View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Explore Bristol Research

Dufour, S., Dohoo, I. R., Barkema, H. W., Descôteaux, L., Devries, T. J., Reyher, K. K., ... Scholl, D. T. (2012). Epidemiology of coagulase-negative staphylococci intramammary infection in dairy cattle and the effect of bacteriological culture misclassification. Journal of dairy science, 95(6), 3110-24. 10.3168/jds.2011-5164

Link to published version (if available):
10.3168/jds.2011-5164

Link to publication record in Explore Bristol Research
PDF-document

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: http://www.bristol.ac.uk/pure/about/ebr-terms.html

Take down policy

Explore Bristol Research is a digital archive and the intention is that deposited content should not be removed. However, if you believe that this version of the work breaches copyright law please contact open-access@bristol.ac.uk and include the following information in your message:

- Your contact details
- Bibliographic details for the item, including a URL
- An outline of the nature of the complaint

On receipt of your message the Open Access Team will immediately investigate your claim, make an initial judgement of the validity of the claim and, where appropriate, withdraw the item in question from public view.

Coagulase-negative staphylococci intramammary infection epidemiology in dairy cattle and impact of bacteriological culture misclassification

Journal:	*Journal of Dairy Science*
Manuscript ID:	JDS-11-5164.R2
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Dufour, Simon; University of Montreal, Pathology and Microbiology Dohoo, Ian; University of P.E.I., Health Management Barkema, Herman; University of Calgary, Faculty of Veterinary Medicine DesCôteaux, Luc; University of Montreal, Faculty of Veterinary Medicine, Clinical Sciences DeVries, Trevor; University of Guelph, Kemptville Campus, Animal & Poultry Science Reyher, Kristen; Atlantic Veterinary College, Health Management; Roy, Jean-Philippe; Université de Montréal, Sciences cliniques Scholl, Daniel; South Dakota State University, SD Agricultural Experiment Station
Key Words:	dairy cow, mastitis, CNS, Misclassification

SCHOLARONE™
Manuscripts

ScholarOne support: (434) 964 4100

1 **INTERPRETIVE SUMMARY**

2 **Coagulase-negative staphylococci intramammary infections epidemiology**. *Dufour.*

3 A longitudinal cohort study was carried out to identify management practices that can be
4 used on dairy farms to prevent acquisition or increase elimination of intramammary
5 infections caused by coagulase-negative staphylococci. The results indicate that the
6 infection acquisition rate is lower in herds using sand or wood product based bedding
7 compared to straw bedding. Quarters of cows with access to pasture also showed lower
8 odds of becoming infected. Ignoring the limitations of bacteriological culture for
9 identification of these IMI resulted in a considerable bias in measures of disease
10 frequency and of association with exposures.

11

For Peer Review

ScholarOne support: (434) 964 4100

12 COAGULASE-NEGATIVE STAPHYLOCOCCI IMI EPIDEMIOLOGY

13

14 **Coagulase-negative staphylococci intramammary infection epidemiology in dairy**

15 **cattle and impact of bacteriological culture misclassification**

16

17 **S. Dufour*[, †], I.R. Dohoo*[, ‡], H.W. Barkema*[, §], L. DesCôteaux*[, #], T. J. DeVries*[, ‖],**

18 **K.K. Reyher*[, ‡], J.-P. Roy*[, #], and D. T. Scholl*[, ¶]**

19

20 *Canadian Bovine Mastitis Research Network, C.P. 5000, St-Hyacinthe, Quebec, J2S

21 7C6, Canada

22 [†]Dept. of Pathology and Microbiology, Faculty of Veterinary Medicine, University of

23 Montreal, C.P. 5000, St-Hyacinthe, Quebec, J2S 7C6, Canada

24 [‡]Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave,

25 Charlottetown, PEI, C1A 4P3, Canada

26 [§]Dept. of Production Animal Health, Faculty of Veterinary Medicine, University of

27 Calgary, 3300 Hospital Drive NW, Calgary, Alberta, T2N 4N1, Canada

28 [#]Dept. of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, C.P.

29 5000, St-Hyacinthe, Quebec, J2S 7C6, Canada

30 [‖]Dept. of Animal and Poultry Science, University of Guelph, Kemptville Campus, 830

31 Prescott St., Kemptville, Ontario, K0G 1J0, Canada

32 [¶]College of Agriculture and Biological Sciences, South Dakota State University,

33 Brookings, SD 57007, USA

34 Corresponding author: Simon Dufour

For Peer Review

ScholarOne support: (434) 964 4100

35 Faculty of Veterinary Medicine, University of Montreal
36 C.P. 5000
37 St-Hyacinthe, Quebec, Canada, J2S 7C6
38 Tel: +1 (450) 773-8521 ext: 0195
39 Fax: +1 (450) 778-8179
40 E-mail: simon.dufour@umontreal.ca
41

For Peer Review

ScholarOne support: (434) 964 4100

42 **ABSTRACT**

43 Objectives of this study were to identify the manageable risk factors associated with the
44 lactational incidence, elimination, and prevalence of coagulase-negative staphylococci
45 (**CNS**) intramammary infections (**IMI**) while taking into account the difficulties inherent
46 to their diagnosis. A second objective was to evaluate the impact of CNS IMI
47 misclassification in mastitis research. A cohort of 90 Canadian dairy herds was followed
48 throughout 2007-2008. In each herd, series of quarter milk samples were collected from a
49 sub-sample of cows and bacteriological culture was performed to identify prevalent,
50 incident and eliminated CNS IMI. Practices used on farms were captured using direct
51 observations and a validated questionnaire. The relationships between herd CNS IMI
52 prevalence and herd incidence and elimination rates were explored using linear
53 regression. Manageable risk factors associated with the prevalence, incidence, or
54 elimination of CNS IMI were identified via semi-Bayesian analyses using a latent class
55 model approach allowing adjustment of the estimates for the imperfect sensitivity and
56 specificity of bacteriological culture. After adjustment for the diagnostic test limitations,
57 a mean CNS IMI quarter prevalence of 42.7 % (95% CI: 34.7, 50.1) and incidence and
58 elimination rates of 0.29 new IMI/quarter-month (95% CI: 0.21, 0.37) and 0.79
59 eliminated IMI/quarter-month (95% CI: 0.66, 0.91), respectively, were observed.
60 Considerable biases of the estimates were observed when CNS IMI misclassification was
61 ignored. These biases were important for measures of association with risk factors, were
62 nearly always toward the null value, and led to both Type I and Type II errors.
63 Coagulase-negative staphylococci IMI incidence appeared to be a stronger determinant of
64 herd IMI prevalence than IMI elimination rate. The majority of herds followed were

For Peer Review

65 already using blanket dry cow treatment and post-milking teat disinfection. A holistic
66 approach considering associations with all 3 outcomes was employed to interpret
67 associations between manageable risk factors and CNS IMI. Sand and wood-based
68 product bedding showed desirable associations with CNS IMI compared to straw
69 bedding. Quarters of cows that had access to pasture during the sampling period had
70 lower odds of acquiring a new CNS IMI and of having a prevalent CNS IMI. Many
71 practices showed an association with only one of the CNS outcomes and should,
72 therefore, be considered with caution.

73

74 ***Key words:*** dairy cow, mastitis, CNS, misclassification

75

For Peer Review

76 ## INTRODUCTION

77

78 Historically, CNS IMI have received less attention compared to IMI caused by
79 major pathogens such as *Staphylococcus aureus*, streptococci, and coliforms. One reason
80 for this is that CNS IMI most often remain subclinical and generally lead to only mild to
81 moderate SCC elevations compared to IMI caused by major mastitis pathogens (Djabri et
82 al., 2002; Sampimon et al., 2010; Supré et al., 2011). With the gradually increasing
83 control of IMI caused by major mastitis pathogens, however, recognition of the
84 importance of CNS IMI and of their potential impact on udder health is rising. In recent
85 studies conducted in different countries, CNS were the most common cause of IMI and
86 were described as emerging mastitis pathogens (Pyörälä and Taponen, 2009; Sampimon
87 et al., 2009a; Tenhagen et al., 2006). In a Dutch study, 10% of the quarters from low
88 SCC cows and 15% of the quarters from high SCC cows had CNS cultured from their
89 milk (Sampimon et al., 2009a). Similarly, in Germany, CNS was cultured from 8 to 11%,
90 depending on parity and stage of lactation, of apparently healthy quarters (Tenhagen et
91 al., 2006). Results from different studies are difficult to compare, though, since different
92 definitions of what constitute a CNS IMI are often used. In addition, regardless of the
93 CNS IMI definition used, the use of bacteriological culture to diagnose CNS IMI always
94 produces a substantial level of IMI misclassification (Dohoo et al., 2011). In much
95 research, misclassification bias is ignored or discussed strictly qualitatively. Nonetheless,
96 relatively mild non-differential misclassification can yield, in some situations, a sizeable
97 bias of the estimates of disease frequency and of association with exposures (Höfler,
98 2005).

For Peer Review

99 Even though each CNS-infected quarter may only show a moderate increase in
100 SCC, the often large proportion of infected quarters in a herd can still have an important
101 impact on the bulk milk SCC (**BMSCC**). In a large field study in the USA, it was
102 estimated that CNS IMI were responsible for 18% of the BMSCC in low BMSCC herds
103 (<200,000 cells/ml), a BMSCC contribution substantially larger than those of any of the
104 so-called major mastitis pathogens (Schukken et al., 2009). These results suggest that, in
105 herds where major mastitis pathogens have been controlled, CNS IMI are an important
106 obstacle impeding further udder health improvement.
107 Although CNS IMI have been shown to have an impact on individual cow SCC
108 and BMSCC, there is still much debate, however, on the harmful effect of acquiring a
109 CNS IMI. In some studies cows or heifers with CNS IMI were shown to have a slightly
110 higher daily milk production when compared to uninfected individuals (Compton et al.,
111 2007; Piepers et al., 2010; Schukken et al., 2009). Milk production losses can be
112 underestimated, however, when infected individuals are compared to healthy herd mates
113 rather than to their own pre-infection milk production (Pyörälä and Taponen, 2009). It is
114 plausible that higher producing cows or heifers would be more at risk of acquiring a CNS
115 IMI than the other way around. In a study conducted by Matthews et al. (1990) CNS-
116 infected quarters had lower odds of acquiring a *S. aureus* IMI than CNS-free quarters. In
117 another study, however, an increase risk of *S. aureus* IMI acquisition was observed in
118 CNS-infected quarters (Dufour et al., In press). It is still unclear whether or not there is a
119 real protective effect of CNS IMI against *S. aureus* IMI. It is also unclear whether a
120 hypothetical beneficial effect resulting from a few potentially averted *S.aureus* IMI

ScholarOne support: (434) 964 4100

For Peer Review

121 would compensate for a higher CNS prevalence. With the available knowledge on CNS
122 IMI, preventing these IMI seems to remain an appropriate recommendation.
123 Preventing new CNS IMI is the key determinant for long-term reduction and
124 control of these IMI. Little is known, however, about effective strategies for CNS IMI
125 prevention. A recent study has investigated risk factors associated with CNS IMI
126 prevalence in early lactation of dairy heifers (Piepers et al., 2011), while another
127 examined the risk factors associated with CNS IMI herd prevalence (Sampimon et al.,
128 2009b). No studies could be found in the literature to have been conducted on risk
129 factors associated with the acquisition or the elimination of CNS IMI during the lactation.
130 The study presented is a longitudinal cohort study on acquisition and elimination
131 of CNS IMI during lactation on 90 Canadian dairy herds. The main objective was to
132 identify manageable risk factors associated with the incidence, elimination, and
133 prevalence of these IMI while taking into consideration the difficulties inherent to the
134 diagnosis of CNS IMI. A secondary objective was to evaluate the impact of CNS IMI
135 misclassification on estimates of disease frequency and on estimates of association with
136 risk factors.
137

138 **MATERIALS AND METHODS**

139

140 The herds selected were members of the National Cohort of Dairy Farms (**NCDF**)
141 of the Canadian Bovine Mastitis Research Network (**CBMRN**). A complete description
142 of the herd selection process as well as of the characteristics of these herds has been
143 published previously (Reyher et al., 2011). Briefly, 91 herds were recruited in 4 regions

For Peer Review

144 of Canada to participate in a 2 yr (2007 and 2008) cohort study. Early in 2007, one herd
145 refused to pursue participation because of the extent of work involved. The study
146 presented in this manuscript was carried out with data from the 90 herds that participated
147 to the NCDF for at least one yr.

148 During the 2 yr course of the study, management practices in place and other
149 important farm conditions were measured on multiple occasions using direct observations
150 and a validated questionnaire (Dufour et al., 2010). These repeated observations were
151 designed to allow the use, in subsequent analyses, of the practices and conditions in place
152 at the beginning of each of 4 different sampling periods rather than merely those
153 employed at the beginning of the cohort study. Management practices under
154 investigation have been described thoroughly elsewhere (Dufour et al., 2010; Dufour et
155 al., 2011a) and could be summarized in 8 categories: 1) milking procedures; 2) milking
156 equipment; 3) stalls and housing; 4) maternity pens; 5) general management and
157 biosecurity; 6) nutrition; 7) clinical mastitis; and 8) demographic and IMI prevalence.
158 Attitudes, motivations, knowledge, and beliefs of dairy producers were also investigated
159 as on-farm conditions that could potentially modify the effect of the practices under
160 investigation. Individual and herd level milk production and SCS data, as well as herd
161 demographic data were obtained from Dairy herd improvement records from 2005 to
162 2009. A complete description of the data collection process as well as the prevalence of
163 use of the selected management practices on the NCDF herds can be found in Dufour et
164 al. (2010).

165 ***Milk Sampling***

For Peer Review

166 At the beginning of each of 4 different sampling periods (March-May 2007, June-
167 August 2007, January-March 2008, and June-August 2008), a sample of 15 apparently
168 healthy lactating cows from each NCDF herd was selected. During each sampling
169 period, series of 3 milk samples were collected from each quarter of the selected cows at
170 intervals of 3 wks by a team of trained technicians using a standardized protocol (Reyher
171 et al., 2011). Signs of inflammation of the quarter and teat end condition scores
172 (Neijenhuis et al., 2000) were recorded. Quarters showing signs of clinical mastitis were
173 excluded. Cows that were treated for conditions other than mastitis were not excluded.
174 Bacteriological culture of the milk samples was carried out using a protocol based on
175 NMC guidelines (Hogan et al., 1999). Ten µl of milk was streaked on a Columbia agar
176 +5% sheep blood plate and incubated aerobically at 35ºC for 24h. The different types of
177 colonies were enumerated (up to 10 colonies) and speciated after 24h using
178 recommended bacteriologic procedures, then re-incubated for another 24h. SCC
179 measurements were obtained for each quarter milk sample using the Fossomatic milk cell
180 counter (Fossomatic 4000 series, Foss Electric, Hillerød, Denmark).

181 Quarter milk samples for which 3 or more pathogen species were cultured were
182 considered contaminated and were excluded. A quarter was considered infected with
183 CNS whenever bacteriological culture yielded ≥ 100 phenotypically identical CNS cfu/ml
184 of milk. This threshold was chosen based on the results from Dohoo et al. (2011). The
185 same threshold was chosen to define IMI due to *S. aureus*, *Corynebacterium* spp,
186 *Streptococcus uberis*, *Streptococcus agalactiae, Streptococcus dysgalactiae*, and other
187 streptococci species (presumably, primarily enterococci). Pathogen-specific quarter,
188 cow, and herd prevalence of IMI at the first sampling of each sampling period were

For Peer Review

189 computed for the previously mentioned pathogens and investigated as explanatory
190 variables.
191 For each outcome (incidence, elimination, and prevalence of CNS IMI) a different
192 dataset was constituted. To investigate CNS IMI incidence and elimination, samples
193 from each series were organized in 2 pairs (1st and 2nd samples, 2nd and 3rd samples) and
194 pairs with incomplete results were discarded (i.e. pairs with a contaminated sample).
195 Only pairs negative for CNS on the 1st sample of the pair were considered at risk of
196 becoming infected and an incident IMI was deemed to have occurred if CNS was
197 cultured from the following sample. Conversely, only pairs where CNS was cultured
198 from the 1st sample of the pair were considered at risk of eliminating an existing CNS IMI
199 which was deemed to have occurred if the following sample was negative. Based on
200 these definitions, outcomes for the incidence and elimination datasets were, respectively,
201 acquisition and elimination of a CNS IMI over a 3-week period (i.e. between milk
202 samples of a pair).
203 For CNS IMI prevalence, the series of quarter milk samples collected during a
204 specific sampling period were considered as one single observation. A prevalent CNS
205 IMI was deemed to be present if 1 or more of the 3 samples collected was found to be
206 positive for CNS. Series where CNS was never cultured were defined as free of CNS
207 IMI. The outcome for the prevalence data set was, therefore, the presence of a CNS IMI
208 in any of the milk samples of a series. Based on these definitions, 3 separate datasets
209 specific to each of the 3 outcomes of interest (CNS IMI incidence, elimination, and
210 prevalence) were generated.
211 ***Analyses***

For Peer Review

212 First, the 2 yr CNS IMI incidence rate, elimination rate, and prevalence were
213 computed for each NCDF herd. Next, the relative impact of incidence and elimination
214 rates on the prevalence of CNS IMI was investigated using a linear regression model with
215 dependent variable (the computed 2 yrs CNS IMI herd prevalence) and explanatory
216 variables (the herd incidence and elimination rates).

217 ***Screening of Explanatory Variables.*** Descriptive analyses were conducted for
218 each variable in each of the 3 datasets to identify distributions and unlikely values. In
219 one herd, pre-milking teat disinfection and wearing gloves during milking were only used
220 by half of the milkers; observations from this herd for these specific variables were,
221 therefore, excluded from subsequent analyses. Only one of the participating herds had
222 not implemented post-milking teat disinfection (PMTD). This practice was, therefore,
223 not retained as an explanatory variable since its measure of association would be
224 perfectly confounded by other characteristics specific to this herd. Back-flush of the
225 milking units between groups of cows was also used in one herd only and was not
226 retained as explanatory variable for the same reason. Finally, maternity pen variables
227 were not considered in the incidence analyses since cows were not exposed to these
228 variables anymore when CNS IMI acquisition was measured during the lactation.

229 Next, for each outcome (acquisition of a CNS IMI over a 3-week period,
230 elimination of an existing CNS IMI over a 3-week period, and presence of a CNS IMI in
231 a series of milk samples), unconditional associations between explanatory variables and
232 occurrence of the outcome were estimated. Explanatory variables at the herd, cow,
233 quarter, and pair (of samples) level were considered. The correlation structure of the data
234 was a hierarchical cross-classified structure. Briefly, although 2 pairs of observations

For Peer Review

ScholarOne support: (434) 964 4100

235 were available per quarter during a sampling period, the definitions used for incident and
236 eliminated IMI precluded correlation of observations per quarter per sampling period.
237 For instance, a quarter acquiring an IMI on the first pair (first sample of the pair negative,
238 second sample positive) would not be considered at risk of acquiring a new IMI for the
239 second pair (first sample of the pair is positive), thus pairs of samples collected on a
240 quarter during a sampling period could be considered independent observations. In the
241 prevalence dataset, only one observation was considered per quarter during a sampling
242 period, therefore precluding any quarter correlation within a sampling period. For the 3
243 outcomes, however, observations were clustered within cow, and, since cows could be
244 randomly selected in multiple sampling periods, observations from some cows could be
245 cross-classified by herd and by sampling period. In all 3 datasets, however, most of the
246 cows were randomly selected for only one sampling period, and only 18%, 2%, and < 1%
247 of cows were selected for respectively 2, 3, and all 4 sampling periods. To facilitate the
248 first stages of the analyses, unconditional analyses were carried out using a hierarchical
249 logistic regression model which accounted only for cow and herd clustering of
250 observations. These analyses were performed with the GLIMMIX procedure of SAS 9.2
251 (SAS Institute Inc., Cary, NC) using Laplace approximation. For continuous variables,
252 linearity was evaluated by visual inspection of the lowess smoothed curve of the
253 relationship between the continuous variable and the log odds of the outcome (Dohoo et
254 al., 2009); variables were categorized whenever the linearity assumption could not be
255 met. Variables with $P \leq 0.20$ (Wald test) were retained as potentially important
256 explanatory variables. Pearson and Spearman correlation coefficients were computed
257 among the retained variables to identify co-linearity issues ($\rho < -0.6$ or $\rho > 0.6$).

For Peer Review

ScholarOne support: (434) 964 4100

258 ***Rough Models Construction.*** For each outcome, a putative causal diagram based
259 on theoretical background was developed with the retained variables to identify
260 potentially important confounders and effect modifiers. A stepwise backward selection
261 strategy was then used to construct a rough model for each of the 3 outcomes using the
262 previously described simplified logistic hierarchical model. In these models, only the
263 retained variables that could theoretically be modified relatively easily (referred to as
264 "manageable risk factors" in the remainder of the manuscript) were tested for inclusion.
265 Initial quarter, cow, or herd prevalence of IMI by pathogens other than CNS were strictly
266 considered in these models as potential confounders or effect modifiers. Initial quarter
267 SCC measurements were treated likewise. A relatively liberal *P* value of 0.10 was
268 chosen as the inclusion criterion so variables that might have been significantly
269 associated with the true outcome (the true unmeasured CNS IMI status) would not be
270 excluded because of the inability to correctly and precisely measure this outcome using
271 routine bacteriological culture. During the selection process, variables identified as
272 potential confounders in the putative causal diagram were included in the model
273 whenever one of the confounded variables was present. For each management practices
274 included in the model, a maximum of three logically-plausible effect modifiers were then
275 tested. These effect modifiers were included in the model if a Wald test conducted on the
276 cross-product terms yielded a *P* value lower than 0.05/n, where n was the total number of
277 effect modifiers tested in the model (Bonferroni adjustment for multiple comparisons).
278 ***Misclassification Adjustment of the Models.*** Estimates from these 3 rough
279 models were then revised to take into account the cross-classified part of the structure and
280 to correct for the likely CNS IMI status misclassification due to the imperfect sensitivity

For Peer Review

281 (**Se**) and specificity (**Sp**) of bacteriological culture. For this last step, a semi-Bayesian
282 approach using a latent class model similar to the one described by McInturff et al.
283 (2004) was used. A latent class model relates an observed variable to a latent
284 unmeasured variable; in this study the IMI status measured using milk bacteriological
285 culture needed to be related to the true but unmeasured quarter IMI status. With the
286 proposed approach, prior distributions for the Se and Sp of the test used to measure the
287 outcome can be used to relate the latent and observed variables. In this study, estimates
288 of Se and Sp of bacteriological culture for an IMI definition based on isolation of ≥ 100
289 CNS cfu/ml, and obtained using NCDF bacteriological isolates (Dohoo et al., 2011) were
290 used to generate prior distributions for CNS IMI misclassification parameters. In this
291 latent class model, misclassification of IMI status was deemed to be independent of the
292 others variables in the model (non-differential misclassification). For instance,
293 misclassification of the CNS IMI status of a quarter milk sample was deemed to be
294 independent of the management practices used on the farms.
295 The impact of misclassification of exposures has been well described by
296 Gustafson (2004) and, in some situations, will also lead to an important and sometimes
297 unpredictable bias of the estimate of association and of its standard error. A validation
298 study was, therefore, conducted with the NCDF participants to obtain Se and Sp estimates
299 of the exposure measurements obtained using a questionnaire compared to direct
300 observations (Dufour et al., 2010). For some exposures that could not directly be
301 observed, estimates of repeatability rather than Se and Sp were available; in this situation
302 the method proposed by Lash et al. (2007) was used to generate Se and Sp estimates.
303 Sensitivity and Sp estimates of the explanatory variables were inspected, and these

For Peer Review

304 variables were further categorized when needed in order to restrict the magnitude of the
305 potential misclassification bias. This bias was minimized by ensuring that moderately
306 observed exposures (30-70%) used in the analyses had both Se and Sp estimates ≥ 0.90,
307 that uncommonly observed exposures (≤30%) had Sp estimate ≥ 0.95, and, finally, that
308 commonly observed exposures (≥70%) had Se estimates ≥ 0.95. These values were
309 chosen based on results from Höfler (2005) to restrict the analyses to situations where the
310 magnitude of exposure misclassification bias was likely to be small.

311 During this last phase of analyses, the complete cross-classified hierarchical
312 structure of the data was taken into account. The informative prior distributions specified
313 for the misclassification parameters (Se and Sp) are described in Table 1. Briefly, uni-
314 modal beta distributions centered on the Se and Sp estimates obtained using NCDF
315 isolates and reported in Dohoo et al. (2011) were chosen for Se and Sp. Furthermore,
316 these distributions were truncated at values of more and less than 5 percentage-points
317 around the reported estimate. This latter restriction was implemented to avoid less
318 probable and sometimes inappropriate Se and Sp combinations and, therefore, improve
319 convergence of the Markov chain Monte Carlo (**MCMC**) chains. In addition, for the IMI
320 prevalence analysis, different Se and Sp prior distributions were used for series where
321 one (n=1,439), 2 (n=4,852), or 3 (n=13,551) culture results were available to account for
322 the increasing Se and decreasing Sp resulting from the parallel interpretation of multiple
323 diagnostic tests (Dohoo et al., 2009). Non-informative prior distributions were used for
324 the risk factors and random effects parameters. To evaluate the impact of using
325 traditional analyses where IMI misclassification is usually ignored, the 3 models were
326 also run with Se and Sp of exactly 100%.

For Peer Review

327 Finally, traditional and misclassification-adjusted estimates of the mean CNS IMI
328 prevalence and incidence and elimination rates were obtained using the same approach.
329 To achieve this, a model with only an intercept (β_0) and random effects was used for each
330 outcome using first Se and Sp estimates of exactly 100% and then the Se and Sp
331 estimates presented in Table 1. Mean estimates of prevalence, incidence rate, and
332 elimination rate were then obtained by transformation of their respective intercepts using
333 the following formula (Dohoo et al., 2009):

334 $$P = \frac{1}{1 + e^{-(\beta 0)}} \quad [1]$$

335 Incidence and elimination rates were then converted to number of events per quarter-
336 month.
337 Inferences presented were obtained using WinBUGS 1.4.3 (MRC Biostatistics
338 Unit, Cambridge, UK). These were based on MCMC samples of size 75,000 composed
339 of 3 different chains with different starting values. Visual inspection of the trajectories
340 and of the evolution of the Gelman-Rubin statistic were used to monitor the convergence
341 of the chains (Ntzoufras, 2009). Plots of the chains autocorrelation were inspected and
342 thinning of the chains was used when appropriate. The WinBUGS code is available from
343 the main author upon request. There were no further attempts to prune off the models
344 from the variables that were not statistically significant after the misclassification
345 adjustment was conducted. In the revised models, explanatory variables with 95%
346 credibility interval not containing the null value (1.0) were considered statistically
347 significant.
348
349 **RESULTS**

For Peer Review

ScholarOne support: (434) 964 4100

350

351 Herds selected in this study milked on average 85 cows (range 32 to 326) and had
352 a mean 305-d milk production of 9,781 kg of milk (range 7,734 to 12,377). A complete
353 description of the NCDF herds can be found in Reyher et al. (2011). Over the 2 yr course
354 of the study, 59,167 quarter milk samples were collected; 67 samples were lost or
355 damaged before bacteriological culture could be realized, 159 samples were excluded
356 because signs of clinical mastitis (mastitis score > 0) were observed, and 7,145 samples
357 were excluded because 3 or more pathogen species were cultured.

For Peer Review

358 The non-adjusted herd CNS IMI quarter prevalence and incidence and elimination
359 rates were all normally distributed with respective medians (25th and 75th percentiles) of
360 58.8% (47.2, 67.3), 0.36 new CNS IMI/quarter-month (0.28, 0.49), and 0.76 eliminated
361 CNS IMI/quarter-month (0.67, 0.86). Both herd CNS IMI incidence and elimination
362 rates were significant ($P \leq 0.05$) predictors of the herd prevalence. Scatter plots of the
363 relationships between herd prevalence and incidence and elimination rates are displayed
364 in Figure 1. The herd incidence rate had a greater impact on the herd prevalence than the
365 elimination rate. An increase of the herd incidence rate by its inter-quartile range (0.21
366 new IMI/quarter-month) was associated with an increase of the herd prevalence of 16.5
367 percentage-points. An equivalent decrease of the herd elimination rate by its inter-
368 quartile range (0.19 eliminated IMI/infected quarter-month) was associated with an
369 increase in the herd prevalence of only 2.3 percentage-points.

370 ***Risk Factors***

371 ***CNS IMI Incidence.*** The incidence data set was composed of 20,354 pairs of
372 milk samples at risk of becoming infected. These pairs were obtained from 11,221

373 quarters belonging to 3,707 cows. A new CNS IMI was identified in 5,009 of these pairs.
374 When correcting for misclassification due to imperfect Se and Sp of bacteriological
375 culture, a CNS IMI incidence of 0.29 new IMI/quarter-month (95% CI: 0.21, 0.37) was
376 observed. In comparison, an incidence rate of 0.36 new IMI/quarter-month (95% CI:
377 0.32, 0.40) was estimated when misclassification was ignored. The direct consequences
378 from the imperfect Se and Sp of bacteriological culture coupled with the observed
379 prevalence of CNS IMI were, therefore, a substantial overestimation of the true CNS IMI
380 incidence rate and an overly narrow confidence interval.

381 Conditional estimates of associations between manageable risk factors and odds
382 of CNS IMI acquisition are presented in Table 2. Quarters of cows that had access to
383 pasture during the sampling period had lower odds of acquiring a new CNS IMI
384 compared to quarters of cows that were confined inside. The type of bedding used in
385 lactating cows' stalls or pens was significantly associated with CNS IMI acquisition; use
386 of sand or wood-based product bedding was associated with lower odds of acquiring a
387 CNS IMI compared to straw bedding. Lower odds of CNS acquisition were observed in
388 herds where milkers received a bonus for milk quality.

389 For the incidence risk factors analysis, ignoring CNS IMI misclassification
390 resulted in a bias toward the null value for all of the computed measures of association.
391 In addition, IMI misclassification lead to narrower interval estimates for these measures.
392 For this analysis, however, ignoring IMI misclassification did not result in any Type I
393 (association wrongfully identified as statistically significant) or Type II (association
394 wrongfully identified as insignificant) errors.

For Peer Review

ScholarOne support: (434) 964 4100

395 ***CNS IMI Elimination.*** The elimination dataset comprised 10,054 pairs of milk
396 samples at risk of eliminating a CNS IMI. These pairs of samples were obtained from
397 7,132 different quarters from 3,304 cows. An elimination of the existing CNS IMI was
398 observed in 5,121 of these pairs. When correcting for imperfect Se and Sp of the
399 bacteriological culture, an estimate of 0.79 eliminated IMI/infected quarter-month (95%
400 CI: 0.66, 0.91) was observed. When misclassification was ignored, an estimate of 0.80
401 eliminated IMI/infected quarter-month (95% CI: 0.75, 0.86) was obtained. Coagulase-
402 negative staphylococci IMI elimination rate was therefore only slightly overestimated
403 when IMI misclassification was present. The width of the associated 95% confidence
404 interval was, however, grossly underestimated.
405 Results from the final model on risk factors associated with CNS IMI elimination
406 are reported in Table 3. Briefly, the use of sand bedding was associated with higher odds
407 of IMI elimination. Higher odds of IMI elimination was also seen for quarters of cows
408 with very dirty lower leg. Lower odds of CNS IMI elimination were seen when straw
409 was used as bedding in maternity pens and when new bedding was added fewer than one
410 time per day in these pens. Lower odds of IMI elimination was also seen in herds where
411 milk conductivity was measured during milking. Finally, higher odds of CNS IMI
412 elimination was seen in herds where cows have been purchased in the preceding 6 mo.
413 Like the incidence analysis, ignoring misclassification lead to bias of the odds
414 ratio toward the null value and to narrower confidence intervals. In addition, a Type II
415 error was made (lower leg cleanliness score) when CNS IMI misclassification was
416 ignored.

For Peer Review

ScholarOne support: (434) 964 4100

417 ***CNS IMI Prevalence.*** The prevalence dataset contained 19,842 series of quarter
418 milk samples. These series of samples were obtained from 15,771 different quarters from
419 3,998 cows. A total of 11,603 CNS-positive series were observed. Of these, 7,054 series
420 (60.8%) had one CNS-positive sample, 3,183 (27.4%) had 2 positive samples, and for
421 1,366 series (11.8%), all 3 samples were positive for CNS. After adjusting for IMI
422 misclassification, the true CNS IMI prevalence was estimated to be 42.7% (95% CI: 34.7,
423 50.1%). When IMI misclassification was ignored, a prevalence of 60.8% (95% CI: 57.1,
424 64.1%) was estimated. Ignoring IMI misclassification, therefore, resulted in a gross
425 overestimation of the true CNS IMI prevalence and, again, in a too narrow 95%
426 confidence interval.

427 Results from the model on the manageable risk factors for CNS IMI prevalence
428 are reported in Table 4. Similar to the incidence model, quarters of cows that had access
429 to pasture during the sampling period had lower odds of having a prevalent IMI. In herds
430 using sand or wood-based product bedding, a lower CNS IMI prevalence was observed.
431 Odds of having a CNS IMI generally increased, although non-significantly, with the
432 initial average herd SCS. This increase was constant across bedding type with the
433 exception of hay bedding, for which a significant and steep decrease of the odds of a CNS
434 IMI was seen with increasing average herd SCS. Lower odds of a prevalent IMI were
435 seen in herds where cows were left in a maternity pen for more than a week following
436 calving. Finally, providing a bonus to milkers for milk quality and drying teats with
437 paper or cloth towels as part of the milking procedures were associated with lower CNS
438 IMI prevalence.

For Peer Review

ScholarOne support: (434) 964 4100

439 For the prevalence analysis, ignoring misclassification resulted, for nearly all
440 measures of association, in a bias toward the null value. For one estimate (sand bedding
441 and herd SCS interaction term; a continuous variable), however, a bias away from the
442 null value was observed. All confidence intervals were narrower when misclassification
443 was ignored and one Type I (feed total mixed ration) and one Type II (milkers receive
444 bonus for milk quality) errors were made.
445

446 **DISCUSSION**

447

For Peer Review

448 This is the first longitudinal study reporting lactational incidence and elimination
449 rates of CNS IMI and the manageable risk factors associated with acquisition and
450 elimination of these in a large sample of herds over an extended period of time. An
451 important strength of this study was the attempt to account for the imperfect Se and Sp of
452 bacteriological culture for identifying CNS IMI. There is still a lack of agreement in the
453 scientific community on what constitutes a CNS IMI, and efforts should therefore be
454 made to link the milk bacteriological culture results interpreted within a given IMI
455 definition to the proper quarter IMI status. Using the method proposed by McInturff et
456 al. (2004) or simpler methods developed for 2x2 tables (Lash et al., 2009) would certainly
457 improve the comparability across studies. In this study, for instance, CNS IMI was
458 identified in 42.7% of apparently healthy mammary quarters. In comparison, a quarter
459 prevalence of 42% was observed in early lactating heifers in Belgium (Piepers et al.,
460 2011) but using a CNS IMI definition requiring ≥ 200 CNS cfu/ml of milk. In Germany
461 (Tenhagen et al., 2006) and in the Netherlands (Sampimon et al., 2009a), using IMI

462 definitions of ≥ 1,000 and ≥ 500 CNS cfu/ml of milk respectively, quarter prevalences of
463 8 to 11% and 10 to 15% were reported. It is difficult indeed to directly compare these
464 results because of the different IMI definitions used and the lack of adjustment for these
465 imperfect definitions.

466 In this study, a CNS IMI definition of ≥ 100 phenotypically identical CNS cfu/ml
467 of milk was used. This less specific but more sensitive definition was chosen to optimize
468 the negative predictive value (**NPV**) of the diagnostic test used to diagnose the outcome,
469 but also to initially select quarter at risk of becoming infected. Essentially, a less
470 sensitive IMI definition would have lead to the incorrect inclusion of a larger number of
471 already infected quarters in the incidence analysis, which was deemed to be the most
472 important part of this study. For instance, assuming a prevalence of CNS IMI of 40%,
473 and using the Se and Sp estimates reported in Dohoo et al. (2011), when requiring ≥ 200
474 CNS cfu/ml of milk, 24% of the recruited quarters would actually be already infected
475 and, thus, wrongly recruited (NPV: 76%). This proportion would be reduced to 13%
476 (NPV: 87%) with a ≥ 100 CNS cfu/ml of milk IMI definition. The ≥ 100 CNS cfu/ml
477 IMI definition was, therefore, chosen to reduce a selection bias that could not be handled
478 analytically. Under the same assumptions, using the ≥ 100 CNS cfu/ml of milk IMI
479 definition to diagnose subsequent acquisition of a new CNS IMI would, however, result
480 in a higher, but not as spectacular, proportion of wrongly identified new IMI (20%), when
481 compared to the ≥ 200 CNS cfu/ml IMI definition (12%). This potentially greater
482 misclassification bias could, however, be handled analytically with the latent class model
483 used to adjust estimates of disease frequency and of association with exposures. In fact,
484 when using such analytical treatment of misclassification bias, the choice of a specific

ScholarOne support: (434) 964 4100
For Peer Review

485 IMI definition over another should not significantly alter the results, as long as well
486 informed Se and Sp distributions can be specified for the chosen definition. To illustrate
487 this point, the presented incidence model was also ran using a ≥ 200 CNS cfu/ml IMI
488 definition to diagnosed acquisition of a new CNS IMI, and using a similar latent class
489 model with Se and Sp distributions centered on 0.56 and 0.95, respectively (the Se and Sp
490 estimates for a ≥ 200 CNS cfu/ml IMI definition reported in Dohoo et al., 2011). When
491 comparing measures of association between the 2 misclassification-adjusted models,
492 measures of association obtained using the ≥ 100 cfu/ml misclassification-adjusted model
493 corresponded, on average, to 95% of those obtained using the ≥ 200 cfu/ml IMI
494 misclassification-adjusted model (data not shown). Using the ≥ 100 cfu/ml CNS IMI
495 definition, therefore, resulted in only very slightly weaker measures of association with
496 exposures and should not impact the results from these analyses.

497 ***Impact of CNS IMI Misclassification***

498 In this study, ignoring CNS IMI misclassification yielded substantial bias of most
499 measures of disease frequency. Usually, investigators tend to rely on intuition to
500 qualitatively discuss how the misclassification bias may affect their results. In the
501 authors' opinion, relying solely on intuition is unlikely to lead to a correct appraisal of the
502 magnitude and direction of the resulting biases. Even for relatively simple analyses, such
503 as estimating IMI prevalence, the resulting bias will be influenced by 3 components: the
504 frequency of the disease in the population; the test Se; and the test Sp. While the bias can
505 very easily be assessed quantitatively, correctly appraising the combined impacts of these
506 3 components qualitatively is very difficult. As observed by Lash (2007), when asked to
507 intuitively appraise such bias, the vast majority usually fail to take into account the

For Peer Review

508 frequency of the disease in the population. In this study, most would have wrongfully
509 guessed, for instance, that the relatively low test Se for CNS IMI would result in an
510 underestimation of the true CNS incidence.

511 Important biases were also seen on measures of association with manageable risk
512 factors. In the incidence and prevalence models, for instance, traditional regression
513 coefficients corresponded, in general, to roughly 50% of the misclassification adjusted
514 coefficients (Table 2 and 4). In the elimination model, they corresponded more or less to
515 30% of the misclassification adjusted ones (Table 3). Although bias away from the null
516 value was seen, the resulting biases were nearly always toward the null value, as would
517 be expected with non-differential misclassification of binary variables. At first sight,
518 Type I errors may seem nearly impossible with a bias toward the null value, but it is not
519 once the grossly underestimated 95% confidence intervals are taken into consideration.
520 Therefore, although direction of the biases was often predictable, these biases were
521 sufficient to lead to either type I or type II errors. In this study, pretending that the
522 outcome was measured perfectly would have lead to different recommendations to dairy
523 producers. Similar findings have been reported before by McGlothlin et al. (2008) and
524 by Tarafder et al. (2011). Finally, estimates of association with exposures reported in the
525 literature are commonly used latter on in economic studies, meta-analyses, or for the
526 computation of other epidemiologic measures such as population attributable fractions.
527 Reporting unadjusted estimates in one scientific manuscript is, therefore, very likely to
528 lead to a certain number of subsequent erroneous recommendations.

529 Results from this study clearly highlight the important impact of ignoring CNS
530 IMI misclassification. The method proposed by McInturff et al. (2004), however, can be

For Peer Review

531 used to handle this problem and offers many particularities that make it extremely
532 interesting for mastitis research: it can correctly estimate both measures of disease
533 frequency and measures of association with exposures; it can easily deal with hierarchical
534 data structure; and, finally, uncertainty around Se and Sp estimates can be built-in.

535 ***CNS Epidemiology***

536 As for many diseases, the rate at which new CNS IMI were acquired seemed to be
537 a stronger determinant of the herd IMI prevalence than the elimination rate. These results
538 would suggest that the control of risk factors associated with CNS IMI incidence would
539 have a greater impact over time than the control of risk factors associated with
540 elimination of existing CNS IMI. Actually, the relatively high CNS IMI incidence rate is
541 certainly a striking feature of CNS IMI epidemiology compared to other common mastitis
542 pathogens. Assuming that CNS IMI acquisitions are evenly distributed across quarters, a
543 healthy quarter would have 29% chance of getting infected in any one-month period
544 which translates into 87% chance of getting infected over a 6 month period. On the
545 NCDF farms, CNS IMI yielded by far the highest incidence rate among the mastitis
546 pathogens reported (S. Dufour, unpublished data). In contrast, *S. aureus* incidence rates
547 of 0.012 (Dufour et al., 2011a) and 0.019 new IMI/quarter-month (Zadoks et al., 2001)
548 have been reported. With the often short duration (Supré et al., 2011; Taponen et al.,
549 2007) and high prevalence of infection reported for CNS, that CNS would have such a
550 high IMI incidence rate was already suspected, and these results only corroborate this
551 general belief. Coagulase-negative staphylococci IMI natural elimination rates have been
552 reported before (Deluyker et al., 2005; McDougall, 1998; Taponen et al., 2006) and were
553 quite variable across the populations studied and across the IMI definitions used.

For Peer Review

554 Although it cannot be directly compared to previously published studies, the CNS IMI
555 elimination rate of 0.79 eliminated IMI/infected quarter-month observed in this study
556 would be considered rather high. This high elimination rate could be the result of
557 specific differences on Canadian farms in either or both the CNS species found and the
558 host characteristics altering the response to these IMI. In a convenient sample of 387 of
559 the NCDF CNS isolates recovered from apparently normal milking cows and speciated
560 using gene sequencing, a large proportion (49.4%) were found to be *Staphylococcus*
561 *chromogenes* (J.R. Middleton, unpublished data). In term of most frequent CNS species,
562 therefore, the CNS isolates in this study would be comparable to those of studies
563 conducted in the U.S. (Gillespie et al., 2009), Belgium (Piessens et al., 2011; Supré et al.,
564 2011), and the Netherlands (Sampimon et al., 2009b), but would differ from those of
565 studies carried out in Sweden (Thorberg et al., 2009; Waller et al., 2011) and Finland
566 (Taponen et al., 2006). Remaining NCDF CNS isolates speciated by gene sequencing
567 were found to be mainly *Staphylococcus simulans* (24.0%), *Staphylococcus xylosus*
568 (8.8%), *Staphylococcus haemolyticus* (4.9%), and 16 other CNS species (12.9%) (J.R.
569 Middleton, unpublished data).

570 One drawback of this study was the consideration of the CNS retrieved from
571 NCDF farms as one homogeneous group. As can be seen from the small sample of CNS
572 isolates that could be speciated, the isolates studied could be further differentiated into a
573 few groups that could potentially show a certain level of heterogeneity in term of
574 incidence and elimination rates, as well as risk factors for these. Because of the large
575 number of isolates involved, identification of the CNS isolates at the species level was
576 not available when the analyses were carried out. Plans for speciation of a larger sample

For Peer Review

577 of the NCDF CNS isolates have been laid and, in future research, this issue will be
578 resolved. The present study should, therefore, be regarded as an exploratory study on the
579 epidemiology of CNS as a group, while keeping in mind the possible heterogeneity of the
580 isolates that constitute this group. In addition, since CNS IMI duration or persistence
581 could not be precisely established, this important aspect was not addressed in this study.
582 The presented study was strictly focused on acquisition and elimination of CNS IMI over
583 3-week periods and on presence of CNS IMI.
584
585 ***Manageable Risk Factors***
586 Many management practices were associated with the odds of having a prevalent
587 CNS IMI. It is important to realize that these associations can only be mediated by an
588 effect on CNS IMI incidence, elimination, or both. In addition, when measures of disease
589 prevalence on their own are used, it is difficult to identify the correct time-order of
590 occurrence between exposure and disease, and this can potentially lead to the
591 identification of spurious associations. For these reasons, less consideration should be
592 given to management practices associated solely with CNS IMI prevalence in particular
593 or with only one outcome in general. For a thorough interpretation of the study's results,
594 the authors suggest consideration of a holistic analysis and interpretation of associations
595 with all 3 outcomes jointly. A conceptual chart of the associations between manageable
596 risk factors and prevalence, incidence, and elimination of CNS IMI based on the results
597 from Tables 2, 3, and 4 is presented in Fig. 2, and should help the reader to bridge this
598 gap.

For Peer Review

599 As a starting point, it is worth mentioning that all risk factors associated with CNS
600 IMI incidence were also associated with IMI prevalence. Conversely, a few of the risk
601 factors associated with CNS IMI prevalence were not associated with IMI incidence.
602 These differences may be explained, in part, by the higher power of the study for the
603 prevalence dataset for which the number of observations and the distribution of the
604 outcome were superior. Only one of the management practices studied - the type of
605 bedding used in stalls or pens - showed similar associations with all 3 outcomes. Matos
606 et al. (1991) have already reported disparities in bacterial load between bedding types and
607 between fresh and used bedding. These researchers observed different distributions of
608 staphylococci species among bedding types and reported these species as common in the
609 cows' environment. Results from the present study suggest that bedding type plays a
610 substantial role in CNS epidemiology and, based on these previously published results,
611 this role is probably mediated through differential selection of CNS species that are more
612 or less competent at causing IMI. When compared to straw bedding, the use of sand
613 bedding showed a desirable association with all 3 outcomes. In the literature, sand
614 bedding has been consistently associated with lower SCC (Dufour et al., 2011b).
615 Compared to organic bedding, very little substrate is available to support bacterial growth
616 in an inorganic bedding, such as sand, and this may explain the lower IMI incidence and
617 prevalence observed. Given that the odds of IMI elimination was greater for sand
618 bedding, it also suggests that more poorly host-adapted CNS species or strains would be
619 found in the environment of sand bedded barns. Our results also suggest that, among the
620 organic bedding used, wood-based product would support either a lower quantity of CNS,
621 less well host-adapted CNS species, or both. This is supported by results from Matos et

For Peer Review

ScholarOne support: (434) 964 4100

622 al. (1991) who reported generally decreasing bacterial counts between hay, straw, and
623 sawdust beddings as well as different CNS species populations between bedding types.
624 In their study, the very different CNS populations found in alfalfa hay could explain the
625 lower odds of IMI elimination observed in the present study.

626 In this study, quarters of cows that had access to pasture during the sampling
627 period had lower IMI incidence and prevalence, which suggests a lower CNS infection
628 pressure from pasture compared to confinement housing. These results are in contrast
629 with those of Sampimon et al. (2009b) who found a higher herd CNS prevalence in herds
630 where cows had access to pasture during the outdoor season. In that Dutch study,
631 however, the yearly herd CNS prevalence was used as the outcome rather than the
632 seasonal prevalence. The direct impact of pasture access, therefore, would be difficult to
633 evaluate. In addition, it is likely that the very different weather and pasture conditions
634 prevailing in the Netherlands compared to Canada could have led to these different
635 observations.

636 The only other manageable risk factor associated with at least 2 of the studied
637 outcomes was to provide a bonus for milk quality to the persons milking. It is difficult,
638 however, to clearly evaluate the direct effect of such practice. Providing a bonus for milk
639 quality could, for instance, motivate the milkers to be more thorough and to follow more
640 closely the recommended milking procedures, which would help prevent acquisition of
641 new CNS IMI. The association seen would then be an indirect effect of this practice. On
642 the other hand, providing such bonus could also be an indication of a more proactive
643 attitude toward udder health in general, which would, in turn, lead to a greater adoption

For Peer Review

644 of other recommended practices. The association observed would then be a spurious
645 association resulting from residual confounding by general attitude toward udder health.
646 A few manageable risk factors related to maternity pen management, cow
647 cleanliness, purchase habits, and monitoring of udder health were associated solely with
648 CNS IMI elimination. Further investigation into these possible risk factors should be
649 undertaken before drawing any conclusions. Similarly, some practices related to milking
650 procedures and maternity pen management were associated with IMI prevalence
651 exclusively. Again, it is recommended that caution be used in drawing conclusions in
652 these cases.
653 Two cornerstones of every mastitis control program, blanket dry cow therapy
654 (**DCT**) and PMTD, were already used by a vast majority of the NCDF herds (88% for
655 blanket DCT, and 99% for PMTD). Because of the low number of dairy producers not
656 using these practices, the power to find significant associations between CNS IMI
657 outcomes and blanket DCT or PMTD was limited. These practices should certainly not
658 be rejected as potential important risk factors for CNS IMI based on the study's results.
659 One should instead consider the manageable risk factors identified in this study as
660 practices that could be use to control CNS IMI in herds already using blanket DCT and
661 PMTD.
662 Finally, as mentioned before, it is still unclear whether or not CNS IMI are indeed
663 detrimental to udder health. The SCC increases that have been generally reported for
664 CNS IMI, though, seem to indicate that prevention of these IMI, at least in low BMSCC
665 herds, is a cautious approach. In addition, most of the manageable risk factors for CNS
666 IMI identified in this study, have shown desirable association in previous studies with

For Peer Review

ScholarOne support: (434) 964 4100

667 other measures of udder health. It would, therefore, be very unlikely that implementing
668 these practices to control CNS IMI would result in a general deterioration of udder health.

669 ***Potential Bias***

670 Like most exploratory studies, many potential biases may have led to the observed
671 estimates of association. First of all, the herds selected were a convenience sample of
672 Canadian dairy herds and, although they shared some similar attributes with the Canadian
673 dairy herd population (Reyher et al., 2011), they may have differed from the target
674 population in terms of CNS IMI burden or of management practices used. The resulting
675 selection bias would affect estimates of CNS IMI prevalence, incidence, and elimination.
676 It would, however, be much less likely to affect estimates of association between
677 manageable risk factors and CNS IMI outcomes.

678 Secondly, although an effort was made to adjust for the most obvious
679 confounders, it is likely that residual confounding still may bias the presented estimates
680 to some extent. In a previously published study on manageable risk factors associated
681 with *S. aureus* IMI (Dufour et al., 2011a), however, and using an extended and thorough
682 investigation procedure to identify confounding, very few of the theoretically identified
683 confounders were actually modifying the reported estimates by a significant amount (S.
684 Dufour, unpublished data). So, in the opinion of the authors, although the direction of
685 residual confounding bias is unpredictable, its magnitude is likely to be small.

686 Finally, despite the use of a latent class model approach to adjust the presented
687 estimates for disease misclassification, and despite the use of Se and Sp thresholds for
688 explanatory variables, a limited degree of misclassification bias probably remains. The
689 level of control of misclassification bias that can be achieve using the latent class model

For Peer Review

690 approach, or any other misclassification adjustment approach, is directly related to the
691 exactitude of the misclassification parameters (the Se and Sp) chosen (Lash et al., 2009).
692 In this study, since the Se and Sp estimates were obtained from an internal validation
693 study using a sample of the studied CNS isolates, the misclassification parameters used
694 are likely to be very close to the true Se and Sp values. Any remaining misclassification
695 bias should, therefore, be fairly small.
696

697 **CONCLUSIONS**

698

699 Like a number of infectious diseases, prevention seems to be the key to long-term
700 CNS IMI control. When an outcome is measured with an obviously imperfect diagnostic
701 procedure, such as bacteriological culture for CNS, determining the direction and
702 magnitude of the resulting bias on estimates of prevalence, incidence, elimination, or on
703 associations with risk factors rapidly becomes intractable. In these situations, using a
704 technique accounting for the test limitations would provide better estimates and would
705 improved comparability between studies. In herds already employing blanket DCT and
706 PMTD, many additional practices can be implemented to prevent acquisition of new CNS
707 IMI. These practices seemed to be mainly related to management of the environment of
708 the cow such as bedding condition or pasture access.
709

710 **ACKNOWLEDGEMENTS**

711

For Peer Review

712 The authors wish to thank all the dairy producers and animal health technicians
713 who participated to this study. This research was financed by the Natural Sciences and
714 Engineering Research Council of Canada, Alberta Milk, Dairy Farmers of New
715 Brunswick, Dairy Farmers of Nova Scotia, Dairy Farmers of Ontario and Dairy Farmers
716 of Prince Edward Island, Novalait Inc., Dairy Farmers of Canada, Canadian Dairy
717 Network, Agriculture and Agri-Food Canada, Public Health Agency of Canada,
718 Technology PEI Inc., Université de Montréal and University of Prince Edward Island,
719 through the Canadian Bovine Mastitis Research Network.

720

721 **REFERENCES**

722

723 Compton, C. W., C. Heuer, K. Parker, and S. McDougall. 2007. Epidemiology of mastitis
724 in pasture-grazed peripartum dairy heifers and its effects on productivity. J. Dairy
725 Sci. 90(9):4157-4170.
726 Deluyker, H. A., S. N. Van Oye, and J. F. Boucher. 2005. Factors affecting cure and
727 somatic cell count after pirlimycin treatment of subclinical mastitis in lactating
728 cows. J. Dairy Sci. 88(2):604-614.
729 Djabri, B., N. Bareille, F. Beaudeau, and H. Seegers. 2002. Quarter milk somatic cell
730 count in infected dairy cows: a meta-analysis. Vet. Res. 33(4):335-357.
731 Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary epidemiologic research. 2nd ed.
732 VER Inc, Charlottetown, PE, Canada.

For Peer Review

ScholarOne support: (434) 964 4100

733 Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing
734 intramammary infections: evaluation of definitions based on a single milk sample.
735 J. Dairy Sci. 94(1):250-261.
736 Dufour, S., H. W. Barkema, L. DesCôteaux, T. J. DeVries, I. R. Dohoo, K. Reyher, J. P.
737 Roy, and D. T. Scholl. 2010. Development and validation of a bilingual
738 questionnaire for measuring udder health related management practices on dairy
739 farms. Prev. Vet. Med. 95(1-2):74-85.
740 Dufour, S., I. R. Dohoo, H. W. Barkema, L. Des Côteaux, T. J. DeVrie, K. K. Reyher, J.-
741 P. Roy, and D. T. Scholl. 2011a. Manageable risk factors associated with the
742 lactational incidence, elimination, and prevalence of *Staphylococcus aureus*
743 intramammary infections in dairy cows. J. Dairy Sci. In press.
744 Dufour, S., A. Frechette, H. W. Barkema, A. Mussell, and D. T. Scholl. 2011b. Invited
745 review: effect of udder health management practices on herd somatic cell count. J.
746 Dairy Sci. 94(2):563-579.
747 Gillespie, B. E., S. I. Headrick, S. Boonyayatra, and S. P. Oliver. 2009. Prevalence and
748 persistence of coagulase-negative Staphylococcus species in three dairy research
749 herds. Vet. Microbiol. 134(1-2):65-72.
750 Gustafson, P. 2004. Measurement error and misclassification in statistics and
751 epidemiology: impacts and Bayesian adjustments. Interdisciplinary statistics.
752 Chapman & Hall/CRC, Boca Raton.
753 Höfler, M. 2005. The effect of misclassification on the estimation of association: a
754 review. Int. J. Methods Psychiatr. Res. 14(2):92-101.

For Peer Review

ScholarOne support: (434) 964 4100

755 Hogan, J. S., R. N. Gonzalez, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey,
756 and K. L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. Madison, WI.
757 Lash, T. L. 2007. Heuristic thinking and inference from observational epidemiology.
758 Epidemiology 18(1):67-72.
759 Lash, T. L., M. P. Fox, and A. K. Fink. 2009. Applying quantitative bias analysis to
760 epidemiologic data. Statistic for biology and health. Springer, New York.
761 Lash, T. L., M. P. Fox, S. S. Thwin, A. M. Geiger, D. S. Buist, F. Wei, T. S. Field, M. U.
762 Yood, F. J. Frost, V. P. Quinn, M. N. Prout, and R. A. Silliman. 2007. Using
763 probabilistic corrections to account for abstractor agreement in medical record
764 reviews. Am. J. Epidemiol. 165(12):1454-1461.
765 Matos, J. S., D. G. White, R. J. Harmon, and B. E. Langlois. 1991. Isolation of
766 *Staphylococcus aureus* from sites other than the lactating mammary gland. J.
767 Dairy Sci. 74(5):1544-1549.
768 Matthews, K. R., R. J. Harmon, and B. A. Smith. 1990. Protective effect of
769 *Staphylococcus chromogenes* infection against *Staphylococcus aureus* infection in
770 the lactating bovine mammary gland. J. Dairy Sci. 73(12):3457-3462.
771 McDougall, S. 1998. Efficacy of two antibiotic treatments in curing clinical and
772 subclinical mastitis in lactating dairy cows. N. Z. Vet. J. 46(6):226-232.
773 McGlothlin, A., J. D. Stamey, and J. W. Seaman, Jr. 2008. Binary regression with
774 misclassified response and covariate subject to measurement error: a bayesian
775 approach. Biom. J. 50(1):123-134.
776 McInturff, P., W. O. Johnson, D. Cowling, and I. A. Gardner. 2004. Modelling risk when
777 binary outcomes are subject to error. Stat. Med. 23(7):1095-1109.

For Peer Review

778 Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. Noordhuizen. 2000.
779 Classification and longitudinal examination of callused teat ends in dairy cows. J.
780 Dairy Sci. 83(12):2795-2804.
781 Ntzoufras, I. 2009. Bayesian modeling using WinBUGS. Wiley series in computational
782 statistics. Wiley, Hoboken, N.J.
783 Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers
784 infected with coagulase-negative staphylococci in early lactation have fewer cases
785 of clinical mastitis and higher milk production in their first lactation than
786 noninfected heifers. J. Dairy Sci. 93(5):2014-2024.
787 Piepers, S., K. Peeters, G. Opsomer, H. W. Barkema, K. Frankena, and S. De Vliegher.
788 2011. Pathogen group specific risk factors at herd, heifer and quarter levels for
789 intramammary infections in early lactating dairy heifers. Prev. Vet. Med. 99(2-
790 4):91-101.
791 Piessens, V., E. Van Coillie, B. Verbist, K. Supre, G. Braem, A. Van Nuffel, L. De
792 Vuyst, M. Heyndrickx, and S. De Vliegher. 2011. Distribution of coagulase-
793 negative Staphylococcus species from milk and environment of dairy cows differs
794 between herds. J. Dairy Sci. 94(6):2933-2944.
795 Pyörälä, S. and S. Taponen. 2009. Coagulase-negative staphylococci-emerging mastitis
796 pathogens. Vet. Microbiol. 134(1-2):3-8.
797 Reyher, K. K., S. Dufour, H. W. Barkema, L. Des Côteaux, T. J. Devries, I. R. Dohoo, G.
798 P. Keefe, J. P. Roy, and D. T. Scholl. 2011. The National Cohort of Dairy Farms-
799 A data collection platform for mastitis research in Canada. J. Dairy Sci.
800 94(3):1616-1626.

For Peer Review

801 Sampimon, O., H. W. Barkema, I. Berends, J. Sol, and T. Lam. 2009a. Prevalence of
802 intramammary infection in Dutch dairy herds. J. Dairy Res. 76(2):129-136.
803 Sampimon, O. C., H. W. Barkema, I. M. Berends, J. Sol, and T. J. Lam. 2009b.
804 Prevalence and herd-level risk factors for intramammary infection with coagulase-
805 negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134(1-2):37-44.
806 Sampimon, O., B. H. van den Borne, I. Santman-Berends, H. W. Barkema, and T. Lam.
807 2010. Effect of coagulase-negative staphylococci on somatic cell count in Dutch
808 dairy herds. J. Dairy Res. 77:318-324.
809 Schukken, Y. H., R. N. Gonzalez, L. L. Tikofsky, H. F. Schulte, C. G. Santisteban, F. L.
810 Welcome, G. J. Bennett, M. J. Zurakowski, and R. N. Zadoks. 2009. CNS
811 mastitis: nothing to worry about? Vet. Microbiol. 134(1-2):9-14.
812 Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De
813 Vliegher. 2011. Some coagulase-negative *Staphylococcus* species affect udder
814 health more than others. J. Dairy Sci. 94(5):2329-2340.
815 Taponen, S., J. Koort, J. Bjorkroth, H. Saloniemi, and S. Pyörälä. 2007. Bovine
816 intramammary infections caused by coagulase-negative staphylococci may persist
817 throughout lactation according to amplified fragment length polymorphism-based
818 analysis. J. Dairy Sci. 90(7):3301-3307.
819 Taponen, S., H. Simojoki, M. Haveri, H. D. Larsen, and S. Pyörälä. 2006. Clinical
820 characteristics and persistence of bovine mastitis caused by different species of
821 coagulase-negative staphylococci identified with API or AFLP. Vet. Microbiol.
822 115(1-3):199-207.

For Peer Review

823 Tarafder, M. R., H. Carabin, S. T. McGarvey, L. Joseph, E. Balolong, Jr., and R. Olveda.
824 2011. Assessing the impact of misclassification error on an epidemiological
825 association between two helminthic infections. PLoS Negl. Trop. Dis. 5(3):e995.
826 Tenhagen, B. A., G. Koster, J. Wallmann, and W. Heuwieser. 2006. Prevalence of
827 mastitis pathogens and their resistance against antimicrobial agents in dairy cows
828 in Brandenburg, Germany. J. Dairy Sci. 89(7):2542-2551.
829 Thorberg, B. M., M. L. Danielsson-Tham, U. Emanuelson, and K. Persson Waller. 2009.
830 Bovine subclinical mastitis caused by different types of coagulase-negative
831 staphylococci. J. Dairy Sci. 92(10):4962-4970.
832 Waller, K. P., A. Aspan, A. Nyman, Y. Persson, and U. G. Andersson. 2011. CNS species
833 and antimicrobial resistance in clinical and subclinical bovine mastitis. Vet.
834 Microbiol. 152(1-2):112-116.
835 White, L. J., T. J. Lam, Y. H. Schukken, L. E. Green, G. F. Medley, and M. J. Chappell.
836 2006. The transmission and control of mastitis in dairy cows: a theoretical
837 approach. Prev. Vet. Med. 74(1):67-83.
838 Zadoks, R. N., H. G. Allore, H. W. Barkema, O. C. Sampimon, G. J. Wellenberg, Y. T.
839 Grohn, and Y. H. Schukkent. 2001. Cow- and quarter-level risk factors for
840 *Streptococcus uberis* and *Staphylococcus aureus* mastitis. J. Dairy Sci.
841 84(12):2649-2663.
842
843

For Peer Review

844 Table 1. Prior distributions used in the latent class model for bacteriological culture
845 sensitivity (Se) and specificity (Sp).

Analysis	Param.[1]	Distribution	Mean[2]	Limits[3]	
				Lower	Upper
Incidence and elimination	Se	Beta(165, 39.0)	0.81	0.76	0.86
	Sp	Beta(145, 22.5)	0.87	0.82	0.92
Prevalence					
Series with 1 culture result	Se	Beta(165, 39.0)	0.81	0.76	0.86
	Sp	Beta(145, 22.5)	0.87	0.82	0.92
Series with 2 results[4]	Se	Beta(92, 4.0)	0.96	0.91	1.00
	Sp	Beta(174, 55.5)	0.76	0.71	0.81
Series with 3 results[4]	Se	Beta(68, 1.5)	0.98	0.93	1.00
	Sp	Beta(172, 89.0)	0.66	0.61	0.71

846 [1] Parameter estimated.
847 [2] All distributions were centered on the parameter estimate obtained using CBMRN
848 isolates and reported in Dohoo et al. (2011)
849 [3] Lower and upper truncation of the distributions were implemented to avoid selection of
850 less probable and sometimes inappropriate Se and Sp combinations and improve MCMC
851 convergence. Lower and upper limits correspond to the parameter estimate reported in
852 Dohoo et al. (2011) ± 5 percentage-points.
853 [4] Whenever CNS IMI status were determined using 2 or 3 bacteriological culture results
854 interpreted in parallel, the Se and Sp estimates reported in Dohoo et al. (2011) were
855 adjusted accordingly.
856

For Peer Review

857 Table 2. Final multivariable cross-classified hierarchical model of the relationship
858 between manageable risk factors and odds of acquisition of new coagulase-negative
859 staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	0.97	0.75	1.3	0.91	0.54	1.5
Bedded pack barn	0.72	0.46	1.1	0.51	0.18	1.3
Outside access						
No outside access	Ref	Ref	Ref	Ref	Ref	Ref
Access to exercise yard	0.92	0.63	1.4	0.81	0.40	1.6
Access to pasture	0.71*	0.61	0.81	0.52*	0.38	0.70
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	0.51*	0.33	0.78	0.27*	0.10	0.64
Wood products	0.73*	0.57	0.94	0.55*	0.31	0.90
Hay	1.0	0.58	1.8	1.0	0.36	3.0
Wood and straw	0.90	0.72	1.1	0.84	0.55	1.3
Milkers receive bonus for milk quality	0.59*	0.36	0.96	0.33*	0.11	0.91
% of clinical mastitis (CM) cases treated						
< 50%	Ref	Ref	Ref	Ref	Ref	Ref
50 to 90%	0.88	0.66	1.2	0.76	0.43	1.3
≥ 90%	1.3	0.98	1.6	1.6	0.98	2.7

860 [a] Median odds ratio estimate

861 [b] Variable kept in the model as confounding variable

862 * OR statistically significant (95% credibility interval not including the null value)

For Peer Review

863 Table 3. Final multivariable cross-classified hierarchical model of the relationship
864 between manageable risk factors and odds of elimination of coagulase-negative
865 staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	1.2	0.94	1.6	1.9	0.80	4.4
Bedded pack barn	1.2	0.79	2.0	2.0	0.51	8.6
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	1.7*	1.1	2.5	4.9*	1.4	21.0
Wood products	1.1	0.92	1.4	1.6	0.79	3.2
Hay	0.91	0.62	1.3	0.67	0.18	2.3
Wood and straw	1.5*	1.2	1.8	3.3*	1.7	7.9
Lower leg cleanliness score						
Very clean	Ref	Ref	Ref	Ref	Ref	Ref
Clean	0.90	0.73	1.1	0.82	0.42	1.6
Dirty	1.1	0.90	1.4	1.7	0.83	3.6
Very dirty	1.4	1.0	1.8	2.9*	1.2	8.1
Distance neckrail-curb						
< 1.7m	Ref	Ref	Ref	Ref	Ref	Ref
1.7 to 1.8m	1.2	0.94	1.5	1.8	0.83	4.3
1.8 to 1.9m	0.93	0.65	1.3	0.76	0.23	2.4
>1.9m	0.91	0.69	1.2	0.74	0.30	1.8
Type of bedding in maternity pens (MP)						
Wood products	Ref	Ref	Ref	Ref	Ref	Ref
Straw	0.59*	0.45	0.76	0.20*	0.07	0.51
Hay	0.45	0.11	1.8	0.06	<0.01	6.6
Wood products and straw	0.65*	0.47	0.90	0.26*	0.08	0.82
Bedding added to MP						
≥ once/d	Ref	Ref	Ref	Ref	Ref	Ref
once/d to once/mo	0.72*	0.60	0.80	0.37*	0.18	0.69
< once/mo	0.72	0.45	1.2	0.46	0.09	2.0
After every calving	0.70*	0.54	0.92	0.34*	0.13	0.81
As needed	1.7	0.58	5.0	9.4	0.24	>100.0

866

867

For Peer Review

868 Table 3. (Continued)

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Measures milk conductivity	0.57*	0.42	0.76	0.16*	0.05	0.41
Ration balanced based on forage analyses	0.63	0.47	1.1	0.32	0.04	1.6
Purchase habits in preceding 6 mo						
Never buys cattle	Ref	Ref	Ref	Ref	Ref	Ref
Usually buy cattle but not in last 6 mo	0.42	0.17	1.1	0.04	<0.01	1.0
Purchased only heifers	1.2	0.94	1.5	1.8	0.84	4.0
Purchased cows	1.3*	1.1	1.5	2.3*	1.4	3.9

869 [a] Median odds ratio estimate

870 [b] Variable kept in the model as confounding variable

871 [c] Median odds ratio estimate and 2.5th and 97.5th percentiles are presented per increase of

872 30 days in milk

873 * OR statistically significant (95% credibility interval not including the null value)

874

For Peer Review

ScholarOne support: (434) 964 4100

875 Table 4. Final multivariable cross-classified hierarchical model of the relationship
876 between manageable risk factors and odds of a prevalent coagulase-negative
877 staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	1.2	0.83	1.7	1.5	0.78	2.9
Bedded pack barn	0.76	0.41	1.4	0.73	0.23	2.5
Herd mean SCS in preceding 24 mo[b]	1.1	0.83	1.4	1.3	0.80	2.1
Outside access						
No outside access	Ref	Ref	Ref	Ref	Ref	Ref
Access to exercise yard	1.3	0.85	1.9	1.5	0.75	3.2
Access to pasture	0.80*	0.68	0.93	0.71*	0.52	0.97
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	0.58*	0.36	0.96	0.39*	0.16	0.96
Wood products	0.70*	0.54	0.92	0.48*	0.29	0.79
Hay	3.9*	1.7	8.7	7.8*	2.0	37.3
Wood and straw	0.72*	0.56	0.91	0.56*	0.36	0.87
Type of bedding by herd SCS						
Straw by herd SCS	Ref	Ref	Ref	Ref	Ref	Ref
Sand by herd SCS	0.93	0.49	1.8	0.95	0.25	3.6
Wood products by herd SCS	1.0	0.76	1.4	1.1	0.65	1.9
Hay by herd SCS	0.21*	0.11	0.41	0.11*	0.03	0.29
Wood and straw by herd SCS	0.97	0.69	1.4	0.91	0.48	1.8
Distance neckrail-curb						
< 1.7m	Ref	Ref	Ref	Ref	Ref	Ref
1.7 to 1.8m	0.85	0.58	1.2	0.63	0.30	1.3
1.8 to 1.9m	0.69	0.40	1.2	0.43	0.15	1.1
>1.9m	1.1	0.73	1.7	0.99	0.43	2.1
Cows left >7d in MP after calving	0.38*	0.18	0.82	0.12*	0.02	0.57
Milkers receive bonus for milk quality	0.59	0.33	1.0	0.27*	0.08	0.89
S. aureus cows milked last or with a specific unit	1.3	0.95	1.8	1.6	0.89	3.3

878

For Peer Review

ScholarOne support: (434) 964 4100

879 Table 4. (Continued)

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Teat drying method						
No drying	Ref	Ref	Ref	Ref	Ref	Ref
Paper towels	0.67*	0.51	0.89	0.51*	0.32	0.85
Reusable cloth towels	0.63*	0.46	0.86	0.39*	0.21	0.73
Feed total mixed ration	1.3*	1.1	1.7	1.6	1.0	2.6
% of clinical mastitis (CM) cases treated						
< 50%	Ref	Ref	Ref	Ref	Ref	Ref
50 to 90%	0.85	0.63	1.1	0.70	0.42	1.2
≥ 90%	1.2	0.88	1.5	1.4	0.85	2.2
Herd SCS at beginning of sampling period	1.2	0.99	1.4	1.3	0.96	1.7

880 [a] Median odds ratio estimate

881 [b] Variable kept in the model as confounding variable

882 * OR statistically significant (95% credibility interval not including the null value).

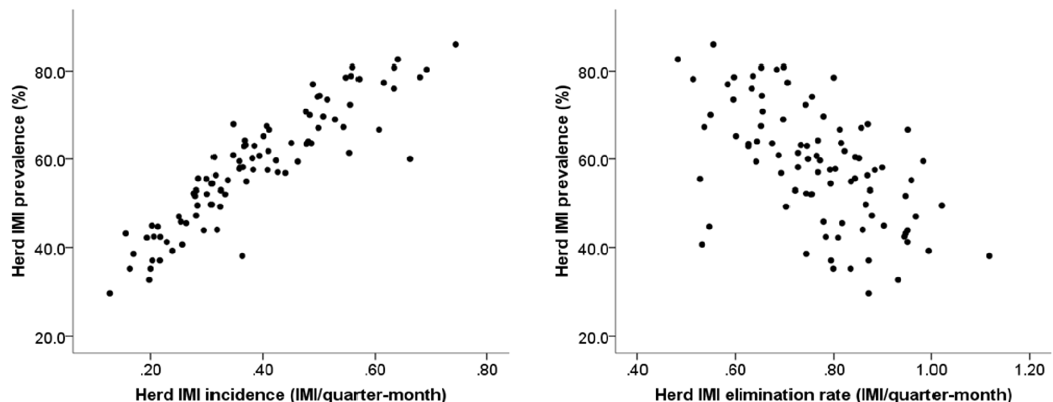
For Peer Review

883 Figure 1. Scatter plots of the herd coagulase-negative staphylococci (CNS) IMI
884 prevalence against herd IMI incidence and elimination rates.
885
886 Figure 2. Conceptual chart of associations between manageable risk factors and
887 coagulase-negative staphylococci (CNS) IMI incidence, elimination, and prevalence.
888

For Peer Review

ScholarOne support: (434) 964 4100

889 Figure 1.

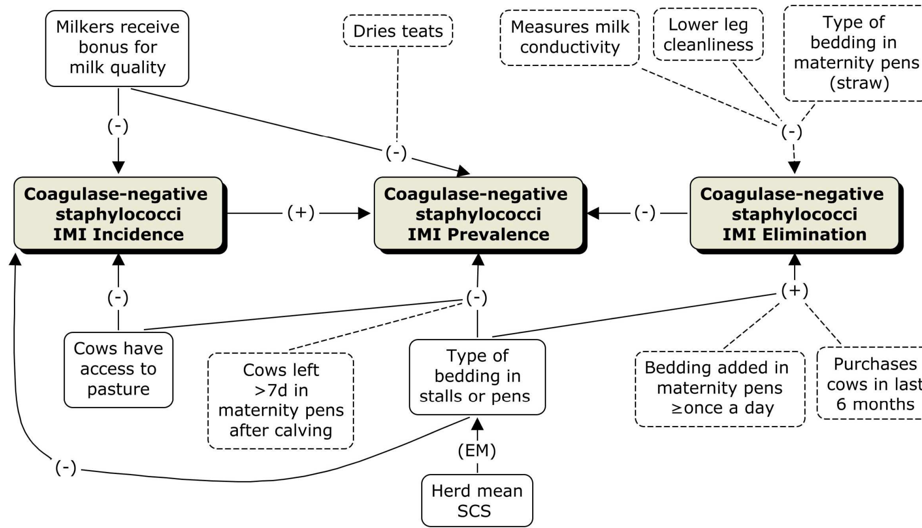


890

891

For Peer Review

892 Figure 2.



893

Peer Review