Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* in dairy cattle in Saskatchewan

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Abstract — Blood was drawn from 1530 dairy cows in 51 herds. For antibodies against bovine leukemia virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum,* 37.4%, 2.7%, and 5.6% of cows were test positive, respectively, while 29.2% of herds had unvaccinated animals with ≥ 1:64 for bovine viral diarrhea virus.

Résumé — Séroprévalence des anticorps contre le virus de la leucémie bovine, le virus de la diarrhée virale bovine, *Mycobacterium avium* sous espèces *paratuberculosis* et *Neospora caninum* chez les bovins laitiers de la Saskatchewan. Du sang a été prélevé chez 1530 vaches laitières de 51 troupeaux. Les tests d'anticorps contre le virus de la leucémie bovine, *Mycobacterium avium* sous espèces paratuberculosis et *Neospora caninum* étaient respectivement positifs chez 37,4 %, 2,7 % et 5,7 % des vaches alors que 29,2 % des troupeaux comprenaient des animaux non vaccinés avec ≥ 1 :64 pour le virus de la diarrhée bovine virale.

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In 1997, individuals and organizations involved in the cattle industry (veterinarians, livestock genetics companies, livestock exporters, and national dairy breed associations) formed the Production Limiting Diseases Committee (PLDC). The committee is interested in maintaining the ability of Canadian cattle producers to sell products domestically and internationally in the future. To achieve this "mission," the PLDC initiated research to estimate the prevalence, risk factors, and economic impact of 4 infectious diseases: neosporosis, caused by *Neospora caninum* (NC); Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP); bovine viral diarrhea (BVD), caused by the bovine viral diarrhea virus (BVDV); and enzootic bovine leukosis (EBL) caused by the bovine leukemia virus (BLV). These 4 infectious diseases have significant health and economic impacts related to lost international market opportunities, lower domestic productivity and production efficiency, the potential for reduced consumer confidence in dairy products, or all 3 (1).

Estimated seroprevalence levels for the agents of these 4 diseases have been determined for Maritime Canada and published previously (2). However, there are considerable regional differences between Saskatchewan and Maritime Canada, which may impact on the levels of infection for these diseases. The purpose of this study was to determine the seroprevalence levels for the agents of these 4 production-limiting diseases in Saskatchewan dairy cattle, thereby expanding knowledge of their prevalence in other parts of Canada. Results of this study will also be combined with seroprevalence, questionnaire, and monthly production data from this and other Canadian provinces in future analyses (work in progress and therefore not presented here) to determine the impacts and risk factors of seropositivity for BLV, BVDV, MAP, and NC in Saskatchewan dairy cattle and nationally.

A stratified 2-stage random sampling procedure (using computer generated random numbers) was employed for this survey. In 2001, dairy herd producers were randomly selected from a census of all dairy farms in Saskatchewan until 51 farmers had agreed to participate in the study. There was sufficient budget to test approximately 50 farms. The response rate among randomly selected eligible participants was 39%. In each participating herd, 30 lactating cows were randomly selected from the entire milking herd for blood sample collection and testing for

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Table 1. Animal and herd level seroprevalence against bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP), and *Neospora caninum* (NC) in 1530 dairy cattle in 51 herds in Saskatchewan

	Animals +[a] p (95% CI)	Herds 1 +[b] p (95% CI)	Herds 2 +[c] p (95% CI)	+ Herds 1 +[d] p (95% CI)	+ in herds 2 +[e] p (95% CI)
BLV	37.4% (28.8% to 46.0%)	89.1% (80.8% to 97.4%)	81.2% (70.7% to 91.7%)	41.8% (32.7% to 50.9%)	45.8% (36.2% to 55.4%)
MAP	2.7% (1.6% to 3.9%)	43.3% (27.4% to 59.3%)	24.3% (9.8% to 38.7%)	6.3% (4.8% to 7.7%)	8.6% (6.7% to 10.6%)
NC	5.6% (4.0% to 7.1%)	71% (57.6% to 84.4%)	44.0% (28.0% to 60.1%)	7.8% (6.02% to 9.6%)	10.5% (8.6% to 12.5%)

p — proportion; CI — confidence interval
[a]Proportion of animals testing positive
[b]Proportion of herds with at least 1 animal testing positive
[c]Proportion of herds with at least 2 animal testing positive
[d]Proportion of animals testing positive in herds with at least 1 animal testing positive
[e]Proportion of animals testing positive in herds with at least 2 animals testing positive

antibodies against BLV, MAP, and NC, regardless of herd size. Herd and animal sample size calculation procedures were similar to those used in the Maritime Canada seroprevalence study (2) to ensure comparability between study results.

Five unvaccinated (for BVDV) cattle more than 6 mo old were also selected for blood sample collection and testing for exposure to BVDV, where possible. In unvaccinated herds, these 5 were part of the 30 cows selected for the other 3 diseases. In vaccinated herds, 5 unvaccinated heifers over 6 mo of age were selected, in addition to the 30 cows. The BVDV sampling technique was based on Houe's study (3), in which it was reported that the herd-level sensitivity was ≥ 95% and the herd-level specificity was ≥ 98% for correctly identifying BVDV-infected and uninfected herds when 5 unvaccinated animals were used.

Serum was harvested from the blood samples and stored at -20°C until all samples had been collected, after which they were submitted for laboratory testing. Testing for antibodies to BLV was done, in duplicate, at the Canadian Food Inspection Agency (CFIA) laboratory in Charlottetown, which was certified as the national laboratory for BLV testing for international trade purposes, by using an ELISA (IDEXX ELISA; Idexx Laboratories, Westbrook, Maine, USA — sensitivity 98.5%, specificity 99.9%) (4). This test required a confirmation of positive tests, by using a sample-to-negative host-cell ratio of ≥ 1.8 (sample optical density divided by negative control optical density — as described on the package insert).

Testing for antibodies to MAP was conducted, in duplicate, at Prairie Diagnostic Services in Regina, certified to have appropriate quality control for MAP ELISA testing by the United States Department of Agriculture when an indirect ELISA (IDEXX ELISA — sensitivity 43.0%, specificity 99.2%) was used (5).

Testing for antibodies to NC was conducted, in duplicate, at a commercial laboratory by using an indirect ELISA (BIOVET ELISA; Biovet Laboratories, 4375 Beaudry Ave, St. Hyacinthe, Quebec — sensitivity 99.0%, specificity 98.4%) (6). An animal was considered to be test positive for BLV, MAP, and NC if the serum-to-positive ratio was ≥ 0.50, ≥ 0.25, and ≥ 0.60, respectively, as recommended by the manufacturers of the various test kits.

Testing for antibodies to BVDV was conducted at the Canadian Food Inspection Agency laboratory in Lethbridge by using virus neutralization to the Type 1 genotype, cytopathic Singer strain (sensitivity 99.6%, specificity 100%) (7).

Seroprevalence estimates and 95% confidence intervals (CIs) were determined for the proportion of cattle and herds that were seropositive for BLV, NC, and MAP by utilizing survey commands in a statistical package (STATA, version 8; Stata Press, College Station, Texas, USA), which adjusted for within herd clustering and sampling weights. Due to the large number of animals tested per herd and the less than perfect specificity for NC and MAP, false positive test results were likely. Therefore, some herds with only 1 seropositive animal may be erroneously considered a positive herd. As a result, herd level seroprevalence was calculated by using 2 definitions of positive herds: 1) a lenient definition — having at least 1 test-positive animal, and 2) a more restrictive definition — having at least 2 test-positive animals. Furthermore, due to substantial inaccuracies of the IDEXX ELISA for identifying MAP-infected animals, the estimated true animal and herd prevalence and 95% CIs, correcting for test sensitivity and specificity, were calculated (8).

Herd level estimates of BVD prevalence were calculated by using 2 definitions for a positive herd: 1) a lenient definition — having at least 1 animal with antibodies against BVDV, and 2) a restrictive definition — having at least 1 animal with a titer of ≥ 1:64 for BVDV. The first definition was based on a minimum titer of 1/2 and was utilized to enable comparisons with the results from Maritime Canada (2). The latter definition was based on Houe's study (3), where this titer was likely to represent recent exposure to a source of BVDV, which could be either an acutely infected or a persistently infected animal. This latter definition is more indicative of active BVDV infection, while the first definition would include both active and historical infection, and therefore, should be interpreted with caution.

Overall, 1530 cows from 51 Saskatchewan dairy herds were included in the final database for BLV, MAP, and

NC. The final database of BVDV test results contained 185 unvaccinated cows and heifers from 36 herds. Of the tested animals, 37.4%, 2.7%, and 5.6% of cattle were positive for BLV, MAP, and NC, respectively. Table 1 shows the proportion (and 95% CI) of seropositive cows, the proportion of herds with at least 1 and 2 seropositive cows, and the average prevalence of infection in herds with at least 1 and 2 seropositive cows.

Overall, 28.1% (15.9% to 40.3%, 95% CI) of the animals were seropositive for BVDV, with only 16.8% (6% to 27.5%, 95% CI) having a titer ≥ 1:64. Infected animals were found in 48.7% (31.4% to 65.9%, 95% CI) of herds, while only 29.2% (13.2% to 45.2%, 95% CI) of herds had animals with a titer ≥ 1:64, indicating more recent or current infection. Therefore, compared with dairy herds in Maritime Canada, fewer cows and dairy herds were seropositive for BVDV, although these differences were not significant (2). Only dairy farms in Prince Edward Island (PEI) had significantly more BVDV infection compared with dairy farms in Saskatchewan, which may have been due to the low proportion of dairy farmers in PEI at the time of testing that utilized BVD vaccinations for protection against transmission of the virus (unpublished data).

The estimated true cow and herd prevalences for MAP, correcting for test sensitivity and specificity, were determined to be 4.5% and 30%, respectively. More dairy herds (24%) in Saskatchewan had at least 2 seropositive cows for MAP, as compared with dairy herds in Maritime Canada (17%), although this difference was not statistically significant at $P < 0.05$ (2). In general, the estimates for MAP seroprevalence for herds in Saskatchewan were very similar to those for herds in the Maritimes in 1998. Perhaps exposure levels to cow manure among calves, an important risk factor for transmission of MAP (9), is similar between the 2 regions, a factor to be examined in a future risk factor study.

Significantly more dairy cows (37%) and herds (89%) in Saskatchewan were seropositive for BLV as compared with dairy cows (21%) and herds (70%) in Maritime Canada (2). These results reflect real differences in the amount of virus transmission occurring among animals and level of exposure to this virus between regions.

Conversely, significantly fewer dairy cows (6%) and herds (44%) in Saskatchewan were seropositive for NC as compared with dairy cows (19%) and herds (79%) in Maritime Canada (10). These differences may be due to different vertical or horizontal transmission rates, related to their respective housing, nutrition, biosecurity, demographics, or frequency of dogs or wild canid populations. Again, future analyses will investigate which of these risk factors is associated with seroprevalence for NC.

The differences in seroprevalence levels for the 4 diseases between Saskatchewan and Maritime Canada were very unlikely to be due to differences in test accuracies, because exactly the same tests at the same laboratories were utilized. However, because the Maritime samples were tested from 1998 to 1999, and the Saskatchewan samples were tested in 2001, temporal differences in test lots or laboratory conditions may have created a systematic bias in the results, leading to some of the differences seen. However, the manufacturers of the tests and the laboratories that used them employ careful quality control efforts, trying to minimize this bias.

Furthermore, a selection bias in the farms surveyed in Saskatchewan may also be responsible for some of the differences seen. The low response rate of 39% may have lead to results for the sample population that would not be representative of the target population, namely all dairy farms in Saskatchewan. The reasons for not participating were varied (uninterested, planning to sell, too busy, no handling facilities for taking blood), making it unclear whether a selection bias would be likely. However, the average herd size and milk production level of the sample population farms were very similar to those of all dairy farms in Saskatchewan, leading one to believe that the bias, if present, was likely small, making comparisons between provinces possible.

In conclusion, compared with cattle in Maritime Canada, higher numbers of cows and herds were seropositive for BLV, but lower numbers of cows and herds were seropositive for NC in Saskatchewan. Only dairy farms in PEI had significantly more BVDV infection compared with farms in Saskatchewan. Overall, 37.4%, 2.7%, and 5.6% of cows were test positive for BLV, MAP, and NC, respectively, while 29.2% of herds had unvaccinated animals with a titer ≥ 1:64 for BVDV.

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References

1. Bradford PS. Large Animal Internal Medicine. 3rd ed. St. Louis, Missouri: Mosby, 2002.
2. VanLeeuwen JA, Keefe GP, Tremblay R, Power C, Wichtel JJ. Seroprevalence of infection with *Mycobacterium avium* subspecies *paratuberculosis,* bovine leukemia virus, and bovine viral diarrhea virus in Maritime Canada dairy cattle. Can Vet J 2001;42:193–198.
3. Houe H. Serological analysis of a small herd sample to predict the presence of animals persistently infected with bovine viral diarrhea virus (BVDV) in dairy herds. Res Vet Sci 1992;53:320–323.
4. Johnson RJ, Kaneene JB. Bovine Leukemia Virus. Part I. Descriptive Epidemiology, Clinical Manifestations, and Diagnostic tests. Compend Contin Educ Pract Vet 1991;13:328.
5. Sockett DC, Conrad TA, Thomas CB, Collins MT. Evaluation of four serological tests for bovine paratuberculosis. J Clin Microbiol 1992;30:1134–1139.
6. 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 2000;41:464–467.
7. Deregt D, Smithson S, Kozub GC. A short incubation serum neutralization test for bovine viral diarrhea virus. Can J Vet Res 1992;56:161–164.
8. Dohoo IR, Martin SW, Stryhn H. Veterinary Epidemiologic Research. Charlottetown, Prince Edward Island: Atlantic Veterinary College. 2003.
9. Muskens J, Elbers AR, van Weering HJ, Noordhuizen JP. Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. J Vet Med B Infect Dis Vet Public Health 2003;50:372–377.
10. Keefe GP, VanLeeuwen JA. Neospora then and now: prevalence of *Neospora caninum* in Maritime Canada in 1979, 1989, and 1998. Can Vet J 2000;41:864–866.