していただきましたものを参考にマイコプラズマ乳房炎発生の原因解明に努力いたします。

根室管内マイコプラズマ乳房炎対策会議
酪農学園大学

Abstract

Application of causal inference and mathematical modelling to control enzootic diseases in Japanese dairy farms

Veterinary Epidemiology
Doctoral Course of Veterinary Medicine
Graduate School of Veterinary Medicine
Rakuno Gakuen University Graduate School
Yuri Fujimoto

The objective of the thesis is to reveal the way to control infectious diseases in Japanese dairy cows by causal inference and mathematical modelling. To achieve the objective, two studies were conducted: analysis of risk factors of associated with an outbreak of *Mycoplasma* mastitis in Nemuro area, Hokkaido, Japan and construction of a simulation model of spread of bovine leukemia virus (BLV) in a dairy herd.

Economic loss caused by livestock infectious diseases was a critical problem for farmers. Several outbreaks of livestock diseases occurred in Japan: foot-and-month disease in 2000 and 2010, highly pathogenic avian influenza from 2004, and classical swine fever from September 2018 to March 2020. These diseases are in the list of monitored infectious diseases and national disease control programs with compensation at culling for the control are in place. However, diseases with milder symptoms are relatively neglected and there are no such national financial supports. Thus, to control such non-highly pathogenic diseases, elucidation of the most effective control method of a disease is necessary to concentrate resources on that.

Epidemiology is defined as the study of disease in populations and of factors that determine its occurrence. In this study, two infectious diseases dairy cows, *Mycoplasma* mastitis and enzootic bovine leukosis by BLV were epidemiologically investigated.

In the Chapter 1, risk factors associated with an outbreak of *Mycoplasma* mastitis occurred in Nemuro area, Hokkaido, Japan from 2014 to 2015 were analyzed at farm- and cow-level. Descriptive epidemiology and causal inference using causal diagrams were selected as

approaches in the chapter. Data collected and analyzed included results of two questionnaire surveys, movement records, dairy herd test records, and clinical history. In the herd-level analysis, tie stall housing for milking cows (odds ratio [OR] = 0.20, 95% CI: 0.07-0.60, *p* = 0.004), consciously wiping of teat openings before milking (OR = 0.15, 95% CI: 0.02-0.76, *p* = 0.030), and use of paper towels to wipe teats (OR = 0.31, 95% CI: 0.09-0.92, *p* = 0.045) were identified as preventive factors, whereas introduction of cattle (OR = 3.43, 95% CI: 1.14-10.86, *p* = 0.030) was a risk factor. In the cow-level analysis, a history of presence in livestock markets (OR = 10.80, 95% CI: 1.12-104.38, *p* = 0.040), higher milk yield 2 months prior to *Mycoplasma* infection (OR = 1.09, 95% CI: 1.02-1.18, *p* = 0.014), and previous diagnosis of acute mastitis without isolation of the causal pathogen (OR = 3.14, 95% CI: 0.86-11.41, *p* = 0.082) were identified as risk factors. These results highlight the importance of proper milking hygiene control and quarantine of introduced cattle to prevent *Mycoplasma* infection.

In the Chapter 2, a simulation model of spread of BLV in a dairy herd was constructed. In the chapter, an individual-based mathematical modelling of infection was used as an approach. The model simulates monthly changes in status of each cow such as age, parity, and infection status. Data obtained by monitoring BLV prevalence of four dairy farms in Hokkaido, Japan was used for and simulation. Probability of seroconversion at a communal pasture largely affected the within-herd prevalence in a farm which sent all their heifers to a communal pasture. This emphasized the importance of BLV control in communal pastures. BLV test sensitivities and test frequencies did not affect the period to eradicate BLV in a herd. The constructed model was released on the Internet. The model should contribute to choosing an effective BLV-control strategy by comparing simulation results under different conditions.

This thesis revealed the way to control the two infectious diseases in dairy cows by epidemiological approaches. The results obtained by two approached adopted in the study, risk factor analysis in the Chapter 1 and infectious disease modelling in the Chapter 2, have strong relationships actually. While constructing the model, selection of events to build in the model and parameter values referred previous studies of risk factors quite a bit. The constructed model can be applied to other infectious disease by modifying parameters. For example, an infectious disease model of *Mycoplasma* mastitis can be made utilizing the result of the Chapter 1. This thesis shows the usefulness of an approach to control and infectious disease by risk factor analysis by causal inference followed by construction of a simulation model using the result the analysis.

Abstract in Japanese (和文要旨)

日本の酪農における常在性疾病制御のための因果推論および数理モデルの応用に関する研究

酪農学園大学大学院獣医学研究科
獣医学専攻博士課程
獣医疫学　藤本悠理

本研究論文の目的は、因果推論および感染数理モデルにより、日本の乳用牛における感染症を制御する方法を明らかにすることである。本目的のため、北海道根室地域におけるマイコプラズマ性乳房炎のリスク要因の解析、および農場内における牛白血病ウイルス（BLV）の広がり方のシミュレーションモデルの開発を行った。

家畜感染症による経済的損失は畜産農家にとって深刻な問題である。近年日本では、2000年および2010年の口蹄疫、2004年から発生が続く高病原性鳥インフルエンザ、2018年9月から2020年3月まで流行した豚熱など、種々の監視伝染病のアウトブレイクが発生した。これらの監視伝染病は殺処分に際して国から手当金が交付される一方、それ以外の比較的症状の軽い家畜感染症には国からの補助金が存在しない。よって、そのような症状の軽い感染症の制御には、最も効果的な対策方法を明らかにし、それにリソースを集中することが肝要である。

疫学とは、集団における疾病及びその発生を規定する要因を明らかにする学問である。本研究では、北海道で飼養される乳用牛における二つの感染症、マイコプラズマ性乳房炎および牛白血病ウイルスによる地方病性牛白血病について、疫学的手法によりその制御方法を明らかにした。

第1章においては、2014年から2015年にかけて北海道根室地域で多発したマイコプラズマ性乳房炎の、農場レベルおよび個体レベルでのリスク要因を解析した。本章においては記述疫学および因果ダイアグラムによる因果推論を行った。当該地域の農場に対する2回の質問票調査の結果、個体の移動履歴、牛群検定成績および治療履歴が解析に用いられた。農場レベルにおいては、搾乳牛の繋ぎ飼い（オッズ比［OR］= 0.20, 95%信頼区間［CI]: 0.07-0.60, $p = 0.004$）、乳頭口の意識的な清拭（OR = 0.15, 95% CI: 0.02-0.76, $p = 0.030$）および乳頭清拭時のペーパータオルの使用（OR = 0.31, 95% CI: 0.09-0.92, $p = 0.045$）が防御因子、牛

の導入（OR = 3.43, 95% CI: 1.14-10.86, $p = 0.030$）がリスク因子であった。個体レベルにおいては、家畜市場にいた経験（OR = 10.80, 95% CI: 1.12-104.38, $p = 0.040$）、高泌乳量（OR = 1.09, 95% CI: 1.02-1.18, $p = 0.014$）および原因菌が分離されなかった急性乳房炎の診断履歴（OR = 3.14, 95% CI: 0.86-11.41, $p = 0.082$）がリスク要因であった。本疾病の防止のためには、適切な搾乳衛生管理および導入牛の隔離・検査が重要であることが示された。

第 2 章においては、BLV の農場内での感染の広がり方のシミュレーションモデルを開発した。本章においては個体レベルでの感染数理シミュレーションモデルを手法として選択した。モデル内では農場内の各牛の状態（年齢、産次数、感染状況など）を月ごとにシミュレーションした。シミュレーションには道内 4 酪農場において BLV 感染状況を継続調査したデータを用いた。公共牧場に子牛を預託している 1 農場について、公共牧場における BLV 陽転率が農場内有病率に大きく影響した。これにより公共牧場における BLV 制御の重要性が示唆された。また、BLV 検査の感度および頻度は農場内における BLV 撲滅までの期間に影響を与えなかった。開発したモデルはインターネット上で公開した。様々な条件下での農場内有病率の変化を本モデルによりシミュレーションし比較することで、より効果的な BLV 制御方法を選択できると考えられ、本モデルの有用性が示唆された。

本研究論文においては、乳用牛における二つの感染症について、疫学的方法を用いてその制御方法を明らかにした。第 1 章における疾病のリスク要因の解析と第 2 章における感染モデルの開発という二つの研究成果は、一見応用範囲が異なっているように思えるが実際は密接に関係している。感染モデルの開発においては、モデル内で考慮すべき要因の選択やパラメーターの決定において、既存のリスク要因解析研究の結果を大きく参考にしている。作成された感染モデルはパラメーターを調整することで他の感染症にも応用可能であり、例えばマイコプラズマ性乳房炎について、第 1 章における解析結果を応用して感染モデルを作成できる。本研究論文は、因果推論によるリスク要因の解析およびその結果を基にした感染モデルの開発というプロセスによる家畜感染症制御の有用性を示すものである。