Special Report

Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States

Michael T. Collins, DVM, PhD, DACVM; Ian A. Gardner, BVSc, MPVM, PhD; Franklyn B. Garry, DVM, MS, DACVIM; Allen J. Roussel, DVM, MS, DACVIM; Scott J. Wells, DVM, PhD, DACVPM

The report provided here contains a simplified set of diagnostic testing recommendations. These recommendations were developed on the basis of research funded by the USDA–Animal and Plant Health Inspection Service–Veterinary Services through a cooperative agreement. The report is intended to provide simple, practical, cost-effective consensus testing recommendations for cattle herds that are not enrolled in the US Test-Negative Program.[1] The information has been reviewed by paratuberculosis (Johne's disease) experts at the USDA and academic centers as well as stakeholders in various segments of the cattle industry. The recommendations were accepted by the National Johne's Working Group and Johne's Disease Committee of the US Animal Health Association during their annual meetings in October 2006.

The report is intended to aid veterinarians who work with cattle producers in the United States. The recommendations are based on information available up to October 2006. There is a paucity of large-scale, high-quality studies of multiple tests conducted on samples obtained from the same cattle. It is understood that there may be special circumstances that require deviation from these recommendations. Furthermore, as new information becomes available and assays are improved and their accuracy is critically evaluated, changes to these recommendations may be necessary.

Abbreviations

NVSL	National Veterinary Services Laboratories
HEY	Herrold's egg yolk

Paratuberculosis (Johne's disease) is caused by *Mycobacterium paratuberculosis* (also known as *Mycobacterium avium* subsp *paratuberculosis*). It is a prevalent and economically important disease that affects cattle and impacts the cattle industry.[2-4] In the past 5 years, special funding from Congress has provided the USDA–Animal and Plant Health Inspection Service–Veterinary Services with more than $70 million to help combat this infectious disease. Most of that money has been allocated to states to strengthen their paratuberculosis control programs and assist herd owners with the cost of risk assessments for herds, management changes, and diagnostic testing.

The past decade has witnessed the development and commercialization of multiple accurate and cost-effective diagnostic tests for paratuberculosis that are based on detection of antibodies to *M paratuberculosis* in serum or milk or detection of the etiologic agent in feces from specific cattle or the farm environment by conventional bacterial culture, bacterial culture by use of an automated liquid culture system, or genetic detection systems (ie, PCR assays). Although the availability of several tests for paratuberculosis provides options for detection and control of the disease, it also causes confusion for practitioners and producers with regard to the test that should be used for a specific purpose.

From the Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI 53706-1102 (Collins); the Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616 (Gardner); the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1678 (Garry); the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475 (Roussel); and the Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108 (Wells).

Supported by the USDA–Animal and Plant Health Inspection Service–Veterinary Services (cooperative agreement No. 05-9100-0996-GR).

Presented in part at the National Johne's Working Group in conjunction with the US Animal Health Association Meeting and the Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, Minneapolis, October 2006.

The authors thank Drs. Michael Carter, Jason Lombard, Elizabeth Manning, Elisabeth Patton, Boyd Parr, Thomas Schomer, and Andrew Schwartz for technical assistance.

This article has not undergone *JAVMA* peer review; opinions expressed are not necessarily those of the AVMA.

Address correspondence to Dr. Collins.

Terms and Definitions

It is necessary to use terms consistently to ensure clarity and prevent misunderstandings. A few terms and definitions are needed.

- Best test or best-test regimen is defined as the scientifically justifiable testing regimen that optimizes cost effectiveness and practicality. When there is no clear-cut best-test regimen, all testing options of equivalent value are listed.
- Tests are the laboratory-based methods used to detect cattle with a high likelihood of infection with *M paratuberculosis*. Only tests of which accuracy has been evaluated and the reports published in peer-reviewed scientific journals or that have passed the NVSL proficiency test (ie, check test) were considered. Test accuracy assumptions, which serve as the foundation of these recommendations, reflect consensus based on the most current high-quality studies (**Table 1**). When all other factors are equal, tests that are available as USDA-licensed commercial kits are preferred. Also, in all cases, the tests are considered best only when performed at NVSL-approved laboratories.[5]
- Commercial dairy indicates commercial dairy herds that do not produce cattle for sale as breeding stock.
- Seedstock dairy indicates dairy herds that produce cattle for sale as breeding stock.
- Beef cow-calf indicates beef herds that do not produce cattle for sale as breeding stock.
- Seedstock beef indicates beef herds that produce cattle for sale as breeding stock.

Tests for Detection of Paratuberculosis in Cattle

Several types of tests are available for use in the detection of paratuberculosis in cattle. These include bacterial culture, gene detection assays, antibody assays, and histopathologic evaluation of tissues.

Tests based on bacterial culture of fecal samples—Testing often includes bacterial culture of fecal samples obtained from individual cattle. Fecal samples are cultured on solid HEY agar medium, and appropriate testing is conducted to confirm that isolates are *M paratuberculosis*.[6-10] Testing of fecal samples obtained from individual cattle can also include bacterial culture by use of any commercial liquid culture system.[a-c] Similar to isolates cultured by use of solid HEY agar, isolates cultured by use of liquid culture systems should be subjected to appropriate testing to confirm that acid-fast isolates are *M paratuberculosis*.[7,11-15]

Bacterial culture can also be performed on pooled fecal samples obtained from several cattle in a herd.[16-20] It is recommended to use 5 fecal samples/pool and to culture on solid HEY agar medium or by use of any commercial liquid culture system.[a-c] Again, appropriate testing should be conducted to confirm that acid-fast isolates are *M paratuberculosis*.

Fecal samples can be collected from areas in which cattle from a herd commingle. Such samples should be collected in accordance with Uniform Program Standards[1] and cultured on solid HEY agar medium or by use of any commercial liquid culture system.[a-c] Appropriate testing should be conducted to confirm that acid-fast isolates are *M paratuberculosis*.

Tests based on detection of *M paratuberculosis* genes—Fecal samples collected from individual cattle can be tested by use of PCR assays.[21-25] Similarly, pooled fecal samples (5 fecal samples/pool) can be tested by use of PCR assays. In addition, PCR assays can be conducted on pooled fecal samples (5 fecal samples/pool) collected in accordance with Uniform Program Standards from areas in which cattle from a herd commingle.

Tests to detect antibodies against *M paratuberculosis*—Serum or milk samples from individual cattle can be tested by use of ELISAs. In this report, ELISA testing of milk samples refers to use of a specific ELISA[d] because no data were available on the accuracy of other ELISAs for testing of milk samples.[26] The complement fixation test can be performed by use of NVSL reagents and in accordance with NVSL procedures on serum samples obtained from individual cattle.[27-31] A USDA-licensed agar-gel immunodiffusion kit[e] can be used to test serum samples obtained from individual cattle.[32,33]

Tests on tissue samples—Biopsy specimens can be obtained surgically. Specimens should include collection of a full-thickness section of ileum (≥ 1.0 g) in addition to ≥ 1.0 g of an ileocecal lymph node or an ileum-associated lymph node.[34] At least 0.5 g of each tissue should be homogenized and cultured separately for *M paratuberculosis* by use of solid or liquid media. In addition, ≥ 0.5 g of each tissue should be processed for histologic examination. Tissue sections should be stained with H&E and acid-fast stains and evaluated by a qualified veterinary pathologist. Specimens obtained during necropsy are the same as the biopsy specimens, although more tissues and larger quantities of each tissue can be collected and tested.

Table 1—Assumptions for test sensitivity and specificity used when selecting the best test for detection of paratuberculosis in cattle.

Test	Sensitivity (%)	Specificity (%)
Bacterial culture of fecal samples obtained from individual cattle	60 ± 5	99.9 ± 0.1
PCR assay of fecal samples obtained from individual cattle	30 ± 5	99.5 ± 0.5
ELISA on serum or milk	30 ± 5	99.0 ± 1.0
Evaluation of biopsy specimens	90 ± 5	100
Necropsy*	100	100

Estimates represent most likely values and plausible ranges. Rounding of values and lack of distinction between solid and liquid culture media or between ELISAs performed on serum and milk reflect an insufficient quality of published data on the sensitivity and specificity of these tests as well as averaging of test performance among diverse populations.

*Necropsy represents the criterion-referenced standard for the purposes of this analysis; therefore, values for sensitivity and specificity of necropsy are each 100%.

Number of Cattle to be Tested (Sampling Frame) for Herd Classification

The number of cattle required for testing of herds to qualify for the US Test-Negative Program is governed by the Uniform Program Standards[1] and thus not the focus of our report. For the information reported here, the number of cattle recommended for testing is considered the minimum number required to attain a defined degree of confidence in the testing outcome with consideration for the specific purpose for the testing and the characteristics of the herd.[35-40] Costs listed here were those that existed at the time of the report and are subject to change in the future (**Table 2**).

Rationale for Recommendations on the Basis of the Purpose for Conducting Testing

The reasons for testing herds of cattle can vary. The purpose for testing will impact the number and type of samples collected and the tests performed. Seven specific testing purposes were considered, and purpose-specific recommendations are made for each of the 4 types of cattle operations (**Table 3**).

Herd classification as infected or not infected—For commercial and seedstock dairy herds, bacterial culture of 6 fecal samples obtained from the environment, according to methods outlined in the Program Standards,[1] is sensitive and the most cost-effective method for determining whether a dairy herd is infected.[41-43] However, finding that all 6 samples yield negative results does not guarantee the herd is not infected. Instead, negative culture results on 6 environmental fecal samples suggest that the dairy herd is not infected or has a low within-herd prevalence. The second best testing option for this situation is PCR assay of fecal samples collected from the environment. The PCR techniques are considered less sensitive than bacterial culture. Owners of herds with negative culture or PCR test results on all 6 samples should be encouraged to enroll their herds in the US Test-Negative Program.[1]

Three basic testing options exist for beef cow-calf and seedstock beef herds. A whole-herd test can be conducted by bacterial culture of fecal samples. Alternatively, a whole-herd test can be conducted by use of an ELISA, and positive results for individual cattle can be confirmed by bacterial culture of fecal samples. Target testing of a particular group of cows within

Table 2—Cost of tests for detection of paratuberculosis in cattle and rapidity for reporting of test results on the basis of data obtained from published literature and a survey of 30 diagnostic laboratory Web sites conducted in January 2006.

Test	No. of laboratories offering the test (n = 30)	Cost/test ($)*		Time to obtain test results (d)
		Median	Range	
Bacterial culture of fecal samples†				
IND-HEY	18	17.00	0.00–38.00	112
IND-LIQ	12	19.00	0.00–30.00	56
POOL-HEY	0	—	—	—
POOL-LIQ	2	18.00	16.00–20.00	56
ENV-HEY	0	—	—	—
ENV-LIQ	1	16.00	16.00	56
PCR assay				
IND	9	25.00	15.00–30.00	7
POOL	0	—	—	—
ENV	0	—	—	—
ELISA				
Serum	29	5.00	0.00–16.75	7
Milk	1	6.00	6.00	7
CF	7	6.00	0.00–12.00	7
AGID	14	8.03	2.50–16.75	7

*Cost/test is listed for laboratory charges billed to a submitting veterinarian for an in-state fee without volume discounts. Evaluation of biopsy specimens and necropsy are also considered tests, but price information is not typically provided by diagnostic laboratories. †For all bacterial cultures, appropriate testing is performed to confirm that acid-fast isolates are *Mycobacterium paratuberculosis*.

IND-HEY = Bacterial culture of fecal samples obtained from individual cattle; fecal samples are cultured on solid HEY agar medium. IND-LIQ = Bacterial culture of fecal samples obtained from individual cattle; fecal samples are cultured by use of any commercial liquid culture system. POOL-HEY = Bacterial culture of pooled fecal samples (5 fecal samples/pool); pooled fecal samples are cultured on solid HEY agar medium. POOL-LIQ = Bacterial culture of pooled fecal samples (5 fecal samples/pool); pooled fecal samples are cultured by use of any commercial liquid culture system. ENV-HEY = Bacterial culture of fecal samples collected in accordance with the Uniform Program Standards[1] from areas in which cattle commingle; fecal samples are cultured on HEY agar medium. ENV-LIQ = Bacterial culture of fecal samples collected in accordance with the Uniform Program Standards[1] from areas in which cattle commingle; fecal samples are cultured by use of any commercial liquid culture system. IND = A PCR assay of fecal samples obtained from individual cattle. POOL = A PCR assay of pooled fecal samples (5 fecal samples/pool). ENV = A PCR assay of pooled fecal samples (5 fecal samples/pool) collected in accordance with Uniform Program Standards from areas in which cattle of multiple herds commingle. CF = Complement fixation. AGID = Agar-gel immunodiffusion. — = Not applicable.

Table 3—Recommended test regimen for the detection of paratuberculosis in cattle on the basis of herd type and testing purpose.

	Dairy		Beef	
Testing purpose	**Commercial**	**Seedstock**	**Cow-calf**	**Seedstock**
Herd classification (infected or not infected)	Bacterial culture by ENV-HEY or ENV-LIQ	Bacterial culture by ENV-HEY or ENV-LIQ	Whole-herd testing, target testing, or bacterial culture by ENV-HEY or ENV-LIQ	Whole-herd testing, target testing, or bacterial culture by ENV-HEY or ENV-LIQ
Precise estimation of within-herd prevalence	NR	NR	NR	NR
Control disease in herd with known infection, high prevalence (> 10% positive results on ELISA), and clinical disease, or owner is concerned	ELISA	Bacterial culture by IND-HEY or IND-LIQ	ELISA	Bacterial culture by IND-HEY or IND-LIQ
Surveillance (estimation of biological burden)	Bacterial culture by ENV-HEY or ENV-LIQ	NR	Confirmatory testing of clinically affected, suspect cattle	NR
Eradication (eliminate *M paratuberculosis* infections from herd)	Bacterial culture by POOL-HEY, POOL-LIQ, IND-HEY, or IND-LIQ	Bacterial culture by POOL-HEY, POOL-LIQ, IND-HEY, or IND-LIQ	Bacterial culture by IND-HEY or IND-LIQ	Bacterial culture by IND-HEY or IND-LIQ
Confirm a clinical diagnosis in herds				
No prior confirmed cases of paratuberculosis in herd	Necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	Evaluation of biopsy specimens, necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	Necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	Evaluation of biopsy specimens, necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND
Prior confirmed cases of paratuberculosis in herd	ELISA, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	Evaluation of biopsy specimens, necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	ELISA, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND	Evaluation of biopsy specimens, necropsy, bacterial culture by IND-HEY or IND-LIQ, or PCR assay of IND
Biosecurity (prepurchase testing)	Decision analysis*	Decision analysis*	Decision analysis*	Decision analysis*

*Decision analyses are illustrated in Figures 1–3.
NR = Not recommended.
See Table 2 for remainder of key.

a herd (eg, those with low body condition scores, such as ≥ 30 thin cows 36 months of age or older) can be conducted by bacterial culture of fecal samples. Alternatively, target testing can be conducted by use of an ELISA, and positive results for individual cattle can be confirmed by bacterial culture of fecal samples. For intensively managed beef cow-calf and seedstock beef herds, bacterial culture of fecal samples obtained from the environment can be used. For extensively managed herds in which congregation of cattle is not common, bacterial culture of environmental fecal samples may not provide a reliable assessment of the infection status of the herd, and whole-herd or target testing is recommended.

Precise estimation of within-herd prevalence—Expert consensus was that pursuing this goal is not recommended from the perspective of controlling paratuberculosis. Precise estimation of within-herd prevalence (which differs from broad prevalence estimation used in herd risk assessments) has limited value for the management of paratuberculosis. To obtain narrow confidence limits for the estimated prevalence value (eg, 95% confidence interval that has a prevalence estimate with an error margin of ± 2%, which corresponds to a 95% confidence interval with a width of 4%), many cattle must be tested.[44-46] For herds that consist of < 300 cattle, every animal must be tested by use of an ELISA, bacterial culture of fecal samples, or PCR assay of fecal samples. For herds that consist of > 1,000 cattle, it is possible to test a statistically determined subset of the herd but the cattle tested must be randomly selected. Tests with lower costs are favored for this testing purpose. To validly document changes in prevalence over time, the

same test must be used for each subsequent herd test. The number of cattle tested must be calculated by use of standard epidemiologic equations that take into consideration estimate confidence, desired margin of error for the prevalence estimate, and sensitivity and specificity of the test.[44-46] Although this may be appropriate in a research setting, it is expensive and of limited value in a clinical setting.

Control of paratuberculosis—Testing by ELISA is recommended for control of paratuberculosis in commercial dairy herds for which the objective is to reduce economic impact of the infection. Current evidence indicates that the greatest negative economic impact is caused by cows with late-stage paratuberculosis (ie, cows with strong-positive ELISA results).[47] The ELISAs have low cost and high specificity and accurately detect the most infective cattle (test sensitivity is approx 85%).[26,48-50] However, when ELISAs are used, producers must act on the results in concert with risk assessment findings. Producers must be willing to cull cattle on the basis of the magnitude of an ELISA result[51] and institute proper management of maternity pens, colostrum, and feeding of calves.[52]

Testing by ELISA is recommended for control of paratuberculosis in infected beef cow-calf herds. However, there are limited data on the economic impact of paratuberculosis in beef cow-calf herds, which makes it difficult to assess whether the costs of testing and control programs are justified.[53] When testing is conducted, test results must be used in the management of the cattle. Options include culling each ELISA-positive cow and her most recent calf. Alternatively, the herd can be segregated into ELISA-negative and ELISA-positive groups. Offspring of ELISA-positive cows should be sold for slaughter.

The overall goal for seedstock dairy or beef herds is to eradicate rather than control paratuberculosis. Until eradication is achieved, owners face substantial liability and risk to their reputation as a consequence of the sale of infected cattle. To eradicate the disease, producers must use the most sensitive and specific tests available on individual cattle, especially for owners who continue to sell seedstock. Accelerated control programs can be achieved by use of bacterial culture of fecal samples alone, ELISA testing of serum samples followed by bacterial culture of fecal samples obtained from ELISA-positive cattle, or real-time PCR assay of fecal samples with concurrent bacterial culture of fecal samples. Producers must aggressively cull cattle that have positive test results. In addition, offspring of cows with positive results should be sold for slaughter.

Disease surveillance—For commercial dairy herds, surveillance represents a herd testing strategy designed to assess the infection pressure in a herd (eg, environmental load or level of premises contamination by *M paratuberculosis*). Corrective actions are instituted when test results indicate that the infection pressure has increased above a specified threshold value. Surveillance is appropriate for commercial dairy herds in which paratuberculosis is controlled and the owner wants a low-cost method of ensuring herd infection pressure remains low without the cost and inconvenience of testing individual animals.

Surveillance in beef cow-calf herds may not be warranted for economic reasons. Recognition of clinical disease events and use of confirmatory bacterial

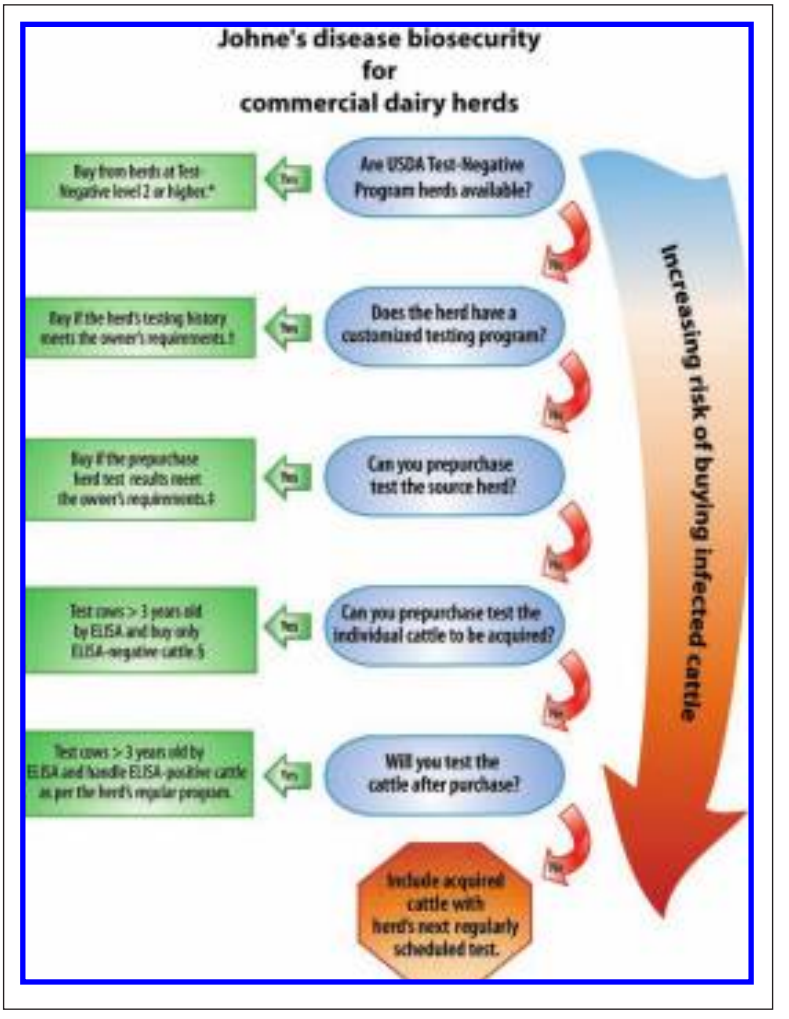

Figure 1—Illustration of the decision analysis for testing recommendations to limit the probability of introducing cattle infected with *Mycobacterium paratuberculosis* (Johne's disease) into commercial dairy herds. The terms "buy," "bought," or "purchase" are used to indicate acquisition of any cattle (eg, purchase, lease, or rent). Overall, the fewer the cattle that are bought, the lower the infection risk for the buyer's herd. All purchased cattle should be included in the regular testing program conducted for the buyer's herd. *Levels in the Test-Negative Program refer to guidelines established in the US Program Standards.[1] †For example, < 3% positive results during a multiple-year period of testing. ‡For example, perform an ELISA on samples obtained from 100 cows that are > 3 years old; consider a maximum of 2 positive results acceptable. §There is limited value in ELISA testing of younger cattle (ie, cattle < 3 years old). When a source herd contains multiple test-positive cattle, do not purchase cattle from that herd.

culture of fecal samples may be sufficient for herd surveillance. When herd owners want to have a more sensitive surveillance program based on laboratory test data, periodic target testing of thin cows or bacterial culture of environmental fecal samples for herds with a high stocking density is recommended.

Expert consensus was that surveillance testing of seedstock dairy and beef herds for *M paratuberculosis* infection is not recommended because the diagnostically detectable amount of infection is likely to be low. Seedstock producers with sufficient premises infection pressure to result in positive results for bacterial culture of environmental fecal samples should be working toward eradication of infection, rather than merely conducting surveillance.

Eradication of paratuberculosis—Eradication is a logical choice only when a herd has a low (< 5%) test prevalence. The best test for this purpose has maximal sensitivity; thus, bacterial culture of fecal samples from individual cattle is the best choice. Producers and veterinarians should understand that there are limited field data that indicate eradication of paratuberculosis is actually possible. Whole-herd testing must be conducted for several years. For commercial herds, eradication may not be a cost-effective option. Culling of all test-positive cattle is necessary, but it must be accompanied by effective changes in herd management. Analysis of data on bacterial culture of pooled fecal samples obtained from dairy cattle indicates that there is limited loss in test sensitivity and significant cost savings, compared with results for bacterial culture of individual fecal samples.[17,18,20] Therefore, bacterial culture of pooled fecal samples is also considered a valid testing option for eradication of paratuberculosis in a dairy herd. Comparable data on bacterial culture of pooled fecal samples are not yet available for beef cattle; thus, the recommended test for eradication programs in beef cattle is bacterial culture of fecal samples obtained from individual beef cattle.

Confirmation of a clinical diagnosis—For herds in which there are no prior confirmed cases of paratuberculosis, it is important to establish a diagnosis of *M paratuberculosis* so that an effective herd management plan can be formulated after performing a risk assessment. The most definitive and sensitive method for use in confirming a diagnosis of paratuberculosis is a complete necropsy, which should include recording gross lesions and obtaining ileal and mesenteric lymph node tissues for bacterial culture and histologic examination. This approach should yield a diagnostic sensitivity of 100%. In addition, necropsies provide the benefit of identifying other disease problems in addition to paratuberculosis. Bacterial culture of fecal samples and PCR assay of fecal samples are acceptable alternatives to necropsy but have lower sensitivity for confirmation of the diagnosis.

In herds in which there are prior confirmed cases of paratuberculosis (ie, herds known to be infected), confirmation of a diagnosis is a useful surveillance tool to establish the proportion of culled cows that are infected with *M paratuberculosis*. Samples can be collected on cows as they are culled from the herd. Use of an ELISA, bacterial culture of fecal samples, and PCR assay of fecal samples are all acceptable methods for confirming a diagnosis in clinically affected cattle.

Prevention through prepurchase testing (biosecurity)—Biosecurity in this context refers to the use of diagnostic tests to limit the risk of acquiring herd replacements that are infected with *M paratuberculosis*.[36,54] Herds in the US Test-Negative Program must adhere to Uniform Program Standards[1] regarding herd additions. For other herds, many testing options are available (**Figures 1–3**). The best way to limit risk of purchasing infected cattle is to buy as few animals as possible. When acquisition of cattle is considered necessary, the risk is best limited through the use of tests that evaluate the infection status of the source herd,

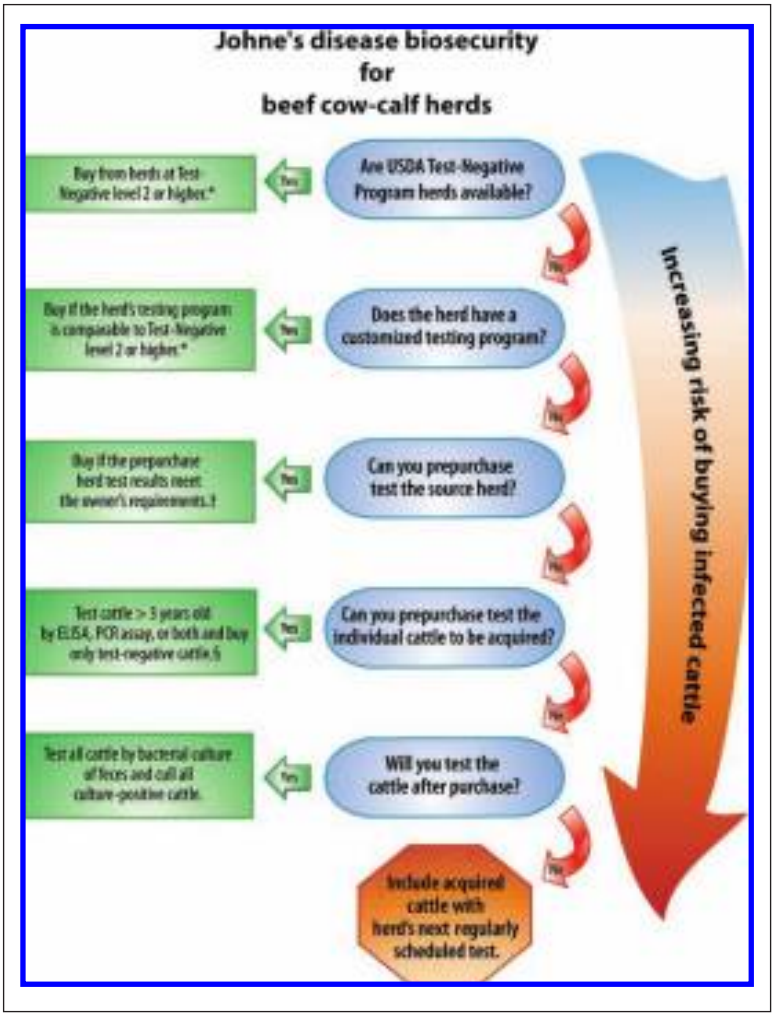

Figure 2—Illustration of the decision analysis for testing recommendations to limit the probability of introducing cattle infected with *M paratuberculosis* (Johne's disease) into commercial beef cow-calf herds. *See* Figure 1 for key.

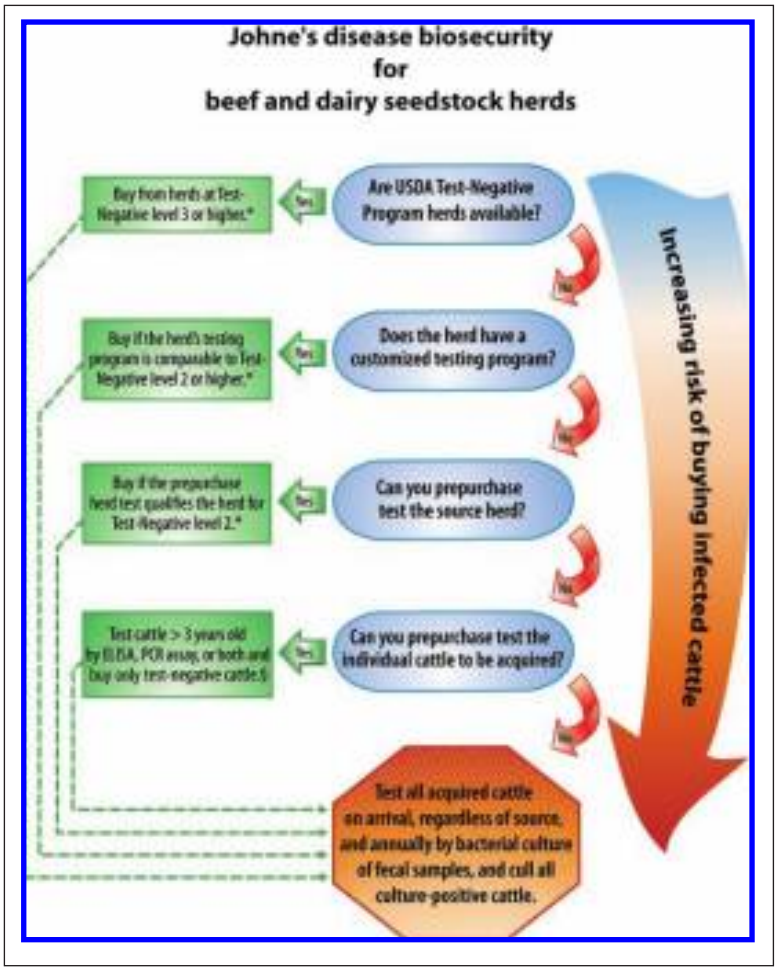

Figure 3—Illustration of the decision analysis for testing recommendations to limit the probability of introducing cattle infected with *M paratuberculosis* (Johne's disease) into dairy or beef seedstock herds. *See* Figure 1 for key.

rather than tests conducted on individual cattle. Cattle should truly represent herds (ie, cattle in contact with each other, under the same style of management, and that share a range or are provided with the same [or similar] feed) and not simply be a group of recently assembled cattle. The situation differs among herds, based in part on the estimated infection prevalence in the buyer's herd, but the goal should be to purchase replacements from herds with a test-positive percentage that is lower than that of the buyer's herd (ideally, the test-positive percentage for the replacement herds should be < 50% of that of the home-raised replacement cattle).

Conclusions

These recommendations are intended for use by veterinary practitioners to simplify selection of diagnostic tests for control of paratuberculosis in cattle. They are purpose-specific, scientifically sound, and economically justifiable. Although some changes may be required as new diagnostic techniques and technologic advances become available, these recommendations are sufficiently generic that they are likely to remain legitimate for many years. Control of paratuberculosis at the state, regional, or national level can only be achieved when a substantial proportion of the veterinary practitioner and cattle producer communities apply directed disease management efforts developed on the basis of scientifically sound control and testing methods.[55]

a. TREK ESP II, TREK Diagnostic Systems Inc, Cleveland, Ohio.
b. BACTEC-12B, BD Diagnostic Systems, Franklin Lakes, NJ.
c. BACTEC-MGIT 960 paraTB, BD Diagnostic Systems, Franklin Lakes, NJ.
d. Antel BioSystems Inc, Lansing, Mich.
e. Rapid Johne's test, ImmuCell, Portland, Me.

References

1. USDA–APHIS. Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program (APHIS 91-45-016). Available at: www.aphis.usda.gov/vs/nahps/johnes/. Accessed Nov 11, 2006.

2. Harris NB, Barletta RG. *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. *Clin Microbiol Rev* 2001;104:489–512.

3. Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. *Prev Vet Med* 1999;40:179–192.

4. Johnson-Ifearulundu YJ, Kaneene JB. Epidemiology and economic impact of subclinical Johne's disease: a review. *Vet Bull* 1997;67:437–447.

5. USDA. NVSL approved laboratories. Available at: www.aphis.usda.gov/vs/nahps/johnes/. Accessed Nov 11, 2006.

6. Crossley BM, Zagmutt-Vergara FJ, Fyock TL, et al. Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* by dairy cows. *Vet Microbiol* 2005;107:257–263.

7. Eamens GJ, Whittington RJ, Marsh IB, et al. Comparative sensitivity of various faecal culture methods and ELISA in dairy cattle herds with endemic Johne's disease. *Vet Microbiol* 2000;77:357–367.

8. Stabel JR. An improved method for cultivation of *Mycobacterium paratuberculosis* from bovine fecal samples and comparison to three other methods. *J Vet Diagn Invest* 1997;9:375–380.

9. Whipple DL, Kapke PA, Andersen PR. Comparison of a commercial DNA probe test and three cultivation procedures for detection of *Mycobacterium paratuberculosis* in bovine feces. *J Vet Diagn Invest* 1992;4:23–27.

10. Whipple DL, Callihan DR, Jarnagin JL. Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardized procedure. *J Vet Diagn Invest* 1991;3:368–373.

11. Grant IR, Kirk RB, Hitchings E, et al. Comparative evaluation of the MGIT and BACTEC culture systems for the recovery of *Mycobacterium avium* subsp. *paratuberculosis* from milk. *J Appl Microbiol* 2003;95:196–201.

12. Whittington RJ, Marsh I, McAllister S, et al. Evaluation of modified BACTEC 12B radiometric medium and solid media for culture of *Mycobacterium avium* subsp. *paratuberculosis* from sheep. *J Clin Microbiol* 1999;37:1077–1083.

13. Whittington RJ, Marsh I, Turner MJ, et al. Rapid detection of *Mycobacterium paratuberculosis* in clinical samples from ruminants and in spiked environmental samples by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR. *J Clin Microbiol* 1998;36:701–707.

14. Sockett DC, Carr DJ, Collins MT. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of *Mycobacterium paratuberculosis* infections in cattle. *Can J Vet Res* 1992;56:148–153.

15. Collins MT, Kenefick KB, Sockett DC, et al. Enhanced radiometric detection of *Mycobacterium paratuberculosis* using filter concentrated fecal specimens. *J Clin Microbiol* 1990;28:2514–2519.

16. Kalis CHJ, Collins MT, Barkema HW, et al. Certification of herds as free of *Mycobacterium paratuberculosis* infection: actual

pooled faecal results versus certification model predictions. *Prev Vet Med* 2004;65:189–204.

17. Kalis CHJ, Hesselink JW, Barkema HW, et al. Culture of strategically pooled bovine fecal samples as a method to screen herds for paratuberculosis. *J Vet Diagn Invest* 2000;12:547–551.

18. van Schaik G, Stehman SM, Schukken YH, et al. Pooled fecal culture sampling for *Mycobacterium avium* subsp. *paratuberculosis* at different herd sizes and prevalence. *J Vet Diagn Invest* 2003;15: 233–241.

19. Wells SJ, Godden SM, Lindeman CJ, et al. Evaluation of bacteriologic culture of individual and pooled fecal samples for detection of *Mycobacterium paratuberculosis* in dairy cattle herds. *J Am Vet Med Assoc* 2003;223:1022–1025.

20. Wells SJ, Whitlock RH, Lindeman CJ, et al. Evaluation of bacteriologic culture of pooled fecal samples for detection of *Mycobacterium paratuberculosis*. *Am J Vet Res* 2002;63:1207–1211.

21. Wells SJ, Collins MT, Faaberg KS, et al. Evaluation of a rapid fecal PCR test for detection of *Mycobacterium avium* subsp. *paratuberculosis* in dairy cattle. *Clin Vaccine Immunol* 2006;13:1125–1130.

22. Stabel JR, Bannantine JP. Development of a nested PCR method targeting a unique multicopy element, ISMap02, for detection of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples. *J Clin Microbiol* 2005;43:4744–4750.

23. Tasara T, Stephan R. Development of an F57 sequence-based real-time PCR assay for detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Appl Environ Microbiol* 2005;71: 5957–5968.

24. Christopher-Hennings J, Dammen MA, Weeks SR, et al. Comparison of two DNA extractions and nested PCR, real-time PCR, a new commercial PCR assay, and bacterial culture for detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine feces. *J Vet Diagn Invest* 2003;15:87–93.

25. Fang Y, Wu WH, Pepper JL, et al. Comparison of real-time, quantitative PCR with molecular beacons to nested PCR and culture methods for detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine fecal samples. *J Clin Microbiol* 2002;40:287–291.

26. Collins MT, Wells SJ, Petrini KR, et al. Evaluation of five antibody detection tests for bovine paratuberculosis. *Clin Diagn Lab Immunol* 2005;12:685–692.

27. Kalis CHJ, Barkema HW, Hesselink JW, et al. Evaluation of two absorbed enzyme-linked immunosorbent assays and a complement fixation test as replacements for fecal culture in the detection of cows shedding *Mycobacterium avium* subspecies *paratuberculosis*. *J Vet Diagn Invest* 2002;14:219–224.

28. Sockett DC, Conrad TA, Thomas CB, et al. Evaluation of four serological tests for bovine paratuberculosis. *J Clin Microbiol* 1992;30:1134–1139.

29. Norris MJ, Spencer TL. The efficacy of the complement fixation test for the detection of *Mycobacterium paratuberculosis* infection in cattle. In: Milner AR, Wood PR, eds. *Johne's disease. Current trends in research, diagnosis and management*. Melbourne: CSIRO, 1989;153–157.

30. Ridge SE, Morgan IR, Sockett DC, et al. Comparison of the Johne's absorbed EIA and the complement fixation test for the diagnosis of Johne's disease in cattle. *Aust Vet J* 1991;68:253–257.

31. de Lisle GW, Seguin P, Samagh BS, et al. Bovine paratuberculosis I. A herd study using complement fixation and intradermal tests. *Can J Comp Med* 1980;44:177–182.

32. Sherman DM, Gay JM, Bouley DS, et al. Comparison of the complement-fixation and agar gel immunodiffusion tests for the diagnosis of subclinical bovine paratuberculosis. *Am J Vet Res* 1990; 51:461–465.

33. Sherman DM, Bray B, Gay JM, et al. Evaluation of the agar gel immunodiffusion test for the diagnosis of subclinical paratuberculosis in cattle. *Am J Vet Res* 1989;50:525–530.

34. Benedictus G, Haagsma J. The efficacy of mesenteric lymph node biopsy in the eradication of paratuberculosis from an infected farm. *Vet Q* 1986;8:5–11.

35. Carpenter TE, Gardner IA. Simulation modeling to determine herd-level predictive values and sensitivity based on individual animal test sensitivity and specificity and sample size. *Prev Vet Med* 1996;27:57–66.

36. Carpenter TE, Gardner IA, Collins MT, et al. Effects of prevalence and testing by enzyme-linked immunosorbent assay and fecal culture on the risk of introduction of *Mycobacterium avium* subsp. *paratuberculosis*-infected cows into dairy herds. *J Vet Diagn Invest* 2004;16:31–38.

37. Gardner IA, Stryhn H, Lind P, et al. Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. *Prev Vet Med* 2000;45:107–122.

38. Gardner IA, Carpenter TE, Collins MT. Risk of introduction of *Mycobacterium paratuberculosis* into dairy herds: effects of prevalence and test sensitivity. In: Juste RA, Geijo MV, Garrido JM, eds. *Proceedings of the 7th International Colloquium on Paratuberculosis*. Madison, Wis: International Association for Paratuberculosis, 2003;516.

39. Jordan D. Aggregate testing for the evaluation of Johne's disease herd status. *Aust Vet J* 1996;73:16–19.

40. Stabel JR, Wells SJ, Wagner BA. Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. *J Dairy Sci* 2002;85:525–531.

41. Berghaus RD, Farver TB, Anderson RJ, et al. Environmental sampling for detection of *Mycobacterium avium* ssp. *paratuberculosis* on large California dairies. *J Dairy Sci* 2006;89:963–970.

42. Lombard JE, Wagner BA, Smith RL, et al. Evaluation of environmental sampling and culture to determine *Mycobacterium avium* subspecies *paratuberculosis* distribution and herd infection status on US dairy operations. *J Dairy Sci* 2006;89:4163–4171.

43. Raizman EA, Wells SJ, Godden SM, et al. The distribution of *Mycobacterium avium* ssp. *paratuberculosis* in the environment surrounding Minnesota dairy farms. *J Dairy Sci* 2004;87: 2959–2966.

44. DiGiacomo RF, Koepsell TD. Sampling for detection of infection or disease in animal populations. *J Am Vet Med Assoc* 1986; 186:22–23.

45. Martin SW, Meek AH, Willeberg P. Measurement of disease frequency and production. In: Martin SW, Meek AH, Willeberg P, eds. *Veterinary epidemiology—principles and methods*. Ames, Iowa: Iowa State University Press, 1987;73–76.

46. Martin SW, Shoukri M, Thorburn MA. Evaluating the health status of herds based on tests applied to individuals. *Prev Vet Med* 1992;14:33–43.

47. Lombard JE, Garry FB, McCluskey BJ, et al. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. *J Am Vet Med Assoc* 2005;227:1975–1981.

48. Sweeney RW, Whitlock RH, Buckley CL, et al. Diagnosis of paratuberculosis in dairy cattle, using enzyme-linked immunosorbent assay for detection of antibodies against *Mycobacterium paratuberculosis* in milk. *Am J Vet Res* 1994;55:905–909.

49. Whitlock RH, Wells SJ, Sweeney RW, et al. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. *Vet Microbiol* 2000;77:387–398.

50. Nielsen SS, Toft N. Age-specific characteristics of ELISA and fecal culture for purpose-specific testing for paratuberculosis. *J Dairy Sci* 2006;89:569–579.

51. Gardner IA, Greiner M. Receiver-operating characteristics and likelihood ratios: improvements of traditional methods for the evaluation and application of veterinary clinical pathology tests. *Vet Clin Pathol* 2006;35:8–17.

52. Collins MT. Clinical approach to control of bovine paratuberculosis. *J Am Vet Med Assoc* 1994;204:208–210.

53. Elzo MA, Rae DO, Lanhart SE, et al. Factors associated with ELISA scores for paratuberculosis in an Angus-Brahman multibreed herd of beef cattle. *J Anim Sci* 2006;84:41–48.

54. Marchevsky N, Held JR, Garcia-Carrillo C. Probability of introducing diseases because of false negative test results. *Am J Epidemiol* 1989;130:611–614.

55. Dorshorst NC, Collins MT, Lombard JE. Decision analysis model for paratuberculosis control in commercial dairy herds. *Prev Vet Med* 2006;75:92–122.