The Society for Veterinary Epidemiology and Preventive Medicine
Madrid, ESP, 20-22th March 2013

Diagnostic accuracy of culture of environmental fecal samples and a real-time PCR assay for bulk for detecting herds with animals infected with *Mycobacterium avium* subsp. *paratuberculosis*

J. Kruze[a], G. Monti[b*], F. Schulze[c], A. Mella[a], S. Leiva[a]

[a] Biochemistry and Microbiology Department, Faculty of Sciences, Universidad Austral de Chile (UACh), [b] Veterinary Preventive Medicine Department, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile

Objective

The aim of the study was to estimate the diagnostic accuracy of culture of environmental fecal samples and a real-time PCR assay for bulk-tank milk samples

Material and Methods

• 150 dairy herds were randomly selected in the main dairying region of Chile (provinces of Valdivia and Ranco in the XIV Region and provinces of Osorno and Llanquihue in the X Region)

•herds were stratified according to herd size defined as large (>200 cows), medium (100-200 cows), and small (<100 cows) and it was proportional to the population of herds in the study area

•From each dairy farm 2 composite environmental fecal samples were collected from different areas of the farm where cows congregate or manure is stored and 100 ml from the bulk tank

•Each composite environmental fecal sample was composed of approximately 20 g of fecal material with bedding or soil from four different sites within each sampling location

•Fecal samples were cultured and positive results were confirmed by PCR.

•Milk samples were analyzed by real- time PCR (SYBR Green) targeting the IS*900* element. All PCR samples were analyzed by melting curve profile and agarose gel electrophoresis (2%)

•A Bayesian model was used considering no gold standard to estimate overall prevalence, sensitivity and specificity of both tests, using the model developed by Branscum *et al.,* (2005), considering that both test were conditional dependent. Priors were:

Test	Se	Sp
Bulk tank PCR	beta(2.06, 2.59)	beta(329.69, 11.16)
Culture of environmental fecal samples	beta(87.11, 31.25)	beta(560.72; 6.53)

•Estimations were performed with the Gibbs sampler, as implemented in the WinBUGS program and 200,000 iterations were run after a burn-in of 10,000 iterations were discarded

Conclusions

Bulk Milk PCR test seems to be a useful test for detecting herds with MAP infected animals and it could be used in control programs given that it Provides results faster and cheaper than culture techniques

Results

The estimations of diagnostic test performance parameters were:

• for bulk-tank milk real-time PCR, the posterior median for
  •Sensitivity was 69.0% (95% Credible Interval 58.4; 78.6) and for
  •Specificity was 96.4% (95% Credible Interval 93.8; 98.1)

•For environmental fecal culture the posterior median for
  •Sensitivity was 60.1% (95% Credible Interval 53.1; 66.9) and for
  •Specificity was 99.0% (95% Credible Interval 97.9; 99.6)

•Confidence intervals of the dependence parameters (either for infected or non-infected herds), all contain the value 0, indicating that independence or mild dependence is not unlikely.

• The Figure displays the posterior predictive density curves for the diagnostic accuracy parameters of each test