PEER REVIEWED

Case report – *Mycobacterium avium* subspecies *paratuberculosis* infection in three generations of beef cattle

Julia M. Smith[1], *DVM, PhD;* **Robert H. Whitlock**[2], *DVM, PhD*
[1]*Department of Animal Science, University of Vermont, Burlington, VT 05405*
[2]*New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA 19348*

Abstract

Diagnostic specimens collected postmortem from a seven-month-old, 275 lb (125 kg), unweaned, emaciated Blonde bull calf born to a Johne's-infected dam confirmed infection with *Mycobacterium avium* subsp *paratuberculosis* (MAP). This case represents the third generation in one line of cattle to be infected with MAP. Transmission of infection from dam to calf is an important means of sustaining Johne's disease in cow-calf herds. Not all submitted tissue samples cultured positive for MAP, illustrating the need to submit an adequate number of samples from appropriate locations to make a positive diagnosis. Positive cultures from composite environmental samples coincided with the presence of the clinically-affected dam of this calf.

Key words: pathology-gastrointestinal tract, herd health, beef cattle, mycobacteria, Johne's disease

Résumé

Des spécimens diagnostiques recueillis suivant la nécropsie d'un veau mâle de race Blonde émacié et non-sevré de sept mois (275 lbs, 125 kg), né d'une mère infectée par la paratuberculose, ont confirmé l'infection avec *Mycobacterium avium* subsp *paratuberculosis* (MAP). Ce cas représente la troisième génération d'une lignée de bovins infectés avec MAP. La transmission de l'infection de la mère au veau est un moyen important de maintenir la paratuberculose dans les troupeaux vaches-veaux. Comme tous les échantillons de tissus soumis ne se sont pas révélés positifs pour MAP, il est important de soumettre un nombre adéquat d'échantillons provenant de localisations appropriées pour émettre un diagnostic positif. Des cultures positives provenant d'échantillons environnementaux composites étaient corrélées avec la présence de la mère cliniquement affectée de ce veau.

Introduction

Transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from dam to calf is the critical factor in the persistence of paratuberculosis in cow-calf herds. While the fecal-oral route is the most important route of transmission of MAP to neonatal and young cattle, in utero transmission does occur when dams are in later stages of infection.[1,15,20] Unlike the situation in most dairy herds, calves in cow-calf operations remain with their dams for several months, increasing potential exposure to manure of adult cattle. Culling clinically affected animals and their immediate offspring is recommended to eliminate high-risk animals from beef herds.[8] Failing to do so can lead to perpetuation of infection, as in this case.

This report documents infection with MAP in three generations of beef cattle. Diagnosis was based on culture of individual cow fecal samples and calf tissue samples collected postmortem. The history of this herd highlights the importance of vertical transmission in maintaining MAP in a cow-calf herd.

Case Description, History, Clinical and Laboratory Findings

A seven-month-old, approximately 275 lb (125 kg), unweaned, emaciated Blonde d'Aquitaine[a] bull calf born to a five-year-old cow was found dead by the owner in late November 2005. A field examination of the calf was conducted about two days after it died to collect tissues to test for MAP. Cold environmental conditions had preserved the carcass. The dam was losing weight and subsequently died two weeks later; a necropsy was not done. The dam had been one of 12 mature cows in a 23-head beef herd pastured on 30 acres, including a rocky hillside, a spring-fed wet meadow, and wooded areas. The herd was enrolled in the Vermont Cattle Health Improvement Program, Vermont's Johne's disease management program, which

followed the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program[19] current at the time.

Samples of feces and multiple gastrointestinal tissues from the calf were submitted for testing at the Johne's research laboratory at the School of Veterinary Medicine, University of Pennsylvania. Tissues were processed using standard methods.[16] *Mycobacterium avium* subspecies *paratuberculosis* was not isolated from one of two ileocecal lymph nodes, liver, or hepatic lymph nodes. Low numbers of MAP were recovered from the feces, distal ileum, and ileocecal valve, while moderate numbers were recovered from the proximal ileum and mesenteric lymph node. The highest numbers of MAP were found in the remaining ileocecal lymph node, and were too numerous to count.

The granddam of this bull calf was the index case for Johne's disease in the herd. She died in the fall of 2002 at 10½ years of age, having entered the herd two years earlier with a heifer calf at her side. The index case was one of six head of Blonde cattle purchased in Ontario, Canada in August 2000. The paratuberculosis status of the source herd was not known prior to purchasing the cattle. The index case was reported to have lost and regained weight several times prior to her death, and had delivered only one other calf which died at less than six months of age. A fecal sample obtained from the index case soon after death, cultured[6] using liquid media,[b] was signal-positive for MAP at 11 days after inoculation. This very short time to detection signal suggests a very high level of MAP shedding, over a million colony forming units per gram of feces.[6]

The heifer calf belonging to the index case when introduced to the Vermont herd was born in July 2000. Once mature, the daughter of the index case reportedly had recurrent cycles of weight loss and gain, and did not conceive until she was over four years old despite continuous exposure to the herd bull. Her only progeny was the bull calf described in this report. In August 2005, four months before the daughter of the index case died, an individual fecal sample was collected from her and composite environmental manure samples were collected around two hay feeders in this cow's paddock on each of two consecutive days.

The environmental composites were created by mixing handfuls of manure collected from fresh fecal patties expected to represent all of the animals in the paddock. These antemortem composite and individual samples were cultured by the same method in liquid media.[6] The fecal sample from the daughter of the index case was signal-positive at 15 days. As in the index case, this rapid time to detection is equivalent to millions of organisms in 1 gram of feces.[6] One environmental composite was positive at 16 days in liquid culture and the other at 24 days. All positive cultures were confirmed by polymerase chain reaction.[5,6] Thirteen fecal samples from individual animals submitted in 2004, as well as three collected concurrently with the other August 2005 samples, were culture-negative. Subsequent composite environmental manure samples collected about a year after the death of the calf and his dam (two samples submitted in both September and December 2006) were negative. The environmental contamination detected in 2005 was likely attributable to the cow with clinical Johne's disease.

Demonstration of moderate to high numbers of MAP from mesenteric and ileocecal lymph nodes and proximal ileum confirms the diagnosis of paratuberculosis in this calf, the third generation in one line of Blonde cattle known to be infected. A complete necropsy was not performed on the unweaned calf, so the exact cause of death is unknown.

Discussion

While not surprising to document MAP infection in the calf of a clinically affected cow, it is impossible to determine with certainty whether the infection occurred in utero or postnatally. The risk of infection in utero from infected dams has been estimated to be 11.3 to 40.7% (95% CI),[14] and has been shown to increase with the level of shedding of the dam.[17] Using the point estimate for in utero infection of 26.4% from Seitz *et al*,[14] Aly and Thurmond[1] estimated that up to 58.2% of infection attributable to being born to a seropositive dam occurs after birth. Teats and colostrum pose a risk for infecting neonatal calves as they may be positive for MAP, even when the dam is uninfected.[11] As seen in the calf in this report, the most commonly affected tissues are proximal ileum and ileocecal lymph nodes.[16] However, sampling only gastrointestinal tract and associated lymph nodes will miss infection in up to 10% of cases, even when many sections are cultured.[10] Sampling fewer tissues will increase the chance of a false negative. Sampling only one ileocecal lymph node may miss 25 to 30% of MAP infections in neonatal calves.[16] Finding organisms too numerous to count in one ileocecal lymph node and none in the other adjacent lymph node, as in this case, illustrates the risk in relying on only one or two tissues to make a diagnosis of infection with MAP.

In this case, the Johne's disease status of the source herd(s) was not known prior to purchasing the cattle. Verifying the Johne's disease status of source herds prior to purchasing additions is an important biosecurity measure to prevent the introduction of Johne's disease into a herd. Thorne and Hardin[18] estimated 28 to 52% of beef herds and 49 to 90% of dairy herds contain at least one seropositive animal. For herd-level assessment of infection status, culture of environmental composite manure samples (comprising four to six samples from one general location) in dairy herds has been shown to be almost as sensitive as fecal pooling.[12] Furthermore, the culture of environmental composite samples or pooled samples is more

sensitive and cost-effective than serology in dairy herds.[12] Environmental surveillance may be useful for monitoring disease status of beef herds with high stocking density.

Early identification and removal of heavy shedders is critical to prevent exposure of susceptible young stock to infective manure or manure-contaminated feed (i.e., pasture) or water. Risk factors of the herd in this report included failure to cull the daughter of the index case at the time the index case was diagnosed, inability to routinely test individual animals, and a stream running through the paddocks.[4,13] The herd owner also failed to cull the index case's daughter when she became clinically ill, thereby increasing the chances of infection being transmitted to the next generation. High stocking density also increased risk of transmission in this herd and may justify surveillance testing.[3] Additional data is needed to quantify and model the risk of MAP transmission from environmental contamination in beef herds.[4] Full-blooded beef herds are potentially at higher risk of maintaining infection once introduced because owners could be less willing to cull valuable genetics. In the long run, however, Johne's disease could lead to the loss of valuable animals, reduced productivity of infected cows, and restrictions on marketing of semen and embryos.[7] Thus, the economic viability of registered and seed stock cattle operations may depend on their paratuberculosis status.

Exposure to MAP in the environment and shared genotypes associated with risk of infection also contribute to increased risk of infection of calves of infected dams.[9] Osterstock *et al*[9] examined pedigrees of Texas Longhorn cattle that were positive and negative for paratuberculosis by ELISA. The odds of an offspring being seropositive were increased by the presence of particular ancestors in the pedigree. Additional studies of genetic linkages and identification of specific genetic elements responsible for differences in disease susceptibility are needed before genetic selection can be applied systematically to reduce the prevalence of paratuberculosis in cattle.

A recent study in Texas found that the voluntary Johne's disease control program could be better promoted to beef producers and their veterinarians.[2] Veterinarians working with beef operations should educate clients about Johne's disease, conduct assessments, and encourage the implementation of management strategies to prevent or control infection with MAP.

Conclusion

Despite the overall low prevalence of MAP in beef herds, the risk of introducing the disease to a herd through the purchase of animals is a valid concern. The MAP infection in the calf reported here illustrates this risk and the consequences of subsequent transmission from cow to calf. Currently this is the only line of cattle in this herd affected with paratuberculosis. To date, whole-herd testing has not been possible, so it is unknown whether MAP infection has been limited to only one cow family in this herd.

Endnotes

[a]Blonde d'Aquitaine; http://blondecattle.org
[b]TREK ESP II, TREK Diagnostic Systems, Inc., Sun Prairie, WI

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References

1. Aly SS, Thurmond MC. Evaluation of *Mycobacterium avium* subsp *paratuberculosis* infection of dairy cows attributable to infection status of the dam. *J Am Vet Med Assoc* 2005;227:450-454.
2. Benjamin LA, Fosgate GT, Ward MP, Roussel AJ, Feagin RA, Schwartz AL. Attitudes towards biosecurity practices relevant to Johne's disease control on beef cattle farms. *Prev Vet Med* 2010;94:222-230.
3. Collins MT, Gardner IA, Garry FB, Roussel AJ, Wells SJ. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. *J Am Vet Med Assoc* 2006;229:1912-1919.
4. Humphry RW, Stott AW, Adams C, Gunn GJ. A model of the relationship between the epidemiology of Johne's disease and the environment in suckler-beef herds. *Vet J* 2006;172:432-445.
5. Kim SG, Kim EH, Lafferty CJ, Miller LJ, Koo HJ, Stehman SM, Shin SJ. Use of conventional and real-time polymerase chain reaction for confirmation of *Mycobacterium avium* subsp *paratuberculosis* in a broth-based culture system ESP II. *J Vet Diagn Invest* 2004;16:448-453.
6. Kim SG, Shin SJ, Jacobson RH, Miller LJ, Harpending PR, Stehman SM, Rossiter CA, Lein DA. Development and application of quantitative polymerase chain reaction assay based on the ABI 7700 system (TaqMan) for detection and quantification of *Mycobacterium avium* subsp *paratuberculosis*. *J Vet Diagn Invest* 2002;14:126-131.
7. Merkal RS, Whipple DL, Sacks JM, Snyder GR. Prevalence of *Mycobacterium paratuberculosis* in ileocecal lymph nodes of cattle culled in the United States. *J Am Vet Med Assoc* 1987;190:676-680.
8. National Johne's Working Group and Johne's Committee of USAHA. *How to do risk assessments and management plans for Johne's disease*, 3rd ed, 2003.
9. Osterstock JB, Fosgate GT, Cohen ND, Derr JN, Manning EJ, Collins MT, Roussel AJ. Familial associations with paratuberculosis ELISA results in Texas Longhorn cattle. *Vet Microbiol* 2008;129:131-138.
10. Pavlik I, Matlova L, Bartl J, Svastova P, Dvorska L, Whitlock R. Parallel faecal and organ *Mycobacterium avium* subsp *paratuberculosis* culture of different productivity types of cattle. *Vet Microbiol* 2000;77:309-324.
11. Pithua P, Well SJ, Godden SM, Stabel JR. Evaluation of the association between fecal excretion of *Mycobacterium avium* subsp *paratuberculosis* and detection in colostrum and on teat skin surfaces of dairy cows. *J Am Vet Med Assoc* 2011;238:94-100.

12. Raizman EA, Wells SJ, Godden SM, Bey RF, Oakes MJ, Bentley DC, Olsen KE. The distribution of *Mycobacterium avium* ssp *paratuberculosis* in the environment surrounding Minnesota dairy farms. *J Dairy Sci* 2004;87:2959-2966.
13. Roussel AJ, Libal MC, Whitlock RL, Hairgrove TB, Barling KS, Thompson JA. Prevalence of and risk factors for paratuberculosis in purebred beef cattle. *J Am Vet Med Assoc* 2005;226:773-778.
14. Seitz SE, Heider LE, Heuston WD, Bech-Nielsen S, Rings DM, Spangler L. Bovine fetal infection with *Mycobacterium paratuberculosis. J Am Vet Med Assoc* 1989;194:1423-1426.
15. Sweeney RW. Transmission of paratuberculosis. *Vet Clin North Am Food Anim Pract* 1996;12:305-312.
16. Sweeney RW, Uzonna J, Whitlock RH, Habecker PL, Chilton P, Scott P. Tissue predilection sites and effect of dose on *Mycobacterium avium* subsp *paratuberculosis* organism recovery in a short-term bovine experimental oral infection model. *Res Vet Sci* 2006;80:253-259.
17. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. *Am J Vet Res* 1992;53:477-480.
18. Thorne JG, Hardin LE. Estimated prevalence of paratuberculosis in Missouri, USA cattle. *Prev Vet Med* 1997;31:51-57.
19. USDA. Uniform program standards for the voluntary bovine Johne's disease control program, 2006.
20. Whittington RJ, Windsor PA. In utero infection of cattle with *Mycobacterium avium* subsp *paratuberculosis*: a critical review and meta-analysis. *Vet J* 2009;179:60-69.