Likelihood ratios provide a rational and practical method for quantitative use of ELISAs for paratuberculosis

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SUMMARY

ELISA optical density (OD) values are a measure of antibody concentration. Transforming OD values to positive or negative interpretations based on a single cut-off value ignores the clinical value of the magnitude of the ELISA result. Likelihood ratios (LRs = [sensitivity/(1-specificity)]) calculated on sera from well characterized cases and controls allows the magnitude of ELISA results to be used to estimate the probability the tested animal has or does not have paratuberculosis. The purpose of this study was to calculate LRs for a wide range of ELISA S/P values. LRs were determined using a serum antibody ELISA for Johne’s disease (IDEXX) on 143 bovine sera from culture- or histopathology-confirmed cases of paratuberculosis and 2,973 bovine sera from animals in herds proven free of Mycobacterium avium subsp. paratuberculosis (Map) infection (herds negative on whole-herd fecal culture a minimum of eight times at intervals of 6 months or more). ELISA ODs were transformed to S/P values as per the kit manufacturer’s directions. Sensitivity and specificity were calculated for S/P values 0.0 to 2.0 in intervals of 0.05 S/P units. LRs ranged from 1 to 583. LRs were plotted against ELISA S/P value cut-off (x-axis). These data pairs fitted a line defined by a power function with an r² value = 0.94. The LR algorithm can be used to estimate the probability of Map infection. Example: a cow with an ELISA S/P of 0.40 has an LR of 41:1 (odds the cow is infected); converted to probability this is 97.6 %. Infection probability estimates are more accurate if the pre-test probability of infection is included in the calculation. Comparison of LR values on the 143 cases of paratuberculosis to other test results catalogued in a repository database showed that LR values are directly related to the percentage of cattle positive on other tests for paratuberculosis.

MATERIALS AND METHODS

Serum samples. Samples originated from two well-defined dairy cattle populations. Sera from 143 subclinically infected Mycobacterium avium subsp. paratuberculosis (Map) cows were part of a previously described specimen repository (8). The case definition for Map-infected cattle was isolation of Map by fecal culture and/or histopathologic evidence of infection. Sera from uninfected cows included 760 samples from U.S. dairy cattle and 2,214 were sera from Dutch dairy cattle. These cattle were from herds free of paratuberculosis as defined a minimum of three negative annual whole-herd fecal cultures.

ELISA. The Map Antibody Test Kit (IDEXX Laboratories, Inc., Westbrook, ME) was used to test all 3,117 sera according to manufacturer’s instructions. With this kit, optical density (OD) values are transformed to S/P ratios based on ODs for the serum sample plus negative and positive controls provided with the kit using the following equation:

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S/P = \frac{(OD \text{ sample} - OD \text{ negative control})}{(OD \text{ positive control} - OD \text{ negative control})}
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Data analysis. Frequency distributions for S/P values on sera from the infected and non-infected populations were tabulated in intervals of 0.05 S/P units. At each interval the sensitivity (Se), specificity (Sp), and LR

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[Se/(1-Sp)] of the ELISA were calculated. Regression analysis was used to determine the equation describing the best fit line for the plot of S/P cut-off value versus LR. The relationship between the magnitude of ELISA S/P value and findings of other diagnostic tests applied to these same Map-infected cattle was based on data contained in the repository (8).

RESULTS

ELISA sensitivity decreased with increasing ELISA S/P cut-off (Figure 1). ELISA specificity increased with increasing ELISA S/P cut-off (Figure 2). Plotting the false-positive rate (1-specificity) makes the effect of cut-off on rate of false-positive results more evident (Figure 3). Plotting LRs (Se/(1-Sp) versus ELISA S/P) showed a relationship that fitted a line described by the power function LR = 265*(S/P value)^2.03 with an r^2 value of 0.94 (Figure 4). When the 143 Map-infected cattle were grouped into five levels of ELISA S/P, a relationship between the magnitude of ELISA S/P and the rate at which cattle tested positive on other tests for paratuberculosis was apparent (data not shown).

DISCUSSION

Control of paratuberculosis in dairy cattle herds requires hygienic measures to limit opportunities for transmission of the infection from cows to calves in combination with management of cattle most likely to be infectious (2,3,12). Infected cattle should not provide colostrum or milk fed to calves and their manure should not be allowed to contaminate feed, water or the environment. This is particularly true for the pens in which calves are born and the location on the farm where calves are raised. In addition, as many of the infected cows as possible should be culled from the herd when economically feasible. Because the majority of Map-infected cattle are infectious (shedding the organism in their manure, colostrum and milk) but clinically normal, laboratory diagnostics are needed to identify them.
Culture of feces to diagnose *Map* infection using conventional culture media, such as Herrold’s Egg Yolk agar requires 8 to 16 weeks (13). Laboratories typically charge $12 - $25 per sample. Liquid culture-based detection system such as the Trek ESP system and the BACTEC system are able to shorten the detection time to 8 weeks but are not less costly than conventional culture (1,4). Genetic *Map*-detection technology coupled with PCR methods theoretically should enhance detection sensitivity and considerably shorten the time to detection. However, commercial tests have yet to attain the analytical sensitivity of culture methods, they are roughly twice as expensive, and they cannot be efficiently used with high sample volumes (14).

SEROLOGY provides a cost-effective alternative to organism detection-based diagnostic methods for bovine paratuberculosis. ELISA-based methods have the highest sensitivity of serologic tests for paratuberculosis (9) plus they offer the kind of low-cost and high-throughput process (>1,000/day) needed to serve the dairy industry. A disadvantage of ELISAs for paratuberculosis is that assay specificity is less than that of fecal culture (5,10,15) (considered to be 100 %) and the economic consequences of mistakenly culling a cow are high (roughly $1,300/cow based on average price of replacement cattle in the U.S. in 2001 and the average salvage value of cull dairy cow).

Traditional ELISA interpretation is dichotomous (positive or negative) based on a single assay cut-off value designed to optimize assay sensitivity and specificity. Use of multi-level LRs capitalize on the assay’s ability to report results on a continuous scale thereby enhancing the amount of diagnostic information gained. They quantify the probability of an accurate diagnosis. Diagnostic probabilities generated from LRs, with or without use of pre-test probabilities, can be integrated with the economic impact of actions taken based on test results such as culling. Thumond *et al.* nicely demonstrated this using an ELISA for *Neospora caninum* infection in dairy cattle (11).

Diagnostic laboratory medicine for animal agriculture is driven more by economics than is companion animal or human diagnostic laboratory medicine. Containment of testing costs and consideration of the economic consequences of the actions resulting from the diagnostic results are critical considerations in deciding which laboratory services to offer. They are factors that veterinary practitioners must consider in deciding what test to use in which circumstances.

The ELISA evaluated in this study produced quantitative results that were directly related to the likelihood a dairy cow was infected with *Map*. While use of LRs in clinical epidemiology have long been advocated (7), few clinicians actually use them to estimate diagnostic probability (6). Acknowledging this, the LR data have been integrated into a simple five category ELISA interpretation and action scheme for paratuberculosis control in dairy herds. This scheme effectively lowers the “cut-off” for identification of high risk cattle to an S/P of ≥0.10 (the manufacturer’s cut-off for a positive test is S/P = 0.25), and couples this with recommendations for low-cost interventions involving cows with such results to limit spread of infection (segregated calving pen and discarding of colostrum). It also raises the “cut-off” for cattle recommended to be culled from the herd to an S/P of ≥1.00 thereby limiting the economic impact on the herd owner cause by sale and replacement of the cow. In this way, veterinary practitioners and dairy producers have a simple scheme for ELISA use in herds that is based on EBM and LRs without need of calculations or nomograms.

CONCLUSIONS

Veterinary practitioners should incorporate pre-test probabilities of infection (estimated within herd paratuberculosis prevalence) together with the magnitude of the *Map* ELISA result, translated into a LR, for more precise estimation of the post-test probability of *Map* infection. Additional information can be found at: http://johnes.org/dairy/diagnosis.html.

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REFERENCES


