PEER REVIEWED

Johne's Disease: Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Florida Beef and Dairy Cattle

Linda L. Keller, *DVM*[1]; **C. Dix Harrell,** *DVM, MS*[2]; **Suzan M. Loerzel,** *DVM, PhD*[3];
D. Owen Rae, *DVM, MPVM*[4] *(corresponding author)*
[1,4]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, PO Box 100136, University of Florida, Gainesville, FL 32610-0136, 352-392-4700 ext 4100, 352-392-7551 (fax), RaeO@mail.ufl.edu*
[2,3]*Area Epidemiologist, Animal Disease Programs and Plant Health Inspection Service, USDA, Gainesville, FL*

Abstract

The objective of this study was to estimate the prevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection among Florida beef and dairy cattle. This was a retrospective seroprevalence study on serum samples from 32,011 cattle originating from 75 herds. Selection was limited to whole herds being tested for diagnostic purposes by owners considering participation in the voluntary Johne's Control Program or the voluntary Florida Johne's Disease Herd Status Program. Data were obtained from the Florida State Veterinary Diagnostic Laboratory and USDA-APHIS statewide submission of specimens for Johne's testing from 1999 to 2001. Specimens were evaluated using a commercial IDEXX ELISA kit with a published sensitivity and specificity of 50 and 99%, respectively. Overall prevalence in the sample population was 6.5%; prevalence in beef and dairy cattle was 7.4 and 6.3%, respectively. Eighty-three percent of herds included in the study had one or more positive cows in the herd. Larger herds (>100 head) had statistically higher herd prevalence than herds with less than 100 cattle. The true prevalence estimate was calculated to be 11.2%. Although within-herd prevalence was lower than previously reported in Florida, seroprevalence appears to be widely distributed and pervasive among Florida cattle. Our findings suggest there could be 168,000 or more cattle in Florida infected with the organism. There is a need for increased awareness of the disease and implementation of control methods appropriate for each individual herd.

Résumé

L'objectif de cette étude était d'estimer la prévalence d'infection par *Mycobacterium avium* sous-espèce *paratuberculosis* chez les bovins laitiers et de boucherie de la Floride. Cette étude rétrospective était basée sur la séroprévalence d'échantillons de sérum obtenus à partir de 32 011 bovins provenant de 75 troupeaux. La sélection a été limitée aux troupeaux testés de façon diagnostic par les producteurs qui pensaient joindre le programme volontaire de contrôle de la maladie de Johne ou le programme volontaire d'évaluation du statut de la maladie de Johne dans les troupeaux de la Floride. Les données ont été obtenues du laboratoire de diagnostic vétérinaire de l'état de la Floride à partir des échantillons soumis dans le cadre du programme USDA-APHIS de contrôle de la maladie de Johne entre 1999 et 2001. Les échantillons ont été évalués avec le test commercial IDEXX ELISA qui possède une sensibilité établie à 50% et une spécificité à 99%. La prévalence globale dans la population échantillonnée était de 6.5% et s'établissait à 7.4% dans les troupeaux laitiers et à 6.3% dans les troupeaux de boucherie. Un total de 83% des troupeaux échantillonnés renfermaient au moins une vache positive dans le troupeau. La prévalence était significativement plus élevée dans les gros troupeaux (> 100 têtes) que dans les plus petits troupeaux. La valeur estimée de la vraie prévalence était de 11.2%. Bien que la prévalence au sein d'un troupeau était moindre que celle rapportée au préalable en Floride, la séroprévalence semble bien

établie et commune chez les bovins de la Floride. Notre étude suggère qu'il pourrait y avoir plus de 168 000 bovins en Floride infectés avec cette bactérie. Il y a un besoin de sensibilisation à l'égard de cette maladie et de mise en place de méthodes de contrôle appropriées à chaque troupeau.

Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) is an acid-fast intracellular bacillus that infects ruminants worldwide, causing a chronic, granulomatous enteritis known as Johne's disease (JD). The disease is characterized by chronic diarrhea and weight loss, despite a good appetite, even on a high plane of nutrition. There is no known cure for the disease and it is eventually fatal. The disease has a significant economic impact on both the dairy and beef cattle industry in the US.[12] In addition to losses from clinical disease, substantial subclinical losses have been documented including decreased milk production, increased culling rates and decreased fertility.[2,9] The organism has been isolated from colostrum and milk, and is transmitted primarily by the fecal-oral route to calves in the first few months of life. There is a long incubation period, and animals rarely show clinical signs until two years of age or more. Infected females are often the source of infection for their calves. Herds become infected by new additions that may be shedding the bacteria and showing no clinical signs. Some evidence suggests that stressors, such as gestation and parturition, may be responsible for the manifestation of the clinical disease.[9]

Control of JD is difficult due to fecal shedding of the subclinical animal. Although much effort has been put into identifying the disease in the early stages, there are currently no reliable tests for detecting early infection. Fecal culture has been considered the gold standard for detecting infection, but the animal must be shedding the organism at the time of sample collection. A major disadvantage of fecal culture is that it can take up to 16 weeks to confirm due to the organism's slow growth rate. The ELISA test has been used in many seroprevalence studies. This test has sensitivity comparable to the fecal culture, and offers a quicker turnaround time (1-3 days). Sensitivity of the ELISA test is approximately 40-50%, but varies from 15-87%, depending on the stage of infection. Higher sensitivities are obtained from heavy shedders of the bacteria. The specificity of the current ELISA test is 99%.[4,15]

Many studies have been done worldwide to estimate the prevalence of infection in ruminants, including wildlife.[9] Prevalence appears to be increasing, but measuring prevalence is challenging, due in part to difficulties in reliable testing. A 1983-1984 US study of cultures of ileocecal lymph nodes from 7,450 culled, clinically normal cattle in slaughterhouses in 37 states, indicated an apparent prevalence of 2.9% in dairy cattle and 0.8% in beef cattle, with an overall prevalence of 1.6%.[10]

To the authors' knowledge, the only previous study done in Florida was published in 1990 by Braun *et al.* ELISA results were obtained from a 1986-1987 survey of Florida cattle, showing a prevalence of 8.6% in beef cattle and 17.1% in dairy cattle.[1] The high prevalence in that study warranted re-evaluation of the prevalence of Johne's disease in the state. The objective of this study was to estimate the apparent prevalence of MAP antibodies in Florida cattle from samples submitted to the State Veterinary Diagnostic Laboratory. Information gained from this study may aid in implementation of control methods to minimize economic losses in Florida and other southeastern states' beef and dairy cattle herds.

Materials and Methods

Data were obtained from the Florida State Veterinary Diagnostic Laboratory and USDA-APHIS statewide submissions of serum samples for Johne's testing from 1999-2001. Samples were tested for the presence of MAP antibodies using the IDEXX *Mycobacterium avium* subspecies *paratuberculosis* ELISA test kit.[a] Sensitivity and specificity of this test is estimated at 50 and 99%, respectively. The data set included 64,413 test results from beef and dairy cattle, goats, sheep and exotic ruminants. Information obtained at sample submission included county from which animals originated, herd size when applicable, breed, age, sex, clinical signs present and reason for the testing.

For the purpose of evaluating seroprevalence in this cattle population, bias in the data was anticipated and addressed. The following exclusion criteria were applied. Samples from species other than cattle were excluded, as well as samples submitted for diagnostic purposes from animals exhibiting clinical signs. Over the two-year data collection period, herd testing was repeated in some herds; in these cases, only the first year's data were used to avoid duplication. Only adults two years of age or older were selected, since the likelihood of finding antibodies in younger animals was considered low, regardless of infection status. Final selection, then, was limited to whole herd samples submitted to the State Veterinary Diagnostic Laboratory for surveillance purposes.

After exclusion of data, the study data set represented 32,011 cattle, of which 25,561 were dairy cattle and 6,450 were beef cattle. These cattle originated from 75 herds in 30 Florida counties. Beef breeds represented in this study were predominantly Brahman crosses. Purebred beef herds included Angus, Brangus, Hereford, Charolais, Red Angus, Limousin, Santa Gertrudis and Senepol breeds, while dairy herds were almost exclusively Holstein. Female gender was specified for 26,604 of the cattle tested, and 40 of 73 herds tested included females only. Five beef herds tested bulls only, and accounted for 105 of the total cattle tested. Both sexes were tested in 28 herds, but numbers of males and females in those herds were not specified (n = 5302).

Distribution of cattle in the study was determined according to county from which the herd originated, the geographical location of that county (north or south) and the number of herds and herd size represented in each county. Cattle were classified according to herd size (<100,100-500, or >500), herd type (beef or dairy) and geographical location (north or south Florida) to assess the association of these factors and prevalence.

Apparent prevalence within the sample population, herd prevalence and ranges were calculated using Microsoft Excel 2000. Herd-level prevalence was defined as the percentage of herds with one or more seropositive animal(s). Apparent prevalence is the proportion of test positive individuals within the herd or population. How well this represents the true prevalence is dependent on the characteristics of the test used. An estimate of the true prevalence of MAP antibodies in Florida cattle was calculated using the apparent prevalence determined for this study population, and the published sensitivity and specificity of the ELISA test used, in the formula: $TP = (AP + Sp - 1)/(Sp + Se - 1)$; where TP = true prevalence, AP = apparent prevalence, Se = sensitivity of the test used (0.50), and Sp = specificity of the test used (0.99).[11]

Variables associated with MAP serologic prevalence in individual animals and herds (i.e., animal type [beef or dairy], herd size [by defined categories], geographical location [north or south] and interaction of these) were evaluated using the general linear model (Proc GLM) procedure of SAS version 8.1. Least squares means and significant P-values (< 0.10) for individual

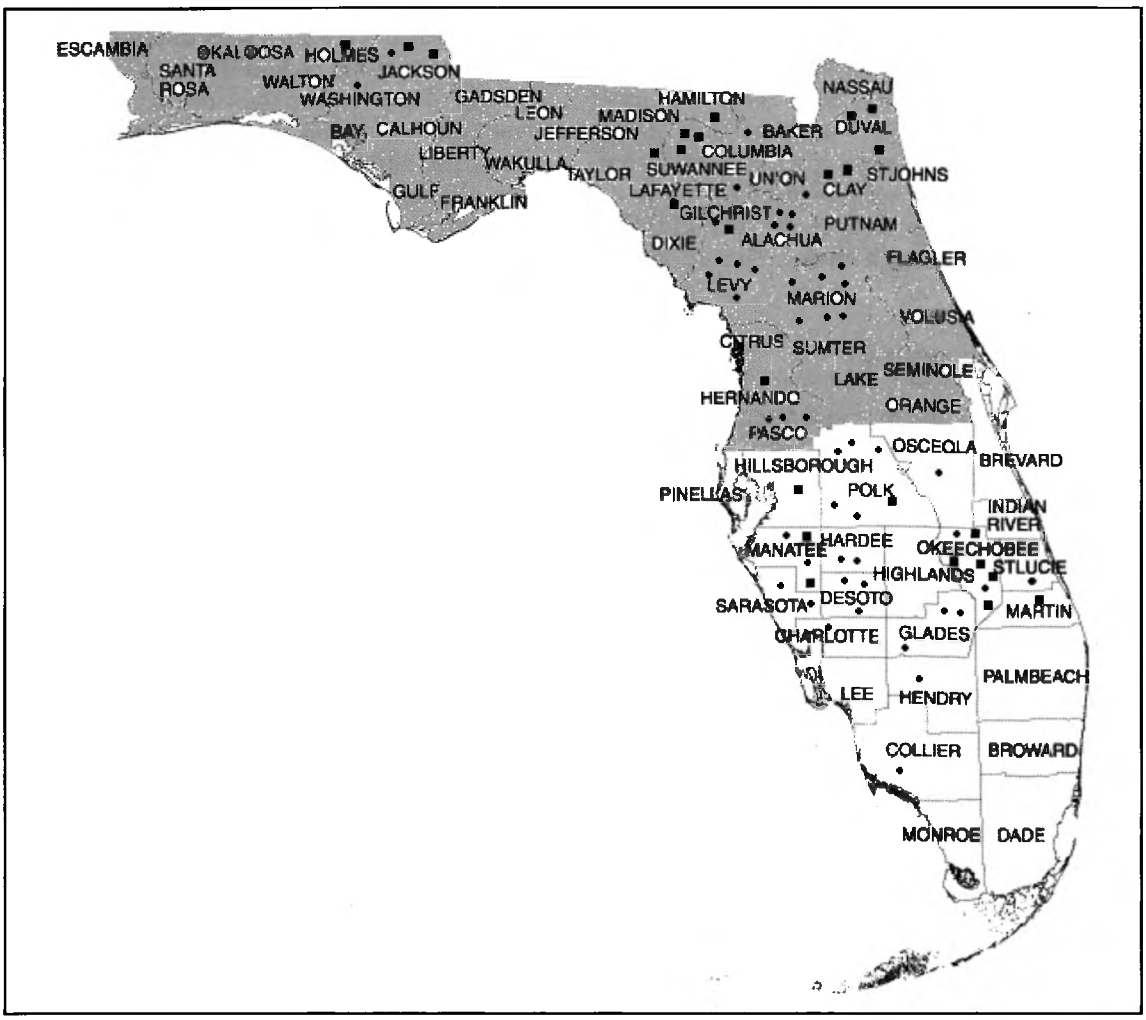

Figure 1. Distribution of cattle herds included in the study according to geographical location. Shaded area designates north Florida; unshaded area designates south Florida. A circle represents beef herds; a square represents dairy herds.

and herd prevalence were reported by animal type for geographical location and herd size categories.

Results

Distribution of the 49 beef and 26 dairy study herds across the state is illustrated in Figure 1. Counties of herd origin, state region, number of cattle and herds tested and herd size category are summarized in Table 1. The estimated apparent prevalence of MAP antibodies in the Florida cattle sampled was 6.5% (2,089 of 32,011 cattle). Seroprevalence in the dairy and beef cattle population was 6.3 and 7.4%, respectively. Of the 75 herds, sixty-two herds (82.7%) had at least one seropositive animal in the herd. The raw data and prevalence estimates for each of the herd categories are listed in Table 2.

Least squares means and *P*-values for prevalence estimates for beef and dairy cattle are summarized in Table 3. Significant differences ($P = 0.02$) in mean herd prevalence existed when comparing beef (75.5 ± 5.3%) and dairy (96.2 ± 7.3%) herds. The mean herd prevalence of herds with less than 100 head was 60.6%, and was significantly lower ($P < 0.001$) than the 100% herd prevalence in herds having 100 or more cattle. There were also differences when comparing beef and dairy herds of less than 100 head with respective herds of greater than 100 head. Using the above formula, the estimated true prevalence in the total Florida cattle population was 11.2%.

Discussion

Other studies have been conducted using the ELISA to test for antibodies to MAP in cattle populations representing various geographical locations. In a 1982-1984 Louisiana study of 1581 beef cattle from 152 herds, seroprevalence was 4.4% in beef cattle, and herd prevalence was 30%.[14] ELISA testing performed on 4990 dairy cattle in Wisconsin found the prevalence of MAP antibody-positive animals to be 4.8%, involving 34% of herds.[3] A Missouri study of serum collected in 1993 and 1994 from 1954 beef and dairy cattle representing 89 herds estimated prevalence in beef cattle to be 5% with 40% of herds infected, and 8% prevalence in dairy cattle with 74% of herds seropositive to MAP antibodies.[13] A 1999 Michigan study of 3886 dairy cattle reported a prevalence of 6.9%, with a statewide herd prevalence of 54%.[8] In 2001, a large ELISA study of 10,371 beef cattle from 380 herds in 21 states concluded the prevalence to be 0.4% (only 40 animals positive) with 7.9% of the herds analyzed having an infected animal.[5] In a recently published Alabama study carried out on a subpopulation of beef cattle, prevalence of seropositive animals was reported to be 8% with a 50% herd prevalence.[6] With the exception of the low prevalence found in the 2001 national study, all regional studies to date have estimated prevalence rates from 4.4 to 17.1%, and herd prevalence from 30 to 74%. Results of our study are in agreement with the findings in these other studies, and support the conclusion that Johne's disease is prevalent throughout the beef and dairy cattle population in the southeast and midwest.

The apparent prevalence of 6.3% in dairy cattle in our study was much lower than previously reported for Florida dairy cattle,[1] which estimated a MAP prevalence of 17.1%. Due to differences in study design and animal selection, it is difficult to determine if the decrease in prevalence is significant. A challenge associated with large seroprevalence studies is to address and attempt to minimize bias. An inherent bias exists in the selection of only whole herd tests from owners considering participation in a voluntary Johne's control program. Because of the nature of this type of voluntary program, only herds that show no conclusive evidence of disease are granted an approved status in the program. It can be argued that owners who had a herd history of clinical disease would be less likely to submit their herds for testing. However, at the time of this study, Johne's disease was a growing concern among cattle producers in the state, and the Florida State Diagnostic Laboratory was offering the test at a reduced price to encourage herd participation. Herd owners were interested in determining their herd prevalence and taking appropriate steps to control the disease. If the animal selection criteria did influence the results of this study, the prevalence may actually have been underestimated. This may explain, in part, the discrepancy between our study and the 1990 Florida study.

The sample population of this study was 32,011 cattle, or approximately 2.8% of the total number of cattle in the state, representing about 16% of the dairy and 0.7% of the beef cattle populations. The 75 sampled herds represented areas of Florida where the beef and dairy industries are concentrated. Sixty-two of these herds (82.7%) had at least one positive animal in the herd, which was higher than the reported herd prevalence from previous studies. A significant finding in this study was the 100% herd prevalence in herds (both beef and dairy) with more than 100 head of cattle. Consequently, the number of larger herds (42) included in the study could have contributed substantially to the higher overall herd prevalence. Owners of larger herds should be aware that there is a higher probability of infected animals in their herds compared to smaller ones. Many calves can become infected from a single cow, ultimately spreading the disease more widely within the herd. Since the majority of the Florida cattle industry is beef cow-calf operations, this could pose a serious problem for the industry in the future if control methods are not implemented.

Table 1. Distribution of Florida cattle included in the study according to county, geographical region and herd size.

County	Region	Cattle (n)	Herds (n)	Herd size		
				<100	100-500	>500
Alachua	North	577	4	2	2	0
Bradford	North	8	1	1	0	0
Charlotte	South	72	1	1	0	0
Clay	North	1,493	2	0	1	1
Collier	South	5	1	1	0	0
Columbia	North	109	2	1	1	0
Desoto	South	1,217	3	2	0	1
Duval	North	1,571	3	0	2	1
Gilchrist	North	72	2	2	0	0
Glades	South	134	3	3	0	0
Hamilton	North	303	1	0	1	0
Hardee	South	436	2	0	2	0
Hendry	South	67	1	1	0	0
Hernando	North	623	1	0	0	1
Hillsborough	South	282	1	0	1	0
Holmes	North	159	1	0	1	0
Jackson	North	296	3	1	2	0
Lafayette	North	358	2	1	1	0
Levy	North	786	5	3	2	0
Manatee	South	2,145	4	1	1	2
Marion	North	611	7	4	3	0
Martin	South	50	1	1	0	0
Okeechobee	South	17,416	7	1	1	5
Osceola	South	299	1	0	1	0
Pasco	North	277	3	2	1	0
Polk	South	1,175	6	3	2	1
Saint Lucie	South	216	1	0	1	0
Sarasota	South	428	2	0	2	0
Suwannee	North	806	3	1	1	1
Washington	North	20	1	1	0	0
Total		**32,011**	**75**	**33**	**29**	**13**

Table 2. Raw data, apparent sample prevalence estimates and herd prevalence estimates of MAP in Florida cattle according to herd size, type and geographical location.

	Total	Herd size			Herd type		Region	
		<100	100-500	≥500	Beef	Dairy	North	South
Cattle (individuals) tested	32,011	1,109	6,918	23,984	6,450	25,561	8,069	23,942
Positive test results	2,089	67	527	1,495	477	1,612	539	1,550
Sample prevalence (%)	6.5	6.0	7.6	6.2	7.4	6.3	6.7	6.5
Prevalence range within herds (%)	0-28.6	0-28.6	0.9-19.2	3.5-15.8	0-28.6	0-15.8	0-28.6	0-20
Herds tested	75	33	29	13	49	26	41	34
Positive herds	62	20	29	13	37	25	31	31
Herd size range	5 - 8,921	5 - 75	102 - 469	544 - 8,921	5 - 1,153	40 - 8,921	7 - 1,171	5 - 8,921
Mean herd size	427	34	239	1,845	132	1,014	197	704
Herd prevalence (%)	82.7	60.6	100	100	75.5	96.2	75.6	91.2

Table 3. Least squares means prevalence estimates obtained from SAS general linear model procedure.

Prevalence category (%)	Beef	Dairy	All cattle
Individual	7.2	7.6	—
Herd	75.5	96.2[a]	—
Herd region			
North	64.0[c]	93.8	75.6[b]
South	87.5	100	91.2
Herd size			
<100 head	58.6[d]	75.0[b]	60.6[d]
100-500 head	100	100	100
>500 head	100	100	100

[a]Values for beef and dairy herd prevalence differ significantly (P=0.02).
[b,c,d]Values for column-wise least squares means comparisons (herd region, herd size) differ significantly; [b]P<0.10, [c]P<0.05 and [d]P<0.01.

The IDEXX ELISA has been used extensively to determine seroprevalence of MAP in cattle populations. Testing, while improved over the years, can still be unreliable. Limitations of the testing method include low sensitivity, particularly in asymptomatic animals, and problems associated with repeatability of test results. An S/P ratio of ≥ 0.25 is recommended by the manufacturer for classification of positive results, and was the cut-off point at which test results were called positive in our study. A recent study has shown that although the estimated specificity of the test is 99%, the repeatability of positive results can be problematic when using 0.25 as the cut-off value.[7] This can cause frustration for both the herd owner and veterinarian since seropositive cattle may be asymptomatic; therefore, there is a reluctance to cull these animals when results are equivocal.

Using the 0.25 cut-off value in our study, it is possible that herds with only one positive animal could have been misclassified as a seropositive herd. However, there were only six herds with only one positive animal, and all of these were small beef herds with 30 or less cattle, indicating that even if these animals were misclassified as false positive it would not significantly change our findings. Herd prevalence estimates for dairy herds and all herds with greater than 100 head in our study would not be affected. A higher S/P ratio (≥ 0.70) may provide a greater positive predictive value of disease prevalence. However, the number of false negatives may increase when using higher S/P ratios as a cut-off value for the classification of a true positive animal. As a result, infected animals could remain in the herd, therefore perpetuating the disease.

Despite inherent inaccuracies, the ELISA is still considered the best herd-screening test. Results are available in a short period, and the test can identify some early infections before bacteria are shed in the feces. Fecal culture should be performed to confirm positive ELISA results, however, fecal cultures can be equally frustrating. Although specificity is excellent, it will not detect the bacteria unless they are being shed by the animal.[4,5]

Results from our study suggest that Johne's disease is pervasive throughout Florida's cattle population, and 168,000 or more cattle in the state of Florida may be infected based on the true prevalence estimate of 11.2%. This is a large number of potentially infected cattle, totaling as many or more cattle than Florida's total current dairy population, estimated at about 160,000. Although this estimate is guarded, and subject to the inherent bias of the sample population and limitations of the testing method, it is a reasonable deduction.

Conclusions

The chronic and insidious nature of JD calls for implementation of biosecurity practices and careful scrutiny of herds to identify and remove infected animals. There must be expanded educational programs about the disease and continued efforts toward more accurate testing. A routine screening program is recommended. Herd owners and veterinarians, taking into consideration the presence of clinical signs, should carefully analyze all results, assess the risks and develop an appropriate management plan for the herds. By increasing awareness, carefully interpreting test results and developing appropriate control measures for each herd, the veterinarian can play a crucial role in preventing the spread of this disease in cattle populations.

Footnote

[a]IDEXX Laboratories, Inc., Westbrook, ME.

References

1. Braun RK, Buerguelt CD, Littell RC, *et al:* Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. *J Am Vet Med Assoc* 196:1251-1254, 1990.
2. Buerguelt CD, Duncan JR: Age and milk production data of cattle culled from a dairy herd with paratuberculosis. *J Am Vet Med Assoc* 173:478-480, 1978.
3. Collins MT, Sockett DC, Goodger WJ, *et al:* Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. *J Am Vet Med Assoc* 204:636-641, 1994.
4. Dargatz DA, Byrum BA, Barber, LK, *et al:* Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. *J Am Vet Med Assoc* 218:1163-1166, 2001.
5. Dargatz DA, Byrum BA, Hennager SG, *et al:* Prevalence of antibodies against *Mycobacterium avium* subsp *paratuberculosis* among beef cow-calf herds. *J Am Vet Med Assoc* 219:497-501, 2001.

6. Hill BB, West M, Brock KV: An estimated prevalence of Johne's disease in a subpopulation of Alabama beef cattle. *J Vet Diagn Invest* 15:21-25, 2003.
7. Hirst HL, Garry FB, Salman MD: Assessment of test results when using a commercial enzyme-linked immunosorbent assay for diagnosis of paratuberculosis in repeated samples collected from adult dairy cattle. *J Am Vet Med Assoc* 220:1685-1689, 2002.
8. Johnson-Ifearulunde Y, Kaneene JB: Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. *Am J Vet Res* 60:589-596, 1999.
9. Jones P: Bovine paratuberculosis: ongoing challenges, renewed concerns. *In Practice* 23:402-411, 2001.
10. Merkel RS, Whipple DL, Sacks JM, *et al: Mycobacterium paratuberculosis* in ileocecal lymph nodes of cattle culled in the United States. *J Am Vet Med Assoc* 190:676-680, 1987.
11. Rogan WJ, Gladen B: Estimating prevalence from the results of a screening test. *Am J Epidemiology* 107: 71-76, 1978.
12. Step DL, Streeter RN, Kirkpatrick JG: Johnes disease update. *Bov Pract* 34:6-12, 2000.
13. Thorne JG, Hardin LE: Estimated prevalence of paratuberculosis in Missouri, USA cattle. *Prevent Vet Med* 31:51-57, 1997.
14. Turnquist SE, Snider TG, Kreeger JM, *et al:* Serologic evidence of paratuberculosis in Louisiana beef cattle herds as detected by ELISA. *Prevent Vet Med* 11:125-130, 1991.
15. Whitlock RH, Wells SJ, Sweeney RW, *et al:* ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. *Vet Micro* 77:387-398, 2000.

Abstract

The Costs of Poor Fertility and What to Do about Reducing Them

Esslemont R.J.
Cattle Practice 11(4):237-250, 2003

The management of fertility in modern dairy herds is proving more difficult because of lack of skilled labour, poor nutrition and the lack of understanding of economics by farmers and veterinarians. The average loss per cow in the UK is estimated to be £183, 3.06p/litre in the 6000 litre cow. It is important to incorporate culling rate for failure to conceive into any index of fertility and to calculate an overall economic loss for fertility (FERTEX). The current choice of voluntary waiting period and number of oestrus cycle allowed to individual animals needs to be determined in advance and appropriate action taken to cope with late calvers in seasonal calved herds who have very short windows of opportunity. The cost of fertility depends on the stage of lactation and the shape of the lactation curve. Cows normally have a curve that loses 8 to 10% per month after peak. Those rare animals losing 4% or so may justify longer calving intervals.