Detection of *Mycobacterium avium* subspecies *paratuberculosis* in naturally exposed dairy heifers and associated risk factors

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ABSTRACT

An observational prospective study was conducted to identify risk factors associated with fecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in naturally exposed dairy heifers. The study population consisted of heifers from 8 dairy herds in Michigan participating in a MAP control demonstration project. Ten heifers from 4 age groups (0 to 3, 4 to 6, 7 to 14, and 15 to 24 mo) were selected from each herd every 4 mo for 28 mo and tested for the presence of MAP by fecal culture (FC). Heifers from dams testing positive for MAP by serum ELISA or FC were preferentially selected, with the remainder of the age cohort filled with randomly selected heifers. Logistic regression using generalized estimating equations to account for clustering of data within herds and repeated measures across heifers was used to evaluate the relationship between MAP FC status of heifers and herd risk factors. In total, 1,842 fecal samples were collected from 1,202 heifers. Thirty-six (2%) fecal samples, representing 27 individual heifers, cultured positive for MAP. Heifers shedding MAP were more likely to occur in herds with adult-cow MAP ELISA prevalence >10% (odds ratio = 4.7; 95% confidence interval: 2.0–11.1) and herds milking >300 cows (odds ratio = 5.7; 95% confidence interval: 2.4–13.4). *Mycobacterium avium* ssp. *paratuberculosis* can be cultured from the feces of naturally infected dairy heifers. The future performance of these MAP FC-positive heifers is unknown and needs to be explored.

Key words: *Mycobacterium avium* ssp. *paratuberculosis* (MAP), MAP fecal shedding, dairy heifer, Johne's disease

INTRODUCTION

Johne's disease (JD), caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP), is a chronic granulomatous enteritis primarily affecting ruminants. It is of particular concern to the US dairy industry with an estimated 68% of the country's dairy farms containing MAP-infected cows (USDA, 2008), resulting in annual economic losses of \$200 to \$250 million (Ott et al., 1999). Additionally, evidence is mounting that MAP plays a role in Crohn disease in people (Feller et al., 2007), making JD a potential public health issue and 1 of the top 3 health issues for dairy cattle (Wells et al., 1998).

Controlling JD is challenging due to its prolonged prepatent period that can last for 2 to 10 yr (Whitlock and Buerge, 1996; Collins, 2003). Cattle generally become infected as young calves through ingestion of bacteria shed by infected cows. For all practical purposes, JD is untreatable in commercial production livestock (St. Jean, 1996). Therefore, control must focus on preventing the infection of susceptible calves, which would be aided greatly by early identification of infected cattle.

Early identification of MAP-infected cattle would allow for their early removal from the herd before the heavy shedding phase (McDonald et al., 1999; Antognoli et al., 2007), thereby reducing the chance of susceptible cohorts becoming infected. Additionally, early detection of infected replacement heifers could result in culling from the herd before significant investments are made (Antognoli et al., 2007). Given that shedding of MAP may be an infrequent event in young heifers (Sweeney, 1996), targeted sampling would increase detection rates, reduce testing costs, and potentially result in decreased MAP transmission on infected farms.

Diagnostic tests for detecting MAP infections suffer from less than ideal sensitivity, especially in subclinically infected cattle in the earliest stages of infection (Collins et al., 2006). This is due to the slow-growing and fastidious nature of MAP, which makes it difficult to culture; intermittent shedding in the early, subclinical stages of the disease (Sweeney, 1996); and an initial

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cell-mediated rather than humoral immune response delaying the detection of antibodies (Whitlock and Buerge, 1996; Whittington and Sergeant, 2001). Culturing MAP from infected tissues is the most definitive method for diagnosing MAP infection (Stabel, 1998; Whittington and Sergeant, 2001), but it is impractical for herd screening purposes. Currently, fecal culture (FC) is considered the most sensitive method for detecting MAP in antemortem cattle (Collins et al., 2006).

Because culture on conventional solid bacteriological media is laborious and slow, liquid culture-based mycobacterial detection systems have become common in diagnostic laboratories, offering advantages of automation and shorter detection times (Motiwala et al., 2005; Rajeev et al., 2006; Alinovi et al., 2009). When combined with additional molecular detection assays such as PCR and acid-fast staining for MAP confirmation, these liquid-culture systems are more sensitive than culture on solid media (Kim et al., 2004; Rajeev et al., 2006). Thus, a liquid culture system (ESP Culture System II, **ESP II**, Trek Diagnostic Systems, Cleveland, OH) was chosen for this study.

The primary objective of this study was to determine risk factors associated with young heifers shedding MAP in their feces.

MATERIALS AND METHODS

This project and the procedures described were approved by the Michigan State University Institutional Animal Care and Use Committee.

Study Design

This was an observational prospective study spanning 28 mo (April 2004 to August 2006). The study population included heifers from 8 commercial dairy herds enrolled in the Michigan Johne's Disease Control Demonstration Project (**MJDCDP**). Herds were enrolled in the MJDCDP based on a confirmed diagnosis of JD in adult cows and had implemented a JD control program before the initiation of this project. Herd-size ranged from 80 to 600 milking cows, and the within-herd apparent JD prevalence based on serum ELISA varied from 0 to 19% over the course of this study.

Ten heifers from each of 4 age groups: 0–3, 4–6, 7–14, and 15–24 mo were selected for testing by FC on each sampling date. The age groups were chosen based on distinct management groups found on most commercial dairies, thereby representing 4 distinct sets of environmental risks. We assumed a priori that identifying FC-positive heifers would be a rare event; therefore, heifers from ELISA-positive [Parachek, Prionics AG,

Schlieren-Zurich, Switzerland; “positive” test cutoff OD $\geq$ (mean of negative controls + 0.1)] or FC-positive (on Herrold's egg yolk medium or ESP II, ESP Culture System II, Trek Diagnostic Systems, Cleveland, OH) dams were preferentially targeted for testing at each sample date, with the remainder of each age cohort filled with randomly chosen heifers. Occasionally, 10 heifers from each age group were not available for testing. This was due to the small herd size with not enough heifers born within the cohort or housing systems that prohibited the safe restraint of heifers for sample collection. In these instances, samples were collected from as many heifers as could be safely restrained. Samples were collected at approximately 4-mo intervals with a maximum of 8 visits per herd over 28 mo.

Fecal samples were collected from the rectum of each heifer by digital palpation using a new latex glove for each heifer. At least 8 g of feces was collected, placed in individual sample containers, labeled, and refrigerated at 5°C. Samples were submitted to the laboratory within 48 h of collection and processed immediately upon arrival at the laboratory.

Laboratory Testing

Fecal samples were cultured for the presence of MAP at the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing) and results reported according to the laboratory's standard MAP culture protocol approved by USDA's National Veterinary Services Laboratory (Ames, IA). This protocol consisted of decontamination of samples using the Cornell method described previously (Stabel, 1997). The samples were then cultured using ESP II according to the manufacturer's recommendations and as described in Tavornpanich et al. (2004) and confirmed to contain MAP by real-time PCR targeting the IS900 sequence and Kinyoun's acid-fast stain. A positive sample was classified as a high shedder if it took 7 to 21 d to signal positive, a moderate shedder if it took 22 to 28 d to signal positive, and a low shedder if it took 29 to 42 d to signal positive.

Data Collection

Data were collected for each heifer tested and included identification, date of birth, dam identification, and the heifer's MAP FC status. The FC status of the heifer was determined based on the culture results reported by the Diagnostic Center for Population and Animal Health as described previously. Annual adult-herd JD test results were used to determine the dam's MAP ELISA test status, the within-herd (adult) prevalence, and mean herd size at the time the heifer was tested.

Each calf was also assigned a JD “risk” score to reflect its risk of being exposed to MAP through its environment. This score was extrapolated from the standardized risk assessment performed annually for each herd (National Johne’s Working Group of the United States Animal Health Association Johne’s Committee, 2003). This risk assessment evaluates 5 areas from which MAP infection may originate and spread: the maternity, preweaned-calf, weaned-calf, bred-heifer, and lactating-cow areas. Each area is assigned a score based on the perceived risk of within-herd JD transmission; the higher the score, the greater the risk of MAP transmission. The heifer’s JD risk score consisted of the summation of the respective herd’s risk assessment score for all areas to which the heifer had been exposed at the time of testing. For example, the JD risk score for heifers in group 1 (0–3 mo of age) was the summation of the herd risk assessment score for the maternity and preweaned-calf areas, whereas that for heifers in group 4 (15–24 mo of age) was the summation of the herd risk assessment scores for the maternity, preweaned, weaned, and bred-heifer areas.

Data Analysis

Data were maintained in and descriptive statistics generated using SAS software (PROC FREQ, version 9.2, SAS Institute Inc., Cary, NC).

To assess the effect of age, dam’s MAP ELISA test status, herd prevalence, herd size, and JD environmental risk on FC status of the heifer, logistic regression was used, accounting for clustering of the data within herds and repeated measures on individual heifers using generalized estimating equations (GEE; PROC GENMOD, SAS version 9.2) in a marginal, or population-averaged, model. The univariable model was specified as follows:

$$\text{Logit} [E (Y_{\text{FC Outcome}})] = \beta_0 + \beta_1 X_{\text{risk factor}},$$

where $\text{Logit} [E (Y_{\text{FC Outcome}})]$ is the expected value of the log-odds given the fecal culture outcome, β_0 is the intercept, β_1 is the regression parameter for a given risk factor, and $X_{\text{risk factor}}$ is the observed value of the risk factor under consideration.

Clusters were defined as repeated measures on heifers nested within herds. Various correlation structures for the model were compared using the quasi-likelihood information criterion (QIC) proposed by Pan (2001). The correlation structure minimizing QIC was used. Any variable with a P -value ≤ 0.15 on univariable analysis was considered in multivariable analyses using backward elimination. Interaction terms were only considered once the main effects were included in the model. Potential

confounding in the multivariable model was assessed analytically by determining the percentage change in the estimates of the remaining variables following the removal of the each respective variable during backward elimination. A percentage change $>25\%$ in the respective estimate of any variable following the removal of another variable in the analysis was considered evidence of confounding necessitating stratification on the confounder and the calculation of the stratum-specific odds ratio. The final multivariable model included only those factors with a P -value ≤ 0.05 .

For analysis purposes, within-herd prevalence was dichotomized into 2 categories: $\leq 10\%$ and $>10\%$, as was herd size: <300 cows milking and ≥ 300 cows milking. Because birthdates were unavailable for heifers tested on one herd, age group was used as a proxy for age in the multivariable model. All pair-wise comparisons between the various age groups (1–4) using Wald Chi-squared analysis revealed no statistical difference between age groups 1 (0–3 mo) and 2 (4–6 mo), or between age groups 3 (7–14 mo) and 4 (15–24 mo); therefore, age group was collapsed into a dichotomous variable (≤ 6 mo and >6 mo).

RESULTS

In total, 1,842 fecal samples were collected from 1,202 heifers. Three hundred ninety-three (33%) heifers were tested at least twice. Thirty-six samples (2%) were reported FC positive for MAP, and 27 individual heifers were FC positive. Seven heifers were FC positive on 2 collection dates, and one heifer was FC positive 3 times. The number of heifers tested by herd, the within-herd adult-cow seroprevalence, and the median herd size during the study are summarized in Table 1. The age at which the heifers tested positive and the level of MAP shedding for FC-positive heifers are summarized in Table 2. Thirty (83%) of the heifers were FC positive at >6 mo of age.

The dam MAP ELISA test status was available for 1,126 (94%) of the heifers tested. One hundred eighty-three heifers had ELISA-positive dams, of which 9 heifers (5%) were FC positive for MAP. Of the 943 heifers that had ELISA-negative dams, only 11 (1%) were FC positive.

Three of the 8 herds had an apparent within-herd MAP ELISA prevalence $>10\%$ throughout the entire study, whereas the other 5 herds had an apparent ELISA prevalence $\leq 10\%$. Fecal culture-positive calves were detected in 7 of the 8 study herds. Twenty-two (81%) of the FC-positive heifers originated from herds with a MAP ELISA prevalence $>10\%$. The 5 remaining FC-positive heifers originated from herds with a MAP ELISA prevalence $\leq 10\%$.

Table 1. Number of heifers tested by herd across age groups along with the within-herd adult-cow seroprevalence grouping and median herd size

Herd	Age group								Adult-cow MAP seroprevalence (%)	Median herd size
	0–3 mo		4–6 mo		7–14 mo		15–24 mo			
	FC positive	Tested	FC positive	Tested	FC positive	Tested	FC positive	Tested		
1	0	22	0	20	11	23	1	10	>10	535
2	0	59	0	60	2	58	2	62	>10	191
3	0	86	0	53	2	59	1	52	≤10	143
4	2	102	1	44	0	64	1	52	>10	417
5	0	61	0	62	0	81	0	76	≤10	93
6	1	58	0	50	0	71	0	64	≤10	178
7	1	124	0	31	2	30	5	113	≤10	504
8	0	64	1	44	1	66	2	21	>10	169
Total	4	576	2	364	18	452	12	450	—	—

¹FC = fecal culture; MAP = *Mycobacterium avium* ssp. *paratuberculosis*.

In the herds in which FC-positive heifers were identified, the proportion of positive samples ranged from 0.4 to 16% with a median of 1.7%. The herd in which 16% of samples were FC positive was the herd with the second highest adult-herd MAP seroprevalence (mean seroprevalence = 12.5% over the course of the study), and consistently had the highest scores for JD transmission in the weaned and bred-heifer areas on the annual risk assessment.

The intracluster correlation structures evaluated included independent, exchangeable, and first-order autoregressive, with independent correlation consistently providing the lowest QIC. Results of the univariable regression analyses are summarized in Table 3, and the results of the final multivariable regression model are provided in Table 4. We found no evidence of confounding during analysis. All possible interaction terms of the main effects in the final model were evaluated, but none was statistically significant.

DISCUSSION

In this study, we were able to detect MAP in the feces of naturally exposed young dairy heifers using the ESPII liquid culture system. Two percent of total

samples, and total heifers, cultured were FC positive for MAP. This proportion is similar to findings of a previous observational study in which 3% of 8-mo-old heifers were MAP FC positive using Herrold's egg yolk solid culture medium. Those calves were from a single herd with an adult-cow MAP seroprevalence of 11% (Antognoli et al., 2007). However, in our study, we found that the proportion of shedding heifers could be much higher (up to 16%) in individual herds. This was an isolated incident in one herd with relatively high within-herd disease prevalence in adult cows coupled with less than optimal management practices to prevent MAP transmission in young heifers.

Of the risk factors assessed in this study, we found that age >6 mo, a >10% adult-herd MAP seroprevalence, and herd size >300 cows all had strong associations with heifers being MAP FC positive.

The age at which infected cattle begin shedding MAP and exhibiting clinical signs of JD is dependent on the age and dose of exposure. The earlier in life the calf is exposed to MAP, as well as with higher and more frequent doses, accelerates the disease process in infected cattle such that they begin shedding bacteria and exhibiting signs at an earlier age (Weber et al., 2010; Windsor and Whittington, 2010). Given the

Table 2. Age at which heifers tested positive for Johne's disease by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) fecal culture (categorized by shedding level)

Age group	Shedding level ¹			Total samples tested
	Low	Moderate	High	
0–3 mo	3	0	1	576
4–6 mo	2	0	0	364
7–14 mo	16	1	1	452
15–24 mo	10	1	1	450
Total	31	2	3	1,842

¹Low = 29–42 d to positive on ESP Culture System II (Trek Diagnostic Systems, Cleveland, OH; ESP II); moderate = 22–28 d to positive on ESP II; high = 7–21 d to positive on ESP II.

Table 3. Results of univariable regression analyses: factors associated with heifers being fecal culture positive for *Mycobacterium avium* ssp. *paratuberculosis* (MAP)

Factor	Odds ratio (95% CI)	Wald P-value
Age at test (mo)	1.09 (1.04–1.15)	0.0009
Age group >6 mo	5.36 (1.94–14.75)	0.0012
Dam ELISA positive	2.26 (0.97–5.26)	0.0596
Herd prevalence >10%	3.82 (1.67–8.73)	0.0015
Herd size >300 cows	3.91 (1.67–9.17)	0.0017
Calf risk score (per 10 points)	1.03 (1.01–1.04)	0.0019

fact that MAP is the slowest growing of the cultivable mycobacteria (Rowe and Grant, 2006), it is reasonable that shedding would not readily be detected for at least several months following infection. Therefore, it is reasonable that most of the FC-positive heifers in this study were >6 mo of age. Another observational study failed to identify fecal shedding in calves up to 3 mo of age (Pithua et al., 2010). Conversely, experimental studies have documented shedding in calves <3 mo of age following MAP exposure (McDonald et al., 1999; van Roermund et al., 2007; Stabel et al., 2009). The identification of MAP in the feces of calves <3 mo of age may be a reflection of “pass-through” bacteria following a large oral dose of MAP and not an active infection. However, it should be noted that all calves in which this pass-through shedding was observed were later confirmed as infected based on histopathological identification or culture of MAP from tissues (McDonald et al., 1999; Stabel et al., 2009). The fact that MAP was identified in the feces of young heifers confirms MAP exposure in these animals and puts them at high risk for becoming infected. It also supports the observation that horizontal calf-to-calf MAP transmission can occur as previously documented (van Roermund et al., 2007).

If repeated exposure to high doses of MAP accelerates disease progression such that infected animals exhibit clinical signs and test positive at an earlier age (Collins, 2003), then it is reasonable that herds with a higher adult-cow JD prevalence will have proportionately more young heifers shedding MAP than herds with lower JD prevalence. This is because the environmental MAP load is higher in herds with high prevalence compared with low prevalence herds, thereby increasing the exposure risk of susceptible cattle in those herds. This trend held true in the current study as well as in 2 previous studies in which MAP was identified in the feces of heifers <12 mo of age in herds with “high MAP risk” (high adult-cow seroprevalence), but not from heifers in herds with “low MAP risk” (Antognoli et al., 2007; Mitchell et al., 2009). All of these studies support the findings of a much more extensive, retrospective study (18,979 cattle from 353 herds) in which the proportion

of cattle shedding MAP in their feces before 2 yr of age increased from 1% in herds with an apparent FC prevalence of <5% to 20% in herds with a prevalence of >20% (Weber et al., 2010).

The association between increased risk of heifers being MAP FC positive and larger herds is likely an extension of the association of higher within-herd JD prevalence in large herds (Wells and Wagner, 2000; Muskens et al., 2003; Hirst et al., 2004; Tavoranpanich et al., 2008). Large herd size has been associated with the purchase of cattle (USDA, 2005), and the addition of purchased cattle is considered a primary method for MAP transmission between herds (Sweeney, 1996). Thus, purchased cattle bring MAP into a herd and increase the herd size. This increases herd density, leading to greater MAP contamination in the environment, which increases the infection pressure on susceptible calves (Muskens et al., 2003) and decreases the age at which infected cattle begin shedding the bacterium as previously discussed.

In this study, the shedding of MAP in feces occurred in heifers in all 4 age groups, but the majority (83%) of positive samples occurred in heifers >6 mo of age, with over half (52%) of the positive samples originating from 7- to 14-mo-old heifers. This finding is similar to that of van Roermund et al. (2007). Considering the time and cost of MAP FC, targeting heifers between 7 and 14 mo of age may be the best use of this resource.

In conclusion, the results of this study suggest a target age (7–14 mo) to start testing for fecal shedding of MAP in naturally exposed dairy heifers, especially in high prevalence herds. Even if MAP shedding in heifers does not progress to clinical JD later in life, the

Table 4. Final multivariable model: factors associated with heifers being fecal culture positive for *Mycobacterium avium* ssp. *paratuberculosis* (MAP)

Factor	Odds ratio (95% CI)	Wald P-value
Age group >6 mo	6.01 (2.07–17.43)	0.0010
Herd prevalence >10%	4.73 (2.01–11.10)	0.0004
Herd size >300 cows	5.65 (2.38–13.41)	<0.0001

potential exists for transmission to pen mates through environmental contamination (van Roermund et al., 2007; Weber et al., 2010). The intermittent and low level of shedding by infected heifers along with the low sensitivity of currently available FC methods likely contributes to an underestimation of the prevalence of heifers shedding MAP. Further investigation needs to be done using serial testing of 7- to 14-mo-old heifers with positive MAP FC to follow their production parameters and clinical outcomes over time, especially in high prevalence herds. This may help determine if we are detecting a true MAP infection and the onset of shedding, or if these were cases of passive shedding. Furthermore, given recent work with pooled fecal samples (Tavornpanich et al., 2004; Eamens et al., 2008), it would be interesting to see if this strategy could be used in this age group of calves to help alleviate costs of individual animal testing.

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REFERENCES

- Alinovi, C. A., M. P. Ward, T. L. Lin, G. E. Moore, and C. C. Wu. 2009. Real-time PCR, compared to liquid and solid culture media and ELISA, for the detection of *Mycobacterium avium* ssp. *paratuberculosis*. *Vet. Microbiol.* 136:177–179.
- Antognoli, M. C., H. L. Hirst, F. B. Garry, and M. D. Salman. 2007. Immune response to and faecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* in young dairy calves, and the association between test results in the calves and the infection status of their dams. *Zoonoses Public Health* 54:152–159.
- Collins, M. T. 2003. Paratuberculosis: Review of present knowledge. *Acta Vet. Scand.* 44:217–221.
- Collins, M. T., I. A. Gardner, F. B. Garry, A. J. Roussel, and S. J. Wells. 2006. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. *J. Am. Vet. Med. Assoc.* 229:1912–1919.
- Eamens, G. J., D. M. Walker, N. S. Porter, and S. A. Fell. 2008. Radiometric pooled faecal culture for the detection of *Mycobacterium avium* ssp. *paratuberculosis* in low-shedder cattle. *Aust. Vet. J.* 86:259–265.
- Feller, M., K. Huwiler, R. Stephan, E. Altpeter, A. Ahang, H. Furrer, G. E. Pflyffer, T. Jemmi, A. Baumgartner, and M. Egger. 2007. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: A systematic review and meta-analysis. *Lancet Infect. Dis.* 7:607–613.
- Hirst, H. L., F. B. Garry, P. S. Morley, M. D. Salmon, R. P. Dinsmore, B. A. Wagner, K. D. McSweeney, and G. M. Goodell. 2004. Seroprevalence of *Mycobacterium avium* ssp. *paratuberculosis* infection in dairy cows in Colorado and herd-level risk factors for seropositivity. *J. Am. Vet. Med. Assoc.* 225:97–101.
- Kim, S. G., E. H. Kim, C. J. Lafferty, L. J. Miller, H. J. Koo, S. M. Stehman, and S. J. Shin. 2004. Use of conventional and real-time polymerase chain reaction for confirmation of *Mycobacterium avium* ssp. *paratuberculosis* in a broth-based culture system ESP II. *J. Vet. Diagn. Invest.* 16:448–453.
- McDonald, W. L., S. E. Ridge, A. F. Hope, and R. J. Condron. 1999. Evaluation of diagnostic tests for Johne's disease in young cattle. *Aust. Vet. J.* 77:113–119.
- Mitchell, R. M., R. H. Whitlock, J. M. Smith, M. Wood, N. S. Gollnick, and Y. H. Schukken. 2009. Calf shedding of MAP on two farms with high and low environmental exposure. *Proc. 10th Int. Colloq. on Paratuberculosis*, Minneapolis, MN. Accessed March 27, 2011. <http://pubs.paratuberculosis.org/index.php/paratb/article/viewFile/821/420>.
- Motiwala, A. S., M. Strother, N. E. Theus, R. W. Stich, B. Byrum, W. P. Shulaw, V. Kapur, and S. Sreevatsan. 2005. Rapid detection and typing of strains of *Mycobacterium avium* ssp. *paratuberculosis* from broth cultures. *J. Clin. Microbiol.* 43:2111–2117.
- Muskens, J., A. R. W. Elbers, H. J. van Weering, and J. P. T. M. Noordhuizen. 2003. Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 50:372–377.
- National Johne's Working Group of the United States Animal Health Association Johne's Committee. 2003. A Guide for Johne's Disease Risk-Assessments and Management Plans for Dairy Herds. 3rd ed. Accessed March 27, 2011. <http://www.johnesdisease.org/Handbook%20for%20Vets%20&%20Dairy%20Producers.pdf>.
- Ott, S. L., S. J. Wells, and B. A. Wagner. 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. *Prev. Vet. Med.* 40:179–192.
- Pan, W. 2001. Akaike's information criterion in generalized estimating equations. *Biometrics* 57:120–125.
- Pithua, P., S. J. Wells, S. Sreevatsan, and S. M. Godden. 2010. Lack of evidence for fecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* in calves born to fecal culture positive dams. *Prev. Vet. Med.* 93:242–245.
- Rajeev, S., W. Shulaw, R. Berghaus, Y. Zhang, and B. Byrum. 2006. A testing scheme for the detection of *Mycobacterium avium* ssp. *paratuberculosis* in bovine feces utilizing the ESP *para*-JEM liquid culture system. *J. Vet. Diagn. Invest.* 18:529–535.
- Rowe, M. T., and I. R. Grant. 2006. *Mycobacterium avium* ssp. *paratuberculosis* and its potential survival tactics. *Lett. Appl. Microbiol.* 42:305–311.
- St. Jean, G. 1996. Treatment of clinical paratuberculosis in cattle. *Vet. Clin. North Am. Food Anim. Pract.* 12:417–430.
- Stabel, J. R. 1997. An improved method for cultivation of *Mycobacterium paratuberculosis* from bovine fecal samples and comparison to three other methods. *J. Vet. Diagn. Invest.* 9:375–380.
- Stabel, J. R. 1998. Johne's disease: A hidden threat. *J. Dairy Sci.* 81:283–288.
- Stabel, J. R., M. V. Palmer, B. Harris, B. Plattner, J. Hostetter, and S. Robbe-Austerman. 2009. Pathogenesis of *Mycobacterium avium* ssp. *paratuberculosis* in neonatal calves after oral or intraperitoneal experimental infection. *Vet. Microbiol.* 136:306–313.
- Sweeney, R. W. 1996. Transmission of paratuberculosis. *Vet. Clin. North Am. Food Anim. Pract.* 12:305–312.
- Tavornpanich, S., I. A. Gardner, R. J. Anderson, S. Shin, R. H. Whitlock, T. Fyock, J. M. Adaska, R. L. Walker, and S. K. Hietala. 2004. Evaluation of microbial culture of pooled fecal samples for detection of *Mycobacterium avium* ssp. *paratuberculosis* in large dairy herds. *Am. J. Vet. Res.* 65:1061–1070.
- Tavornpanich, S., W. O. Johnson, R. J. Anderdon, and I. A. Gardner. 2008. Herd characteristics and management practices associated with seroprevalence of *Mycobacterium avium* ssp. *paratuberculosis* in dairy herds. *Am. J. Vet. Res.* 69:904–911.

- USDA-APHIS:VS, CEAH. 2005. Johne's Disease on U.S. Dairy Operations, 2002. #N27.0205. National Animal Health Monitoring System, USDA-APHIS:VS, CEAH, Fort Collins, CO.
- USDA-APHIS:VS, CEAH. 2008. Johne's Disease on U.S. Dairies, 1991–2007. #N521.0408. National Animal Health Monitoring System, USDA-APHIS:VS, CEAH, Fort Collins, CO. Accessed March 27, 2011. http://www.jdip.org/index.php?option=com_docman&task=doc_download&gid=339.
- van Roermund, H. J. W., D. Baker, P. T. J. Willemsen, and M. C. M. de Jong. 2007. Horizontal transmission of *Mycobacterium avium* ssp. *paratuberculosis* in cattle in an experimental setting: calves can transmit the infection to other calves. *Vet. Microbiol.* 122:270–279.
- Weber, M. F., J. Kogut, J. de Bree, G. van Schaik, and M. Neilen. 2010. Age at which dairy cattle become *Mycobacterium avium* ssp. *paratuberculosis* faecal culture positive. *Prev. Vet. Med.* 97:29–36.
- Wells, S. J., S. L. Ott, and A. Hillberg Seitzinger. 1998. Key health issues for dairy cattle—New and old. *J. Dairy Sci.* 81:3029–3035.
- Wells, S. J., and B. A. Wagner. 2000. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventative measures. *J. Am. Vet. Med. Assoc.* 216:1450–1457.
- Whitlock, R. H., and C. Buergelt. 1996. Preclinical and clinical manifestations of paratuberculosis (including pathology). *Vet. Clin. North Am. Food Anim. Pract.* 12:345–356.
- Whittington, R. J., and E. S. G. Sergeant. 2001. Progress towards understanding the spread, detection and control of *Mycobacterium avium* ssp. *paratuberculosis* in animal populations. *Aust. Vet. J.* 79:267–278.
- Windsor, P. A., and R. J. Whittington. 2010. Evidence for age susceptibility of cattle to Johne's disease. *Vet. J.* 184:37–44.