Experience with the Danish Mix-ELISA in the United States

Michael J. Daniels[1], Yuhua Zhang[1], Matthew M. Erdman[2], Isabel Turney Harris[2]

1: Department of Statistics, Iowa State University, 303 Snedecor Hall, Ames, IA 50011, United States, Phone: +1-515-294-7775, Fax: +1-515-294-4040, E-mail: mdaniels@iastate.edu 2: Dept. of Microbiology, Iowa State University, Ames, IA 50011, United States.

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Summary

This article details some of our experiences with Danish mix-ELISA (DME) testing on herds in the United States. In contrast to Denmark, clinical outbreaks of *Salmonella* Choleraesuis occur in the United States. We examine the appropriateness of the current cut-off of OD%>=40 for U.S. herds by examining serum and fecal samples collected from individual pigs and tested with the DME and culture, respectively. We report the estimated sensitivity and specificity of the DME using the original and possibly modified cut-off values. The 30% cutoff was deemed optimal with a sensitivity of .57 (.45, .82) and a specificity of .84 (.68, .98) for the first set of samples and .69 (.49, .93) and .63 (.53, .76) for the second set of samples. A major use of these tests is for monitoring herds for Salmonella exposure over time. Information on the sensitivity and specificity of the DME is helpful in determining how many animals in a herd to sample and how often.

Keywords

culture, Hui-Walter paradigm, salmonella, sensitivity, specificity

Introduction

DME assay of meat juice in Denmark has enabled producers there to categorize herds as to level of Salmonella exposure, a 25% herd prevalence level corresponding to a 10% carcass contamination rate at slaughter (Sorensen et al., 2000).Culture lacks sensitivity in the live animal shedding the organism intermittently, while the serologic response is determined in part by the time of exposure relative to the sampling time and if and to what extent a serologic response develops to the serotypes present in the herd (5,8). The main goal of this study was to determine the sensitivity and specificity of the DME for U.S. pig herds. This facilitates choosing an optimal cutoff for the test and allows for the estimation of the true prevalence of Salmonella in a herd. This would allow high prevalence herds to be identified and targeted for reduction, and enable the assessment of intervention strategies.

Materials and Methods

Culture and the Danish Mix-ELISA test were conducted on two sets of individual rectal swabs and serum samples collected 3 weeks apart from 148 finishing pigs. The procedure was repeated on a second group of 142 pigs. Sera was assayed using the DME (5). Rectal swabs were cultured for Salmonella after pre-enrichment with buffered peptone water, and transferring to RV broth then XLD agar (2).These data were then collected into four 2x2 tables. To estimate the sensitivity and specificity of the DME compared with culture, we use the Hui-Walter paradigm (3) and a Bayesian estimation approach within this paradigm (1) This paradigm requires the two diagnostic tests to be conditionally independent given true infection status which should be applicable here as one test is based on serum and one on culturing fecal samples. The data in the 2x2 tables are not independent because each of the two samples were tested at two different times. Thus, we computed the sensitivity and specificity separately using the first time for each sample and the second time for each sample. Due to lack of prior knowledge on the parameters, Beta(1,1) priors were used for all the parameters with the condition that the sum of the sensitivity and specificity for each test was greater than or equal to 1. The gibbs sampler was used to sample from the posterior distribution. The sensitivity and specificities were computed using cut-offs of 40%, 30%, and 20%.

Results

The results are presented in Table 1. The estimated sensitivity and specificity for culture and the prevalences were quite stable
as we change the cut-off for the DME; in theory, these should not change as we change the DME cut-off. Specificity for the DME was lower than for culture, however, culture has much lower sensitivity.

Table 1: Posterior means and 95% credible intervals for the first sampling time (left of the slash) and the second sampling time (right of slash). Se sensitivity; Sp, specificity; P, prevalence; Cul, culture; and 1 and 2 first and second samples, respectively.

	20%	30%	40%
Se(DME)	.92(.79 , .99)/.60(.13,.94)	.69(.49 , .93)/57(.45,.82)	.45(.25 , .71)/.49(.38,.66)
Sp(DME)	.69(.53 , .92)/.59(.20,.86)	.63(.53 , .76)/.84(.68,.98)	.62(.39 , .83)/.91(.77,1.0)
Se(Cul)	.26(.16 , .44)/.29(.01,.76)	.39(.16 , .83)/.29(.20,.39)	.34(.01 , .86)/.29(.21,.39)
Sp(Cul)	.98(.94 , 1.0)/.83(.65,.97)	.96(.90 , 1.0)/.88(.77,.99)	.88(.37 , 1.0)/.92(.80,.99)
P 1	.24(.07 , .47)/.59(.03,.98)	.16(.01 , .40)/.85(.45,1.0)	.29(.01 , .98)/.86(.58,.99)
P 2	.64(.41 , .85)/.34(.02,.89)	.40(.12 , .84)/.33(.07,.58)	.43(.10 , .86)/.37(.10,.57)

For the first set, the specificity was stable as we decreased the cut-off value whereas for the second set, there was a large decrease from the 30 to 20% cut-off. Using a 30%, as opposed to the 40%, cut-off, would suggest a sensitivity of around 60% at a cost of a small decrease in specificity. The lack of consistency of the values of the culture test and prevalence for the 20% cut-off results from the 2x2 tables having sparse cells for that case.

Discussion

The data available for this study suggested a decrease in the DME cutoff to at least 30 OD% for use in the United States. This coincides with recent recommendations from Denmark lowering the cutoff from 40% (5). Both the sensitivity and specificity of the test at the individual pig level appear very sensitive to other factors in the herd under examination as evidenced by the varying sensitivity and specificity in the two sets of data and the specificity being very low relative to experience in experimentally infected animals (5). Knowledge of the sensitivity and specificity provides the ability to estimate the true prevalence given the seroprevalence by solving the following equation for the prevalence, seroprevalence = sensitivity * prevalence + specificity * (1 - prevalence). Sample size calculations can then be undertaken to determine the number of animals to be sampled and how often, given the size of the herd and the true prevalence of which it is a concern to detect (8). This study provides some preliminary guidance in using the DME to monitor Salmonella levels in U.S. herds. However, more experience is needed and more data needs to be collected to completely appreciate and understand how to optimally use the DME in the United States.

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