Biosecurity risks of cattle for *Salmonella* Dublin infections on dairy farms in Alberta, Canada

Master thesis

Marlinde de Pater **August 2024**
Veterinary Medicine Utrecht University
Student number 6663168

Dutch supervisor Dr. Ruurd Jorritsma R.jorritsma@uu.nl
Population Health Sciences and Farm Health department Utrecht University
Canadian supervisor PhD candidate Waseem Shaukat Waseem.shaukat@ucalgary.ca
Epidemiology and Economics of Animal Health department Calgary University

I. Abstract

Infectious diseases are a significant danger to the sustainable production of high-producing animals such as cattle. *Salmonella enterica* serovar Dublin (*S.* Dublin) is a zoonotic pathogen of interest on dairy farms as it is associated with high levels of morbidity, mortality and economic losses.

S. Dublin is an emerging bacterium in Canada. Risk factors have been identified for Ontario, but because of the possible effect of demographic factors, there exists a knowledge gap on risk factors for Alberta and Western Canada as a whole (Perry et al., 2023).

We therefore performed a case-control study to identify the biosecurity risk factors related to herd positivity for *S.* Dublin in Alberta. The potential risk factors in this study were based on questionnaires and observations done in 2023 on 52 farms. The herd positivity of 13.5% was based on at least one positive test (>35% positivity on ELISA) for antibodies against *S.* Dublin in four quarterly bulk tank milk samples in 2021 and 2022.

Results from this study indicated that contact between adult cow feces and weaned calves (OR=8.2; P<0.001) and between adult cow feces and breeding heifers (OR=6.9; P<0.001) increased the risk for dairy farms to be infected with *S.* Dublin. We also found the association between *S.* Dublin positive herds and manure contact between different age groups through boots or tools (OR=7.6; P=0.024) or manure management equipment (OR=3.6; P=0.026). Lastly, this study demonstrated that buying hay or silage from other producers (OR=3.9; P=0.008) or not cleaning and sanitizing the calving pen after each use (OR=7.3; P<0.001) can also be associated with herd positivity for *S.* Dublin. Dairy farmers in Alberta should minimize indirect contact of manure between different age groups and farms to reduce the risk of *S.* Dublin infections.

II. Acknowledgements

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Furthermore, I would also like to take this opportunity to acknowledge that this research has been done on the traditional territories of the people of the Treaty 7 region in Southern Alberta. The City of Calgary is also home to Métis Nation of Alberta, Region III.

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1. Introduction

Infectious diseases are a threat to sustainable dairy industry. Not only the health and welfare of cattle is affected, infected cattle can also show a significant decrease in production (Doeschl-Wilson et al., 2021). Due to the decrease in production, the dairy industry faces considerable economic loss because of infectious diseases (Aghamohammadi et al., 2018).

Certain conditions and limitations in dairy farms can increase the risk of cattle acquiring infectious diseases (Loh et al., 2015). These conditions and limitations are typically exacerbated in larger herds due to an increase in contact between animals, which makes them more susceptible to transmitting infections (Lindström et al., 2012).

Besides herd size and housing type influencing pathogen transmission, climate change has been linked to the emergence of infectious diseases (Ogden & Gachon, 2019). A rise in temperature, for instance, can contribute to the spread of infectious diseases (Anwar et al., 2019). This is why research is needed on emerging infectious diseases.

Salmonella enterica serovar Dublin (*S.* Dublin) is a pathogen of interest in dairy farms. *S.* Dublin is a host-adapted serotype that can cause septicemia and respiratory disease in younger animals. In young calves, *S.* Dublin has been linked to an increased morbidity and higher mortality rates (Srednik et al., 2021a). In mature cattle *S.* Dublin has been associated with gastroenteritis, increased abortions and decreased production (Hezil et al., 2021; Salaheen et al., 2020; Velasquez-Munoz et al., 2023a). *S.* Dublin, however, is also present in animals without clinical symptoms (Mir & Rautela, 2020).

Besides the economic and cattle health impact associated with *S.* Dublin, it is also a zoonotic disease. Zoonotic diseases can cross species and affect human health. *S.* Dublin therefore is a threat to public health (Mir & Rautela, 2020). Human infection of *S.* Dublin can lead to severe illness, including bacteremia, potentially being lethal. Transmission to humans is possible by direct contact with animals or by indirect contact via feces or consuming unpasteurized dairy products. (Srednik et al., 2021a; Velasquez-Munoz et al., 2023b)

Treatment is necessary for some animals that are infected with *S.* Dublin. This treatment can consist of fluid restoration for all cattle and antibiotics for calves because of the risk of bacteremia (Holschbach & Peek, 2018). *S.* Dublin is found as one of the most multidrug resistant serotypes in cattle (Fritz et al., 2022). The use of antibiotics can create multidrug resistant strains of S. Dublin that are resistant against sulfonamides, tetracyclines and beta-lactams (Srednik et al., 2021b). These antibiotics are important for treating severe illnesses in humans and are becoming less effective due to growing resistance. Because of the far-reaching consequences of *S.* Dublin infections in cattle, and the potential impact in human, it is important to find ways to reduce the risk of *S.* Dublin transmission.

S. Dublin is an emerging pathogen in Canada (Mangat et al., 2019; Uyama et al., 2022). It was isolated in Quebec in 2011 and one of the most isolated *Salmonella* serotype in Ontario in 2022 (Perry et al., 2023). The Cattle Health Surveillance System (CHeSS) project demonstrated that 77 (16%) Alberta dairy herds tested positive for antibodies against *S.* Dublin on at least one of the quarterly bulk tank milk (BTM) sample tests in 2021 and 2022. The government of Alberta has classified *S.* Dublin as a reportable disease because of the economic importance and risks for public health (Provincially Regulated Animal Diseases | Alberta.Ca, n.d.).

1.1 Biosecurity

Biosecurity can influence the emergence of infectious diseases such as *S.* Dublin. Biosecurity measures are aimed to prevent introduction and spread of infectious diseases (Brennan & Christley, 2012). This involves being aware of risks regarding diseases and playing an active role in disease prevention. There have been made frameworks to test biosecurity measurements on farms which may help to identify risk factors (Pedersen et al., 2023). In Canada, the proAction program is a national quality assurance program, initiated by the Dairy Farmers of Canada, through which dairy farmers are required to meet certain biosecurity targets. The biosecurity module was introduced in the proAction program in 2019.

Whether the biosecurity targets are met is related to demographic factors, farm characteristics and the willingness of farmers to meet targets (Denis-Robichaud et al., 2020). Studies in Denmark concluded that biosecurity measures within or between herds to minimize the spread of infection were not always applied (Kristensen & Jakobsen, 2011; Oliveira et al., 2018). A study on Canadian dairy farms regarding biosecurity and herd health management practices indicated that less than half of the farms required clean boots and coveralls for farm visitors (Denis-Robichaud et al., 2019). Furthermore, most farms did not always take measurements cleaning the calving pen after calving. It is therefore important to note that not all biosecurity measures that are indicated as useful are executed at farms.

1.2 Risk factors

While *S.* Dublin is an emerging pathogen in Canada, there is a lack of knowledge about the involved risk factors for cattle to be infected with *S.* Dublin in Alberta. This knowledge is available for other regions in and outside Canada.

A previous study in Ontario indicated that farms that introduced purchased animals within the last two years and herds with animals leaving the premises and returning were at greater risk for *S*. Dublin (Perry et al., 2023). Research carried out in Denmark and the Netherlands demonstrated that poor hygiene created a risk for increased *S.* Dublin infections (Nielsen et al., 2012; Nielsen & Dohoo, 2012; Vaessen et al., 1998). This included poor hygiene of the maternity barn, pre-weaned area and tools used to feed or treat sick animals. Research in Denmark also demonstrated that contact between youngstock and older cattle increased the risk of obtaining *S.* Dublin (Nielsen et al., 2012). Lack of isolation facilities for sick animals is identified in America as a risk factor for *S.* Dublin infections on dairy farms (Fossler et al., 2005). Research on infectious diseases in the Netherlands indicated that *S.* Dublin infected farms less often made professional visitors wear protective clothing compared to non-infected farms (Van Schaik et al., 2002).

Demographic factors might influence the risk factors for *S.* Dublin infections that apply to a specific region (Shortall et al., 2017). To investigate whether the same risk factors apply to *S.* Dublin infections on dairy farms in Alberta, it is necessary to perform research.

The aim of this study is to assess biosecurity practices of Alberta dairy herds and their association with *S.* Dublin infections. The identification of risk factors for *S.* Dublin provides an opportunity to devise evidence-based strategies for preventing and controlling *S.* Dublin infections. This may lead to fewer farms testing positive for *S.* Dublin on BTM samples, and therefore fewer herds being infected with *S.* Dublin in Alberta. The risk of transmission from cattle to humans is also expected to decrease as the number of *S.* Dublin infections in cattle decreases.

1.2 Objectives

The objective of this case-control study is to identify biosecurity risks associated with herd positivity for *S.* Dublin tested with BTM screening in Alberta.

This study is part of the project "Uncovering the impact of *Salmonella* Dublin – an emerging threat to the Alberta dairy industry". The project aims to improve the understanding of the prevalence, risk factors, economic impact and antimicrobial resistance profile of *S.* Dublin in Alberta dairy herds.

1.3 Hypothesis

We hypothesized that not wearing protective clothing for visitors creates a risk for *S.* Dublin infections on dairy farms in Alberta. Besides this, it is expected that sharing manure management equipment between different age groups is a risk factor for *S.* Dublin to appear as indirect animal contact is increased by this.

The study was approved by the Animal Care Committee (AC21–0070) of the University of Calgary (Calgary, AB, Canada).

2. Materials and methods

2.1 Sample size

We did no sample size calculations, but used data from 52 dairy farms that participated voluntarily in a larger project on biosecurity. Out of the 52 farms that participated in this study, there were seven farms (13.5%) that tested positive for antibodies against *S.* Dublin at least once in the four BTM samples. Therefore, a 6:1 ratio of control versus cases was used in this study.

2.2 Bulk tank milk samples

Additional bulk tank milk samples were collected at all active dairy farms participating in the CHeSS project and frozen in December 2021 (n=489), April 2022 (n=487), July 2022 (n=487), and October 2022 (n=480). The cream layer was removed from the sample after the samples were centrifuged. The samples were stored at -20°C until they were tested after approximately 142, 17, 5 and 18 days for timepoint 1, 2, 3 and 4 samples, respectively. The samples were tested for antibodies against *S.* Dublin using an indirect ELISA test (Thermo Fischer Scientific, n.d.). (Shaukat et al., 2024)

Percentage positivity of the samples was calculated with the following formula (Equation 1). To quantify the corrected optical density (OD), the negative control OD was subtracted from the sample OD.

$$PP\% = \left(\frac{Corrected\ OD\ samples}{Corrected\ OD\ Positive\ Controls}\ x\ 100\right) - 10 \qquad (1)$$

We used cut-off values recommended by the manufacturer to promote repeatability (Um et al., 2022). Thus, a percentage positivity of 35% and higher was considered positive while a percentage positivity lower than 35% was considered negative (Thermo Fischer Scientific, n.d.). This consideration gives a specificity of 97.5% and a sensitivity of 16.3%. Farms that tested positive on at least one BTM sample were considered positive in this study. This means that farms were classified as negative if they tested negative during all four quarterly BTM sample tests.

2.2 Questionnaire and observations

The data used for this study was retrieved through a sub-set of the larger data collection consisting of three questionnaires which can be found in appendix one.

First, we used part of the results from a biosecurity assessment administered on paper during the summer of 2023. In the selection, the questions about possible risks for dairy farms to test positive on *S.* Dublin were included. We included all questions containing risk factors that were found relevant in other publications on Salmonella positivity for *S.* Dublin antibodies (Ågren et al., 2017; Jorritsma & Hofste, 2011; Pedersen et al., 2023; Perry et al., 2023). While taking the questionnaires no advice or opinions were given to the farmers.

Second, we used two questions from the most recent Dairy Farm Biosecurity General Risk Assessment of each farm performed under proAction. This assessment is a mandatory requirement for every herd to be done by the herd veterinarian every two years. The Dairy Farm Biosecurity General Risk Assessments were filled in between 2021 and 2023.

Finally, we included the results of three observations on management practices. Those observations were filled in on a paper questionnaire by the researchers during the farm visit in 2023.

The data from the questionnaires and observations from the case-control study was collected on paper. We digitalized the data in Microsoft Excel spreadsheet for structuring, comparison and analysis.

2.3 Statistical analysis

In this case control study, we performed a univariate analysis using two-way contingency tables and the Fisher's exact test for the fifteen independent variables included in this study. All variables that discussed management practices relevant to *S.* Dublin infections in the survey and had results of at least 5 positive tested farms were included. The Fisher's exact test was used to test for a significant association between herds tested positive for *S.* Dublin antibodies and possible risk factors. Odds ratios (OR) were manually computed using the contingency table using the formula below:

$$OR = \left(\frac{ad}{bc}\right) \qquad (2)$$

Only farms where the *S.* Dublin infection status in 2021 and 2022 was known and that were visited in the biosecurity project were included in the statistical analysis. Variables of which the odds ratio came with a P value of <0.05 were considered as being statistically significant. Missing data was not included.

3. Results

3.1 Observations and questionnaire answers

Figure 1: Results regarding the farm distribution based on answers and observations in a questionnaire performed on 52 dairy farms in Alberta in 2023.

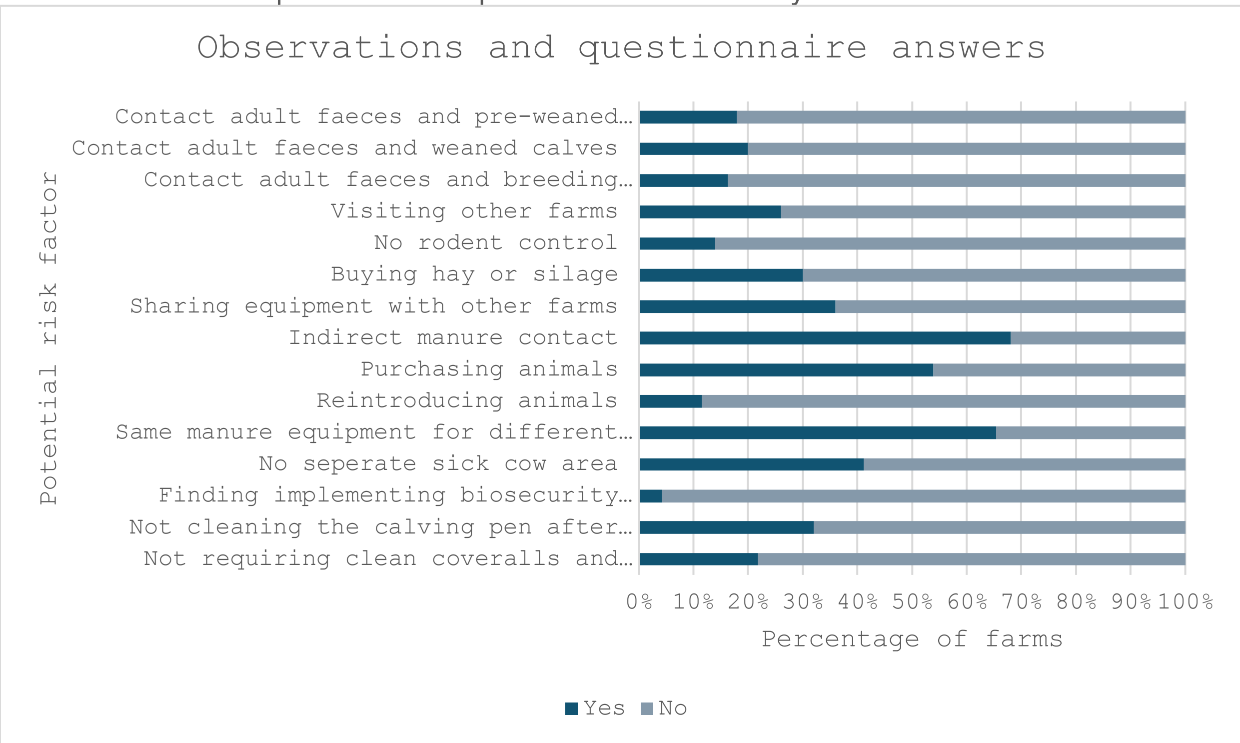

Figure 1 shows the proportion of farms in this study that contained potential risk factors or implemented certain biosecurity practices. As demonstrated in figure 1, it appears that almost all farms agreed to the statement that implementing biosecurity practices, in general, is useful to control diseases on their farm (96%).

From the Dairy Farm Biosecurity General Risk Assessment of proAction, it appeared that most farms in this study cleaned the calving pen after each use (68%) and that most farms required all workers, visitors and farm service providers to wear clean or disposable coveralls and boots on the farm (78%).

Based on observations, we found that the minority of the farms (18-20%) had the possibility of contact between adult cow feces and other cattle groups on the farm.

Table 1: Results from contingency tables and Fisher's exact test based on questionnaires and observations on 52 dairy farms in Alberta in the summer of 2023 and quarterly BTM samples in 2021 and 2022.

Variable	n	Odds ratio	*P* value
Possibility of contact between adult cow feces and pre-weaned calves			
No possibility	41	Reference	
Possibility	9	2.1	0.072
Possibility of contact between adult cow feces and weaned calves			
No possibility	40	Reference	
Possibility	10	8.2	<0.001
Possibility of contact between adult cow feces and breeding heifers			
No possibility	36	Reference	
Possibility	7	6.9	<0.001
Farm personnel also frequently visiting other cattle farms			
Not visiting other farms	37	Reference	
Visiting other farms	13	1.2	0.243
Controlling rodents in the farm			
Self control	43	Reference	
No control	7	0.3	0.782
Buying hay or silage from other producers			
Not buying	35	Reference	
Buying	15	3.9	0.008
Sharing either vehicles or equipment with another farm			
Not sharing	32	Reference	
Sharing	18	1.4	0.179
Possibility of indirect contact with adult cow excrement or manure through boots or tools			
No possibility	15	Reference	
Possibility	32	7.6	0.024
Purchasing animals for the herd			
No purchase of animals	24	Reference	
Purchased animals	28	6.0	0.629
Animals leaving the farm and reintroducing them into your herd			
No animals leaving and reintroducing	46	Reference	
Leaving and reintroducing animals	6	1.3	0.166
Using the same manure management equipment for different age groups			
Not using the same	18	Reference	
Using the same	34	3.6	0.026
Having a seperate pen or area on the farm to keep sick cattle			
Having a seperate pen	30	Reference	
Not having a seperate pen	21	2.1	0.066
Finding implementing biosecurity practices, in general, useful to control diseases on the farm			
Useful	45	Reference	
Not useful	2	8.0	0.256

Table 1 Continued

Variable	n	Odds ratio	*P* value
Cleaning and sanitizing the calving pen after each use			
Cleaning	34	Reference	
Not cleaning	16	7.3	<0.001
Requiring wearing clean or disposable coveralls and boots on the farm			
Requiring	43	Reference	
Not requiring	12	0.6	0.513

3.2 Potential risk factors for *S.* Dublin herd positivity

Table 1 demonstrates 15 variables that are potential risk factors for dairy farms to test positive on *S.* Dublin. Also, the odds ratio and *P* value calculated from the Fisher's exact test is shown. Table 1 indicates six risk factors to be significantly associated with a positive BTM result for *S.* Dublin. The variable that can be associated is the possibility of contact between adult cow feces and weaned calves (OR=8.2; *P*<0.001) or breeding heifers (OR=6.9; *P*<0.001). Buying hay or silage from other producers is another variable that shows higher odds for positive BTM samples (OR=3.9; *P*=0.008). Other variables with increased odds for *S.* Dublin infections are the possibility of indirect contact with adult cow excrement or manure through boots or tools (OR=7.6; *P*=0.024) and using the same manure management equipment (automatic scrapper, tractor or shovel) for four months old to first calving heifers and for adult cows (OR=3.6; *P*=0.026). Lastly, Table 1 demonstrates that not cleaning and sanitizing the calving pen after each use increases the odds of BTM samples on *S.* Dublin to be positive for dairy farms in Alberta (OR=7.3; *P*<0.001).

4. Discussion

There were 52 farms included in this study spread all over Alberta, without the consideration of infection status. Farms were considered positive with one or more positive BTM samples out of four quarterly BTM samples. Due to the high specificity of the test (97.5%), it is expected that farms indeed were infected when one or more BTM samples out of the four quarterly BTM samples came back as positive. This study therefore provides insights on certain risk factors and effective biosecurity measures that can also be relevant for dairy farms across Alberta and Western Canada.

The potential risk factors, however, have different origins. Two of the variables that have been used in this study were based on observations done by the research team, which makes the data consistent. Other risk factors were based on the opinion or behavior of the farmer. Risk factors based on the farmers management methods are likely to be more reliable than risk factors based on the motivation of the farmer as management methods can be proved better than motivation. This might mean that the variables in this study based on opinion might not be as reliable compared to variables based on methods.

Another consideration in this study is that the 52 farms that were included in this study were based on voluntary participation without the consideration of infection status. The farmers in this study therefore are motivated to join in a study about biosecurity. Being motivated to participate in a biosecurity study might be related to already being more aware about biosecurity measurements on the farm. It is therefore possible that other risk factors also are associated with herd positivity for *S.* Dublin which are not significant in this study. Caution should therefore be used while applying the findings of this study for other dairy farms in Alberta.

Additionally, the ELISA test used in this study is not perfect as the reported herd sensitivity is 16.3%. Consequently, there is a high probability of false negatives leading to influencing the results of the estimated association with the management and biosecurity practices explored in this study. In such scenarios, more complex data analysis methods including Bayesian statistics are useful which are beyond the scope of this thesis. It is likely that an underestimation of the actual number of *S.* Dublin infected herds has been used in this study. Because of the potential underestimation, it is possible that dairy herds that are infected with *S.* Dublin do introduce purchased animals but are considered negative in this study. This is why risk factors in this study should we used with precaution. It is more likely that the risk factors apply to farms with a high prevalence of *S.* Dublin as the ELISA test in this study considered farms positive if they had a percentage positivity of 35% and higher (Thermo Fischer Scientific, n.d.-b). Sample results from individual cows could have increased the reliability of the infection status of the farms.

The positive sample size in this study was low. Out of the 14.9% dairy farms in Alberta that were included in this study, there were seven farms that tested positive on one or more out of four quarterly BTM sample tests for antibodies against *S.* Dublin. Research indicated that there is little gain in having more than four controls to one case (Hormuzd A. et al., 2023). Having a low sample size and a high number of variables made a multivariable logistic model less suitable for the analysis. This type of analysis with a small sample size could result in overestimating the effect measure(Sabyasachi et al., 2021). This is why an univariable model with a Fisher's exact test has been used in this study.

Another subject that should be considered in this study is the time of taking the questionnaire and the observations from the proAction Dairy Farm Biosecurity General Risk Assessment. The questionnaire from the Biosecurity project was taken in the summer of 2023. At that time, the most recent version of the Dairy Farm Biosecurity General Risk Assessment was used for analysis. This questionnaire could not be older than two years. This means that there may be a difference of two years between the proAction assessment and the observations that were done in this study. This may have resulted in differences between the management practices of the farms in those two years.

Earlier studies in Europe indicated poor hygiene as an import risk factor for *S.* Dublin infections (Nielsen et al., 2012; Nielsen & Dohoo, 2012). This study demonstrated that possible indirect contact with adult cow excrements or manure through boots or tools is a significant risk factor. This result shows in similarity with earlier research that lack of hygiene can indeed be a risk factor for dairy farms to test positive on BTM sample for *S.* Dublin. Contrarily, the results of this study determined that not wearing protective clothing for visitors is not as significant as other variables while it was hypothesized as a significant risk factor. The reason for this may be that *S.* Dublin infected farms that are aware of their infection status change practices such as asking visitors to wear protective clothing. This therefore might reduce the demonstrated odds in this study of herd positivity for *S.* Dublin on farms that do not require visitors to wear protective clothing.

Also, other studies indicated that less than half of the farms applied hygiene measurements for farm visitors (Denis-Robichaud et al., 2019). This study contradicted this indication as 78% of the farms required to wear clean of disposable coveralls and boots for workers, visitors and farm service providers. This might demonstrate that more attention is being paid to hygiene of visitors and workers on farms in Alberta compared to earlier studies. Therefore, not wearing protective clothing might not be indicated as a risk factor in this study.

Besides this, an earlier study indicated that introducing purchased cattle within the last two years and having these animals leaving and returning to the premises creates a higher risk for the farm to be infected with *S.* Dublin (Perry et al., 2023). A reason why we did not find this in our study, may be because we did not discriminate between the purchase of a high or a very small number of animals and whether those animals were tested for infectious diseases. Thus, situations in which only one bull was purchased that was tested for infectious diseases were treated similarly as situations in which many untested animals were introduced to a farm. In hindsight, we think that this may have caused a distorted result in whether the variable of introducing purchased animals was a significant risk factor.

The risk factors that have been identified in this study can contribute to reducing the spread of *S.* Dublin infections in cattle in Alberta and beyond. Farmers could be informed about the importance of minimizing contact between adult cow feces and younger cattle groups and preventing manure on vehicles and equipment from being transferred between different groups. The advice, furthermore, can be given to clean calving pens after each use. All those measurements may result in fewer farms being infected with *S.* Dublin and therefore improved cattle and human health.

Without proper identification of the risk factors associated with *S.* Dublin, it is likely that the spread of *S.* Dublin will proceed. An increase in *S.* Dublin rates poses an economic risk to affected herds, as well as affecting the health of the herd, in addition to the health of the humans that have contact with infected animals and their products.

The identification of risk factors associated with *S.* Dublin in dairy farms of Alberta raises new questions. Regarding the infection status of the farms, a test with a higher sensitivity or within herd testing of groups that are not represented in the BTM sample can be used to get a more accurate overview of what farms are infected with *S.* Dublin. More accurate infection data of the farms can be used to point out more reliable risk factors for *S.* Dublin infections. More recent BTM samples would also be relevant to investigate if the potential risk factors in this study can be related to dairy farms that tested positive on *S.* Dublin for the first time. Lastly, it is possible to include more farms in the study and have them randomly selected to have a more accurate analysis of the risk factors that are of importance for dairy herds to be infected with *S.* Dublin.

In this study, six risk factors were identified for dairy herds to become infected with *S.* Dublin. It appears that knowledge about infectious diseases is not always implemented in an effective way (Oliveira et al., 2018). Knowledge about infectious diseases might therefore not be used efficiently. Further research could be performed to determine the best way to implement effective biosecurity practices as identified in this study. Also, the prevention of risk factors can be tested in the future. By researching the implementation of measures against risk factors, farmers will be able to actively contribute to the decreasing the transmission of *S.* Dublin within and between farms.

5. Conclusion

In conclusion, this study identified six variables as significant risk factors for *S.* Dublin positivity on dairy farms in Alberta. Those risk factors can be combined to having the possibility of manure contact between different age groups (through manure on boots, tools and other manure management equipment), buying hay or silage from other producers and not cleaning the calving pen after each use.

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Appendices

Appendix 1 questionnaires

Biosecurity questionnaire

1. Does any of the farm personnel also work or frequently visit other cattle farms?
    - No
    - Yes
    - I do not know
2. Do you hire a professional for controlling rodents in your farms?
    - No, I do not have a rodent control strategy
    - No, I do it myself
    - Yes
3. Do you buy or silage from other producers?
    - No
    - Yes
4. Did you share either vehicles or equipment with another farm that could have been in contact with animals, their feed, or manure?
    - No
    - Yes
5. Is there a risk of indirect contact with adult cow excrements or manure through boots or tools?
    - No
    - Yes
6. Have you purchased animals for your herd?
    - No
    - Yes
7. Did your animals left the farm and were then reintroduced into your herd?
    - No
    - Yes
8. Do you use the same manure management equipment (automatic scrapper, tractor, shovel) for 4 months old to first calving heifers and for adult cows?
    - No
    - Yes
9. Is there a separate pen/area on your farm to keep sick cattle?
    - No
    - Yes
10. Statement: Implementing biosecurity practices, in general, is useful to control diseases on my farm
    - 1=strongly disagree
    - 2=disagree
    - 3=neither agree nor disagree
    - 4=agree
    - 5=strongly agree

Observations

11. Is there a possibility of contact between adult cow feces and and the following animals? Pre-weaned animals
    - Yes
    - No
12. Is there a possibility of contact between adult cow feces and and the following animals? Weaned animals
    - Yes
    - No
13. Is there a possibility of contact between adult cow feces and and the following animals? Heifers
    - Yes
    - No

Dairy Farm Biosecurity General Risk Assessment

14. Do you clean and sanitize the calving pen after each use?
    - 1=Always or Yes >95%
    - 2=Almost always 70-95%
    - 3=Sometimes 1-69%
    - 4=Never or No 0%
15. Do you require all workers, visitors, and farm service providers to wear clean or disposable coveralls and boots on your farm?
    - 1=Always or Yes >95%
    - 2=Almost always 70-95%
    - 3=Sometimes 1-69%
    - 4=Never or No 0%