PEER REVIEWED

Seroprevalence and Productivity Effects of Infection with Bovine Leukemia Virus, *Mycobacterium avium* Subspecies *paratuberculosis,* and *Neospora caninum* in Maritime Canadian Dairy Cattle

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Abstract

To determine the effect of subclinical infection with bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC) on milk production in dairy cattle in three Maritime Canadian provinces, 90 dairy herds (all on monthly milk recording) were randomly recruited. Within each herd, a serum sample was obtained from approximately 30 randomly selected lactating animals. Samples were tested for antibodies against the agents using commercially available enzyme linked immunosorbent assays (ELISA). Associations were tested between 305-day milk production and test results for each pathogen using linear regression and controlling for sampling weights, within-herd clustering, provincial stratification, lactation number and linear score somatic cell count. Overall, 20.8, 2.6 and 20.3% of cattle were test-positive for exposure to BLV, MAP and NC, respectively. MAP test positive cows had significantly lower 305-day milk production than MAP test negative cows, when data were pooled for all lactations, and specifically for lactations one (1260 lb; 573 kg) and five (3020 lb; 1373 kg). There was a negative trend toward lower 305-day milk production for MAP test positive cows in lactation two and four compared with MAP test negative cows. In lactation three, MAP test positive cows had significantly higher 305-day milk production (3248 lb; 1476 kg) than MAP test negative cows. No significant differences in production were observed for NC or BLV positive animals. Being test positive for one pathogen was not associated with being test positive for the other pathogens, and no interactive effects on 305-day milk production among pathogen test results were observed.

Résumé

Quatre-vingt dix troupeaux laitiers, tous avec rapports mensuels de production, ont été choisis aléatoirement dans le but de déterminer l'effet de l'infection sous-clinique causée par trois agents pathogènes, le virus de la leucémie bovine (BLV), *Mycobacterium avium paratubercolosis* (MAP) et *Neospora caninum* (NC), sur la production de lait chez des vaches laitières de trois provinces maritimes canadiennes. Dans chaque troupeau, un échantillon de sérum a été prélevé chez environ 30 vaches en lactation choisies aléatoirement. Les échantillons ont été soumis à des tests ELISA d'anticorps disponibles commercialement contre les trois agents pathogènes. L'association entre la production de lait projetée à 305 jours et les résultats des tests a été analysée pour chaque agent pathogène avec la régression linéaire en prenant en ligne de compte la taille de l'échantillonnage, le troupeau, la province d'origine, le numéro de lactation et le compte linéaire de cellules somatiques. Globalement, 20.8% des vaches ont testées positif pour le BLV, 2.6% pour MAP et 20.3% pour NC. Sur l'ensemble des lactations, la production de lait projetée à 305 jours était plus faible chez les vaches qui testèrent positif à MAP que chez les vaches qui testèrent négatif avec une différence plus marquée à la première (1260 lbs; 573 kg) et à la cinquième lactation (3020 lbs; 1476 kg). Pour la seconde et la quatrième lactation, la production de lait projetée à 305 jours chez les vaches qui testèrent positif pour MAP était marginalement plus faible que chez celles qui testèrent négatif. A la troisième lactation, les vaches avec un test positif pour MAP avait une production de lait projetée à 305 jours plus élevée (3248 lbs; 1476 kg) que les vaches

avec un test négatif. Il n'y avait pas de différence significative au niveau de la production chez les animaux qui testèrent positif pour NC et le BLV. Le fait de tester positif pour un agent pathogène n'était pas associé à un test positif pour un autre agent pathogène et il n'y avait pas d'interaction sur la production de lait projetée à 305 jours entre les résultats des tests pour les différents agents pathogènes.

Introduction

Infection with bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC), the causative agents of Enzootic Bovine Leukosis, Johne's Disease (JD), and Neosporosis, respectively, are believed to have significant health and economic impacts on the cattle industry. These effects may include lost international market opportunities, lower domestic productivity and production efficiency, and the potential for reduced consumer confidence in dairy products.[6-8,11,20] The World Trade Organization's new trading rules state that health certification standards for imported cattle, semen and embryos cannot exceed those required under domestic regulatory programs.[24] As a result, there is renewed interest in many countries to determine the prevalence, regional distribution and productivity effects of these diseases (both clinical and subclinical) on their cattle industries, and to determine how these effects vary with lactation number and other factors affecting milk production.

There have been conflicting reports regarding how these agents affect milk production. For BLV, 102 seropositive and seronegative cattle pairs were matched by age and herd in one study. Seropositive cows produced 3.5% (2471 lb [1123 kg]/cow, $p < 0.05$) less milk than seronegative cows.[3] However other studies[9,13] have indicated little or no relationship between seropositive cows and milk production. Whether this effect varies with lactation number or co-infection with other pathogens in a population of randomly sampled herds is unclear.

For MAP, numerous studies have examined the impact of being fecal culture-positive on milk production. In three northern California dairy herds, clinically normal cattle that were MAP fecal culture positive produced 1838 lb (835 kg; 15%) less milk on a 305-day mature equivalent basis compared to fecal culture-negative herdmates.[1] Wilson *et al*[23] found that culture-positive cows in the third or greater lactation produced 1300 and 2800 lb (590 kg and 1273 kg) less milk than did age-matched culture-negative herdmates in third and fourth lactations, respectively. Less research has focused on the relationship between seropositive cows and milk production. In two previous studies, there was no significant impact on milk production among MAP test positive cows,[14,18] whereas a third study found a 829 lb (377 kg)/lactation[15] decrease in milk production in seropositive cows compared to seronegative cows. In two herds, Spangler *et al*[18] found no significant effect on milk production in MAP seropositive cows, but found an 18.8% reduction in milk production in 84 MAP culture-positive cows compared to culture-negative herdmates. The impact on milk production appears to be higher for MAP culture-positive cows than MAP seropositive cows because fecal culturing is not expected to yield false positive cows due to specificity of the test being 100%.[22]

For NC, milk production effects ranged from no effect on the first three test days,[5] to 2.8 to 3.6 lb (1.27 to 1.64 kg) less milk per cow per day by seropositive cows compared to seronegative cows.[7,20] Whether the effects of NC or MAP infection on milk production also vary with lactation number or co-infection with other pathogens in a population of randomly sampled herds is unclear.

The objective of this project was to determine the effect on milk production of being seropositive for bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC) in 90 randomly selected herds in Maritime Canada, and whether there were any synergistic effects on milk production among animals that were seropositive for more than one agent. A secondary objective was to examine these effects by lactation group. With increasing use of quick serological tests for infectious diseases in the dairy industry, it is important to determine if cows seropositive for various diseases produce less milk (and therefore less income) compared to seronegative cows, and whether milk production effects are related to parity, providing guidance regarding culling decisions.

Materials and Methods

Serum Sample Collection

A stratified two-stage random sampling procedure was employed for the survey. Participating herds were randomly selected (using computer generated random numbers) until 90 herds were recruited, 30 from each Maritime province: Prince Edward Island (PEI), New Brunswick (NB) and Nova Scotia (NS). Only herds that were enrolled in a monthly, individual cow milk testing program through the Atlantic Dairy Livestock Improvement Corporation (ADLIC) were eligible for participation. Response rate among randomly selected eligible participants was greater than 90% for all provinces, producing an externally valid, unbiased sample population of herds (sampled herds had similar demographics compared to regional industry averages with respect to herd size, milk production and days in milk). The sample size formula to determine the number of required herds assumed 250 herds per province on ADLIC, a seroprevalence of 10%, an allowable error of 10%, and a

confidence level of 95%. The lowest expected seroprevalence of any of the investigated diseases was 10%, therefore this seroprevalence was used to calculate sample size.

A serum sample was obtained from 30 randomly selected (using computer-generated random numbers) lactating animals in each herd. At least 30 cattle were needed to be tested in each herd to detect at least one infected animal in a herd, based on a within-herd prevalence estimate of 10%, confidence of 90%, average herd size of 45, and sensitivity of the enzyme linked immunosorbent assay (ELISA) test for MAP of 43.0%.[17]

Laboratory Analysis and Milk Production Data Collection

Serum samples were stored at -4°F (-20°C) until all samples were collected. Approximately 30 cow samples per herd were subsequently assessed for antibody against: BLV using an ELISA[a] (sensitivity 98.5%, specificity 99.9%);[10] MAP using an ELISA[b] (sensitivity 43.0%, specificity 99.0%),[17] tested in duplicate; and NC using an ELISA[c] (sensitivity 99.0%, specificity 98.4% based on manufacturer's estimates).[2] An animal was considered to be infected with BLV, MAP or NC if the serum-to-positive ratio on the ELISA was ≥0.50, ≥0.25, and ≥0.60, respectively, as recommended by the manufacturers of the various test kits. The BLV ELISA test requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥1.8.

For each tested animal, the actual 305-day milk production data for the lactation during which each animal was blood tested were gathered electronically from a central milk recording database.

Statistical Analysis

Laboratory and production data were merged for statistical analyses using a commercial statistical software package.[d] The svyreg command in STATA was used for each pathogen to determine linear regression associations ($p \leq 0.05$) between 305-day milk production and test results for each pathogen, controlling for sampling weights (the inverse of the probability of the cow and herd being sampled), within-herd clustering and provincial stratification. Each regression analysis also included test status for the other two pathogens and their interaction terms to determine synergistic effects on milk production due to co-infection with more than one pathogen, and lactation number and linear score somatic cell count to control for their effects.

Results

Overall, 20.8, 2.6 and 20.3% of cattle were test-positive for exposure to BLV, MAP and NC, respectively. Details of these seroprevalence levels have been reported elsewhere.[12,21] In total, 2454 cows had 305-day production records for the lactation during which they were blood tested. Due to technical difficulties with reading certain serum samples, only 2445, 2395 and 2425 of these cows had laboratory test results in the final database for BLV, MAP and NC, respectively. Average milking herd size among the sampled herds was 42 milking cows per herd (range of 17-145), with average herd 305-day milk production being 17,679 lb (8036 kg)/cow (range of 11,336 - 24,939 lb [5173 - 11,336 kg]/cow), with more than 90% of the herds being Holstein.

Table 1 shows the 305-day production for cows seropositive and seronegative for BLV in various lactation categories. After adjusting for clustering within herd, and weighting observations according to sampling probability, BLV seropositive cows did not have significantly lower 305-day milk production compared to BLV seronegative cows in any of the lactations, or when all groups were pooled for analysis.

The 305-day production for cows seropositive and seronegative for MAP in various lactation categories are shown in Table 2. Significantly lower 305-day milk production was found for MAP seropositive cows compared to seronegative cows when all lactation groups were pooled ($p<0.001$), and for lactations one and five ($p<0.05$), after controlling for the effect of significant covariates. There was a trend toward decreased 305-day milk production in MAP seropositive cows in lactation two and four. MAP test-positive cows produced 1260, 497, 1099 and 3020 lb (573, 226, 500 and 1373 kg)/cow/305-day lactation less milk, on average, than MAP test-negative cows for lactations one, two, four and five, respectively.

Table 1. Mean 305-day milk production for cows seropositive and seronegative for bovine leukosis virus (BLV), by lactation group.

Lactation No.	Adjusted 305-day milk production (lb) BLV positive	N	BLV negative	N
1	15892.7 (SE 451.6)[a]	115	15323.5 (SE 382.0)[a]	637
2	17809.6 (SE 406.8)[a]	136	17850.9 (SE 484.9)[a]	434
≥3	19263.0 (SE 502.9)[a]	278	18695.3 (SE 438.1)[a]	845

[a,b]values with different superscript within lactation comparisons are significantly different ($p<0.05$)

In lactation three, MAP seropositive cows produced 3248 lb (1476 kg) more milk per cow per 305-day lactation than MAP seronegative cows ($p<0.05$).

Table 3 shows the 305-day production for cows seropositive and seronegative for NC in various lactation categories. There was no significant effect of being test positive for NC on 305-day milk production for any of the lactations, despite the slightly numerically higher milk production for NC seropositive cows.

Being seropositive for one pathogen was not associated with being seropositive for the other pathogens, and there were no interactive effects observed on 305-day milk production among pathogen test results.

Discussion

Milk production is the net effect of a complex interaction of a large number of variables, some operating at the cow level and others at the herd level. Our analyses for all three pathogens controlled for sampling weights, within-herd clustering, provincial stratification, lactation number and linear score somatic cell, which likely removed some of the cow and herd level confounding effects on milk production. However, some residual confounding may remain due to unmeasured confounding variables. This residual confounding (and for MAP, in combination with the imprecise test for MAP) may also explain the unexpected higher milk production by seropositive cows in lactation three.

With respect to BLV, there is little or no relationship between laboratory test results and 305-day milk production, which supports earlier findings.[9,13] The current Maritime BLV apparent prevalence levels are similar to those found in other parts of North America.[10] However, the level is higher than a similar survey of PEI dairy farms in 1987,[16] and much higher than some European Community member countries with mandatory BLV eradication programs, where prevalence has been reduced to less than 2%.[10] The trend toward higher production in the seropositive animals is difficult to explain, and because it is not statistically significant, may simply be due to chance. It is possible that seropositive animals are not culled if they have substantially higher production than their herdmates. The lack of association would support the conclusion of Johnson and Kaneene that most economic losses associated with subclinical BLV infection are attributable to importation restrictions of infected cattle.[11]

The weighted apparent prevalence of MAP in this study was relatively low (2.6%). Using the reported sensitivity and specificity data of the ELISA test,[17] the estimated true prevalence can be calculated to be approximately 3.8%. The relatively small number of seropositive cows in this study (n=56) may have limited the power of the study to discern statistically significant differences in production, particularly when individual lactation groupings were analyzed.

Our MAP findings conflict with the results reported by Wilson *et al.*[23] They found that fecal culture-positive

Table 2. Mean 305-day milk production for cows seropositive and seronegative for *Mycobacterium avium* subspecies *paratuberculosis* (MAP), by lactation group.

Lactation No.	Adjusted 305-day milk production (lb) MAP positive	N	MAP negative	N
1	14136.1 (SE 811.15)[a]	8	15396.0(SE 374.17)[b]	740
2	17352.5 (SE 847.54)[a]	15	17849.8 (SE 469.94)[a]	553
3	21902.3 (SE 1158.35)[a]	12	18654.2 (SE 548.51)[b]	415
4	18170.9 (SE 208.76)[a]	12	19269.9 (SE 536.84)[a]	303
5	16054.2 (SE 1419.77)[a]	5	19074.7 (SE 699.81)[b]	173
≥6	19385.3 (SE 1042.96)[a]	4	18374.0(SE 420.85)[a]	155

[a,b]values with different superscript within lactation comparisons are significantly different ($p<0.05$)

Table 3. Mean 305-day milk production for cows seropositive and seronegative for *Neospora caninum* (NC), by lactation group.

Lactation No.	Adjusted 305-day milk production (lb) NC positive	N	NC negative	N
1	15570.1 (SE 411.29)[a]	152	15384.1 (SE 375.42)[a]	593
2	18476.0 (SE 419.62)[a]	107	17842.9 (SE 469.21)[a]	455
≥3	19628.0 (SE 547.37)[a]	203	18786.0 (SE 424.65)[a]	915

[a,b]values with different superscript within lactation comparisons are significantly different ($p<0.05$)

cows in third and fourth lactations produced 1300 and 2800 lb (591 and 1273 kg) less milk, respectfully, than did culture-negative herdmates of similar parity. This difference may be due to 1) misclassification of uninfected cows into our seropositive group, and 2) fecal culture-positive cows being in a later stage of disease than seropositive infected cows, resulting in greater effects on milk production. With a test specificity of 99%, when used on 2454 animals with a estimated true prevalence of 3.8%, approximately 24 of the 56 (43%) test positives were likely false positives, and 42 of the 2398 (1.8%) test negatives were likely false negatives. Based on the pathophysiology of JD, sensitivity of the ELISA test to detect subclinically infected cattle is poor, ranging from 15% in young "lightly" infected cattle to 87% in old "heavily" infected cattle. Therefore, misclassification bias is likely to occur more frequently in young cattle than old cattle. This misclassification, particularly with the small number of positive cows in our study, may also explain why MAP seropositive first-lactation cows had significantly less 305-day milk production compared with seronegative first-lactation cows, and why this negative impact on milk production was numerically (not statistically) larger in first-lactation cows than in second-lactation cows.

Fecal culture for MAP could have been used to confirm infection in seropositive cows in our study. However, due to insufficient funds and storage facilities at the time of sampling for the large number of sampled cows, fecal samples were not obtained for culture. Also, the primary goal of the research was to determine the seroprevalence of the pathogens, and the impact of seroprevalence on milk production. The main advantage to including fecal culture confirmation in our study would have been the determination of a population of truly infected cows. However, Stabel *et al*[19] found that only 11 of 49 (22%) ELISA-positive cows were also fecal culture-positive. Therefore, using fecal culture confirmation would likely have reduced our population of test positive cows from 56 to 13, reducing the already limited power to detect a statistically significant difference between test positive and negative cows, particularly when cows were stratified by parity. Furthermore, the non-shedding, truly infected, seropositive cows (some portion of the 43 fecal culture-negative cows) would be misclassified as "non-infected". The gains achieved by eliminating false positives from the test positive group could be outweighed by the addition of false negatives to the test negative group. Also, the results would be interpreted as the impact on milk production of being seropositive and culture-positive, not just being seropositive. Therefore, for cost, objective and interpretation reasons, fecal culture confirmation of ELISA-positive cows was not undertaken.

We did not find an association between NC test results and 305-day milk production, which corresponds with a study of 25 non-randomly selected Ontario dairy herds which showed no significant impact of being NC-positive on milk production for the first three test-days of a group of cow's lactation.[5] In a second Ontario study, milk production was reduced in herds with high abortion rates, but unaffected in herds with normal abortion rate histories.[4] However, in a study of a single large herd in California, NC seropositive primiparous animals were found to produce 3.6 lb (1.6 kg) less fat-corrected milk than did their seronegative primiparous herd mates.[20] The herds in our study represent the regional industry and were a cross-section of herd size and management styles. Our NC findings would have only minimal misclassification bias because of the good test sensitivity (99.0%) and specificity (98.4%) (although these values are based on the manufacturer's estimates which may be somewhat biased), good sample size and 20.3% NC seroprevalence. NC association with milk production would require further evaluation of differences in specific management and culling practices between positive and negative cows.

Seropositivity, while frequently utilized and commonly equated with infection, is only a superficial indicator of infection, and in particular, stage of infection (particularly so for MAP with its low test sensitivity). Stage of infection, particularly for MAP, is an important determinant of whether physiological and pathological effects on milk production are developing from infection with a pathogen. Without any underlying disease induction, there will not be an effect on production. Therefore, seroprevalence studies are challenged with the prospect of detecting milk productivity effects of subclinical infection when some subclinically infected animals have not advanced to a stage of infection when physiological and pathological effects are detectable. Only profound negative effects on productivity will be detected. Further studies are needed that investigate specific hypotheses about herd-level and cow-level conditions and stage of infection under which subclinical infections reduce production.

Conclusions

It appears that there could be substantial milk production losses in MAP seropositive cows, even during their first lactation. This finding requires further investigation with a larger sample size. Bovine practitioners may want to consider the potential for milk production losses of MAP seropositive cows in their culling decisions, particularly on farms with a confirmed history of JD infection or farms with a seroprevalence of 10% or more, where test-positive cows are likely to be truly infected cows. No significant differences were determined for NC or BLV when data were stratified by lactation, and when all lactations were combined. Therefore, NC or BLV test-positive cows should not be culled

for milk production reasons. Being test-positive for one pathogen was not associated with being test-positive for other pathogens, and therefore being infected with one pathogen does not appear to predispose cows to infection with the other pathogens. There were no interactive effects on 305-day milk production among pathogen test results. Therefore, being seropositive for JD and either of the other two diseases does not amplify the milk production losses seen by JD itself.

Footnotes

[a]IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA
[b]IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA
[c]BIOVET ELISA - BIOVET Inc. - St. Hyacinthe, Quebec, Canada
[d]STATA® - version 7 - Stata Press, College Station, Texas, USA

References

1. Abbas B, Rieman H, Hird D: Diagnosis of Johne's diasease (paratuberculosis) in northern California cattle and a note on its economic significance. *California Vet* 8:20-24, 1983.
2. Bergeron N, Fecteau G, Pare J, Martineau R, Villeneuve A: Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Quebec. *Can Vet J* 41:464-467, 2000.
3. Brenner J, Van Haam M, Savir D, Trainin Z: The implication of BLV infection in the productivity, reproductive capacity and survival rate of a dairy cow. *Vet Immunol Immunopathol* 22:299-305, 1989.
4. Duffield TF, Hobson J, Kelton DF, Peregrine AS, McEwen B, Hietala S, Lissemore KD, Leslie KE, Cramer G: *Neospora caninum* and milk production - a theory based on a comparison of the effect in two populations of Ontario dairy herds. *Proc Am Assoc Bov Pract* 34:178, 2001.
5. Duffield TF, Peregrine AS, McEwen B, Hietala S, Bagg R, Dick P: Seroprevalence of *Neospora caninum* infection in 25 Ontario dairy herds and its association with periparturient health and production. *Bov Pract* 35:8-12, 2001.
6. Fidler HM, Thurrell W, Johnson NM, Rook GA, McFadden JJ: Specific detection of *Mycobacterium paratuberculosis* DNA associated with glaucomatous tissue in Crohn's disease. *Gut* 35:506-510, 1994.
7. Hernandez J, Risco C, Donovan A: Association between exposure to *Neospora caninum* and milk production in dairy cows. *J Am Vet Med Assoc* 219:632-635, 2001.
8. Hutchinson LJ: Economic impact of paratuberculosis. *Vet Clin North Am Food Anim Pract* 12:373-381, 1996.
9. Jacobs RM, Heeney JL, Godkin MA, Leslie KE, Taylor JA, Davies C, Valli VE: Production and related variables in bovine leukemia virus-infected cows. *Vet Res Commun* 15:463-474, 1991.
10. Johnson R, Kaneene JB: Bovine leukemia virus. Part 1. Descriptive epidemiology, clinical manifestations, and diagnostic tests. *Compend Contin Educ Pract Vet* 13:315-325, 1991.
11. Johnson RJ, Kaneene JB: Bovine Leukemia Virus. Part IV. Economic impact and control measures. *Compend Contin Educ Pract Vet* 13:1727-1737, 1991.
12. Keefe GP, VanLeeuwen JA: Neospora then and now: prevalence of *Neospora caninum* in Maritime Canada in 1979, 1989, and 1998. *Can Vet J* 41:864-866, 2000.
13. Langston A, Ferdinand GA, Ruppanner R, Theilen GH, Drlica S, Behymer D: Comparison of production variables of bovine leukemia virus antibody- negative and antibody-positive cows in two California dairy herds. *Am J Vet Res* 39:1093-1098, 1978.
14. McNab WB, Meek AH, Martin SW, Duncan JR: Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. *Can J Vet Res* 55:356-361, 1991.
15. Nordlund KV, Goodger WJ, Pelletier J, Collins MT: Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. *J Am Vet Med Assoc* 208:1872-1876, 1996.
16. Richardson GF, MacAulay EB: The prevalence of bovine leukemia virus infection in dairy cows on Prince Edward Island. *Can Vet J* 33:188-189, 1992.
17. Sockett DC, Conrad TC, Thomas CB, Collins MT: Evaluation of four serological tests for bovine paratuberculosis. *J Clin Microbiol* 30:1134-1139, 1992.
18. Spangler E, Bech-Nielsen S, Heider LE: Diagnostic performance of two serologic tests and fecal culture for subclinical paratuberculosis and associations with production. *Prev Vet Med* 13:185-195, 1992.
19. Stabel JR, Wells SJ, Wagner BA: Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. *J Dairy Sci* 85:525-531, 2002.
20. Thurmond MC, Hietala SK: Effect of *Neospora caninum* infection on milk production in first- lactation dairy cows. *J Am Vet Med Assoc* 210:672-674, 1997.
21. VanLeeuwen JA, Keefe G, Tremblay R, Power C, Wichtel J: Seroprevalence of infection with Johne's Disease, Enzootic Bovine Leukosis, and Bovine Viral Diarrhea in Maritime dairy cattle. *Can Vet J* 42:193-198, 2001.
22. Whitlock RH, Wells SJ, Sweeney RW, Van Tiem J: ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. *Vet Microbiol* 77:387-398, 2000.
23. Wilson DJ, Rossiter C, Han HR, Sears PM: Association of *Mycobacterium paratuberculosis* infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. *Am J Vet Res* 54:1851-1857, 1993.
24. World Trade Organization. Final Act of the 1986-1994 Uruguay Round of trade negotiations. http://www.wto.org/english/docs_e/legal_e/ursum_e.htm. 1994.

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