Article

Leptospirosis in beef herds from western Canada: Serum antibody titers and vaccination practices

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Abstract — One study described the frequency of pre-breeding vaccination for leptospirosis in 205 cow-calf herds from across western Canada and the prevalence of positive *Leptospira* antibody titers in unvaccinated, weaned calves from 61 of these herds. The percentages of herds vaccinated for leptospirosis were 13.7% in 2001 and 8.4% in 2002. Of 1539 calves examined, 13 (0.8%) had a positive antibody titer for a *Leptospira* serovar; the most common serovar detected was hardjo. A second study examined the prevalence of positive *Leptospira* antibody titers during the summer grazing season in 313 vaccinated and 478 unvaccinated cows from 40 cow-calf herds in southern Saskatchewan. Antibody titers for 7 *Leptospira* serovars were measured during the grazing season. Of the non-vaccinated cows, 9.6% were positive in the spring for serovar pomona, 6.7% for serovar grippotyphosa, and 6.1% for serovar icterohaemorrhagiae; the corresponding percentages for the fall were 5.5%, 3.0%, and 1.3%, respectively. Of 781 vaccinated and unvaccinated cows that were sampled twice, 11.3% of vaccinated cows and 2.3% of unvaccinated cows had increases in *Leptospira* antibody titers during the grazing season.

Résumé — Leptospirose chez les troupeaux bovins de l'Ouest canadien : titres d'anticorps sériques et pratiques de vaccination. Une étude a décrit la fréquence de la vaccination avant l'accouplement pour la leptospirose dans 205 troupeaux de vaches et de veaux de l'Ouest canadien et la prévalence des titres d'anticorps positifs envers *Leptospira* chez des veaux non vaccinés et sevrés provenant de 61 de ces troupeaux. Les pourcentages des troupeaux vaccinés pour la leptospirose étaient de 13,7 % en 2001 et de 8,4 % en 2002. Parmi les 1539 veaux examinés, 13 (0,8 %) avaient un titre d'anticorps positif pour un sérotype de *Leptospira;* le sérotype le plus communément détecté était hardjo. Une deuxième étude a examiné la prévalence de titres d'anticorps positifs envers *Leptospira* pendant la saison de pâturage d'été chez 313 vaches vaccinées et chez 478 vaches non vaccinées provenant de 40 troupeaux vaches-veaux dans le sud de la Saskatchewan. Des titres d'anticorps pour 7 sérotypes de *Leptospira* ont été mesurés durant la saison de pâturage. Parmi les vaches non vaccinées, 9,6 % étaient positives au printemps pour le sérotype pomona, 6,7 % pour le sérotype grippotyphosa et 6,1 % pour le sérotype icterohaemorrhagiae; les pourcentages correspondants pour l'automne étaient de 5,5 %, de 3,0 % et de 1,3 %, respectivement. Parmi les 781 vaches vaccinées et non vaccinées pour lesquelles des prélèvements ont été effectués à deux reprises, 11,3 % des vaches vaccinées et 2,3 % des vaches non vaccinées ont présenté des hausses des titres d'anticorps de *Leptospira* durant la saison de pâturage.

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Introduction

Efforts to determine the cause of poor pregnancy rates and early abortion losses in extensively managed beef herds are often frustrated by the retrospective nature of the investigation. Problems with infectious agents such as bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis (IBR), neosporosis, and leptospirosis, can be particularly difficult to diagnose given the time lag between infection and pregnancy assessment. In herds using pasture breeding, pregnancy testing and recognition of reproductive failure often occur 4 to 6 mo or more after the start of the breeding season. When other causes are ruled out, the veterinarian's only option for evaluating the role of many infectious diseases is to examine the herd's serological profile. Recent studies have described the prevalence of serum antibodies to BVDV, IBR, and *Neospora caninum* in

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mature cows and weaned calves and explored the association between antibody titers and herd reproductive performance (1,2). However, no recent studies in western Canada have examined the prevalence of *Leptospira* antibody titers and the relative likelihood of leptospirosis as a differential diagnosis in cases of poor reproductive performance.

Leptospirosis can cause abortions, birth of stillborn and weak calves, and infertility in cattle. Disease-causing *Leptospira* can be classified as host-adapted, infecting maintenance host animals, or non-host-adapted, infecting accidental host animals. *Leptospira borgpetersenii* serovar hardjo is the most common leptospiral cause of abortions and infertility in cattle in North America (3,4). Hardjo is a host-adapted serovar for cattle, which can become chronic carriers of hardjo and serve as reservoirs for infection of other cattle and humans (5). *L. interrogans* serovars pomona, icterohaemorrhagiae, and grippotyphosa are non-host adapted for cattle and sporadically infect cattle as accidental hosts, causing acute disease and abortion (6,7).

Commercial vaccines are the most practical and commonly used options for control of leptospirosis. While vaccination is often encouraged for cattle with a history of reproductive problems, or for herds using communal grazing, almost no relevant regional information is available to assist veterinarians in making cost-effective and evidence-based recommendations for vaccine use. The prevalence of chronically infected animals and annual incidence of new infections are unknown in western Canada.

This paper presents 2 complementary studies examining the prevalence of positive *Leptospiral* titers in western Canadian beef herds. The first study reports data describing the frequency of vaccination for leptospirosis in cow-calf herds from across western Canada, and the prevalence of positive *Leptospiral* antibody titers in unvaccinated beef calves in the fall of the year. The second study measured *Leptospiral* antibody titers in beef cows at the start and end of the grazing season in community pastures in southern Saskatchewan. Paired serum samples were used to determine the change in antibody titers during the summer grazing season. The factors associated with positive antibody titers for the most common leptospiral serovars and changes to these titers during the grazing season were examined in addition to the association between *Leptospiral* titers and fall pregnancy status.

Materials and methods

Study 1

Study population. Participants were selected in the fall of 2002 from a group of 205 cow-calf herds enrolled in an extensive investigation of factors affecting productivity in northeastern British Columbia, Alberta, and Saskatchewan (8). Each participant was asked to report whether the cow herd was vaccinated for leptospirosis before the breeding season in the spring of 2001 or 2002, and whether or not the calves were vaccinated for leptospirosis in the spring of 2002 before being turned out on summer pasture.

The criteria for selection of a subset of these 205 herds to examine infectious diseases affecting calf losses, as previously described (2), were chosen to maximize the range of herd risk of calf loss in the final sample and the power to detect pathogens potentially associated with that loss. All herds were ranked before fall calf processing on the basis of total calf losses, including abortions, reported by the herd owner. Those in the highest ($>$ 10% total loss) and lowest quartiles ($<$ 6% total loss) were listed in random order. Herd owners from these lists were then approached sequentially until 30 herd owners from each group agreed to participate. Blood samples were collected from a systematic random sample (9) of 30 calves from each participating herd in the fall of 2002 after the cow-calf pairs had been removed from summer pasture.

Individual calf, herd, and pasture data. Additional data for each enrolled cow and herd included calf birth date, calf sex, date of sample collection, cow age, and cow breed. All land locations used by the herds were electronically mapped and classified by ecoregion (as defined by Agriculture and Agri-Food Canada) (10), using a geographic information system (GIS) (ArcView GIS 3.2, ESRI, Redlands, California, USA). Total accumulated precipitation data from the 2002 growing season were obtained for the period from April 1 to August 31, 2002 from the Drought Watch Web site, maintained by Agriculture and Agrifood Canada (11).

Study 2

Study population. Data were collected for an observational study of risk factors for non-pregnancy in beef cows. Researchers presented the objectives of the study and requirements for participation to herd owners. Cows were enrolled from 40 commercial beef herds that grazed cattle on 5 Prairie Farm Rehabilitation Administration (PFRA) community pastures (12) in southern Saskatchewan from May to October, 2008.

The first 20 cows from each participating herd that entered the handling system were selected for sampling on arrival at the pasture in May 2008. If the herd owner sent fewer than 20 cows to pasture, then the entire allotment of cows was sampled. A second set of blood samples was collected in October 2008 from all available spring-sampled cows.

Individual cow, herd, and pasture data. Each herd owner completed a written survey in June 2008 documenting age and calving date information for the 20 enrolled cows, overall herd size, and vaccination program to control infectious diseases that could affect reproductive health (BVDV, IBR, *Leptospira,* and *Campylobacter fetus*) (13).

Each pasture manager completed a written survey for each field with enrolled cows, including the bull vaccination program, bull identification, bull and cow numbers, breeding season dates, field size and location, and a description of the water sources present on the fields with enrolled cows. Each bull used on the community pastures was required to have a satisfactory semen evaluation according to published standards (14), and each bull $\geq$ 2 y that was placed with cows enrolled in this study was tested to confirm negative status for *Tritrichomonas foetus* and *C. fetus* (13). While the breeding soundness examinations and *T. foetus* testing are standard management practices, the *C. fetus* testing was done to minimize potential biases by controlling other variables among herds and pastures.

In October 2008, the enrolled cows were palpated transrectally to determine their pregnancy status. Researchers also assessed cow dentition in October to classify the age of animals

when age records from the producer were not available (15). Five herds had some or all cows aged in this manner.

Collection of blood samples and laboratory analysis

Blood samples from both studies were collected by jugular venipuncture using 10 mL vacutainer tubes with no additive. The blood was allowed to clot, and the serum separated and frozen at −70°C (Study 1) or −20°C (Study 2) within 24 h of collection and then stored at −70°C until it was transported to a commercial laboratory [Animal Health Laboratory, Guelph, Ontario (AHL)] for analysis of antibodies to 7 *Leptospira* serovars.

Leptospira microscopic agglutination tests (MATs) used to detect antibodies were harmonized with WHO recommendations. The reference strains and controls were obtained from KIT Biomedical Research (Royal Tropical Institute, Amsterdam, Netherlands) and included serovar autumnalis — strain Akiyami A; serovar bratislava — strain Jez Bratislava; serovar canicola — strain Hond Utrecht IV; serovar grippotyphosa — strain Moskva V; serovar hardjo — strain Hardjoprajitno; serovar icterohaemorrhagiae — strain M20; and serovar pomona — strain Pomona.

Samples were initially screened at a 1:50 dilution; those samples that produced ≥ 50% agglutination compared with the control antigen were considered reactive and subjected to quantitative MAT (AHL). Quantitative MATs were performed by making 2-fold serial dilutions of reactive sera to determine the antibody end-point titers, defined as the highest dilution of serum showing at least 50% agglutination. The end-point antibody titer was the reciprocal of the highest dilution with a positive reaction.

Statistical analysis

All data were examined and summarized using a commercial software program (Microsoft Excel; Microsoft Corporation, Redmond, Washington, USA). Antibody titers for each serovar were categorized into binary variables according to laboratory cut-offs; antibody titers ≥ 100 and < 100 were classified as positive and negative, respectively (AHL).

Data for Study 2 were further analyzed using MLwiN version 2.11 (Centre for Multilevel Modeling, University of Bristol, Bristol, United Kingdom). Associations between potential risk factors and each outcome of interest were examined using generalized linear mixed models with a binomial distribution, logit link function, and random intercepts to account for repeated measures on individual animals and clustering by herd of origin and pasture (9). The strength of each association was reported as an odds ratio (OR) with a 95% confidence interval (95% CI) determined using penalized quasi-likelihood estimates.

Association between potential risk factors and detection of positive *Leptospira* antibody titers (Study 2). The first series of models examined associations between potential risk factors and detection of a positive *Leptospira* antibody titer for serovars with a prevalence of > 10% in the spring. Three potential risk factors were examined after accounting for repeated measures on individual animals and clustering within herd of origin and pasture: season of sample collection (spring or fall), cow age category (1 to 3, 4 to 9, or 10 to 14 y), and whether or not a *Leptospira* vaccination was given in 2008 before the grazing season.

Multivariable models were developed using manual backwards elimination from the group of risk factors that were unconditionally associated ($P < 0.20$) with the presence of a positive antibody titer. Variables were retained in the final model for each serovar if statistically significant ($P < 0.05$) or important as confounders (if their removal changed the model output by ≥ 10%). Biologically reasonable first-order interaction terms were tested after establishing a main effect model. The residuals were examined for the presence of outliers and influential observations. Data from the final models were used to calculate the proportion of remaining variance in positive titers accounted for by herd:

$$[\rho = \sigma^2_h/(\sigma^2_c + \sigma^2_h + \sigma^2_p + \pi^2/3)] \quad \textit{Equation 1}$$

and then by pasture (9):

$$[\rho = \sigma^2_p/(\sigma^2_c + \sigma^2_h + \sigma^2_p + \pi^2/3)] \quad \textit{Equation 2}$$

Association between potential risk factors and the odds of increasing antibody titers during the grazing season (Study 2). A second series of models examined unconditional associations between both vaccination and age and the odds of increasing antibody titers in cows during the grazing season for the leptospiral serovars with a prevalence > 10% in the spring. These models also accounted for clustering within herd of origin and pasture.

Association between potential leptospiral risk factors and the odds of non-pregnancy (Study 2). A third series of models examined the associations between the odds of non pregnancy and 1) whether or not the herd was vaccinated for leptospirosis, 2) *Leptospira* antibody status at the start of the breeding season, and 3) an increase in *Leptospira* antibody status during the breeding season. Only *Leptospira* serovars with a spring prevalence > 10% were evaluated. Three other previously reported risk factors for non-pregnancy in these herds (13) were included in all models examining the odds of non-pregnancy: pre-breeding body condition (< 2.5/5 or ≥ 2.5/5), age category (1 to 3, 4 to 9, or 10 to14 y), and calving to start of breeding season interval (≤ 50 or > 50 d). All models accounted for clustering of non-pregnancy within herd of origin and pasture.

Results

Study 1

Participants in the baseline study reported vaccinating 13.7% (28/205) of herds for leptospirosis before the start of the breeding season in the spring of 2001 and 8.4% (17/203) in 2002. All reported leptospirosis vaccines contained serovars canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona. Cows from 27% of herds in 2001 and 59% in 2002 grazed on communal pastures. No calves from these herds were vaccinated for leptospirosis in the spring of either year.

Individual calf, herd, and pasture data. Blood samples were collected from 1782 calves from 61 herds in the fall of 2002. Most calves were between 6 and 8 mo old [mean: 227 d; standard deviation *(s)* ± 43 d] when blood samples were collected in the fall of 2002; 41.1% were steers and 52.7% were

Table 1. The frequency of positive antibody titers (≥ 100) for 7 *Leptospira* serovars in May and October 2008 for cows in Study 2 that were and were not vaccinated[a] for *Leptospira*

	Number of vaccinated cows[b]				Number of non-vaccinated cows[c]			
		MAT antibody titer				MAT antibody titer		
Leptospira serovar	Positive MAT	100	200	400 to 800	Positive MAT	100	200	400 to 800
Autumnalis								
Spring	0	0	0	0	0.6%	2	1	0
Fall	0.3%	0	1	0	0.2%	0	1	0
Bratislava								
Spring	0	0	0	0	0	0	0	0
Fall	0	0	0	0	0	0	0	0
Canicola								
Spring	9.6%	18	11	1	5.2%	14	10	1
Fall	0.6%	2	0	0	0.4%	2	0	0
Grippotyphosa								
Spring	23.6%	31	38	5	6.7%	14	17	1
Fall	8.0%	18	6	1	1.3%	5	1	0
Hardjo								
Spring	1.6%	5	0	0	0.2%	0	1	0
Fall	0	0	0	0	0	0	0	0
Icterohaemorrhagiae								
Spring	28.1%	62	24	2	6.1%	12	16	1
Fall	3.5%	6	4	1	3.0%	9	4	1
Pomona								
Spring	37.4%	49	40	28	9.6%	23	11	12
Fall	13.2%	34	6	1	5.5%	18	7	1

MAT — microscopic agglutination test.
[a] Based on herd vaccination records from January 1 until placement on grazing pasture in 2008.
[b] 313 vaccinated cows in spring; 311 vaccinated cows in fall.
[c] 478 unvaccinated cows in spring; 470 invaccinated cows in fall.

heifers (remaining 6.2% not reported). Breed type information was available for the calves' dams; 40.1% were primarily British breed, 47% were primarily continental breeds, and the remainder were mixed breed (7.5%) or not recorded (5.4%).

The median herd size was 146 breeding females at pregnancy testing in 2002 (range: 40 to 486). The average reported risk of calf loss, including; abortions, stillbirths, and calf deaths, for the 61 herds was 9.7% ($s \pm 6.2\%$). Cows from 58 of these herds were pregnancy tested in the fall of 2002 and the mean herd non-pregnancy risk was 8.6% ($s \pm 5.6\%$). The growing season of 2002 was unusually dry in most areas of western Canada. Half of the herds (31/61) were pastured on land that received $<$ 250 mm precipitation from April 1 to August 31; only 1 herd was pastured on land that received $>$ 300 mm of precipitation.

Antibody titers in individual calves and herds. Sufficient serum was available for analysis from 1539 of the sampled calves representing all 61 herds; 13 (0.8%) calves from 5 herds had antibody titers of 1:100 or greater to at least one *Leptospira* serovar. One Alberta herd had 6 calves test positive for serovar hardjo (3 — 1:100; 2 — 1:200; 1 — 1:400), and 1 Saskatchewan herd had 4 calves positive for hardjo (1:100; 1:200; 1:800; 1:1600); both of these herds were in the Aspen Parkland ecosystem. The remaining 3 Alberta herds had 1 calf each that was seropositive: 1 for grippotyphosa (1:100) from the Moist Mixed Grasslands region, 1 for pomona (1:100) from the Mixed Grassland region, and 1 for autumnalis (1:100) from the Aspen Parkland. The herds with calves positive for hardjo, grippotyphosa, and pomona were pastured in areas receiving $<$ 250 mm precipitation during the grazing season.

Study 2

Individual cow, herd, and pasture data. Blood samples were collected from 791 cows in May 2008 and 781 of the same 791 cows in October 2008. Twenty-two percent (171/791) of the enrolled cows were 1 to 3 y old, 63% (500/791) were 4 to 9 y old, and 15% (115/791) were 10 to 14 y old. Age records were missing for 5 animals. The median size of participating herds on January 1, 2008 was 133 breeding females (range: 11 to 370).

Owners administered a pentavalent *Leptospira* vaccine, in combination with BVDV and IBR antigens, to 40% (16/40) of the herds and 40% (313/791) of cows, between February 10 and May 21, 2008. The vaccine was administered to 13 herds in May, 2 herds in March, and 1 herd in February; the median time from vaccination to sample collection was 21 d. No producer reported using a second dose of vaccine.

Thirty-five of the 40 herds (88%) were mixed on community pasture fields with cattle from at least 1 other herd. All bulls used on the 5 community pastures were vaccinated against BVDV, IBR, and *C. fetus* before the start of the 2008 breeding season; 2 of the 5 pasture managers used a vaccine that included *Leptospira* antigens. The median cow to bull ratio for the community pasture breeding fields was 27:1. The median length of the breeding season was 72 d (range: 64 to 126 d). There were

Table 2. The frequency of increases in antibody titers for 7 *Leptospira* serovars during the 2008 grazing season for cows in Study 2 that were and were not vaccinated[a] against *Leptospira*

	Number of individual cows[b]				
		Change in MAT titer			
Leptospira serovar	Increase in titer	0 to 100	100 to 200	0 to 200	200 to 400
All serovars					
Vaccinated	11.3%	22	7	5	1
Unvaccinated	2.3%	7	2	1	1
Pomona					
Vaccinated	5.5%	12	4	1	0
Unvaccinated	1.3%	4	1	1	0
Grippotyphosa					
Vaccinated	3.5%	8	0	2	1
Unvaccinated	0	0	0	0	0
Icterohaemorrhagiae					
Vaccinated	1.9%	1	3	2	0
Unvaccinated	0.9%	3	0	0	1
Canicola					
Vaccinated	0.3%	1	0	0	0
Unvaccinated	0	0	0	0	0
Autumnalis					
Vaccinated	0	0	0	0	0
Unvaccinated	0.2%	0	1	0	0

MAT — microscopic agglutination test.
[a] Based on herd vaccination records from January 1 until placement on grazing pasture in 2008.
[b] 311 vaccinated cows and 470 non-vaccinated cows.

Table 3. The frequency of Study 2 herds and pastures with at least 2 cows with positive antibody titers (≥ 100) against the 3 most common *Leptospira* serovars in May or October 2008 and at least 2 cows with increases in *Leptospira* serovars during the 2008 grazing season

	Number of herds		Number of community pastures	
Leptospira serovar	≥ 2 cows with serum antibody titers ≥ 1:100	≥ 2 cows with an increase in antibody titer	≥ 2 cows with serum antibody titers ≥ 1:100	≥ 2 cows with an increase in antibody titer
Pomona				
Vaccinated	11/16 (68.8%)	3/16 (18.8%)	3/5 (60%)	2/5 (40%)
Unvaccinated	7/24 (29.2%)	2/24 (8.3%)	2/5 (40%)	2/5 (40%)
Icterohaemorrhagiae				
Vaccinated	11/16 (68.8%)	2/16 (12.5%)	3/5 (60%)	1/5 (20%)
Unvaccinated	4/24 (16.7%)	0/24	2/5 (40%)	0/5
Grippotyphosa				
Vaccinated	9/16 (56.3%)	4/16 (25%)	2/5 (40%)	2/5 (40%)
Unvaccinated	3/24 (12.5%)	0/24	2/5 (40%)	0/5

263/771 cows (34%) exposed to bulls for ≤ 84 d; the remainder of the cows were exposed for > 84 d.

The 5 community pastures with enrolled cows contained 31 primary water sources, including dugouts, sloughs, dams, and creeks. The mean total precipitation received by each pasture from April 1 to October 31, 2008 was 307 mm (range: 263 to 406 mm).

In October, 93% (726/781) of the cows were pregnant. Ten cows were missing from the fall roundup because they had died on pasture, had been removed early from pasture, or could not be located.

Antibody titers in individual cows, herds, and pastures. Positive antibody titers to *Leptospira* serovars pomona, icterohaemorrhagiae, and grippotyphosa were the most prevalent of the 7 serovars examined. No sample reacted beyond a 1:800 dilution for any of the *Leptospira* serovars examined. Detectable antibody titers for serovar hardjo were rare in all cows, regardless of vaccination status (Table 1).

Of the 781 cows sampled in both May and October 2008, 11.3% of vaccinated cows and 2.3% of non-vaccinated cows had increases in *Leptospira* antibody titers during the grazing season. Increases were most commonly seen with serovars pomona, grippotyphosa, and icterohaemorrhagiae (Table 2).

More than 12% of herds and 20% of pastures had 2 or more unvaccinated cows with a positive MAT to one of the 3 most common serovars. Two of the 24 unvaccinated herds located in 2 of 5 pastures had 2 or more cows with an increase in pomona antibody titers during the grazing season; none of the

Table 4. Summary of the associations between vaccination status, season, cow age, and positive antibody titers to the 3 most common *Leptospira* serovars observed in Study 2 during the 2008 grazing season ($n = 781$ cows from 40 herds)

Leptospira serovar	Risk factor comparison	Odds ratio	Lower 95% CI	Upper 95% CI	*P*-value
Pomona	Vaccinated: unvaccinated in spring	9.2	1.9	45	0.006
	Vaccinated: unvaccinated in fall	3.6	0.7	19	0.13
	Spring: fall in vaccinated cows	6.0	3.8	9.4	0.001
	Spring: fall in unvaccinated cows	2.4	1.3	4.3	0.006
	Old cows: mature cows	2.4	1.4	4.1	0.002
	Old cows: young cows	3.5	1.7	7.1	0.004
	Mature cows: young cows				0.19
Grippotyphosa	Vaccinated: unvaccinated in spring	5.7	1.0	33	0.05
	Vaccinated: unvaccinated in fall	1.4	0.2	10	0.71
	Spring: fall in vaccinated cows	14	7.2	27	0.001
	Spring: fall in unvaccinated cows	3.5	1.5	8.4	0.005
Icterohaemorrhagiae	Spring: fall	4.8	2.5	9.3	0.001
	Old cows: mature cows	2.3	1.3	4.2	0.001
	Old cows: young cows	4.1	1.7	9.7	0.001
	Mature cows: young cows				0.09

95% CI — 95% confidence interval.

[a] The odds ratios describe the relative differences in the odds of being positive among the categories of each risk factor. The odds ratios were derived from models accounting for repeated measures from individual cows and clustering within herd of origin and pasture, as well as considering relevant interaction terms.

unvaccinated herds had detectable antibody titers for icterohaemorrhagiae and grippotyphosa in 2 or more cows (Table 3).

Association between potential risk factors and detection of positive *Leptospira* antibody titers. The only consistent finding among the 3 most common serovars was a decrease in antibody titers from the spring to the fall (Table 4). After adjusting for identified risk factors for each serovar, 47.3% of the remaining variance in whether a cow had a positive antibody titer for pomona was accounted for by the herd of origin and 5.0% by differences between the community pastures; for icterohaemorrhagiae, 38.9% was accounted for by herd and 17.9% by community pasture; and for grippotyphosa, 17.4% was accounted for by herd and 49.3% by community pasture.

Association between potential risk factors and the odds of increasing antibody titers during the grazing season. The antibody titers for serovars pomona ($P = 0.01$) and grippotyphosa ($P = 0.001$) were more likely to increase during the grazing season in vaccinated cows than in unvaccinated cows, but this was not the case for icterohaemorrhagiae ($P = 0.86$). Age was not associated with the odds of an increase in antibody titers for any of the serovars (pomona $P = 0.75$; icterohaemorrhagiae $P = 0.73$; grippotyphosa $P = 0.84$).

Association between potential leptospiral risk factors and the odds of non-pregnancy. After accounting for cow age, body condition, and calving to breeding interval, neither vaccination status of the cows ($P = 0.53$) nor positive antibody status at the start of the grazing season [serovar pomona ($P = 0.46$), serovar icterohaemorrhagiae ($P = 0.92$), or serovar grippotyphosa ($P = 0.35$)] was associated with the odds of non-pregnancy in these herds.

After accounting for cow age, body condition, and calving to breeding interval, increases in antibody titers during the 2008 grazing season for serovars pomona (OR: 3.27; 95% CI: 0.86 to 12.4; $P = 0.08$), icterohaemorrhagiae (OR: 3.39; 95% CI: 0.64 to 2.88; $P = 0.15$), or grippotyphosa (OR: 4.63; 95% CI: 0.89 to 3.18; $P = 0.07$) were more closely associated with the odds of non-pregnancy, although none were statistically significant.

Discussion

There was little serological evidence of widespread exposure to either host-adapted or non-adapted leptospirosis in either of the 2 populations examined in this report. Few animals and herds in either study had positive antibody titers to *Leptospira* serovar hardjo. The small titer increases observed with seroconversion to the non-adapted serovars pomona, icterohemorrhagiae, and grippotyphosa during the 2008 grazing season were not indicative of recent natural infection with these agents.

Weaned calves from only 2 of the 61 herds examined in 2002 had convincing serologic evidence of herd infection with serovar hardjo. A previous 1984–1985 survey of beef cows in AB reported much more variable herd prevalences for hardjo titers ranging among municipal divisions from 0% to 54% (16). Our study might have underestimated the risk of herd exposure to serovar hardjo by measuring serum antibody levels in weaned calves. Prepubertal calves that are widely dispersed on grazing pasture would be at low risk for 2 common transmission mechanisms for hardjo: natural breeding and direct contact with a carrier animal's urine. However, < 2% of the breeding females examined in our 2008 community pasture study had antibodies to serovar hardjo, despite hardjo being the most commonly reported *Leptospira* serovar in surveys of beef and dairy cattle in North America (3,4,6).

The prevalence of natural infection with hardjo could also have been underestimated due to limitations of the MAT. Cattle with chronic hardjo infections have low or no agglutinating antibody titers (17,18), possibly because the lipopolysaccharides on the bacterial surface are poor activators of B lymphocytes (19,20). Supplementing the MAT with a more sensitive diagnostic test to identify hardjo bacteria in urine or the genital

tract of chronic carriers (21) should be considered in future prevalence surveys.

None of the herds sampled in 2002 from either Alberta or Saskatchewan had titers suggesting substantial exposure of weaned calves to the non-adapted serovars examined in this study. Kingscote (16) had reported focal infections with serovar pomona in an earlier study in southeastern Alberta. Cattle acquire infections with non-adapted serovars by contact with an animal that is actively shedding the bacteria or contact with a contaminated environment, often water or feed. The severe drought conditions during 2001 and 2002 in western Canada may have limited the potential for transmission of non-host adapted serovars through contaminated surface water sources. Many high risk water sources disappeared during the extremely dry grazing season.

A surprisingly high frequency of unvaccinated cows on community pastures in 2008 had positive antibody titers to non-adapted *Leptospira* serovars. Since more unvaccinated cows with positive antibody titers for serovars pomona, icterohaemorrhagiae, and grippotyphosa were detected in the spring than in the fall, it is possible that natural exposure to these serovars occurred on the farms of origin before the animals came to pasture. However, the relatively low positive antibody titers described in unvaccinated cows may not indicate recent natural exposure. Old cows were more likely to be positive for serovars pomona and grippotyphosa than young or mature cows. The increased likelihood of positive antibody titers for non-adapted *Leptospira* serovars in old cows is consistent with an increased likelihood of exposure over time and could be due to previous natural infections or previous vaccination. Previous studies (3,7) have also reported an increased prevalence of positive antibody titers for hardjo, pomona, and icterohaemorrhagiae in old beef and dairy animals compared to young animals.

Although antibody titers for the *Leptospira* serovars pomona and icterohaemorrhagiae increased during the grazing season in a small percentage of the unvaccinated cows on Saskatchewan community pastures, no cow experienced more than a 2-fold increase in MAT for any serovar. These relatively small antibody increases are not typical of acute infection with non-adapted serovars, regardless of the presence of clinical disease (16,17,22), and could simply represent laboratory variation in the reported MAT results. However, the potential for natural exposure to pomona and icterohaemorrhagiae cannot be completely ruled out because of the long time period between taking the first and second samples. Antibody concentrations may have peaked earlier in the grazing season and declined before the cows were sampled in October.

The observed increases in antibody titers for pomona, icterohaemorrhagiae, and grippotyphosa during the grazing season had a tendency to be associated with increased odds of non-pregnancy in the fall, although the associations were not statistically significant. Widespread transmission of a non-adapted serovar through a herd can cause large numbers of abortions, but these events appear rare in western Canada with few published reports of their occurrence (22). The potential for contact between wildlife species and cattle on grazing pastures would likely have varied across the study areas, and the spread of infection through a cow herd during the summer grazing season could be limited by the low stocking density imposed by the short grass environments of southern Saskatchewan and Alberta.

A relatively low proportion of the cow herds received leptospirosis vaccine in 2001 (14%) and 2002 (8%) compared with 2008 (40%). The differences in vaccination rates between the studies could potentially be explained by increased awareness of the risk of infectious diseases associated with the use of communal pastures. All producers who vaccinated their herds in both studies reported using a pentavalent *Leptospira* vaccine in combination with BVDV and IBR antigens. The *Leptospira* vaccine contains 5 serovars: canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona and is reported to protect against disease caused by these serovars (23,24). A decline in diagnosed cases of abortion due to pomona infections has been attributed to use of these vaccines (17). However, the pentavalent *Leptospira* vaccines do not consistently induce antibodies to serovar hardjo (19,23,24), potentially explaining the lack of positive antibody titers for hardjo in the vaccinated cows.

Most of the vaccinated cows did not have positive antibody concentrations in the spring for the serovars contained in the pentavalent *Leptospira* vaccines, possibly due to the short time between vaccination and spring sampling. Brown et al (23) report that animals vaccinated with pentavalent leptospiral vaccines had lower antibody titers at 1 mo than at 2 mo post-vaccination and all serovar antibody titers were < 400 at 2 mo post-vaccination. Not surprisingly, fewer vaccinated cows had positive antibody concentrations in the fall than in the spring. This observation corresponds with previous reports of relatively low (MAT < 400) and short-lived (1 to 3 mo) vaccine-induced MATs in most cattle (17,25–27).

These 2 studies suggest leptospiral infection in western Canadian cow-calf herds is uncommon. While infections with serovar hardjo may have been underestimated in both cows and calves, a 2002 field study of 183 aborted fetuses from this region found no evidence of leptospirosis (28). Similarly, a regional diagnostic laboratory reported no cases of abortion due to leptospirosis in either 2002 (29) or 2004 (30). Given the widespread use of *Leptospira* vaccines and other factors, including; age, season, geographic location that may be associated with antibody titers, the usefulness of the MAT to examine the prevalence of leptospiral infections in western Canada is limited and other testing methods should be incorporated into future research. Further information is necessary to make sound vaccine recommendations for leptospirosis in cow-calf herds in western Canada.

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