RESEARCH ABSTRACT

PORK SAFETY

Title: Rapid detection of *Yersinia enterocolitica* in pigs using the TaqMan System - **NPB #98/160**

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ABSTRACT: The TaqMan assay, or 5' nuclease PCR assay, is a second-generation PCR detection system, which is reportedly more sensitive than conventional PCR tests. We have developed and evaluated a 5' nuclease PCR assay for the detection of *Y. enterocolitica.* The assay targets the chromosomally encoded *ail* (**a**dhesion **i**nvasion **l**ocus) gene.

Three primer/probe sets, (TM1, TM2, and TM3) amplifying different, yet overlapping, regions of *ail* were examined for specificity and sensitivity. The TM1 set displayed the highest specificity, accurately detecting each of the 26 *Y. enterocolitica* strains and none of the 21 non-*enterocolitica* strains. TM1 set detected ~0.5 pg (10^{-12} grams) of purified *Y. enterocolitica* DNA. The TM2 set was the most sensitive and detected ~ 0.25 pg of purified DNA. However, it failed to recognize 10 of the *Y. enterocolitica* strains used in this study. For TM3, sensitivities comparable to TM1 were achieved; cross-reaction with non-*enterocolitica* strains was not observed. However, TM3 did not identify all of the *Y. enterocolitica* strains tested.

The optimized TaqMan assay was compared with bacteriological culture methods and the first-generation multiplex PCR for the rapid detection of pathogenic *Y. enterocolitica* in market weight hogs (n=240) and pork products (n=650). *Y. enterocolitica* was not detected by bacteriological culture in any of the hog tissues tested (nine samples per hog) but was detected by multiplex PCR (2.0%) and TaqMan (45.6%) assays. In addition, ground pork (n=300 samples) and chitterlings (n=350) were screened for *Y. enterocolitica*. By standard culture, *Y. enterocolitica* was detected in chitterlings (8%), but not in ground pork (0%). By multiplex PCR, *Y. enterocolitica* was identified in ground pork (12%) and chitterlings (27%). In contrast, the highly specific TaqMan assay identified *Y. enterocolitica* in ground pork (52%) and chitterlings (79%).

The results of this study indicate that the TaqMan probes and primers for the *ail* gene (TM1) are more specific for pathogenic strains of *Y. enterocolitica* than either bacteriological culture or first-generation multiplex PCR assays.