



Using ELISA Adjusted Optical Density (OD) Measures to Predict *Mycobacteria avium subsp. paratuberculosis* Shedding Status of Individual Dairy Cattle



Roxanne Pillars, DVM, MS,^{1,2} John B. Kaneene, DVM, MPH, PhD,^{1,2} Daniel L. Grooms, DVM, PhD²

¹ The Population Medicine Center and ²Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA

Abstract

Fecal culture remains the standard for diagnosing *Mycobacterium avium subsp. paratuberculosis* (MAP) in individual cattle, however it requires 6-8 weeks to obtain results. The enzyme-linked immunosorbent assay (ELISA) has a rapid turn-around time, although its sensitivity is poor, especially in subclinically infected cattle. It is generally accepted that as infection progresses, **mean ELISA response (what is this?? Do we need to be more clear??)** and sensitivity increases. Cattle infected with MAP have a long prepatent period in which no shedding of the bacterium occurs followed by intermittent then continuous shedding **increasing in volume (??)** as the disease progresses. While any cow infected with MAP is undesirable, only cattle shedding the organism pose an immediate threat to other **susceptible** cattle. Cattle shedding MAP typically have higher ELISA adjusted optical density (OD) values compared to cattle not shedding MAP. Due to the quicker turn-around time and reduced cost of ELISA testing compared to fecal culture, it would be helpful to reliably predict if an individual cow was shedding MAP based on her ELISA adjusted OD value. The objective of this study was to develop a calculator to determine the probability that a cow is shedding MAP given her ELISA adjusted OD score and the prevalence of MAP shedding within her herd. A database consisting of fecal culture results and concurrent serum ELISA adjusted OD values from 2,578 adult cattle from six Michigan dairy herds over two years was used as the foundation for calculating the positive likelihood ratio (PLR) for a given ELISA adjusted OD value. Herd prevalence was converted to a pre-test odds ratio then multiplied by the PLR resulting in a post-test odds ratio. The post-test odds ratio was then converted back to a rate to estimate the probability that a cow with the given ELISA adjusted OD value is shedding MAP. Upon further development, this calculator can be used to predict the probability that any individual cow is shedding MAP based on her ELISA adjusted OD score and the known prevalence of MAP shedding within the cow's herd.

Introduction

While fecal culture remains the standard for diagnosing *Mycobacterium avium paratuberculosis* (MAP) in individual cattle, the serum ELISA is becoming the preferred test in the field due to its reduced cost and quicker availability of results. Generally the OD value of the ELISA test is provided along with the lab's interpretation of "Positive" or "Negative". The OD value, once adjusted for the negative control for that particular batch, can be used as the cutoff value for calculating a likelihood ratio when applied to a large database.

A likelihood ratio expresses the odds that a given finding or test result occurs in an animal with a **condition of interest** compared to an animal without the condition of interest. In this case a positive likelihood ratio (PLR) would provide the odds that a cow with a particular adjusted ELISA OD value is shedding MAP as opposed to not shedding MAP.

The PLR can be multiplied by the pre-test odds to provide the post-test odds. Pre-test odds is simply a derivation of the prevalence of MAP shedding within the herd of interest. Post-test odds is the estimate of the likelihood that the cow question is shedding MAP. Notice that the above calculations are all based on odds of MAP shedding, a **ratio**, while most people generally think in terms of probability, or prevalence, of MAP shedding, a **rate**. For the above calculation it is a simply a matter of converting the prevalence (rate) into an odds (ratio) and back again to provide a probability estimate that is more consistent with current thinking.

These principles can be applied to a dataset within a spreadsheet to create a calculator in which one would only have to input a cow's ELISA adjusted OD score and the herd prevalence to obtain an estimate of the probability that the cow is shedding MAP.

Materials and Methods

Database.

Fecal culture results and concurrent serum ELISA adjusted OD values from 2,578 adult cattle from six Michigan dairy herds over two years formed the database for the calculator. The herds are part of the ongoing Michigan Johne's Disease Control Demonstration Project. Prevalence of MAP in these herds based on fecal culture ranged from zero to 42% with an average of 9.8%.

Spreadsheet.

An excel spreadsheet was developed using the above database and the following principles.

Calculating Positive Likelihood Ratio (PLR).

A 2 x 2 table using the fecal culture results and a given ELISA cutoff to calculate the PLR.

		Fecal Culture	
		Positive	Negative
ELISA Adjusted OD Value	≥ Cutoff	A	B
	< Cutoff	C	D

$$\text{Positive Likelihood Ratio} = \frac{\text{true positive rate}}{\text{false positive rate}} = \left(\frac{A/(A + C)}{B/(B + D)} \right)$$

Converting prevalence/probability to odds and back again.

The conversion between the probability, or prevalence (**rate**), of disease to the odds (**ratio**) of disease and back again is simply a mathematical problem. In a rate the numerator is included in the denominator, while in a ratio the numerator is not included in the denominator. The relationship between probability and odds of disease can be expressed as follows:

$$\text{odds of shedding} = \frac{\text{probability of shedding}}{1 - \text{probability of shedding}} \qquad \text{probability of shedding} = \frac{\text{odds of shedding}}{\text{odds of shedding} + 1}$$

Calculation of the probability of MAP shedding from ELISA adjusted OD Values.

$$\text{pretest odds} \times \text{PLR} = \text{post test odds}$$

Results

Calculating Probability Cow is Shedding MAP Based on Adjusted ELISA OD Score	
Adjusted OD Score	0.1
Estimated Herd Prevalence	10%
Probability Cow is Shedding	44.9%

Discussion and Conclusion.

This calculator model, while still in development, could be applied to a much larger national or international database and provide a valuable tool for veterinarians and producers in making management decisions for an individual cow based on her ELISA adjusted OD value.