Occurrence of *Mycobacterium avium* subspecies *paratuberculosis* and *Neospora caninum* in Alberta cow-calf operations

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**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) and *Neospora caninum* (NC) are two pathogens causing important production limiting diseases in the cattle industry. Significant impacts of MAP and NC have been reported on dairy cattle herds, but little is known about the importance, risk factors and transmission patterns in western Canadian cow-calf herds. In this cross-sectional study, the prevalence of MAP and NC infection in southwest Alberta cow-calf herds was estimated, risk factors for NC were identified, and the reproductive impacts of the two pathogens were assessed.

Blood and fecal samples were collected from 840 cows on 28 cow-calf operations. Individual cow and herd management information was collected by self-administered questionnaires and one-on-one interviews. Bayesian estimates of the true prevalence of MAP and NC were computed, and bivariate and multivariable statistical analysis were done to assess the association between the NC serological status and herd management risk factors, and the clinical effects of the two pathogens.

Bayesian estimates of true prevalence indicated that 20% (95% probability interval: 8–38%) of herds had at least one MAP-positive cow, with a within-herd prevalence in positive herds of 22% (8–45%). From the Bayesian posterior distributions of NC prevalence, the median herd-level prevalence was 66% (33–95%) with 10% (4–21%) cow-level prevalence in positive herds. Multivariable analysis indicated that introducing purchased animals in the herd might increase the risk of NC. The negative association of NC with proper carcass disposal and presence of horses on ranch (possibly in relation to herd monitoring and guarding activities), may suggest the importance of wild carnivores in the dynamics of this pathogen in the study area. We also observed an association between MAP and NC serological status and the number of abortions. Additional studies should be done to further examine specific risk factors for MAP and NC, assess the consequences on the reproductive performances in cow-calf herds, and evaluate the overall impact of these pathogens on cow-calf operations.

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**Keywords:** Mycobacterium avium subspecies paratuberculosis Neospora caninum Ranch management Biosecurity Bayesian prevalence estimate Risk factors Beef cattle

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1. Introduction

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC) are two important production-limiting pathogens of cattle (Chi et al., 2002). MAP is responsible for a chronic infection of the digestive tract of ruminants (Nielsen and Toft, 2009), often silent during a latent period ranging from 2 to 10 years (Whitlock and Buergeit, 1996). The clinical stage of the disease, known as Johne's disease, is characterized by chronic enteritis, diarrhea and active shedding in cattle (Behr and Collins, 2010). Johne's disease has a high economic impact due to its consequences for milk production (Kudahl et al., 2004), premature culling and reduced slaughter value (Benedictus et al., 1987; McKenna et al., 2006). It was estimated that MAP infection cost US dairy industry US$200–250 million annually (Ott et al., 1999). In Georgia (USA), MAP infection cost beef producers US$1.8–4.9 million in 2000, assuming a loss of US$75–100 per infected adult beef cow in the herd (Pence et al., 2003). Control of MAP infection usually involves reducing the risk of introduction to the herd, test-and-cull strategies, and ranch management practices related to hygiene at calving, reduction of feed and water contamination, and manure management (McKenna et al., 2006; Canadian Animal Health Coalition, 2009; Roussel, 2011). Alberta has the highest reported prevalence in dairy cattle across Canada (Tiwari et al., 2006) with a cow-level prevalence of 8% and a herd-level prevalence of 27% as determined by serum ELISA (Sorensen et al., 2003). There are only a few reports of Johne’s disease in beef cattle in Alberta, indicating a lower cow-level prevalence (1.5%) than in dairy herds and providing little information on risk factors in cow-calf herds (Scott et al., 2007). Given the impact on production, together with the potential correlations between MAP infection and Crohn’s disease in humans (Behr and Kapur, 2008; Hermon-Taylor, 2009; Barkema et al., 2010), and the high incidence and prevalence of Crohn’s disease in North America and more specifically in Alberta (Moledgey et al., 2012), it is essential to better assess the status of the beef cattle production system regarding MAP infection in the province.

Neosporosis, caused by the protozoan parasite *N. caninum*, is another important production limiting disease of dairy and beef cattle. The estimated cost of NC infection in beef cattle herds in Texas (USA) was estimated at US$23–35 per head or US$978–1479 per 42-head infected herd with a 20% prevalence of NC infection (Kasari et al., 1999). This parasite is found as tachyzoites or tissue cysts in muscles, nervous tissues and reproductive tracts of infected ungulates, and can cause infertility, abortion and birth of weak or chronically infected calves (vertical transmission) (Dubey, 1999). Wild and domestic carnivores, definitive hosts of this parasite, are infected by consumption of tachyzoites or tissue cysts from infected intermediate hosts (Rosypal and Lindsay, 2005; Dubey et al., 2007). The parasite reproduces sexually in the gastrointestinal tract of the carnivore and releases oocysts in the feces. These oocysts sporulate in the environment and can infect cattle through oral exposure (horizontal transmission) (Dubey and Lindsay, 1996). Therefore, horizontal transmission between definitive and intermediate hosts is associated with a predator–prey life cycle and often related to the presence on farm of dogs (Dijkstra et al., 2002) and wild carnivores (Wapenaar et al., 2006; Dubey et al., 2011) having access to infected tissues and abortion material. In contrast, vertical transmission (from dam to fetus) involves other herd management risk factors such as the purchase of replacement heifers and control measures such as culling of infected cows (Dubey et al., 2007). Presence of NC was previously reported in beef cattle in the province (Waldner et al., 2001; Scott et al., 2007; Waldner and Kennedy, 2008), but it was suggested that more information about the risk factors, the relative importance of horizontal or vertical transmission, and impact of NC was required in the different provinces of Canada (Haddad et al., 2005). With a high density of cow-calf herds, wild ungulates and wild carnivores, southwestern Alberta is a particularly interesting area to investigate the occurrence, transmission patterns and risk factors of NC (Morehouse and Boyce, 2011; Pruvot et al., 2014).

The objectives of this cross-sectional study were to describe the prevalence of MAP and NC infection in south-west Alberta cow-calf herds, identify risk factors associated with NC, assess their reproductive impact, and report on ranch management practices relevant to NC, MAP, and the occurrence of infectious diseases in cow-calf operations.

2. Materials and methods

2.1. Herd selection and sampling

Cow-calf operations maintaining more than 100 adult cattle were selected in the municipal districts of Pincher Creek, Crowsnest Pass, Willow Creek, and Cardston, Alberta, Canada. Among 54 operations initially identified by local veterinarians and preliminary ranch visits, 30 agreed to participate. Because the incubation period of MAP is long (Whitlock and Buergeit, 1996), cows of second parity or greater were selected to increase the probability of detection of MAP. Thirty such cows from each ranch were selected by systematic sampling during handling operations in Fall/Winter 2011 (1 sample every *N* cows, with *N* being the total number of cows meeting the selection criteria divided by 30, the first cow being randomly picked among the *N* first cows using a random number table). From each sampled cow, a fecal sample was collected from the rectum and a blood sample from the coccygeal vein. This sample size allowed for the detection of a percentage of positive individuals of at least 10% with 95% confidence. At sampling, the following individual cow data were recorded: animal ID, sex, age, breed, origin (born on ranch or purchased), pregnancy status (if a pregnancy diagnosis was performed by the veterinarian), parity, and history of abortion. The protocol of this study was reviewed and approved by the Animal Care Committee of the University of Calgary under the certification file M09123.

2.2. Ranch management data collection

Previously published risk factors for the introduction, intra-herd transmission, and maintenance of MAP and NC
were identified from peer-reviewed literature (Obasanjo et al., 1997; Daniels et al., 2002; McKenna et al., 2006; Dubey et al., 2007; Norton et al., 2009; Behr and Collins, 2010; Vanleeuwen et al., 2010a). Management data were collected from ranchers using a self-administered questionnaire. The questionnaire consisted of 51 questions on pasture/feed/water management, health management and history, reproduction, biosecurity, and contact with other livestock and wildlife species, of which 20 were relevant to the purpose of this paper. In particular, the numbers of abortion and calf mortality over the last 4 years were documented. Given the absence of accurate data on wild carnivore densities and other wildlife, we also asked the respondents to score coyote, wolf, rabbit and crow relative densities on a 0–4 continuous scale. The overall wild carnivore density was estimated by adding the coyote and wolf scores for each ranch. Additional data on pasture rotation, feed, water management were collected in follow-up one-on-one interviews and mapped on a Geographic Information System software. Prior to the start of the study, questionnaire and interview material (Supplementary document 1) were internally and externally reviewed, and field-tested on individuals similar to the target population to ensure the material was easily understandable and could accurately measure the variables of interest. This material was approved by the University of Calgary Conjoint Faculties Research Ethics Board (file no. 6598).

The elk density on each ranch was estimated by averaging the values of a kernel density estimate (in elk per km²) obtained from elk winter surveys (provided by Alberta Sustainable Resource Development/Alberta Conservation Association) over the land of each ranch in ArcGIS 10 (ESRI, Redlands, CA, USA).

2.3. Laboratory procedures

2.3.1. MAP serology and fecal culture

Serum samples were tested in duplicates with the IDEXX Mycobacterium paratuberculosis Antibody Test Kit (IDEXX, Westbrook, Maine, USA) according to manufacturer’s instructions. Results were expressed as a mean sample-to-positive ratio (S/P ratio), and the manufacturer-recommended cut-off of 40% was used. The manufacturer-reported sensitivity (SeMAP) and specificity (SpMAP) of this test against a culture “gold standard” are 51.4% (95% confidence limit 45.2–57.6%) and 99.3% (95% confidence limit 98.3–99.7%) (Milner et al., 1990), respectively. However, later reports indicated lower sensitivity (Nielsen and Toft, 2008; Whitlock et al., 2000).

For each ranch, fecal samples were sorted by increasing cow age and pooled by five (150 pools) (Kalis et al., 2000). Fecal sample pools were cultured with the TREK ESP® Culture System and reagents (TREK diagnostic systems, Cleveland, OH, USA) as previously described (Forde et al., 2012). All samples from positive pools were subsequently cultured individually. DNA was extracted from the broth of all fecal cultures (pool cultures, and subsequent individual culture of positive pools) and all extracts were tested by polymerase chain reaction (PCR) of the target sequence IS900 (Vary et al., 1990).

2.3.2. NC serology

Serum samples were tested in duplicates using a commercial NC competitive ELISA kit (Neospora caninum Antibody Test Kit, CELISA, VMRD, Inc., WA, USA) according to the manufacturer’s recommendations. Results for each sample were expressed as a mean percentage of inhibition, and the manufacturer-recommended cut-off of 30% was used. The manufacturer-reported sensitivity (SeNC) and specificity (SpNC) of this test are 96% and 99%, respectively (VMRD, Inc., WA, USA; Baszler et al., 2001).

2.4. Statistical analyses

2.4.1. Prevalence estimate

True prevalence was estimated using a Bayesian prevalence estimation model in WinBUGS (code available on www.epi.ucdavis.edu/diagnostictests/software.html) to account for the uncertainty around sensitivity and specificity of MAP and NC tests. The model is based on a mixture model, where a herd can be either infected or not, according to a probability equal to the herd prevalence (HPMAP and HPNC). If it is infected, the intra-herd prevalence follows a prevalence distribution (PDMAPl and PDNC) of mean μ (μMAP and μNC) and variance-related measure ψ ((ψMAP and ψNC) (Branscum et al., 2004). Peer-reviewed literature was used to inform prior distributions for HPMAP, μMAP, HPNC, and μNC (Scott et al., 2007), and MAP ELISA test SeMAP and SpMAP (Branscum et al., 2004). SeNC and SpNC of the NC ELISA were informed by the manufacturer documentation. The variance-related measures ψMAP and ψNC were adapted from Branscum et al. (2004) to allow more uncertainty (Table 1).

2.4.2. Ranch management practices and risk factor analysis

The ranch characteristics were extracted from the questionnaires and compared to averages in Alberta and Saskatchewan (Table 2) to assess the external validity of our study. Information on ranch management practices across the participating ranches and sampled cows was described based on their status for MAP and NC (Table 3). Associations between NC serological results and possible risk factors were explored using bivariable mixed effect models to account for the hierarchical structure of the data: multi-level logistic regression with the ranch as a grouping variable (random effect) on the binary outcome (positive vs. negative).

Multi-level logistic regression was also used in multivariable analysis on the NC binary outcome. To avoid introducing spurious (or specific to the dataset) associations in the multivariable model, associations found in the exploratory univariable analysis did not influence the introduction of variables in the full model, as recommended by Burnham and Anderson (2002). For NC, the initial selection of relevant variables was, therefore, based on previously published risk factors and other variable of biological relevance, and included: herd size, cow age, breed (indicated by whether the cow was purebred or not, and the overall proportion of pure breed in the herd), the provenance of animals (indicated at the ranch level: closed herd or not; and at the individual level: cow born
on ranch or purchased), the presence of dogs on ranch, and the presence of other susceptible species on ranch, including horses, wild carnivores and wild cervids. Multivariable models with all possible combinations of this relevant set of variables were computed by the R package MuMIn (R Core Team, 2013) while the ranch random effect was kept in all models to account for the data clustering. Models were ranked by increasing value of corrected Akaike information criterion (AICc) to select the best model. Subsequent model averaging was performed on all models, weighted by Akaike weights in order to capture the model selection uncertainty. The importance of each variable was obtained by summing the Akaike weights over all models in which the variable appeared (Burnham and Anderson, 2002).

For comparison, a manual backward variable selection procedure was also applied to the initial set of variables, with an exclusion threshold of $P=0.05$, unless the variable significantly improved the model as evaluated by a likelihood ratio test. Additionally, the fit of the best model was assessed with its $R^2$ and the area under its receiver operating characteristic (ROC) curve.

### 2.4.3. Analysis of MAP and NC reproductive effects

The association between serological status for both pathogens and pregnancy (among cows tested for pregnancy by the veterinarian) was assessed using Fisher’s exact test. Similarly, the association between infection with the two pathogens and previous abortion or infertility events was assessed based on individual cow reproduction history and parity.

At the herd level, the associations between the number of abortions and calf deaths over the last 4 years, and the ranch status for NC and MAP were assessed using Poisson regressions controlling for the herd size.

### 3. Results

#### 3.1. Prevalence estimates

Of the 30 ranches enrolled in the study, two dropped out after the beginning of the sampling period and could not be replaced. The characteristics of the 28 ranches were compared to provincial and regional averages (Table 2). Among the 840 cows tested from 28 ranches, 7 were MAP ELISA-positive (apparent seroprevalence 0.8%, 95% confidence interval (CI): 0.2–1.4% from 6 ranches (21% of ranches). In total, only two cows tested positive by MAP fecal culture (one of which was also seropositive), and these came from the same ranch. The fecal culture positive cows were confirmed by PCR, and all other samples were negative by PCR. Fifty–three cows tested positive by NC ELISA (apparent seroprevalence 6.3%, 95% CI: 4.6–7.9%) in 15 ranches (54% of ranches).

The median of the posterior distributions of MAP herd prevalence ($HP_{MAP}$) and prevalence distribution in positive herds ($PD_{MAP}$) indicated that 20% of herds had at least one positive cow, with a within–herd prevalence in positive herds of 22% (8–45%). The median herd-level prevalence of NC ($HP_{NC}$) was 66% (Table 1). Posterior estimates did not change significantly when changing the prior distribution of the variance–related measure $\psi$ ($\psi_{MAP}$ and $\psi_{NC}$) (data not shown). The posterior predictive probabilities of a randomly picked herd in the area to be free of MAP and NC were 79% and 36%, respectively (Table 1).

### Table 1

Prior and posterior distributions of *Mycobacterium avium* subspecies paratuberculosis (MAP) and *Neospora caninum* Bayesian prevalence estimates. Prior distributions of the test sensitivity (Se) and specificity (Sp), herd prevalence (HP), prevalence distribution (PD) mean $\mu$ and PD variance–related measure $\psi$. Bayesian posterior estimates are given for HP, PD mean $\mu$, and probability that a random herd has a prevalence lower than 5% ($P(\pi^* \leq 0.05|y_j|)$) and null ($P(\pi^* = 0|y_j|)$).

<table>
<thead>
<tr>
<th>Prior distributions</th>
<th>Posterior estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>MAP</td>
<td>(58.8, 174.5)</td>
</tr>
<tr>
<td>Neospora caninum</td>
<td>(19.1, 1.75)</td>
</tr>
</tbody>
</table>

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*a* $\beta$ (58.8, 174.5) indicates a Beta distribution of parameters (58.8, 174.5).

*b* $\beta$ (5.1) indicates a Gamma distribution of parameters (5.1).

*c* Prevalence estimate [95% confidence interval].

*d* Posterior predictive probability.

### Table 2

Comparison of surveyed ranch characteristics with averages in Alberta and Alberta/Saskatchewan.

<table>
<thead>
<tr>
<th>Surveyed ranch mean (95% confidence interval)</th>
<th>Alberta average$^a$</th>
<th>Alberta/Saskatchewan average$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of wintered cows</td>
<td>191 (136–247)</td>
<td>144</td>
</tr>
<tr>
<td>Number of cows per bull</td>
<td>27 (21–33)</td>
<td>25</td>
</tr>
<tr>
<td>Conception rate</td>
<td>92% (90–94)$^c$</td>
<td>91.7</td>
</tr>
<tr>
<td>Calving rate</td>
<td>99% (99–100)$^d$</td>
<td>98.2</td>
</tr>
<tr>
<td>Weaning rate</td>
<td>99% (98–99)</td>
<td>97.3</td>
</tr>
<tr>
<td>Calf crop</td>
<td>85% (80–90)</td>
<td>87.3</td>
</tr>
<tr>
<td>Open cows</td>
<td>7.7% (6.0–10.1)</td>
<td>8.0</td>
</tr>
<tr>
<td>Death loss of calves</td>
<td>3.3% (1.9–4.2)$^e$</td>
<td>2.9</td>
</tr>
</tbody>
</table>

---

$a$ From Kalied and Perillat (2003).

$b$ From Millang (2003).

$c$ Calculated from the subgroup of cows that were checked for pregnancy; 93% (92–95) if calculated from questionnaire–reported values.

$d$ Calculated from questionnaire–reported calf mortality, likely underreported.

$e$ Calculated from questionnaire–reported abortions over 4 years, likely under-reported.

$f$ na = not available.
Table 3: Descriptive statistics of variables by cow- and herd-status for *Neospora caninum* and *Mycobacterium avium* subspecies *paratuberculosis*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neospora caninum status</th>
<th>Mycobacterium avium subspecies <em>paratuberculosis</em> status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− (n=788)</td>
<td>+ (n=53)</td>
</tr>
<tr>
<td>Cow age (years)</td>
<td>Mean (median)</td>
<td>S.E.</td>
</tr>
<tr>
<td>Cow purchased (%)</td>
<td>6.2 (6)</td>
<td>0.1</td>
</tr>
<tr>
<td>Purebred cows (%)</td>
<td>17.1 (32.0)</td>
<td>1.3</td>
</tr>
<tr>
<td>Pregnancy (%)</td>
<td>52.2 (47.2)</td>
<td>1.8</td>
</tr>
<tr>
<td>Abortion history (%)</td>
<td>3.6 (2.1)</td>
<td>0.7</td>
</tr>
<tr>
<td>Herd level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd size (number adult cows)</td>
<td>215 (184)</td>
<td>49</td>
</tr>
<tr>
<td>Calving period (days)</td>
<td>56 (9.0)</td>
<td>5</td>
</tr>
<tr>
<td>Proportion purebred at herd level (%)</td>
<td>45.5 (30.0)</td>
<td>14.4</td>
</tr>
<tr>
<td>Manure fertilization (%) of herds</td>
<td>54.5 (52.9)</td>
<td>15.7</td>
</tr>
<tr>
<td>Sick pen for sick animals only (%) of herds</td>
<td>18.2 (35.3)</td>
<td>12.2</td>
</tr>
<tr>
<td>Calving-weaning interval (days)</td>
<td>165 (169)</td>
<td>7</td>
</tr>
<tr>
<td>Dog on ranch (%)</td>
<td>81.8 (12.2)</td>
<td>12.2</td>
</tr>
<tr>
<td>Carcass removal by company (%) of herds</td>
<td>18.2 (12.1)</td>
<td>5.8</td>
</tr>
<tr>
<td>Horse number</td>
<td>7 (3.2)</td>
<td>1</td>
</tr>
<tr>
<td>Wild carnivore score (scale 0–8)</td>
<td>5.1 (4.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Wild elk density (per km²)</td>
<td>2.2 (1.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>Segregation of sick cows (%) of herds</td>
<td>100 (82.4)</td>
<td>9.5</td>
</tr>
<tr>
<td>Closed-management herd (%) of herds</td>
<td>72.7 (41.2)</td>
<td>14.1</td>
</tr>
<tr>
<td>Dead calves (total over 4 years)</td>
<td>6.9 (5.5)</td>
<td>2.1</td>
</tr>
</tbody>
</table>

3.2. **Ranch management practices and risk factor analysis**

Thirteen of 28 ranches (46%; 95% CI: 36–56%) purchased replacement heifers every year. In univariable analysis, purchased animals had 2.3 (95% CI: 1.2–4.3) times higher odds of being seropositive (*P* = 0.006). Individuals from ranches that did not purchase animals (closed management) had a 6.4 times lower odds of being seropositive for NC (95% CI: 2.2–27; *P* = 0.0008) than ranches regularly purchasing cows. A quarantine period was only conducted by one of the 13 ranches purchasing replacement heifers. Given the importance of carnivores in the cycle of NC, it is noteworthy that 75% (95% CI: 67–83%) of ranchers owned at least one dog, of which 55% (95% CI: 44–66%) were cattle working dogs, and 95% (95% CI: 90–100%) had access to parturition or abortion materials. Only 29% (95% CI: 20–38%) of ranches reported disposing carcasses in a way that reduced access by wild carnivores (burning, burying, rendering companies, composting). Carcass removal was negatively associated with the NC serological titer (*P* = 0.03). Note that if a Bonferroni adjustment was used for multiple hypothesis testing, the significance level would have to be α = 0.004.

Despite the vast pasture land available for cattle grazing, with an average operation size of 14.8 km² (95% CI: 10.2–19.4 km²), 96% (95% CI: 89–100%) of ranchers reported that their cattle have contact over the fence with neighboring cattle. Sixty-four percent of ranchers (95% CI: 45–83%) reported that they themselves or their employees handle cattle outside their ranch, and 57% (95% CI: 38–77%) reported sharing equipment with other ranches, while 69% (95% CI: 43–94%) of ranchers who shared equipment did not perform any cleaning/disinfection procedures. Three (of 28) respondents reported requesting biosecurity measures when receiving visitors involved with cattle in other operations.

The variables used in the multivariable analysis are described by cow and herd status for the two pathogens in Table 3. For NC in multivariable analysis, the relative importance of each parameter and their multi-model averaged coefficients, as well as the best models according to AICc ranking or obtained by manual backward selection are presented in Table 4.

Multi-model averaged model of the NC multi-level logistic regression indicated that closed herd management had a high relative importance and was negatively associated with the risk of cows being NC ELISA-positive. In addition, larger herds had higher odds of containing NC ELISA-positive cows, while the number of horses on the ranch was associated with a decreased risk of having seropositive animals. The best model in the backward selection procedure included closed herd management and...
number of horses, while AICc-based selection additionally included the herd size (AICc = 394.4; $R^2 = 37\%$ and area under the ROC curve was 0.84).

### 3.3. Reproductive impact of MAP and NC

The pregnancy status of cows that were checked during sampling was not associated with the NC or MAP serological status. However, at the ranch level, the number of abortions was associated with the ranch status for both NC ($P=0.009$) and MAP ($P=0.0001$) in Poisson regressions adjusting for herd size. The number of calf deaths was not associated with the presence of NC-positive cows on the ranch ($P=0.45$), but was associated with the presence of MAP-positive cows ($P=0.001$).

### 4. Discussion

In this study, we found several risk factors for MAP and NC occurrences in beef cattle herds of southwestern Alberta. Ranch recruitment relied on collaboration with local veterinarians, preliminary ranch visits, and thereafter, ranchers’ interest in the study, as there was no comprehensive listing of cow-calf operations available to us due to privacy protection reasons. This may have introduced biases in our sample. However, the ranch characteristics were consistent with benchmark data of Alberta and Saskatchewan cow-calf operation characteristics as illustrated in Table 2 (Kaliel and Perillat, 2003; Millang, 2003). Moreover, we had a particular interest in the southwestern Alberta for the primary objective of a larger project, and thus restricted our recruitment to this study area. Therefore, interpretations and generalization of these results should be made cautiously.

Cow-level MAP prevalence was low in the sampled cow-calf herds. This was consistent with previous studies in Alberta that reported a 1.5% (95% CI: 0.9–2.5%) cow prevalence of MAP, with 28.5% (95% CI: 17.3–43.0%) of cow-calf herds having at least one seropositive cow and 7.9% (95% CI: 2.3–23.4%) with at least two, although it varied across agro-ecological sub-regions (Scott et al., 2007). Our Bayesian estimate of true MAP herd prevalence is consistent with this report, and additionally, our estimate of prevalence distribution gives a clearer indication of the extent to which infected herds may be affected. Our results also suggest potential reproductive consequences of MAP infection in cattle, particularly on cow abortion and calf mortality. This has previously been reported (Smith et al., 2010), however, this association is often inconsistent across studies (McKenna et al., 2006). The low prevalence in our study and the risk of false-positive reactions should encourage cautious interpretations of these possibly spurious associations.

Our ability to identify risk factors associated with MAP occurrence was impaired by the low sample size and low prevalence. Our findings for NC (herd prevalence: 66%; cow prevalence in infected herds: 10%) were similar to previous studies in Alberta where in cow-calf herds there was a 9.7% (95% CI: 7.6–12.3%) prevalence in adult cows, and a 91.0% (85.4–94.6) herd prevalence (Scott et al., 2007) and a 6.5% (95% CI: 5.1–8.2) seroprevalence was reported in feedlot steers and bull calves (Waldner et al., 2004). Whereas these studies did not investigate specific herd management risk factors, our study identified associations between NC serological response and ranch management practices.

The level of biosecurity was associated with NC occurrence in our study, consistent with previous reports (Vanleeuwen et al., 2010a); introduction of purchased animals was associated with the presence of positive animals at the ranch level ($P=0.01$), and purchased cows also tended to be more likely positive ($P=0.06$; Table 4). In contrast, a study conducted in Texas indicated that ranches that raised their own replacement heifers were at higher risk of NC (Barling et al., 2000). These variations may be related to differences in the predominant transmission patterns (horizontal or vertical) between study areas.

The bivariable analysis suggested that carcass disposal method might be related to NC occurrence. Bone-yards and carcasses left on pasture attract wild carnivores (Morehouse and Boyce, 2011). Proper carcass disposal has
been complicated by new regulations after the occurrence of bovine spongiform encephalopathy cases and the new costs associated with carcass removal by rendering companies. However, this may have deleterious effects by increasing the attraction of wild carnivores on pastures and by increasing the circulation of NC. Further research should investigate the role of bone-yards in NC circulation.

The number of horses on the ranch was negatively associated with NC occurrence in our study ($P = 0.009; \text{Table 4}$), despite the competence of horses as intermediate hosts in North America (Dubey et al., 1999). In Texas, a negative association between NC occurrence and the presence of domestic dogs on farm was also speculated to be related to the exclusion of wild carnivores by domestic dogs (Barling et al., 2001). Both dogs and horses are involved in ranch management activities (such as guarding and herd monitoring) that have been shown to reduce the presence of wild carnivores (Gese, 2003; Alberta Agriculture and Rural Development, 2010). It is, therefore, difficult to conclusively interpret the biological mechanism underlying this association, but it may suggest the importance of horizontal transmission by wild carnivores in this study area. Unfortunately, the subjective scoring of the relative density of wild carnivores by ranchers may have been insufficiently accurate to assess the effect of wild carnivores on NC occurrence.

We did not detect any association between the serological status for NC and pregnancy status in cows, however, at the herd level, ranches with NC-positive individuals experienced higher number of abortions. This is consistent with the well-known reproductive effects of NC in dairy and beef cattle (Thurmond and Hietala, 1997; Dubey, 1999; Vanleeuwen et al., 2010b), and previous reports in Alberta where NC seropositive cows were at lower odds of being pregnant (OR = 0.61; 95% CI: 0.37–1.00) (Scott et al., 2007). Additionally, in a 2001 survey on northern Alberta cow-calf herds conducted at the auction, seropositive cows were more likely to be open than seronegative cows (Waldner et al., 2001). The absence of association at the cow-level may be due to a lack of statistical power, or to the usual practice in cow-calf herds to cull or sell “open” or “late” cows every year. Although this practice may provide some level of control by removing cows having had abortion or early embryonic death, it may reduce the apparent prevalence on ranch and thus hide the impact of NC (that is, open cows are culled, but the underlying cause of non-pregnancy is not diagnosed). Note that culling occurred after our sampling period and, therefore, did not bias our sample. Better understanding how much NC is contributing to premature culling will be essential to truly assess the economic impact of this parasite on cow-calf herds. Additionally, understanding the relative role of vertical and horizontal transmission, and domestic and sylvatic cycles are essential challenges that will enable to tailor control efforts to the local characteristics of this complex system.

Our study presented serological evidence of NC circulation, and a low prevalence of MAP in cow-calf operations in Alberta. It highlighted the impact of the introduction of purchased animals and possibly of herd guarding/monitoring for NC occurrence. Additional studies should be done to further our understanding of risk factors for MAP and NC in cow-calf herds. Moreover, it is important to assess the consequences on the reproductive performances of cow-calf operations and more generally the impact of these infections on the beef cattle production.

**Conflict of interest**

Authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2014.09.003.

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