RESEARCH ABSTRACT

PORK SAFETY

Title: Identification of diagnostic proteins of *Toxoplasma gondii* by targeted proteomic analysis **NPB# 00-025**

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Abstract:

Humans become infected with the ubiquitous protozoan parasite, *Toxoplasma gondii*, by congenital transmission from mother to fetus, through ingestion of tissue cysts (cyst structure found in animal tissues which contains the bradyzoite [=slow] form) in under cooked or uncooked meat, or by ingesting food or water contaminated with sporulated oocysts (cyst structure found in cat feces which contains the sporozoite [=reproductive] form) from infected cat feces. Pigs become infected by the same routes, resulting in meat products containing tissue cysts which could infect consumers. Reduction of risk of human and swine infection with *T. gondii* is hampered by a number of factors, making epidemiological studies which could lead to the development of strategies to reduce infection in humans and pigs difficult. Though experimentally infected pigs elicit a strong antibody response to the tachyzoite (rapidly dividing, non-encysted tissue form) stage of the parasite, available serological assays which utilize a crude tachyzoite extract as the antigen failed to detect nearly 30% of naturally exposed pigs killed at a commercial abattoir in which tissue cysts were later detected. In addition, there is a lack of epidemiological data documenting the predominant routes of infection (oocyst versus tissue cyst consumption) in horizontally transmitted toxoplasmosis. Existing serological assays can determine previous exposure to the parasite, but there are no tests which can differentiate between oocyst ingestion versus tissue cyst ingestion as the infection route. In this study, we have used surface enhanced laser desorption/ionization time of flight-mass spectrometry (SELDI-TOF-MS) in combination with 1 and 2 dimensional electrophoresis to identify stage specific proteins in *T. gondii* sporulated oocysts, tachyzoites, and bradyzoites. Specific, reproducible, mass spectra protein profiles were produced for each sample. Western blots of 2-D gels of the *T. gondii* oocyst proteins using pooled sera from 10 pigs with acute oocyst-induced *T. gondii* infection were used to select a single 18.3 kDa protein which matched the mass of one of the stage specific peaks identified in the SELDI spectral analysis. One dimensional Western blots revealed a single protein band of Mr 18,350 which was recognized by sera from pigs infected orally with *T. gondii* oocysts and not by sera from pigs infected orally with *T. gondii* tissue cysts. The protein was also recognized by chronically infected pigs (pigs infected with oocysts 1 year earlier), indicating the persistence of antibodies to the 18.3kDa oocyst-specific protein and suggesting that this protein could be useful even in chronic, long standing infections to determine the original infection route. A serological test based upon this 18.3 kDa antigen may provide epidemiological evidence of the predominant infection route in humans and swine, and may make the development of control strategies for *Toxoplasma* infection feasible. Proteins that appeared to be specific for tachyzoites and bradyzoites by SELDI analysis were isolated from 2D gels and are currently being analyzed for amino acid sequence information. These proteins will be cloned and tested in ELISA assays for sensitivity and specificity for detection of chronic *T. gondii* infection in pigs.