Risk Factors Associated with Transmission of *Mycobacterium avium* subsp. *paratuberculosis* to Calves within Dairy Herd: A Systematic Review


**Background:** Paratuberculosis has a worldwide distribution and many countries have implemented control programs to prevent transmission among and within herds. For these programs to be efficient, knowledge of the risk factors involved in transmission is essential.

**Objectives:** Systematically review the scientific literature concerning risk factors associated with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) transmission to dairy calves.

**Study Design:** Systematic review.

**Methods:** An electronic search was done in PubMed and CAB to retrieve references relevant to answer at least 1 of the 5 questions concerning neonatal environment, colostrum, milk, housing of calves, and contact of calves with adult cow feces as risk factors in MAP transmission. A 1st screening was done using titles only, then abstracts, and finally full-length articles were reviewed for relevance. From the articles selected, risk factors and presence of a significant association between these risk factors and MAP transmission were recorded.

**Results:** Twenty-three articles from 11 different countries and published in 12 different journals were reviewed. The most common study design was cross-sectional (n = 16). The case definition and diagnostic tests used were very variable among studies, but serum ELISA was used in most studies (n = 14). The study unit was the herd in 18 studies.

**Conclusions and Clinical Importance:** The contact of calves with adult cow feces is the most important risk factor in MAP transmission. The 5 categories of risk factors are linked to one another.

**Key words:** Control; Johne’s disease; Management; Prevention.

Paratuberculosis or Johne’s disease is a chronic enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The bacteria is mostly transmitted by the feco-oral route, but also can be excreted in colostrum and milk from subclinically infected or clinically affected cows. The infection also can be transmitted in utero. Age susceptibility of cattle recently has been studied in a systematic review with meta-analysis using 11 experimental studies published between 1938 and 2006 (n = 140 cattle). It was concluded that 73.7% of calves exposed to MAP before the age of 6 months developed lesions of Johne’s disease, whereas only 19.3% of cattle exposed after 12 months of age developed lesions.

Prevention is the key to control paratuberculosis because the long incubation period (2–10 years) and low sensitivity of most diagnostic tests make early detection of infected animals difficult. It has been suggested by simulation models that improving calf management was more efficient to decrease MAP prevalence in a herd than a test and cull strategy. These are reasons why control programs should emphasize prevention of MAP transmission, especially to the more susceptible young stock.

The objective of this study was to systematically review the scientific literature concerning risk factors related to MAP transmission to calves.

**Materials and Methods**

The guidelines for conducting a systematic review were based on “A Guide to Conducting Systematic Reviews in Agri-Food Public Health.”

**Search Strategy**

The electronic databases PubMed Medline (1950–2010) and CAB (1973–2010) were searched in January 2011. The systematic search addressed 5 specific questions related to risk factors for transmission of MAP to calves:

1. Is there a relationship between the characteristics of the immediate neonatal environment and the risk of MAP transmission?
2. What is the risk of MAP transmission to neonatal calves through colostrum ingestion?
3 What is the risk of MAP transmission to neonatal calves through milk ingestion?
4 Does group-housing calves increase the risk of MAP transmission?
5 Is there an increased risk of MAP transmission when calves have contact with adult cow feces?

The following Medical Subject Headings (MeSH) were used for the search in PubMed Medline: “Paratuberculosis/epidemiology” [MeSH] OR “Paratuberculosis/prevention and control” [MeSH] OR “Paratuberculosis/transmission” [MeSH] OR “Paratuberculosis/veterinary” [MeSH] AND “Cattle.”

The following key words were used for the search in CAB: Cattle OR Bovine AND Paratuberculosis OR Johne’s OR mycobacterium avium paratuberculosis OR mycobacterium avium subsp paratuberculosis OR mycobacterium avium subspecies paratuberculosis AND Transmission OR Control OR Prevention OR Risk Factors OR Strategies OR Management AND Milk OR Colostrum OR Calves OR Calf OR Calving OR Housing OR Environment.

**Identification of Relevant Studies**

Only studies published in peer-reviewed journals were included. English, French, and Spanish manuscripts were considered. If at least 1 of the 5 questions was potentially answered in the publication, it was deemed relevant. The 1st selection was based only on the title. Citations discarded based on the title concerned diagnostic tests, vaccine, economics, productivity, species other than bovine, Crohn’s disease, pharmacology, pathophysiology, genomics, immunology, transmission in utero, by embryo transfer or semen, and in vitro studies. The abstracts then were reviewed and more manuscripts were discarded for similar reasons. Citations concerning prevalence studies or beef cattle were not discarded based on the title, but after reading the abstract if deemed irrelevant. The remaining articles were reviewed in totality by 3 authors (E.D., J.P., G.F.) to ensure they addressed at least 1 of the 5 questions. Articles concerning only theoretical mathematical models were discarded.

**Data Extraction**

The following information was collected from each article: first author, journal, and year of publication, country (US state or Canadian province if applicable) where the study was done, study design, unit of interest and number of animals or farms, farm or individual case definition, and diagnostic test used when applicable. Relevant risk factors studied for each question and statistical analysis used (univariate or multivariate analysis and significance threshold) were recorded.

For each article, conclusions drawn with regard to the questions of interest were recorded. The possible conclusions were (1) significant association between factor and risk of MAP transmission in the univariate analysis, (2) significant association between factor and risk of MAP transmission in the multivariate analysis (3), no significant association detected. It also was noted when the association was contrary to common knowledge. The level of significance was the threshold used in each manuscript.

**Study Appraisal**

The study appraisal process was done by 2 authors independently (E.D., J.P.). We used a qualitative checklist derived from Sanderson et al. The internal and external validities of the studies were evaluated to determine support for causal association between risk factor and paratuberculosis infection. The validities were described separately, but with the same scale: low, moderate, or high. The following stepwise criteria were used to evaluate internal validity: (1) study design and (2) quality of the study (specifically case definition and diagnostic test reliability). Study designs for which time of exposure could not be ascertained (case-control, cross-sectional) could not be classified as high. A qualitative score was given as follow: + for case-control and cross-sectional studies, ++ for longitudinal, follow-up prevalence and retrospective cohort studies, and +++ for experimental and randomized-controlled clinical trial. The case definition had to be stated and clear. Diagnostic tests and threshold used also were important considerations. Fecal culture to identify MAP has a stronger diagnostic value than a serologic test (ELISA) to detect the immune response. More specifically, requiring 2 positive ELISA results to call a herd infected is a more reliable criterion than requiring 1 positive ELISA result to call an individual cow infected. A qualitative score was given as follows according to the diagnostic method used: 0 for identification of a case with clinical signs by owners or veterinarians, 1 if detection of humoral response on an individual animal was used, 2 for more than 1 individual animal with positive humoral response to consider a herd positive, and 3 if the test used was aimed at detecting MAP directly.

The internal validity was high for study design ++ or ++++, with a clear case definition and diagnosis based on MAP isolation. The support for causal association was judged moderate if the study design was +, and isolation of MAP was used for diagnosis or with a study design ++ with detection of antibody response for diagnosis. All other study designs were considered to provide low support for causal association.

The criteria used to determine external validity were sample size and how sampling was done. Studies with <40 herds or cows enrolled in a voluntary control program were judged to have a low-external validity. If the sample size was ≥40 and the sampling was done randomly, the external validity was judged high. Moderate external validity was used to describe studies with ≥40 enrolled cows in a control program, or studies with fewer herds for which the sampling was done randomly.

Also, how the herds were selected, the time lag between evaluation of risk factors by a questionnaire, and testing of the herd for MAP and type of possible biases were recorded.

**Results**

The PubMed Medline search yielded 441 citations. Thirty-four citations were not in English, French, or Spanish. The remaining citations (n = 407) were screened using the title only, and 268 were considered irrelevant. After reading the abstract (n = 139), 84 more were discarded. Fifty-five articles were read completely and reviewed, and 20 were kept for data extraction.

The CAB search yielded 346 citations that were screened using their titles, and 257 were discarded. After review of 89 abstracts, 36 were discarded because they did not address any of the questions, 11 because they were not in English, French, or Spanish, and 33 were duplicates from the PubMed search. Nine articles were reviewed, and 3 were kept for data extraction.

Twenty-three articles were included in the study. The year of publication varied from 1992 to 2010 and articles originated from 12 different journals. The work was done in 11 different countries. The most common
study design was cross-sectional (n = 16). The study unit was the herd (n = 18) or the cows (n = 5). The number of farms studied varied from 1 to 2,953. The definition of a positive animal or a positive herd was variable. The most common diagnostic test used was serum ELISA (n = 14). Details of the data extracted for the 23 articles are presented in Table 1. Study appraisal is presented in Table 2. The characteristics of the immediate neonatal environment and the risk of MAP transmission (question 1) were addressed in 1812–29 of 23 manuscripts (Table 3). Eleven articles reported a significant association between the immediate neonatal environment and MAP infection. Contamination of udders with manure,12 group-housing of periparturient cows,29 and presence of more than 1 cow in the maternity pen28 were 3 factors that increased the risk of being a MAP-infected herd. Benedictus et al13 found a relationship between lifelong infection status of calves born from negative dams and calving pen contamination. Calves exposed to a contaminated calving pen by infected cattle or shedding cattle between 3 and 10 days of life were more likely to become infected by MAP, and to have a positive fecal culture compared with calves not exposed. Odds ratio varied from 2.2 to 3.9, if infected cattle contaminated the calving pen and varied from 3.6 to 6.6, if shedding cattle were in the calving pen. Ridge et al in 200525 found an increased risk of infection when calving occurred in a shed or a calving pad compared with a paddock. Ridge et al in 201026 found that calving in a paddock or in a shed compared with a calving pad increased the risk of MAP transmission.

Çetinkaya et al15 demonstrated a protective effect of calving in an individual pen when the cows are at grass. Goodger et al19 identified that scores in the newborn calf care category, which includes time of removal from the dam, were significantly correlated with the apparent prevalence of MAP in dairy herds. Nielsen and Toft23 found that proper management of the calving area (proper hygiene of the feeding area, more straw) reduced the odds of MAP infection at the herd level. Cashman et al14 found that the probability of having a MAP-positive culture was significantly decreased as the percentage of the calvings that were attended increased.

A case-control study by Johnson-Ifearulundu and Kanene20 reported an association that was contrary to common thinking. In this study, washing cows’ udders before parturition was associated with an increased risk of infection with MAP.

The risk of MAP transmission through colostrum ingestion (question 2) was addressed in 1112,14,18,19,21,22,27,28,30–32 of 23 manuscripts (Table 4). A significant association between the type of colostrum fed to calves and MAP transmission was detected in 4 manuscripts. Dieguez et al18 found that feeding colostrum from ELISA-positive cows increased the risk of being an infected herd by MAP. Nielsen’s study31 was specifically designed to study colostrum as a risk factor for MAP infection in dairy cattle. The risk of a cow having a positive ELISA is greater for cows that, when they were calves, had been fed pooled colostrum from multiple cows than it was for cows that had been fed colostrum from their own dams. Calves fed pooled colostrum from multiple cows were at greater risk of testing positive, once adult to an in-house milk ELISA compared with calves fed colostrum only from their own dam. In Stabel’s30 randomized-control trial, 6 calves were fed colostrum from their dam (DC) and 5 calves received pasteurized colostrum (PC). The DC calves were allowed to nurse their dam for 8 hours after birth, received milk from their dam for 3 weeks, and milk replacer for the next 3 weeks. The PC calves were separated from their dam immediately after birth before they could nurse and were fed milk replacer for 6 weeks. After weaning, the 11 calves were housed together until 1 year of age. There was no significant difference among serum ELISA results, fecal shedding of MAP, and positive culture of MAP from postmortem tissues of the 2 groups. The only significant difference noted was that IFN-γ secretion was higher in DC calves compared with PC calves at 5 months of age. In Goodger’s study,19 newborn care was significantly associated with the prevalence of MAP in dairy herds. Newborn calf care included colostrum management factors: cleanliness of udder and bottles, and if the colostrum was pooled.

The risk of MAP transmission through milk ingestion (question 3) was addressed in 1312,14,15,17,21,22,24,28,30,31 of 23 manuscripts (Table 5). A significant association between the type of milk fed to calves and MAP transmission was detected in 4 manuscripts. Nielsen’s study31 was specifically designed to study colostrum and milk as risk factors for MAP transmission in dairy cattle. Calves suckling with foster cows had an odds ratio of 2.012 (95% CI: 1.370–2.956) to be ELISA positive compared with calves fed milk replacer. In-house milk ELISA was repeated up to 4 times a year on all lactating cows. Ridge et al25 demonstrated that feeding waste milk to calves was significantly associated with increased occurrence of MAP infection based on serum ELISA and clinical cases. In a subsequent study, Ridge et al26 found that feeding waste milk to calves decreased the risk of MAP transmission. In McNab’s case-control study,21 being a high-risk herd, based on the herd mean LAM-ELISA optical density and the distribution of individual LAM-ELISA results among the herds, was positively associated with the proportion of newborn calves fed no raw milk.

Group-housing calves as a risk of MAP transmission (question 4) was addressed in 1113–16,20,21,23,24,28,29,33 of 23 manuscripts (Table 6). Four studies found a significant association between group-housing of preweaned calves and MAP transmission. Tiwari et al28 found that group-housing preweaned calves in winter was associated with the number of ELISA-positive cows in a herd. Benedictus et al13 confirmed in a 20-year longitudinal study the risk of transmission of MAP to calves raised with a future high shedder (>100 colony forming units of MAP/g of feces on culture). Calves born within 90 days after the birth of a future high shedder were 19.1 times more likely to become
Table 1. Data extracted from the 23 articles included in the systematic review.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Country</th>
<th>Study Design</th>
<th>Number Animals/Herds (Study Unit in Bold)</th>
<th>Case Definition</th>
<th>Diagnostic Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correia-Gomes et al</td>
<td>2010</td>
<td>Portugal</td>
<td>Cross-sectional</td>
<td>5,294 cows/122 herds</td>
<td>Milk ELISA+ = cow+</td>
<td>Milk ELISA(^b)</td>
</tr>
<tr>
<td>Ridge et al</td>
<td>2010</td>
<td>Australia</td>
<td>Retrospective cohort</td>
<td>137 herds</td>
<td>ELISA+ or clinical case of JD after 2nd whole herd test = herd+</td>
<td>ELISA (not specified)</td>
</tr>
<tr>
<td>Ansari-Lari et al</td>
<td>2009</td>
<td>Iran</td>
<td>Cross-sectional</td>
<td>110 herds</td>
<td>PCR+ on bulk-tank milk = herd+</td>
<td>IS900-PCR</td>
</tr>
<tr>
<td>Norton et al</td>
<td>2009</td>
<td>New Zealand</td>
<td>Cross-sectional</td>
<td>427 herds</td>
<td>Perception of clinical case by veterinarians, farmers' records</td>
<td></td>
</tr>
<tr>
<td>Phihua et al</td>
<td>2009</td>
<td>United States</td>
<td>Randomized-control clinical trial</td>
<td>497 calves/12 JD endemic herds</td>
<td>ELISA, FC or both at 30, 42 or 54 months of age</td>
<td>ELISA IDEXX(^e) HEYM</td>
</tr>
<tr>
<td>Tiwari et al</td>
<td>2009</td>
<td>Canada</td>
<td>Cross-sectional</td>
<td>7,689 cows/257 herds</td>
<td>ELISA = cow+</td>
<td>ELISA IDEXX(^e)/BIOCOR(^d)</td>
</tr>
<tr>
<td>Benedictus et al</td>
<td>2008</td>
<td>United States</td>
<td>Longitudinal</td>
<td>6400 cows from 1 herd over 20 years</td>
<td>Count ELISA+ cows/farm</td>
<td>HEYM</td>
</tr>
<tr>
<td>Cashman et al</td>
<td>2008</td>
<td>Ireland</td>
<td>Cross-sectional</td>
<td>59 herds</td>
<td>PCR or culture+ of milk sock filters = herd+</td>
<td>PCR, culture</td>
</tr>
<tr>
<td>Dieguez et al</td>
<td>2008</td>
<td>Spain</td>
<td>Cross-sectional</td>
<td>5,528 cows &gt;1 year old/101 herds</td>
<td>Herd--; no ELISA+ cows or only one ELISA+ but no cows with CS</td>
<td>ELISA IDEXX(^e)</td>
</tr>
<tr>
<td>Nielsen et al</td>
<td>2008</td>
<td>Denmark</td>
<td>Cross-sectional</td>
<td>93,994 cows/799 herds</td>
<td>ELISA+ = cow+</td>
<td>In-house ELISA(^\text{a})</td>
</tr>
<tr>
<td>Stabel et al</td>
<td>2008</td>
<td>United States</td>
<td>Randomized-controlled clinical trial</td>
<td>6 DC calves</td>
<td>FC+, ELISA+ or ↑ INF-(^-\gamma) Monthly Culture or PCR+ on necropsy tissues at 12 months of age</td>
<td>HEYM, ELISA(^\text{a}), INF-(^-\gamma) IS900 PCR</td>
</tr>
<tr>
<td>Tavornpanich et al</td>
<td>2008</td>
<td>United States</td>
<td>Cross-sectional</td>
<td>60 lactating cows per herd/21 herds</td>
<td>Low to zero seroprevalence herd: ≤ 2 ELISA+ cows; high seroprevalence: ≥ 3 ELISA+ cows</td>
<td>ELISA IDEXX(^e)</td>
</tr>
<tr>
<td>Nielsen and Toft</td>
<td>2007</td>
<td>Denmark</td>
<td>Follow-up prevalence</td>
<td>All lactating cows/97 herds</td>
<td>Continuous OD milk ELISA</td>
<td>In-house ELISA(^\text{a})</td>
</tr>
<tr>
<td>van Roermund et al</td>
<td>2007</td>
<td>The Netherlands</td>
<td>Experimental</td>
<td>(A) 2 × 5 calves 1w old + 2 × 6 cows (B) 2 × 5 donor calves + 2 × 5 receiver calves</td>
<td>Culture+ (facs or necropsy tissues), ELISA+ or ↑ INF-(^-\gamma) Animals kept until 43–48 months of age</td>
<td>Culture,(^\text{b}) ELISA Pourquier,(^\text{b}) INF-(^-\gamma)</td>
</tr>
<tr>
<td>Ridge et al</td>
<td>2005</td>
<td>Australia</td>
<td>Cross-sectional</td>
<td>All cows over 2 years tested annually/54 herds</td>
<td>ELISA+ or clinical case of JD after 2nd whole herd test</td>
<td>ELISA(^\text{i})</td>
</tr>
<tr>
<td>Muskens et al</td>
<td>2003</td>
<td>The Netherlands</td>
<td>Cross-sectional</td>
<td>All cows ≥ 3 year old/370 herds</td>
<td>ELISA+ in herd ≥ 34 cows and ≥ 2 cows</td>
<td>ELISA IDEXX(^e)</td>
</tr>
<tr>
<td>Wells and Wagner</td>
<td>2000</td>
<td>United States</td>
<td>Cross-sectional</td>
<td>31,745 cows/967 herds</td>
<td>ELISA+ in herd ≥ 34 cows = herd+</td>
<td>ELISA IDEXX(^e)</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Country</th>
<th>Study Design</th>
<th>Study Unit in Bold</th>
<th>Case Definition</th>
<th>Diagnostic Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson-Ifearulundu and Kaneene</td>
<td>1998</td>
<td>United States</td>
<td>Case-control</td>
<td>46 case herds-37 control herds</td>
<td>≥ 2 ELISA+ cows = herd+</td>
<td>ELISA IDEXX&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Obasanjo et al</td>
<td>1997</td>
<td>England</td>
<td>Cross-sectional</td>
<td>2,953 herds</td>
<td>Reporting a case in 1993 or in 1994</td>
<td>Not done</td>
</tr>
<tr>
<td>Goodger et al</td>
<td>1996</td>
<td>United States</td>
<td>Cross-sectional</td>
<td>33 herds</td>
<td>Herds already known to have MAP: 1 clinical case/year or ≥ 2 + FC in past year</td>
<td>HEYM</td>
</tr>
<tr>
<td>Collins et al</td>
<td>1994</td>
<td>United States</td>
<td>Cross-sectional</td>
<td>4,990 cows/158 herds</td>
<td>ELISA+ = cow+</td>
<td>ELISA&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>McNab et al</td>
<td>1992</td>
<td>Canada</td>
<td>Case-control</td>
<td>56 case herds-58 control herds</td>
<td>Herd mean LAM-ELISA OD and distribution of individual LAM-ELISA</td>
<td>LAM-ELISA</td>
</tr>
</tbody>
</table>

CS, clinical signs; DC, dam colostrum; FC, fecal culture; HEYM, Herrold’s egg yolk medium; INF-γ, interferon-gamma; JD, Johne’s disease; LAM-ELISA, lipoarabinomannan enzyme-immuno-assay; OD, optical density; PC, pasteurized colostrum; PCR, polymerase chain reaction.

<sup>a</sup>Study design in accordance with description of the study in materials and methods not always according to author definition.

<sup>b</sup>Institut Pourquier, Montpellier, France.

<sup>c</sup>IDEXX Herdcheck ELISA; IDEXX Laboratories, Westbrook, ME.

<sup>d</sup>BIOCOR Parachek ELISA; BIOCOR Animal Health Inc, Omaha, NE.


<sup>f</sup>Parachek, PRIONICS AG, Schlierten-Zurich, Switzerland.

<sup>g</sup>Bovigam, Prionics, Lincoln, NE.


<sup>i</sup>Bovigram, CSL Ltd, Parkville, Australia.

<sup>j</sup>Johne’s Absorbed EIA Kit; CSL Ltd.

<sup>k</sup>Herdcheck Mpt Ab, Idexx Skandinavia AB, Sweden.

<sup>l</sup>IDEXX Laboratories, Portland, ME.

<table>
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<tr>
<th>Authors</th>
<th>Year</th>
<th>Herds Selection</th>
<th>Time Lag between Questionnaire and Testing</th>
<th>Potential Bias</th>
<th>Internal Validity</th>
<th>External Validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridge et al [26]</td>
<td>2010</td>
<td>Random selection, from program</td>
<td>6 months for visits; testing over 18 years (May 1990–Mar 2008)</td>
<td>Misclassification</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Ansari-Lari et al [22]</td>
<td>2009</td>
<td>From 3 districts with more than 80% of dairies in the region</td>
<td>Mar–Aug 2006</td>
<td>Misclassification</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Pitua et al [12]</td>
<td>2009</td>
<td>Endemic herds</td>
<td>N/A</td>
<td>N/A</td>
<td>High</td>
<td>Moderate</td>
</tr>
<tr>
<td>Ridge [25]</td>
<td>2005</td>
<td>From program</td>
<td>N/A</td>
<td>N/A</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Tavornpanich et al [27]</td>
<td>2008</td>
<td>From clientele of a practice</td>
<td>Questionnaire Aug–Nov 2001</td>
<td>Misclassification</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Nielsen and Toft [23]</td>
<td>2007</td>
<td>Not random, part of a project higher in size</td>
<td>Questionnaire from Aug 1999 to Dec 1999</td>
<td>Misclassification</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>van Roermund et al [13]</td>
<td>2007</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Wells and Wagner [29]</td>
<td>2000</td>
<td>Stratified random sample</td>
<td>Questionnaire Jan–May 1996 (practices currently used and 3 years prior)</td>
<td>Misclassification</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Obasanjo et al [34]</td>
<td>1997</td>
<td>From program</td>
<td>Phone interviews Jul–Oct 1993</td>
<td>Misclassification</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

(Continued)
infected with MAP. Wells and Wagner\textsuperscript{29} found that group-housing of calves before weaning increased the risk of being a herd infected with MAP. Cashman et al\textsuperscript{14} found that herds raising calves in individual pens had decreased odds of a positive culture for MAP on the milk sock filter residue.

The contact between calves and adult cow feces and the risk of MAP transmission (question 5) was addressed in 14\textsuperscript{14,17–20,22,25,27–29,33,34}\textsuperscript{–29,33,34} of 23 manuscripts (Table 7). A significant association between MAP transmission and contact between calves and adult cow feces was detected in 5 manuscripts. Norton et al\textsuperscript{24} found an almost dose-response relationship between the frequency of grazing calves in a hospital paddock and the odds of being a high incidence herd. Herds where calves were housed with adults before 6 months of age were more likely to be infected by MAP.\textsuperscript{18} Obasanjo et al\textsuperscript{34} found a similar association in herds for which calves between 0 and 6 weeks of age were exposed to adult feces. An experimental study\textsuperscript{33} demonstrated that calves in contact with adult fecal shedders are at higher risk of becoming infected. In Goodger's study,\textsuperscript{19} a regression analysis identified that newborn care was significantly associated with the prevalence of MAP in dairy herds. Herds having high score for management practices were less likely to be infected with MAP. Questions relevant to evaluate the contact between calves and adult cow feces were included in the manure-handling category and were manure equipment not used for feeding, young stock not near adult manure, and barn cleaner not near calves.

**Discussion**

Based on the present systematic review, contact with adult cow feces appears to be the most important risk factor for MAP transmission. Contact of calves with feces from adult cows was a risk factor for MAP transmission with high odds ratio (range, 4.59–30.5). Contact with adult cow feces represented a specific question, but appeared to be addressed by a surrogate variable in other questions. Calving environment, colostrum, milk, or housing might be a surrogate measure of fecal contamination. For example, it was found that suckling of foster cows (question 3: milk) increased the risk of being MAP infected. In this situation, the exact role of MAP (milk or feces on the teats) cannot be determined with certainty. Another example is question 1, concerning the neonatal environment. Most of the risk factors were closely related to fecal contamination (hygiene), for example, cleanliness of the calving area and udder washed before collection of colostrum.

The source of colostrum or milk as a risk factor for MAP transmission appeared to be of less relevance because most of the studies with moderate or high support for a causal association did not find an association between these risk factors and MAP transmission. There were 3 studies designed specifically to examine the impact of colostrum source. Of the 2 studies classified as high in the study appraisal, 1 did not find...
Table 3. Manuscripts that studied risk factors concerning neonatal environment (question 1): results from univariate and multivariate analyses.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Risk Factors Examined</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correia-Gomes17</td>
<td>(1) CA: yes or no; (2) hours to separate dam-calf: ≤ 6, 7–12, &gt;12 hours</td>
<td>NS</td>
<td>Not included in final model</td>
</tr>
<tr>
<td>Ridge26</td>
<td>CA: calving pad versus shed versus paddock</td>
<td>CA: <em>P</em> = .259</td>
<td>Calving in a paddock HR 2.94 (CI 1.289–6.708) or in a shed HR 6.61 (CI 1.693–25.786) increased risk of MAP transmission <em>P</em> &lt; .01</td>
</tr>
<tr>
<td>Ansari-Lari12</td>
<td>(1) Calves with dam ≥3 hours; (2) separate CA; (3) contaminated udders of periparturient cows with manure</td>
<td>Udder contaminated with manure (3) <em>P</em> &lt; .2</td>
<td>Contamination of udders of periparturient cows with manure (3) OR 6.38 (CI 1.29–31.49) <em>P</em> = .02</td>
</tr>
<tr>
<td>Norton24</td>
<td>(1) Calf-dam contact after birth; (2) CA: main herd or separated</td>
<td>NS</td>
<td>CA included in model for biologic importance but NS</td>
</tr>
<tr>
<td>Tiwari28</td>
<td>CA/management: (1) time with dam; (2) cleanliness of teat area; (3) use for sick cows; (4) location; (5) number of cowsa</td>
<td>More than 1 cow in maternity pen (5) CR 1.5 (SE 0.26)</td>
<td>More than 1 cow in maternity pen (5) CR 1.7 (CI 1.2–2.2) <em>P</em> &lt; .01</td>
</tr>
<tr>
<td>Benedictus13</td>
<td>CA contamination for calves born from MAP negative dam (shedding/infected animals versus negative and unknown)</td>
<td>Calves between 3 and 13 days of age in a contaminated CA <em>P</em> &lt; .05</td>
<td>ND</td>
</tr>
<tr>
<td>Cashman14</td>
<td>(1) Calf-dam separation; (2) calving attendance; (3) time allowed to suckle their dam; (4) precalving udder clipping</td>
<td>Probability of positive culture significantly reduced as % calving attended increased (2) <em>P</em> &lt; .05</td>
<td>ND too small data set</td>
</tr>
<tr>
<td>Dieguez18</td>
<td>(1) Separate CA; (2) time of separation calf-dam; (3) udder/teat washed before collection or calf suckling</td>
<td>NS</td>
<td>Not included in model</td>
</tr>
<tr>
<td>Tavornpanich27</td>
<td>(1) Time separation calf-dam; CA: (2) separated from sick cows/lactating; (3) frequency of bedding changes; (4) group versus separate pen</td>
<td>NS</td>
<td>Not included in model</td>
</tr>
<tr>
<td>Nielsen23</td>
<td>CA: (1) used for sick cows; (2) no specific CA; (3) 7 aspect of CA hygiene</td>
<td>ND</td>
<td>Hygiene in feeding area (3) OR 2.70 (CI 1.04–6.8) for 3rd best versus best Amount of straw in bedding (3) OR 3.0 (CI 1.21–8.1) for worst versus best</td>
</tr>
<tr>
<td>Ridge25</td>
<td>(1) CA: calving pad versus shed versus paddock; (2) time before calf removed from dam</td>
<td>ND</td>
<td>Calving in paddock decreased risk of MAP transmission (1) <em>P</em> = .047</td>
</tr>
<tr>
<td>Muskens22</td>
<td>(1) Separate CA; (2) ≥90% cows calved in CA; (3) ≥90% cows calved in clean CA; (4) noncalving cattle in CA; (5) calf removed from dam immediately</td>
<td>Separate calving area (1) <em>P</em> = .06; ≥90% cows calved in CA (2) <em>P</em> = .08; Noncalving cattle in CA (4) <em>P</em> = .05</td>
<td>NS</td>
</tr>
<tr>
<td>Wells29</td>
<td>(1) With dam &gt;24 hours; (2) CA for sick cows; (3) bedding for CA; (4) group-housing periparturient; (5) teats and udder washed beforecolostrum collected or calves suckle</td>
<td>Group-housing periparturient (4) <em>P</em> = .01</td>
<td>Group-housing periparturient (4) OR 1.5 (CI 1.0–2.3) <em>P</em> = .06</td>
</tr>
<tr>
<td>Johnson-Ifearu1ndu20</td>
<td>(1) Separation (in hours) calf-dam; (2) washing cows’ teats and udder before parturition; Maternity pen: (3) used for calving; (4) used for sick cows; (5) frequency of cleaning</td>
<td>(2) Washing teats and udder; (3) Maternity pen for parturition</td>
<td>Washing udders before parturition (2) OR 8.66 (CI 1.87–40.08) <em>P</em> = .006</td>
</tr>
</tbody>
</table>

(Continued)
a significant association between ingestion of maternal colostrum and risk of MAP transmission and the other one found only an increase in INF-γ in DC calves. The 3rd study, classified as low in the study appraisal, found an increased risk of MAP transmission when colostrum from multiple cows was fed but with a small OR of 1.243 (95% CI: 1.089–1.418).
The most common study design used was cross-sectional study. This type of observational study provides little evidence of causality and is considered moderately relevant to “real-world” situations. When a management practice is identified to be more prevalent in MAP-infected herds, it does not provide any evidence that it is a risk factor involved in MAP transmission. Also there is no certainty that the risk factor was present before the exposure to MAP in cross-sectional studies. The same applies to case-control studies. Wells and Wagner studied not only associations between risk factors and herd status for Johne’s disease but also associations with the herd manager’s familiarity with Johne’s disease and the prior diagnosis of Johne’s disease in a herd. In herds where managers were familiar with Johne’s disease, the cows were 1.5 times more likely to have their teats and udder washed before colostrum was collected or the calf was allowed to suckle, compared with herds where managers were not familiar with the disease. Also, in herds with a previous diagnosis of Johne’s disease, newborn calves were 3.4 times more likely to be separated from the dam less than 1 hour after birth compared with herds where the disease was not previously diagnosed. This may explain why some studies found associations that seemed opposite to what might be expected according to common knowledge. In cohort studies, where the risk factor or exposure is recorded before disease occurs, an inverse association can be found if the cohort is not followed long enough, because Johne’s disease has a very long incubation period. This can be especially true for retrospective or historic cohort studies in which recalling historical management practices can be very subjective and a source of recall bias.

Ridge published 2 different studies on herd management practices and transmission of Johne’s disease in dairy herds in Victoria, Australia. The relation between some management practices and Johne’s disease transmission in the herds differed between the 2 studies. For example, in the 2005 study, feeding waste milk to calves was a significant risk factor for Johne’s transmission, but, surprisingly, was found to be a protective factor in the 2010 study. The 1st survey to assess calf rearing practices was done in 2002, and the 2nd one between July 2005 and January 2006. The herd status for MAP (clinical cases or ELISA-positive cows) was updated in March 2008. The author stated that between the 2 surveys, several changes had been made concerning calf management practices. Once again, because of the long incubation period of Johne’s disease, a herd that was being fed waste milk in the past could find infected cows several years after the

Table 5. Manuscripts that studied risk factors concerning milk (question 3): results from univariate and multivariate analyses.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Risk Factors Examined</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correia-Gomes et al.</td>
<td>Exclusively dam milk: yes or no</td>
<td>NS</td>
<td>Not included in final model</td>
</tr>
<tr>
<td></td>
<td>Antibiotic and waste milk</td>
<td></td>
<td>Protective effect of feeding antibiotic/waste milk HR 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(CI 0.247–0.720) P &lt; .001</td>
</tr>
<tr>
<td>Ridge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ansari-Lari et al.</td>
<td>Unpasteurized milk</td>
<td>ND (all calves fed</td>
<td>ND (all calves fed unpasteurized milk)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unpasteurized milk</td>
<td></td>
</tr>
<tr>
<td>Norton et al.</td>
<td>(1) Penicillin milk; (2) nurse cows</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tiwari</td>
<td>(1) Pooled all cows; (2) pooled MAP neg. cows; (3) mastitic (clinic or high SCC) or antibiotic residuea</td>
<td>NS</td>
<td>Not in final model</td>
</tr>
<tr>
<td>Cashman</td>
<td>(1) Milk mixed with colostrum; (2) pooled milk</td>
<td>NS</td>
<td>ND too small data set</td>
</tr>
<tr>
<td>Nielsen</td>
<td>(1) Different sources milk: (a) replacer, (b) bulk tank,</td>
<td>Descriptive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c) pooled high SCC; (d) bulk tank if insufficient milk high SCC; (2) foster cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stabel</td>
<td>Milk from infected dam</td>
<td>ND</td>
<td>Not discussed</td>
</tr>
<tr>
<td>Tavornpanich et al.</td>
<td>Unsalable milk</td>
<td></td>
<td>Caudal probability for risk factor = 0.712 NS but ASSOCIATION</td>
</tr>
<tr>
<td>Ridge</td>
<td>Replacer, whole, whole + colostrum, whole + antibiotic residues</td>
<td>Descriptive</td>
<td>Feeding “antibiotic milk” P &lt; .001 increased risk of MAP infection</td>
</tr>
<tr>
<td>Muskens</td>
<td>Only fed milk replacer</td>
<td>P = .04 sero + 25.2%</td>
<td>NS</td>
</tr>
<tr>
<td>C¸etinkaya</td>
<td>Pooled, milk replacer</td>
<td>NS</td>
<td>Included in model for biologic importance but NS</td>
</tr>
<tr>
<td>Mc Nab</td>
<td>Raw milk</td>
<td>Descriptive</td>
<td>Newborn calves to weaning fed no raw milk more in high-risk (case) herds P = .02</td>
</tr>
</tbody>
</table>

CI, 95% confidence interval; ND, not done; NS, not significant; OR, odds ratio; SCC, somatic cell count.
aDetails of questionnaire obtained from the author, not in the manuscript.
management practice was discontinued because a cow may develop the disease several years later. In this case, a selection bias is possible in terms of the survey constructs with regard to the farmers’ knowledge of the status of the herd and adjusting their behaviors according to their status.

Systematic review is the study design that gives the strongest level of evidence. The search method and criteria for inclusion are transparent and repeatable. In this study, gathering information from good primary studies allows summarizing knowledge to answer questions on a specific topic. We searched a major medical database and an additional agricultural database that only added 3 different articles to the review. Some studies never get published, and the language of publication can be a barrier for accessing the results. We searched for studies written in 3 different languages and only found relevant studies written in English. Studies that found significant results are more likely to get published and may be published in peer-reviewed journals. So, we might have created a bias toward studies that found significant results. Also, because of the timelines of a systematic review, we did not search nonpeer-reviewed journals and proceedings from conferences.

Most systematic reviews focus on only 1 research question. We decided to study 5 different questions concerning risk factors for MAP infection of dairy calves to cover all the different possibilities of MAP transmission. It might have been less laborious to review the literature with only 1 specific question, but we may not have identified the close relationship among risk factors. Moreover, we would have minimized the importance of contact with adult cow feces in MAP transmission because that risk factor was identified by many questions as discussed previously.

Information concerning the different risk factors of MAP transmission to calves was summarized qualitatively. With this systematic review, it was not possible to compile quantitative data of the different studies.

Table 6. Manuscripts that studied risk factors concerning group-housing of calves (question 4), results from univariate and multivariate analyses.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Risk Factors Examined</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton24</td>
<td>Individual versus group</td>
<td>NS</td>
<td>Not included in final model</td>
</tr>
<tr>
<td>Tiwari28</td>
<td>For preweaned: group/individual pens, hutches (summer versus winter)</td>
<td>Group-housing in winter P = .15</td>
<td>Group-housing in winter CR 2.0 (CI 1.3–2.8) P &lt; .01</td>
</tr>
<tr>
<td>Benedictus13</td>
<td>Calf-to-calf transmission (exposure to future high shedder) calves from test-neg dam and no contamination in calving area</td>
<td>4/14 infected were born within 90 days after birth of future high shedder ($\chi^2 = 12.7$; $P = .0004$; Fisher 2-sided: $P = .0064$; OR 19.1) 3/4 born 30–90 days after ($\chi^2 = 6.91$; $P &lt; .0086$; Fisher 2-sided: $P = .036$; OR 9.7)</td>
<td>ND</td>
</tr>
<tr>
<td>Cashman14</td>
<td>Individual calf pens</td>
<td>Individual pens → odds milk sock filter culture + significantly lower $P &lt; .05$ (OR 0.21; CI 0.04–1.0)</td>
<td>ND (too small data set)</td>
</tr>
<tr>
<td>Nielsen23, van Roermund33</td>
<td>Calves &lt;2 months: single pen or other Infection calf-to-calf</td>
<td>ND</td>
<td>NS</td>
</tr>
<tr>
<td>Wells29</td>
<td>Group-housing for calves before weaning during preceding year</td>
<td>$P = .05$</td>
<td>$P = .04$ if grouped OR 1.5 (CI 1.0–2.3)</td>
</tr>
<tr>
<td>Johnson-Ifealbumunlu20</td>
<td>Individual calves hutches or not</td>
<td>NS</td>
<td>Not included in final model</td>
</tr>
<tr>
<td>Çetinkaya21</td>
<td>Individual pens (never, first 30 days, more 30 days)</td>
<td>$P \leq .25$</td>
<td>NS</td>
</tr>
<tr>
<td>Collins36</td>
<td>Calves housing before weaning (calf barn, hutches, pens in cow barn, other)</td>
<td>Calf housing before weaning $P = .33$</td>
<td>Not included in final model</td>
</tr>
<tr>
<td>McNab21</td>
<td>Housing newborn calves to weaning: individual tied, indoor pens, outdoor hut or pen</td>
<td>Descriptive</td>
<td>Heifer calves, weaning to 8 months, individually tied in summer $P = .02$ (not neonatal calves)</td>
</tr>
</tbody>
</table>

ND, not done; NS, not significant; CR, count ratio; OR, odds ratio; CI, 95% confidence interval.
Table 7. Manuscripts that studied risk factors concerning contact with adult cow feces (question 5): results from univariate and multivariate analyses.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Risk Factors Examined</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correia-Gomes17 Norton24</td>
<td>Pasture share with cows (1) Age at 1st contact with adult (2) Calves in hospital paddock</td>
<td>$P &lt; .25$</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes = low incidence (OR 1.98; CI 1.25–3.15); frequently = high incidence (OR 4.53; CI 1.62–2.69) (2)</td>
<td>Paddock frequently = high incidence (2) OR 5.92 (CI 1.37–25.48)</td>
</tr>
<tr>
<td>Tiwari28 Cashman14</td>
<td>Equipment for manure and heifer feed</td>
<td>NS</td>
<td>Not in final model</td>
</tr>
<tr>
<td></td>
<td>Slurry spread on calf pasture, calves grazed adult pasture (animal waste and hygiene management)</td>
<td>NS</td>
<td>ND too small data set</td>
</tr>
<tr>
<td>Dieguez18</td>
<td>(1) &lt;6 months housed with adults (2) Fed pasture or herbage treated with manure</td>
<td>Housing with adult &lt;6 months (1) ($P &lt; .001$); herbage with manure (2) ($P &lt; .001$)</td>
<td>Replacement animals housed with adult cattle &lt;6 months (1) ($P = .026$; OR 4.59 (CI 1.20–17.6);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tavornpanich27</td>
<td>(1) Heifers ≤ 6 months exposed to adult manure (2) Manure-handling equipment to feed (3) Lagoon</td>
<td>Manure-calves (1) $P = .09$ Manure-feed (2) $P = .19$ Lagoon (3) $P = .13$</td>
<td>NS causal probability; Manure-calves (1) 0.68; Manure-feed (2) 0.662; Lagoon (3) 0.680</td>
</tr>
<tr>
<td>Nielsen23</td>
<td>Calves &lt;2 months separated from cows (Y versus N)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>van Roermund33</td>
<td>Cow-calves transmission</td>
<td>MLE estimator for R (3 months) is 2.7 with CI 1.1–6.6; 1-sided test H0: $R \leq 1 \rightarrow P = .019$ (H0 rejected), H0: $R \geq 1 \rightarrow P = .99$</td>
<td>ND</td>
</tr>
<tr>
<td>Ridge25</td>
<td>Unweaned calves housing: adequately separated from adult cattle and their effluent</td>
<td>NS suggestive association between adequate separation of the calf shed from adult cattle, feces of adults or effluent and reduced BJD incidence $P = .07$</td>
<td></td>
</tr>
<tr>
<td>Muskens22</td>
<td>Age in months when calves no longer housed separately from adults</td>
<td>Average: sero– = 8.6 ± 8.7; sero+ = 10.2 ± 8.4 $P = .11$</td>
<td>NS</td>
</tr>
<tr>
<td>Wells29</td>
<td>Heifers &lt;12 months: (1) Common feed or water sources with adult (2) Equipment to handle manure and their feed</td>
<td>Heifers &lt;12 months sharing feed/water with adults was associated with JD status (1) but inverse direction...spurious association</td>
<td>Not included in model</td>
</tr>
<tr>
<td>Johnson-Ifearulundu20</td>
<td>Common equipment for feed and manure; common feed and water source between calves and adults; feed for calves on fields manure spread</td>
<td>$P \leq .95$ and 70 or more observations for 3 factors so offered to multiple regression</td>
<td>NS</td>
</tr>
<tr>
<td>Obasanjo34</td>
<td>(1) Exposure calves 0–6 weeks to adults feces (2) Young stock contact adult feces from same equipment used for clean (3) Feces spread on forage fed to any age group</td>
<td>(1) OR 8.3 (CI 1.4–47.5); (2) OR 6.4 (CI 1.0–38.8); (3) OR 10.3 (CI 1.8–60)</td>
<td>Any practice leading to exposure of calves 0–6 weeks to feces of adult cows (1) OR 30.5 (1.2–808.7)</td>
</tr>
<tr>
<td>Goodger79</td>
<td>(1) Milk-fed calf care = pens void of adult manure (2) Manure handling = young stock not near adult manure, barn cleaner not near calves, manure equip. for feeding</td>
<td>Descriptive</td>
<td>$R^2 = 0.90$; high score for manure handling significantly associated with MAP apparent prevalence (2) (main effect $P &lt; .001$ and interactions terms significant)</td>
</tr>
</tbody>
</table>

CI, 95% confidence interval; ND, not done; NS, not significant; OR, odds ratio.
and conduct a meta-analysis. Different study designs were used, the case definition for a MAP-positive cow or MAP-positive herd was variable, the diagnostic tests to detect a MAP infection differ, and finally, the statistical analysis of the data varied. For all of these reasons, a meta-analysis was not realistically feasible. Instead, we decided to classify qualitatively the studies according to the strength of the causal association between risk factor and MAP using a checklist of 3 different criteria. Although we attempted to make this appraisal repeatable, such classification remains subjective and can be viewed as a potential bias because systematic reviews are meant to be objective studies. On the other hand, it enables us to weigh the results, significant or not, in the different studies.

Paratuberculosis was first diagnosed in 1885 in Germany, and the first report in North America was in 1908 in Pennsylvania. For almost a hundred years, studies were focused on understanding the pathophysiology of the disease. Interestingly, studies relevant to any of the 5 questions were published only in the past 20 years. Epidemiology and risk factor studies are relatively recent science. Although multiple studies have been done to find risk factors involved in MAP transmission, to our knowledge, this is the 1st systematic review on risk factors associated with transmission of MAP to calves.

From this study, we can conclude that the contact of calves with adult cow feces is the most important risk factor for MAP transmission, because all 5 questions studied were addressing the fecal-oral route of transmission.

Acknowledgments

The study was supported by the Programme de Soutien à l’Innovation en Agroalimentaire du ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec.

References


