Evaluation of a Commercial Enzyme-Linked Immunosorbent Assay for Johne’s Disease

M. T. COLLINS,1* D. C. SOCKETT,1 S. RIDGE,2 AND J. C. COX3

School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin 53706,1 and Department of Agriculture and Rural Affairs, Veterinary Research Institute, Attwood,2 and Commonwealth Serum Laboratories, Parkville,3 Victoria, Australia

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A new commercial kit for diagnosis of bovine paratuberculosis (Johne’s disease), called the Johne’s Absorbed EIA (enzyme immunoassay; Commonwealth Serum Laboratories, Parkville, Victoria, Australia), was evaluated by using serum specimens from the National Repository for Paratuberculosis Specimens. The evaluation was specifically designed to measure test sensitivity and specificity for detection of dairy cattle with subclinical paratuberculosis. The case definition of subclinical bovine paratuberculosis was isolation of Mycobacterium paratuberculosis from fecal samples or internal organs of cattle without diarrhea or chronic weight loss. Animals designated as free of the disease originated exclusively from four herds in Wisconsin that were certified to be free of disease. The kit had a sensitivity of 47.3% for serum specimens from 150 infected cattle. The test detected 59.7% of animals that shed M. paratuberculosis in their feces, as defined by conventional fecal culture, at the time of serum collection. Testing of 196 serum specimens from cattle without paratuberculosis yielded two false-positive results; the test specificity was thus 99.0%. Decision analysis procedures on the economics of using the kit in a test-and-cull disease control program indicated it would be cost-effective in any herd with a true paratuberculosis prevalence of ≥3%. Comparison of the sensitivity and specificity of the Johne’s Absorbed EIA with those of other tests for detection of subclinical paratuberculosis indicated that it may be the most accurate commercially available test at present and better than the standard complement fixation test used in the United States.

For the past decade, the majority of the research effort on Johne’s disease (paratuberculosis) has been directed at development of improved diagnostic tests for the disease. Several new techniques have recently been described that improve culture detection of the causative agent, Mycobacterium paratuberculosis (9, 11, 16, 21, 48), use gene probes (15, 22, 44), or are based on serological methods (25, 39, 43, 51). Among the serological techniques, enzyme-linked immunosorbent assays (ELISAs) that use absorption of sera to reduce nonspecific reactions have shown considerable promise (24–26, 49–51). This technique was commercialized by Commonwealth Serum Laboratories, Parkville, Victoria, Australia, and was released in March 1990 as a kit called the Johne’s Absorbed EIA (enzyme immunoassay) for diagnosis of bovine paratuberculosis. In this report we describe the evaluation of the diagnostic accuracy of this kit by using serum specimens from the National Repository for Paratuberculosis Specimens.

MATERIALS AND METHODS

Specimens. Serum and fecal specimens were collected concurrently from 856 dairy cattle in 13 Wisconsin herds. Nine of the herds, containing a total of 660 dairy cattle, were infected with M. paratuberculosis and had prevalence rates that ranged from 5 to over 60%. Four of the herds, containing a total of 196 cattle, were certified to be free of paratuberculosis by the state of Wisconsin Department of Agriculture, Trade and Consumer Protection based on a regular program of annual whole-herd testing for paratuberculosis by the conventional fecal culture method (10). These specimens constituted a part of the National Repository for Paratuberculosis Specimens housed at the University of Wisconsin—Madison (39a).

An extra effort was made to identify animals with the full spectrum of M. paratuberculosis infection severity. At the time of serum collection fecal specimens were collected and cultured both by conventional methods (46) and by a new radiometric technique (9). In addition, serum specimens from all animals were recultured three or more times during the next 18 months by conventional methods at the Wisconsin Animal Health Laboratory. In Wisconsin, for the conventional method we used 0.25% hexadecyltrimethylammonium chloride and two tubes of Herrold egg yolk (HEY) agar with mycobactin and one tube without mycobactin. Cultures were incubated and observed for 3 months. To recover tissues for histopathology and culture, surgery was performed on animals that were culture negative at every test but that were positive for antibodies to M. paratuberculosis by an absorbed ELISA (Allied Laboratories, Inc.). A full-thickness (1 by 2 cm) piece of terminal ileum, taken 10 to 20 cm proximal to the ileocecal junction, and an ileocecal lymph node were obtained through a right-flank laparotomy incision. Histopathology with both hematoxylin-eosin and acid-fast stains was done on half of each tissue, and the remainder was homogenized and cultured for M. paratuberculosis by the radiometric method (9).

A total of 856 serum specimens (0.5-ml aliquots) from the repository were shipped on dry ice to the Australian Animal Health Laboratory, Geelong, Victoria. All serum specimens were those collected concurrently with fecal specimens from individual animals at the time of first sampling at each farm. Testing was performed blind by technical staff of the Veterinary Research Institute.

Johne’s Absorbed EIA. Assays were performed with the commercial kits according to the directions of the manufac-
EVALUATION OF AN ELISA FOR JOHNE'S DISEASE

The Johne's Absorbed EIA was highly specific. Only 2 of 196 cows in four herds certified to be free of paratuberculosis tested positive, and the resulting test specificity estimate of 99.0% was not significantly different from the value of 99.8% found when the kit was used to test 1,000 serum specimens from cattle in Western Australia, where paratuberculosis does not occur (10b), nor from the absorbed ELISA specificity of 98.9% ± 0.6% reported by Yokomizo (49).

For specificity estimates of serological tests for paratuberculosis, cattle that are unequivocally free of the disease have often not been used. Instead, animals residing in infected herds have been used and three negative fecal cultures obtained at intervals of ≥6 months have been relied upon to classify animals as noninfected (30, 38, 40, 42). Problems surrounding the use of an imperfect standard for the disease-free classification have been described previously (33) and were illustrated in the present study. Among 488 animals in
the *M. paratuberculosis*-infected herds that were negative on all three conventional fecal culture attempts. 24 were found to be infected either by radiometric culture of feces or by radiometric culture of surgically collected tissues. Had those 488 animals been used for test specificity determination, the result would have been 95.1%. This underestimation of test specificity would have adversely affected adoption of the test by laboratories because of the concern about the economic consequences to farmers associated with false-positive test results.

The stage of *M. paratuberculosis* infection significantly affects diagnostic test sensitivity for paratuberculosis. As a case definition of subclinical paratuberculosis, many investigators have used animals without signs of diarrhea and weight loss from which *M. paratuberculosis* was isolated from fecal specimens collected two or three times over a period of roughly 18 months (8, 14, 15, 38). The case definition in the present study was more inclusive, as indicated by the fact that 25 of the 150 infected animals classified as having paratuberculosis were negative on three or more fecal culture attempts (conventional methods). The diagnosis was established in 11 of these animals by radiometric culture of filter concentrated fecal specimens (9) and in the remaining 14 animals by culture of biopsy tissues.

The diagnostic ‘gold standard’ that we used ensured a more rigorous definition of diagnostic test sensitivity than has previously been attempted. The sensitivity of the Johne’s Absorbed EIA for detection of both fecal culture-positive and -negative cattle was 47.3% ± 8.2%. Alternatively, if only animals that were culture positive by conventional methods at the time of serum collection were defined as having subclinical paratuberculosis, the test ‘sensitivity’ would be 59.7%. With clinically affected animals, the sensitivity of the Johne’s Absorbed EIA was 87.5% (unpublished data).

Detection of conventional culture-positive cattle by the Johne’s Absorbed EIA (59.7%) was the same as the 60% organism excreter detection rate found by Yokomizo (49) when 58 culture-positive cattle were tested by an absorbed ELISA. Using a positive fecal culture as the diagnostic criterion for Johne’s disease, Sherman et al. (38) reported the sensitivity and specificity of the agar gel immunodiffusion kit (Rapid Johne’s Test; ImmuCell Corp., Portland, Maine) to be 18.9 and 99.4%, respectively. They also reported the sensitivity and specificity of the standard complement fixation test (28) to be 10.8 and 97.4%, respectively, when titers of ≥1:32 were classified as positive, or 31.1 and 90.3%, respectively, when titers of ≥1:16 were considered positive. The greater sensitivity of ELISA-based techniques over those of gel diffusion methods for the serodiagnosis of Johne’s disease was also reported by Tsai et al. (43). While the sensitivity measures reported by Sherman et al. (38) may be in error for the reasons described above, absorbed ELISA techniques appear to achieve greater diagnostic sensitivity without compromising diagnostic specificity, making them more accurate than the other available serological tests.

The McNemar test (34) indicated that conventional fecal culture and the Johne’s Absorbed EIA are not significantly different in diagnostic accuracy. The kappa statistic, however, was relatively low, suggesting that the two tests detected different groups of animals. Fecal culture detects animals that are excreting viable *M. paratuberculosis* in their feces. Serological tests, such as the Johne’s Absorbed EIA, potentially detect animals prior to initiation of the infectious stage (organism excretion). Some fecal excreters were missed by the serological test, however.

Detection of fecal excreters of *M. paratuberculosis* may be of more importance to paratuberculosis control efforts than is detection of serologically positive cattle. It is not known whether all seropositive cattle progress to organism excretion, clinical disease, or both (7). The stage of Johne’s disease in which economically significant reduction of animal productivity occurs has been shown to correspond with detection of *M. paratuberculosis* in fecal specimens (2), providing an economic argument for organism detection-based tests. Long-term prospective studies are needed to determine whether cattle that become infected and serologically positive always progress to the infectious stage of disease, shedding *M. paratuberculosis* in their feces, and then clinical disease and whether serologically positive but fecal culture-negative animals have reduced productivity.

Johne’s disease has been shown in several studies to cause significant reductions in the productivity of dairy cattle (1, 2, 4, 23, 47). Decision analysis indicated that use of the Johne’s Absorbed EIA as the basis for culling of presumptively diseased cows would be economical whenever the true prevalence of Johne’s disease in the herd was ≥3%. That is, the benefits of eliminating infected cattle from a herd outweigh the cost of testing and the costs associated with test errors. The test’s high specificity would minimize the cost of culling animals because of false-positive test results.

By using the test sensitivity and specificity reported here, the kit can be used to estimate the true Johne’s disease prevalence from the observed (apparent) prevalence rate of test-positive animals in a population (18, 37). This would make the serological survey data obtained by use of the kit more meaningful. For example, Braum et al. (3) recently reported that 17.1% of dairy cattle in Florida tested positive by an absorbed ELISA (apparent prevalence) but made no estimate of the accuracy of their test nor the true prevalence of Johne’s disease. If their test had the same sensitivity and specificity as those of the Johne’s Absorbed EIA kit, the true prevalence of Johne’s disease in Florida’s dairy cattle is 34.8%. Estimation of true prevalence is also a useful clinical tool for advising farmers on the infection status of their herds and the economics of paratuberculosis control.

The kit from Commonwealth Serum Laboratories is the first commercially available kit for serodiagnosis of Johne’s disease by using absorbed ELISA techniques. It differs from other absorbed ELISAs that have been reported, in that absorption of cross-reactive antibodies is effected by using soluble *Mycobacterium phlei* antigens simply by dilution of test sera in *M. phlei* antigen-containing diluent and incubation for 30 min before the assay is performed. This eliminates the need for overnight absorption and centrifugation of serum specimens (25, 49). A standardized paratuberculosis serodiagnostic assay is important for development of state or national control programs and the international trade of cattle. Use of absorbed ELISA procedures without standardized reagents can result in significantly different diagnostic accuracies between laboratories, as shown by Lopez et al. (17), and thus should be evaluated carefully before adoption.

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REFERENCES


