Case Report  Rapport de cas

A high-morbidity outbreak of Johne’s disease in game-ranched elk
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Abstract — Following an outbreak of Johne’s disease on an elk farm in northern Alberta, Canada, fecal culture, fecal polymerase chain reaction (PCR), and serum enzyme-linked immunosorbent assay (ELISA) tests were performed on individual animals. The magnitude of the outbreak is described and the challenges associated with poor test agreement, as well as herd management options, are discussed.

Résumé — Éclosion à morbidité élevée de paratuberculose chez des wapitis d’élevage. Après une écllosion de paratuberculose dans un élevage de wapitis du Nord de l’Alberta, au Canada, une coproculture, une réaction d’amplification en chaîne par la polymérase (ACP) et des tests sérologiques immunoenzymatiques (ELISA) ont été réalisés chez les animaux individuels. L’ampleur de l’écllosion est décrite et les défis associés à une mauvaise concordance des tests ainsi que les options de gestion du groupe sont discutés.

Johne’s disease (JD) is a well-known production-limiting disease of livestock, caused by the bacterium Mycobacterium avium subspecies paratuberculosis (MAP). This disease is characterized by granulomatous enteritis that leads to chronic weight loss and often diarrhea in affected animals. Although MAP infection has been sporadically reported in a wide range of species, clinical disease is usually limited to adult domestic (1) and wild ruminants (2). Over the past few decades there have been several reports of JD in cervids, including farmed elk (3), cervids kept in zoos or wildlife parks, and occasionally in wild reindeer, axis deer, tule elk, and white-tailed deer (4). Johne’s disease is well-documented in farmed red deer (Cervus elaphus), and is of particular importance for the industry in New Zealand, where losses include increased mortality in young stock, occasional deaths among adults, reduced growth, and lower pregnancy rates (5). Red deer are closely related to North American elk (Cervus canadensis), an important wildlife and game-ranched species for Canada and Alberta in particular, where there are approximately 45 000 farmed elk (6).

From research focused on cattle, common belief has been that calves younger than 6 mo are most susceptible to MAP infection; however, recent studies have shown that calves up to 1 y of age can become infected with low doses of the bacterium (7). After becoming infected, cattle can remain sub-clinically infected for months to years before beginning to shed the bacterium in their feces (infectious) and later displaying clinical signs (affected) (8). In red deer, although an age-related resistance to infection was not found, animals infected at a younger age (3 mo) were significantly more likely to develop clinical disease than animals infected as yearlings or adults (9). Certain noteworthy differences in the clinical profile of JD exist among the most common ruminant hosts (Table 1). In cervids, intrauterine infection plays a more significant role in the spread of MAP than is described in the literature for cattle (12). Additionally, elk and red deer as young as 8 mo of age develop clinical disease which progresses rapidly and tends to appear in the form of an outbreak (3,5).

There is no treatment available for JD, and vaccines are rarely used in North America, primarily due to lack of availability and to cross-reactivity with the caudal fold skin test for bovine tuberculosis (15). There are currently no approved vaccines against JD available in Canada [Alberta Agriculture and Rural Development (AARD), personal communication]. Therefore preventing the introduction of MAP to farms and management strategies that limit its spread in infected herds are of paramount importance. We explore these management options in the context of an outbreak of JD on an elk farm in northern Alberta.

Case description

In February 2012, a veterinarian in northern Alberta diagnosed JD in 2 game-farmed elk by postmortem examination, supported by histological examination and polymerase chain reaction (PCR) on fixed tissues performed by AARD, Edmonton. A first suspicion of JD was noted in this herd in 2009, when...
1 animal developed diarrhea leading to weight loss and death. In 2011, approximately 10 animals aged 1 to 2 y died in similar conditions suggestive of JD, although no additional diagnostic tests were performed. Following the 2 deaths in February, it was decided that the extent of MAP infection on the farm should be determined to help evaluate potential management options.

Detailed information on herd management practices was collected in April, 2012. At this time there were 60 bull and 52 cow elk on the farm, as well as 19 yearlings and 16 weaned calves. The elk were kept in 4 pasture groups, separated into cows with calves, yearling males, yearling females, and bulls. The total pasture area of 65 hectares was divided into 8 separate enclosures. Rotational grazing was practiced with supplemental feeding of hay, as grass became limited in dry years. The herd was handled once annually in a single working area, at which time animals were treated with moxidectin (applied topically) and an 8-way clostridial vaccine; otherwise, no treatments or vaccinations were implemented. The herd was not closed, and females have been regularly brought in and synchronized for artificial insemination in the fall; eight 2- to 3-year-old females had just been purchased and placed on-farm (April, 2012). No other ruminants were kept on the property or have been since elk were first introduced in 2008. Cattle were grazed on the same pastures for a generation until a few months prior to the introduction of the elk. Direct contact with wild ruminants such as elk and deer occurred across the fence line, and potential environmental contamination could occur since a large wild elk herd resides in the area and has been observed in near proximity to the game-ranched elk.

Given the existence of multiple pasture areas on the farm, the eventual possibility of separating at-risk groups was perceived to be feasible. In April 2012, individual diagnostic testing was performed on all 52 adult cow elk (aged 3 to 12 y; median = 4 y) and three 6-year-old bulls used for breeding. Feces were collected from all 55 adult elk using a new glove for direct fecal extraction or culture if Ct (cycle threshold at which a fluorescent signal is observed) values < 37 were obtained for both IS900 and F57. The DNA for PCR was directly extracted from fecal samples using the commercially available MagMAX total nucleic acid isolation kit (Applied Biosystems, Carlsbad, California, USA) and incubated for 5 wk, with DNA extracted from the culture broth as previously described (18). No-template controls were also included throughout the culture process. All DNA samples (from culture or direct fecal extraction) were tested using PCR targeting the IS900 insertion sequence and confirmed using the F57 gene (17). A sample was considered positive for either direct DNA extraction or culture if Ct (cycle threshold at which a fluorescent signal is observed) values < 37 were obtained for both IS900 and F57.

Using the IDEXX ELISA kit modified for use with elk serum, 8 adults were positive (15%), and 17 were designated as “suspect” (31%) (Table 2). Only 2 of the samples that were positive using the IDEXX kit were also positive using the ID Vet ELISA; both these samples had S/P ratios > 0.8 using the IDEXX kit.

Table 1. Comparison of the profile of Johne’s disease among different ruminant species

<table>
<thead>
<tr>
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<th>Cattle</th>
<th>Sheep</th>
<th>Cervids</th>
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<tbody>
<tr>
<td>Importance of intra-uterine transmission</td>
<td>9% fetuses of subclinically infected cows; 39% fetuses of clinically affected cows (10)</td>
<td>2% fetuses of subclinically infected ewes (11)</td>
<td>78% fetuses of infected hinds (12)</td>
</tr>
<tr>
<td>Age of clinical onset</td>
<td>1 to 10 years (13)</td>
<td>2 years and older (14)</td>
<td>Outbreaks with high mortality in 8-month to 2-year-olds; sporadic cases in adults (5)</td>
</tr>
<tr>
<td>Clinical portrait</td>
<td>Chronic weight loss, diarrhea (starts as intermittent), appetite maintained, decreased milk production (13)</td>
<td>Chronic weight loss, diarrhea only in terminal stages (14)</td>
<td>Adults: chronic weight loss; Juveniles: rapid loss of weight and muscle mass, poor coat quality, diarrhea (5)</td>
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* Studies conducted on dairy cows.
The first necropsied animal was positive on both ELISA tests, with S/P ratio > 2.0. No additional samples tested positive using the ID Vet ELISA. Seven of the 16 juveniles were positive using the IDEXX ELISA (44%) (Table 3), and of the next 2 necropsied animals, 1 was positive and 1 was “suspect.”

All fecal samples were PCR-positive for MAP DNA extracted directly from the feces except for 1 adult female; this animal was ELISA positive. For the 55 adults, all positive IS900 PCR Ct values ranged between 30 to 33, which corresponds to the range associated with dairy cows shedding moderate levels of MAP in their feces. All samples were confirmed positive with F57. For the 15 fecal samples tested from juveniles, direct PCR values ranged from Ct = 17 to Ct = 34, suggesting that some of the younger animals had higher levels of shedding, as lower Ct values are associated with higher initial levels of MAP DNA in the sample. Fourteen of 55 adults (25%) and 8 of the 15 juveniles (53%) were fecal culture positive (Tables 2 and 3). All tissues tested from the necropsied animals were culture positive.

A kappa test was used to evaluate the level of agreement between the IDEXX serum ELISA and fecal culture test results. There was no agreement for the adult elk and only fair agreement (Kappa = 0.34) between the test results for juveniles.

**Discussion**

This case clearly highlights some of the important differences in JD among host species. The outbreak-type presentation of JD that can occur in juvenile elk is in sharp contrast to JD in cattle, in which clinical signs in young animals are hardly ever observed. Even in adult cows, clinical signs beyond decreased milk production are rare in North American dairy operations, as most cows are culled before progressing to advanced clinical stages of the disease. In this case, higher than expected morbidity was observed in 1- to 2-year-olds leading to euthanasia. This is similar to descriptions of JD outbreaks in juvenile red deer in New Zealand which involved up to 20% of animals in that age group (5), and an early onset of clinical signs has also been seen in experimentally infected deer (9). The early onset of JD in cervids may be partially due to the fact that intra-uterine transmission plays a particularly important role in MAP transmission in these species (12).

Some of the most important management strategies used by dairy cattle producers, which include limiting contact between calves and their dams, are clearly not viable options on cervid farms. Preventing the introduction of MAP infection into a herd, by maintaining a closed herd or by requesting test results from source herds, is the most cost-effective management strategy for JD (19). Once infection is established in a herd, the management of JD in farmed deer is based on the prevention of new infections and the elimination of infected individuals (test-and-cull strategy) (5,19). Prevention strategies are based on separation of infected animals, manure management, and colostrum and milk management. Elimination strategies consist of disinfection and a test-and-cull program, in which culling of affected and/or test-positive animals (including calves born from infected dams) is done to limit the spread of infection. The effectiveness of these strategies is limited by the fact that diagnostic tests for detecting sub-clinically infected individuals have low sensitivity, and that MAP can potentially survive in the environment for over a year (20). These limitations make the eradication of MAP from an infected herd nearly impossible to achieve (5). However, these management strategies may progressively reduce the prevalence of JD and its impact on production to economically viable levels.

Antemortem tests for detecting MAP infection in subclinically infected animals are notorious for their lack of sensitivity. This is primarily due to the chronic nature of infection, during the early stages of which the targets for diagnostic tests (i.e., antibodies for ELISA, bacteria shed in the feces for direct PCR and culture) are not yet present. There does not appear to be a consistent temporal relationship between the onset of fecal shedding and the development of a serological immune response in cattle (8), and a weak correlation between serum ELISA reactivity and fecal shedding has previously been noted in red deer (21).

In this case, despite the poor agreement among the diagnostic tests used, the results were sufficient to confidently communicate to the elk farmer and the attending veterinarian that MAP infection was highly prevalent on the farm. Based on fecal culture, at least 1/4 of the adults and 1/2 of the juveniles on the farm were infectious (i.e., shedding MAP in their feces) at the time of sampling. This test is considered to be 100% specific, although the sensitivity varies greatly depending on the stage of infection. It can be as high as 70% in affected individuals, or as low as 25% for subclinically infected dairy cows (8), due to the late onset of shedding and the inability of the assay to detect low levels of MAP in the sample. In a herd of tule elk in California, fecal culture was positive in 4/10 animals confirmed infected using tissue culture (22). Given the high proportion of animals with detectable fecal shedding in this case, it is likely that most of the animals on the farm were truly infected.

The results of the modified IDEXX ELISA also strongly suggested that a large proportion of the elk herd was infected. Forty-four percent of the juveniles had high antibody titers, while 15% of adults were ELISA positive when taking the

| Table 2. Fecal culture and serum ELISA test results for *Mycobacterium avium* subspecies *paratuberculosis* performed on adult elk (*n* = 55) |
|-----------------|-----------------|-----------------|
|                | Culture positive | Culture negative | Total |
| ELISA positive  | 2               | 6               | 8     |
| ELISA positive/suspect | 4           | 13              | 17    |
| ELISA negative  | 8               | 22              | 30    |
| Total           | 14              | 41              | 55    |

| Table 3. Fecal culture and serum ELISA test results for *Mycobacterium avium* subspecies *paratuberculosis* performed on juvenile elk (*n* = 15) |
|-----------------|-----------------|-----------------|
|                | Culture positive | Culture negative | Total |
| ELISA positive  | 5               | 2               | 7     |
| ELISA negative  | 3               | 5               | 8     |
| Total           | 8               | 7               | 15    |
The poor concordance between fecal PCR and culture is more perplexing. Our laboratory has several years of experience with this direct PCR assay, and although false positives due to cross-contamination can occur during sample processing, it cannot explain the fact that all samples but 1 tested positive. Given our initial surprise at these results, samples were processed a second time, with identical results. Additionally, all negative controls were negative; therefore, we are confident that these are true positive results. In some cases, direct PCR has been shown to be more sensitive than culture (23), and PCR is also capable of detecting both live and dead bacteria. In heavily contaminated environments the phenomenon of passive shedding may occur, wherein animals ingest the bacterium from the environment and subsequently pass it in their feces without becoming infected (24). However, the importance of passive shedding remains contested, and the relative extent to which this might contribute to positive PCR and culture results is unknown.

In the case presented, after initial diagnosis of JD in 2 elk, the main motivation for herd-level testing was to determine the feasibility of managing the infection to reduce within-herd spread of MAP and limit further production losses. Prior to diagnostic testing, the location and use of all enclosures on the farm over the past 2 y was mapped (i.e., which groups of animals were kept in each enclosure). Pastures were identified that would allow groups at different risk levels to be kept separately without direct contact (e.g., separate infectious and/or sero-reactive animals). Unfortunately, given the extent of the problem on the farm, the management options in this case were limited. The major options identified were:

i) Choose to do nothing. The risk of continuing to lose a large proportion of animals to disease would be high, given the likely extent of environmental contamination on-farm and the strong likelihood that many of the breeding females are infected and could pass on the infection to their offspring in-utero. This option would also be of concern for the welfare of the animals.

ii) Prevent new infections by culling infectious individuals (i.e., those shedding MAP in their feces based on culture results), any individuals that develop clinical signs, and possibly high sero-reactors (infected individuals). In this case, this strategy would involve culling 14/55 adults, or 20/55 if high sero-reactors are included. Given the high number of infectious animals at the time of testing, it is likely that there would be significant environmental contamination of the pastures grazed by these animals. Our evaluation of the recent use of the grazing pens indicated that it was unlikely that any of the pens were free of MAP contamination. The potentially long environmental survival of MAP could be sufficient to maintain a high infection pressure, even after infectious animals are culled. Therefore, although a test-and-cull strategy of this type could be considered as a viable option, it was difficult to ensure the cost-effectiveness in this context.

iii) Depopulate the herd. This was the choice that was opted for given the increasing incidence of clinical disease and suspected high level of environmental contamination. If the farmer chooses to re-populate, it would be recommended to wait at least 2 y to reduce the likelihood of re-infection due to environmental persistence (5). Similarly, it would be ill-advised to graze other ruminant species on the premises during this time, as they would be at a similar risk of contracting the infection and they could contribute to the maintenance of environmental contamination.

Vaccination was not presented as an option since there are no approved vaccines against JD for any species in Canada. Vaccines for use in deer are available in other countries and are effective in reducing the clinical signs associated with MAP infection. When available, vaccination can therefore be an important element of JD management strategies (25).

A final consideration is that of the potential role of wildlife in the epidemiology of MAP. Although unlikely, the possibility that wildlife in the surrounding area could have become infected by this herd should be considered, as it could eventually act as a source of re-infection (spill-back) were the herd to be re-populated at a later date (26). It has been shown that MAP can be disseminated in surface water runoff (27). Heavily contaminated pastures could therefore act as a source of infection for wildlife, even beyond a high fence line. However, the risk of spill-back infection from wildlife is believed to be low, and MAP infection tends to result in only sporadic clinical cases in wild populations.

This case illustrates the impact MAP infection can have on farmed cervids. With no treatment or effective vaccine available, control of JD is focused on preventing the introduction of MAP into a herd, or controlling its spread within infected herds by preventing new infections and removing infectious animals. Given the particular challenges of epidemic JD in farmed cervids, an economic evaluation into test-and-cull control strategies for cervid farms in North America would be of great value in providing farmers and veterinarians with the necessary background to make informed management decisions. This case also highlights the challenges associated with individual animal diagnostics for JD, especially with exotic species, and provides the veterinary and farming community with more insight into the different manifestations of this disease.

Acknowledgments

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References


