

# Article

## Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis*, *Neospora caninum*, *Bovine leukemia virus*, and *Bovine viral diarrhea virus* infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity

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**Abstract** — A province-wide, cross-sectional seroprevalence and agroecological risk factor study of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Neospora caninum* (NC), *Bovine leukemia virus* (BLV), and *Bovine viral diarrhea virus* (BVDv) genotypes 1 and 2 (BVDv1 and BVDv2) infection in dairy cattle herds in Alberta was conducted. Among adults, the seroprevalence of MAP, NC, and BLV was 9.1%, 18.5%, and 26.9%, respectively. For MAP, based on a herd test cutpoint of 2 or more seropositive cows, 58.8% of herds were infected. Herd-level seroprevalence for NC and BLV was 98.7% and 86.7%, respectively, based on a herd-test cutpoint of 1 seropositive cow. Among unvaccinated dairy heifers, the seroprevalence for BVDv1 and BVDv2 infection was 28.4% and 8.9%, respectively, while herd-level infection was 53.4% and 19.7%. Seroprevalence for MAP varied moderately by agroecological region, whereas that for NC, BLV, and BVDv1 and BVDv2 did not. For MAP, aridity and soil pH (correlated features of the region) were also important.

**Résumé** — Séroprévalence des infections à *Mycobacterium avium* sous-espèce *paratuberculosis*, à *Neospora caninum*, au virus de la leucémie bovine et au virus de la diarrhée bovine virale parmi les bovins et les troupeaux laitiers de l'Alberta et facteurs de risques agroécologiques associés à la séropositivité. Une étude de séroprévalence transversale et de facteurs de risques agroécologiques a été menée à la grandeur de l'Alberta sur l'infection des troupeaux laitiers par *Mycobacterium avium* sous-espèce *paratuberculosis* (MAP), *Neospora caninum* (NC), le virus de la leucémie bovine (VLB) et le virus de la diarrhée bovine virale (VDB<sub>v</sub>) génotype 1 et 2 (VDB<sub>v1</sub> et VDB<sub>v2</sub>). Parmi les adultes, la séroprévalence au MAP, NC et VLB était respectivement de 9,1, 18,5 et 26,9 %. Pour le MAP, avec un seuil de 2 vaches positives ou plus par troupeau, 58,8 % des troupeaux étaient infectés. Le niveau de séroprévalence de troupeaux pour NC et le VLB était respectivement à 98,7 et 86,7 %, avec un seuil d'une vache séropositive par troupeau. Parmi les génisses laitières non vaccinées, la séroprévalence pour les infections au VDB<sub>v1</sub> et VDB<sub>v2</sub> était respectivement de 28,4 et 8,9 % alors que le taux d'infection des troupeaux était de 53,4 et 19,7 %. La séroprévalence du MAP varie modérément selon les régions agroécologiques alors que pour NC, le VLB et le VDB<sub>v1</sub> et VDB<sub>v2</sub> la séroprévalence ne variait pas. Pour MAP, l'aridité et le pH du sol (caractéristiques combinées des régions) étaient également importants.

(Traduit par Docteur André Blouin)

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### Introduction

Losses due to infectious diseases of livestock generally fall into 3 categories: 1) mortality, morbidity, impaired productivity, or all three, 2) lack of livestock market access for regional, national, or international trade, and 3) public

perceptions of food safety/public health risks associated with consumption of food products from infected animals or herds. In recent years, while focus has increasingly been on infectious diseases with implications for either national or international trade, or public health/food safety risks,

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concern has remained among various livestock industries about the so-called “production-limiting diseases.” In response to these ongoing concerns, national and provincial agencies and cattle livestock industry groups formed a Production Limiting Diseases Committee (PLDC), whose initial efforts to quantify and characterize the prevalence and risk factors for 4 major diseases in Canada have led directly to the present study. Specifically, initial efforts to support inquiries into the magnitude of impacts from infections due to 1) *Mycobacterium avium* subspecies *paratuberculosis* (MAP — the causative agent of Johne’s disease [JD]), 2) *Neospora caninum* (NC), 3) *Bovine leukemia virus* (BLV), and 4) *Bovine viral diarrhoea virus* (BVDv) have been underway since 1998 nationally (1). Recent efforts to reinvigorate the Johne’s Disease Working Group and enact a voluntary Johne’s disease control program in Alberta have led to a multipronged approach to education and training, research, and extension (2).

Johne’s disease is a chronic, insidious disease of ruminants worldwide (3,4). Besides causing significant death, culling, and production and reproduction losses due to clinical and subclinical disease (3,5–7), MAP has also recently been implicated as a cause of Crohn’s disease in humans (8,9). While this association remains unproven and contentious (10), public perception of a causal link represents one of the most important economic risks to the milk and meat industries. In addition, paratuberculosis (another term for Johne’s disease) is on the list of “multiple species diseases” notifiable to the World Organization for Animal Health (4). A previous prevalence study in Alberta (11) suggested that levels of MAP infection in dairy herds are at least as high as in other jurisdictions in Canada and the United States (1,12–15). Some U.S. studies also have pointed to soil type and farm management as being associated with an increased prevalence of MAP (16–19).

Neosporosis is an infectious disease caused by NC, which can result in abortion or embryonic death in infected cattle. The disease is world-wide in distribution and is most commonly acquired via point-source exposure (3). No recent studies of the prevalence of NC in dairy cattle have been reported for Alberta. A recent study of beef cattle in northern Alberta found the seroprevalence to be 9.0%, compared with 13.5% of 260 samples collected earlier in the 1980s (20). Other historical data, this time from the Maritime Provinces (Maritimes), show that the seroprevalence in dairy cattle of those areas has also decreased in recent years (21). No published data were available for dairy herds in Alberta.

*Bovine leukemia virus* causes enzootic bovine leukosis (EBL), characterized by lymphosarcoma in cattle. Clinical signs of the disease include weight loss, pallor, decrease in milk production, and enlargement of all lymph nodes (3,4,22). Infection is often subclinical and can occur at any age, though tumors are typically found in animals over the age of 3 y (4). No recent prevalence studies of BLV have been reported for dairy herds in Alberta. In 1980, Samagh and Kellar (23) found that 12.5% of the dairy herds in Alberta harbored cows infected with BLV. In a 1997 study in Ontario, several farm management-related factors, including purchasing animals from outside sources, average weaning age, and housing practices for calves, were found to increase the likelihood of seropositivity for antibodies

to BLV (24): 1330 cows from 102 dairy herds were tested: 23% of the cows tested were positive for BLV, and 69.6% of herds had at least 1 seropositive cow (24). Recently, in the Maritimes, the overall prevalence of BLV infection was found to be 20.8%, with a within-herd prevalence of 30.9% among infected herds; 70% of the herds tested had at least 1 seropositive cow (1).

Bovine viral diarrhoea (BVD) is a multifaceted disease, variably characterized by fever, diarrhoea, and oral erosions, caused by BVDv. The disease is world-wide in distribution and often subclinical in young cattle (3). Cattle are typically infected in 1 of 2 ways: acutely via infection shortly after birth or persistently via prenatal infection (25). Persistently infected cattle are the most insidious for causing transmission within and between farms, in that they are often subclinically infected (3,25). In the Maritimes, vaccination against BVDv was found to be associated with low herd level prevalence of infection (26). Other studies have shown that the most important farm-management practice that resulted in increased risk of disease was the presence of persistently infected cattle and calves not receiving adequate colostrum (3,27). There are no published estimates of BVDv prevalence among unvaccinated heifers in dairy herds in Alberta. However, in the Maritimes, 37.8% of unvaccinated calves > 6 mo of age were seropositive (titer  $\geq$  1:64) for antibodies to BVDv, and 46.1% of herds tested had at least 1 seropositive calf (1,26).

The objectives of the present study were as follows: 1) to estimate (a) the individual-level seroprevalence of antibodies to MAP, NC, and BLV among adult dairy cattle and to BVDv1 and BVDv2 among unvaccinated dairy heifers in Alberta, and (b) the herd-level seroprevalence of antibodies to MAP, NC, BLV, BVDv1, and BVDv2 among dairy herds in Alberta, and 2) to provide a preliminary examination of the major agroecological factors associated with seroprevalence of antibodies to MAP, NC, and BLV among individual dairy cattle and to MAP, NC, BLV, BVDv1, and BVDv2 among herds in Alberta. It is expected that this information will help to prioritize and direct future research and control programs in the province and, further, will be integrated into any national PLDC research and control campaigns.

## Materials and methods

### Study populations and sampling procedures

A stratified, 2-stage random sampling procedure was used to obtain valid estimates of individual- and herd-level seroprevalences for antibodies to the pathogens of interest in Alberta. Comprehensive individual cow as well as dairy herd sampling frames were unavailable. Instead, sampling was stratified, based on eligible and willing veterinarians (who subsequently randomly selected herds and collected random samples within herds), as described below.

The target population for this study comprised cattle in dairy herds in Alberta. The study population was limited to cattle in herds serviced by Johne’s disease accredited veterinarians; that is, veterinarians accredited by the Alberta Johne’s Control Program as of January 2002. The sampling frame was the listing of all dairy cattle owned by the client base of the accredited veterinarians.

The list included 102 veterinarians working throughout Alberta. A letter of introduction, a basic information packet, and enrollment forms for dairy and beef studies were mailed to each accredited veterinarian to gauge interest in the study. Sixty accredited veterinarians expressed interest in participating; of these, 27 indicated that their client base included dairy herds. All respondents indicated that they also had beef herds in their client base.

Twenty-four veterinarians formally agreed to participate in the study. Each veterinarian was asked to provide the number of dairy herds in his or her practice. The sampling frame comprised cattle in 288 dairy herds that were eligible to be selected into our study. To estimate the prevalence of herds expected to be infected with MAP, within  $\pm 10\%$  (of 40%, based on prior work [11]), 75 herds (adjusted for finite population) were required. The number of animals sampled per herd was consistent with prior work by PLDC investigators (1). The number of herds to be sampled per veterinarian was determined by researchers using probability proportional to size (weighted by the number of herds in the practice), with the exception being that each veterinarian would sample at least 1 herd. Where more than 1 accredited veterinarian from a practice volunteered, the number of herds in the practice was split evenly for the purposes of the weighted sampling. As a result, each of the veterinarians was asked to sample as few as 1 and as many as 14 dairy herds.

We provided each participating veterinarian with a further information package that included random selection procedures for dairy herds and cattle within herds. The participating veterinarians used a coding system to protect the herd identity and all research communication with the herd owners (reporting of results) was through the accredited veterinarian. The study ran from early February 2002 through January 2003.

The sampling protocol for randomly selecting the dairy herds was as follows: each veterinarian was advised to select a specified number of participating herds; herds were required to have more than 30 adult cattle (females, 2nd lactation and older; males, 3 y and older) in order to participate; herds were randomly selected from ordered client lists, which were assigned random number lists by the researchers; if a herd owner did not wish to participate, the veterinarian simply moved — in order — to the next random number until he or she reached his or her quota of eligible herds.

The sampling protocol for selecting the animals within each dairy herd was as follows. The clinic veterinarian(s) used systematic random sampling protocols ( $n/30$  sampling interval between animals, with a random starting point) to select 30, 2nd lactation and older cows (or males over 36 mo), 7, 1st lactation cows, and 5 unvaccinated (for BVDv) heifers  $> 6$  mo of age from the list of eligible animals in each of the selected herds.

Blood samples were collected from the randomly selected animals into serum-separator tubes. The veterinarian collected 5–8 mL of blood/vial from the median caudal (tail) vein of each selected animal (4 vials for adults, 2 vials for juveniles). The animal's identification was recorded on each vial and on the submission form, along with the age, sex, and breed. Samples were packaged and submitted on ice (4°C) to the Alberta Agriculture,

Food and Rural Development — Food Safety Division (AAFRD–FSD) Edmonton laboratories within 24 h after sampling.

### Laboratory procedures and assays

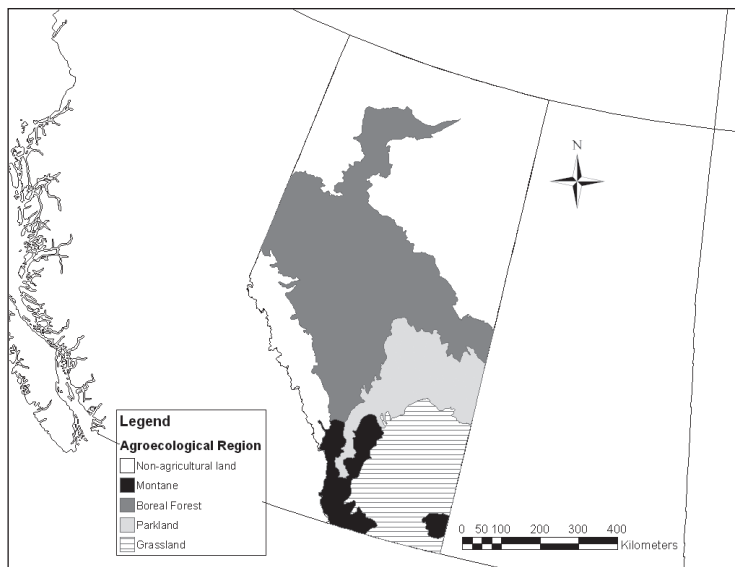
Upon arrival at the AAFRD-FSD laboratories, blood samples were centrifuged and serum samples were harvested. Serum samples from individual test animals were assigned a unique bar code number for tracking purposes and stored frozen at  $-72^{\circ}\text{C}$  until use. One vial of adult serum was stored for future confirmatory testing (if needed), 1 vial was sent for testing for antibodies to BLV at the laboratory of the Canadian Food Inspection Agency (CFIA) in St. Hyacinthe, Quebec, and 2 vials were forwarded to the AAFRD-FSD serology laboratory for testing for antibodies to MAP and NC. Sera from the dairy heifers only were shipped first to the CFIA laboratory in Lethbridge for testing for antibodies to BVDv1, and subsequently forwarded to the University of Guelph for testing for antibodies to BVDv2. Serological laboratory assays for MAP and NC were conducted in sequence as the samples arrived, and results reported to the herd veterinarians soon afterwards. Serological laboratory assays for BLV, BVDv1, and BVDv2 were conducted en masse, once all samples had been received.

An enzyme linked immunosorbent assay (ELISA) (BIOCOR® Parachek® ELISA; BIOCOR Animal Health, Omaha, Nebraska, USA) was used to analyze the samples for the presence of antibody to MAP. The procedure employed followed the directions in the product insert, except that the capacity, efficiency, and accuracy of the test was increased by using an automation workstation (Beckman Biomek; Beckman Coulter, Fullerton, California, USA). The test sample result for the ELISA was determined by using the optical density (O.D.) and the cut-off value. The cut-off value was the mean O.D. of the negative control plus 0.100. A positive result was an O.D. value  $>$  the cut-off value. A negative result was an O.D. value  $\leq$  the cutoff value. The sensitivity and specificity of this test were estimated at 47.3% and 99%, respectively (28).

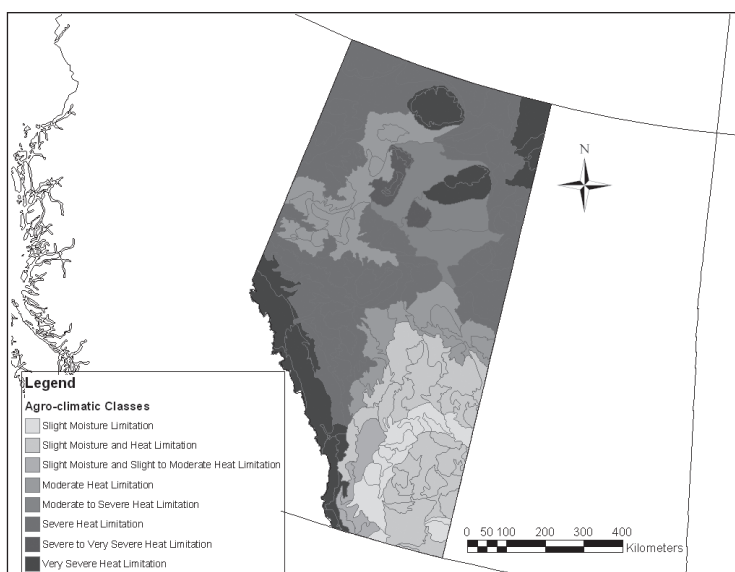
An ELISA kit (IDEXX® Herdchek® ELISA kit; IDEXX Laboratories, Westbrook, Maine, USA) was used to determine the presence of antibodies to NC. The procedure followed the manufacturer's guidelines with the following exceptions: 1) the procedure was automated by using an automation workstation (Beckman Biomek 2000; Beckman Coulter); and 2) if the sample-to-positive (S/P) ratio was  $\geq 0.4$ , the sample was classified as positive for antibodies to NC (0.5 is the S/P cutpoint recommended by the manufacturer). The 0.4 S/P test cutpoint has been validated in the laboratory with sensitivity estimated at 97.6% and specificity at 99.5% (29).

An ELISA kit for determining the presence of antibodies to BLV (IDEXX® Herdchek® Anti-BLV ELISA kit; IDEXX Laboratories) was used according to the manufacturer's instructions, with sensitivity and specificity reported as 98.5% and 99.9%, respectively (30). The testing for antibodies to BLV was performed by the same CFIA Laboratory of Excellence utilized previously (1).

To determine the presence of BVDv1, a standard virus neutralization (VN) test (using the cytopathic Singer strain)



**Figure 1.** Major agroecological regions in Alberta.



**Figure 2.** Agro-climatic classes representing heat and moisture limitations in Alberta.

involving initial screenings of sera at dilutions of 1/2 and 1/4 was used (31). Sera found positive at this stage were subsequently assayed by using dilutions of 1/2 to 1/256. The sensitivity (99.6%) and specificity (100%) were as described previously (31). To determine the presence of BVDv2, a standard VN assay (using the NVSL125 strain) was used in the Animal Health Laboratory, University of Guelph (32,33). Titers > 1:2 were considered positive.

### Agroecological risk factors

Farm and ranch locations were for the “quarter-section” of land representing the location of the dairy barn. The land locations — provided by the dairy producers — were converted to latitude and longitude with projection based on the North American datum of 1983 (NAD83) (an earth-centered datum based on the Geodetic Reference System of 1980). These locations were used to relate the herd seroprevalence data to: 1) agroecological regions (boreal

forest, parkland, grassland, montane [Figure 1]); 2) agro-climatic features (heat and moisture limitations [Figure 2]); 3) landscape features (plains, valleys, uplands); and 4) soil features (soil orders, texture, pH) (34). By using the algorithms described in an Alberta Soil Database (34), these features were joined and related for agricultural regions of Alberta and then appended to each of the dairy herds in a software program (ArcMap 8.1; Environmental Systems Research Institute, Redlands, California, USA) as follows. Each farm was nested in the most refined polygon for each of the climate and soil landscape feature data sets. Where multiple features were applicable (multiple soil types per polygon), the following decision tree was followed: 1) the value for the uppermost soil horizon was always chosen over that for lower horizons; 2) the value for disturbed soils was chosen over that for undisturbed or natural soils; and 3) the dominant soil type was chosen over lesser soil types. In the end, a single soil feature representing the dominant



**Table 1. Individual animal and herd seroprevalence for *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Neospora caninum* (NC), *Bovine leukemia virus* (BLV), *Bovine viral diarrhoea virus* genotype 1 (BVDv1), and *Bovine viral diarrhoea virus* genotype 2 (BVDv2) among dairy cattle and herds in Alberta**

Pathogen	Animal type	Individual (seropositive) % (95% CI)	Herd <sup>a,b</sup> (≥ 1 seropositive) % (95% CI)	Herd <sup>a,b</sup> (≥ 2 seropositive) % (95% CI)
MAP	All adult cows ( <i>n</i> = 2819 animals in 77 herds)	9.1 (6.3–13.0)	70.2 (53.7–86.6)	58.8 (42.2–75.4)
	— 2nd and greater lactation ( <i>n</i> = 2311 animals in 77 herds)	10.9 (7.4–15.7)	—	—
	— 1st lactation ( <i>n</i> = 508 animals in 73 herds)	0.4 (0.1–1.5)	—	—
NC	All adult cows ( <i>n</i> = 2816 animals in 77 herds)	18.5 (12.0–27.5)	98.7 (97.5–99.9)	88.5 (77.7–99.2)
	— 2nd and greater lactation ( <i>n</i> = 2308 animals in 77 herds)	19.0 (11.9–29.2)	—	—
	— 1st lactation ( <i>n</i> = 508 animals in 73 herds)	15.9 (11.3–21.9)	—	—
BLV	All adult cows ( <i>n</i> = 2814 animals in 77 herds)	26.9 (22.1–32.2)	86.7 (78.5–92.1)	77.7 (67.2–88.2)
	— 2nd and greater lactation ( <i>n</i> = 2306 animals in 77 herds)	28.3 (23.1–34.1)	—	—
	— 1st lactation ( <i>n</i> = 508 animals in 73 herds)	19.8 (12.6–29.6)	—	—
BVDv1 <sup>c</sup>	Unvaccinated heifers ( <i>n</i> = 179 animals in 36 herds)	28.4 (14.6–48.0)	53.4 (30.4–75.0)	32.2 (9.9–54.4)
BVDv2 <sup>c</sup>	Unvaccinated heifers ( <i>n</i> = 179 animals in 36 herds)	8.9 (3.7–20.1)	19.7 (7.4–43.0)	9.2 (1.3–17.2)

<sup>a</sup>Herd positivity for MAP, NC, and BLV is based on the entire sample including cows of all lactations

<sup>b</sup>Herd positivity for BVDv1 and BVDv2 is based on the 5 younger animals tested in each herd

<sup>c</sup>Based on a titer of ≥ 1:2

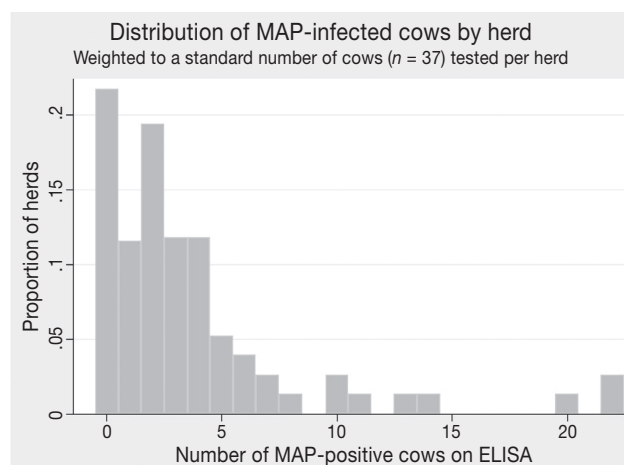
soil class for the upper horizon in disturbed (cultivated or pastured) agricultural soils was applied to the herd database, along with the appropriate agroecological region and climatic features.

### Statistical analyses

All analyses were performed by using standard statistical software packages (SPSS version 12.0; SPSS, Chicago, Illinois, USA); (Stata release 8; Stata Corporation, College Station, Texas, USA), or (SAS version 9.2; SAS Institute, Cary, North Carolina, USA).

Descriptive statistics were compiled as follows. The stratified 2-stage sampling scheme required that prevalence estimates and standard errors be adjusted for strata (veterinarian), primary sampling unit (herd), and sampling weights (inverse of the product of the probability of herd and cows being sampled) (35). The 'svyset' command (Stata release 8; Stata Corporation) permitted the combined adjustment of these and for correlated observations within herd. Individual- and herd-level seroprevalence (+/- 95% confidence interval [CI]) for infection with each of MAP, NC, BLV, BVDv1, and BVDv2 were estimated using 'svyprop' and 'svytab' (Stata release 8; Stata Corporation) commands (36,37). Because of the low sensitivity of the serological test for MAP, an estimate of the true individual-level prevalence (adjusted for imperfect sensitivity and specificity) was calculated (38).

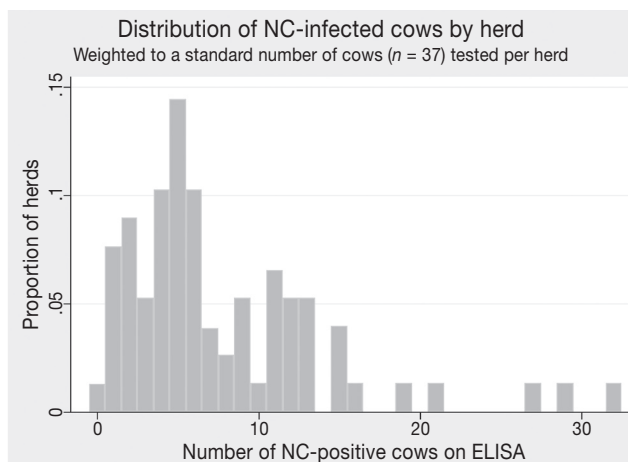
In exploratory analyses, the bivariable association of the individual- and herd-level serological status for each of MAP, NC, BLV, BVDv1, and BVDv2 with each of the agroecological risk factors was modelled in a generalized linear model (GLM) (39) framework (SAS PROC GENMOD — SAS Institute) by using a binomial error distribution and a logit link function. Ratio statistics assessing the odds of seropositivity (individual or herd) for each level of the agroecological risk factor versus a baseline level were calculated. Agroecological risk factors significantly associated (based on likelihood ratio [LR] statistics at *P* < 0.05) with outcomes in bivariable analyses were then further assessed in multivariable models. For agroclimatic risk-factor analysis purposes, heat limitations



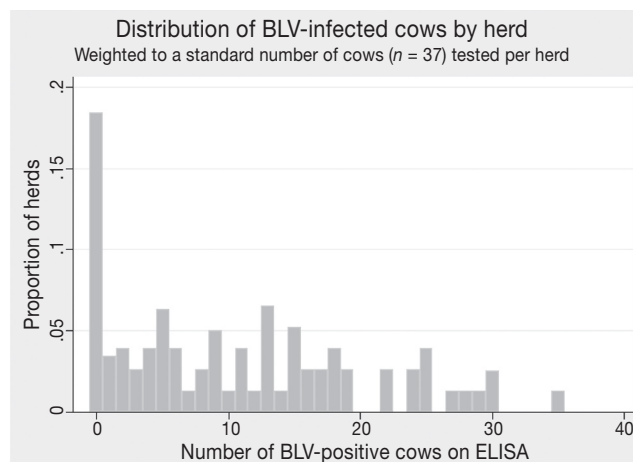
**Figure 3.** Distribution of MAP-infected cows by herd (*n* = 77 herds tested).

for crop growth and climate aridity were broken-out and treated as separate risk factors.

Multivariable model-building approaches were as follows. Herd size (dichotomized at the median — 90 cows) was assessed in each model. Individual animal age, or demographic age structure of the herd, was not included in the models as a potential confounder, since an equal number of 1st (*n* = 7), and 2nd and greater (*n* = 30), lactation animals were drawn from each herd. Subsequently, saturated models were created by using all variables significant in the bivariable analyses. Variables (or groups of indicator variables) then were removed in a backwards-elimination strategy (model-based likelihood ratio statistics at *P* < 0.05). Contingency tables and basic chi-square tests illustrated the joint frequency distribution of agroecological risk factors by region at the farm level. Since the individual-level binary outcome responses were grouped (clustered) within herds, a generalized estimating equation (GEE) (40) within the GLM framework was used to assess the significance of each of the variables, based on robust estimates of the standard errors. Indicator variables with more than 2 levels ('r' levels) were retained in the



**Figure 4.** Distribution of NC-infected cows by herd ( $n = 77$  herds tested).



**Figure 5.** Distribution of BLV-infected cows by herd ( $n = 77$  herds tested).

final models when the  $P$ -values were less than 0.05 for the generalized 'score' statistic 'T', based on a chi-square distribution with 'r-1' df.

## Results

### Descriptive analyses

Of the 81 herds, there were 77 herds with complete samples and data for 2nd and greater lactation adult cattle and land location. Of these 77 herds, there were 4 herds from which no blood samples from the 7 randomly selected 1st lactation cows were received. There was a minimum of 30 and a maximum of 405 adult cows (mean = 110.9) on the sampled farms. In addition, there was a minimum of 0 and a maximum of 180 heifers (mean = 44.6) on these same farms. There was a total of 2819 adult dairy cows (2311 — 2nd and greater lactation; 508 — 1st lactation) sampled (no bulls were selected). For dairy heifers, 36 herds meeting the inclusion criteria were sampled for a total of 179 pre-BVD-vaccinated heifers.

The seroprevalence of antibodies to MAP was 9.1% (95% CI: 6.3–13.0) for the adult dairy cows (Table 1). For 2nd and greater lactation cows the seroprevalence was 10.9%, while 1st lactation cows exhibited only 0.4% (Table 1). Based upon the imperfect performance of the serological test (47.3% sensitivity, 99% specificity), the true overall adult individual-level prevalence of MAP infection (38) was estimated to be 17.5%. Herd-prevalence estimates (dichotomized as infected herds and noninfected herds) based on cutpoints of both 1 or more (1+), and 2 or more (2+) ELISA positive cows are presented in Table 1. To minimize the possibility of false-positives at the herd-level, the 2+ cutpoint has been more widely applied in MAP seroprevalence studies (1,11,12), and on this basis, 58.8% of dairy herds in Alberta were estimated to harbor infected animals. The within-herd frequency distribution of MAP-positive cows (weighted to a standard of 37 cows tested per herd) is presented in Figure 3.

The province-wide seroprevalence of antibodies to NC was 18.5% (95% CI: 12.0–27.5) among all adult dairy cows (Table 1). There was no significant difference between 2nd and greater lactation and 1st lactation cows

**Table 2. Joint prevalence and standard errors of the mean ( $s_{\bar{x}}$  for infection/coinfection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Neospora caninum* (NC), and *Bovine leukemia virus* (BLV) among adult dairy cattle in Alberta ( $n = 2813$ )**

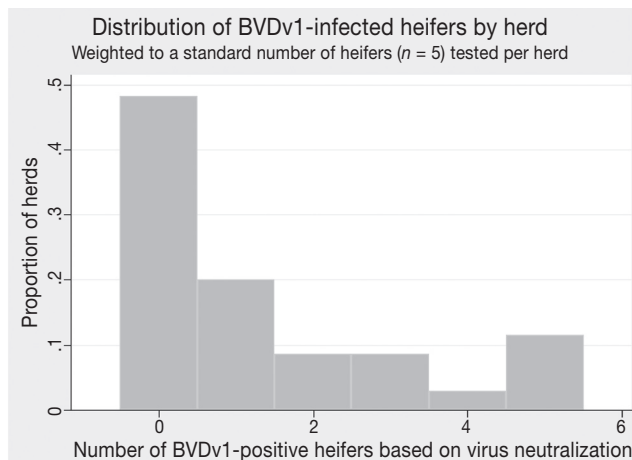
MAP	NC	BLV	Observations	Joint Probability	$s_{\bar{x}}$
0	0	0	1431	0.509	0.0094
0	0	1	580	0.206	0.0076
0	1	0	345	0.123	0.0062
0	1	1	173	0.062	0.0045
1	0	0	152	0.054	0.0043
1	0	1	58	0.021	0.0027
1	1	0	58	0.021	0.0027
1	1	1	16	0.006	0.0014

(19.0% and 15.9%, respectively), though older cows had a slightly higher seroprevalence level (Table 1). All but one of the herds (98.7%) in the province had at least 1 cow with antibodies to NC; 88.5% of herds had 2+ ELISA positive animals (Table 1). The within-herd frequency distribution of NC-positive cows is presented in Figure 4.

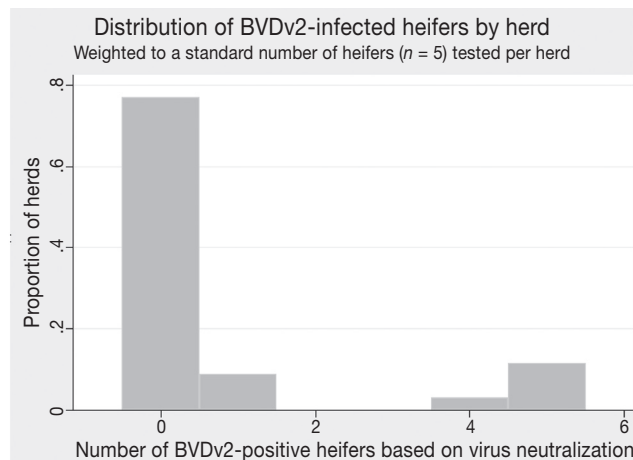
Seroprevalence of infection with BLV was 26.9% (95% CI: 22.1–32.2 [Table 1]). Cattle of 2nd and greater lactation were at a nonsignificantly ( $P > 0.05$ ) higher risk of infection (28.3%) than 1st lactation cattle (19.8%) (Table 1). Most herds (86.7%, 95% CI: 78.5–92.1) had at least 1 infected animal in the herd; 77.7% had 2+ infected animals (Table 1). Beyond the 13.3% of herds with no BLV-infected animals, the within-herd frequency distribution of BLV-positive cows is uniform between 1 and 30 animals (Figure 5).

The joint frequency distribution of apparent prevalence of infection with each of MAP, NC, and BLV among adult cattle is tabulated in Table 2. Of 2813 cattle with valid test results for all 3 assays, 50.9% of the animals were serologically negative for all 3 infectious agents, only 16 were positive for all 3.

Among the smaller number of herds ( $n = 36$  out of 77 total) that had not vaccinated their heifers before 6 mo of age, the seroprevalence for BVDv1 infection



**Figure 6.** Distribution of BVDv1-infected heifers by herd ( $n = 36$  herds tested).



**Figure 7.** Distribution of BVDv2-infected heifers by herd ( $n = 36$  herds tested).

**Table 3. Contingency table illustrating association of ecological risk factors with the aggregated index of agroecological region at the herd-level ( $n = 77$  herds)**

Ecological risk factor	Level of risk factor	Grassland	Agroecological region			Significance ( $P$ -value) <sup>a</sup>
			Montane	Parkland	Boreal forest	
pH	< 7.0	6	4	30	13	< 0.001
	≥ 7.0	14	5	3	2	
Arid climate	No	0	6	28	15	< 0.001
	Yes	20	3	5	0	
Heat limitation	Area 2	14	3	30	2	< 0.001
	Areas 3 & 4	6	6	3	13	

<sup>a</sup> $P$ -values were derived from asymptotic Pearson chi-square 2-sided tests of association comparing agroecological region to each of the other ecological risk factors

was 28.4% (95% CI: 14.5–48.0 [Table 1]). The herd-level seropositivity (at least a single heifer out of 5 sampled was positive) was 53.4% (32.2% based on a cutpoint of 2+) (Table 1). Considering only BVDv genotype 2 infection, the seroprevalence was markedly lower at 8.9% overall (95% CI: 3.7–20.1), with 19.7% of the herds containing infected calves (9.2% using a 2+ cutpoint) (Table 1). Within-herd frequency distributions of infected heifers (weighted to a standard sample of 5 animals per herd) for BVDv genotypes 1 and 2 are presented in Figures 6 and 7, respectively.

### Agroecological risk-factors

Agroecological region (Figure 1) is a composite summary index of a variety of climatic, soil, and vegetative features of the landscape in Alberta. Several of the ecological risk factors, such as climatic aridity, heat limitations for plant growth, and soil pH, were highly associated ( $P < 0.001$ ) with region (Table 3). Serological evidence of MAP infection at the herd level (2 or greater seropositive cows) was significantly associated with agroecological region in bivariable analysis ( $P = 0.026$ ) and multivariable analysis adjusted for herd size ( $P = 0.034$ ). For the agroecological region, with grassland as the referent level (Figure 1), the unadjusted odds ratios (OR) for the other regions were montane (OR: 4.78, 95% confidence interval [95% CI]: 0.74–30.93), parkland (OR: 5.85, 95% CI: 1.62–21.10), and boreal forest (OR: 1.44, 95% CI: 0.37–5.57). None of the other agroecological risk factors was associated

( $P < 0.05$ ) with the herd-level risk of infection with MAP in bivariable analyses. Owing to NC's wide distribution (seroprevalence of 98.7% of herds based on 1 or greater infected animals out of 37 tested), there were no agroecological factors associated with its herd-level risk of infection. Likewise, for BLV, BVDv1, and BVDv2, none of the agroecological risk factors was found to be significantly associated ( $P < 0.05$ ) with the herd-level risk of infection.

At the individual level, there were several agroecological risk factors associated ( $P < 0.05$ ) with the risk of MAP seroprevalence in bivariable analyses. These included agroecological region, terrain morphology, soil pH, climate aridity, and heat limitations. When adjusted for herd size and within-herd dependence of the outcome variable, only agroecological region, soil pH, and climate aridity remained significant ( $P < 0.05$  [Table 4]). Because several of the features (soil pH, aridity) within the indexed regions were associated (nesting), the 3 ecological risk factors could not be included in the same model. The 3 competing models (I, II, and III), representing agroecological region, climatic aridity, and soil pH, respectively, are presented in Table 4.

In bivariable analysis, individual-level seroprevalence to NC infection was significantly associated ( $P < 0.001$ ) with agroecological region in a similar pattern to that seen for MAP. However, when adjusted for within-herd dependence of outcomes, agroecological region was no longer important ( $P = 0.21$ ).

**Table 4. Three alternative models of associations of ecological risk factors with individual MAP seroprevalence among dairy cattle in Alberta. Model I: agroecological region, Model II: climate aridity, and Model III: soil pH. All models are generalized estimating equation-adjusted for within-herd dependence of outcomes. Intercept not shown**

Risk factor	Level of risk factor	Odds ratio (OR)	95% confidence interval (OR)	<i>P</i> -value <sup>a</sup>
Model I:				
Herd size	Small (< 90 cows)	0.51	0.27–0.96	0.05
	Large (≥ 90 cows)	—	—	
Agroecological region	Grassland	0.33	0.14–0.76	0.05
	Montane	0.55	0.32–0.95	
	Boreal forest	0.87	0.37–2.06	
	Parkland	—	—	
Model II:				
Herd size	Small (< 90 cows)	0.55	0.30–1.01	0.07
	Large (≥ 90 cows)	—	—	
Arid climate	No	1.94	1.05–3.57	0.04
	Yes	—	—	
Model III:				
Herd size	Small (< 90 cows)	0.54	0.29–0.98	0.06
	Large (≥ 90 cows)	—	—	
pH	< 7.0	1.92	1.14–3.22	0.03
	≥ 7.0	—	—	

<sup>a</sup>*P*-value was derived from a Type III generalized estimating equations (GEE) analysis in SAS (40) assessing the overall significance of each risk factor over all levels of the variable

MAP = *Mycobacterium avium* subspecies *paratuberculosis*

There were no significant agroecological factors associated with BLV, BVDv genotype 1, or BVDv genotype 2 seroprevalence at the individual level.

## Discussion

The sampling frame and study design employed in this investigation were generally similar to those used in other published PLDC seroprevalence studies (1,41), with several exceptions. Whereas the herds eligible for inclusion in the earlier studies had to be Dairy Herd Improvement (DHI) subscribers, the herds in the present study were restricted to dairy clients of Johne's control program veterinarians in Alberta, and not limited by DHI subscriber status. Our sampling frame represented approximately 288 out of the 817 herds licensed to operate as of January 1, 2002, with an average herd size of 111 adult cows among our random sample of 77 herds.

Another difference between the present and past PLDC studies was in the numbers and age of animals selected for inclusion. In the previous PLDC studies, any adult cow (1st or greater lactation) was eligible to be randomly selected to be 1 of the 30 animals bled. In the present study, 30, 2nd or greater lactation animals were randomly selected. To aid in future national prevalence comparisons, 7 additional 1st lactation cows were also randomly selected and will be used to randomly replace 7 of the 30 older cattle in a regional comparison of seroprevalence for MAP, NC, and BLV in Canada. Finally, the ELISAs used in the present study for MAP and NC differed from those used in previous studies (1,41). However, estimates of true prevalence can be used to compare results between studies.

The MAP seroprevalence estimated in this study is very similar to that estimated in a recent seroprevalence

study of dairy herds in Alberta (11). This previous study utilized 1500 serum samples from adult dairy cattle in 50 herds tested with an ELISA test kit (IDEXX<sup>®</sup> Herdchek<sup>®</sup> ELISA kit; IDEXX Laboratories); 105 samples from 20 herds were positive for MAP for an apparent herd level prevalence of 40.0%, when a cutpoint of 2 or more positive test results was used for a herd to be classified as positive. The same study also included evaluation of pooled fecal cultures, giving a herd-level prevalence estimate ranging from 27.6% to 57.1%, depending on how many samples in each pool were positive — this was unknown (11).

Compared with historical data from other parts of Canada (1,41), the United States (17,42), Belgium, and The Netherlands (43,44), herd-level seroprevalence generally appears to be somewhat higher among dairy herds in Alberta. There are obvious differences in study design and diagnostic tests that should be considered when comparing these studies, such as the selection of older cows (2nd and older lactation) in the present study, which might shift the probability of obtaining positive test results higher, depending on the underlying demographic structure of herds in other jurisdictions.

Studies conducted elsewhere in Canada have found somewhat lower prevalence estimates than in this study (1,13,41). In 3 Maritime provinces (excluding Newfoundland), 30 dairy herds were randomly selected from each province, and 30 cows were randomly selected from each herd. These cows were surveyed by ELISA for the presence of antibodies to MAP; 2.6% of cows tested positive, for an apparent herd seroprevalence of 16.7%, assuming that a minimum of 2 positive cows constituted a positive herd (1). In a similar study in Saskatchewan, where 1530 cattle were tested, the province-wide seroprevalence for MAP was 2.7% and the herd prevalence (using the 2 or



more positive cows cutpoint) was 24.3% (41). These results suggest that dairy cattle and herds in Alberta may exhibit a greater seroprevalence of MAP infection than that of other provinces in Canada.

In the United States, results have been almost uniformly of similar or lower seroprevalence than in the present study, with 1 major exception. In Colorado, dairy cows were surveyed using an ELISA, and 424 out of 10 280 (4.12%) cows were classified as seropositive; the within-herd prevalence was estimated to be up to 7.82% (42). In that study, of 15 large herds tested, only a single herd was classified as negative, based on seropositivity. However, the size and demographic dynamics of western large-scale dairy herds in the United States make comparisons with Canadian dairy herds difficult at the herd level. In Michigan, where the dairy industry is more similar to that in Alberta, many dairy cattle and herds also tested positive, with 55% of 121 herds tested having at least 2 positive cows (17). Nationally, however, the 1996 National Animal Health Monitoring System (NAHMS) study estimated that only 22% of U.S. herds were infected with MAP (12).

Northern European dairy production practices are somewhat similar to those in Canada. In The Netherlands, 2.5% of tested cows were seropositive, while 28% of herds had at least 2 seropositive cows (44). In Belgium, an absorbed ELISA kit was used on a stratified random sample of adult cattle 24 mo or older. The median seroprevalence of MAP infection among all herds was found to be 2.9%. The individual-animal seroprevalence was 0.87% — the herd seroprevalence was 18% (43).

The performance of the diagnostic and screening tests for MAP infection remains one of the most critical roadblocks to successful control of Johne's disease. Commercial ELISAs are commonly used to identify antibodies to MAP in blood samples from cattle. One issue with this type of test is that its sensitivity greatly depends on the stage of the disease in infected animals (14,45–47). Most seroprevalence studies that attempt to classify both cattle and herds rely on previous estimates of sensitivity (47.3%) and specificity (99%) that may or may not be appropriate. It is important to understand that all seroprevalence studies rely on screening tests, such as ELISA, which is the apparent prevalence presented; that is, even if a “true” prevalence is reported, it is an estimate calculated from assay performance criteria reported in the literature.

Some of the agroecological risk factors for seroprevalence identified in this study have been examined elsewhere (16,18,19). Two major inhibitors to MAP organism survival outside the host (increased soil pH and aridity) were noted in this study as moderately associated with a decreased risk of seropositivity. However, pH was not associated with risk in a strictly linear relationship, as noted elsewhere (16). Dichotomized pH (at 7.0) revealed a sparing effect on the risk of MAP seropositivity associated with more alkaline soils. This suggests that there may be a critical cutpoint of effect on organism viability, rather than a dose-response on a continuous linear scale. While Johnson-Ifearulundu and Kaneene (16) in Michigan and Ward and Perez (19) in Indiana found soil features — including pH — to be associated with risk of MAP seroprevalence, it is important to note that different approaches to assessing exposure were used in each of these studies as compared with those used

herein. In the present study, more northern agroecological regions appeared to favor increased seroprevalence, possibly due to prolonged indoor confinement during winter months and calving periods, and variable freeze-thaw episodes during spring months. However, both soil pH and arid climatic conditions are correlated with agroecological region and latitude, and these effects could not be separated out in the present analysis.

In this study, seroprevalence for infection with NC was estimated at levels that are comparable with those for the seroprevalence of NC in other regions of Canada (20,21,48,49). Overall, the seroprevalence in Canada is similar to the prevalence in the United States and Great Britain (50,51). Almost all herds tested in Alberta have some evidence of infection. Agroecological regional associations with the risk of seropositivity to NC (though nonsignificant when adjusted for within-herd dependence) were similar in direction between MAP and NC, in that northern ecological regions had somewhat higher seroprevalences than southern regions for both pathogens. However, neither soil type nor pH had an effect on NC seroprevalence. This is compatible with known biological features of the disease that include both domestic and wild canid hosts and a point source infection. Certainly, the northern regions of Alberta exhibit greater forested lands, which could support a larger wild canid population, as well as bringing cattle and wildlife into direct contact more often in wooded grazing lands.

The seroprevalence of BLV in Alberta is virtually the same as that estimated for other regions of Canada (1,23,41). However, the seroprevalence in Canada is generally lower than that in the United States (52). It was not surprising to find no significant ecological risk factors for BLV seroprevalence, given that infection is generally associated with management practices pertaining to cattle purchases and use of dirty needles, gloves, and other objects that are contaminated with blood (53), rather than macroecological risk factors (climate, soil type).

In our study, the seroprevalence of BVDv1 infection of unvaccinated dairy heifers in Alberta was very similar to that in the Canadian Maritime Provinces (1,26) and Saskatchewan (41).

The unique aspect of the present round of PLDC studies is that comparisons and contrasts may be more readily made among provinces. However, even with the relative consistency in study design and application, there remain some differences in methodology, laboratory assays, and the time period during which the studies were performed. Therefore, comparisons between provinces should be made with some caution. That said, these data will provide a rich addition to further efforts to quantify the risks associated with these 4 diseases and to prioritize and customize control programs in Canada.

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processing, MAP, and NC testing), as well as those of the Canadian Food Inspection Agency [CFIA] laboratories in St. Hyacinthe, Quebec, (BLV testing) and Lethbridge, Alberta (BVDv1 testing). The Animal Health Laboratory at the University of Guelph is acknowledged for the BVDv2 testing. Geographical information systems (GIS) and AGRASID 3.0 Alberta soil data analysis and support were graciously provided by resource specialists from the Conservation and Development Division of AAFRD and from Agriculture and Agri-food Canada. CVJ

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## Book Reviews

### Comptes rendus de livres

#### Veterinary Anatomy of Domestic Mammals: Textbook and Colour Atlas

König HE, Liebich H-G. Schattauer, Stuttgart, 2004, 681 pp. ISBN 3-7945-2101-3, £195.

**Y**ou can't read this book in bed: it weighs 4.7 kg. But it is a masterpiece of the publisher's art, produced in Germany on the finest chlorine-free paper in full color throughout. You are immediately struck by the high quality of the illustration, design, and printing. It is spectacular. There are many entirely new illustrations: mainly photographs of dissections, body slices, and corrosion casts of vessels and joint cavities. There are also many reworked classic diagrams, taken mainly from the long German tradition of anatomical illustration. These look bright, clean, and modern, courtesy of digital processing; a few have been politically rectified: for instance, that crop-eared dog with an uncompromising expression, whose muscular front view first appeared in Ellenberger in 1901, is now a full-eared softie that could lick you anytime.

According to the preface, this book is intended to “combine the didactic character of a textbook with the informative character of a colour atlas.” It is also intended to give a “consistent overall view of structure and function in one unit.” In this, it only partially succeeds. There is much good microanatomical and some fine histological material, but the embryologic and physiologic information is often rather scant. The account of penile erection, for instance, is misleading and not a patch on that given over 300 years earlier by Regnier de Graaf. In general, the text lacks the erudite flow of Dyce, Sack, and Wensing (*Textbook of Veterinary Anatomy*, Elsevier, 2002).

This might appear to be a multiauthor text but though there are contributors from Berlin, Munich, Vienna, Brno, Toulouse, and Barcelona, their segments are almost invariably coauthored by one or both the primary authors. The original German version is now in its 3rd edition and has been translated into Portuguese, Czech, Slovak, Spanish, Italian, and now English. Ay, there's the rub.

The English translation is idiosyncratic to say the least; it is also ungrammatical and misleading. What follows is a bad patch but not truly exceptional: “Thus can arrangement exists to prevent shoulder flexion, involving isometric contraction of

the supraspinous muscle of the forearm and more importantly the biceps brachii muscles, in which tension increases as the shoulder tends to flexes” (page 258). Where was the copy editor? But this is not the worst of it.

Officially agreed anatomical terms are listed in Latin and those of us whose daily language is not Latin are encouraged to translate terms into the vernacular where this improves understanding. English being the practical, perverse, and beautiful thing that it is, there is a long tradition of how this should be done; but the details are not written down anywhere. The translators of this book know nothing of this and make their own haphazard Latin translations that are not even internally consistent. Referring to the free part of the ruminant spleen as the “liberal” part has a certain charm, but other examples are just annoying. In the text, we find the conventional Latin term “zona pellucida,” which everyone understands, but the same structure is labelled “pellucid zone” in the adjacent diagram. Pellucid zone could mean any clear area and that is why anatomical writers stick to the Latin for this structure. In the same diagram of an ovarian follicle, the translators advisedly use the Latin “corona radiata” but mistranslate and mislabel cumulus oöphorus as “ovarian cumulus.” Why not egg cloud, I wonder? This reminds me of those cloud-shaped black structures on the margins of the pupils in ungulates. They are called “negroid bodies” in a figure label, “iridial granules” in the caption to this figure, and “granula iridica (corpra [sic] nigra)” in the adjacent text. The pars ceca retinae is confusingly labelled “Blind spot of retina.” And what could be more awkward than the “Albuginous tunic” or the “Ulnar flexor muscle of the carpus?” But at least “milk wall” for milk well is a bit of joke.

I could go on and on. There is no excuse, other translators of German texts have managed superbly (Wolfgang Sack, you are sorely missed).

In conclusion, serious morphologists will revel in the artwork and photography; neophytes risk serious confusion. It is a big price to pay for confusion.

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