

PORK SAFETY

Title: Development of Methods for Surveillance of Retail Meat for *Toxoplasma gondii* – NPB# 00-024

Investigator: J. P. Dubey, Microbiologist

Institution: USDA, ARS, ANRI, PBESL, Bldg. 1040, Rm. 103, BARC-East, Beltsville, MD 20705, Phone: 301-504-8300, Fax: 301-504-6273, Email: jdubey@anri.barc.usda.gov

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I. Abstract: *Toxoplasma gondii* is a single-celled organism that causes mental retardation and loss of vision in children born infected with this parasite. Humans become infected with *T. gondii* by ingesting undercooked meat from infected animals or by ingesting food and water contaminated with oocysts excreted by cats. The ingestion of infected meat is considered to be the main source of human infections in the U. S. Among food animals, pigs are considered the major source of *T. gondii* infections for humans in the U. S. However, there has been no systematic effort to evaluate the level of contamination of retail meats with *T. gondii*, and there are no reliable, simple methods to detect viable *T. gondii* in meat. Among all methods available to detect *T. gondii* in meat, bioassay of meat samples in laboratory-raised cats and mice and detection of the parasite DNA have been used.

In this project funded by NPPC, scientists from ARS, USDA have found that bioassay of meat in cats is the most sensitive method to detect *T. gondii* in meat. Cats fed as few as 1 infective *T. gondii* (called bradyzoites) shed millions of oocysts in their feces whereas 10-100 bradyzoites were needed to infect mice. A sensitive PCR method using unique primers was developed that detected DNA from as few as 4 bradyzoites. These results will now be used to conduct a survey of *T. gondii* in meat samples from retail meat stores.

II. Introduction: *Toxoplasma gondii* can be considered an emerging pathogen from the standpoint of public awareness and our current understanding of the economic impact of this parasite. While cats were long thought to be the major, if not exclusive source of human exposure, it is now recognized that meat also plays a significant role. Among food animals, pigs are considered the major source of *Toxoplasma gondii* infections for humans in the U. S. Humans become infected with *T. gondii* by ingesting food or water contaminated with oocysts excreted in feces of infected cats or by ingesting tissue cysts in uncooked meat. There are no test which can determine the proportion of people that become infected via oocysts or via meat.

Among food animals, *T. gondii* has never been isolated from beef in the U. S. and little is known about its prevalence in poultry. However, serious surveys of these

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For more information contact:

National Pork Board, P.O. Box 9114, Des Moines, Iowa USA

800-456-7675, Fax: 515-223-2646, E-Mail: porkboard@porkboard.org, Web: <http://www.porkboard.org/>

food animals have not been conducted. In contrast, *Toxoplasma* in pigs is well documented. In a national serologic survey conducted on 11,842 swine sera collected in 1983-1984, the prevalence of *T. gondii* was 23% in market hogs and 42% in sows. More recent data indicate that the prevalence of *T. gondii* may be decreasing. Using the same serologic test in 1992-1994, the prevalence of *T. gondii* was 2.5 % in 6,137 market age pigs and 18.8% in 7, 697 sows. How this relatively high prevalence in live animals translates into risk of exposure in retail meats is largely unknown. Only one study examined the presence of *T. gondii* in meat from retail stores in the U. S. *Toxoplasma gondii* was isolated from 16 of 50 (32%) pork loins, 2 of 50 (4%) lamb chops, and 0 of 50 beef loins in samples from Palo Alto, California. This often cited survey is more than 30 years old and is not likely representative of the current status of the prevalence of *T. gondii* infection in retail pork. There have been no investigations of meat in a retail environment by anyone and this vital information is needed to link data on the presence of *T. gondii* in live animals with risk of exposure from retail meats.

III. Objectives: The objective is to develop accurate methods for determining the prevalence of viable *Toxoplasma* in retail pork, beef and poultry. The tools developed and validated here will form the basis of methods to be used conduct a statistically valid survey of retail pork, beef and poultry. We anticipate funding and/or field support of this larger effort through the CSREES, National Research Initiative, the Centers for Disease Control, the Food Safety and Inspection Service and the Agricultural Research Service.

IV. Procedures: Procedures used are described in detail in 2 papers published from the results obtained with this grant. (J. P. Dubey, Journal of Parasitology 87(1): 215-219, 2001 reprint attached) and Jauregui, Higgins, Zarlenga, Dubey and Lunney (Journal of Clinical Microbiology 39: 2065-2071, 2001- grants 00-132 and 00-024) preprint attached.

V. Results: Two important finding from this research were: 1. Bioassay meat in cats is the most sensitive method to detect viable *Toxoplasma* in meat because cats fed even few bradyzoites shed millions of oocysts which were easily detected in cat feces and as much 500 grams of test meat could be fed to a cat. 2. A sensitive Toxo-Taqman procedure was developed using unique primers that detects DNA from as few as 4 bradyzoites.

VI. Importance of the results to pork procedures. These results will be applied to conduct a statistically valid survey of retail pork, beef, and poultry.