Preventive Veterinary Medicine 150 (2018) 162–167

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

# Diagnosing intramammary infection: Controlling misclassification bias in longitudinal udder health studies

Denis Haine[a,d,*], Ian Dohoo[b,d], Daniel Scholl[c], Simon Dufour[a,d]

[a] Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, St-Hyacinthe, Québec, Canada J2S 2M2
[b] Atlantic Veterinary College, Centre for Veterinary Epidemiological Research, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3
[c] College of Agriculture and Biological Sciences, South Dakota State University, Box 2207, South Dakota Brookings, SD 57007, USA
[d] Canadian Bovine Mastitis and Milk Quality Research Network, St-Hyacinthe, Québec, Canada J2S 2M2

ARTICLE INFO

*Keywords:*
Misclassification
Dairy cow
mastitis
Bias
Epidemiologic methods

ABSTRACT

Using imperfect tests may lead to biased estimates of disease frequency and of associations between risk factors and disease. For instance in longitudinal udder health studies, both quarters at risk and incident intramammary infections (IMI) can be wrongly identified, resulting in selection and misclassification bias, respectively. Diagnostic accuracy can possibly be improved by using duplicate or triplicate samples for identifying quarters at risk and, subsequently, incident IMI.

The objectives of this study were to evaluate the relative impact of selection and misclassification biases resulting from IMI misclassification on measures of disease frequency (incidence) and of association with hypothetical exposures. The effect of improving the sampling strategy by collecting duplicate or triplicate samples at first or second sampling was also assessed.

Data sets from a hypothetical cohort study were simulated and analyzed based on a separate scenario for two common mastitis pathogens representing two distinct prevailing patterns. *Staphylococcus aureus*, a relatively uncommon pathogen with a low incidence, is identified with excellent sensitivity and almost perfect specificity. Coagulase negative staphylococci (CNS) are more prevalent, with a high incidence, and with milk bacteriological culture having fair Se but excellent Sp. The generated data sets for each scenario were emulating a longitudinal cohort study with two milk samples collected one month apart from each quarter of a random sample of 30 cows/herd, from 100 herds, with a herd-level exposure having a known strength of association. Incidence of IMI and measure of association with exposure (odds ratio; OR) were estimated using Markov Chain Monte Carlo (MCMC) for each data set and using different sampling strategies (single, duplicate, triplicate samples with series or parallel interpretation) for identifying quarters at risk and incident IMI.

For *S. aureus* biases were small with an observed incidence of 0.29 versus a true incidence of 0.25 IMI/100 quarter-month. In the CNS scenario, diagnostic errors in the two samples led to important selection (40 IMI/100 quarter-month) and misclassification (23 IMI/100 quarter-month) biases for estimation of IMI incidence, respectively. These biases were in opposite direction and therefore the incidence measure obtained using single sampling on both the first and second test (29 IMI/100 quarter-month) was exactly the true value.

In the *S. aureus* scenario the OR for association with exposure showed little bias (observed OR of 3.1 versus true OR of 3.2). The CNS scenario revealed the presence of a large misclassification bias moving the association towards the null value (OR of 1.7 versus true OR of 2.6). Little improvement could be brought using different sampling strategies aiming at improving Se and/or Sp on first and/or second sampling or using a two out of three interpretation for IMI definition.

Increasing number of samples or tests can prevent bias in some situations but efforts can be spared by holding to a single sampling approach in others. When designing longitudinal studies, evaluating potential biases and best sampling strategy is as critical as the choice of test.

* Corresponding author at: Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, St-Hyacinthe, Québec, Canada J2S 2M2.
*E-mail address:* denis.haine@umontreal.ca (D. Haine).

https://doi.org/10.1016/j.prevetmed.2017.11.010
Received 31 January 2017; Received in revised form 31 October 2017; Accepted 9 November 2017

## 1. Introduction

A cohort study is the standard method to estimate the incidence of diseases and identify their natural history, by analyzing the association between a baseline exposure and risk of disease over the follow-up period. A disease-free population is identified, i.e. subjects with the outcome at baseline are excluded from the follow-up, while new, incident cases of exposure are identified. Therefore, it is assumed that prevalent and non-prevalent cases can be differentiated with no error so that only susceptible individuals are included in the follow-up. Incident cases are likewise supposed to be correctly identified.

However, using imperfect tests may lead to biased estimates of disease frequency and of association with exposure. For instance in longitudinal udder health studies in which bacteriological culture is commonly used for diagnosis of intramammary infections (IMI), both quarters at risk of becoming infected and, later on, incident IMI can be wrongly identified. If the wrong classification at baseline and at follow-up are both misclassification biases, in the former the bias resulting from IMI misclassification could be considered a selection bias, as the wrong (diseased) subjects are included in the cohort (Rothman et al., 2012) while in the latter, it would be commonly defined as misclassification bias (Delgado-Rodriguez and Llorca, 2004). Different methods can be used to limit or address these biases, such as study design, improving the diagnostic procedures for identifying quarters at risk and incident IMI, or addressing the biases analytically (McInturff et al., 2004; Dufour et al., 2012a). Improvement of the diagnostic procedures is commonly achieved in udder health studies by using duplicate or triplicate samples in order to improve the sensitivity (Se) and/or specificity (Sp) of the bacteriological cultures. With series interpretation, animals are declared positive only if they test positive to both tests, resulting in an increased Sp but decreased Se. With parallel interpretation, a positive result in either test is sufficient to declare the animal positive, increasing Se but decreasing Sp. Regarding test characteristics for IMI diagnostic, Dohoo et al. (2011a) reported that series interpretation of duplicate samples provided the highest Sp but lowest Se, whereas parallel interpretation of duplicate samples resulted in the highest Se but lowest Sp. If triplicate samples provided the best combination of Se and Sp compared with a single sample, the gain in Sp was modest and there were little or no gain in Se.

Little is known about the relative impact in cohort studies of the selection bias resulting from misidentification of quarters at risk of becoming infected compared to the more traditional misclassification bias. Furthermore, the impact on measures of association of any reduction of this selection bias using more accurate diagnostic procedures is unknown. With the often limited resources available for milk samples analyses, a better understanding of the relative impact of these biases would allow a more appropriate distribution of these resources in a manner that would optimize the balance between cost and precision of a study.

The objectives of this study were to evaluate the relative impact of selection and misclassification biases resulting from IMI misclassification on measures of disease frequency (incidence) and of association with a hypothetical exposure. The effect of improving the sampling strategy was also assessed.

## 2. Materials and methods

Data sets from a hypothetical cohort study were simulated and analyzed based on a separate scenario for two common mastitis pathogens studied in udder health researches and representing two distinct prevailing patterns, *Staphylococcus aureus* and coagulase negative staphylococci (CNS). *S. aureus*, a relatively uncommon pathogen (prevalence $< 5\%$) with a low incidence (1 NIMI/100 quarter-month), can be identified with excellent Se ($\sim 90\%$) and almost perfect Sp ($> 99\%$, at 100 CFU/ml) by bacteriological culture (Zadoks et al., 2001; Dohoo et al., 2011b; Dufour et al., 2012b). Coagulase negative staphylococci are more prevalent (10–30%), with a high incidence ($\sim 30$ NIMI/100 quarter-month), and with milk bacteriological culture having a fair Se ($\sim 60\%$) but an excellent Sp (95%, at 200 CFU/ml; Dohoo et al., 2011b; Dufour et al., 2012a).

The generated data sets for each scenario were emulating a longitudinal cohort study with two milk samples collected one month apart from each quarter of a random sample of 30 cows/herd, from 100 herds, with a herd-level exposure having a known strength of association. The true IMI status ($S_1$) on first milk sample collection was used to identify quarters at risk of IMI at the beginning of the cohort, while the second ($S_2$) was used to identify the true outcome (acquisition of a new IMI). A hypothetical exposure at the herd-level with known strength of association (OR $\sim$ 3.0) was generated at baseline ($S_1$). To make the scenario more realistic, exposure was equally associated with odds of a prevalent IMI on the first milk sample as with odds of IMI acquisition on the second sample (as observed in Dufour et al., 2012a). Exposure was randomly associated with the odds of eliminating an existing IMI. Correlation of these two specific types of IMI by cow and by herd were obtained from Dufour et al. (2012a,b) to produce realistic datasets. As demonstrated in Dufour et al. (2012a,b), IMI incidence has a much greater effect on IMI prevalence than the elimination rate. The parameters to generate these data sets are given in Table 1. For each scenario, 100 data sets were generated.

Sensitivity and Sp to diagnose *S. aureus* and CNS were represented as Beta distributions with the following shape parameters: (46.8, 6.09) and (45, 30.3) for Se; and (1, i.e. uniform distribution) and (4.26, 1.17) for Sp, for *S. aureus* and CNS, respectively. Analyses for each of the two scenarios were conducted separately. On each datasets new $S_1'$ and $S_2'$ variables were generated by applying the scenario misclassification parameters to the $S_1$ and $S_2$ samples. Incidence and measures of association with the hypothetical exposure were computed using first the $S_1'$ and $S_2'$ variables (total bias), then $S_1'$ and $S_2$ (selection bias only), and finally the $S_1$ and $S_2'$ variables (misclassification bias only). If the selection and misclassification biases were deemed important, the effect of improving Se and Sp on the first and/or second sampling(s) was assessed by applying different sampling strategies having the objective to improve Se and/or Sp. This is commonly achieved in udder health studies by carrying on duplicate or triplicate samplings with parallel or

**Table 1**
Parameters used to generate the simulated data sets (CNS: coagulase negative staphylococci).

| Parameters | *S. aureus* | CNS |
|---|---|---|
| Exposure distribution (0–1) of the binary herd-level predictor | 0.5 | 0.5 |
| Exposure distribution (0–1) of the binary cow-level predictor | 0.5 | 0.5 |
| Exposure distribution (0–1) of the binary quarter-level predictor | 0.5 | 0.5 |
| Herd-level variance for prevalence of intramammary infection (IMI) | 0.14 | 0.363 |
| Cow-level variance for prevalence of IMI | 2.25 | 0.294 |
| Intercept for IMI prevalence; aiming at a prevalence of 2.5% | −6.7 | |
| Intercept for IMI prevalence; aiming at a prevalence of 40% | | −2.15 |
| odds ratio (OR) of association between herd-level variable and IMI prevalence | 3 | 3 |
| Herd-level variance for incidence of IMI | 0.838 | 0.27 |
| Cow-level variance for incidence of IMI | 2.926 | 0.256 |
| Intercept for IMI incidence, aiming at an incidence of 1 IMI/100 quarter-month | −8.3 | |
| Intercept for IMI incidence, aiming at an incidence of 30 IMI/100 quarter-month | | −2.4 |
| OR of association between herd-level variable and IMI incidence | 3 | 3 |
| Herd-level variance for elimination of IMI | 0.15 | 0.112 |
| Cow-level variance for elimination of IMI | 2.246 | 0.7 |
| Intercept for IMI persistency, aiming at 61 IMI/100 quarter-month | −0.6 | |
| Intercept for IMI persistency, aiming at 21 IMI/100 quarter-month | | 1.6 |