Associations between Dairy Production Indices and Lipoarabinomannan Enzyme-immunoassay Results for Paratuberculosis

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ABSTRACT

Data from an epidemiological study in Ontario, involving 304 dairy herds, were used to identify associations between selected production indices and lipoarabinomannan antigen serological test results for paratuberculosis (LAM-ELISA). Analyses were conducted at both the herd and individual cow levels of organization. After analytically controlling for management and cow factors in the respective regression models, positive serological paratuberculosis status (as defined by the LAM-ELISA test), was associated with higher milk somatic cell counts at both the herd average ($p < 0.01$), and individual cow levels of organization ($p < 0.0001$). In contrast, LAM-ELISA test results were consistently not associated with calving intervals in either the herd average or individual cow level analyses. Associations between LAM-ELISA results and milk production were inconsistent. No associations were found at the herd level of organization, and LAM-ELISA results were not associated with a change in breed class average (BCA) for milk, between the previous and the most recent lactations of individual cattle. However, at the individual cow level, LAM-ELISA results were positively associated with higher milk production as measured by the current BCA ($p < 0.05$), and individual cow average kg of milk produced per year of life since two years of age ($p < 0.0001$).

RÉSUMÉ

Les associations entre les indices de production et le résultat du test sérologique d'enzymo-immunocaptation de l'antigène lipoarabinomannan (LAM-ELISA) pour la paratuberculose furent mesurées à partir d'une étude épidémiologique conduite en Ontario dans 304 troupeaux de bovins laitiers. Les analyses furent effectuées au niveau de l'individu et du troupeau. Un résultat positif pour ce test fut associé à un comptage de cellules somatiques élevé au niveau du troupeau ($p < 0,01$) et au niveau de l'individu ($p < 0,0001$) après que l'on ait ajusté le modèle de régression en tenant compte des facteurs régie et vache. Par contre, les résultats du test ne furent pas associées à l'intervalle vêlage-vêlage, que ce soit au niveau du troupeau ou de l'individu. La relation entre les résultats du test et la production laitière fut variable. Il n'y avait pas d'association au niveau du troupeau. Au niveau individuel, les résultats du test ne furent pas associés à un changement de la moyenne de la classe pour la race (MCR) pour le lait entre la lactation précédente et la lactation la plus récente pour la vache. Par contre, les résultats du test furent associés positivement à une production laitière élevée mesurée par le MCR en cours ($p < 0,05$) et par la moyenne individuelle de production annuelle depuis que la vache a atteint l'âge de deux ans ($p < 0,0001$). (*Traduit par Dr Josée Daigneault*)

INTRODUCTION

Several authors have reported that bovine paratuberculosis is responsible for significant production losses (1–7). Crude estimates of its annual economic impact on regional dairy industries have been reported as $52 million for Wisconsin (8), $5.8 million for Pennsylvania (9), $15.4 million for the New England states (10), and $1.5 billion for the United States (11). However, the accuracy and precision of these estimates is unknown. They are based on extrapolations from available impact studies conducted at the herd and cow levels of organization, involving relatively few cattle or herds.

Merkal *et al* (12) followed animals to slaughter from one dairy herd and observed that subclinical infection with *Mycobacterium paratuberculosis* was significantly associated with the reason for disposal being perceived as mastitis or infertility. Abbas *et al* (13) noted a statistically significant 15% decrease in mature equivalent milk production and a 1.7 month increase in average calving interval, among infected cattle, relative to noninfected cattle, in one herd. Buergelt and Duncan (14) noted a statistically significant 15.9% decrease in mature equivalent milk production among clinically affected relative to noninfected culled cattle from one herd. However, the 7.7% decrease in production they observed among subclinically infected, relative to noninfected culls, was not statistically significant. Whitlock *et al* (9)

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traced cull animals from an abattoir survey back to their farm of origin, and observed a statistically significant 12 lb per day decrease in average daily milk production among infected cattle relative to noninfected culls. However, they observed no significant difference in total last lactation, lifetime, or herd average production. None of the above studies attempted to control potential individual or herd level confounding variables. Spangler *et al* (6) observed a statistically significant decrease in mature equivalent milk production among fecal culture positive relative to fecal culture negative cattle, after controlling for somatic cell count score and herd of origin. They found no significant difference in production among enzyme-linked immunosorbent assay (ELISA) positive relative to ELISA negative cattle, from three endemically infected Ohio dairy herds.

There is a need for additional studies that formally compare production indices of infected and noninfected cattle that are representative of the target population, and that control for potential confounding variables. This paper is one of a series of publications that present results arising from a large epidemiological study of paratuberculosis in dairy cattle in Ontario (15–17). The objective of this paper is to identify associations between selected dairy production indices and paratuberculosis status as defined by the lipoarabinomannan (LAM) antigen ELISA.

MATERIALS AND METHODS

SAMPLING AND DATA COLLECTION

Samples and data were collected in a survey of dairy farms in Ontario, as previously described in detail (15). The survey was conducted in two phases. Herd level sampling for the first phase was cross-sectional. Blood samples were collected from 14,923 dairy cattle over two years of age from 304 randomly selected farms. All samples were tested for paratuberculosis using the lipoarabinomannan antigen ELISA test (LAM-ELISA) at the Agriculture Canada, Animal Diseases Research Institute (ADRI), Nepean, Ontario (16).

Data on farm management were collected by a mail survey in which all 304 producers completed a 31 question, multiple choice questionnaire (15,17). The questionnaire captured information concerning winter and summer farm management practices, including: calving and calf management, contact between heifers and the adult herd, dry cow housing and exercise, milking cow management, water sources, waste management, and general farm information.

Production data were down-loaded directly from the Ontario Dairy Herd Improvement Corporation (ODHIC) mainframe computer. Both herd average and individual cow records were obtained from the January 1987 and January 1988 ODHIC herd reports including data on cow identification, age, breed, days in milk, breed class average production indices (BCAs), kg of milk produced, and calving intervals. Milk somatic cell count data were available for a subset of 150 herds.

Herd level sampling for the second phase of the farm survey was done in a case control manner. Herds were ranked in descending order of risk of paratuberculosis based on phase 1 LAM-ELISA test results (15). The 60 highest and the 60 lowest risk herds willing to continue on the study were visited for sample and data collection. Blood samples from 2,943 selected animals were tested using the LAM-ELISA at the ADRI.

Phase 2 management data were collected using a detailed 28 page interview questionnaire that was administered by a technician during each farm visit (17). The questionnaire recorded winter and summer management practices, including housing, feeding, watering, interaction between age groups and general information. The questionnaire was divided into sections to capture information concerning: (A) calving and dropped calves, (B) newborn calves to weaning, (C) heifer calves, weaning to approximately eight months of age, (D) heifers, eight months of age to entry into the adult herd, (E) milking cows, (F) adult herd nutrition, (G) dry cows, (H) water sources, (I) waste management, (J) general farm enterprise information, and (K) farm paratuberculosis history. Each section of the interview was conducted on location while observing the housing area of concern. (A copy of each questionnaire is available on request from the senior author.)

Phase 2 production information was obtained as described for phase 1.

ANALYSIS

The LAM-ELISA results, ODHIC production records and farm management data were collated in microcomputer data files, and verified both electronically and manually as described previously (15).

Associations between LAM-ELISA results and four production outcomes; namely, BCA for milk, kg of milk produced per cow year, calving interval and mean natural log of somatic cell counts per mL of milk, were investigated.

A total of twelve models were generated, three for each of the above four production outcomes, by using the three data sources; namely, (A) phase 1 herd average data and mail questionnaire, (B) phase 2 herd average data and the detailed phase 2 interview questionnaire, and (C) phase 2 individual cow data. Throughout these analyses, the LAM-ELISA results were left as a continuous optical density (OD) outcome, to conserve information and statistical power.

(A) Phase 1 herd average data and mail questionnaire

Herd average production indices were regressed on herd average LAM-ELISA results (i.e. mean optical density, MOD), and phase 1 mail questionnaire data, using least squares regression (SAS Institute Inc., Cary, North Carolina, 1985).

The phase 1 mail questionnaire consisted of 29 multiple choice questions with categorical outcomes, plus two open questions with continuous outcomes. In order to reduce the number of variables required to code the categorical data, selected responses to the multiple choice questions were collapsed into biologically related responses, or deleted from the data base. Decisions concerning data collapsing were based on the frequency of selection of each option. That is, where

possible and with few exceptions, options selected by less than 5% of the owners were grouped with similar responses. Questionnaire options selected by less than 2% of the owners were either grouped with similar options, or deleted from the data set across all records. As a result, the 101 options available from the multiple choice questions were collapsed in such a way that they could be coded by 40 "dummy" variables. In addition, categorical and continuous variables describing herd size, breed, geographic location, and LAM-ELISA MOD were included in the analyses. A total of 46 variables were made available for entry into each of the four herd level production models.

The LAM-ELISA MOD results were available for entry at all times during the development of each model. Variables were initially offered for entry at the 15% level. Following stepwise examination for confounding and interaction, variables significant at the 15% level were made available for entry into a model at the 5% level. One way interaction terms were created between variables significant at the 5% level and offered for entry. Simple variables and one way interaction terms significant at the 5% level (including their main effects components) were then forced into the model and all other variables were again made available, one at a time at the 5% level. Residuals from the final models were examined graphically for homoscedasticity and normality.

(B) Phase 2 herd average data and interview questionnaire

Many of the data collected in the phase 2 questionnaire were recorded as continuous variables. In order to reduce the number of variables available for phase 2 regression analyses, responses to the phase 2 questionnaire were collapsed into biologically related responses, or deleted from the data, depending on the mean proportion of cattle exposed to each management practice. In addition, categorical and continuous variables describing herd size, breed, geographic location, and phase 1 LAM-ELISA MOD were included in the analyses. Phase 1 MOD results were used in these analyses because they better represented the herd's overall status than the phase 2 sampling which was weighted in favor of higher risk individual animals (15).

In order to further reduce the number of variables, preliminary models were first generated for each age group section of the phase 2 questionnaire, at the 15% level of significance, for each production outcome. Final models were subsequently developed at the 5% level as described for phase 1 by offering variables from all sections of the questionnaire that had been identified at the 15% level during each sectional analysis. Specifically, 33, 24, 18 and 13 variables were made available for the milk BCA, milk production per cow year, calving interval, and somatic cell count models respectively, after the sectional analyses. Investigations for interaction and analysis of residuals were conducted as described for phase 1.

(C) Phase 2 cow level analyses

Preliminary analyses at the cow level of organization were conducted using ordinary least squares regression models (SAS Institute Inc., Cary, North Carolina, 1985). Four separate models were generated with outcomes: (i) current BCA for milk, (ii) average kg of milk produced per year of life since two years of age, (iii) most recent calving interval, and (iv) mean of the $\log_e$ of somatic cell counts of up to the six most recent milk tests. Each outcome was regressed on available individual cow data including age, breed, days in milk at milk test, phase 1 LAM-ELISA optical density, body condition score, cleanliness score, and whether or not the individual animal was raised in the host herd.

Following the development of production models using ordinary least squares regression, the estimates were adjusted for intraherd correlation by forcing the ordinary least squares models into a weighted least squares regression program Super-Carp® (18–21) (Iowa State University, Ames, Iowa, 1980).

Benedictus *et al* (4), reported a significant decrease in mature equivalent milk production between lactations before culling and the lactation at culling, among animals that were histopathologically positive for paratuberculosis at slaughter. To investigate the possibility of a similar trend in the present study, the difference between the current projected BCA (or most recently completed BCA) and the BCA from the preceding completed lactation was regressed on LAM-ELISA OD, age, breed, days in milk, condition, cleanliness and origin as described above.

RESULTS

(A) Phase 1 herd average data and mail questionnaire

The model with herd level somatic cell count as the outcome indicates that MOD was positively associated with higher somatic cell counts after controlling for dry cow treatment, teat dipping, number of years farming experience and calf housing (Table I).

TABLE I. Regression analysis between rolling herd average $\log_e$ somatic cell count[a] and herd average LAM ELISA optical density (MOD), adjusted for farm management practices entering the model

Variable	Coefficient	SEM	p
Intercept	+12.333	0.2731	0.0001
LAM ELISA MOD herd mean optical density	+ 1.223	0.4135	0.0037
Routinely "dry cow" treat > 70% cows	− 0.447	0.1408	0.0018
"Dry cow" treat only specific cows	− 0.331	0.1472	0.0262
No. years farming experience by manager	+ 0.008	0.0033	0.0130
Routinely "teat dip" after milking	− 0.305	0.1279	0.0185
Calves < 4 mo old housed in room separate from adults during summer	+ 0.170	0.0806	0.0370

[a]Six month mean of $\log_e$ of monthly herd average somatic cell count per mL of milk
n = 143 herds, model R^2 = 0.237

This model accounted for 23.7% of the variation in the outcome. After controlling for questionnaire management factors entering each model, no significant associations were identified between MOD and herd average milk production per cow year, BCA for milk, or herd average calving interval. The farm management practices entering the latter three models are described elsewhere (17).

(B) Phase 2 herd average data and interview questionnaire

After controlling for mangement factors entering each model, no significant associations were identified between MOD and herd average milk production per cow year, BCA for milk, herd average calving interval, or average $\log_e$ of monthly herd average somatic cell counts. The four farm management models generated are described in detail elsewhere (17).

(C) Phase 2 cow level analyses

Tables II, III and IV summarize weighted least squares regression results at the individual cow level, for three different production indices, regressed on individual animal LAM-ELISA optical density (OD) and cow level information. The cow level coefficients are adjusted for herd level covariance.

Table II indictes that LAM-ELISA OD was positively associated with milk production per year of life since two years of age, after controlling for age, breed, days in milk (current lactation), body condition and an interaction term between age and days in milk.

Table III indicates that LAM-ELISA OD was positively associated with BCA for milk after controlling for days in milk in the lactation. The LAM-ELISA was not significantly associated with the change in BCA from the previous to the most recent lactation.

TABLE II. Weighted least squares regression analysis between cow average milk production[a] and cow LAM ELISA optical density adjusted for cow characteristics entering the model

Variable	Coefficient	SEM	p
Intercept	+3812.88	221.41	< 0.0001
Age (yr)	+ 290.02	24.25	< 0.0001
Non-Holstein breed	−1466.15	265.11	< 0.0001
Days in milk (1988)	+ 6.17	0.66	< 0.0001
LAM ELISA optical density	+ 667.21	124.77	< 0.0001
Body condition score	− 175.87	49.78	0.0004
Interaction term			
Age x days in milk	− 0.67	0.11	< 0.0001

[a]Milk production kg/cow/yr of life since 2 yr of age
n = 2014 cows, model R^2 = 0.121

TABLE III. Weighted least squares regression analysis between cow BCA for milk[a] and LAM ELISA optical density adjusted for cow characteristics entering the model

Variable	Coefficient	SEM	p
Intercept	+137.665	2.2028	< 0.0001
Days in milk (BCA)	+ 0.029	0.0052	< 0.0001
LAM ELISA optical density	+ 5.155	2.2136	0.0200

[a]Breed class average index (BCA) for milk
n = 1974 cows, model R^2 = 0.0143

TABLE IV. Weighted least squares regression analysis between cow average $\log_e$ somatic cell count[a] and LAM ELISA optical density adjusted for cow characteristics entering the model

Variable	Coefficient	SEM	p
Intercept	+10.391	0.1152	< 0.0001
Age (yr)	+ 0.130	0.0115	< 0.0001
LAM ELISA optical density	+ 0.663	0.1165	< 0.0001
Days in milk	+ 0.001	0.0003	< 0.0001

[a]Per cow mean of $\log_e$ of monthly somatic cell count per mL of milk
n = 971 cows, model R^2 = 0.182

Table IV indicates that LAM-ELISA OD was positively associated with a cow's mean natural log of monthly somatic cell counts of up to six of the most recent milk tests, after controlling for age and days in milk. The association between LAM-ELISA results and somatic cell counts was consistent wih that described in Table I from phase 1 herd level analysis.

After controlling for age and days in milk, LAM-ELISA OD was not significantly associated with calving interval. The details of the calving interval model are presented elsewhere (17).

DISCUSSION

Although it is generally accepted that paratuberculosis reduces the production efficiency of dairy cattle (1-7,12-14), relatively few analytic studies have been reported investigating the impact of subclinical infection. Furthermore, most previously reported studies have involved relatively few cattle from a few known paratuberculosis problem herds and did not attempt to control potentially confounding management practices beyond within-herd analyses (4,9,12-14).

The primary advantages of the present study are its inclusion of a large number of test positive and negative animals from three hundred herds believed to be representative of the target population (15), its attempt to analytically control potentially confounding herd management practices, and its analyses at both the herd and individual cow levels of organization.

Ordinary least squares regression at the cow level of organization assumes cattle to be independent. However, two stage sampling was used to select individual cows for testing during the second phase of the study. Analyzing the data as if cows were independent may lead to an underestimation of variance among clustered data and erroneous declarations of significance (18). In this situation, nested least squares regression, weighted in proportion to cluster (herd) correlation, may be used to adjust the overall estimate of variance (18-20). Therefore, following the development of production models using ordinary least squares regression, the estimates were

adjusted for intraherd correlation by forcing the ordinary least squares models into a weighted least squares regression program Super-Carp® (21) (Iowa State University, Ames, Iowa, 1980).

The generally poor R^2 values achieved in the regression analyses suggest that only a small proportion of outcome variability has been accounted for by the models and that important information is missing. Also, it is important to note that associations identified between production and LAM-ELISA results do not prove a causal impact of paratuberculosis on production. They are measures of association only. Strict causal relationships cannot be verified by the present study's cross-sectional and case control sampling methods because the temporal ordering of events cannot be confirmed within this type of study.

However, in a comparison with previous studies, the findings of the present study agree with those of Merkal (12) concerning mastitis, but not with those concerning infertility and calving intervals. Regarding production, reports by Buergelt and Duncan (14), Abbas *et al* (13), and Whitlock *et al* (9), all suggested that cull animals, in more advanced stages of paratuberculosis, experienced decreased milk production relative to other animals.

Benedictus *et al* (4) followed animals to slaughter that had been culled for paratuberculosis. They noted that the earlier production capacity of these animals was greater than herd average. They also noted significantly lower production during the lactation at culling, relative to the previous lactation, among histopathologically positive cattle. The change in production within these animals was not compared to the change in production within culled animals that were negative for paratuberculosis nor to negative or subclinically infected animals remaining in the herd.

In the present study, after controlling for management and cow characteristic factors entering the respective models, risk of paratuberculosis as defined by the LAM-ELISA, was associated with higher milk somatic cell counts both at the herd average and individual cow levels of organization. In contrast, LAM-ELISA results were consistently not associated with calving intervals in any of the herd average or individual cow level analyses in data from either phase 1 or phase 2. Association between LAM-ELISA results and production were inconsistent. No associations were found at the herd level in either phase 1 or 2. However, LAM-ELISA results were positively associated with production (both BCA and kg milk/cow year) at the individual level, but were not associated with a change in BCA between the most recent lactation and the one previous to it. These results suggest that the negative impact of subclinical paratuberculosis may not be as great as was feared, based on previous studies involving cull animals. The positive association between cow level subclinical status and cow level milk production that was identified in the present study and by Benedictus *et al* (4), may suggest that cattle with higher production potential may be at greater risk of later being culled for paratuberculosis. The absence of an association betweeen herd level LAM-ELISA results and herd level production, may indicate that this relatively subtle but positive cow level association was lost in pooled herd average analysis.

Previous studies have tended to investigate production impact among more advanced cases of paratuberculosis as defined by culture or histopathology at culling, whereas, the present study used the LAM-ELISA serological test (16) to predict subclinical infection status among randomly selected animals, to investigate the impact of subclinical paratuberculosis within the general population. These differences in design may account for the differences in results between studies.

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