

Case Report - Johne's Disease: The Recipient Risk

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Abstract

This is the first report of intrauterine *Mycobacterium paratuberculosis* transmission from a bovine embryo recipient to the resultant calf. The recipient was ELISA and fecal culture test-negative before embryo placement, but became ELISA-positive during the pregnancy. Johne's disease was confirmed at necropsy subsequent to Caesarean section. Her embryo transfer calf was diagnosed with Johne's disease two years later. Strict research biosecurity protocols under which the embryo transfer calf was raised eliminated routes of post-natal exposure to *M. paratuberculosis*. The recipient cow had been purchased from a herd at risk for *M. paratuberculosis* infection for which no Johne's disease surveillance was performed.

To minimize the likelihood of purchasing an infected recipient cow or obtaining *M. paratuberculosis*-contaminated colostrum and thus jeopardizing valuable embryos, recipient and colostrum donor purchases should be made from a source herd with no or low, whole-herd Johne's disease test prevalence. The risk of purchasing an animal with *M. paratuberculosis* infection can be better assessed with whole-herd infection prevalence data than with test results for a single animal only.

Résumé

Ceci constitue le premier rapport de transmission intra-utérine de *Mycobacterium paratuberculosis* d'une receveuse d'embryon bovin au veau transféré. La receveuse, qui testait négatif selon le test ELISA et la culture des fèces avant la transplantation de l'embryon, devint positive selon le test ELISA durant la gestation. La présence de la maladie de Johne fut confirmée à la nécropsie suite à la césarienne de la vache. Le veau transféré a aussi été diagnostiqué avec la maladie de Johne deux années plus tard. Le respect de protocoles stricts en recherche au niveau de la biosécurité pendant l'élevage du veau transféré élimine les chances d'un con-

tact post-natal avec *M. paratuberculosis*. La vache receveuse provenait d'un troupeau à risque pour la maladie de Johne dans lequel aucun programme de surveillance pour la maladie ne prenait place.

Dans le but de minimiser les chances d'acheter une vache receveuse infectée ou d'obtenir du colostrum contaminé avec *M. paratuberculosis*, mettant ainsi en péril des embryons de valeur, les achats de vaches receveuses et de donneuses de colostrum devraient être faits dans des troupeaux où la prévalence de la maladie de Johne telle qu'observée au niveau du troupeau en entier est absente ou très faible. Le risque d'acheter un animal infecté avec *M. paratuberculosis* s'évalue plus aisément avec les données de prévalence au niveau du troupeau en entier plutôt qu'au niveau d'une vache individuelle.

Case Description

A female Holstein approximately one year of age was purchased in October 1998 from a large dairy herd in the Midwest. Heifers were frequently bought from and sold into this herd through livestock dealers, and the cull rate was high. This herd was at considerable risk of infection with *Mycobacterium paratuberculosis*, given its animal management protocols and the fact that the prevalence of Johne's disease for dairy herds milking at least 300 cows in the United States has been estimated at 40%.⁴ No Johne's disease diagnostic test results were available for the herd.

The recipient heifer was test-negative for Johne's disease by both ELISA and fecal culture assays completed just prior to purchase. Three subsequent ELISAs during the next nine months were negative. The heifer was to serve as an embryo transfer (ET) recipient for genetically valuable embryos, and a successful ET was made in June 1999. The pregnancy proceeded normally. In December 1999 during the sixth month of pregnancy, another blood sample was taken and the ELISA result was interpreted as strong positive (S/P ratio of 1.3).

At the time of Caesarean section in March 2000 the recipient had a body condition score of 2.5 and intermittent mild diarrhea. Due to positive serology results, the heifer was necropsied at the completion of the Caesarean section. Gross evidence of Johne's disease was found, including irregular corrugation of the jejunum, thickening of the mucosa, dilated lymphatics and mildly enlarged mesenteric lymph nodes. Histopathologic lesions consistent with Johne's disease were also present (*i.e.*, giant cells and macrophages containing few to numerous acid-fast bacilli in the ileum, jejunum, cecum and colon). *M. paratuberculosis* was isolated through radiometric culture of two tissue samples (mesenteric lymph node and ileum).

The female ET calf born to the recipient was removed from the dam immediately after the Caesarean section and fed four quarts of previously frozen colostrum within 2 hours. The colostrum had been aseptically collected from a clinically normal cow at least 4 years of age. The colostrum donor was twice ELISA and fecal culture test-negative within 12 months prior to freshening, and ELISA-negative and clinically normal one month after freshening. Concurrent fecal culture and ELISA tests for all adult cattle in the colostrum donor herd yielded entirely negative fecal culture results and 13% ELISA-positive results. The risk that the colostrum was contaminated with sufficient *M. paratuberculosis* to establish an infection in the ET calf was believed to be low (but not zero) since the colostrum was collected from a healthy multiparous test-negative cow in a herd free of cattle shedding the organism.

The organization managing the ET calf was comprised of separate physical facilities, each with a different function: animal isolation, quarantine, embryo transfer, gestation and calf-raising. The two closest facilities were one-half mile apart. No adult cattle had been held at the calf-raising facility, and no adult manure had been spread on pasture used for grazing for more than two years. Thus, the calves were not exposed to adults or adult manure and sufficient time had passed to eliminate any *M. paratuberculosis* that may have been on the premises previously.³

Strict sanitation protocols were observed, *e.g.*, cement floors were cleaned daily, manure was removed from the property, farm staff boots were dedicated to the facility, visitors wore plastic boots and had no physical contact with the animals, no trucks entered the pasture or the calf hutch area. Hay fed to the calves was harvested from the farm where the calves were housed and no manure was spread on the hay fields during the same season as the hay was harvested. An on-site well supplied automatic waterers. Under these conditions, the likelihood of acquiring *M. paratuberculosis* through contaminated feed or water was very low.

The calf was housed in its own calf hutch at the facility used to raise fewer than 30 calves. The other

calves at the facility had been delivered by Caesarean section from repeatedly test-negative ET recipients. The calf was bottle-fed milk replacer until approximately two-months of age, calf-starter was provided within a few days of birth and hay was added to its diet at weaning at 6-8 weeks. After weaning, 2-3 calves shared a cement floor barn pen, and by approximately seven months of age they were put on pasture (10 calves/pasture). Under these conditions, the possibility of the presence and subsequent horizontal transmission of the organism was very low.

The ET heifer was moved at 20 months of age to another facility with comparably strict sanitation protocols and housed in cement lots with 19 other ET cattle of the same age. These animals had been obtained by Caesarian section from ET recipients that were test-negative before and after calving, and were raised under the same biosecurity protocols as the calf in question. None of these other ET cattle have subsequently demonstrated any clinical signs of *M. paratuberculosis* infection, nor have they ever tested positive on any Johne's disease diagnostic assay. There were no other cattle on the premises at the time and no other cattle had been housed on these premises for a year. Again the exposure risks for acquiring the organism were very low, and at this point the calf was beyond the most susceptible age range for infection (6 months of age).

The ET heifer annually received dairy standard vaccines for respiratory, leptospiral and clostridial diseases. Three TB skin tests were completed in 1999 and all were negative. The heifer was Johne's disease ELISA and fecal culture-negative at 24 months of age when it was euthanized due to management considerations.

Clinically, the ET heifer was normal and in good body condition. At necropsy, however, gross findings included moderately enlarged mesenteric lymph nodes, ruggation of small intestinal loops and modest thickening of mucosal intestinal tract surfaces. Multinucleated giant cells and activated macrophages were noted in Peyer's patches with rare intracellular acid-fast organisms. The pathologic diagnosis was granulomatous enterocolitis with intralesional bacteria (*i.e.*, Johne's disease). *M. paratuberculosis* was not isolated from the only tissue sample (ileum) available for radiometric culture.

Discussion

Embryo transfer technologies are powerful tools for improving the genetics of a herd, but the process can be costly. It is tempting to minimize costs where it seems feasible, such as purchasing an inexpensive recipient to carry the embryo. However, the entire endeavor can be jeopardized if this theoretically cost-saving animal carries an infectious disease. The false-negative test results for the recipient in this case are typical of *M. paratuberculosis*-infected cattle under two years of age.

Diagnostic or clinical indications that an ET calf was infected during gestation may not appear until years after birth, thus wasting not only the ET costs but calf-raising and adult animal expenditures as well.

While *in-utero* transmission of *M. paratuberculosis* has been previously reported,⁵ this is the first report of *in-utero* transmission from an embryo transfer recipient to the fetus. Due to the biosecurity protocols under which the embryo transfer calf was raised, the post-natal routes of *M. paratuberculosis* transmission under typical dairy husbandry conditions (Table 1) were eliminated. The recipient dam thus represented the greatest transmission risk, significantly outweighing all other factors. This was especially true since she was in late-stage Johne's disease as evidenced by high ELISA results,² visible lesions at necropsy and isolation of the organism from tissue. Colostrum was the only other factor with any risk potential as it was collected from a cow in a seropositive, but fecal culture negative, herd. While the epidemiology of *M. paratuberculosis* transmission through contaminated colostrum is not completely understood, the risk that frozen colostrum aseptically collected from a repeatedly test-negative, clinically normal cow raised in a herd with no detectable shedding of the organism is thought to be low.

Although *M. paratuberculosis* was not cultured from the ileum of the ET heifer, it is unlikely that the pathology described was due to any member of the mycobacterial family other than *M. paratuberculosis*. While the most definitive diagnosis requires genetic identification of an acid-fast organism isolated from tissue, given the confirmed disseminated *M. paratuberculosis* infection in the dam the predictive value of the pathologic lesions in the ET calf is high.

Conclusions

To safeguard the embryo transfer investment and to minimize the risk of infection to a valuable embryo transfer calf, it is important to select an embryo recipient with the greatest probability of being free of *M. paratuberculosis* infection. Attention to biosecurity is important when selecting a colostrum donor as well. While animals may be purchased at a young age when currently available testing methods for Johne's disease are insensitive (due to the absence of antibody production or minimal fecal shedding at early phases of the infection), it is still possible to make an informed decision about the likelihood of infection by assessing Johne's disease prevalence in the source herd. To achieve the highest level of confidence that the ET recipient and colostrum donor cows are not infected with *M. paratuberculosis*, the best approach is to obtain them from a herd certified as Johne's disease test-negative. If this is not possible, the next best approach is

Table 1. Risk factors for transmission of *M. paratuberculosis* to the ET calf.*

Risk factor	Risk level*
Contact with infected cattle/manure	Negligible to none
Environmental contamination	Negligible to none
Horizontal transmission	Negligible to none
Contaminated feedstuff	Negligible to none
Contaminated water	Negligible to none
Human/vehicle borne	Negligible to none
Contaminated colostrum	Low
Intrauterine exposure	High

*Per the risk assessment worksheets used by the NAHMS 2001 dairy survey for use in herds with prior evidence of Johne's disease. These can be obtained at <http://www.johnes.org/handouts>.

to establish the test prevalence in a herd free of clinical cases that follow good Johne's disease control practices (*e.g.*, uses milk replacer instead of waste milk, separates calves from dams at birth, limits calf exposure to adult manure, etc.) The test prevalence can be based on either fecal culture or ELISA assays completed for the adult herd. If whole-herd testing is not fiscally feasible, completing assays on a subset of the herd (minimum 30 cows three years or older) can still provide confidence about the true infection status of the herd.² If the assay results demonstrate a low test prevalence, clinically healthy test-negative cows (born to healthy dams that are test-negative as well) are the most "biosecure" choices to serve as embryo recipients and colostrum donors.

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