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

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Abstract — Of 1204 dairy cows and 1425 beef cows sampled, 60.8% and 10.3% were seropositive for *Bovine leukemia virus,* 4.5% and 1.7% were seropositive for *Mycobacterium avium* subspecies *paratuberculosis,* and 8.3% and 9.1% were seropositive for *Neospora caninum,* respectively, while 28.1% of dairy herds had unvaccinated animals with titres ≥ 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 de boucherie et laitiers au Manitoba. Des 1204 vaches laitières et 1425 vaches de boucheries échantillonnées, 60,8 % et 10,3 % étaient séropositives pour le virus de la leucémie bovine, 4,5 % et 1,7 % étaient séropositives pour *Mycobacterium avium* sous-espèces paratuberculosis et 8,3 % et 9,1 % étaient séropositives pour *Neospora caninum,* respectivement, alors que 28,1 % des troupeaux laitiers avaient des animaux non vaccinés avec un titre de ≥ 1 :64 pour le virus de la diarrhée virale bovine.

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In 1997, the Production Limiting Diseases Committee initiated research to estimate the prevalence, risk factors, and economic impact of 4 infectious diseases of cattle: neosporosis, caused by *Neospora caninum* (NC); Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP); bovine viral diarrhea (BVD), caused by bovine viral diarrhea virus (BVDV); and enzootic bovine leukosis (EBL) caused by 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 cattle products, or all 3 of these (1).

Estimated seroprevalence levels for the agents of these 4 diseases have been published for dairy cattle in maritime Canada (1) and Saskatchewan (2). This paper reports the seroprevalence levels for exposure to the agents of these 4 production-limiting diseases in randomly selected dairy and beef cattle in Manitoba. These 2 related but separate surveys provide an excellent opportunity to identify differences in seroprevalences in dairy and beef cattle, using similar tests and sampling protocols within the same area in Canada during a similar time frame.

A stratified 2-stage random sampling procedure was employed for these surveys. In 1999, a total of 360 cow-calf producers were randomly selected from a list of 10 250 herds kept by the Manitoba Cattle Producers Association in order to produce 49 cow-calf producers who agreed (14% response rate) to supply blood samples for testing. In 2002, a total of 75 dairy producers were randomly selected from a list of 290 herds enrolled with the Western Canada Dairy Herd Improvement Services in order to produce 40 dairy producers who agreed (53% response rate) to supply blood samples for testing. There were 527 dairy farms in the province at the time of the herd recruitment.

In each participating beef and dairy herd, up to 30 cattle (if available) were randomly selected by researchers for blood collection and testing for antibodies against BLV, MAP, and NC. Five unvaccinated (for BVDV) cattle more than 6 mo old were also selected for blood collection and testing for exposure to BVDV, where possible. In herds that were not vaccinated for BVDV, these 5 animals were selected from the 30 cows selected for the other 3 diseases; in BVDV-vaccinated herds, 5 unvaccinated heifers over 6 mo of age were selected, in addition to the 30 cows. For the beef samples, local veterinarians were asked to draw the blood samples, whereas blood sampling of dairy cattle was arranged through provincial veterinary staff.

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Table 1. Animal and herd level seroprevalences for bovine leukemia virus (BLV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC) in 1425 beef and 1204 dairy cattle in 49 beef and 40 dairy herds in Manitoba

		BLV	MAP	NC
Animals test +ve %	Dairy	60.8%	4.5%	8.3%
(95% confidence interval)		(51.8%–69.9%)	(2.8%–6.2%)	(5.1%–11.6%)
	Beef	10.3%	1.7%	9.1%
		(2.5%–18.0%)	(0.02%–3.5%)	(6.1%–12.1%)
Herds with ≥ 1 test +ve %	Dairy	97.4%	68.4%	59.8%
(95% confidence interval)		(93.5%–100%)	(52.5%–84.2%)	(41.8%–77.9%)
	Beef	47.9%	29.2%	85.7%
		(28.0%–68.0%)	(10.5%–47.9%)	(73.5%–97.8%)
Herds with ≥ 2 test +ve %	Dairy	97.4%	43.1%	37.0%
(95% confidence interval)		(93.5%–100%)	(24.9%–61.4%)	(19.9%–54.3%)
	Beef	32.6%	11.0%	65.2%
		(13.8%–51.3%)	(0.0%–27.9%)	(48.2%–82.2%)
Animals test +ve in herds	Dairy	62.5%	6.6%	13.4%
with ≥ 1 test +ve %		(53.5%–71.4%)	(4.6%–8.6%)	(9.3%–17.4%)
(95% confidence interval)	Beef	21.7%	6.0%	10.6%
		(8.6%–34.7%)	(2.3%–9.6%)	(7.4%–13.7%)
Animals test +ve in herds	Dairy	62.5%	8.7%	17.4%
with ≥ 2 test +ve %		(53.5%–71.4%)	(6.3%–11.1%)	(11.8%–23.0%)
(95% confidence interval)	Beef	29.0%	9.9%	12.9%
		(12.4%–45.7%)	(0.0%–26.3%)	(9.1%–16.6%)

Herd and cow sample size calculation procedures were similar to those used in maritime Canada (1) and Saskatchewan (2) to ensure comparability between study results. The allocated funds from the beef industry limited the number of herds recruited for the cow-calf survey to 49 herds, making them less representative of the large beef industry in Manitoba than the 40 herds sampled for the 527 dairy farms in Manitoba.

Sera from enrolled beef and dairy cattle were harvested from the blood samples and stored at -20°C until all samples had been collected in 1999 and 2002, respectively, after which, they were submitted for laboratory testing. All BLV testing was done, in duplicate, at the Canadian Food Inspection Agency laboratory in Charlottetown, using an enzyme linked immunosorbant assay (ELISA) (IDEXX ELISA; IDEXX Laboratories, Westbrook, Maine, USA), sensitivity (Se) of 98.5% and specificity (Sp) of 99.9% (3). An animal was considered to test positive for BLV, if the serum-to-positive (S/P) ratio was ≥ 0.50, as recommended by the manufacturer.

All MAP testing was done, in duplicate, using an indirect ELISA (IDEXX ELISA; IDEXX Laboratories), Se of 43.0% and Sp of 99.2% compared with fecal culture results (4) but Se of 7% and Sp of 98% compared with tissue culture results (5). Testing was conducted at the Provincial Veterinary Services Branch laboratory in Winnipeg for the sampled beef cattle, while dairy cattle samples were tested at Prairie Diagnostic Services in Regina, in compliance with a national dairy seroprevalence survey to ensure comparability of dairy results between provinces. At both laboratories, an animal was considered to be test positive for MAP, if the S/P ratio was ≥ 0.25, as recommended by the manufacturer.

For the sampled beef cattle, NC testing was conducted, in duplicate, at the Provincial Veterinary Services Branch laboratory in Winnipeg, using an ELISA (IDEXX ELISA; IDEXX Laboratories), Se of 98% and Sp of 92% (6). For the sampled dairy cattle, NC testing was conducted, in duplicate, at the BIOVET Inc. laboratory in Quebec City, using another ELISA (BIOVET ELISA), Se of 99.0% and Sp of 98.4% (7), again in compliance with a national dairy seroprevalence survey to ensure comparability of dairy results between provinces. An animal was considered to be test positive for NC, if the S/P ratio was ≥ 0.50 (beef cattle) and ≥ 0.60 (dairy cattle), as recommended by the manufacturers of the respective test kits.

All BVDV testing was conducted at the Animal Diseases Research Institute laboratory in Lethbridge, using virus neutralization to the Type 1 genotype, cytopathic Singer strain, Se of 99.6% and Sp of 100% (8).

Seroprevalence estimates and confidence intervals were determined for the proportion of cattle and herds that were seropositive by utilizing survey commands in a statistical package (STATA, version 8; Stata Press, College Station, Texas, USA) that adjusted for within herd clustering and sampling weights. Herd level estimates of BVD seroprevalence 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. Herd level seroprevalence for BLV, NC, and MAP was calculated 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. Due to substantial inaccuracies of the ELISA used for identifying MAP infected animals, the estimated true herd prevalences for beef and dairy cattle were calculated, correcting for test Se and Sp (5).

Table 1 shows the proportion of seropositive cows, the proportion of herds with at least 1 and 2 seropositive cows, and the average seroprevalence in herds with at least 1 and 2 seropositive cows for each pathogen. Of the 1204 dairy cows and 1425 beef cows in the final dataset, there were significantly ($P < 0.05$) more BLV-seropositive dairy cattle (60.8%) compared with beef cattle (10.3%), as seen by the 95% confidence intervals (CI) not overlapping. Based on a

definition of an infected herd having at least 1 seropositive cow, there were significantly more BLV-seropositive dairy herds (97.4%) than beef herds (47.9%). Furthermore, in these infected herds, the within herd seroprevalence was significantly higher in dairy herds (62.5%) than in beef herds (21.7%). Based on a definition of an infected herd having at least 2 seropositive cows, there were significantly more BLV-seropositive dairy herds (97.4%) than beef herds (32.6%). Again, in those infected herds, the within herd seroprevalence was significantly higher in dairy herds (62.5%) than in beef herds (29.0%).

There appeared to be more MAP-seropositive dairy herds than beef herds and more NC-seropositive beef herds than dairy herds. However, the observed differences in NC- and MAP-seroprevalences between dairy and beef cattle may have been partly due to differences in tests (NC), laboratories (NC and MAP), or both (NC), making statistical comparisons inappropriate. There did not appear to be differences in cow-level MAP- and NC-seroprevalences between dairy and beef herds, or differences in within herd seroprevalences between MAP- and NC-infected dairy herds and beef herds, despite the differences in testing protocols.

The final database of BVDV test results contained 128 unvaccinated dairy cows and heifers from 26 dairy herds (65% of sampled herds). No comparisons to beef herds in Manitoba could be made, due to the very low number of participating beef farms having 5 unvaccinated heifers over 6 mo of age, producing a very unrepresentative sample. In dairy herds, 16.4% (4.2% to 28.6%, 95% CI) of animals were seropositive for BVDV, with 15.6% (3.4% to 27.9%, 95% CI) having titers $\geq$ 1:64. Seropositive animals were found in 32.0% (12.5% to 51.5%, 95% CI) of herds, while 28.1% (9.3% to 47%, 95% CI) of herds had animals with titers $\geq$ 1:64. This cut-off value was utilized to enable comparisons to be made between the results in Manitoba and those in other Canadian provinces where this cut-off indicated a natural break-point in the data, a cut-off that appeared to indicate recent or current exposure to transiently or persistently infected animals (1).

Regarding comparisons of seroprevalence confidence intervals in other provinces in Canada, significantly more dairy animals in Prince Edward Island (45%) were seropositive (titer $\geq$ 1:64) for BVDV infection (1) compared with dairy animals in Manitoba (16%). With the same laboratory and same test being used for these 2 provinces, the difference in seroprevalence may have been due to the lower proportion of dairy farmers in Prince Edward Island that vaccinated (61% versus 82%) against BVDV infection (unpublished data).

Using the same laboratory and test, there were significantly more MAP-seropositive dairy cows in Manitoba than in Prince Edward Island (1). Differences in calf and manure management or in agroecological factors between provinces may explain these differences, factors to be examined in a future risk factor study. However, agroecological factors are unlikely to be the reason that dairy herds in Manitoba were more likely to be MAP-seropositive compared with beef herds in Manitoba (if this difference was not just due to differences in testing protocols), considering that the farms were located in proximity to each other. One possible reason may be due to differences in length of time that purchased animals are kept on farm. On dairy farms in Canada, milking cows are the animals most commonly purchased (unpublished data), and it is not unusual for dairy cattle to be kept until they are 8 to 10 y of age, long enough for those infected with MAP to advance to the later stages of infection when they shed MAP in their manure and thus infect other cattle. Conversely, on beef farms in Canada, young bulls are the animals most frequently purchased (unpublished data), and to avoid inbreeding, they are frequently culled before reaching an advanced age when they are more likely to shed MAP. Again, future research may clarify this speculation. A survey of beef cattle in 1999 on community pasture in Saskatchewan found that 0.8% of cows and 15.2% of herds were MAP-seropositive (9), similar seroprevalences to those found in beef cattle in Manitoba, using the same test but different laboratories.

Dairy herds in Manitoba had significantly more BLV-seropositive cows (61%) compared with dairy herds in New Brunswick (29%), Nova Scotia (16%), Prince Edward Island (17%), and Saskatchewan (37%), using the same lab and test (1,2). However, it is unclear why only Prince Edward Island (63%) and Nova Scotia (70%) had significantly fewer BLV-seropositive dairy herds compared with Manitoba (97%) (1,2). In a national survey in 1981 (10), 40% of dairy and 31% of beef herds in Manitoba were found to be BLV-seropositive, substantially lower than the prevalences found in the current study. Other Canadian provinces have also seen substantial increases in cow and herd level BLV-seroprevalences in dairy cattle over the last 20 to 25 y (1,2).

In 2001, using the BIOVET ELISA, 5.2% of 2484 beef cows in western Canada were NC-seropositive (11), which is substantially lower than the sampled beef herds in Manitoba, although the difference could be due to the different laboratories and tests used. Conversely, with the same test and laboratory utilized, dairy herds in Saskatchewan had similar cow and herd level seroprevalences to dairy herds in Manitoba (2), with seroprevalences from both prairie provinces being significantly lower than those for dairy herds in maritime Canada (12). Future analyses will investigate test, laboratory, ecological, or management reasons for these apparent differences in seroprevalence.

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Book Review
Compte rendu de livre

Arthropod-borne Infectious Diseases of the Dog and Cat

Shaw SE, Day MJ. Lippincott, Williams & Wilkins, Baltimore, Maryland, USA, 2005, 152 pp. ISBN 0-7817-9014-X. CDN$93.50.

In Canada, the risk of arthropod-transmitted infections in dogs and cats is considerably less than in other parts of the world. However, in light of the increasing ease with which dogs and cats can travel globally, the interest in adopting pets from international locations (currently from the southeast USA), and the predicted climate change, it is increasingly likely that veterinarians in Canada will see dogs or cats with arthropod-borne diseases. This book provides succinct, up-to-date, information on the epidemiology, pathogenesis, clinical presentation, diagnosis, treatment, prevention, and zoonotic risk of each of the major arthropod-borne diseases of dogs and cats — anaplasmosis, babesiosis, bartonellosis, borreliosis, cytauxzoonosis, *Dirofilaria immitis* (heartworm) infection, ehrlichiosis, hepatozoonosis, leishmaniosis, and rickettsial infections. Other, less common, infections are also briefly reviewed. Prior to discussing each of these diseases, the authors have provided a useful, practical review on the arthropod vectors and wildlife reservoirs involved in the epidemiology of each pathogen. In addition, since immune-mediated disease is a common occurrence with many of these infections, current information on the immune mechanisms underlying each disease is clearly described. In later sections, this information is used to justify the most appropriate diagnostic tests and treatment options for each disease. Finally, a useful chapter is included that discusses the utility of the various diagnostic tests for arthropod-borne diseases that are currently available in commercial diagnostic laboratories.

Each of the chapters is well-written by experts on the diseases from around the world and, unlike other texts on the same topic, provides current information on the global risk of infection for both dogs and cats. In light of the newly emerging nature of some of these diseases in specific parts of the world, such information is particularly helpful.

The authors have included a large number of good quality color pictures that are visually appealing, informative, and greatly enhance the readability of the book, consistently throughout the chapters. The clinical pathology pictures are of consistently high quality. However, it would have been helpful for the reader if the authors had included the names of the stains that were used to generate many of the pictures. The only other weakness identified concerned the section on heartworm. Compared with other chapters, the text could have been a little more clinically relevant and, in some places, a little more up-to-date. However, overall, the text was very informative.

In summary, at approximately CDN$93, this book is excellent value for money. Furthermore, the text is well-suited for both veterinary students and veterinary practitioners with a specific interest in vector-borne diseases of companion animals.

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