Mediation Analysis of Milk Losses Associated With Clinical Mastitis

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ABSTRACT: Milk losses associated with mastitis can be attributed to pathogens (direct losses) or to the immune response triggered by infection (indirect losses). The level of infection is often unknown making it difficult to estimate direct losses, whereas indirect losses can be approximated by measuring the association between SCC and milk. An alternative is to perform a mediation analysis. We applied this method on data collected during a survey of clinical mastitis which included milk and SCC, bacteriological cultures, and cow characteristics. Inversely to direct changes in milk, losses mediated by SCC were significantly different from zero for all bacterial species. Therefore, we inferred that products to boost the immune system would not sustain milk production and that, preventive measures, such as genetic selection for cattle resistant to infection, able to clear an infection with a low SCC, or both would be more appropriate.
Keywords: Mastitis; Mediation analysis; Tolerance; Milk loss

Introduction

It is well known that milk losses associated with intramammary infection (IMI) are important. One way to decrease such losses would be to select cows tolerant to the infection, i.e., cows able to withstand the infection without losing a great quantity of milk. Tolerance can be further classified as direct tolerance, i.e., the ability to reduce the damages caused by pathogens, and indirect tolerance, i.e., the ability to reduce the damages caused by the immune response triggered by the infection. The distinction is important, as selective breeding would be appropriate if genetic correlations between direct and indirect mechanisms of tolerance are favorable. If it is not, improving one type of tolerance mechanisms would worsen the other, thereby negating any benefit. Moreover, if the majority of losses were indirect, then the priority would be to select cattle resistant to IMI, or those able to clear an IMI with low SCC or with a short-duration of high SCC. Conversely, if the majority of total losses due to clinical mastitis were not associated with changes in SCC, then the inflammatory response should be boosted, either therapeutically or by selecting cattle with a high SCC in the absence of infection.

Direct and indirect tolerance can be measured by randomly regressing milk yield against an increasing number of pathogens (Kause et al.(2011)) and an indicator of the intensity of the immune response to these pathogens, respectively (Detilleux (2013)). In bovine mastitis, the information on the number of disease-causing pathogens is usually missing. As an alternative, we performed a mediation analysis (VanderWeede (2012)) in an attempt to apportion decreases in milk production (associated with clinical mastitis) into its direct and indirect components.

Data

The dataset consists of records collected during a survey of clinical mastitis (Barkema et al. (1998)). Information for the present study includes identification of cows, test-day recordings of milk and SCC, culture clinical mastitis cases, stage of lactation (days in milk), parity, and dates of calving, of milk recording and of mastitis diagnosis. Bacteriological findings included *Escherichia coli* (ECO), *Staphylococcus aureus* (SAU), *Streptococcus uberis* (SUB), coagulase-negative staphylococci (CNS), *Streptococcus dysgalactiae* (DYS), or streptococci other than *Strep. dysgalactiae* and *Strep. uberis* (SOV).

Methods

Test-day milk yields and test-day SCC, before ($T_j^i \leq 0$) and after ($T_j^i > 0$) the date of clinical diagnosis ($T_j^i = 0$), were analyzed with the following segmented structural equation model with random regression coefficients.

If $T_j^i \leq 0$, the model is:

$$C_j^i = (h_0 + h_1 T_j^i + X_j^i p) + (h_0^i + h_1^i T_j^i + e_j^i)$$
$$P_j^i = (g_0 + g_1 T_j^i + g_3 C_j^i + g_4 CT_j^i + X_j^i p) + (g_0^i + g_1^i T_j^i + g_3^i C_j^i + g_4^i CT_j^i + f_j^i),$$

If $T_j^i > 0$, the model is:

$$C_j^i = (h_0 + h_2 T_j^i + X_j^i p) + (h_{0i} + h_2^i T_j^i + k_j^i$$
$$P_j^i = (g_0 + g_2 T_j^i + g_3 C_j^i + g_5 CT_j^i + X_j^i p) + (g_0^i + g_2^i T_j^i + g_3^i C_{ij} + g_5^i CT_j^i + l_j^i).$$

In both equations, i indexed the case, j indexed the time with respect to the date of clinical mastitis, C_j^i = ln(SCC*10³/mL), P_j^i = milk (kg), T_j^i = time (days) relative to clinical mastitis time, p = vector of class variables for herd, season, month in milk , and parity. Random regression coefficients and error terms were assumed to be normally and independently distributed.

Direct (DE) and indirect (IE) effects were estimated according to their counterfactual definitions (Valeri and Vanderweele (2013)): DE = $\hat{g}_1$ and IE = $\hat{g}_3 \hat{h}_1$ if $T_j^i \leq 0$; and DE = $\hat{g}_2$ and $IE_t = \hat{g}_3 \hat{h}_2$ if $T_j^i > 0$. Then, DE measures the daily differences in P_j^i when C_j^i are kept at their values under no clinical mastitis, and IE measures the daily differences in P_j^i when C_j^i are kept at values under clinical mastitis *vs* no clinical mastitis. Total effects (TE) are: TE = DE + IE.

Results and Discussion

Averages of observed and estimated test-day SCC (as ln(SCC*10^3/mL)) and milk (kg) relative to detection of clinical mastitis are given in Figure 1, suggesting a good fit of the model.

Estimated direct, indirect and total daily effects are presented in Table 1. Indirect losses, mediated by SCC increase, were significantly different from zero for all bacterial species, whereas direct changes, not mediated by SCC, were significant only for coagulase-negative staphylococci and before diagnosis. This suggests that products or treatments to boost the migration of neutrophils in the gland would not sustain milk production. In contrast, we predict that preventive measures, such as genetic selection for cattle resistant to IMI, able to clear an IMI with a low SCC, or both, would be more appropriate.

Table 1. Estimates of direct and indirect daily losses in test-day milk yield (kg), before and after detection of clinical mastitis.

Species	Before detection		After detection	
	Direct	Indirect	Direct	Indirect
CNS	-0.072	-0.004	-0.010	0.002
DYS	-0.007	-0.008	-0.015	0.007
ECO	0.013	-0.033	-0.026	0.012
SAU	-0.014	-0.008	0.004	0.002
SOV	-0.043	-0.012	-0.004	0.007
SUB	0.061	-0.025	-0.028	0.012

Bacterial species are *Escherichia coli* (ECO), *Staphylococcus aureus* (SAU), *Streptococcus uberis* (SUB), coagulase-negative staphylococci (CNS), *Streptococcus dysgalactiae* (DYS) and streptococci other than *Strep. dysgalactiae* and *Strep. uberis* (SOV).

Although our model was useful to estimate direct and indirect effects, it was, however, unable to determine whether the indirect effect was strictly due to neutrophil migration and whether the direct effect was only associated with bacterial virulence. Mathematical modeling of the inflammatory process (Detilleux et al. (2012)) and detailed biological evidence could help in validating the results.

The present model also assumes the same level of resistance (same time trend in bacterial growth) for all animals. To deal with this issue, a nonlinear segmented regression is currently under investigation, in which time of clinical diagnosis is not considered as the junction point between pieces of the model but is estimated from the data. The junction point can be considered as a function of the individual level of resistance. Indeed, resistant cows mount a faster inflammatory response than less resistant ones, as was shown for example in *E. coli* mastitis (Burvenich et al. (1998)). A fast inflammatory response is translated in a fast increase in SCC and an early return to normal SCC values. Results from this model are currently under investigation.

Conclusion

Here, we showed how mediation analysis can be used to decompose milk losses due to infection by various mastitis pathogens into direct and indirect components. Indirect losses were significantly different from zero for all bacterial species, before and after clinical diagnosis. Therefore, we inferred that products or treatments to boost the migration of neutrophils in the gland would not sustain milk production. In contrast, we predicted that preventive measures, such as genetic selection for cattle resistant to IMI, the ability to clear an IMI with a low SCC, or both, would be more appropriate. We are currently testing a nonlinear segmented regression to release several assumptions of the model.

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Figure 1. Average of observed (dot) and estimated (line) test-day milk (lozenge) and SCC (square) per week with respect to time of mastitis detection for each bacterial species.