RESEARCH ABSTRACT

PORK SAFETY

Title: Development of a sensitive molecular assay to detect *Toxoplasma gondii* DNA in biological samples **NPB #00-132**

Investigator: Joan K. Lunney

Institution: Immunology and Disease Resistance Lab and Parasite Biology, Epidemiology and Systematics Lab, ANRI, ARS, USDA, B.1040, Beltsville, MD 20705

.

Co-Investigators: Luis Jauregui*, and J.P. Dubey

2

Date Received: 9/6/2001

4

Abstract:

A quantitative molecular technique was developed and standardized to detect *Toxoplasma gondii* (Tg) DNA in biological samples. This real time fluorogenic Tg assay is a highly sensitive and specific method to reproducibly detect and quantitate Tg burden in animal products. Assay specificity was confirmed against a panel of DNA samples from different isolates of Tg, from other common protozoa, as well as from host animal tissues. The real time fluorogenic Tg assay uses polymerase chain reaction (PCR) primers proven to be specific only for Tg with detection based on a fluorogenic probe using the real-time TaqMan PCR technology. Specificity, or lack of cross reactivity, was checked by testing DNA samples from closely related parasites and from muscle tissues prepared from uninfected pigs and mice.

The sensitivity limits for this real time fluorogenic Tg assay were defined in several ways: 1) test serial dilutions of Tg DNA alone; 2) assess detection sensitivity for Tg DNA in pig tissues artificially infected with different doses of Tg, and 3) compare fluorogenic Tg assay results versus Tg bioassay in mice. All results were statistically analyzed. The real time fluorogenic Tg assay was able to detect as little as 0.1 pg of Tg genomic DNA, which is equivalent to 1 Tg bradyzoite. The assay has a dynamic range of detection over 6-7 log of Tg DNA concentration (from 100 ng to 100 fg). Tissues from Tg experimentally infected mice and pigs, as well as bradyzoite-spiked pig muscle samples, were used to test and standardize this technique. Positive signals were obtained with Tg parasite concentrations, ranging from as few as 4 parasites to $3.7x10^5$ parasites per gram of spiked pig tissue, with excellent linearity (R^2= 0.9776). All Tg infected animals were correctly identified using this technique. Results indicate this assay is applicable to testing swine carcasses and commercial pig products, is compatible with automation technology for potential slaughterhouse usage, and will enable scientists to diagnose and quantitate Tg in animal tissues. Such a sensitive fluorogenic Tg assay will speed results to producers and regulators, and will enable researchers to directly test pork products for possible Tg contamination.