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Seroprevalence of Toxoplasma gondii IgG antibodies and behavioral risks of exposure among women of childbearing age from the Knoxville, Tennessee area

Vina Rachel Diderrich
University of Tennessee

To the Graduate Council:

I am submitting herewith a dissertation written by Vina Rachel Diderrich entitled "Seroprevalence of Toxoplasma gondii IgG antibodies and behavioral risks of exposure among women of childbearing age from the Knoxville, Tennessee area." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

Sharon Patton, Major Professor

We have read this dissertation and recommend its acceptance:

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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SEROPREVALENCE OF *TOXOPLASMA GONDII* IGG ANTIBODIES AND BEHAVIORAL RISKS OF EXPOSURE AMONG WOMEN OF CHILDBEARING AGE FROM THE KNOXVILLE, TENNESSEE AREA

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Vina Rachel Diderrich
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ABSTRACT

This study investigates if women of childbearing age (pregnant and nonpregnant) engage in behaviors that may increase their risk of exposure to *Toxoplasma gondii* and how these behaviors differ between age groups. Risky behaviors were identified as activities that could result in accidental ingestion of either the tissue stage in edible portions of meat or the environmental oocyst stage shed in feline feces. Serum samples and interview data were collected by a convenience sampling of 829 women between 18-53 years. The seroprevalence of *T. gondii* was 7.0%. Most women, 93.7%, were meat eaters with 8.7-50.2% preferring to eat some meats less than well-done. Those in the age groups of 18-24 years were more likely to eat meat less than well-done compared to most older age groups. Those 18-19 years old were more likely not always to wash vegetables and fruits before eating or wash hands before handling or eating food. Most women, 56.8%, garden at least occasionally, with those 30 years and older more likely to garden. Analysis of the behaviors of seronegative women (93.0%) show that many women may engage in more than one of these risky behaviors. In addition to published recommendations, educational programs for the prevention of *T. gondii* during pregnancy need to reinforce the importance of consuming only well-done meats, washing fruits and vegetables, and hand washing to reduce risk of exposure to tissue cysts and oocysts to 18-24 year olds. In women more likely to garden, >30 years old, the risk of oocyst exposure needs to be emphasized. These behaviors should be emphasized during prenatal classes.

Risky behaviors for *T. gondii* infection were evaluated using the odds ratio; however, the data collected in this cross-sectional study cannot be used to prove a causal relationship between specific behaviors and *T. gondii* seropositivity. Time is a confounding factor in the analysis because chronic infections cannot be attributed to current behavior. The modified agglutination test was used as the screening tool to detect IgG antibodies to *T. gondii.*

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
Ag	Agriculture
C	Celcius
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
df	degrees of freedom
DH	Definitive Host
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbant assay
F	Farenheit
FDA	Food and Drug Administration
FIV	Feline Immunodeficiency virus
FSIS	Food Safety Inspection Service
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IH	Intermediate Host
IHA	Indirect hemagglutination test
IL	Interleukin
INF	Interferon
IQ	Intelligence quotient
kDa	kilodalton
LAT	Latex agglutination test
lbs	pounds
LD_{50}	Lethal Dose that kills 50% of test subjects
LD_{100}	Lethal Dose that kills 100% of test subjects
MAT	Modified Agglutination Test
ME	Mercaptoethanol
MHC	Major histocompatibility complex
MSDS	Material safety and data sheet
NAHMS	National Animal Health Monitoring System
NHANES III	National Health and Nutrition Examination Survey III

NK	Natural killer cell
NO	Nitric oxide
ml	milliliters
OR	Odds ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Th	T-helper cell
TNF	Tumor necrosis factor
μg	micrograms
μl	microliters
μm	micrometers
USDA	United State Department of Agriculture
UT	University of Tennessee
VTH	Veterinary Teaching Hospital
$	United States dollars

CHAPTER 1

INTRODUCTION

The seroepidemiology of *Toxoplasma gondii* exposure and infection in people in the United States has not been studied in recent years, and has never been studied in the Knoxville, Tennessee area. The occurrence and frequency of behaviors for *T. gondii* exposure are not known for people in the United States. Although infection is known to occur by three means of exposure (transplacental passage of *T. gondii* tachyzoites, ingestion of undercooked meat containing *T. gondii* cysts, and ingestion of *T. gondii* oocysts) the contribution of each to the total burden of infection in unknown (Hershy and McGregor, 1987). Some researchers have suggested that the consumption of infected pork is the primary vehicle of foodborne transmission (Dubey, 1986a). This hypothesis is based on pork's prevalence in the American diet, and because animal studies indicate much higher rates of transmission from infected pork than from other infected meats (Dubey, 1986a).

This epidemiological investigation of women of childbearing age in the Knoxville, Tennessee area estimates the seroprevalence of *T. gondii* antibodies and the frequency of risky behaviors for exposure to the parasite. The actions of all women of childbearing age are important to evaluate for the development of educational materials designed to reduce *T. gondii* infection and birth defects. Primary infection with *T. gondii* during pregnancy may cause lost pregnancies, infant birth defects, and ocular problems in children that survive congenital infection. If exposure and infection are prevented, these consequences are avoided.

Thrusfield, a veterinary epidemiologist, recognized that animals serve as reservoirs of infection to human populations: "An important factor that determines the occurrence of acquired zoonoses is the amount of disease in domestic animals" (Thrusfield, 1986). To address the role of pork as a reservoir of *T. gondii* infection to people in the United States, the epidemiology of *T. gondii* on swine farms was also investigated (Diderrich et al., 2001). By investigating swine and swine farms, a measurement of the magnitude of infection in animals used for human consumption was measured. With the focus of food safety in the forefront of United States domestic policy, this information was complementary to the primary research focus of examining risky behaviors for *T. gondii* exposure in women of childbearing age which included the consumption of meat.

CHAPTER 2

EPIDEMIOLOGY OF *TOXOPLASMA GONDII*

Life Cycle Biology

Toxoplasma gondii is an obligate, intracellular, single-celled Coccidian parasite. Unlike other host-specific protozoan parasites such as malaria, *T. gondii* is not host specific and infects a wide variety of warm-blooded hosts (Dubey and Beattie, 1988). It is able to invade and multiply in many types of nucleated mammalian cells. There are three forms of the parasite that play a role in transmission from one host to the next: 1) the oocyst containing sporozoites, 2) the proliferative intracellular forms called tachyzoites, and 3) the latent intracellular forms called bradyzoites which are grouped together in a tissue cyst. The natural cycle of *T. gondii* transmission occurs between cats and between cats and their prey, primarily rodents and birds. Domestic and wild felidae are the definitive hosts (DH), and the only hosts in which the parasite undergoes gametogony (sexual multiplication) within the epithelial cells of the small intestine. At the end of the sexual phase, the resulting extracellular zygote is enclosed in a thin cell wall which is eliminated in the feces as an oocyst; no further development occurs within the gut of the cat (Frenkel et al., 1970; Hutchison et al, 1970). After 24 hours incubation in the environment, the zygote divides into two sporocysts. Two further divisions take place to produce four sporozoites within each sporocyst. This fully sporulated oocyst is infective when ingested. If ingested by a second (previously uninfected) cat, the sporozoites exit the oocyst and begin the enteroepithelial cycle in this cat's small intestine. An infected feline may excrete millions of oocysts per day for a period of seven to 21 days (Dubey and Frenkel, 1972). Cats that have excreted oocysts usually do not have a second episode of oocyst shedding after reinfection (Dubey and Frenkel, 1974; Dubey, 1995b). Immunosuppression by administration of greater than therapeutic doses of corticosteroids will cause some chronically infected cats to reexcrete *T. gondii* oocysts (Dubey and Frenkel, 1974). Intestinal immunity is strong in cats that have shed oocysts (Frenkel and Smith, 1982; Dubey, 1995b). Under controlled conditions, cats that are challenged within three to six months of primary infection do not excrete oocysts again. However, four of

nine cats reshed oocysts when challenged with a heterologous strain of *T. gondii* six years after previous infection (Dubey, 1995b). The duration of immunity to oocyst excretion and the frequency of repetitive shedding of oocysts is unknown in pet and feral cats (Dubey, 1995b). Infections with feline immunodeficiency virus (FIV) in cats chronically infected with *T. gondii* do not cause the reactivation of *T. gondii* oocyst excretion or development of clinical toxoplasmosis (Lappin et al., 1992; 1996). Primary infections with FIV or feline leukemia virus prior to *T. gondii* infection do not appear to enhance oocyst excretion (Patton et al., 1991; Davidson et al., 1993; Lappin et al., 1992; 1996; Lin et al., 1992).

Countless hosts may be infected from the contaminated environment resulting from one infected feline. The oocysts are very resistant to environmental degradation and may remain viable for a year or more after the feces have broken down and disappeared (Dubey and Beattie, 1988). Lower temperatures slow the rate of sporulation to between two and 21 days; and oocysts will not sporulate below 4°C (39°F). Soil-associated arthropods, insects, or annelids may come in contact with decomposing feces and pick-up oocysts on their exoskeleton or skin and then act as short-term mechanical vectors by transferring oocysts to food (Wallace, 1973; Frenkel et al., 1975; Ruiz and Frenkel, 1980b). Coprophagic arthropods also may harbor infective oocysts within their digestive tract and then release them onto food when they defecate or vomit (Wallace, 1971). Recently, researchers have demonstrated that earthworms can play a role in the transmission of *T. gondii* by carrying infective oocysts in their digestive tract (Bettiol et al., 2000).

When sporulated oocysts are ingested by avians or mammals, including felidae, wild carnivores and herbivores, wild and domestic food animals, pets, and people, the sporozoites emerge from the oocyst, penetrate or are phagocytized and multiply asexually by schizogony (asexual multiplication) in tissue cells and macrophages (Dubey and Beattie, 1988). Asexual multiplication occurs in the intermediate host (IH). Felidae may be both an IH and DH at the same time with both schizogony occurring in tissue cells and gametogony occurring in intestinal cells. Asexual reproduction occurs within a parasitophorus vacuole of the host cell with endodyogeny of the parent cell yielding two daughter cells. Endodyogeny occurs every four to six hours. The host cell's cytoplasm eventually fills and ruptures. These rapidly dividing stages, called tachyzoites, are present during the acute phase of infection. Once released from the cell, tachyzoites either invade new cells or are phagocytized by host cells. The dissemination of tachyzoites causes a

systemic parasitemia where tachyzoites can be passed in semen and milk, or cross the placenta to infect the fetus. During this stage of infection, the parasite destroys host cells and tissue damage occurs. Tachyzoites are capable of infecting both a new feline DH and another IH when tissues of an infected IH are consumed; the parasite does not need to go back to the feline host and can be cycled from one IH to another. In the intestine of the new host, tachyzoites invade cells, divide, and disseminate throughout the body. At about one week post-infection, tachyzoites slow metabolically and enter a resting stage. The infected cell membrane does not rupture, tachyzoites are retained in the parasitophorous vacuole, and a cyst-like structure is formed (Ferguson and Hutchinson, 1987). These resting stages of the parasite, called bradyzoites, form when the infection proceeds to the chronic stage. Although they may be found in every organ, these tiny cyst-like structures are most commonly found in the brain, heart, and skeletal muscles. The infection is passed to the next host when bradyzoite tissue cysts are ingested in raw or undercooked meat (Dubey and Beattie, 1988). In the small intestine, enzymes in the digestive juices disrupt the cyst wall and bradyzoites emerge from the tissue cyst, invade new cells, replicate as tachyzoites, and then follow the same pattern of acute and chronic infection. People can also be infected by the passage of tachyzoites in blood transfusions or by receiving an organ transplant where tachyzoites and/or bradyzoites are present in the donated organ (Frenkel, 1990). Infections last for the life of most hosts because after the acute phase, the parasite is not eliminated and the chronic phase is characterized by the development of persistent, viable bradyzoite-filled tissue cysts (Frenkel, 1988).

Immune Response to *Toxoplasma gondii* Infection

Knowledge of the immune response to *T. gondii* infection in both the intermediate host and the cat can be applied to the development of appropriate vaccine strategies against *T. gondii*. Vaccines to prevent fetal damage and abortion and those that limit the number of cysts in meat products or reduce oocyst excretion by cats will have significant beneficial public and veterinary health implications.

The development of immunity is important in tissue cyst formation. Ending tissue destruction by *T. gondii* tachyzoites depends on the development of both cell-mediated and humoral immunity. The principal mechanism of human resistance to *T. gondii* is through activation of macrophages by gamma-

interferon (IFN-γ) produced by CD4+ and CD8+ T cells and natural killer (NK) cells when stimulated by interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF-α) (both produced by macrophages) (Beaman et al., 1992; 1994). This is characteristic of a predominantly Th1-type response to infection with an intracellular parasite. The rapidly dividing tachyzoite stage is controlled by IFN-γ-induced formation of nitric oxide (NO) from macrophages, which destroys tachyzoites and also triggers parasite conversion to the bradyzoite stage (Bohne et al., 1994). NO may accelerate stage conversion by slowing down the replication of tachyzoites. Recently, the discovery of heat shock proteins produced by virulent strains of *T. gondii*, which interfere with production of NO, may explain the prolonged proliferation of tachyzoites in these strains (Miller et al., 1999). Protection and survival of the host to *T. gondii* infection appears to be dependent upon an early Th1-type response with NO and IFN-γ production (Candolfi et al., 1996). However, this initial response needs to be down-regulated and replaced by a Th2-type reaction later. The precise timing of Th1- and Th2-type cytokine production influences *T. gondii* pathogenicity. The host that does not shift from cell-mediated (IFN-γ and IL-2) to humoral (IL-4 and IL-10) response may experience excessive inflammation (Gazzinelli et al., 1996). In mice, immunity during different phases of infection is under genetic control of at least five different genes, one of which is located in the major histocompatibility complex (MHC) (McLeod et al., 1989; Blackwell et al., 1993). The switching of MHC expression from MHC class I to class II does change the pathway for restricted processing and presentation of peptides to CD4+ and CD8+ T cells, thereby switching immunity from a Th1- to a Th2-type response (McLeod et al., 1996).

Humoral immunity does limit the progression of infection and the development of new lesions. In the mouse model, both a rising antibody titer and tissue cyst formation occur around six days post-infection (Dubey and Frenkel, 1976; Dubey, 1998). Continued tissue destruction may occur in those sites where antibodies typically do not circulate (central nervous system and eye). Antibodies bind and neutralize cellular attachment sites on the parasite's surface and, in the presence of complement, lyse extracellular parasites. Also, if parasites are phagocytized after antibody binding, lysosomes are allowed to fuse with the parasitophorous vacuole (Roitt et al., 1996). These actions prevent the spread of tachyzoites to new host cells. In chronic infections, the action of antibody prevents recrudescence of disease after tissue cyst

rupture. Leakage of antigen from tissue cysts may be the stimulus for persistent antibody titers in chronically infected hosts (Huldt, 1971).

Cellular and humoral immunity are depressed during pregnancy leading to increased susceptibility to intracellular pathogens such as *T. gondii* (Luft and Remington, 1982). Studies in the early 1990s suggested that the predominant response to parasites during pregnancy was Th2-like, which may increase susceptibility to intracellular parasites such as *T. gondii* (Candolfi et al., 1996). Recently, low levels of NK cells were found in pregnant women with primary *T. gondii* infection indicating a suppression of the Th1 response (Nigro et al., 1999). In the mouse model, pregnant mice infected with *T. gondii* had low levels of IL-2 and IFN-γ and were more susceptible to infection than non-pregnant controls (Shirihata et al., 1993; Haque et al., 1994; Candolfi et al., 1995). Suppression in the Th1 response was resolved when IL2 was administered. Pregnancy in mice did not appear to decrease antibody production (Candolfi et al., 1996).

The state of premunition and immunity to reinfection, which is assumed to be lifelong, is a valuable guide to follow for characterizing the protective immune response. Infection with *T. gondii* most commonly occurs via *ingestion* of any of the three life cycle stages, oocyst, tachyzoite, or bradyzoite with specific antibodies produced to each stage during the course of the infection (Frenkel et al., 1969). The mechanism(s) for resistance in a chronically infected host against recrudescence or additional infections develop as the parasite converts from one stage to another. As previously discussed in mice, the immune responses against early infection are not identical to those later responses that control cyst burdens (McLeod et al., 1989). Tachyzoite and bradyzoite-cyst antigens recognized by serological techniques are stage-specific with much of the immunological activity directed against tachyzoite antigens and little recognition of cyst antigens (Zhang and Smith, 1995; Zhang et al., 1995). In mice, immunization with the tachyzoite stage surface protein, P30 (molecular weight of 30 kDa), provided nearly total protection against challenge with a moderately virulent *T. gondii* strain (Bulow and Boothroyd, 1991). In mice, P30 is the major antigen recognized by IgA antibodies in the milk of acutely infected mothers. IgA also may play an important role against *T. gondii* infection after ingestion of the parasite and subsequent penetration of the intestinal epithelium. Induction of the mucosal immunity may be an important factor in the development of a vaccine against infection via the oral route (McLeod et al., 1988).

Vaccines for Controlling *Toxoplasma gondii* Infection

A commercially available vaccine (Toxovax, Agresearch, New Zealand, www.agvax.co.nz), utilizing an attenuated tachyzoite (over 3,000 passages in mice), has been successful in preventing abortion in sheep in New Zealand and the United Kingdom (Buxton, 1993). A similar vaccine has been tested in pigs (Lindsey et al., 1993; Dubey, 1994; Dubey et al., 1994). These vaccines reduced cyst burdens following challenge or natural infection, but did not completely prevent infections. Vaccination of cats to prevent oocyst excretion is a desirable objective (Frenkel and Smith, 1982; Frenkel et al., 1991). In the cat, five asexual stages of *T. gondii* precede the formation of oocysts in the intestine. Therefore, developing a vaccine for the prevention of toxoplasmosis in cats is difficult because it requires antigenic components from all stages of the coccidian cycle of *T. gondii*. Vaccine trials with laboratory animals failed to prevent oocyst excretion when challenged with a different strain (Freyre et al., 1993). A field trial that tested the effectiveness of vaccinating cats on a farm to reduce *T. gondii* exposure to swine reported that vaccinated felines excreted fewer oocysts (Mateus-Pinella et al., 1999). During the study, there was a significant decrease in the seroprevalence of *T. gondii* in finishing pigs, suggesting that vaccinating cats reduced *T. gondii* exposure for pigs (Mateus-Pinella et al., 1999). Vaccination with live organisms in people is unlikely for ethical reasons because reactivation of parasites may occur if the person becomes immunocompromised later in life. The development of sub-unit, recombinant antigens, or synthetic peptides are being tested in mice and are more likely to be approved for use in people in the future (Alexander et al., 1996).

Diagnosis of *Toxoplasma gondii* Infection

The modified agglutination test (MAT), which uses formalin-fixed tachyzoites, is the most sensitive and specific test available for the detection of antibodies to *T. gondii* (Patton et al, 1990; Dubey, et al., 1995b; Dubey, 1997a). To evaluate the sensitivity and sensitivity of the MAT, it was compared to the other traditional tests, 1) the "gold standard" dye test developed in 1948 (Sabin and Feldman, 1948) which uses live tachyzoites, 2) the indirect hemagglutination test (IHA) which uses red blood cells tagged with *T. gondii* antigen, 3) the latex agglutination test (LAT) which uses latex beads coated with *T. gondii* antigen,

and 4) an ELISA (enzyme-linked immunosorbant assay) test which uses tachyzoite lysate (Dubey, 1997a). The MAT was more sensitive at 82.9%, than the other assays in detecting antibodies in 1000 naturally exposed sows confirmed through the bioassay using mouse inoculations (Dubey et al., 1995a). The specificity of the MAT was 90.29% (Dubey, 1997a).

In the bioassay test, body fluids or tissues from a person or animal suspected of being infected with *T. gondii* are tested by intraperitoneal inoculation into a laboratory mouse (confirmed uninfected) (Dubey and Beattie, 1988). Mice develop antibodies to *T. gondii* between three and 21 days after infection depending upon the strain of *T. gondii*, dose, and stage of the parasite inoculated. The bioassay test is sensitive for both virulent strains, where only one organism causes an LD_{100}, and nonvirulent strains, where LD_{50} values may range from 10^2 to $>10^5$ organisms (Sibley and Howe, 1986). Bioassays in cats are used to detect viable *T. gondii* in meat because larger volumes of tissue can be fed to cats than can be assayed in mice (Dubey and Beattie, 1988). After tissue cysts are ingested, *T. gondii* multiplies in the intestine of the cat, eventually leading to excretion of numerous oocysts in feces. Only cats raised in confinement and negative for previous infection are used in the bioassay.

Recent advances in the field of molecular biology have allowed for the development of recombinant antigen and synthetic peptide ELISA tests that detect specific immunoglobulins, such as IgG, IgM and/or IgA antibodies in humans (Johnson and Illana, 1991; Tenter and Johnson, 1991; Jacobs et al., 1999) and in swine (Andrews et al., 1997; Gamble et al., 2000). Researchers have the potential to select antigens that are characteristic for the acute or chronic stages of infection by discriminating between tachyzoite and bradyzoite stages (Acebes et al., 1994; Knoll and Boothroyd, 1998). These have not, however, surpassed the MAT in sensitivity and specificity. These tests are not suitable for large epidemiological studies because they are expensive, labor-intensive, and only available in specialized laboratories.

Early diagnosis of acute infection in pregnant women is important for efficient timing of anti-parasitic therapy and treatment. Diagnosis is based on serological tests that detect IgM and IgG antibodies, and various techniques have been used to attempt to distinguish between acute infection versus chronic infection (Danneman et al., 1990). The use of sensitive methods to detect IgM antibodies has complicated

the interpretation of serological results. Acute toxoplasmosis is characterized by specific IgM antibodies, but these sensitive assays continue to detect IgM long after acute infection is resolved and may even cross-react with other IgM antibodies. This has led the Food and Drug Administration (FDA) to publish a public health advisory regarding the limitations of *Toxoplasma* IgM commercial test kits (Burlington, 1997). A single positive test result should be interpreted with caution to avoid unnecessary treatment regimes or termination of pregnancy. Serological detection of IgA antibodies in the mother and fetus or infant was found in association with acute infections (Decoster, 1996). IgA antibodies disappear between six to twelve months after infection and do not persist serologically as seen with IgM antibodies (Decoster, 1996). Testing for both IgA and IgM antibodies in the mother is recommended for the diagnosis of possible congenital toxoplasmosis of the fetus (Burlington, 1997).

A number of polymerase chain reaction (PCR) tests targeting different gene sequences of *T. gondii* have been developed which amplify parasite DNA (deoxyribonucleic acid) to detectable levels. These are used to confirm the diagnosis of acute infections in HIV (human immunodeficiency virus) patients and pregnant women because the luxury of time for diagnosis via a rising antibody titer does not exist. Toxoplasmic encephalitis in AIDS (acquired immune deficiency syndrome) patients can be life threatening and a simple blood sample can be used for diagnosis by PCR (Burg et al., 1989; Khalifa et al., 1994). The use of PCR for testing amniotic fluid provides an alternative method to the more invasive collection of umbilical cord blood from suspect congenitally-infected fetuses (Remington et al., 1995). Currently, only research laboratories are conducting PCR testing for *T. gondii* (Hohlfeld et al., 1994; Weiss, 1995; Guy et al., 1996; Pelloux et al., 1998). Again, use of the time and labor-intensive PCR test for the detection of antigen is not suitable for epidemiological studies where the prevalence of infection can be estimated by a simple serological test for antibodies, such as the modified agglutination test (MAT).

Epidemiology of *Toxoplasma gondii* Transmission to People

Toxoplasma gondii is one of the most widespread zoonotic protozoan parasites of people infecting between 30-60% of the world's population, or about two billion people, in both temperate and tropical countries (Dubey and Beattie, 1988; Frenkel, 1990). This variation of prevalence throughout the world are

attributed to factors associated with: a) the parasitic stage of infection, b) the geographic and climatic location, c) the cultural practices and physical make-up of the population, d) pattern of seroprevalence based on age of host, and e) prenatal infections via infection of the mother. Immunocompetent people with anti-*Toxoplasma* IgG antibodies (indicating previous infection) are presumed protected from experiencing a second infection. A mother who is seropositive before becoming pregnant is presumed not be at risk of transferring subsequent infections to her fetus; therefore, congenital toxoplasmosis does not occur with these mothers (Feldman, 1974). However, a seronegative mother who experiences primary infection during pregnancy is in danger of passing the infection to her developing baby (Feldman, 1974).

Pattern of Transmission to Humans

Two common sources of *T. gondii* infection in people based on the parasitic stage are 1) oocysts in the environment and 2) tissue cysts in the meat of food animals. As most of these studies have found, cats play a quintessential role in the transmission of *T. gondii* to human populations (Frenkel, 1988; Frenkel et al., 1995). This is documented in studies that have ruled-out undercooked meat as a source of infection in both meat eating and non meat-eating populations (Wallace, 1969; Frenkel and Ruiz 1980; 1981). Currently there is no test to distinguish infections from oocysts as opposed to tissue cysts (Dubey, 2000). Epidemiological surveys remain the most useful way of investigating the importance of different sources of *T. gondii* infection in people (Dubey, 2000).

Infected felines leave a concentration of 2-20 million oocysts per 20 grams stool (Frenkel et al., 1975), and in soil, sporulated oocysts remain infective for up to 18 months. Free-ranging cats often use soft surface dirt and sand as outdoor litter boxes creating a focus of oocyst contamination (Wallace, 1971). Children and adults become infected when sporulated oocysts are accidentally ingested from the environment (Frenkel, 1990). This can happen when cleaning the cat litter box, or during outdoor activities where humans have close contact with contaminated soil, such as playing sports, gardening, landscaping, and farming. For example, patrons of a riding stable in Atlanta, Georgia became ill with toxoplasmosis after inhalation and ingestion of oocysts after contaminated dust was stirred up by horses in an enclosed arena (Teutsch et al., 1979). Other sources of oocyst contamination include children's sandboxes (Fleck et al., 1972) and dogs (Frenkel and Parker, 1996). Dogs can serve as mechanical vectors after rolling in cat

feces and having infective oocysts attached to their fur (Frenkel et al., 1995; Frenkel and Parker, 1996; Lindsay et al., 1997b). When adults and children play in the sandbox or pet the dog, oocysts can be transferred to their hands, lodge under fingernails, and become accidentally ingested if hands are not washed thoroughly before eating meals.

Fruits and vegetables taken directly out of the soil may be contaminated with sporulated oocysts of *T. gondii* (Wallace, 1969; Frenkel, 1990). Also, arthropod mechanical vectors may transfer oocysts to fresh fruits and vegetables (Frenkel et al., 1975; Ruiz and Frenkel, 1980b). Oocyst contamination of drinking water in Victoria, British Columbia, Canada, was suspected in the world's largest outbreak of waterborne toxoplasmosis in 100 people (Bowie et al., 1997). The municipal water supply was taken from the surface of a large reservoir and piped for use, unfiltered but chloraminated. Both domestic cats and cougars were common around the edge of the reservoir. Heavy rainfall may have washed large numbers of oocysts into the surface waters. Researchers developed a special filter cartridge, but were not able to recover oocysts from reservoir water (Aramini et al., 1999).

On the farm, food animals may become infected when they ingest sporulated oocysts from pastures, feed, or water contaminated by cat feces (Dubey et al., 1986b; Underwood and Rook, 1992). Tissue cysts containing bradyzoites present in the meat of food animals are capable of infecting people and other animals when they are consumed in poorly cooked meat or acquired from surfaces contaminated with infected raw meat (Dubey and Beattie, 1988).

Pattern of Oocyst Transmission Based on Geographic/Climatic Conditions

Environmental conditions present in different geographical areas of a country may influence the ability of *T. gondii* oocysts to serve as a source of infection to the people living there. Infection in people is more prevalent in warm, humid climates and in low-lying areas compared with cold, dry climates and mountainous regions where oocyst survival is impeded (Dubey and Beattie, 1988).

Gibson and Coleman (1958) found those residents of the Guatemalan and Costa Rican lowlands had significantly higher titers to *T. gondii* compared to residents of the Guatemalan highlands. In two culturally identical communities in Panama, a 60% seroprevalence in people living in tropical lowland areas was statistically higher compared to a 27% seroprevalence in people living in a temperate community

only 15 miles down the road, but 5,000 feet higher in elevation (Walton et al., 1966). Also in Panama, a high seroprevalence in children was associated with living in houses with oocyst-contaminated dirt floors (Etheredge and Frenkel, 1995). Definite variations of antibody prevalence in children from different cities across El Salvador were found (Remington et al., 1970). These differences in prevalence may reflect a higher incidence rate in some children <5 years old exposed to soil. In Costa Rica where the overall prevalence of antibody titers in seven communities was 61%, warm, moist environmental conditions allowed for the maturation and survival of oocysts in the soil which proved to be the source of infection during the crawling and dirt-playing periods of early childhood (Frenkel and Ruiz, 1981; Ruiz and Frenkel, 1980a). In Africa, studies in Somalia reported similar results with significantly higher prevalences in villages on river boarders, situated in humid zones, compared to the arid area around Mogadishu (Zardi et al., 1980).

Higher prevalences in people from rural environments compared to those in urban environments have been reported from Brazilian military recruits (Lamb and Feldman, 1968), pregnant women in Norway (Stray-Pederson and Lorentzen, 1979; Stray-Pederson et al., 1979), and children from Nova Scotia (Pereira et al., 1992). Some studies did not show this difference between rural and urban environments, however (Kimball et al., 1960; Ricciardi et al., 1975).

Islands are geographically isolated communities where the epidemiology of *T. gondii* transmission can be traced (Wallace et al., 1972). Inhabitants (people and other animals) of 2 out of 3 islets on a Pacific atoll had antibodies to *T. gondii*, whereas the third islet did not have any cats and all of the animals were seronegative. In Chilean archipelagos, seroprevalence in cats was 85.6% and in humans was 42.3% (Stutzin, et al., 1989). On these islands, oocyst contamination of the environment was evident in the seroprevalence of wild herbivores such as rabbits at 8.0% and goats at 75.0%. Some islands are toxoplasmosis naïve. Wallace (1969) found that the absence of cats on one of three atolls was correlated to the low prevalence of *T. gondii* antibodies in both humans and animals. On three of nine islands off the eastern Panamanian coast, no antibodies were detected in either children or cats (Etheredge and Frenkel 1995). A study from the island of Iceland concluded that the severity of the Icelandic winter did not

prevent the survival of *T. gondii* oocysts where 18.3% of Icelanders exposed to cats were seropositive (Woodruff et al., 1982).

Pattern of Transmission Based on Socioeconomic Status and Cultural Habits

Socioeconomic status, hygienic and cultural habits of the population may also vary for each geographic location (Feldman and Miller, 1956). In Italy, lifestyle conditions caused by low socioeconomic status such as residential crowding, close contact with domestic animals, improper handling of food, and consumption of undercooked meat were associated with increased risk of infection in children and teenagers (Moschen et al., 1991). In some cultures, the preference for undercooked meat is a culturally acquired habit (Frenkel, 1990). In France, antibody prevalence ranging from 60-80% has been attributed to the consumption of raw or undercooked meat (Desmonts et al., 1965). Foodborne illness from *T. gondii* infection has been associated with the consumption of raw or undercooked pork, lamb, beef, and other meats including wild game (Dubey and Beattie, 1988). Wallace (1976) found ethnic groups on Pacific Islands that enjoy raw meat, especially pork, have a high prevalence of infection. In the United States, a group of college students and a group of hunters became ill with toxoplasmosis after preferential consumption of undercooked hamburgers (accidentally contaminated with ground pork) (Kean et al., 1969) and venison (Sacks et al., 1983) respectively.

For vegetarians, those who do not consume meat, the ingestion of oocysts is the only way *T. gondii* transmission can occur. Prior to the discovery of the oocyst excreted by felines in 1969 (Frenkel et al., 1969), many researchers could only speculate on how vegetarians and herbivorous animals became infected (Jacobs et al., 1960). At that time, transmission by tissue stages in meat was well documented for laboratory animals. Rawal (1959) first attempted to relate eating behavior in people to seroprevalence of toxoplasmosis. This first study of vegetarians was conducted in India, a nation with a large proportion of Hindu vegetarians. He found no difference in the seroprevalence of antibody titers between strict vegetarians and meat eaters in Bombay, which was 37.8% and 37.4% respectively. He also sampled healthy people from the British community of Sheffield for comparison, and found approximately the same proportion of people infected at 35.7% prevalence. Bowerman (1991) recently conducted a similar study in rural India and also did not find an association with seropositivity and the consumption of meat. In

Canada, a community study in Vancouver sampled different ethnic groups, some of which were vegetarians (Proctor and Banerjee, 1994). Statistical associations were found between seropositivity and the ingestion of meat and the consumption of unpasteurized milk. A recent cross-sectional seroprevalence study in the United States found that Seventh Day Adventist vegetarians had a significantly decreased risk of *T. gondii* infection compared to control community volunteers (Roghmann et al., 1999). These epidemiological studies show that vegetarianism is not protective against infection, but a diet lacking meat may reduce the seroprevalence in some populations by reducing exposure. Oocysts in the environment are still a source of infection for vegetarians despite dietary preferences excluding food animal products.

Pattern of Transmission Based on Age

Seroprevalence of *T. gondii* generally increases with age because opportunities for infection are always present over time (Feldman and Sabin, 1949). Two patterns of transmission are observed that contribute to the wide variation of prevalence in people reported across the world: 1) primary infection starting in early childhood and 2) transmission in adults after age 20 years (Frenkel, 1990).

Infections acquired in early childhood are observed in many developing countries such as Chile (Apt, 1985; Schenone et al., 1990a; Schenone et al., 1990b), El Salvador (Remington et al., 1970), French West Indies (Barbier et al., 1983), Colombia (Juliano-Ruiz et al., 1983), Costa Rica (Frenkel and Ruiz, 1980), Panama (Sousa et al., 1988), and Trinidad (Lunde and Jacobs, 1958). In most of these countries, greater than 50% of children may be infected by the age of 10 years. This high prevalence reflects the abundance of stray cats, a climate favoring oocyst sporulation, and playing in oocyst-contaminated soil (Frenkel and Ruiz, 1980). In Panama, a high correlation with seroconversion in children was associated with dog contact (Frenkel et al., 1995). Dogs that eat or roll in cat feces may mechanically transmit oocysts to young children (Frenkel et al, 1995; Frenkel and Parker, 1996). In Africa, 44% of Somalian females were positive by age 10 years (Ahmed et al., 1988). Many researchers suggest that infection in females before reproductive age may be desirable because this "natural vaccination" will ensure that primary infection will not occur during pregnancy (Moorhouse, 1977; Ahmed et al., 1988); therefore, even though contamination of the environment is high, congenital toxoplasmosis is rare in these countries.

In developed countries such as the United States, infections are usually acquired after the age of 10 years (Roberts and Frenkel, 1991), most likely from ingestion of undercooked or raw meat (Frenkel, 1990). Based on research conducted on 891 people from four states in the United States, it was found that the rate of infection increases in adolescence and continues to rise throughout early adulthood (Feldman and Miller, 1956). In a rural ethnic population of Fayette County, Tennessee, the overall seroprevalence was 21.8% (Gibson, et al., 1956) with an increase in prevalence in the older population. Seropositivity was 33.8% in 20-39 year olds and only 17.3% in 10-19 year olds. This trend of higher seroprevalence with age is true for most other developed countries, except for France where early childhood infection is observed. This finding in France is probably related to the culturally acquired habit of eating raw meat in portions of this population (Frenkel, 1990).

Epidemiology of *Toxoplasma gondii* Transmission in the United States

Early studies examining the prevalence of antibody titers to *T. gondii* have been used as the basis for understanding transmission in the United States (Jacobs et al., 1954; Feldman and Miller; 1956; Gibson et al., 1956; Jacobs, 1957; Kimball et al., 1960; McCulloch, et al., 1963; Remington et al., 1963; Schnurrenberger et al., 1964; Feldman; 1965; Kessel et al., 1965; Warren and Dingle, 1966; Walls et al., 1967; Lamb and Feldman, 1968; Southern, 1968). These studies from the 1950s and 60s, although useful and still cited in modern literature, are outdated for estimating the seroprevalence of *T. gondii* in Americans today. Most seroprevalence studies conducted in the United States, up to 1988, have been previously listed in *Toxoplasmosis of Animals and Man* (Dubey and Beattie, 1988).

A variety of epidemiological studies were conducted in the 1970s, 80s, and 90s from various states. An investigation of *T. gondii* seroprevalence in persons living on farms explored the role of occupation as a risk factor for infection (French et al., 1970). Agricultural workers had a higher prevalence (39.6%) than cattle feedlot employees (9.4%) or county employees that worked in the health and sheriff departments (14.4%). This study, actually conducted in 1967, was one of the last human epidemiological studies to be published before the discovery of the oocyst excreted by felines (Frenkel et al., 1969). Speculations about occupational conditions closer to soil and the influence of vectors, including

mosquitoes, illustrates the frustration these early researchers encountered when trying to interpret and connect their findings to some unknown source of infection.

Some studies were conducted on defined populations, such as university personnel, hospital patients, or military recruits. The first of such studies to be published was conducted at a mental institution in Montana (Mackie et al., 1971). No difference in antibody prevalence was found between mentally retarded people and community controls. Many studies explored the association of cat contact with seroprevalence after cats were found to be the definitive host. At Washington State University, researchers found that 20% of cat owners were positive for *T. gondii* antibodies compared to only 9% positive in those that did not own cats (Peterson et al., 1972). A study in California found that there was no difference in *T. gondii* seroprevalence between veterinarians (43.7%) in daily contact with cats and those with other occupations, such as teachers and secretaries (44.0%), that do not contact cats (Behymer et al., 1973). In 1974, a study at the School of Veterinary Medicine, Davis, California tested both employees and students (Riemann et al., 1974). The overall seroprevalence was 9.9%, and no association between seropositivity and exposure to animals or dietary habits was found. Researchers at Iowa State University tested various groups within the College of Veterinary Medicine (Zimmermann, 1976). Overall, 26.0% (65/250) of staff and students were positive. In veterinary students, 20.4% were positive and 33.3% of personnel from the research institute were positive. No relationships were found between age, sex, primary residence, food habits (eating rare beef and smoked pork products), and the extent of animal contact. A study at the State College of Buffalo, New York, measured the antibody prevalences in both local veterinary personnel exposed to cats and control student volunteers who did not live or work with cats (Sengbusch and Sengbusch, 1976). None of the 60 student volunteers were positive, whereas 18% (11/48) of veterinary personnel were positive. In 1990, a study in a university population examined the prevalence of *T. gondii* antibodies and associated risk factors for infection (DiGiacomo et al., 1990). Of the 116 employees tested from various departments in the University of Washington School of Medicine, 36% were positive. Seropositivity was not associated with exposure to cats. In military recruits, a downward trend in antibody prevalence was noted from 14% seroprevalence in 1962 (Feldman, 1965) to 9.6% seroprevalence in 1989 (Smith et al., 1996). In Maryland, 265 people from a community in Washington County who had

previously participated in an ocular histoplasmosis study agreed to be tested for toxoplasmosis and also answer census questions about their homes and lifestyle (Ganley and Comstock, 1980). The seroprevalence was 38.5%. No associations were found between having a cat on the premises and being antibody positive; however, there was a higher *T. gondii* seroprevalence in rural residents who lived on a farm or had exposure to farm animals, compared to those in an urban residence. Recently, a study demonstrated that people living and working on swine farms may be at increased risk for *T. gondii* infection because of oocyst contamination of the facilities and environment by farm cats (Weigel et al., 1999). The seroprevalence of residents and workers was 31% (54/174). A study of Seventh Day Adventist-vegetarians and healthy meat-eaters in Maryland found the overall seroprevalence was 31% in this community (Roghmann et al., 1999)

Some epidemiological studies in the United States were done in conjunction with outbreaks of toxoplasmosis in animals or humans, as previously mentioned with the consumption of undercooked hamburger by college students (Kean et al., 1969), undercooked venison by hunters (Sacks et al., 1983), and in the outbreak in an Atlanta, Georgia riding stable involving 37 people (Teutsch et al., 1979). Behymer et al. (1985) investigated a flock of ewes in northern California that had a history of infertility. The seroprevalence of the ewes was 59%. As part of their investigation, they tested all 6 members of the rancher's family and found that 2 (33%) were positive. The rancher's wife and daughter, who were involved with lambing, had high titers. The other family members that were not involved in lambing were seronegative. It was concluded that direct contact with fluids from the infected ewes might be the source of the infections. Three cases of human toxoplasmosis are linked to the consumption of raw goat's milk. In 1975, a 7-month old infant with high fever, vomiting and sore tongue, and mouth was admitted to a California hospital (Rienmann et al., 1975). The patient was seropositive for *T. gondii* and four out of ten goats providing milk for the baby were also seropositive. In northern California in 1978, 10 of 24 members of an extended family experienced acute toxoplasmosis with retinochoroiditis in the index case (Sacks et al., 1982). All 10 seropositive persons had consumed raw goat's milk from the family herd compared to the 14 negative persons who did not. The third case occurred in Kentucky. A female who had cared for goats and drank unpasteurized milk from a positive herd developed toxoplasmosis early in her pregnancy.

She delivered a premature baby that did not survive and *T. gondii* was isolated from the infant's brain (Patton et al., 1990). A family from New York, acquired retinochoroiditis after a common source of rare lamb meat was consumed; five of seven members experienced febrile illness and high antibody titers (Masur et al., 1978). United States Military recruits from Fort Bragg, North Carolina, experienced a febrile illness approximately two weeks after returning home from a three-week training course in the jungles of Panama (Benenson et al., 1982). One platoon squad received only chlorinated drinking water that was flown-in, while other squads obtained drinking water either from a small stream with stagnant pools or from a rapidly flowing stream in addition to the provided chlorinated water. The squad that used only chlorinated water did not have any illness. However, soldiers became ill in all the other squads, even though the stream water was treated with iodine. Clinical signs and a rise in antibody titer confirmed *T. gondii* infection (Sulzer et al., 1986). Although researchers were not able to find *T. gondii* oocysts in the stream water, they believe that jungle cats, present in this area, had contaminated the stream water with oocysts. In jungle cat feces from Belize (another Central American country), Patton et al. (1986) identified *T. gondii*–like oocysts in 11.1% (5/45) samples. These findings and the outbreak in Canada (Bowie et al., 1997) support the implication of non-domestic felidae as sources of *T. gondii* infection to people.

Infection in Women

In developed countries including the United States, studies show a lower seroprevalence in young children and teenagers with infection and immunity to *T. gondii* deferred until 20-40 years of age, the childbearing years for most women (Kean, 1972; Dubey and Beattie, 1988). Other seroprevalence studies in the United States have been aimed at women of childbearing age with special attention to the effect on the incidence of congenital infections. The first epidemiological studies and early estimates of toxoplasmosis during pregnancy were done in New York City in the 1970s (Kimball et al., 1971a; 1971b; 1974). At that time in New York City, congenital toxoplasmosis occurred approximately once in every 1500 births (Kimball et al., 1971a) compared to the estimated occurrence in the United States today of once in every 1000-10,000 births (Remington et al., 1995). In Oregon, an 8.1% seroprevalence was found in 95,929 pregnant women (Beach, 1979). When tested for recent seroconversion, 18.4% had IgM titers. This study reported that one in every 200 women contracts toxoplasmosis during pregnancy in Oregon.

Hershey and McGregor (1987) found a low prevalence of *T. gondii* titers in pregnant women in Colorado. Only four of 120 women (3%) were positive, which is the lowest reported for any state. The low prevalence was attributed to the high-altitude, low temperatures and an arid climate with high levels of ultraviolet radiation that shortens oocyst survival in the environment. Also in this study, no differences in seropositivity were found between women that were exposed to cats or those that consumed undercooked meat as answered on a questionnaire. In Santa Clara County, California, 147 women were tested in conjunction with 158 felines seen at local veterinary hospitals (MacKnight and Robinson, 1992). The prevalence of antibodies in women was 42.9% and was 34.8% in cats. Analysis of questionnaire information indicated that seropositivity in women was associated with: 1) eating rare-medium cooked beef, 2) exposure to cats, and 3) working in an outside garden. No other recent epidemiological studies of *T. gondii* infection in women are reported from the United States.

Recently, *T. gondii* seroprevalence estimates were compiled using serum samples collected during the 1988-1994 National Health and Nutrition Examination Survey (NHANES III) conducted by the Centers of Disease Control and Prevention (CDC, 1999e; 2000a). The surveys were designed to obtain nationally representative information on the health and nutritional status of the population of the United States through interviews and direct physical examination (CDC, 1997c). The overall *T. gondii* seroprevalence in all persons, two months and older, was 23%. In women of childbearing age (12-49 years), *T. gondii* seroprevalence was 14% (CDC, 2000a). Based on this prevalence, approximately 86% of women of childbearing age in the United States were negative and susceptible to *T. gondii* infection. Toxoplasmosis in the seronegative pregnant mother may result in serious birth defects in her child because this parasite is capable of crossing the placenta and infecting the developing fetus if the mother is infected for the first time during pregnancy. This national seroprevalence study does not have questionnaire information regarding the occurrence of behaviors that put people at risk for *T. gondii* exposure. The United States currently does not have either a government-supported educational program informing women about the importance of preventing *T. gondii* infection during pregnancy or a maternal screening program that monitors for *T. gondii* seroconversion.

In the United States, of the 3.7 million live births, approximately 9,500 children are born each year with congenital toxoplasmosis (Roberts and Frenkel, 1991). With fetal infection, 9% of the infants die and 30% may have severe birth defects such as hydrocephalus, intracerebral calcification, retinochoroiditis, and mental retardation (Roberts and Frenkel, 1991). Over 60% of congenital cases are subclinical at birth, but many develop ocular complications months to years after birth (O'Connor and Frenkel, 1974; Remington et al., 1995). Infection during pregnancy may also result in miscarriage or stillbirth. The trimester of pregnancy at the time of infection determines both the chance of transmission to the fetus and, if transmitted, the severity of damage (Desmonts et al., 1985; Daffos et al., 1988). Infections during the first and second trimesters have lower transmission rates; however, severe damage is more likely to occur in the embryo and fetus. In contrast, infections during the third trimester have higher transmission rates to the fetus, but children may be asymptomatic at birth (Remington et al., 1995).

Pregnant women that acquire acute toxoplasmosis within the first 21 weeks of gestation are treated with the drug spiramycin (Rhone-Poulenc Pharmaceuticals) (Remington et al., 1995). Spiramycin can be used until term, if the fetus is not infected. Treatment with spiramycin reduces, but does not eliminate, the risk of transmission to the fetus (Hohlfeld et al., 1994). Spiramycin does not cross the placental barrier, so it does not "cure" an already infected fetus (Desmonts and Couvreur, 1974). This drug is not currently approved for use in the United States, but it can be acquired by special request to the United States Food and Drug Administration (Daffos et al., 1988). Once fetal infection is documented or after the 18^{th} week of gestation, a combination of pyrimethamine and sulfadizine can be used until the birth of the infant. These two drugs act synergistically against *T. gondii* infection at eight times the expected activity if their effects were merely additive (Remington et al., 1995). In dosage studies in pregnant rats, pyrimethamine caused teratogenic effects on fetuses ranging from stunting to cranial bone defects, incomplete brain development, and death. Therefore, during the early stages of pregnancy when organogenesis occurs (<18 weeks), it is not recommended for treatment in human fetuses. In addition, these drugs pose a risk of bone marrow suppression for both the mother and fetus; however, these effects may be deminished by supplementing with folinic acid (Remington et al., 1995). Infants, older children, and adults with active chorioretinitis

caused by *T. gondii* can be treated with a pyrimethamine and sulfadizine combination plus corticosteroids (Remington et al., 1995).

In rodents, chronic infections have been associated with alterations in behavior patterns, especially in behaviors that would enhance feline predation such as increased activity and diminished fear of novel scents (Holliman, 1997). *T. gondii* infection is not believed to cause clinical consequences in people; however, some studies have suggested that chronic infections with *T. gondii* may lead to changes in behavior (Flegr et al., 1996) and a decrease in IQ (Wilson et al., 1980). Recent studies in the Czech Republic of men and women naturally infected with *T. gondii* suggest that infection may induce shifts in personality profiles regarding higher anxiety, aggressiveness, and physical activity (Flegr et al., 1996; Flegr and Havlicek, 1999).

Educational, Maternal Screening, and Neonatal Screening Programs

Current programs for the control of toxoplasmosis involves three approaches: a) mandatory maternal screening for seroconversion during pregnancy, b) screening of newborns to detect infections in infants, and c) educating women on preventing infection. France and Austria have mandatory serological screening programs for all women of childbearing age followed by monitoring previously uninfected women for seroconversion during pregnancy. Denmark and the United States have conducted newborn screening studies, and Canada and Belgium have implemented education programs for women (CDC, 2000a). Recently in the United States, Georgia and Minnesota have implemented mandatory maternal screening programs that test for *T. gondii*.

United States

Over the past 30 years, many researchers have advocated the recognition of congenital toxoplasmosis as a priority area in medical research with the need for improved education, methods of diagnosis, and treatment (Feldman, 1968a; 1968b; 1974; Frenkel, 1974; Remington and Desmonts, 1976; Wilson and Remington, 1980; Hughes, 1985; McCabe and Remington, 1988; Wong and Remington, 1994); however, advances in diagnosis and treatment were delayed until *T. gondii* infection became a priority in HIV-related disease control (McCabe and Remington, 1988; Hohlfeld et al., 1994). Today, the

implementation and merits of screening for infection by seroconversion during pregnancy are controversial issues in the United States, because incidence is low and complications during pregnancy are rarely seen (McCabe and Remington, 1988; Thorp et al., 1988; Wong and Remington, 1994). Additionally, many doctors do not recognize the importance of controlling congenital toxoplasmosis compared to other congenital infections such as rubella (Wilson and Remington, 1980). Three types of screening programs are considered as viable options for controlling congenital toxoplasmosis in the United States: 1) primary prevention, designed to prevent maternal infection through education, 2) secondary prevention, consisting of treatment of maternal infections to reduce or prevent disease in the fetus or infant, and 3) tertiary prevention, involving early diagnosis and treatment of congenital infection in newborn infants or only treating congenitally-infected children as they present with symptoms (Hall, 1992).

The value of neonatal screening and treatment was evaluated in Massachusetts with 635,000 infants tested (Guerina et al., 1994). One hundred infants tested positive; however, only 50 infants were confirmed with congenital toxoplasmosis, and these were clinically normal. This group concluded that routine testing does allow for the identification of subclinical congenital infections and, with early treatment, severe long-term sequelae may be prevented. This study was criticized by some researchers because screening for antibodies in infants with immature immune responses may fail to identify infected neonates; therefore they advocated screening of the mother instead (Potasman et al., 1994). Other criticisms included no untreated control groups, and cost ($30,000 per infant identified) was too high (Schoen et al., 1994).

One recent study used a theoretical model to evaluate three strategies for the antepartum management of congenital toxoplasmosis: 1) no testing, 2) perform screening when incidental abnormalities noted on ultrasound (current practice in the United States), or 3) universal screening followed by amniocentesis to diagnose fetal infection when maternal seroconversion is detected (Bader et al., 1997). Based on the model of 4 million births per year, universal screening did reduce the total number of cases of congenital toxoplasmosis compared to no testing or targeted screening. However, the model showed that the rigors of testing during universal screening could cause an additional loss of 18.5 pregnancies for each case of toxoplasmosis avoided. Even though screening reduced the number of cases of congenital

toxoplasmosis, the clinical cost (theoretical loss of uninfected fetuses caused by amniocentesis) was too high. The study concluded that universal maternal screening involving amniocentesis should not be performed in the United States, because diagnostic tests and treatment have limited effectiveness. In the United States, no studies have been conducted that evaluate the value of an educational program designed to teach women how to avoid *T. gondii* infection during pregnancy.

Other Developed Countries

The European Research Network on Congenital Toxoplasmosis created working groups to contribute data that can be used to establish programs for prevention, screening, diagnosis, and treatment (Petersen et al., 2001). One recent finding to emerge from the working group on epidemiology is that risk factors vary from area to area in six large European cities, but that overall, infection from meat consumption may contribute between 30% and 63% of all infections, and soil contact may contribute between 6% to 17% based on a study of women who seroconverted during pregnancy (Cook et al., 2000).

Great Britain

The seroprevalence of *T. gondii* in pregnant women in London was 18.8% (Gilbert et al., 1993). Ethnic origin and country of birth did affect seroprevalence in the study population. The high seroprevalence among some women of foreign birth compared to British born women may reflect differences in the consumption of undercooked meat or unpasteurized milk. This study concluded that these women were probably infected before immigration to Great Britain.

Norway

Maternal screening programs and epidemiological studies conducted in Norway have found that the incidence of *T. gondii* is less than 1% (Stray-Pedersen and Lorentzen-Styr, 1979; Stray-Pederson et al., 1979; Stray-Pederson and Jenum, 1992; Jenum et al., 1998). In a recent study, a serologic screening program was used to identify potentially preventable risk factors most likely to have the greatest impact on the incidence of *T. gondii* infection in pregnancy (Kapperud et al., 1996). The seroprevalence of *T. gondii* infection among Norwegian women of childbearing age is low, consequently, the percentage of women at risk of acquiring infection during gestation is high. A total of 37,000 women participated in the screening program and 63 were identified with recent primary *T. gondii* infection either on initial screen or by

seroconversion. The following six factors were independently associated with an increased risk of maternal infection: 1) eating raw or undercooked minced meat products, 2) eating unwashed fruits and vegetables, 3) eating raw or undercooked mutton, 4) eating raw or undercooked pork, 5) cleaning the cat litter box, and 6) infrequent washing of kitchen knives after preparation of raw meat prior to handling another food item. The study concluded that prenatal screening has proved to be a valuable tool for preventing congenital toxoplasmosis in Norwegian women.

France

Studies conducted in France in the 1970s are the basis of our understanding the connection between meat-eating and acquiring toxoplasmosis, where Desmonts and Couvreur (1974) reported infection rates of 6.3 per 100 pregnancies. Prenatal screening programs in France, which included ultrasound, amniocentesis, and fetal blood sampling, have been very effective (Desmonts and Couvreur, 1974; Desmonts et al., 1985; Daffos et al., 1988). Today, approximately 4900 cases of primary *T. gondii* infection occur in pregnant women which is two per 100 pregnancies (Baril et al., 1999). Risk factors for infection were investigated in 80 women who had seroconverted during pregnancy. Compared to 80 seronegative controls, positive women had poor hand hygiene, consumed undercooked beef, owned a cat, frequently ate raw vegetables outside the home, and consumed undercooked lamb. These researchers suggested that along with a serological screening program, prevention campaigns should focus on eating habits, hand washing, and cat care (Baril et al., 1999).

Recently, the value of prenatal diagnosis and early postnatal diagnosis was reviewed in 110 women with *T. gondii* seroconversion during pregnancy (Robert-Gangneux et al., 1999). Twenty of 27 infected fetuses were born alive and all twenty babies had persistent IgG titers for more than one year of life, indicating true infection and not maternal antibodies. The other seven fetuses either died *in utero* or the pregnancy was terminated. All women with prenatal diagnosis received treatment with an antiprotozoal drug and most of the babies were asymptomatic. These results support the role of monitoring negative pregnant women for seroconversion followed by treatment and postnatal testing for the prevention of congenital toxoplasmosis.

Austria

In Austria, a toxoplasmosis-screening program was implemented in 1975. Pregnant women were initially screened and, if negative for *T. gondii* antibodies, were tested again in the second and third trimesters. If a woman seroconverted during this time, she was treated immediately. A dramatic decrease in the incidence of congenital toxoplasmosis from the 1970s at 50-70 cases per 10,000 births to one per 10,000 births in the early 1990s was achieved (Aspock and Pollak, 1992).

Canada

In Canada, the prevalence of antibody titers to *T. gondii* in women from Toronto was 12.9% (Ford-Jones et al., 1996) and from Victoria was 25% (Karim and Trust, 1977). Congenital toxoplasmosis affects between 70-280 newborns in Canada each year (Carter and Frank, 1986). In an effort to control this rate of infection by practical means, an educational program was developed in Ontario to teach women appropriate behavior to avoid infection during pregnancy (Carter et al., 1989). The effectiveness of the program was tested on pregnant participants in a publicly funded prenatal class. A case-control study was designed that involved completing a questionnaire at the first and last class, with one prenatal class receiving a three page handout and a one hour workshop that focused on cat care, food handling, and personal hygiene. The other prenatal class received routine class material that did not mention toxoplasmosis. Responses on the questionnaires were scored based on appropriate behavior modification. Cat owners in classes where cat care was included in the educational materials, had modified their behavior as reflected in higher scores. Additionally, these women also improved their cooking methods for meat as reported on the post-questionnaire. It was concluded that this educational program was effective by modifying behaviors that decreased the risk of *T. gondii* exposure, and it should be offered to all pregnant women to reduce incidence of congenital toxoplasmosis, instead of developing an expensive maternal screening program monitoring for seroconversion.

In a survey of 4,136 pregnant women from Montreal, Quebec, 40.8% were seropositive during a maternal screening program (Veins et al., 1977). This primarily French Canadian population had a much higher prevalence of antibodies than the populations from Toronto (Proctor and Banerjee, 1994; Ford-Jones et al., 1996) or Victoria (Karim and Trust, 1977) indicating that their diet may reflect the French preference

for undercooked meat. Only two out of 52 mothers had documented seroconversion, even though all presented with symptoms of toxoplasmosis. Despite the benefit to the children, researchers said it was difficult to justify the burden of a three-week course of anti-protozoal treatment to the mother (Veins et al., 1977).

An epidemiological study on Inuit women from northern Quebec was conducted after a cluster of four women seroconverted during pregnancy, as identified during an ongoing maternal screening program (McDonald et al., 1990). A questionnaire was given to 22 women who had delivered babies during the previous year. Seroconversion was significantly associated with skinning animals for fur and frequent consumption of Caribou meat or seal liver. Compared to seronegative women, seropositive women were four to six times more likely to have eaten dried seal meat and liver and eight times more likely to have consumed raw caribou meat more than once a week. This information was used to develop guidelines for the prevention of toxoplasmosis in seronegative pregnant women in this arctic region.

Switzerland

In Switzerland, women who had given birth were given a questionnaire on potential risk factors for *T. gondii* infection (Sturchler et al., 1987). Seropositivity in mothers was associated with: 1) farm work and flower production, 2) consumption of lamb meat and/or improperly heated beefsteak, and 3) cat ownership. Researchers determined the incidence of congenital toxoplasmosis was one in 1000 live births. The authors pointed out that information from this study should be used to promote measures for the prevention of *T. gondii* infection in pregnant women.

Italy

In southern Italy, researchers found that locally relevant risk factors were important to evaluate when developing educational materials for this region (Buffolano et al., 1996). Recent infection in pregnant women from Naples was strongly associated with the consumption of cured pork or raw meat. The risk of infection increased three times if these meats were consumed at least once a month.

Belgium

In contrast to the above studies that recommend educational programs for women, one study from Belgium that assessed the value of an educational program, found that the reduction in the incidence of

congenital toxoplasmosis was not significant (Foulon et al., 1988). Although the percentage of seroconversion dropped by 34%, the reduction in incidence was viewed as a normal fluctuation in incidence over time and probably was not related to the prophylactic measures taught to pregnant women.

Developing Countries

Columbia

A recent study in Columbia used a simple questionnaire about known risk factors to find pregnant women at risk for *T. gondii* infection (Gomez-Marin et al., 1997). Columbia does not have a maternal screening program and congenital toxoplasmosis is the second most important cause of congenital blindness. Because serologic screening is expensive, researchers wanted to identify and test only those women whose responses to the questions identified them as at risk for congenital infection. In addition to clinical signs, pregnant women who ate rare meat, an unusual habit in this region, were identified as a group of high-risk mothers recommended for serological testing. Other epidemiological questions, such as contact with cats, were less predictive. This study showed that a questionnaire including clinical and epidemiological risk factors could be used as a selective filter to target the highest risk population for serologic testing in a country where incomes are low and incidence rates are high.

Thailand

In Thailand, a prospective, cross sectional study was conducted on 1,200 pregnant women that were tested for antibodies to both *T. gondii* and HIV as part of an antenatal care clinic (Chintana et al., 1988). Demographic information, dietary history, and contact with cats were recorded on a questionnaire. This information was analyzed for associations with seropositivity. The prevalence of *T. gondii* antibodies in this population was 13.2%. Most women were poor and came from the central or northeastern part of the country. Thai people have strong cultural food habits that include the consumption of many undercooked meat dishes. Women that ate undercooked meat were more than twice as likely to be infected than those that ate cooked meat. Also, Thai women that allowed cats inside their house were also twice as likely to be infected. It was recommended that health education preventing the transmission of *T. gondii* be promoted in this country along with antenatal diagnosis and treatment in mothers that are at risk for infection.

Yugoslavia

In Yugoslavia, where there is no maternal screening program, researchers identified habits that could be targeted in health education (Bobic et al., 1998). Women of reproductive age from Belgrade were tested for *T. gondii* antibodies and asked to complete a questionnaire with demographic information, consumption of meat, exposure to soil, and exposure to cats. Even though the overall prevalence was high at 77.4%, there was a statistical difference between the youngest age group, 15-19 years, at 57.1% compared to the oldest, >40 years, at 92.6%. Seropositivity was associated with undercooked meat consumption. In women younger than 20 years, exposure to soil also was associated with seropositivity. This study recommended that in the absence of a maternal screening program, women in this region could benefit from an educational program to prevent congenital toxoplasmosis.

CHAPTER 3

TOXOPLASMA GONDII AND FOODBORNE ILLNESS

In May 1997, President Clinton announced the new National Food Safety Initiative designed to reduce foodborne illness and improve the safety of the nation's food supply. In "Food Safety from Farm to Table", *Toxoplasma gondii* was included as an important food pathogen along with *Escherichia coli* O157:H7, *Salmonella* ssp., and *Cryptosporidium parvum*, that threatens the nation's food supply (FDA et al., 1997). This nationwide plan will focus on points of food production, manufacturing, and transportation where contamination could occur (Hingley, 1997). Farm-level interventions are the most effective control strategy for parasitic infections, such as *T. gondii*, in food animals (Roberts et al., 1994). Unlike bacterial contaminants, parasites do not multiply in food products once the animal is harvested; therefore, *T. gondii* infection in food animals occurs only on the farm (Roberts et al., 1994).

Epidemiologists with the Centers for Disease Control and Prevention (CDC) estimate that over 2,000 of the 9,500 yearly cases of congenital toxoplasmosis in the United States were caused by foodborne illness in the mother (Schantz and McAuley, 1991; Roberts et al., 1993). Even though *T. gondii* accounts for only 0.8% (112,500/13,814,924) of all illness caused by known foodborne pathogens, the severe consequences of infection in the fetus and HIV-infected patients, made it responsible for 20.7% (375/1,809) of foodborne deaths (Mead et al., 1999). *T. gondii* was third only to *Listeria monocytogenes* and *Salmonella* spp. which caused 27.6% and 30.6% of foodborne deaths, respectively (Mead et al., 1999). At this time, deaths caused by toxoplasmosis may be inflated by illness and death primarily in HIV-infected patients. In the future, advances in HIV prevention and treatment should reduce these toxoplasmosis-related deaths (Mead et al., 1999).

The cost of caring for congenitally infected children is economically devastating. In the mid-1990s, estimates for their specialized care was approximately $3.2 to $8.8 billion dollars a year (Buzby and Roberts, 1997). This figure includes medical costs, income losses, and costs for special education and residential care (Roberts et al., 1993; Buzby and Roberts, 1997). Encephalitis caused by *T. gondii* is among the three most common AIDS-related opportunistic infection of the central nervous system in both men and

women (Luft and Remington, 1988; CDC, 1999d). The cost of treating toxoplasmosis in AIDS-patients in the United States ranges between $23-106 million dollars each year (Roberts, et al., 1993; Buzby and Roberts, 1997). If these estimates are accurate, toxoplasmosis is responsible for higher costs than any other foodborne pathogen, including *Escherichia coli* O157:H7 and *Salmonella* ssp. which were $0.3-2.2 billion and $0.9-12.2 billion, respectively. With the new CDC estimates of food-related pathogen illness and death (Mead et al., 1999), these costs are probably higher. These monetary figures cannot, however, reflect the emotional burden endured by those involved in the daily care of handicapped infants, children, and adults that survived congenital infection.

Toxoplasma gondii Transmission by Meat Consumption

Food animals, especially swine, have been circumstantially implicated as the main source of transmission of *T. gondii* to people today through the ingestion of undercooked meat and/or the contamination of hands, food preparatory surfaces, and food handling utensils from contact with raw meat (Dubey, 1986a). Prior to 1969 [before the life cycle was elucidated showing cats were the definitive host which shed the oocyst stage into the environment with its feces (Frenkel, et al, 1969)], the only documented mode of transmission in human beings was transplacental passage of the parasite to the developing fetus causing congenital infections (Sabin, 1941; 1953). However, many researchers wondered how the mother or any other adult or child acquired the infection (Kass et al., 1952; Weinman, 1952; Cole et al., 1953; Jacobs, 1953). During this time, researchers maintained the parasite in laboratory animals such as mice, rabbits, hamsters, guinea pigs, and pigeons by intracerebral, intradermal, subcutaneous, intranasal, and intraperitoneal inoculations of infected tissue (Jacobs, 1953).

The possibility that infections could be transmitted by ingestion of infected tissue was investigated by many researchers (Eichenwald, 1948; Adams et al., 1949; Van Thiel, 1949; Cowen and Wolf, 1950; Jacobs et al., 1950; Dienst and Verma, 1965). Infections could be passed by feeding infected tissue to a new host, and using tissues from animals with chronic infections worked best in feeding experiments (Jacobs, 1953). The "pseudocyst" (bradyzoites in tissues present in chronic infections) was resistant to digestion by gastric juice and trypsin, compared to free tachyzoites (present in acute infections), which

were not resistant (Kozar et al., 1952; Jacobs et al., 1960). Jacobs (1953) was one of the firsts to recognize the role of consuming infected flesh and acquiring *T. gondii*. With the finding of toxoplasmosis in swine in 1952 (Farrell et al., 1952), researchers started to make the connection between the ingestion of undercooked pork and human infection (Jacobs, 1953; Weinman and Chandler, 1956; Jacobs, 1957). Weinman and Chandler (1956) were the first to present data supporting a "meat-to-man" hypothesis of *T. gondii* transmission. They found that pigs became infected by cannibalism or by feeding on infected rodents. They believed that the organism remained viable after the pig was harvested and the pork arrived in the kitchen. They also noted that humans were more likely to have antibody titers to *T. gondii* if they ate undercooked pork. In 1965, the incidence of *T. gondii* infection was studied among children in a French hospital. The custom of this hospital was to serve undercooked meat, primarily mutton (Desmonts et al., 1965). Although none of the children developed clinical signs, a higher incidence of infection was observed among this population when they increased the amount of undercooked mutton in the diet. Without knowing about environmental contamination of oocysts from infected cats at this time, none of these researchers could explain the widespread infection of *T. gondii* in herbivores, grain/seed eaters, or in people that did not eat pork or other meats.

Toxoplasma gondii Transmission and the Role of Pork Today

Today, the three most commonly consumed meats in the United States are beef, poultry, and pork (NLSMB, 1994). Medical research has linked food-borne illness in people from *T. gondii* to the consumption of raw or undercooked pork, lamb, beef, venison, and other meats, including other wild game (Dubey and Beattie, 1988). Early studies isolated *T. gondii* from tissues of pigs include the brain, salivary gland, and saliva (Eyles et al., 1959). Tissue stages of *T. gondii* were experimentally isolated from commercial cuts of pork, mutton (sheep), chevon (goat), and horsemeat (Jacobs and Melton, 1957; Jacobs et al., 1960; Remington, 1968; Dubey, et al., 1986c; Dubey, 1988). Research conducted in the 1950s and 60s are the only sampling of supermarket meat for *T. gondii* in the United States. Today, the risk of infection from mutton, chevon, and horse is considered low because these meats are not commonly consumed in most households in the United States (Dubey and Beattie, 1988). Recently, serum samples

from horses slaughtered for food in Mexico, Canada, and the United States were tested for *T. gondii* antibodies (Dubey et al., 1999). Antibody prevalence ranged between 3 to 7%, depending on type of serological assay used. Overall, a low seroprevalence was found and the study concluded that the risk for acquiring toxoplasmosis from eating horsemeat is probably low (Dubey et al., 1999). Beef is not considered a source of infection because tissue cysts do not persist in cattle or beef products (Dubey, 1986b; Dubey and Thulliez, 1993). The role of poultry has not been studied; however, some experimentally infected chickens produced viable tissue cysts in leg muscles when tested by the bioassay in mice (Dubey et al., 1993).

In the United States, infected pork has been implicated as the main source of food-borne transmission of *T. gondii* to people (Dubey, 1986a). Research supporting this hypothesis includes: 1) high seroprevalence in sows (42%) and market pigs (23%) (Dubey et al., 1991), 2) recovery of infective tissue stages from pork meat when bioassayed in mice (Dubey, 1986a), 3) persistence of infective tissue cysts in live pigs for 875 days (Dubey et al., 1986c), 4) only a few oocysts are required to produce *T. gondii* infection in pigs (Dubey et al., 1996), and 5) virulent strains of *T. gondii* have essentially the same genotype and are comprised from a single clonal lineage, regardless of their host or geographic origin (Sibley and Boothroyd, 1992).

Toxoplasma gondii, Food Safety, and Consumer Lifestyles

An important part of foodborne disease surveillance involves identifying common errors in food handling that can lead to a foodborne disease outbreak (Collins, 1998). In the bacterial foodborne disease outbreaks from 1983-1992, improper storage temperatures and poor personal hygiene of the food handler were the most common food handling errors. This occurred despite educational efforts about proper food handling (control the temperatures) and sanitation methods (keep it clean) that are dispersed to workers in the commercial food industry (Collins, 1998). These same types of improper food-handling behaviors could impact how *T. gondii* becomes a foodborne illness. Even though specific guidelines for preventing *T. gondii* infection are published in a variety of professional journals (Frenkel, 1974; Lindsay et al., 1997a), lay publications (Sietsema, 1995), and on the World Wide Web (Hughes, 1985; AVMA, 1996; CDC,

1997a; 2000a; 2000b; MOD, 1997), consumer knowledge and the level of compliance with these guidelines are unknown, but are believed to be poor based on general foodborne illness prevention practiced by consumers (PFSE, 1997). This is especially interesting and important in the wake of increasing risk for foodborne illness and higher incidence of foodborne disease (Mead et al., 1999).

Changing consumer lifestyles have had an impact on the emergence and reemergence of foodborne pathogens (Collins, 1998). Food hygiene practices are traditionally learned by years of conditioning, observation, and reinforcement from mothers and grandmothers. With more women in the workforce, there is less time to spend on food preparation. Most spend less than 30 minutes preparing every meal which may not be enough time for internal temperatures of raw meat to reach at least 58°C (136°F). While adults are at work, many children and teenagers are preparing food for themselves (Collins, 1998). Many consumers (38%) are using convenience foods and quick methods of preparation, including partially cooked foods that can be purchased at a take-out location, but eaten at home (FSIS, 1996b). For today's consumers, convenience and saving time are more important than proper food handling and preparation (Collins, 1998).

Present knowledge and practices of meat handling, dietary preferences, and food preparation are important factors that influence the probability of eating *T. gondii* infected food. For example, some assume that it is okay to serve pork rare because the risk for *Trichinella spiralis* has become infrequent in pork (Bailey and Schantz, 1990). Specialists answering calls on the Meat and Poultry Hotline stress that cooking is necessary to destroy any pathogens that might be present, "from trichinae, to salmonellae to *Toxoplasma gondii*" (FSIS, 1996a).

Reducing the Viability of *Toxoplasma gondii* Tissue Cysts

Processing of raw meat by heating, freezing, salting, smoking and drying is a common practice in both commercial and home-based operations that may or may not affect the viability of *T. gondii* tissue cysts in consumable meats. In an experiment to determine the effect of high temperature on the infectivity of *T. gondii* tissue cysts, a mixture of homogenized pork and infected mouse brains were heated to various water-bath temperatures. Heating to temperatures higher than 61°C (142°F) for 3.6 minutes rendered the

tissue cysts nonviable when fed to mice (Dubey et al., 1990). Using the same homogenate recipe, *T. gondii* tissue cysts were inactivated when frozen at -12°C (10 °F) under experimental conditions (Kotula et al., 1991). When commercial cuts of pork from experimentally infected pigs were tested, tissue cysts were nonviable after three days at –12°C (10 °F) (Dubey, 1988).

Curing with sodium chloride (NaCl) (salting) preserves meat without further refrigeration (Lawrie, 1991). Although this practice was once common with all meats, especially pork, beef, and mutton, the organoleptic quality of cured pork allows it to remain popular while the other cured meats are not (Lawrie, 1991). The characteristics of the muscles of pigs (low myoglobin content, amount of intermuscular fat, and relative fast rate of post-mortem conditioning or tenderizing) make cured pork attractive (Lawrie, 1991). For curing, fresh sides of meat are soaked in a 25% brine solution (Lawrie, 1991). Although equilibrium between the outward flow of water and soluble proteins and inward flow of salt is reached in 48 hours at 3 to 7°C (44°F), the sides are usually submerged for four to five days. Additional salting by injection or pumping of brine can also be done with many more aging steps in the curing process to turn these sides into bacon or ham (Lawrie, 1991). Navarro et al. (1992) used varying concentrations of salt on fresh sausage prepared with experimentally infected pork. In mouse bioassays, sausage treated for less than 24 hours did not affect the viability of the parasite. However, after 48 hours, salt concentrations of 2.00% and 2.50% effectively killed the parasite. Dubey (1997b), using the mouse bioassay, found that tissue cysts from rodent brains were inactivated when suspended in 6.0% aqueous NaCl solutions and stored at temperatures ranging from 4 to 20°C (39-68 °F). Lower concentrations of NaCl (0.85%, 2.0%, and 3.3%) had a lesser effect in this temperature range with viability lasting three to 56 days. Based on these studies, salted pork products such as bacon, ham, and some kind of salty sausages, if infected, should contain only nonviable *T. gondii* tissue cyst, if the curing process is done properly (Dubey, 1997b).

Smoking at temperatures exceeding 58°C (136 °F) should kill tissue cysts of *T. gondii* (Lunden and Uggla, 1992). However, drying at ambient temperatures to make jerky does not have an effect on the viability of *T. gondii* because the parasite is able to survive for several days after death of the animal, even in cases where the tissues have started to decay and are foul-smelling (Dubey and Beattie, 1988). Lunden and Uggla (1992) cooked four mutton steaks in the microwave to see if tissue cysts were inactivated. Two

of the four steaks remained infective to mice. Therefore, microwaving is not recommended for cooking raw pork because of uneven heating (Dubey and Beattie, 1988; Lunden and Uggla, 1992). Perhaps irradiation of meat will remove the burden of safe cooking and freezing from the consumer. Gamma radiation does kill the tissue stages of *T. gondii* (Dubey et al., 1986a; Dubey and Thayer, 1994). The penetrating properties of gamma rays allow for the feasibility of whole carcass irradiation to kill parasites in pork (Dubey et al., 1986a).

Epidemiology and Control of *Toxoplasma gondii* on Swine Farms

Prior to the national seroprevalence studies conducted in the 1980s and 1990s, surveys of *T. gondii* antibodies in pigs in the United States were small samplings and probably reflected local prevalence which ranged for <1 to 69% (Dubey, 1990). Less than 5000 pigs were tested in 30 years of investigations and these prevalences are not considered representative of the overall swine population of the United States (Dubey, 1990). Similar surveys on small swine farms have been recently conducted in Hawaii (Dubey et al., 1992) and New England (Gamble et al., 1999).

Using larger statistically valid surveys based on population sampling, reliable estimates of the seroprevalence of *T. gondii* on swine farms in the United States have been calculated. During 1983-84, national seroprevalence in sows at slaughter was 42% (257/613) (Dubey et al., 1991). Surveys in the 1990s showed that in sows from Iowa, 22% (222/1000) (Dubey et al., 1995c) and 14.3% (39/273) (Smith et al., 1992) were positive for *T. gondii* antibodies. Weigel et al. (1995a) found that 20.8% (1056/5080) of sows from Illinois were positive. Using sera collected from sows for the 1990 USDA NAHMS survey, Patton et al. (1996) reported 20% (679/3472) of sows seropositive for *T. gondii*. In sows from Tennessee, 36% (1130/3841) were positive for *T. gondii* antibodies (Assadi-Rad et al., 1995). Zimmerman et al. (1990) using an ELISA tested sows from the 1985 NAHMS and found 11.4% positive. These high prevalences reflect farm management practices of keeping sows outdoors part of the year rather than in total confinement (Lubroth et al., 1983; Zimmerman et al., 1990; Smith et al., 1992; Assadi-Rad et al, 1995). The number of juvenile cats on farms may also increase the risk of infection to sows (Weigel et al., 1995b).

Seroprevalence in market-weight pigs has also been investigated. The national seroprevalence in market-weight pigs was 23% (2583/11229) in 1983-84 (Dubey et al., 1991) by the MAT. Zimmerman et al. (1990) using an ELISA test found 5.4% of finishing pigs positive. Two recent studies found that infection in market pigs has decreased and is considerably lower than sows. In North Carolina, finishing pigs sampled within one month of slaughter had a low prevalence of 0.6% (13/2238) (Davies et al., 1998), and in Tennessee, the seroprevalence was 3% (12/473) (Patton et al., 1996). Both studies used the MAT. These figures reflect the effect of farm management practices, such as total confinement operations, which deny access of birds, rodents, cats, and dogs, decreases the risk for *T. gondii* infection for pigs (Lubroth et al., 1983; Assadi-Rad et al., 1995; Dubey et al., 1995c; Davies et al., 1998).

Commercial cuts of pork, such as pork chops and tenderloin, are derived from market-weight pigs whereas, breeding sows are processed into ready-to-eat meats, such as hot dogs and bologna, that are heated to a temperature that kills *T. gondii.* Infection from raw or undercooked pork, therefore, seems unlikely because of the low prevalence in market-weight pigs (Patton et al., 1996; Davies et al., 1998). These serological studies of swineherds are particularly important to consider when investigating the implication of pork as the source of infection to humans. Over the past 30 years, education of the producer regarding the transmission of both *T. gondii* and *Trichinella spiralis* have led to changes in the way swine farms are managed, such as the development of total confinement operations. These changes may account for the declining prevalence of *T. gondii* observed in Japan and Europe (Dubey, 1986c) and may also account for the observed decline seen in the United States today.

CHAPTER 4

OBJECTIVES

Women of childbearing age are a vulnerable population for *Toxoplasma gondii* infection, because infection in a seronegative pregnant woman may cause serious birth defects if a fetus is infected *in utero*. Published guidelines for preventing infection are available; however, how closely these guidelines are followed is not known. There have been no recent studies in humans in the United States that 1) estimate the proportion of women of childbearing age with antibody titers and 2) identify the occurrence and frequency of behaviors that increase risk of exposure to *T. gondii*.

Hypothesis:

Women of childbearing age engage in behaviors that put them at risk of *T. gondii* exposure and the frequency of these behaviors varies among women of different age groups.

Objectives:

Specific Aim 1

Estimate the seroprevalence of *T. gondii* IgG antibodies in women of childbearing age from the Knoxville, Tennessee area.

Specific Aim 2

Investigate the frequency of present behaviors in this population that increase the risk of *T. gondii* exposure such as: 1) eating less than well done meat, 2) improper kitchen hygiene practices followed in food preparation, 3) cat ownership, and 4) occupation or hobby with exposure to soil.

Specific Aim 3

Develop guidelines for reducing exposure to *T. gondii* based on behaviors of women in different age groups.

CHAPTER 5

BACKGROUND STUDIES

Numerous lay publications and web sites related to women's health issues report that the prevalence of *T. gondii* antibody titers in women range from 10-40% (Winn Foundation, 1997; MOD, 1997; CDC, 2000a). A woman that becomes infected and develops immunity at least six to nine months before pregnancy is in little danger of passing the infection to her baby. However, the population of seronegative women of childbearing age is vulnerable to *T. gondii* infection as a cause of lost pregnancies, birth defects, and problems in children that survive congenital infection. The same lay publications and web sites also list guidelines for preventing *T. gondii* infection, and caution pregnant women or those considering pregnancy to avoid known sources of infection. Studies of Norwegian women that seroconverted during pregnancy show that handling or eating raw meat, raw vegetables or fruit, cat ownership, and improper washing of kitchen utensils were risky behaviors for infection (Kapperud et al., 1996). No recent studies have been conducted in the United States that investigate the frequency of risky behaviors for *T. gondii* infection in women of childbearing age.

In September 1998, the National Workshop on Toxoplasmosis: Preventing Congenital Toxoplasmosis, convened by the Centers for Disease Control and Prevention in Atlanta, Georgia, identified research priorities for preventing toxoplasmosis in the United States (CDC, 2000a). Congenital toxoplasmosis is not a reportable disease; therefore, no national data are available regarding its occurrence. Extrapolated data from regional studies indicate that 400-4,000 cases occur in the United States each year. The panel of experts at the Workshop defined approaches to reduce the prevalence of congenital toxoplasmosis through critical research and prevention efforts for the future. One approach to determining the burden of toxoplasmosis is through serosurveys of the general population and complementary evaluation of potential foodborne, catborne, or soilborne transmission (CDC, 2000a). Recently, Georgia and Minnesota have started screening for toxoplasmosis in pregnant women.

In 1995, a preliminary study was conducted to estimate the prevalence of *T. gondii* in a convenience sampling of pregnant women from the Knoxville area. These serum samples originated from

the University of Tennessee Medical Center's Biochemical and Molecular Genetics Laboratory. All identifiers were removed from the samples, so that only sex, age, ethnicity, and pregnancy status were known. Serum samples of 123 pregnant women, 14-42 years old, were tested for IgG antibodies to *T. gondii*. Eleven percent (14/123) were seropositive indicating that approximately 89% (those that were seronegative) were at potential risk for infection with *T. gondii* during pregnancy and transferring the infection to their unborn babies. Without additional information about their lifestyle behaviors that increase or decrease the risk of exposure, recommendations for behavior modifications could not be developed for women in the Knoxville area.

This study investigates if women of childbearing age (pregnant and nonpregnant) in the Knoxville area engage in behaviors that may increase their risk of exposure to *T. gondii* and how these behaviors differ between age groups. Risky behaviors were identified as activities that could result in accidental ingestion of either 1) the stage in edible portions of meat or milk or 2) the environmental oocyst stage shed by felines in feces.

CHAPTER 6

MATERIALS AND METHODS

Experimental Design

The experimental design used for this research was a cross-sectional study as defined in conventional epidemiological research (Mausner and Kramer, 1985). Observations of a study population were made during a cross-sectional slice of time by using clinical tests and interviews (questionnaire) as measurements of exposure to determine the prevalence of a particular health effect (Mausner and Kramer, 1985). The purpose of such studies is to compare the prevalence of a defined health effect among subgroups of a population with varying exposures, age/gender compositions, or personal habits. For this study, the prevalence of *T. gondii* antibodies was compared among subgroups of the Knoxville study population. These subgroups were defined in terms of demographic identifiers and personal habits and knowledge regarding *T. gondii* exposure and infection. The information collected in this cross-sectional study cannot be used to prove a causal relationship between specific behaviors and *T. gondii* seropositivity because a seropositive person is chronically infected and presumably tests positive for life, regardless of when *T. gondii* infection occurred.

Choice of Subjects

Women, 18-53 years of age, were recruited from two sources. Pregnant females seeking prenatal medical care from an Obstetric-Gynecology office located at the University of Tennessee Medical Center and non-pregnant females donating blood for Medic Regional Blood Center and Blood Assurance. "Childbearing age" for this study was defined as age 18-53 years. Eighteen years was the youngest age able to participate without parental consent. Participation in the study was strictly voluntary and economic incentives were not used to stimulate cooperation. Women meeting the age criteria for inclusion were asked if they wanted to participate, thus these data represent a convenience sampling and participation was not based on systematic population sampling techniques.

Each participant was asked to contribute a blood sample and to complete a questionnaire about present behaviors that are considered risky for exposure to *T. gondii*. The samples were provided anonymously from participating females; therefore, all blood collection tubes and questionnaires were coded with unique matching numbers, rather than by the name of the participant. The choice of subjects and method of sampling via an informed consent statement and questionnaire were reviewed by the University of Tennessee Office of Research by an Internal Review Board (Figures A1, A2 and A4). An approved Form B is on file in the Office of Research. All signed consent forms were stored separately from questionnaires in a file cabinet at the University of Tennessee Parasitology Laboratory, room A233. Participants were offered an information sheet about *T. gondii* published by the March of Dimes (Figure A3).

Blood Collection

Trained medical personnel, medical technologist, nurse, nurse practitioner, or phelbotomist at the University of Tennessee Medical Center or at Medic Regional Blood Center and Blood Assurance blood drives, collected whole blood samples using standard techniques. Five milliliter (ml) red-top, Vacutainer Brand tubes (Becton Dickinson, Franklin Lakes, New Jersey) were used for blood collection in conjunction with compatible hypodermic needles. There were no additional risks over those risks known for providing a blood sample for medical evaluation or for blood donation caused by participation in this study.

Development of the Questionnaire

Questions were designed to investigate for four types of information: 1) demographic information, 2) frequency of current behaviors that are considered unhygienic or risky for exposure to *Toxoplasma gondii*, 3) current knowledge of *T. gondii*, and 4) knowledge and attitudes regarding meat safety. The first questionnaire developed asked about both current risky behaviors and risky behaviors of the past and was used by the first 25 participants (Figure A4). The second questionnaire had the questions about risky behaviors of the past removed and included only the questions about current risky behaviors (Figure A2). This version of the questionnaire was used by 796 participants.

Participants were asked to identify childhood residence and current residence so that the classification of "urban" or "rural" environments could be made. The specific location of the residence was not requested. Education level was divided into six categories, three regarding high school and three regarding college. Participants born in the United States versus those born in another country were identified by asking place of birth. Asking if the participant was a vegetarian identified meat eaters and non-meat eaters. Life-style habits specific for transmission of *T. gondii* (consumption of undercooked meat, exposure to soil via gardening, and exposure to cats) were defined by identifying the frequency of such behaviors as *always, frequently, occasionally*, or *never*. These responses were grouped for analysis. For a "risky behavior", responses of *always, frequently* and *occasionally* were grouped and compared to *never*. For a "hygienic behavior", responses of *frequently, occasionally*, and *never* were grouped and compared to *always*.

Sampling at University of Tennessee Medical Center

Pregnant women that came to their doctor's office at the University of Tennessee Medical Center for gestational diabetes screening were consented by office medical personnel for participation in this study. Samples were collected from June 28, 1999 to January 22, 2000. The diabetes screening consists of consuming a glucose solution and a waiting period of 30 minutes before blood is drawn for blood sugar analysis. During the waiting period of the diabetes screening, women had time to complete the questionnaire for this study. When blood was drawn for the diabetes screening analysis by office medical personnel, an extra 5 ml red top tube of blood was drawn for this study, so a second needle stick was not needed. Completed questionnaires and tubes of blood were carried to the Biochemistry and Molecular Genetics Laboratory of the University of Tennessee Research Center. Samples were held in a refrigerator in the Genetics Laboratory. Once a week, samples were transported to the Parasitology Laboratory of the University of Tennessee Veterinary Teaching Hospital. After separation of serum from the blood clot, approximately two equal aliquots of serum were transferred to one-milliliter cryovials (Corning, Inc., Acton, Massachusetts) for storage at –20°C (–4°F).

Collection at Medic Regional Blood Bank and Blood Assurance

Samples were collected from blood drives within the Knoxville and Chattanooga area from January 1999 through April 2000 (Table B2). Only those women that passed the rigorous healthy standards for blood donation at Medic and Blood Assurance were included in the study (Figure A13). Female blood donors within the age limits (18-53 years) were asked during the blood donor screening process if they would be interested in participating in this study and consented at that time. When the donor was near the end of the blood collection process, the phlebotomist drew an extra tube of blood before the needle was pulled, so a second needle stick was not needed. Questionnaires usually were completed after donation. Occasionally, questionnaires were completed as donors waited for an open phlebotomy table at some of the busier blood drives.

After collection, blood tubes were placed in a Styrofoam cooler labeled with biohazard warnings. Frozen ice packs were used to preserve blood samples for transport to the Parasitology Laboratory at the Veterinary Teaching Hospital. After separation of the serum from the blood clot, two approximately equal aliquots of serum were transferred to Corning cryovials for storage at –20°C (–4°F).

Testing of Serum by the Modified Agglutination Test

For this study, the modified agglutination test (MAT) was used as the screening tool to detect IgG antibodies to *T. gondii* (Desmonts and Remington, 1980) (Figure A5). One aliquot of stored frozen serum pairs was thawed and assayed for IgG antibodies. Sera were treated with 2-mercaptoethanol to reduce cross-reacting IgM antibodies. Formalin-fixed tachyzoite stages of the parasite were used as antigen (bioMerieux, Lyon, France). These natural antigens originated from *T. gondii* grown in the peritoneal cavity of mice (Thulliez, et al., 1986). This test is simple to perform and suited for testing large numbers of samples (Wilson et al., 1990). In a 96-well microtiter plate, serum that is positive for *T. gondii* antibodies causes agglutination of parasites, whereas negative samples form a button in the bottom of the well with no agglutination of parasites. This serological method is capable of detecting *T. gondii* antibodies in serum, plasma, aqueous humor, and breast milk, and does not require species specific conjugates used in other immunology assays like the enzyme linked immunosorbent assay (ELISA) and the immunofluorescent

assay (IFAT). Experimental studies have demonstrated that an antibody titer of 32 or greater by the MAT indicates infection with *T. gondii* (Patton and Funk, 1992). This assay has been used to test many species of animals, including human populations and has performed as well or better than the dye test "gold standard" (Desmonts and Remington, 1980; Dubey et al., 1985).

Questionnaire Analysis

Questionnaire information was entered into an Epi Info version 6 (Dean, 1994) database file. The statistical methods used for analysis of the data were those of descriptive epidemiology in which attributes of the analytical units are summarized by frequencies and proportions. Statistical tests of significance were performed for each of the categorical variables (Tables A6, A7, A8, A9, and A10). Risk factors for *T. gondii* infection were evaluated using the odds ratio. The odds ratio is a mathematical expression of the chances in favor of being infected given the presence of the risk factor versus the chances against being infected given the absence of the risk factor (Mauser and Kramer, 1985). For this study a risk factor was defined as any personal, behavioral, or environmental characteristic associated with an increased probability of *T. gondii* infection (Mausner and Kramer, 1985). An odds ratio whose 95% confidence interval does not include one is considered statistically significant (Gillings and Douglas, 1985). For all statistical tests of significance, the probability of falsely rejecting the null hypothesis of no difference in the infection status of women relative to the tested variable was 0.05 (p-value). In some analyses, the odds ratio could not be calculated because a number was too small or a value of zero was entered for cross tabulation. An incalculable odds ratio was recorded on the data tables as "insufficient data". No conclusions were drawn from these analyses. This analysis cannot be used to prove a causal relationship between risky behaviors and *T. gondii* seropositivity because chronically infected people test positive regardless of when infection occurred.

Questionnaire data were also sorted and analyzed for frequency of risky behaviors across age categories regardless of infection status. Some questionnaires from blood drives were completed before blood donation was attempted, subsequently, if the donor was unable to complete the blood donation process, a blood sample was not obtained. Questionnaire data were entered into the database and *T. gondii*

antibody status was listed as unknown. These questionnaires were included in the age-stratified analyses. Data were analyzed using Stat Calc (Epi Info version 6) (Dean, 1994).

Open-ended Questions

Responses to open-ended questions regarding washing of cutting boards, knives and hands after handling raw meat were judged as adequate or inadequate and then treated as a categorical variable for associations to seropositivity and for age category analysis (Table A8). Answers to the remaining open-ended questions were reviewed and coded for summarization (Tables A10 and A11).

For the open-ended question, "How can you tell that meat has been cooked to a well done state?", responses were judged as right, wrong or incomplete (Table A11). Reasons given that pork should be cooked to well done were coded as right answers if a microorganism or health reason was cited, or coded as a wrong answer if they did not think that pork should be cooked to well done. Answers were recorded as "don't know" if a non-specific reason was given. Applying these reasons to the cooking of other meats, such as chicken or wild game, were coded as applying to both, chicken only, wild game only, pork only, or another reason.

Perceptions regarding the safety of the meat supply were divided into reasons believing it is safe because of personal control versus because of government regulations (Table A11). For those that did not believe the meat supply was safe, the reasons were divided into believing that all meat is inherently unclean versus the government has failed to keep the meat supply safe.

The question about knowledge of the organism, *T. gondii*, was coded by source of information and then coded by identification as an infectious agent and sources of infection. Women were also asked if they had been tested for *T. gondii* and the reason for testing (Table A12).

Age Stratified Analysis

Ages were grouped into categories for analysis of behaviors across these age groups 18-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, and 50-53. The primary statistical procedure used for comparisons between age groups and categorical variables was the chi-square test for non-random associations. The chi-square test was used to look for non-random associations between age groups and frequency of behaviors within each age group. The statistical test of significance, the probability of falsely rejecting the

null hypothesis of no difference in the behaviors of women relative to age group was 0.05 (p-value). Odds ratios were used to determine the strength of associations between age groups and behaviors. All age groups were compared to the youngest age group, 18-19 year olds. Data were analyzed using Stat Calc (Epi Info version 6) (Dean, 1994).

Analysis of Seronegative Women

An analysis of seronegative women was conducted to summarize the frequency of behaviors that increase their risk of exposure to *T. gondii*. Eight of the most frequently occurring risky behaviors were combined for analysis. Other combinations of risky behaviors were analyzed such as eating meat, gardening, and cat ownership. For each combination, risky behaviors were selected from a subset of the previous behavior. Data were analyzed using Epi Info version 6 by the "select" command (Dean, 1994).

CHAPTER 7

RESULTS

Twenty-seven blood drives were attended from January 1, 1999 to April 8, 2000, with 753 serum samples and 769 questionnaires collected (Table B2). Additionally, 52 serum samples and questionnaires were collected from women seeking prenatal care at an OB/GYN office at the University of Tennessee Medical Center. Thirty nine percent (314/805) of samples came from small blood drives within the Knoxville, Maryville, and Chattanooga areas (Table B2). Twelve percent (96/805) of the samples were collected during small blood drives held at the University of Tennessee campus and 43% (344/805) of the samples were collected during the Blue-Orange Blood Drive. Most samples, 55%, were collected from the University of Tennessee campus consisting of both students and staff.

A total of 828 women participated in the study by providing a blood sample and/or completing a questionnaire (Table B1). Of these women, 821 completed a questionnaire and age was known; seven serum samples did not have a corresponding questionnaire. Twenty-three women did not provide a blood sample, but completed a questionnaire. These questionnaires were included in demographic descriptions and age-stratified analysis of risky behaviors. Overall, 7.0% (56/805) of all serum samples were positive for *T. gondii* IgG antibodies.

Demographic Information

Analysis of demographic information and *T. gondii* results are summarized in Table 1.

Age

Participant ages ranged from 18 to 53 years with all years represented (Table B3). A total of 821 participants identified their age. The age most frequently sampled was 19 years with 80 (9.7%) participants. The ages with the fewest participants was 51 and 53 years with five (0.6%) participants each. The median age of the study group sampled was 25 years. When age was stratified into five-year groups, 20-24 years was the largest age group with 245 (29.8%) participants and 50-53 years was the smallest age group with 29 (3.5%) participants (Table B5).

Table 1. Summary of seroprevalence of *Toxoplasma gondii* results by age, ethnicity, education, income current residence, childhood residence, and country of birth.

Demographic Information (n=number)	*T. gondii*-seropositive (Percent)	*T. gondii*-seronegative (Percent)
Age group (n=798)	**48 (6.0)**	**750 (94.0)**
18-19 (n=150) 18.8%	5 (3.3)	145 (96.7)
20-24 (n=235) 29.4%	9 (3.8)	226 (96.2)
25-29 (n=101) 12.7%	9 (8.9)	92 (91.1)
30-34 (n=85) 10.6%	12 (14.1)	73 (85.9)
35-39 (n=61) 7.6%	2 (3.3)	59 (96.7)
40-44 (n=75) 9.4%	4 (5.3)	71 (94.7)
45-49 (n=63) 7.9%	7 (11.1)	56 (88.9)
50-53 (n=28) 3.5%	7 (25.0)	21 (75.0)
Ethnicity (n=787)	**53 (6.6)**	**734 (93.4)**
American Indian (n=5) 0.6%	1 (20.0)	4 (80.0)
Asian (n=11) 1.4%	0 (0.0)	11 (100.0)
Black (n=19) 2.4%	1 (5.3)	18 (94.7)
Hispanic (n=10) 1.3%	1 (10.0)	9 (90.0)
White (n=740) 94.0%	50 (6.8)	690 (93.2)
Mixed Race (n=2) 0.3%	0 (0.0)	2 (100.0)
Education (n=794)	**54 (6.8)**	**739 (93.2)**
Less than High School (n=1) 0.1%	0 (0.0)	1 (100.0)
High School-no diploma (n=11) 1.4%	1 (9.1)	10 (90.9)
High School diploma or GED (n=117) 14.7%	12 (10.3)	105 (89.7)
College-no diploma (n=372) 47.0%	19 (5.1)	353 (94.9)
College diploma (n=210) 26.4%	15 (7.1)	195 (92.9)
Advanced degree (n=82) 10.3%	7 (8.5)	75 (91.5)
Income (n=763)	**51 (6.7)**	**712 (93.3)**
Under $12,499 (n=129) 16.9%	4 (3.1)	125 (96.9)
$12,500-19,999 (n=70) 9.2%	5 (7.1)	65 (92.9)
$20,000-27,499 (n=74) 9.7%	4 (5.4)	70 (94.6)
$27,500-34,999 (n=60) 7.9%	2 (3.3)	58 (96.7)
$35,000-44,999 (n=85) 11.1%	8 (9.4)	77 (90.6)
$45,000-74,999 (n=190) 25.0%	19 (10.0)	171 (90.0)
$75,000-124,999 (n=107) 14.0%	5 (4.7)	102 (95.3)
$125,000+ (n=48) 6.3%	4 (8.3)	44 (91.7)
Current Residence (n=797)	**55 (6.9)**	**741 (93.1)**
Rural (n=137) 17.2%	10 (7.3)	127 (92.7)
Farm (n=19) 2.4%	6 (31.6)	13 (68.4)
Small Town (n=100) 12.5%	6 (6.0)	94 (94.0)
Suburban (n=329) 41.4%	21 (6.4)	308 (93.6)
Urban (n=211) 26.5%	12 (5.7)	199 (94.3)
Childhood Residence (n=753)	**54 (7.2)**	**699 (92.8)**
Farm or Rural (n=244) 32.4%	33 (13.5)	211 (86.5)
Without Farm or Rural (n=509) 67.6%	21 (4.1)	488 (95.9)
Place of Birth (n=785)	**53 (6.8)**	**732 (93.2)**
Other Country (n=28)3.6%	3 (10.7)	26 (89.3)
USA (n=756) 96.3%	50 (6.6)	706 (93.4)

Not every age had a positive woman. Ages without positive individuals were 23, 29, 36, 38, 39, 40, 41, 43, 47, and 50 years (Table B4). When divided into five-year age groups, all age groups had at least one positive woman (Table B6). In the 18-19 year age group and the 35-39 year age group, 3.3% of women were positive, which was the lowest for any group. Conversely, 25% of 50-53 year olds were positive, the highest for any group. Other significant associations were in the 30-34 year age group and in the 50-53 year age group, which were more likely to be infected than the other five-year age groupings. When age was divided by the median age of 25 years, those in the older ages, 25-53 years, were more likely to be infected than those in the youngest division, 18-24 years (Table B7).

Ethnicity

Each ethnic group listed on the questionnaire was represented (Table B8). A total of 811 participants identified their ethnic group. Most participants, 93.5%, identified their ethnicity as white. The other ethnic groups identified were black at 2.7%, Asian or Pacific Islander at 1.7%, Hispanic at 1.2% and American Indian or Native American at 0.6%. Two participants (0.2%) were of mixed ethnicity. Not every ethnic group had positive women (Table B9). No Asian/Pacific Islander or mixed race women were positive. Twenty percent of American Indian women were positive. Because few women were sampled from most ethnic groups, no testing of associations between *T. gondii* seropositivity and ethnicity was conducted.

Education

Educational background was divided into six categories, three regarding high school educations and three regarding college educations (Table B10). Most participants, 47.9%, were attending college and 25.9% had a college diploma or technical school degree. Only one participant did not have a high school diploma or GED equivalent. Those with only a high school diploma/GED had the highest percentage positive for *T. gondii* antibodies at 10.3% (Table B11). No statistical association existed between this group and seropositivity for *T. gondii* (Table B12).

Income

Most participants at 25.2% had a household income range of $45,000-74,999 (Table B13). The $45,000-74,999 income group had the highest percentage positive at 10.5% (Table B14). No testing of

associations between income level and *T. gondii* seropositivity was conducted because many college students volunteered the information that they listed their parent's income instead of their personal income.

Current Residence

At the time of sampling, most participants, 41.1% lived in a suburban setting and only 2.3% resided on a farm (Table B15). Length of time at current residence ranged from one month to 55 years, with the mean at 10.5 years. Participants with the highest seropositivity are those that currently live on a farm, with 31.6% positive (Table B16) which was statistically significant (Tables B16 and B17).

Childhood Residence

At any time during their childhood (<18 years), 244 (32.4%) participants had lived on a farm or in a rural environment in addition to a small town, suburban, or urban environment; whereas, 509 (67.6%) had lived only in a small town, suburban, or urban environment (Table B18). Participants that resided on a farm or in a rural environment at any time during their childhood (18 years) were more likely to be infected than those that never lived in a farm or rural setting (Table B19).

Place of Birth

Most participants, 96.0%, were born in the United States (Table B20). There was no difference between those born in the United States and those born outside of the United States and *T. gondii* status (Table B21).

Knowledge, Attitudes, and Practices Regarding *Toxoplasma gondii* Transmission

Knowledge of Toxoplasmosis

When asked "Have you ever heard of toxoplasmosis?" 38.9% of women marked "yes" (Table A12). Most, 61.9%, had learned about toxoplasmosis in school or other independent reading. Some, (28.7%) had previous experience with the disease either personally or through a family member or friend. A few, 9.4%, had learned about this disease from their doctor or veterinarian. Most women (93.1%) were able to correctly associate the disease with cats/cat feces, meat, unwashed fruit, or vegetables, or label it as a disease of pregnant women that may cause damage to a developing fetus. There were 22 women that had been tested before for *T. gondii*, of those 13 reported their test result as negative and two were positive, and

seven could not remember or did not know their result. Most of these women had been tested because they were pregnant and one was considering pregnancy.

Opinions About Meat Safety

When asked if they thought the meat supply was safe, most (69.8%) thought that it was safe (Table A11). When asked why they thought it was safe, two types of reasons were given: 1) safety as a result of their actions, including handling and cooking meat properly (65.9%) and 2) safety as a result of trust in government actions including regulations and inspections of meat processing facilities (34.1%). For those that did not think that the meat supply was safe (7.1%), 2 types of reasons were also given: 1) not safe because all meat has inherent diseases (as news programs have indicated) (63.9%) and 2) not safe because of lost trust in the government to inspect meat and meat processing areas properly (36.1%). There were 23.0% that answered "don't know".

Criteria for Well Done Meat

Most, 79.4%, were able to list at least one of the criteria for knowing that meat has been cooked to well done (Table A11). Some answered the question generally, such as, "cutting into it, color, texture, or taste". A few, 0.4%, admitted that they did not know and 1.2% answered incorrectly by saying that well-done meats would "still have a hint of pink".

Why Cook Pork?

Most, 88.1%, could give a reason to cook pork well including any disease from bacteria to parasites (Table A11). A few, 0.44%, believed that pork did not have to be cooked to well done, and 11.5% did not know why pork should be cooked to well done.

Do Reasons Apply to Other Meats?

Most, 81.2%, said that the reasons to cook pork to well done do apply to other meats such as chicken and wild game (Table A11). The association between chicken and contamination with *Salmonella* was mentioned by 5.1% of women. Some, 11.1%, did not believe that these reasons apply to all meats and 7.6% did not know.

Meat Consumption

Most participants, 93.7% were meat eaters (Table B122). There were no differences across age groups in this behavior. There were no associations with *T. gondii* seropositivity and being a meat eater (Table B123).

Pork Consumption

Analysis of pork consumption and *T. gondii* seroprevalence is summarized in Table 2.

Sausage

Of meat eaters, 75.8% ate sausage (Table B22). When sausage consumption was stratified by age group, 18-19 and 20-24 year olds were less likely to eat pork sausage than the older age groups, but there were no differences in the frequency of consumption (monthly vs. yearly) between age groups (Table B24). The youngest age groups, 18-19 and 20-24 years, were more likely to eat sausage that is less than well-done (Table B26). There were no associations between *T. gondii* seropositivity and eating pork sausage (Tables B23, B25, and B27).

Chops

Of meat eaters, 85.1% ate pork chops (Table B28). Age groups less than 40 years were less likely to eat pork chops than those 40-44 years old when consumption was stratified by age. There were no differences between age groups in frequency of pork chop consumption (Table B30). However, 18-19 year olds were more likely to eat pork chops less than well done than 6 other age groups, 20-24, 25-29, 30-34, 35-39, 40-44, and 45-49 years (Table B32). Eating pork chops was statistically associated with *T. gondii* seropositivity (Table B29). Frequency of consumption of pork chops (Table B31) and cooking preference (Table B33) were not associated with seropositivity for *T. gondii*.

Roast

Of meat eaters, 73.5% ate pork roast (Table B34). There were no differences between age groups in the consumption or frequency of consumption of pork roast (Table B36). Those in the youngest age groups, 18-19 and 20-24 years, were more likely to eat pork roast less than well done than four other age groups, 25-29, 30-34, 35-39.and 40-44 years (Table B38). There were no associations with *T. gondii* seropositivity and consumption of pork roast (Tables B35, B37, and B39).

Table 2. Summary of analysis of pork consumption and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Pork Consumption (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% CI)	p-value
Sausage (n=741)	n=54 (7.3)	n=687 (92.7)		
Yes (n=563) 76.0%	46 (8.2)	517 (91.8)	1.89 (0.84-4.43)	0.10
No (n=178) 34.0%	8 (4.5)	170 (95.5)		
Frequency of consumption (n=562)	n=45 (8.0)	n=517 (92.0)		
Monthly (n=212) 37.7%	18 (8.5)	194 (91.5)	1.11 (0.57-2.15)	0.74
Yearly (n=350) 62.3%	27 (7.7)	323 (92.0)		
Cooking Preference (n=529)	n=43 (8.1)	n=486 (91.9)		
Less than well done (n=46) 8.7%	2 (4.3)	44 (95.7)	0.49 (0.08-2.17)	0.33
Well done (n=483) 91.3%	41 (8.4)	442 (91.5)		
Chops (N=743)	n=52 (7.0)	n=689 (93.0)		
Yes (n=636) 85.6%	52 (8.2)	584 (91.8)	4.67 (1.09-28.18)	0.02*
No (n=107) 14.4%	2 (1.9)	105 (98.1)		
Frequency of consumption (n=636)	n=51 (8.0)	n=585 (92.0)		
Monthly (n=263) 41.3%	23 (8.7)	240 (91.3)	1.18 (0.64-2.18)	0.57
Yearly (n=374) 8.7%	28 (7.5)	345 (92.5)		
Cooking Preference (n=596)	n=49 (8.2)	n=547 (91.8)		
Less than well done (n=84) 14.1%	7 (8.3)	77 (91.7)	1.02 (0.04-2.47)	0.97
Well done (n=512) 85.9%	42 (8.2)	470 (91.8)		
Roast (n=742)	n=54 (7.3)	n=688 (92.7)		
Yes (n=547) 73.7%	45 (8.2)	502 (91.8)	1.85 (0.85-4.15)	0.10
No (n=195) 26.3%	9 (4.6)	186 (95.4)		
Frequency of consumption (n=546)	n=44 (8.1)	n=502 (91.9)		
Monthly (n=168) 30.8%	15 (8.9)	153 (91.1)	1.18 (0.58-2.36)	0.62
Yearly (n=378) 69.2%	29 (7.7)	349 (92.3)		
Cooking Preference (n=512)	n=42 (8.2)	n=470 (91.8)		
Less than well done (n=80) 15.6%	6 (7.5)	74 (92.5)	0.89 (0.33-2.31)	0.80
Well done (n=432) 82.6%	36 (8.3)	396 (91.7)		
Tenderloin (n=742)	n=54 (7.3)	n=688 (92.7)		
Yes (n=530) 71.4%	41 (7.7)	489 (92.3)	1.28 (0.65-2.58)	0.45
No (n=212) 28.6%	13 (6.1)	199 (93.9)		
Frequency of consumption (n=529)	n=40 (7.6)	n=489 (92.4)		
At least monthly (n=164) 30.9%	15 (9.1)	149 (90.9)	1.37 (0.67-2.79)	0.36
Yearly (n=366) 69.1%	25 (6.8)	340 (93.2)		
Cooking Preference (n=496)	n=39 (7.9)	n=457 (92.1)		
Less than well done (n=82) 16.5%	6 (7.3)	76 (92.7)	0.91 (0.33-2.38)	0.84
Well done (n=414) 83.5%	33 (8.0)	381 (92.0)		
Country Ham (n=743)	n=54 (7.3)	n=689 (92.7)		
Yes (n=517) 69.6%	44 (8.5)	473 (91.5)	2.01. (0.95-4.34))	0.05
No (n=226) 30.4%	11 (4.8)	216 (95.2)		
Frequency of consumption (n=516)	n=43 (8.3)	n=473 (91.7)		
Monthly (n=133) 25.8%	10 (7.5)	123 (92.5)	0.86 (0.44-1.89)	0.69
Yearly (n=383) 74.2%	33 (8.6)	350 (91.4)		
Cooking Preference (n=485)	n=42 (8.7)	n=443 (91.3)		
Less than well done (n=60) 12.4%	3 (5.0)	57 (95.0)	0.52 (0.12-1.83)	0.28
Well done (n=425) 87.6%	39 (9.2)	386 (90.8)		

*Statistically significant by Chi Square (p=0.1, 1df)

Tenderloin

Of meat eaters, 71.0% ate pork tenderloin (Table B40). The youngest age groups, 18-19, 20-24, and 25-29 years, were less likely to eat pork tenderloin compared to 30-34 year olds (Table B42). Those in the youngest age group, 18-19 years, were more likely to eat pork tenderloin less than well done than 35-39 year olds (Table B44). There were no associations with *T. gondii* seropositivity and consumption of pork tenderloin (Tables B41, B43, and B45).

Country Ham

Of meat eaters, 69.7% ate country ham (Table B46). The youngest age groups, 18-19, 20-24, 25-29, 30-34, and 35-39 years were more likely to eat country ham than those 40-44 and 45-49 years. Those 18-19 years were more likely to eat it at least monthly than those 30-34 and 35-39 years (Table B48) and also were more likely to eat it less than well done compared to those 35-39 years (Table B50). There were no associations with *T. gondii* seropositivity and consumption of country ham (Tables B47, B49, and B51).

Beef Consumption

Analysis of beef consumption and *T. gondii* seroprevalence is summarized in Table 3.

Hamburger

Of meat eaters, 95.3% ate hamburger (Table B52). There were no differences between the age groups in the consumption of hamburger (Table B52) or the frequency of consumption (Table B54). The youngest age group, 18-19 years, were more likely to eat it less than well done than those 45-49 years (Table B56). There were no associations with *T. gondii* seropositivity and consumption of hamburger (Tables B53, B55, and B57).

Steak

Of meat eaters, 91.1% ate steak (Table B58). There were no differences between age groups for the consumption of steak (Table B58), the frequency of consumption (Table B60), or the preference for doneness (Table B62). Approximately one half of steak eaters prefer to eat it less than well- done (Table B62). There were no associations with *T. gondii* seropositivity and consumption of steak (Tables B59, B61, and B63).

Table 3. Summary of analysis of beef consumption and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Beef Consumption (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% CI)	p-value
Hamburger (n=744)	n=54 (7.3)	n=690 (92.7)		
Yes (n=710) 95.4%	52 (7.3)	658 (92.7)	1.26 (0.28-7.85)	0.75
No (n=34) 4.6%	2(5.9)	32 (94.1)		
Consumption Frequency (n=709)	n=51 (7.2)	n=658 (92.8)		
At least monthly (n=585) 82.5%	40 (7.0)	545 (93.0)	0.75 (0.36-1.61)	0.43
Yearly (n=124) 17.5%	11 (8.9)	113 (91.1)		
Cooking Preference (n=674)	n=50 (7.4)	n=624 (92.6)		
Less than well done (n=171) 25.4%	8 (4.7)	163 (95.3)	0.54 (0.23-1.22)	0.11
Well done (n=503) 74.6%	42 (8.3)	461 (91.7)		
Steak (N=742)	n=54 (7.3)	n=688 (92.7)		
Yes (n=678) 91.4%	52 (7.7)	626 (92.3)	2.58 (0.60-15.66)	0.18
No (n=64) 8.6%	2 (3.1)	62 (96.9)		
Consumption Frequency(n=678)	n=51 (7.5))	n=627 (92.5)		
At least monthly (n=468) 67.0%	39 (8.3)	429 (91.7)	1.50 (0.74-2.73)	0.23
Yearly (n=210) 31.0%	13 (5.7)	198 (94.3)		
Cooking Preference (n=641)	n=49 (7.6)	n=592 (92.4)		
Less than well done (n=321) 50.1%	22 (6.9)	299 (93.1)	0.80 (0.43-1.49)	0.45
Well done (n=320) 49.9%	27 (8.4)	293 (91.6)		

Poultry Consumption

Analysis of poultry consumption and *T. gondii* seroprevalence is summarized in Table 4.

Chicken

Of meat eaters, 98.8% ate chicken (Table B64). There were no differences between the age groups in the consumption of chicken (Table B64) or the frequency of consumption (Table B66). The younger age groups, 18-19 and 20-24 years, were more likely to eat it less than well done than those were in the five older age groups, 25-29, 30-34, 35-39, 40-44, and 45-49 years (Table B68). There were no associations with *T. gondii* seropositivity and consumption of chicken (Tables B65, B67, and B69).

Turkey

Of meat eaters, 88.0% ate domestic turkey (Table B70). There was no difference between the age groups in the consumption of turkey. The youngest age groups, 18-19 and 20-24 years were more likely to eat it at least monthly compared to all the older age groups (Table B72) and were more likely to eat it less than well done than those 25-29, 35-39, 40-44, and 45-49 years (Table B74). There were no associations with *T. gondii* seropositivity and consumption of turkey (Table B71, B73, and B75).

Other Meat Consumption

Analysis of other meat consumption and *T. gondii* seropositivity is summarized in Table 5.

Lamb

Of meat eaters, 14.6% ate lamb (Table B76). There were no differences between the age groups in the consumption of lamb (Table B76), the frequency of consumption (Table B78), or the preference of doneness (Table B80). There were no associations with *T. gondii* seropositivity and consumption of lamb (Tables B77, B79, and B81).

Mutton

Of meat eaters, 4.2% ate mutton (Table B82). There were no differences between the age groups in the consumption of mutton (Table B82), the frequency of consumption (Table B84), or the preference of doneness (Table B86). There were no associations with *T. gondii* seropositivity and consumption of mutton (Tables B83, B85, and B87).

Table 4. Summary of analysis of poultry consumption and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Poultry Consumed (n=number)	**Seropositive (Percent)**	**Seronegative (Percent)**	**Odds Ratio (95% CI)**	**p-value**
Chicken (n=739)	n=54 (7.3)	n=685 (92.7)		
Yes (n=730) 98.8%	54 (7.4)	676 (92.6)	Insufficient data	0.40
No (n=9) 1.2%	0 (0.0)	9 (100.0)		
Frequency of consumption (n=730)	n=53 (7.3)	n=677(92.7)		
At least monthly (n=648) 88.8%	46 (7.1)	603 (92.9)	0.69 (0.30-1.65)	0.36
Yearly (n=82) 11.2%	8 (9.8%)	74 (90.2)		
Cooking Preference (n=688)	n=51 (7.4)	n=637 (92.6)		
Less than well done (n=69) 10.0%	3 (4.3)	66 (95.7)	0.54 (0.13-1.87)	0.31
Well done (n=619) 90.0%	48 (7.8)	571 (92.2)		
Domestic Turkey (n=736)	n=52 (7.1)	n=684 (92.9)		
Yes (n=647) 87.9%	46 (7.1)	601 (92.9)	1.06 (0.42-2.85)	0.90
No (n=89) 12.1%	6 (6.7)	83 (93.3)		
Frequency of consumption (n=645)	n=46 (7.1)	n=599 (92.9)		
At least monthly (n=191) 29.6%	13 (6.8)	178 (93.2)	0.93 (0.45-1.89)	0.83
Yearly (n=454) 70.4%	33 (7.3)	421 (92.7)		
Cooking Preference (n=592)	n=42 (7.1)	n=550 (92.9)		
Less than well done (n=70) 11.8%	5 (7.1)	65 (92.9)	1.01 (0.34-2.81)	0.99
Well done (n=522) 88.2%	37 (7.1)	485 (92.9)		

Table 5. Summary of analysis of other meat consumption and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Other Meats Consumed (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% CI)	p-value
Lamb (n=740)	n=53 (7.2)	n=687 (92.8)		
Yes (n=110) 14.9%	7 (6.4)	103 (93.6)	0.86 (0.35-2.06)	0.72
No (n=630) 85.1%	46 (7.3)	584 (92.7)		
Frequency of consumption (n=110)	n=7 (6.4)	n=103 (93.6)		
At least monthly (n=10) 9.1%	1 (10.0)	9 (90.0)	1.74	0.62
Yearly (n=100) 90.9%	6 (6.0)	94 (94.0)	(Insufficient data)	
Cooking Preference (n=99)	n=7 (7.1)	n=92 (92.9)		
Less than well done (n=32) 32.3%	2 (6.3)	30 (93.7)	0.83 (0.10-5.25)	0.83
Well done (n=67) 67.7%	5 (7.5)	62 (92.5)		
Mutton (sheep) (n=740)	n=53 (7.2)	n=687 (92.8)		
Yes (n=31) 4.2%	2 (6.5)	29 (93.5)	Insufficient data	0.88
No (n=709) 95.8%	51 (7.2)	658 (92.8)		
Frequency of consumption (n=31)	n=2 (6.5)	n=29 (93.5)		
At least monthly (n=1) 3.2%	0 (0.0)	1 (100.0)	Insufficient data	0.79
Yearly (n=30) 96.8%	2 (6.7)	28 (93.3)		
Cooking Preference (n=30)	n=2 (6.7)	n=28 (93.3)		
Less than well done (n=10)	0 (0.0)	10 (100.0)	Insufficient data	0.30
Well done (n=20)	2 (10.0)	18 (90.0)		
Chevon (goat) (n=742)	n=53 (7.1)	n=689 (92.9)		
Yes (n=21) 2.8%	1 (4.8)	20 (95.2)	0.64 (0.03-4.66)	0.67
No (n=721) 97.2%	52 (7.2)	669 (92.8)		
Frequency of consumption (n=21)	n=1 (4.8)	n=20 (95.2)		
At least monthly (n=1) 4.8%	0 (0.0)	1 (100.0)	Insufficient data	0.82
Yearly (n=20) 95.2%	1 (5.0)	19 (95.0)		
Cooking Preference (n=20)	n=1 (5.0)	n=19 (95.0)		
Less than well done (n=4) 20.0%	0 (0.0)	4 (100.0)	Insufficient data	
Well done (n=16) 80.0%	1 (6.3)	15 (93.7)		
Wild Game (n=744)	n=54 (7.3)	n=690 (92.7)		
Yes (n=159) 21.4%	11 (6.9)	148 (93.1)	0.94 (0.44-1.94)	0.85
No (n=585) 78.6%	43 (7.3)	542 (92.7)		
Frequency of consumption (n=100)	n=10 (10.0)	n=90 (90.0)		
Monthly (n=20) 20.0%	2 (10.0)	18 (90.0)	1.00 (0.13-5.83)	1.0
Yearly (n=80) 80.0%	8 (10.0)	72 (90.0)		
Cooking Preference (n=94)	n=10 (10.6)	n=84 (89.4)		
Less than well done (n=15) 16.0%	2 (13.3)	13 (86.7)	1.37 (0.18-8.31)	0.71
Well done (n=79) 84.0%	8 (10.1)	71 (89.9)		

Chevon (Goat)

Of meat eaters, 2.8% ate chevon (Table B88). There were no differences between the age groups in the consumption of chevon (Table B88), the frequency of consumption (Table B90), or the preference of doneness (Table B92). There were no associations with *T. gondii* seropositivity and consumption of chevon (Tables B89, B91, and B93).

Wild Game

Of meat eaters, 13.7% ate wild game (Table B94). There were no differences between the age groups in the consumption of wild game (Table B94), the frequency of consumption (Table B96), or the preference of doneness (Table B98). There were no associations with *T. gondii* seropositivity and consumption of wild game (Table B95, B97, and B99).

Hygienic Behaviors and *Toxoplasma gondii* Seropositivity

Analysis of hygienic behaviors and *T. gondii* seropositivity is summarized in Table 6.

Hygienic Behaviors

Cleaning of Cutting Board, Knife, and Hands After Cutting/Handling Raw Meat

Most women did wash cutting boards (95.8%) (Table B100) and knives (94.7%) (Table B102) in the dishwasher, or with soap and/or bleach after cutting raw meat. Also, a very high percentage, 98.9%, said they washed their hands with soap after handling raw meat (Table B104). There were no differences across age groups in this behavior. There were no associations with *T. gondii* seropositivity and not washing cutting boards, knives, or hands after contact with raw meat (Tables B101, B103, and B105).

Wash Vegetables and Fruits Before Eating

Most always washed vegetables (72.3%) (Table B106) and fruits (64.7%) (Table B108) before eating. The youngest age group, 18-19 years, was more likely not always to wash vegetables and fruits compared to those 30-34 and 45-49 years. There were no associations with *T. gondii* seropositivity and not always washing vegetables and fruits before eating (Tables B107 and B109).

Table 6. Summary of analysis of hygienic behaviors and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Hygienic Behaviors (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% CI)	p-value
Wash Cutting Board with Soap or Bleach after Cutting Raw Meat (n=653)	50 (7.7)	603 (92.3)		
Yes (n=626) 95.9%	48 (7.7)	578 (92.3)	Insufficient data	0.96
No (n=27) 4.1%	2 (7.4)	25 (92.6)		
Wash Knife with Soap or Bleach after Cutting Raw Meat (n=653)	53 (8.1)	600 (91.9)		
Yes (n=674) 94.5%	51 (7.6)	623 (92.4)	0.72 (0.12-3.19)	0.65
No (n=36) 5.5%	2 (5.6)	34 (94.4)		
Wash Hands After Handling Raw Meat (n=724)	54 (7.5)	670 (92.5)		
Yes (n=716) 98.9%	54 (7.5)	663 (92.5)	0.00 (0.00-8.70)	0.42
No (n=8) 1.1%	0 (0.0)	8 (100)		
Wash Vegetables Before Eating (n=790)	53 (6.7)	737 (93.3)		
Always (n=570) 72.2%	44 (7.7)	526 (92.3)	0.51 (0.23-1.11)	0.07
Frequently, Occasionally, or Never (n=220) 27.8%	9 (4.1)	211 (95.9)		
Wash Fruit Before Eating (n=790)	53 (6.7)	737 (93.3)		
Always (n=509) 64.4%	35 (6.9)	474 (93.1)	0.93 (0.49-1.73)	0.80
Frequently, Occasionally, or Never (n=281) 35.6%	18 (6.4)	263 (93.6)		
Wash Hands Before Handling Food (n=792)	53 (6.7)	739 (93.3)		
Always (n=561) 70.8%	40 (7.1)	521 (92.9)	0.78 (0.39-1.54)	0.44
Frequently, Occasionally, or Never (n=231) 29.2%	13 (5.6)	218 (94.3)		
Wash Hands Before Eating (n=790)	54 (6.8)	737 (93.2)		
Always (n=459) 58.1%	35 (7.6)	424 (92.4)	0.70 (0.37-1.30)	0.23
Frequently, Occasionally, or Never (n=331) 41.9%	18 (5.4)	313 (94.6)		
Wash Hands After Handling Pets (n=788)	54 (6.8)	735 (93.2)		
Always (n=426) 54.1%	31 (7.3)	395 (92.7)	0.82 (0.45-1.50)	0.05
Frequently, Occasionally, or Never (n=362) 45.9%	22 (6.1)	340 (93.9)		
Drink Unpasteurized Milk (n=786)	51 (6.5)	735(93.5)		
Always, Frequently, Occasionally (n=47) 6.0%	4 (8.5)	43 (91.5)	1.37 (0.04-4.21)	0.56
Never (n=739) 94.0%	47 (6.4)	692 (93.6)		
Drink Unpasteurized Goat's Milk (n=788)	53 (6.8)	735 (93.2)		
Always, Frequently, Occasionally (n=10) 1.3%	0(0.0)	10 (100.0)	0.00 (0.00-7.42)	0.39
Never (n=778) 98.7%	53 (6.8)	725 (93.2)		
Eat Raw Eggs (found in raw cookie dough) n=790	53 (6.7)	737 (93.3)		
Always, Frequently, Occasionally (n=392) 49.6%	24 (6.1)	368 (93.9)	0.83 (0.46-1.50)	0.51
Never (n=398) 50.4%	29 (7.3)	369 (92.7)		
Vegetarian (n=797)	55 (6.9)	742 (93.1)		
Yes (n=50) %	1 (2.0)	49 (98.0)	3.82 (0.55-75.82)	0.16
No (n=747) %	54 (7.3)	693 (92.7)		

Wash Hands Before Handling Food

Most, 70.4% always washed hands before handling food (Table B110). The youngest age group, 18-19, was more likely not always to wash their hands before handling food compared to the three oldest age groups, 40-44, 45-49, and 50-53 years. There was no association with *T. gondii* seropositivity and not always washing hands before handling food (Table B111).

Wash Hands Before Eating

Only 42.1% of women always washed their hands before eating (Table B112). The youngest age group, 18-19 years, was more likely not always to wash their hands before eating compared to all other older age groups. When 18-19 year olds were compared to 50-53 year olds, they were more than 11 times more likely not to wash their hands before eating. There was no association with *T. gondii* seropositivity and not always washing hands before eating (Table B113).

Wash Hands After Handling Animals

Over half, 54.1%, always washed hands after handling animals (Table B114). There were no differences across age groups in this behavior. There were no associations with *T. gondii* seropositivity and not always washing hands after handling animals (Table B115).

Dietary Preferences

Drink Unpasteurized Milk

Most did not drink unpasteurized cow (94.2%) (Table B116) or goat (98.8%) (Table B118) milk. There were no differences across age groups in this behavior. There were no associations with *T. gondii* seropositivity and drinking unpasteurized milk (Table B117 and B119).

Eat Raw Eggs (found in raw cookie dough)

Almost half (49.8%) of the women ate raw eggs that may be found in raw cookie dough (Table B120). Those in the youngest age group, 18-19 years, were more likely to eat raw eggs compared to those 40-44 and 50-53 years. There were no associations with *T. gondii* seropositivity and eating raw eggs (Table B121).

Vegetarian Lifestyle

Only 6.5% of women surveyed were vegetarians (Table B122). There were no differences across age groups in this behavior. There were no associations with *T. gondii* seropositivity and following a vegetarian diet (Table B123).

Risky Behaviors That Increase Exposure to Oocysts (Environmental Stage) of *Toxoplasma gondii*

Analysis of risky behaviors and *T. gondii* seropositivity is summarized in Table 7.

Risky Occupation or Hobby

Only 25.4% of women surveyed had an occupation or hobby that may be risky for *T. gondii* exposure (Table B124). Those in the youngest age groups, 18-19 and 20-24 years, were less likely to have a risky occupation or hobby compared to those 25-29, 30-34, and 45-49 years. There was not an association with *T. gondii* seropositivity and having a risky occupation or hobby (Table B 125).

Keep a Garden

When flower and vegetable gardening was looked at as a specific risky occupation or hobby, 56.8% of women gardened occasionally (Table B126). Those in the youngest age groups, 18-19, 20-24, and 25-29 years were less likely to keep a garden, at least occasionally, compared to those in the five older age groups, 30-34, 35-39, 40-44, 45-49, and 50-53 years. There was not an association with *T. gondii* seropositivity and keeping a flower or vegetable garden (Table B127). Of those that gardened, 16.3% always wore gloves (Table B128). Those in the youngest age groups, 18-19 and 20-24 years, were more likely to wear gloves than those 30-34 years. There was no association with *T. gondii* seropositivity and not always wearing gloves while gardening (Table B129). Most, 99.8% do not always wear a mask while gardening (Table B130), but this was not associated with *T. gondii* seropositivity (Table B131).

Cat Ownership

Less than half (45.0%) of women surveyed owned a cat(s) (Table B132). There were no differences across age groups for cat ownership. There were no association with *T. gondii* seropositivity and owning a cat (Table B133). Only a few women, 1.8%, had cats that did not use an indoor litter box (Table B134). There was an association with *T. gondii* seropositivity and having a cat that used the outside for defecation (Table B135). Of women that had cats that used a litter box, 73.1% of these women scooped

Table 7. Summary of analysis of risky behaviors and seroprevalence of *Toxoplasma gondii* in women of childbearing age from the Knoxville, Tennessee area.

Risky Behavior (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% CI)	p-value
Risky Occupation or Hobby (n=780)	52 (6.7)	728 (93.3)		
Yes (n=200) 25.6%	14 (7.0)	186 (93.0)	1.07 (0.54-2.10)	0.83
No (n=580) 74.4%	38 (6.6)	542 (93.4)		
Garden (n=789)	51 (6.5)	738 (93.5)		
Always, Frequently, Occasionally (n=455) 57.7%	33 (7.3)	422 (92.7)	1.37 (0.73-2.59)	0.29
Never (n=334) 42.3%	18 (5.4)	316 (94.6)		
Wear Gloves While Gardening (n=455)	34 (7.5)	421 (92.5)		
Frequently, Occasionally, or Never (n=380) 83.5%	30 (7.9)	350 (92.1)	1.52 (0.49-5.26)	0.44
Always (n=75) 16.5%	4 (5.3)	71 (94.7)		
Wear Mask While Gardening (n=454)	34 (7.5)	420 (92.5)		
Frequently, Occasionally, or Never (n=453) 99.8%	34 (7.5)	419 (92.5)	Insufficient data	
Always (n=1)	0 (0.0)	1 (100.0)		
Own cat (n=785)	49 (6.2)	736 (93.8)		
Yes (n=355) 45.1%	22 (6.2)	333 (93.8)	0.99 (0.53-1.83)	0.96
No (n=431) 54.9%	27 (6.3)	403 (93.7)		
Cat Uses Litter Box (n=275)	16 (5.8)	259 (94.2)		
No (n=5) 1.8%	2 (18.9)	3 (81.2)	12.19 (1.29-101.53)	<0.01*
Yes (n=270) 98.2%	14 (5.2)	256 (94.8)		
I Scoop Cat's Litter (n=272)	14 (5.1)	258 (94.8)		
Yes (n=199) 73.1%	11 (5.5)	188 (94.5)	1.37 (0.34-6.37)	0.64
No (n=73) 26.9%	3 (4.1)	70 (95.9)		
Chew Fingernails (n=786)	53 (6.7)	733 (93.3)		
Always, Frequently, Occasionally (n=440) 55.9%	27 (6.1)	413 (93.9)	0.80 (0.44-1.46)	0.44
Never (n=346) 44.1%	26 (7.5)	320 (92.5)		
Pets Sleep in Bed (n=709)	49 (6.9)	660 (93.1)		
Always, Frequently, Occasionally (n=351) 49.4%	20 (5.7)	331 (94.3)	0.69 (0.36-1.28)	0.21
Never (n=358) 50.6%	29 (8.1)	329 (91.9)		
Play in Children's Sandbox (n=780)	53(6.8)	727 (93.2)		
Always, Frequently, Occasionally (n=299) 38.3%	17 (5.7)	282 (94.3)	0.75 (0.39-1.40)	0.33
Never (n=481) 61.7%	36 (7.5)	445 (92.5)		

*Statistically significant by Chi Square (p=0.1, 1df)

the litter box (Table B136). Those in the youngest age groups, 18-19 and 20-24 years, were less likely to scoop the litter box compared to the five next oldest groups, 25-29, 30-34, 35-39, 40-44, and 45-49 years. There was no association with *T. gondii* seropositivity and scooping the litter box (Table B137).

Other Activities

More than half of women surveyed, 55.9%, chewed their fingernails (Table B138). Those 18-19 years were twice as likely to chew their fingernails compared to those 40-44 years. There was not an association with *T. gondii* seropositivity and chewing fingernails (Table B139).

Almost half of women surveyed, 49.3%, allowed pets to sleep in their bed (Table B140). Those 18-19 years were less likely to let pets sleep in their bed than those 25-29 years. There was not an association with *T. gondii* seropositivity and letting pets sleep in their bed, at least occasionally (Table B141).

A few women, 38.0%, played in a child's sandbox (Table B142). Those in the youngest age group, 18-19 years, were more likely to play in a child's sandbox, at least occasionally, compared to all other age groups except those 35-39 years. There was not an association with *T. gondii* seropositivity and playing in a child's sandbox (Table B143).

Behaviors of Seronegative Women

Risky behaviors for *T. gondii* exposure identified most frequently in seronegative women (749) were eating meat (94.3%), gardening (57.2%), and chewing fingernails (56.3%). There were 13 (1.7%) women that participated in the eight most frequent risky behaviors (Table 8). When eating meat, gardening, and owning a cat were combined, 189 women did all three behaviors (Table 9). Of those that ate meat, 181 women did not wash fruits and vegetables prior to consumption (Table 10). Of women that owned a cat, 115 did not wash hands after handling animals and they chewed their fingernails (Table 11). Among women that gardened and did not wear gloves, 200 chewed fingernails, 152 did not wash hands before eating, and 107 did not wash hands before handling food (Table 12). In those that gardened, owned a cat, did not wear gloves while gardening, and let cat defecate outside, 33 chewed fingernails, 25 did not wash hands before eating, and 18 did not wash hands before handling food (Table 13).

Table 8. Combinations of the eight most frequent risky behaviors for *Toxoplasma gondii* exposure by seronegative women of childbearing age from the Knoxville, Tennessee area. Each square is a subset of the square to the left and square below.

	Eat meat	Garden	Chew fingernails	Let pets sleep in bed	Own cat	Play in child's sandbox	Don't always wash fruits before consumption	Don't always wash vegetables before consumption
Eat meat	693	388	217	100	70	37	18	13
Garden		422	231	109	78	38	18	13
Chew fingernails			413	190	131	51	22	17
Let pets sleep in bed				331	226	79	36	30
Own cat					333	120	52	41
Play in child's sandbox						282	104	81
Don't always wash fruits before consumption							262	200
Don't always wash vegetables before consumption								211

Table 9. Combination of three risky behaviors for *Toxoplasma gondii* exposure by seronegative women of childbearing age from the Knoxville, Tennessee area.

	Eat meat	Garden	Own cat
Eat meat	693	388	189
Garden		422	209
Own cat			333

Table 10. Combination of three risky behaviors for *Toxoplasma gondii* exposure associated with eating meat and kitchen hygiene by seronegative women of childbearing age from the Knoxville, Tennessee area.

	Eat meat	Don't always wash fruits before consumption	Don't always wash vegetables before consumption
Eat meat	693	236	181

Table 11. Combination of three risky behaviors for *Toxoplasma gondii* exposure associated with cat ownership by seronegative women of childbearing age from the Knoxville, Tennessee area.

	Own cat	Don't wash hands after handling animals	Chew fingernails
Own cat	333	188	115

Table 12. Combination of risky behaviors for *Toxoplasma gondii* exposure associated with gardening by seronegative women of childbearing age from the Knoxville, Tennessee area.

	Garden	Do not always wear gloves while gardening	Chew fingernails
Garden	422	350	200

			Do not always wash hands before eating
Garden	422	350	152

			Do not always wash hands before handling food
Garden	422	350	107

Table 13. Combination of risky behaviors for *Toxoplasma gondii* exposure associated with gardening and cat ownership by seronegative women of childbearing age from the Knoxville, Tennessee area.

	Garden	Own cat	Do not always wear gloves while gardening	Let cat defecate outside	Chew fingernails
Garden	422	209	175	68	33
					Do not always wash hands before eating
Garden	422	209	175	68	25
					Do not always wash hands before handling food
Garden	422	209	175	68	18

CHAPTER 8

DISCUSSION

The incidence of congenital toxoplasmosis in the United States is estimated as high as 4,000 cases with approximately $5.2 billion spent annually on care for congenitally infected children (Roberts and Frenkel, 1991). In 1998, the Centers for Disease Control and Prevention recommended that the first approach for controlling the "burden of congenital toxoplasmosis" be through surveys that investigate how foodborne or soil transmission may contribute to infection in the mother. The evaluation of behaviors in women of childbearing age from the Knoxville, Tennessee area contributes to an overall understanding of the occurrence of risky behaviors for *T. gondii* exposure in the United States today. This information can be used to develop age-specific guidelines that modify behaviors and decrease exposure to *T. gondii*.

Study Design

The data collected in this study represent a convenience sample of women who consented to be in the study. The selection of this study group was biased because not every woman of childbearing age from the Knoxville, Tennessee area had an equal chance of being included in the study. Because this was a convenience sampling, it should not necessarily be considered representative of all Knoxville women. Women that agreed to be in the study came from two sources: 1) those that were both willing and able (healthy) to donate blood to Medic Regional Blood Center and Blood Assurance and 2) those that were pregnant during the sampling period and also seeking prenatal care at The University of Tennessee Medical Center. The ages of women sampled in this study generally reflects "childbearing age" because most women in this study were less than 35 years and, in the United States, those less than 35 years have a higher birth rate than those 35 years and older. The ethnic identities of women sampled was 93.5% white with less than three percent of other ethnic groups sampled. Ethnicity was not sampled in proportion to those actually giving birth in Tennessee or the United States where approximately 76% of white, 22% of black, and 1% of both American Indian and Asian/Pacific Islander women account for all recorded births. According to this guideline, blacks were under represented in this study.

Currently there is no test to distinguish infections from oocysts as opposed to tissue cysts (Dubey, 1995a; 2000). Therefore, epidemiological surveys remain the only way to assess the relative importance of different sources of *T. gondii* infection to people (Dubey, 2000). In the Knoxville study, data were analyzed to identify associations between *T. gondii* seropositivity and behaviors that increase exposure to *T. gondii*. A causal relationship between current behavior and seropositivity cannot be established with prevalence studies because these results only represent a "snap-shot" in time (Mausner and Kramer, 1985). Time is a confounding factor in this study because infections may have occurred many years before sampling so a temporal sequence of events (when and how infection occurred) cannot be established. For example, a woman infected as a child would have a life-long measurable antibody titer that cannot be attributed to her current behavior as an adult. This Knoxville study and other published *T. gondii* seroprevalence studies cannot be used to prove causality between a specific behavior and *T. gondii* seropositivity in chronically-infected persons because it is impractical to follow seronegative people through time and determine which behavior caused *T. gondii* infection. These studies do, however, reveal behaviors in at-risk populations, such as seronegative women, that increase the likelihood of exposure to *T. gondii*.

This study was designed as a point prevalence study to identify the occurrence of risky behaviors for *T. gondii* exposure in women of childbearing age in the Knoxville, Tennessee area. Women 18-19 years old were assumed to behave in a manner that increased their risk of exposure because they were younger and had not accumulated life experiences. They were compared to women in all other age groups for statistical analysis. For this study consuming less than well done meat, unhygienic kitchen practices, some personal habits, contact with cats, and behaviors that allowed contact with soil were considered risky behaviors for exposure to *T. gondii*. Previous studies established that some of these behaviors were associated with seroconversion in women during pregnancy (Kapperud et al., 1996; Baril et al., 1999). A seronegative mother that seroconverts during pregnancy is in danger of passing the infection to her developing baby. The analysis of risky behaviors of seronegative women from this Knoxville study can be used to supplement existing educational guidelines for modifying risky behaviors in women aimed at preventing birth defects caused by *T. gondii* infection during pregnancy. A mother who is seropositive

before becoming pregnant is presumed not at risk of transferring subsequent infections to her fetus (Feldman, 1974). Recommendations for preventing *T. gondii* exposure do not apply to seropositive mothers; however, most women do not know their antibody status and these behavioral modifications may be protective against other foodborne or environmentally-acquired pathogens in addition to *T. gondii.*

Childbearing Age and *Toxoplasma gondii* Seropositivity

The overall seroprevalence in this study group of women of childbearing age, 18-53 years, from the Knoxville, Tennessee area is low at 7.0% compared to the 10-15% national seroprevalence of women of childbearing age, 15-45 years identified in the NHANES III study (CDC, 2000a). The NHANES III study was designed as a probability sampling to obtain nationally representative information on the health and nutrition status of the population of the United States. The lower seroprevalence of the Knoxville study group of mostly college-aged women with a median age of 25 years may reflect selection bias. As expected, when participants in the Knoxville study group were divided by the median age of 25 years, participants in the older ages, 25-53 years, were three times more likely to be seropositive than those 18-24 years (OR = 2.91). When stratified by age, 18-19 year olds were statistically less likely to be infected than 30-34 and 50-53 year olds. In developed countries such as the United States, infections are usually acquired after the age of 10 years (Roberts and Frenkel, 1991) with an increase in the rate of infection during adolescence which continues to rise throughout early adulthood (Feldman and Miller, 1956). This also appears to be the pattern in the Knoxville study group. The source of *T. gondii* infection is unknown for these positive women; however, *T. gondii* exposure and infection can occur at any age.

The primary childbearing ages according to the National Vital Statistics Reports published by the CDC, were 25-29 years with a birth rate of 113 pregnancies per 1,000 women (CDC, 1999a). Birth rates were also high (83-110 per 1,000) among older teenagers (18-19 years), women in the early 20s (20-24 years), and those in the early 30s (30-34 years). In the Knoxville sample, women 30-34 years of age were more likely to be seropositive compared to those in younger age groups. This natural trend for seroconversion during the peak childbearing ages for women has important implications. Primary *T. gondii* exposure and infection coincide with pregnancy causing congenital infection approximately two to four

times in every 1000 births (Roberts and Frenkel, 1991). In the Knoxville study group, seroconversion appears to be occurring in the early adult years starting in the 25-29 year age group (8.9% seropositive) and the 30-34 year age group (14.1% seropositive) compared to the youngest age groups, 18-24 years with <4% seropositive. Although, the current birth rate for middle-aged women, those aged 35-49 years tend to be lower (63-0.4 per 1,000) these rates have increased over the past 10 years, with many of these women experiencing a first birth (CDC, 1999a). Nationally, 20% of babies are born to mothers over 40 years of age (CDC, 1999a). Although the birth rate is lower in women over 35 years, there is still a possibility that primary infection and pregnancy may occur at the same time. In the Knoxville study group, 93% of all women tested were seronegative and at risk of *T. gondii* infection. This, unfortunately, may coincide with pregnancy.

Residence

In the Knoxville study group, *T. gondii* seropositivity was associated with a rural/farm childhood residence or currently living on a farm. Residents living in a rural or farm environment, often have "chores" and other activities that bring them into close contact with soil that is potentially contaminated with *T. gondii* oocysts from resident farm or feral cats (Weigel et al., 1999). Studies from different geographical areas have also shown a statistically significant association between rural residence and *T. gondii* seropositivity (Ganley and Comstock, 1980; Weigel et al., 1999). Participants in the Knoxville study with a childhood rural/farm residence were almost four times more likely to be seropositive (odds ratio 3.63). Although only 12.3% of women lived on a farm, 31.6% of them were seropositive for *T. gondii* compared to 6.1% who were positive and lived in urban or suburban environments (80.4% of women sampled). Those currently living on a farm were almost seven times more likely to be seropositive (odds ratio 6.86) than those living in any other environments. This association does not prove a causal relationship between seropositivity and currently living on a farm because infections may have occurred while living elsewhere by various sources of exposure. Seronegative women (1.6%) that lived on farms should take precautions to prevent *T. gondii* exposure during activities associated with soil exposure. Most college women listed their residence as urban and may live in dormitories, apartments, or condominiums

and may not have activities that bring them into contact with the outdoors and potentially contaminated soil; however, some of these women may travel to rural or farm homes. Because these age groups (18-24 years) are less likely to be seropositive, they are at risk for first-time exposure to *T. gondii*, and should be cautioned about possible contaminated farm environments.

Consumption of Undercooked Meat

Approximately half (50.2%) of women in the Knoxville study preferred rare beef, and 8.7-33.3% preferred the other meats listed on the questionnaire less than well done. Remarkably, a high percentage (17.6-75.0%) of those in the youngest age group, 18-19 years, also preferentially consumed the other meats, pork, poultry, small ruminants, and wild game, less than well done. This finding is surprising in the wake of recent outbreaks of *Escherichia coli* O157:H7 in undercooked hamburger (Riley et al., 1983; Bell et al., 1994, Olsen et al., 2000) and Salmonellosis from chicken (CDC, 1987). This list of other foodborne illnesses acquired by meat consumption confirms that people do eat meat undercooked, sometimes accidentally. When undercooked meat is consumed, *T. gondii* infection can occur. In this study, a few participants, 5.1%, volunteered that *Salmonella* sp. contamination of chicken is a reason to cook meat to well done. Most women were able to identify the qualities of cooked meat such as clear juices and an internal temperature of at least 150°F, so it is assumed that they prefer to eat meat less than well done. Foodborne illness from *T. gondii* infection has been attributed to the consumption of raw or undercooked pork, lamb, and other meats including wild game (Dubey and Beattie, 1988). Currently, beef is not considered a source of infection because experimental studies found that tissue cysts do not persist in cattle or beef products (Dubey, 1986b; Dubey and Thulliez, 1993); however, this Knoxville study and other studies (Kimball et al., 1974; Kapperud et al., 1996; Baril et al., 1999; Cook et al., 2000) show that women commonly consume rare beef. This finding may be important for understanding *T. gondii* transmission if future research identifies that beef is a source of *T. gondii*. Also, adulteration of ground beef with other meats, such as pork, may expose people to *T. gondii*; therefore, eating beef should be considered a source of *T. gondii* exposure (Dubey, 2000). Preferences for undercooked chicken and turkey also may play a role

in the transmission of *T. gondii* because viable tissue cysts were isolated from leg muscles of experimentally infected chickens (Dubey et al., 1993).

Most seronegative women (94.3%) did eat meat. Even if people prefer well-done meat, they may unintentionally consume undercooked meat and risk exposure to *T. gondii*. This study shows that educational materials designed to inform women about *T. gondii* need to emphasize the infective potential of under-cooked meat, especially with the overwhelming preference of undercooked meats in this study group. As Kapperud et al. (1996) pointed out, attempts to distinguish between beef, pork, or mutton would only complicate recommendations and women should be advised not to eat any undercooked meat regardless of type.

Pork has been identified as a potential source of *T. gondii* transmission to people in the United States because of its prevalence in the American diet (Dubey, 1986a). Consumption of pork was identified as a risk factor for infection in women that seroconverted during pregnancy (Kapperud et al., 1996). In the Knoxville study group, the consumption of pork chops was statistically associated with *T. gondii* seropositivity. Women who ate pork chops were almost five times more likely to be seropositive (odds ratio 4.67) than women that did not. No other pork products, sausage, roast, tenderloin, or country ham was associated with *T. gondii* seropositivity. Sausage usually originates from older animals, which are more likely to be seropositive for *T. gondii* (Patton et al., 1996; Diderrich et al., 2001). However, this product is sometimes highly seasoned or salted during the grinding process and this may reduce the viability of *T. gondii* tissue cysts (Navarro et al., 1992). The other pork meats, roast, tenderloin, country ham, and pork chops, are not ground and remain as a dense meat product. These choice cuts of meat usually originate from younger market-age pigs (6-9 months), which are less likely to be seropositive for *T. gondii* (Patton et al., 1996; Diderrich et al., 2001). Oven cooking of roast and tenderloin allows the internal temperature to reach 150°F for at least one minute, which is sufficient to kill infective tissue cysts (Dubey et al., 1990). The salting and smoking process of preparing a country ham may also reduce the viability of tissue cysts (Lunden and Uggla, 1992). Although consumption of pork is not the only source of *T. gondii* exposure, these cooking and preservation processes may explain why there was not an association with consumption of these pork meats (roast, tenderloin, or country ham) and *T. gondii* seropositivity in this

study group. Pork chops are commonly cooked in a frying pan or on a barbecue grill and even though the outside has the appearance of being well-cooked, the density of the meat may prevent the inside tissues and areas near the bone from reaching 150°F. One local restaurant offers pork chops cooked less than well done (personal experience). These cooking methods for pork chops and changing consumer preferences for undercooked pork (in addition to beef) may contribute to the association of pork chop consumption and *T. gondii* seropositivity in the Knoxville study group. This finding does not imply a causal relationship between pork consumption and *T. gondii* infection because there are other non-meat sources of exposure; however, this statistically significant association may reflect the role of pork as an important source of *T. gondii* infection to people. In the Knoxville study, 91.8% of seronegative women ate pork chops. Again, educational materials designed for women considering pregnancy must emphasize the health hazards associated with consumption of undercooked meat.

Other Dietary Preferences

Only 2.0% of vegetarians in this study were seropositive for *T. gondii*. However, only 2.5% of participants were vegetarians and this sample size is too small for meaningful statistical comparisons. A recent study in the United States found that Seventh Day Adventist vegetarians were at decreased risk for *T. gondii* infection when compared to an age matched population of meat eating community volunteers (Roghmann et al., 1999). That study did not prove a causal relationship between meat consumption and seropositivity; however, the absence of meat consumption may reduce exposure to *T. gondii*. The Knoxville study supports this assumption because seroprevalence in vegetarians (2.0%) was lower compared to meat eaters at 7.2%. For vegetarian women, the risk of infection from meat consumption has been eliminated; however, oocyst contaminated fruits and vegetables remain potential sources of *T. gondii* exposure.

Drinking unpasteurized goat's milk has been associated with *T. gondii* infection in people (Rienmann et al., 1975; Sacks et al., 1982; Patton et al., 1990). Most women in this study did not drink unpasteurized milk because it may only be available to those that live on or near farms. Perhaps women have become cautious of drinking unpasteurized beverages because of recent foodborne outbreaks in the

United States. These outbreaks were associated with the consumption of unpasteurized beverages contaminated with *E. coli* O157:H7 in milk (Keene et al., 1997) and apple juice/cider (Cody et al., 1999; Hilborn et al., 2000), *Cryptosporidium* in apple cider (Millard et al., 1994), and *Salmonella* in orange juice (Cook et al., 1998). These other foodborne illnesses document that people do drink unpasteurized beverages, even if it is rarely and/or accidentally. Educational materials for pregnant women or women considering pregnancy need to stress the risk of infection of *T. gondii* and other foodborne illnesses from unpasteurized beverages (CDC, 2001).

The consumption of raw cookie dough, was a common habit of almost 50% of women. Cookie dough may contain raw eggs and most women are probably not aware of this. Women in the 18-19 year age group were more likely to engage in this practice compared to older age groups. There was not an association with *T. gondii* seropositivity and consuming raw eggs. In an early study, Kimball et al. (1960) also found that consumption of eggs was not associated with seropositivity. Currently, insufficient data are available regarding the role, if any, of raw eggs in the transmission of *T. gondii*, especially if it is consumed in foods such as raw cookie dough, homemade egg nog or shakes, homemade mayonnaise, Caesar salads, or desserts with meringue topping. Outbreaks of Salmonellosis have been associated with the consumption of raw eggs (Hedberg et al., 1993). Although currently not considered a source of *T. gondii* exposure, this practice should be addressed in educational materials for women of childbearing age for the prevention of problems during pregnancy associated with other infectious diseases (CDC, 2001).

Kitchen Hygiene and Food Handling Practices

Only 5% of women did not wash cutting boards and knives with soap and/or bleach after cutting raw meat which may allow for the cross-contamination of *T. gondii* tissue cysts from these kitchen utensils to other food items. In a Norwegian study, infrequent washing of kitchen knives after preparation of raw meat prior to handling another food item was found to be a risk factor for infection with *T. gondii* (Kapperud et al., 1996). In multistate surveillance in the United States, approximately 70 to 80% of women wash cutting boards and knives with soap and/or bleach after cutting raw meat (CDC, 1998; 2001).

Women should be urged to disinfect all surfaces after contact with raw meat, not only to prevent *T. gondii* exposure, but also other foodborne pathogens.

Washing dirt from fruits, vegetables, and hands may also remove *T. gondii* oocysts from contaminated surfaces. Consumption of unwashed fruits and vegetables and transfer of dirt to other foods by unwashed hands are ways oocysts become accidentally ingested on contaminated foods. Approximately 30-40% of women in this study acknowledged that they did not wash fruits and vegetables before consumption and, 40-60% of women reported that they did not wash hands before eating or handling food. Consumption of unwashed fruits and vegetables was identified as a major risk factor for infection with *T. gondii* in Norwegian women (Kapperud et al., 1996). Recent outbreaks of *Cyclospora* sp. (another fecally transmitted parasite of human origin) in the United States and Canada were linked to the consumption of fresh raspberries shipped from a Central American country (Herwaldt et al., 1999). Widespread media coverage of these outbreaks of *Cyclospora* sp. should increase awareness regarding the importance of washing fruits and vegetables to prevent foodborne illness. Additionally, other foodborne bacteria and viruses may be present on the surface of unwashed fruits and vegetables such as *Salmonella* sp. on tomatoes (Hedberg et al., 1999) and alfalfa seeds/sprouts (Van Beneden et al., 1999), and Hepatitis A on strawberries (CDC, 1997b). Pregnant women and women considering pregnancy should be cautioned about consuming raw or unwashed fruits and vegetables and be encouraged to wash their hands before eating or handling food to reduce *T. gondii* exposure.

Approximately 30% of 18-19 year old women acknowledged that they did not always wash hands after handling animals. This has potential implications for the transfer of oocysts from the fur of animals to hands (Lindsey et al., 1997b). Women who own dogs may unknowingly be at risk because they think that *T. gondii* is only a cat disease (Frenkel et al., 1995; Lindsay et al., 1997b). Dog fur may be contaminated with oocysts if a dog is prone to roll in dirt and/or cat feces (Frenkel and Parker, 1996). Dogs that eat cat feces containing *T. gondii* oocysts, may shed these in their feces (Frenkel and Parker, 1996; Lindsay et al., 1997b). In clinically healthy cats, fecal matter is not usually found on fur because of cat's meticulous grooming behaviors (Dubey, 1995a). However, a sick cat with diarrhea, which sometimes occurs when cats shed *T. gondii* oocysts, may allow fecal material to stick to its perineal area, (Dubey, 1995a). In a

recent phone survey by CDC, only 54% of women said they washed hands after petting a dog or cat (Mayo Clinic, 2000). Recommendations for hand washing after petting animals for prevention of *T. gondii* infections should be included in educational materials for women of childbearing age.

Cat Ownership

The association of cats and the transmission of *T. gondii* to humans is difficult to investigate because oocysts are transmitted by soil exposure and not usually by direct contact with cats (Dubey, 2000). In this study, 45% of all the women owned cats, and 42% of seronegative women owned cats. Pet cats kept indoors and fed dried, canned, or cooked food are at low risk for *T. gondii* oocyst shedding because they do not depend on hunting rodents or birds (intermediate hosts) for food, and this reduces the likelihood of owners becoming infected from their pet cats (Lindsay et al., 1997a; CDC, 1999c). However, in addition to feral cats, pet cats that are allowed outdoors also may be a risk of *T. gondii* infection to people because of "hidden deposits" of oocysts in gardens or flowerpots and cat litter boxes. Of seronegative cat owners, 56% scooped the litter box themselves, which may expose them to *T. gondii* oocysts. In this study, cat ownership was not associated with *T. gondii* seropositivity; however, seropositivity was associated with cat owners whose cats defecated outside. The risk of exposure from a pet cat allowed outdoors may be more important because women whose cats defecated outside were 12 times more likely to be seropositive (odds ratio 12.19) than women whose indoor cats only used a litter box. This finding does not prove a causal relationship between outdoor cats and seropositivity in women in the Knoxville study group, because the source and time of infection in seropositive people are unknown; however, oocyst contamination of the environment by outdoor cats may increase a seronegative woman's risk of exposure (Fleck et al., 1972; Ulmanen and Leinikki, 1975; Teutsch et al., 1979; MacKnight and Robinson, 1992; Weigel et al., 1999). Pet cats allowed outdoors should wear a collar with a bell to limit their ability to catch mice and birds (Dubey 1995a).

The bond between pets and humans has been associated with both emotional and health benefits (Beck and Meyers, 1996). It has been debated whether pet cats should be euthanized or, at least, banished from the household for the duration of pregnancy, as seen in the 1995 movie *Nine Months* (20th Century

Fox) where an expectant father does not want to get rid of his cat. Some researchers feel that keeping a potentially "loaded *T. gondii* gun" is a great risk for *T. gondii* infection during pregnancy (Hartley and Munday, 1974; Spencer, 1992). Other researchers point to limited incriminating evidence in the literature that pet cats are potential sources of infection to their owners, including owners with HIV or those with other immune system compromises (Wallace et al., 1993; Winn Foundation, 1997; CDC, 1997a; 1999c; 2000b). Weighing the well being of an unborn child versus keeping a pet to which an owner is emotionally bonded, is a personal matter for the *informed* mother to decide (Spencer, 1992). Banishment or euthanasia of the pet cat are not warranted if precautions are followed (Dubey, 1995a). Pet cats that test serologically positive (infected) are safer pets because the presence of immunity usually prevents secondary oocyst shedding (Dubey, 1995a). Other precautions include keeping the cat inside so it cannot hunt, changing the litter box daily or having another person do it, and feeding the cat only canned/dried commercial food or well-cooked table food, and not raw or undercooked meats (CDC, 1999c). Educational materials for women should emphasize the risk of adopting or handling stray cats while pregnant because these animals may have recently consumed mice or birds and could shed *T. gondii* oocysts (CDC, 1999c).

Environmental Activities

Other Risky Behaviors

Some risky hobbies or occupations that may put a woman in close contact with a contaminated environment include horseback riding (Teutsch et al., 1979), playing in children's sandboxes (Fleck et al., 1972), being a veterinarian (Sengbusch and Sengbusch, 1976), and farming (Behymer et al., 1985; Weigel et al., 1999). In the Knoxville study, having a risky occupation/hobby was not associated with *T. gondii* seropositivity. Only 25.4% of all women and 25.5% of seronegative women reported that they had a risky hobby or occupation. Most participants were from the University of Tennessee community, and 10.9% of these samples were collected from the agricultural campus. Women on the agriculture campus may have jobs or tasks that put them in close contact with raw meat, animals, including cats, or with soil; therefore, they may be considered at high risk for *T. gondii* exposure. These women did have a higher seroprevalence at 9.1% compared to the 6.7% seroprevalence from all other collection sites; however, the difference was

not significant. Pregnant women or those considering pregnancy should be cautioned about occupations/hobbies that involve risky behaviors (handling raw meat, animals, or soil) and the need for adequate protection (wear gloves, mask) and frequent hand washing.

Habits such as chewing finger nails or letting pets sleep in the bed may increase a woman's exposure to *T. gondii* by increasing her exposure to oocysts wedged under finger nails or on animal fur. In the Knoxville study, 49.3% and 55.9% of women engaged in these two behaviors, respectively. Women should be cautioned about putting hands to their mouth after touching anything that may have come into contact with cat feces. Washing hands after outdoor activities or after handling pets is recommended for the prevention of *T. gondii* oocyst exposure.

Gardening

More than half (57.2%) of seronegative women surveyed gardened at least occasionally. Women in the oldest age groups were more likely to garden than those in the youngest. Only 16.3% of these women always wore gloves while gardening and only one woman said she always wore a mask while gardening. Gloves and masks protect women from contacting and breathing dirt and oocysts while gardening. This study did not find an association between gardening and *T. gondii* seropositivity. In Norwegian women, seroconversion was not associated with gardening (Kapperud et al, 1996); however, in a study of women in Californian, those that gardened were twice as likely to be infected (MacKnight and Robinson, 1992). Although these studies do not prove a causal relationship, gardening is considered a risky behavior for *T. gondii* exposure because cats bury their feces in soft sands and surface soils commonly found in gardens. All foods raised in a garden and other objects such as trowels, spades, and gloves can potentially become contaminated with oocysts when the soil is worked. Oocysts may be transferred to food items and accidentally ingested if hands are not washed properly before handling food. Oocysts can also become airborne in dust where they may be accidentally inhaled and ingested. Women should be advised not to garden during pregnancy because gardening places them at risk for contacting soil potentially contaminated with cat feces and *T. gondii* oocysts from outdoor cats.

Gardening and Age Patterns

Women in the 30-34 year age group had a higher seroprevalence to *T. gondii* than women in most other age groups. This may be a result of sampling error and merely an artifact, or it may reflect some risky behavior that was not analyzed by this survey. Perhaps there is a behavior in which 30-34 year olds engage now that exposes them to the infective stages of *T. gondii* that was not common in this age group in the past. When behaviors from this survey were explored, women 30-34 years old were not more likely to live on a farm, eat pork chops, own a cat, have a risky occupation/hobby, or chew nails than the other age groups. However, women in the older age groups (30-53 years) were more likely to garden compared to younger age women. In this study group, 80% of seropositive 30-34 year olds gardened at least occasionally. National marketing surveys have also found that Baby Boomers (approximately 30-50 years) were more likely to garden as a hobby compared to Generation Xers (18-29 years) (Anon., 1997; Weissman, 1999; Anon., 2000). Traditionally, gardening has been a leisure time hobby for Americans 60 years and older. Current marketing trends show that middle-aged women are excellent targets for products that help them maintain well-ordered lives, such as gardening gadgets and tools (Anon, 2000). These demographic surveys also show that owning a home is one of the best predictors of the likelihood of gardening (Anon, 1997). Women considering pregnancy should be aware that changes in lifestyle, such as purchasing a house, followed by home improvement and beautification by gardening, are circumstances that increase exposure to an environment potentially contaminated with *T. gondii* oocysts. The higher seroprevalence in the 30-34 year age group may reflect this new trend where women have started gardening at a younger age rather than waiting until they are retirement age. In the Knoxville study group, 71.5% (736/750) of seronegative women in the peak childbearing years, aged 18-34, are projected to age into the prime gardening years in the next century (Anon, 1997).

Behaviors of Seronegative Women

In this study group, 93% of women were seronegative and susceptible to *T. gondii* infection. Analysis showed that some of these women engage in more than one risky behavior at the same time, which may increase their risk of exposure by some unknown magnitude. When the eight most frequently

occurring risky behaviors were combined, 13 of the 749 seronegative women may be at higher risk of exposure because they did all eight behaviors, compared to the 693 seronegative women that only ate meat (Table 8). Grouping behaviors associated with eating meat, gardening, and cat ownership created practical scenarios of activities that could lead to *T. gondii* exposure. For example, women that do not wear gloves while gardening, plus own a cat that defecates outside, and do not always wash hands before handling food have not only contacted potentially contaminated soil, but also failed to prevent transfer of dirt and oocysts from hands to food (Table 13). This combination of behaviors was true for 18 seronegative women. This analysis showed that the risk of *T. gondii* exposure is multidimensional for many women. Educational guidelines for decreasing exposure to *T. gondii* in women of childbearing age should include modifying activities such as farm chores and gardening, letting pets sleep in bed, not washing fruits and vegetables before consumption and eating undercooked meat.

Education

This Knoxville study contributes to understanding the type and frequency of risky behaviors for *T. gondii* exposure among women of childbearing age. For the group of seronegative women, modifying behaviors to decrease their risk of exposure to *T. gondii* needs to be emphasized in community health educational programs. Many researchers have recognized that education may be the single most powerful and practical approach to the control of congenital toxoplasmosis (Desmonts and Couvreur, 1974; Frenkel, 1990; Leighty, 1990; Grant and Olsen, 1999; Holliman, 2000). Developing educational materials is costly and time-consuming; therefore, guidelines for decreasing the occurrence of risky behaviors for *T. gondii* exposure must be appropriate for the target audience (Leighty, 1990). A perspective on developing effective educational guidelines can be gained by looking at the folic acid campaign. March of Dimes funding for folic acid awareness/research was $11 million in 1999, while funding for *T. gondii* awareness/research has totaled less than $500,000 since 1984 (MOD, 1999). The March of Dimes has used advertisements in magazines, newspapers, radio, and television, and recently cereal boxes to educate women of childbearing age about the benefit of taking folic acid (a B-vitamin) to prevent birth defects (CDC, 1992). As a result, 13% of women surveyed knew that folic acid helps prevent birth defects (CDC,

1999b). In contrast, no such effort has been made to educate women of childbearing age about the dangers of *T. gondii* infection. In the Knoxville study, few women identified consuming unwashed fruits/vegetables and undercooked meat as sources of *T. gondii* infection. Interestingly, in the United States, both conditions affect approximately 4000 babies every year. However, for preventing birth defects, it may be easier to tell a woman to take a vitamin rather than to give her a long list of behaviors she should not do to prevent *T. gondii* exposure.

Surprisingly, none of the women surveyed identified the World Wide Web as a tool for accessing *T. gondii* information. Although, some of the information about *T. gondii* on the internet is misleading, widespread access to computers and electronic networking enable women to communicate with other women from all over the world and to share their pregnancy experiences. One such group is the "mid-life mommies" (www.midlifemommies.com). This population of women has created a sub-culture of older (over 35 years), usually professional-women that have delayed childbearing because time was spent developing careers, a later marriage, years of infertility, or the unexpected arrival of a second family after the first children have grown. These women are able to discuss life experiences including the risk of *T. gondii* infection during pregnancy. In the Knoxville study group, women in similar age groups (over 30 years) were more likely to wash fruits and vegetables before consumption and more likely always to wash hands before handling food, eating and after handling animals. They also preferred to eat well-done meat. As previously discussed, because women in this age group were more likely to garden, educational materials should inform them that gardening may put them in contact with oocyst contaminated environments.

In the Knoxville study group, only one woman in 10 identified their physician or veterinarian as a source of information regarding *T. gondii*. Physicians may not wish to alarm or burden their pregnant patient and may be reluctant to describe all possible hazards of pet ownership (Leighty, 1990). Most veterinarians would prefer to convey a positive image of the benefits of pet ownership (Leighty, 1990). In a recent survey of physicians and veterinarians in Wisconsin, animal associated pathogens were not discussed frequently with clients (Grant and Olsen, 1999). Physicians were less comfortable advising patients on the role of animals in the transmission of zoonotic agents and felt that veterinarians should play

an equal or greater role in advising patients. However, patients do not view veterinarians as a source of zoonotic disease information. Interestingly, physicians listed *T. gondii* as the zoonotic disease pathogen of greatest concern, especially in immunocompromised persons, whereas veterinarians listed *Salmonella* sp. because it is associated with owning and handling reptiles. The reluctance of physicians to educate women was also found in the survey by the March of Dimes (CDC, 1999b). Only one in five women learned about folic acid from their health care provider. Both the Knoxville *T. gondii* study and the folic acid study have revealed an alarming trend among those that provide health care to women. Physicians rarely recommend folic acid to their patients, and they, along with veterinarians, rarely convey the risk of *T. gondii* exposure to women of childbearing age. In a recent Knoxville study by the March of Dimes, some physicians did not believe that it was their responsibility to make women aware of the benefits of folic acid (Geiser, 2000). Physicians probably do not discuss with these women the benefits of preventing *T. gondii* infection either.

Teenagers and Healthy People 2000, 2010

In the Knoxville study group, most women learned about *T. gondii* in school. Educational efforts should be focused towards teenagers and young adults because women in these age groups were more likely to participate in risky behaviors for *T. gondii* exposure, such as consuming less than well-done meat (Tables B26, B32, B38, B68, B74), not washing fruits and vegetables (Tables B106 and B108), and not always washing hands after handling pets or before handling or eating food (Tables B110, B112, and B114). Knowledge of proper dietary and hygiene behaviors is a priority listed in *Healthy People 2000*, along with many other topics of concern for teenagers ranging from suicide prevention to increasing communication skills (Public Health Service, 1995). As a result of this government initiative, the CDC has identified important topics for primary and secondary school health education programs. These guidelines for training teachers and topics for school health programs have helped students develop the knowledge and skill needed to adopt healthy lifestyles (Grunbaum et al, 1998). With the continuation of this program with *Healthy People 2010* (ODPHP, 2000) and the use of existing health education classes in primary and secondary schools, there exists easy and inexpensive educational approach for including guidelines for the prevention of *T. gondii* infection in the United States today.

CHAPTER 9

SUMMARY AND CONCLUSIONS

Seroprevalence

The seroprevalence of *Toxoplasma gondii* in this Knoxville study group of women of childbearing age was 7.0%. This is lower than the 15% seroprevalence of women of childbearing age from the United States sampled in the NHANES study by the CDC (CDC, 2000b) and the 15-60% seroprevalence reported by many web sites (Winn Foundation, 1997, MOD, 1997). The chance of infection during pregnancy may seem remote if it is assumed that one out of three (~33%) American women are already immune to *T. gondii*, with seroprevalence higher in cat owners (www.allHealth.com). These estimates may lead people to believe that most women are seropositive and not at risk of *T. gondii* infection. However, the results of this study show that 13 out of 14 women (93.0%) in this study group from the Knoxville, Tennessee area were susceptible to *T. gondii* infection, and they reported behaviors that increased their risk of exposure. The lower seroprevalence of the Knoxville study group of mostly college-aged women may reflect selection bias where the median age was 25 years and these women were less likely to be seropositive; therefore, the sample was not representative of all Knoxville women.

Knowledge of Risk Factors for *Toxoplasma gondii* Infection

Most women identified cat feces as potential sources of *T. gondii* exposure; however, not as many women in this study group identified handling and consumption of meat as potential sources of *T. gondii* exposure. When undercooked meat is consumed, *T. gondii* infection may occur. Most seronegative women (94.3%) do eat meat. Even if they prefer well-done meat, they may unintentionally consume undercooked meat and risk exposure to *T. gondii*. The role of pork as a source of *T. gondii* has been investigated through surveys of seroprevalence in slaughter pigs and sows (Patton et al., 1996; Diderrich et al., 2001). The seroprevalence in animals used for whole meats (slaughter pigs) was 3.2%, however, in older animals (sows) used for processed meats, the seroprevalence was 15-20%. Pork is consumed by most

Americans and changing preferences for rare meats, including pork, put people at risk for *T. gondii* infection. Educational materials designed to inform women about *T. gondii* risk need to emphasize the infective potential of undercooked meat, as indicated by the overwhelming preference of undercooked meats in the Knoxville study group.

Women that are not cat owners cannot assume that they are not at risk for *T. gondii* exposure. Outdoor cats from the neighborhood may defecate in household gardens and flowerbeds where people can be exposed to *T. gondii* oocysts. In the Knoxville study, women in the older age groups (30-53 years) were more likely to garden compared to those in the younger age groups. Women should be advised not to garden during pregnancy because gardening places them at increased risk of contact with a concentrated source of oocysts (focus of cat feces).

Dog owners may unknowingly be at risk because they think that *T. gondii* is a cat disease. Dogs that roll in cat feces may carry infective oocysts on their fur. Oocysts can be transferred to hands or clothes when dogs are petted or allowed to sleep in the owner's bed. Approximately 50% of women did not wash hands after handling pets and approximately 50% allowed pets to sleep in their bed. Recommendations for washing hands after handling pets should be included in educational materials for women of childbearing age.

Approximately 30-40% of women in this study acknowledged that they did not wash fruits and vegetables before consumption and 40-60% of women reported that they did not wash hands before eating or handling food. Pregnant women and women considering pregnancy should be cautioned about consuming raw or unwashed fruits and vegetables and the risk of *T. gondii* infection. They also should be encouraged to wash their hands before eating or handling food

In the Knoxville study group, 93% of women were seronegative and susceptible to *T. gondii* infection. This analysis showed that the risk of *T. gondii* exposure is multidimensional for many women in that some of these women engage in more than one risky behavior at the same time, which may increase their risk of exposure by some unknown magnitude.

Recommendations for Modifying Risky Behaviors

There are many lists of behavioral modifications for preventing *T. gondii* infection that are published in a variety of professional journals (Frenkel, 1974; Lindsay et al., 1997a), lay publications (Sietsema, 1995), and on the World Wide Web (Hughes, 1985; AVMA, 1996; CDC, 1997a; 2000c; MOD, 1997). This is the first study to investigate the difference between age groups in the frequency of behaviors that increase risk of exposure to *T. gondii* in women of childbearing age. The results of this study shows the necessity to reinforce to teenagers and women in their early 20s hygienic behaviors that decrease the risk of exposure to *T. gondii* (Table 14). Additionally, because women over 30 years are more likely to garden, they need to be aware of oocyst contamination of soil associated with outdoor activities (Table 14).

Future Research

More studies are needed that investigate the occurrence of behaviors in women of childbearing age in the United States. Once identified, deficits in understanding *T. gondii* transmission can be addressed in existing community-health educational programs. Women of different ages and hobbies identified in the Knoxville study whose risky behaviors should be modified included teenagers, gardeners, and pet owners. Many types of educational models will need to be developed because there were combinations of behaviors that contributed to a woman's risk of exposure in the Knoxville study group. Effective educational tools may include cereal boxes, meat wrappers, cat-litter bags, seed packets, magazine advertisements, or television commercials. For example, ways to reach teenagers may include advertisements on music television stations (MTV or VH1). Curriculum for veterinary education and health care providers should include classes on zoonotic diseases, so that veterinarians and physicians feel both qualified and obligated to educate clients about eating only well-done meat, hand washing, and other hygienic measures that reduce the risk of *T. gondii* transmission. Opportunities to educate clients include small signs in exam rooms, zoonotic disease brochures in reception areas, comments in newsletters, and seminars to community groups Also, parasitologists should interact with local health authorities and medical providers to educate them about changing trends in perceptions and understanding of zoonotic disease transmission. For example, *T. gondii* is now considered an important waterborne parasite along with *Cryptosporidium* sp. that has public

Table 14. Guidelines for reducing *Toxoplasma gondii* exposure based on the most frequently reported risky behaviors of women in different age groups. Recommendations for all women to prevent toxoplasmosis during pregnancy by avoiding known sources of infection are listed in the center. An "X" identifies behaviors that need emphasis in each age group.

Teenagers & 20-29 Years	30-50 Years	Recommendations to prevent toxoplasmosis during pregnancy by avoiding known sources of infection
X		1. Wash hands before eating and before handling food.
X		2. Wash hands after contact with raw meat.
		3. Wash cutting boards and any utensils that contact raw meat.
X		4. Always cook meat to well done (internal temperature of 160°F throughout).
X		5. Wash raw fruits and vegetables before eating.
X		6. Do not chew fingernails.
X		7. Drink only pasteurized milk.
X		8. Do not eat raw cookie dough.
X	X	9. Wash hands after outdoor activities and contact with soil or sandboxes.
	X	10. If pregnant, do not garden.
	X	11. Wear gloves while gardening.
X		12. Wash hands after handling pets.
		13. Change cat's litter box daily.
	X	14. If pregnant, do not empty the cat's litter box. Have someone else do it.
		15. Do not feed cat raw meats and keep cat indoors to prevent it from hunting birds and rodents.

health significance (Thompson, 1999). Hopefully, the development of the Congenital Toxoplasmosis Working Group by the CDC will lead to the development of educational programs for health care providers and prenatal classes for pregnant women.

Testing of supermarket meats for the presence of *T. gondii* tissue cysts has not been conducted in the United States for almost 40 years. Tissue cyst viability from different sources (beef, pork, chicken) and types (hamburger, sausage, whole cut) of meat could identify important sources of *T. gondii* infection for meat eaters today. The prevalence in slaughter pigs is low, but because of the large volume of animals going to market, many seropositive pigs are used for food. The safety of the meat supply begins with on-farm production strategies. *T. gondii* infection of food animals occurs on the farm. Unlike some other foodborne pathogens, it does not enter and multiply in meat after the animal is harvested. For the production of pork, farm management strategies such as total confinement operations, help reduce the transmission of *T. gondii* to market animals (Diderrich et al., 2001). According to this swine production research, the seroprevalence of *T. gondii* in sows has decreased between 1990 and 1995. The seroprevalence in breeding animals (sows) was significantly higher than in young finishing pigs, which reflect age-associated seropositivity (Diderrich et al., 2001). The difference in seroprevalence also reflects different management strategies where some breeding animals are confined and others are kept on pasture. Pigs on pasture may ingest either *T. gondii* oocysts shed by cats into the soil or infected intermediate hosts such as birds and rodents. Sows and finishers in total confinement had a significantly lower seroprevalence compared to swine kept on pasture. Total confinement operations reduce the access of cats and other animals, including birds and rodents, to the facilities which reduces *T. gondii* exposure from these sources.

Infections with *T. gondii* are important in many areas of infectious disease epidemiology. *T. gondii* causes birth defects and foodborne illness and is recognized as a zoonotic disease from pets. *T. gondii* is also recognized as an environmentally acquired disease for both people and food animals. Community health educational programs designed to prevent *T. gondii* infection must include preventing *T. gondii* transmission by modifying risky behaviors for exposure.

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APPENDICES

APPENDIX A

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INFORMED CONSENT STATEMENT

Seroprevalence of *Toxoplasma gondii* antibodies in females of childbearing age living in East Tennessee

INTRODUCTION

You have been asked to participate in a research study involving women of childbearing age (18-53 years) and the disease toxoplasmosis. This age group is important to study because pregnant women may be at risk for possible infection with this organism resulting in severe birth defects such as blindness, deafness, and mental retardation or death of the fetus. Over 9500 babies are born in the United States each year with congenital toxoplasmosis. Information from this study can be used to prevent infection through education of women.

The purpose of this study is:

1. To estimate the percent of females of childbearing age from the Knoxville, Tennessee area that have been exposed to the organism that causes toxoplasmosis.
2. To investigate the role of diet, food safety practices, and soil contact that may increase risk of toxoplasmosis.

Your participation in this study will consist of providing a blood sample and answering a questionnaire. Blood samples will be collected by a nurse, medical technician, or phlebotomist. Approximately 5 milliliters of blood, which is equal to 1 teaspoon, will be collected. Collecting the blood sample will take approximately 10 minutes, and completing the questionnaire will take approximately 10-15 minutes.

RISKS

The risks to you as a result of your participation in this project are considered minimal in that they do not exceed those encountered in donating blood or in providing blood samples for medical evaluation. You cannot get toxoplasmosis by giving a blood sample.

BENEFITS

Participating individuals will not experience any direct benefit.

CONFIDENTIALITY

Your name will not appear on the questionnaire, so your anonymity will be maintained. Consequently, the results of your blood test for toxoplasmosis will not be available to you. Your signed informed consent document and completed questionnaire will be kept for the duration of the project and for at least three years thereafter. These will be stored securely in a locked file cabinet at the UT Veterinary Teaching Hospital, and will be used only by the principal investigator and the faculty advisor. When the consent forms and questionnaires are disposed, they will be destroyed by shredding.

COMPENSATION

You will not be offered any incentives or remuneration.

Figure A1. Informed consent statement.

CONTACT INFORMATION

If you have questions at any time about the study or the procedures, you may contact the researcher, Vina R. Diderrich, at UTCVM, PO Box 1071, Knoxville, TN 37901 and (423) 974-5645. If you have questions about your rights as a participant, contact the Compliance Section of the Office of Research at (423) 974 3466.

PARTICIPATION

Your participation is voluntary and you may discontinue participation (providing a blood sample or completing the questionnaire) at any time. If you are seeking medical attention at a physician's office, you will not be denied care if you choose not to participate. However, we hope you will assist us in this study.

CONSENT

I have read the above information. I have received a copy of this form. I agree to participate in this study.

Participant's signature _____ Date _____

Investigator's signature _____ Date _____

Figure A1. (continued).

Seroprevalence of *Toxoplasma gondii* antibodies in females of childbearing age living in East Tennessee

Please take the time to answer these questions that are important for the health of women and children. With your responses, we will be able to study the role of the environment and eating meat in the transmission of this organism.

1. Current setting of home
   [ ] rural (in the country)
   [ ] on a farm
   [ ] small town (incorporated population center)
   [ ] suburban (neighborhood on outskirts of a city)
   [ ] urban (near a metropolitan downtown)

2. For how many months or years have you lived in this setting?_____

3. Childhood home (<18 years). Can be more than one place.

Setting of home	Years lived there
Rural (in the country)	
On a farm	
Small Town (incorporated population center)	
Suburban (neighborhood on outskirts of a city)	
Urban (near a metropolitan downtown)	

4. Are you a vegetarian? Yes No
   If yes, for how many years?_____
   What type of vegetarian are you (ovo, lacto, etc.)?_____

5. Does your religion limit or prohibit the consumption of any type of meat? Yes No
   If yes, which meats?

   Do you comply with these? Always Sometimes Never

6. What religion are you?_____

Figure A2. The seroprevalence of *Toxoplasma gondii* antibodies in females of childbearing age living in East Tennessee Questionnaire, Version 2.

7-14. Please answer these questions about the frequency of these activities.
Mark the box (X) that applies.

Questions	Never	Occasionally	Frequently	Always
7. Drink un-Pasteurized Cow's milk				
8. Drink un-Pasteurized Goat's milk				
9. Eat raw eggs (found in fresh egg nog or raw cookie dough				
10. Wash fresh fruits before eating them				
11. Wash fresh vegetables before eating them				
12. Wash your hands before you eat				
13. Wash your hands before handling food				
14. Wash your hands after going to bathroom				

If you currently eat meat or prepare meat in your home or work, please answer questions 15-36. Otherwise go onto question 37.

15. When raw meat is prepared in my home, it is
[ ] used fresh from the store
[ ] stored frozen and then prepared
[ ] both
[ ] Only buy cooked meat

16. If a cutting board is used in my home to cut raw meat and is going to be used to chop another food, the board is:

17. If a knife is used in my home to cut raw meat and is going to be used to chop another food, the knife is:

18. The last time I handled raw meat, I cleaned my hands afterwards by:

Figure A2. (continued).

19-32. For the meats below, how often do you include these in your meals and how do you prefer they be cooked before you eat them?

Mark the box (X) that applies.

MEAT TYPE	How often do you eat this meat?				Cooking Preference		
	Never	At least 1 time a year	At least 1 time a month	At least 1 time a week	Rare	Medium	Well
19. Pork Sausage							
20. Pork chops							
21. Pork roast							
22. Pork tenderloin							
23. Country Ham							
24. Hamburger							
25. Steak							
26. Chicken							
27. Domestic turkey							
28. Chevon (goat)							
29. Mutton (sheep)							
30. Lamb							
31. Wild game – list							
32. Other							

33. How can you tell that meat has been cooked to a well done state?

34. List reasons why pork should be cooked to well done.

35. Do the above reasons apply to other meats such as chicken and wild game?

36. Overall, do you think the meat supply is safe to eat? Yes No Don't Know
If yes, why? If no, why?

Figure A2. (continued).

37-42. Mark the box (X) that applies to the frequency of this activity.

Questions				
	Never	**Occasionally**	**Frequently**	**Always**
37. Keep a flower or vegetable garden				
38. Wear gloves while gardening				
39. Wear mask while gardening				
40. Play with children in a sandbox				
41. Chew fingernails				
42. Wash hands after handling animals				

If you own a cat, please answer 43-44. If not, go on to question # 45.

43. Do you own a cat? Yes No
If yes, does it
[ ] use a litter box
[ ] defecate ("go") outside only:

If uses a litter box: Who changes the litter box?
[ ] I do
[ ] Spouse or significant other
[ ] Roommate
[ ] Children
[ ] Parents
[ ] Other _____

How often is it scooped? _____times per _____(i.e. day, weeks, month)
How often is it changed? _____times per _____(i.e. day, weeks, month)
(discarded and replaced)

If you change the litter box, do you wash your hands after changing it? Yes No

44. Does your cat eat: (can mark more than one answer)
[ ] commercial food
[ ] Hunts wild rodents &/or birds
[ ] Undercooked kitchen scraps

45. Do you have any other pets? Yes No
If yes, list.

46. Do you let pets sleep in your bed?
Never Occasionally Frequently Always

47. Do you have an occupation or hobby(ies) (besides owning a cat) that may cause you to come in contact with cat feces, soil (dust), or hay/bedding (farm animal environment)? Such as a veterinarian, work in a pet shop, landscaper or gardener, farmer, ride horses, etc.
Yes No
If yes, list.

Figure A2. (continued).

48. Are you pregnant now? Yes No Suspect, but don't know

49. Have you ever heard of toxoplasmosis? Yes No
If yes, how?

What is it?

50. Have you ever been tested for toxoplasmosis? Yes No
If yes,
When (month and year)?

Result? Positive Negative Unknown

Reason for being tested?

51. How old are you now? _____

52. What is your cultural or ethnic identity?
[ ] Hispanic or Latino
[ ] White
[ ] Black/African American
[ ] Asian or Pacific Islander
[ ] American Indian or Native American
Other:_____

53. In what range is the YEARLY INCOME of your household?
[ ] Under $12,499
[ ] $12,500-$19,999
[ ] $20,000-$27,499
[ ] $27,500-$34,999
[ ] $35,000-$44,999
[ ] $45,000-74,999
[ ] $75,000-$124,999
[ ] $125,000+

54. Where is your place of birth: _____(City, State, Country)

55. Educational background
[ ] No High School
[ ] Some High School-No diploma
[ ] High School Graduate-diploma or GED
[ ] College-No diploma
[ ] College-diploma
[ ] Advanced Degree

Thank You For Your Time and Cooperation!

Figure A2. (continued).

Toxoplasmosis

March of Dimes Resource Center

Toxoplasmosis is a widespread parasitic infection that, when contracted by a pregnant woman, can pose serious risks to her unborn baby. Up to one in 1,000 babies in this country are born infected with toxoplasmosis. Fortunately, a pregnant woman can follow some simple precautions that can reduce her chance of becoming infected. These precautions should be followed by all women who could become pregnant, since more than half of all pregnancies are unintended.

A pregnant woman who contracts toxoplasmosis for the first time during pregnancy has about a 40 percent chance of passing the infection on to her fetus. However, the risk and severity of the baby's infection depend partly on the timing of the mother's infection. Studies suggest that, when mothers are infected in the first trimester, 15 percent of fetuses become infected, as compared to 30 percent in the second trimester and 65 percent in the third. However, the earlier in pregnancy the infection occurs, the more severe the fetal infection.

What risks does toxoplasmosis pose to the baby?

Although up to 90 percent of infected babies appear normal at birth, 80 to 90 percent will develop sight-threatening eye infections months to years after birth. Some also will develop hearing loss, hydrocephalus (water on the brain), mental retardation, learning disabilities or seizures. Toxoplasmosis during pregnancy also can result in miscarriage or stillbirth.

About one in 10 infected babies has a severe Toxoplasma infection that is evident at birth. These newborns often have severe eye infections, an enlarged liver and spleen, jaundice (yellowing of the skin), pneumonia and other problems. Some die within a few days of birth. Those who survive sometimes suffer from mental retardation, severely impaired eyesight, cerebral palsy, seizures and other problems.

What causes toxoplasmosis?

Toxoplasmosis is caused by a parasite called Toxoplasma gondii. It is most often picked up through exposure to cat feces or by eating raw or undercooked meat that is contaminated with the parasite. Other sources of infection may include raw goat's milk, raw eggs, and insects such as flies and cockroaches that may have been in contact with cat feces.

Cats often become infected when they eat an infected rodent or bird. The parasite reproduces in the cat's intestine, and a form of the parasite ends up in the cat's litter box, sand or soil. This form of the parasite becomes infectious within days, and is resistant to most disinfectants. Under certain temperature and humidity conditions, the parasite may live in soil for more than a year. Infected cats usually appear healthy.

Who gets toxoplasmosis?

Toxoplasmosis is one of the most common infections in the world. Most cases go undiagnosed. Symptoms, if any, tend to resemble flu.

Active infection normally occurs only once in a lifetime. Although the parasite remains in the body indefinitely, it is generally harmless and inactive unless the immune system is not functioning properly. If a woman develops immunity to the infection at least six to nine months before pregnancy, there rarely is any danger of passing it on to her baby.

Can a pregnant woman find out if she is immune?

About 60 to 85 percent of American women of childbearing age have never had toxoplasmosis and are susceptible to it during pregnancy. There is a blood test for Toxoplasma antibodies. The pattern of the test results indicate whether infection was acquired recently or in the past.

However, testing for Toxoplasma antibodies when a woman is pregnant can be complicated and worrisome. If a woman is pregnant and her blood tests show a certain kind of antibodies called IgG antibodies, additional blood tests are needed to determine whether the results indicate an old infection (which generally does not threaten the safety of the fetus) or a very recent one (which would put the fetus at risk). Women planning to become pregnant should discuss with their health care provider whether they should have this blood test before pregnancy.

How can a woman prevent toxoplasmosis during pregnancy?

She can prevent it by avoiding the known sources of infection. Here are some safeguards that may help:

- Don't empty the cat's litter box. Have someone else do this.
- Don't feed the cat raw or undercooked meats.
- Keep the cat indoors to prevent it from hunting birds or rodents.
- Don't eat raw or undercooked meat, especially lamb or pork. Meat should be cooked to an internal temperature of 160° F throughout.
- If you handle raw meat, wash your hands immediately with soap. Never touch your eyes, nose or mouth with potentially contaminated hands.
- Wash all raw fruits and vegetables before you eat them.
- Wear gloves when gardening, since outdoor soil may contain the parasite from cats. Keep your hands away from your mouth and eyes, and wash your hands thoroughly when finished. Keep gloves away from food products.
- Avoid children's sandboxes. Cats may use them as litter boxes.

How is toxoplasmosis diagnosed and treated during pregnancy?

If a blood test before or during pregnancy shows no Toxoplasma antibodies, a woman is at risk if she is exposed to the parasite. The presence of antibodies in a blood test indicates that infection is present or has already occurred.

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Your Source *of information on* pregnancy *and* birth defects

Figure A3. March of Dimes *Toxoplasmosis* information sheet.

If a health care provider suspects that a pregnant woman has an active Toxoplasma infection, a special blood test can be done that detects antibodies produced by the body soon after infection. If a pregnant woman has an active Toxoplasma infection, the next step is to test to see if the fetus is infected. Prenatal tests including amniocentesis and ultrasound usually can tell if the fetus is infected and how severely infected it may be. Fetuses found to be infected are treated by giving the mother two medications, pyrimethamine and sulfadiazine. This approach appears to reduce the severity of the baby's symptoms at and after birth.

In a French study, all pregnant women diagnosed with toxoplasmosis were treated with the antibiotic spiramycin. The researchers reported that spiramycin lessened the likelihood that the parasite would reach the fetus. (Spiramycin has not yet been approved for use in this country by the Food and Drug Administration. However, doctors who are interested in obtaining the drug can contact the FDA at 301/443-4280.)

While these results are encouraging, the safety, effectiveness and feasibility of prenatal toxoplasmosis screening, diagnosis and treatment are not yet established. For these reasons, in the United States, most pregnant women are not screened for toxoplasmosis.

How are infected newborns treated?

Infected babies should be treated as soon as possible after birth with pyrimethamine and sulfadiazine which, as mentioned earlier, can help prevent or reduce the disabilities associated with toxoplasmosis.

The March of Dimes has supported an ongoing national study to determine the effectiveness of these and other medications in preventing or reducing the disabilities associated with toxoplasmosis. The results will enable researchers to devise the best treatment for infected babies.

References

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Remington, J.S., McLeod, R., Desmonts, G. Toxoplasmosis, in Remington, J.S., Klein, J.O. (eds.): Infectious Diseases of the Fetus and Newborn Infant, 4th edition, Philadelphia, W.B. Saunders, 1995, pages 140-267.

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Saving babies, together

Figure A3. (continued)

Seroprevalence of *Toxoplasma gondii* antibodies in females of childbearing age living in East Tennessee

Please take the time to answer these questions that are important for the health of women and children. With your responses, we will be able to study the role of the environment and eating meat in the transmission of this organism.

1. Current setting of home
   [ ] rural (in the country)
   [ ] on a farm
   [ ] small town (incorporated population center)
   [ ] suburban (neighborhood on outskirts of a city)
   [ ] urban (near a metropolitan downtown)

2. For how many months or years have you lived in this setting?_____

4. Childhood home (<18 years). Can be more than one place.

Setting of home	Years lived there
Rural (in the country)	
On a farm	
Small Town (incorporated population center)	
Suburban (neighborhood on outskirts of a city)	
Urban (near a metropolitan downtown)	

4. Are you a vegetarian? Yes No
   If yes, for how many years?_____
   What type of vegetarian are you (ovo, lacto, etc.)?_____

5. Does your religion limit or prohibit the consumption of any type of meat? Yes No
   If yes, which meats?
   1.
   2.
   3.
   4.

   Do you comply with these? Always Sometimes Never

   What religion are you?_____

Figure A4. The seroprevalence of *Toxoplasma gondii* antibodies in females of childbearing age living in East Tennessee Quesitonnaire, Version 1.

6-13. Please answer these questions about **Present AND Past** activities. Mark the box (X) that applies.

Questions	Presently		In the Past	
	Yes	No	Yes	No
6. Drink un-Pasteurized Cow's milk				
7. Drink un-Pasteurized Goat's milk				
8. Eaten raw eggs (found in fresh egg nog or raw cookie dough				
9. Wash fresh fruits before eating them				
10. Wash fresh vegetables before eating them				
11. Wash your hands before you eat				
12. Wash your hands before handling food				
13. Wash your hands after going to bathroom				

If you eat meat, please answer questions 14-47. Otherwise go onto question 48.

14. If a cutting board is used in my home to cut raw meat and is going to be used to chop another food, the board is:
    [ ] used as is
    [ ] wiped with a damp cloth or rinsed with water
    [ ] washed with soap and hot water
    [ ] sanitized with a mild chlorine bleach solution

15. If a knife is used in my home to cut raw meat and is going to be used to chop another food, the knife is:
    [ ] used as is
    [ ] wiped with a damp cloth or rinsed with water
    [ ] washed with soap and hot water
    [ ] sanitized with a mild chlorine bleach solution

16. The last time I handled raw meat, I cleaned my hands afterwards by:
    [ ] wiping them on a towel
    [ ] rinsing them under cold, warm or hot tap water
    [ ] washing with soap and warm water

17. When raw meat is prepared in my home, it is
    [ ] used fresh from the store
    [ ] stored frozen and then prepared
    [ ] both
    [ ] Only buy cooked meat

Figure A4. (continured)

18-47. Please answer these questions about **Present AND Past** activities.
For the meats below, how do you prefer they be cooked before you eat them?
Mark the box (X) that applies.

	PRESENTLY			
MEAT TYPE	**Cooking Preference**			**Don't eat this kind of meat**
	Rare	**Medium**	**Well**	
18. Pork Sausage				
19. Pork meat (other than sausage)				
20. Hamburger				
21. Steak				
22. Chicken				
23. Domestic turkey				
24. Chevon (goat)				
25. Mutton (sheep)				
26. Lamb				
27. Deer				
28. Rabbit				
29. Bear				
30. Wild hog				
31. Wild turkey				
32. Grouse or quail				

	IN THE PAST			
MEAT TYPE	**Cooking Preference**			**Never ate this kind of meat**
	Rare	**Medium**	**Well**	
33. Pork Sausage				
34. Pork meat (other than sausage)				
35. Hamburger				
36. Steak				
37. Chicken				
38. Domestic Turkey				
39. Chevon (goat)				
40. Mutton (sheep)				
41. Lamb				
42. Deer				
43. Rabbit				
44. Bear				
45. Wild pig				
46. Turkey				
47. Grouse or quail				

Figure A4. (continued).

48. How can you tell that meat has been cooked to a well done state? (May answer as Don't Know)

49. List reasons why pork should be cooked to well done. (May answer as Don't Know)
    1.
    2.
    3.
    4.

50. Do the above reasons apply to all meats including wild game? Yes No Don't Know
    If no, which do they not apply? Why?

51. Overall, do you think the meat supply is safe to eat? Yes No Don't Know
    If yes, why? If no, why?
    1.
    2.
    3.
    4.

52-59. Please answer these questions about Present AND Past activities.
Mark the box (X) that applies.

Questions	Presently		In the Past	
	Yes	No	Yes	No
52. Keep a flower or vegetable garden				
53. Wear gloves while gardening				
54. Wear mask while gardening				
55. Play in children's sandbox				
56. Chew fingernails				
57. Wash hands after handling animals				
58. Own a dog				
59. Allow pets to sleep in your bed				

60. Do you own a cat? Yes No
    If yes, does it
    [ ] use a litter box
    [ ] defecate ("go") outside only:

    If uses a litter box:
    Who changes the litter box?
    [ ] Spouse or significant other
    [ ] Roommate
    [ ] Children
    [ ] Parents
    [ ] Other _____

How often is it changed? _____times per _____(i.e. day, weeks, month)
If you change the litter box, do you wash your hands after changing it? Yes No

Figure A4. (continued).

61. Does your cat eat: (can mark more than one answer)
    [ ] commercial food
    [ ] Hunts wild rodents &/or birds
    [ ] Undercooked kitchen scraps

62. Do you have any other pets? Yes No
    If yes, list pets, indicate (X) whether indoor or outdoor pets, and years of ownership.

Type of Pet (dog, bird, etc.)	Exclusively Indoor	Exclusively Outdoor	Indoor/ Outdoor	Years of ownership
1.				
2.				
3.				
4.				

63. Do you have an occupation or hobby(ies) (besides owning a cat) that may cause you to come in contact with cat feces? Such as a veterinarian, work in a pet shop, landscaper, farmer, or gardener.
    Yes No
    If yes, list.
    1.
    2.
    3.
    4.

If you are pregnant or have been pregnant, please answer questions 64-77 about behaviors that you may have changed. Otherwise go to question 78.

64-77. Mark the box (X) that applies.

Questions		
	Yes	No
64. Stopped eating meat		
65. Cooked meat to well-done		
66. Stopped gardening		
67. Wear gloves while gardening		
68. Wear mask while gardening		
69. Began washing fruits before eating		
70. Began washing vegetables before eating		
71. Chew fingernails		
72. Wash hands after handling animals		
73. Own a dog		
74. Allow pets to sleep in your bed		
75. If own cat, stopped changing litter box		
76. Stopped drinking alcohol		
77. Stopped smoking		

Figure A4. (continued).

78. Are you pregnant now? Yes No Don't Know

79. If you have been pregnant, your age during your first pregnancy _____.

80. Have you ever heard of toxoplasmosis before? Yes No
If yes, how?

What is it?

81. Have you ever been tested for toxoplasmosis before? Yes No
If yes,
When (month and year)?

Result? Positive Negative Unknown

Reason for being tested?

82. How old are you now? _____

83. What is your cultural or ethnic identity?
[ ] Hispanic or Latino
[ ] White
[ ] Black/African American
[ ] Asian or Pacific Islander
[ ] American Indian or Native American
Other:_____

84. In what range is the YEARLY INCOME of your household?
[ ] Under $12,499
[ ] $12,500-$19,999
[ ] $20,000-$27,499
[ ] $27,500-$34,999
[ ] $35,000-$44,999
[ ] $45,000-74,999
[ ] $75,000-$124,999
[ ] $125,000+

85. Where is your place of birth: _____(City, State, Country)

86. Educational background
[ ] No High School
[ ] Some High School-No diploma
[ ] High School Graduate-diploma or GED
[ ] College-No diploma
[ ] College-diploma
[ ] Advanced Degree

Thank You For Your Time and Cooperation!

Figure A4. (continued).

Modified Agglutination Test (MAT)

Purpose: Serological test for the detection of *Toxoplasma gondii* IgM and IgG antibodies in a variety of avian, marsupalian, and mammalian samples.

Byproducts produced and their hazards: Undiluted Mercaptoethanol is a respiratory irritant and skin irritant with prolonged contact. Trypan Blue is a vital stain that is capable of crossing cell membranes of living organisms. It is carcinogenic and teratogenic in laboratory animals.

Disposal: The dilute serum, saline, Mercaptoethanol, Trypan Blue waste products generated by this procedure are disposed of in designated chemical waste containers for pick up by a UT Environmental Health and Safety team. The microtiter plates and pipette tips are discarded in an approved sharps container which is autoclaved prior to disposal.

Special precautions required: Individuals performing this procedure should be aware that the reagents used in this test are associated with adverse health effects. The MSDS for the chemicals noted above should be consulted to answer any questions about health risk and safety precautions. Additionally, many agents (parasitological, microbiological, and bacterial) present in blood samples may be infectious to humans. Gloves and lab coats should be worn to minimize exposure to these agents.

Procedure:

1. Add 25 *ul* of buffered saline to each of 12 wells on the microtiter plate. Do this for each sample to be tested including the positive and negative control samples.

2. Add 25 *ul* of the serum to be tested in well #1 of the microtiter plate. Repeat for each sample including the positive and negative controls.

3. Make 2-fold serial dilutions of the test sera by transferring 25 *ul* of the contents of well #1 (test serum + buffered saline) to well #2. Mix well contents thoroughly and continue transferring 25 *ul* from well #2 through well #12. Discard 25 *ul* from well #12. Repeat this procedure for each additional specimen, including the controls. Dilutions for the negative control can terminate at well #10 to provide open wells for the antigen control.

4. Add 25 *ul* of 0.2 mol/l 2-Mercaptoethanol (2-ME) to each well (all 12) of the microtiter plate for all samples. For the positive control, it is only necessary to add 2-ME to well numbers 6, 7, 8, 9, and 10. For the negative control, it is only necessary to add 2-ME to well numbers 4 and 8.

5. Well numbers 3 through 12 are the test wells and represent the following dilutions:

Well #3 = 1:16	Well #6 = 1:128	Well #9 = 1:1024
Well #4 = 1:32	Well #7 = 1:256	Well #10 = 1:2048
Well #5 = 1:64	Well #8 = 1:512	Well #11 = 1:4096
		Well #12 = 1:8192

6. Add 50 *ul* of *Toxoplasma* antigen diluted 1:5 to each well of the microtiter plate for all samples. For the positive control it is only necessary to add antigen to well numbers 6, 7, 8, 9, and 10 to test the reactivity of the positive control sample, and establish its endpoint. For the negative control, it is only necessary to add antigen to wells 4 and 8 to test the reactivity of the negative control.

Figure A5. Procedure for the Modified Agglutination Test.

7. Prepare the antigen control in well 12 of the negative control as follows:
   25 *ul* 2-Mercaptoethanol (0.2mol/l)
   25 *ul* PBS (already in well from step 1)
   50 *ul* of *Toxoplasma* antigen diluted 1:5

8. Cover plate and place in incubator overnight.

Reagent Preparation: PBS buffer for use in MAT (Biomerieux Toxoscreen DA)
36.0 g NaCl
7.4 g Na_2HPO_4 (anhydrous)
2.15 g KH_2PO_4 (anhydrous)

This can be dissolved in 5 liters of distilled water or in 1 liter of distilled water. The latter is easier to keep free of bacterial contaminaton. If dissolved in 1 liter of distilled water, it needs to be diluted prior to use.

Reference: Handbook of Experimental Immunology; Third Edition edited by D.M. Weir, Blackwell Scientific Publications pp 20.7.

Figure A5. (continued)

Table A6. Demographic Information requested on the questionnaire. The number of women that responded is listed.

Demographic Information	**Number of Responses**
Age	
By each year	*821*
By 5-year age groups	*821*
Ethnicity	810
Education Level	816
Income	785
Current Residence	819
Childhood Residence	753
Country of Birth	808

Table A7. Meat consumption requested on the questionnaire. The number of women that responded is listed.

Meat Consumption	Number of Responses
Pork	
Sausage	*761*
Roast	*762*
Chops	*763*
Tenderloin	*762*
Country Ham	*763*
Beef	
Hamburger	*764*
Steak	*762*
Poultry	
Chicken	*769*
Domestic Turkey	*756*
Small Ruminants	
Lamb	*760*
Mutton	*760*
Chevon	*762*
Wild Game	764

Table A8. Hygienic behaviors requested on the questionnaire. The number of women that responded is listed.

Hygienic Behaviors	Number of Responses
Wash cutting board after contact with raw meat	816
Wash knife after contact with raw meat	808
Wash hands after handling raw meat	743
Wash vegetables before consumption	813
Wash fruit before consumption	813
Wash hands before handling food	815
Wash hands before eating	813
Wash hands after handling animals	811
Wash hands after going to the bathroom	812

Table A9. Dietary information requested on the questionnaire. The number of women that responded is listed.

Dietary Behaviors	Number of Responses
Drink Unpasteurized Cow Milk	809
Drink Unpasteurized Goat Milk	811
Eat Raw Eggs (found in cookie dough)	813
Vegetarian	819

Table A10. Risky Behaviors requested on the questionnaire. The number of women that responded is listed.

Risky Behaviors	Number of Responses
Risky Occupation or Hobby	803
Garden	812
Wear gloves	*461*
Wear mask	*460*
Own cat	808
Cat uses litter box	*284*
Scoop litter box	*272*
Chew Nails	809
Let Pets Sleep in Bed	732
Play in Children's Sandbox	803

Table A11. Knowledge and opinions regarding meat safety. The number of women that responded is listed.

Knowledge and Opinions Regarding Meat Safety	Number of Responses
Criteria for well-done meat	757
Correct	*601*
Incorrect/Don't Know	*12*
Non-specific Answer	*144*
Why cook pork to well-done?	681
Correct	*600*
Don't Know	*81*
Do same reasons apply to other meats such as chicken and wild game?	700
Yes	*569*
Due to Microorganisms	*37*
Chicken and Wild Game	*532*
No	*78*
Chicken Only	*36*
Wild Game Only	*2*
Pork Only	*2*
Don't Know	*53*
Is meat supply safe?	769
Don't Know	*177*
Why Safe?	*343*
Personal Effort	*226*
Government Effort	*117*
Why not safe?	*61*
Meat is Unclean	*39*
Poor Government Effort	*22*

Table A12. Knowledge of *Toxoplasma gondii*. The number of women that responded is listed.

Knowledge of *T. gondii*	**Number of Responses**
Know of *T. gondii*	820
Yes	*319*
No	*501*
Source learned	*223*
School/Reading	*138*
Personal Experience	*64*
Doctor/Veterinarian	*21*
Able to identify causative agent	231
Yes	*215*
No	*16*
Been tested before?	819
Yes	*22*
Result	
Positive	*2*
Negative	*13*
Unknown	*7*
Reason for testing	*19*
Pregnancy	*16*
Job	*2*
Sick	*1*
No	*797*

Table A13. Eligibility criteria used by Medic Regional Blood Bank and Blood Assurance to screen potential blood donors.

Attribute	Criteria for Eligibility
Blood Hematocrit	38 or greater
Pulse	50-100/minute
Blood Pressure	~180/100 (normal range)
Body Temperature	Less than 37°C
Body Weight	Greater than 110 lbs
Travel in a malarious region	NO
Personal behaviors related to increase risk of HIV	NO
Exposure to Hepatitis B or C	NO

APPENDIX B

QUESTIONNAIRE ANALYSIS: SUMMARY TABLES

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Table B1. Distribution of questionnaires and serum samples obtained from women of childbearing age from the Knoxville, Tennessee area. Not all participants completed a questionnaire and not all questionnaires had an accompanying serum sample.

Sample Information	Seropositive samples	Seronegative samples	Totals
Serum Collected			
Completed questionnaire	55	743	
No questionnaire	1	6	7
TOTALS (Percent)	56 (7.0)	749 (93.0)	805
No Serum Collected			
Questionnaire only			23
			820
Total Participants			**829**

Table B2. Dates of collection from Medic blood drives and from The University of Tennessee Medical Center

Collection Sites	Date	Serum Collected	Questionnaires Collected	Positive (Percent)
Medic				
UT Ag Campus-VTH	1/26/99	15	15	1 (6.7)
UT Dormitory-Clement Hall	2/10/99	9	10	0 (0.0)
Blount Co. Memorial Hospital	2/12/99	39	38	6 (15.4)
St. Mary's Hospital	7/6-8/99	51	48	8 (15.7)
UT Ag Campus-Plant & Soil Science	8/31/99	22	22	2 (9.1)
Blount Co. Memorial Hospital	9/17/99	40	39	2 (5.0)
Baptist Hospital	10/4/99	12	11	2 (16.7)
Blount Co. Memorial Hospital	10/8/99	19	18	1 (5.3)
*UT Student Center**	11/15/99	58	64	4 (6.9)
*UT Student Center**	11/16/99	55	64	1 (1.8)
*UT Ag Campus-Plant & Soil Sciences**	11/16/99	29	29	5 (17.2)
*UT Student Center**	11/17/99	66	67	3 (4.5)
*UT Student Center**	11/18/99	65	66	3 (4.6)
*UT Student Center**	11/19/99	71	76	0 (0.0)
Cariten &Matsushita	1/17&19/00	29	29	1 (3.4)
UT Ag Campus-VTH	1/25/00	22	22	0 (0.0)
Mormon Church, Fountain City	2/2/00	20	20	3 (15.0)
Second Baptist Church	2/9/00	11	11	2 (18.2)
Methodist Medical Center	2/11/00	20	20	4 (20.0)
UT Dormitory-South Carrick Hall	2/15/00	28	28	0 (0.0)
Grace Lutheran Church	2/16/00	19	19	2 (10.5)
Blount Co. Memorial Hospital	2/18/00	33	33	1 (3.0)
Seventh-Day Adventist Church-Knoxville	4/1/00	11	11	0 (0.0)
Blood Assurance				
Seventh-Day Adventist Church-Collegedale	4/8/00	9	9	0 (0.0)
UT Medical Center	6/28/99 -1/22/00	52	52	5 (9.6)
TOTALS		805	821	56 (7.0)

*Denotes Blue/Orange Blood Drive

Table B3. Age distribution of women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Age (n=821)		
18	78	9.5
19	80	9.7
20	74	9.0
21	65	7.9
22	51	6.2
23	25	3.0
24	30	3.7
25	22	2.7
26	23	2.8
27	26	3.2
28	21	2.6
29	11	1.3
30	10	1.2
31	22	2.7
32	26	3.2
33	14	1.7
34	15	1.8
35	8	1.0
36	14	1.7
37	16	1.9
38	11	1.3
39	12	1.5
40	11	1.3
41	19	2.3
42	19	2.3
43	10	1.2
44	16	1.9
45	22	2.7
46	15	1.8
47	13	1.6
48	7	0.9
49	6	0.7
50	10	1.2
51	5	0.6
52	9	1.1
53	5	0.6

Table B4. Seroprevalence of *T. gondii* and age distribution of women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number))	Seropositive (Percent)	Seronegative (Percent)
Age (years) (n=798)		
18 (n=74)	2 (2.7)	72 (97.3)
19 (n=76)	3 (3.9)	73 (96.1)
20 (n=69)	2 (2.9)	67 (97.1)
21 (n=60)	3 (5.0)	57 (95.0)
22 (n=51)	3 (5.9)	48 (94.1)
23 (n=25)	0 (0.0)	25 (100.0)
24 (n=30)	1 (3.3)	29 (96.7)
25 (n=21)	3 (14.3)	18 (85.7)
26 (n=22)	1 (4.5)	21 (95.5)
27 (n=26)	3 (11.5)	23 (88.5)
28 (n=21)	2 (9.5)	19 (90.5)
29 (n=11)	0 (0.0)	11 (100.0)
30 (n=10)	1 (10.0)	9 (90.0)
31 (n=22)	2 (9.1)	20 (90.9)
32 (n=24)	4 (16.7)	20 (83.3)
33 (n=14)	1 (7.1)	13 (92.2)
34 (n=15)	4 (26.7)	11 (73.3)
35 (n=8)	1 (12.5)	7 (87.5)
36 (n=14)	0 (0.0)	14 (100.0)
37 (n=16)	1 (6.2)	15 (93.8)
38 (n=11)	0 (0.0)	11 (100.0)
39 (n=12)	0 (0.0)	12 (100.0)
40 (n=11)	0 (0.0)	11 (100.0)
41 (n=19)	0 (0.0)	19 (100.0)
42 (n=18)	3 (16.7)	16 (83.3)
43 (n=10)	0 (0.0)	10 (100.0)
44 (n=16)	1 (6.2)	15 (93.8)
45 (n=22)	3 (13.6)	19 (86.4)
46 (n=15)	2 (13.3)	13 (86.7)
47 (n=13)	0 (0.0)	13 (100.0)
48 (n=7)	1 (14.3)	6 (85.7)
49 (n=6)	1 (16.7)	5 (83.3)
50 (n=10)	0 (0.0)	10 (100.0)
51 (n=4)	1 (25.0)	3 (75.0)
52 (n=9)	5 (55.6)	4 (44.4)
53 (n=5)	1 (20.0)	4 (80.0)

Table B5. Distribution of age of participants by five year age groups in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Age Group (years) (n=821)		
18-19	158	19.2
20-24	245	29.8
25-29	103	12.6
30-34	87	10.6
35-39	61	7.4
40-44	75	9.1
45-49	63	7.7
50-53	29	3.5

Table B6. Seroprevalence of *T. gondii* by five-year age groups in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% C.I.)	p-value
Age Group (years) (n=798)	48 (6.0)	750 (94.0)		
18-19 (n=150)	5 (3.3)	145 (96.7)	1	
20-24 (n=235)	9 (3.8)	226 (96.2)	0.87 (0.25-2.90)	0.80
25-29 (n=101)	9 (8.9)	92 (91.1)	0.35 (0.10-1.20)	0.06
30-34 (n=85)	12 (14.1)	73 (85.9)	0.21 (0.06-0.67)	<0.01*
35-39 (n=61)	2 (3.3)	59 (96.7)	1.02 (0.17-7.81)	0.98
40-44 (n=75)	4 (5.3)	71 (94.7)	0.61 (0.14-2.81)	0.47
45-49 (n=63)	7 (11.1)	56 (88.9)	0.28 (0.07-1.02)	0.02
50-53 (n=28)	7 (25.0)	21 (75.0)	0.10 (0.03-0.41)	<0.01*

Odds Ratio 0.55<0.93<1.54, Chi Square 0.04, p=0.83
*Statistically significant by Chi Square (p< .01, 1df)

Table B7. Chi Square of seroprevalence of *T. gondii* and median age in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of seropositivity and those greater than the median age. The exclusion of one from the 95% confidence interval indicates that women older than the median age of 25 years are 2.91 times more likely to be infected compared to those younger than the median age.

Age (years) (n=798) (n=number)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
25-53 (n=413)	41*	372
18-24 (n=385)	14	371
Odds Ratio	2.91	P<0.01
Upper 95% confidence limit	5.72	
Lower 95% confidence limit	1.51	

*Statistically significant by Chi Square (p< 0.10, 1df)

Table B8. Ethnic identities of participants in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Ethnicity (n=810)		
American Indian	5	0.6
Asian/Pacific Islander	14	1.7
Black/African American	22	2.7
Hispanic	10	1.2
White	757	93.5
Mixed Race	2	0.2

Table B9. Seroprevalence of *T. gondii* antibodies and ethnic identity in women of childbearing age from the Knoxville, Tennessee area.

Demographic information (n=number)	Seropositive (Percent)	Seronegative (Percent)
Ethnicity (n=787)	53 (6.6)	734 (93.4)
American Indian (n=5)	1 (20.0)	4 (80.0)
Asian/Pacific Islander (n=11)	0 (0.0)	11 (100.0)
Black/African American (n=19)	1 (5.3)	18 (94.7)
Hispanic (n=10)	1 (10.0)	9 (90.0)
White (n=740)	50 (6.8)	690 (93.2)
Mixed Race (n=2)	0 (0.0)	2 (100.0)

Table B10. Education of women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Education (n=816)		
Less than high school	1	0.1
High school-no diploma	11	1.3
High school diploma or GED	119	14.6
College-no diploma	390	47.8
College diploma	212	26.0
Advanced degree	83	10.2

Table B11. Seroprevalence of *T. gondii* and education in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Seropositive (Percent)	Seronegative (Percent)
Education (n=794)	54 (6.8)	739 (93.2)
Less than high school (n=1)	0 (0.0)	1 (100.0)
High school-no diploma(n=11)	1 (9.1)	10 (90.9)
High school diploma or GED (n=117)	12 (10.3)	105 (89.7)
College-no diploma (n=372)	19 (5.1)	353 (94.9)
College diploma (n=210)	15 (7.1)	195 (92.9)
Advanced degree (n=82)	7 (8.5)	75 (91.5)

Table B12. Chi-square of seroprevalence of *T. gondii* and education in women of childbearing age from the Knoxville, Tennessee area. No association was found between seropositivity and education.

Education (n=793)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Attended High School (n=129)	13	116
Attended College (n=664)	41	623
Odds Ratio	1.70	p=0.11
Upper 95% confidence limit	3.41	
Lower 95% confidence limit	0.84	

Table B13. Income of women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Income (n=785)		
Under $12,499	134	17.1
$12,500-19,999	71	9.0
$20,000-27,499	75	9.6
$27,500-34,999	61	7.8
$35,000-44,999	87	11.1
$45,000-74,999	197	25.1
$75,000-124,999	110	14.0
$125,000+	50	6.4

Table B14. Seroprevalence of *T. gondii* and income in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Seropositive (Percent)	Seronegative (Percent)
Income (n=763)	51 (6.7)	712 (93.3)
Under $12,499 (n=129)	4 (3.1)	125 (96.9)
$12,500-19,999 (n=70)	5 (7.1)	65 (92.9)
$20,000-27,499 (n=74)	4 (5.4)	70 (94.6)
$27,500-34,999 (n=60)	2 (3.3)	58 (96.7)
$35,000-44,999 (n=85)	8 (9.4)	77 (90.6)
$45,000-74,999 (190)	19 (10.0)	171 (90.0)
$75,000-124,999 (n=107)	5 (4.7)	102 (95.3)
$125,000+ (n=48)	4 (8.3)	44 (91.7)

Table B15. Current residence in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Current Residence (n=819)		
Farm	19	12.3
Rural	137	6.7
Small Town	102	12.5
Suburban	336	41.0
Urban	225	27.5

Table B16. Seroprevalence of *T. gondii* and current residence in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Seropositive (Percent)	Seronegative (Percent)	Odds Ratio (95% C.I.)	p-value
Current Residence (n=796)	55 (6.9)	741 (93.1)		
Farm (n=19)	6 (31.6)	13 (68.4)	1	
Rural (n=137)	10 (7.3)	127 (92.7)	5.86 (1.58-21.55)	<0.01*
Small Town (n=100)	6 (6.0)	94 (94.0)	7.23 (1.73-30.84)	<0.01*
Suburban (n=329)	21 (6.4)	308 (93.6)	6.77 (2.05-21.77)	<0.01*
Urban (n=211)	12 (5.7)	199 (94.3)	7.65 (2.15-26.95)	<0.01*

Odds Ratio 3.70<6.82<12.61, Chi Square 50.03, p<0.01
*Statistically significant by Chi Square (p< .01, 1df)

Table B17. Chi-square of seroprevalence of *T. gondii* and current residence in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of seropositivity and current residence. The exclusion of 1 from the 95% confidence interval indicates that those that live on a farm are 6.86 times more likely to be seropositive than those that do not.

Current Residence (n=796)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Farm (n=19)	6*	13
Other (n=777)	49	728
Odds Ratio	6.86	p<0.01
Upper 95% confidence limit	20.46	
Lower 95% confidence limit	2.21	

*Statistically significant by Chi Square (p< 0.10, 1df)

Table B18. Childhood residence in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	Number	Percent of Population
Childhood Residence (n=753)		
Residency or mixed residency with farm or rural settings	244	32.4
Residency or mixed residency without farm or rural settings	509	67.6

Table B19. Chi-square of seroprevalence of *T. gondii* and childhood residence in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of seropositivity and childhood residence. The exclusion of 1 from the 95% confidence interval indicates that those with a farm or rural childhood residence were 3.63 times more likely to be seropositive than those that did not.

Childhood Residence (n=753)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Residency with farm or rural settings (n=244)	33*	211
Residency without farm or rural settings (n=509)	21	488
Odds Ratio	3.63	p<0.01
Upper 95% confidence limit	6.68	
Lower 95% confidence limit	1.99	

*Statistically significant by Chi Square (p< .01. 1df)

Table B20. Country of birth in women of childbearing age from the Knoxville, Tennessee area.

Demographic Information (n=number)	**Number**	**Percent of Population**
Country of birth (n=808)		
Foreign birth	32	4.0
USA birth	776	96.0

Table B21. Chi-square of country of birth and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those not born in the United States. No association was found

Country of birth (n=785)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Foreign birth (n=28)	3	26
USA birth (n=756)	50	706
Odds Ratio	1.63	p=0.43
Upper 95% confidence limit	5.91	
Lower 95% confidence limit	0.38	

Table B22. Eating pork sausage in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Pork Sausage	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=761)	577 (75.8)	184 (24.2)		
18-19 (n=141)	91 (64.5)	50 (35.5)	1	
20-24 (n=221)	150 (67.9)	71 (32.1)	0.86 (0.54-1.38)	0.51
25-29 (n=95)	78 (82.1)	17 (17.9)	0.40 (0.20-0.77)	<0.01*
30-34 (n=86)	73 (84.9)	13 (15.1)	0.32 (0.15-0.67)	<0.01*
35-39 (n=60)	55 (91.7)	5 (8.3)	0.17 (0.05-0.47)	<0.01*
40-44 (n=72)	64 (88.9)	8 (11.1)	0.23 (0.09-0.54)	<0.01*
45-49 (n=61)	43 (70.5)	18 (29.5)	0.76 (0.38-1.53)	0.41
50-53 (n=25)	23 (92.0)	2 (8.0)	0.16 (0.02-0.74)	<0.01*

OR 0.35<0.45<0.58, Chi Square 40.50, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B23. Chi-square of eating pork sausage and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork sausage. No association was found.

Eat Pork Sausage (n=741)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=563)	46	517
No (n=178)	8	170
Odds Ratio	1.89	p=0.10
Upper 95% confidence limit	4.43	
Lower 95% confidence limit	0.84	

Table B24. Frequency of eating pork sausage in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of eating Pork Sausage	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=576)	219 (38.0)	357 (62.0)		
18-19 (n=91)	32 (35.2)	59 (64.8)	1	
20-24 (n=150)	56 (37.3)	94 (62.7)	0.91 (0.51-1.62)	0.73
25-29 (n=78)	28 (35.9)	50 (64.1)	0.97 (0.49-1.91)	0.92
30-34 (n=73)	25 (34.2)	48 (65.8)	1.04 (0.52-2.09)	0.90
35-39 (n=55)	22 (40.0)	33 (60.0)	0.81 (0.39-1.72)	0.56
40-44 (n=63)	30 (47.6)	33 (52.4)	0.60 (0.29-1.21)	0.12
45-49 (n=43)	15 (34.9)	28 (65.1)	1.01 (0.44-2.32)	0.97
50-53 (n=23)	11 (47.8)	12 (52.2)	0.59 (0.21-1.64)	0.26

OR 0.66<0.85<1.10. Chi Square 1.41, p=0.24

Table B25. Chi-square of frequency of eating pork sausage and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork sausage at least monthly. No association was found.

Frequency of Eating Pork Sausage (n=562)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=212)	18	194
Yearly (n=350)	27	323
Odds Ratio	1.11	p=0.74
Upper 95% confidence limit	2.15	
Lower 95% confidence limit	0.57	

Table B26. Cooking preference of pork sausage in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Pork Sausage	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=542)	49 (9.0)	493 (91.0)		
18-19 (n=83)	18 (21.7)	65 (78.3)	1	
20-24 (n=141)	18 (12.8)	123 (87.2)	1.89 (0.87-4.12)	0.08
25-29 (n=71)	5 (7.0)	66 (93.0)	3.66 (1.18-12.03)	0.01*
30-34 (n=71)	4 (5.6)	67 (94.4)	4.64 (1.38-17.21)	<0.01*
35-39 (n=52)	0 (0.0)	52 (100.0)	Insufficient data	
40-44 (n=62)	2 (3.2)	60 (96.8)	8.31 (1.74-54.20)	<0.01*
45-49 (n=41)	1 (2.4)	4097.6)	11.08 (1.46-231.06)	0.01*
50-53 (n=21)	1(4.8)	20 (95.2)	5.54 (0.70-118.09)	0.07

OR 2.66<4.43<6.55, Chi Square 45.72, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B27. Chi-square of cooking preference of pork sausage and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork sausage less than well done. No association was found.

Cooking Preference for Pork Sausage (n=529)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=46)	2	44
Well Done (n=483)	41	442
Odds Ratio	0.49	p=0.33
Upper 95% confidence limit	2.17	
Lower 95% confidence limit	0.08	

Table B28. Eating pork chops in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Pork Chops	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=763)	649 (85.1)	114 (14.9)		
18-19 (n=141)	115 (81.6	26 (18.4)	1	
20-24 (n=221)	175 (79.2)	46 (20.8)	1.16 (0.66-2.06)	0.58
25-29 (n=95)	84 (88.4)	11 (11.6)	0.58 (0.25-1.31)	0.16
30-34 (n=87)	79 (90.8)	8 (9.2)	0.45 (0.18-1.10)	0.06
35-39 (n=60)	56 (93.3)	4 (6.7)	0.32 (0.09-1.02)	0.03
40-44 (n=72)	67 (93.1)	5 (6.9)	0.33 (0.11-0.96)	0.02*
45-49 (n=61)	49 (80.3)	12 (19.7)	1.08 (0.47-2.46)	0.84
50-53 (n=26)	24 (92.3)	2 (7.7)	0.37 (0.06-1.77)	0.18

OR 0.49<0.66<0.90, Chi Square 7.04, p=0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B29. Chi-square of eating pork chops and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork chops. The exclusion of 1 from the 95% confidence interval indicates that those that eat pork chops are 4.67 times more likely to be seropositive than those that do not.

Eat Pork Chops (n=743)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=636)	52*	584
No (n=107)	2	105
Odds Ratio	4.67	p=0.02
Upper 95% confidence limit	28.18	
Lower 95% confidence limit	1.09	

*Statistically significant by Chi Square (p<0.01, 1df)

Table B30. Frequency of eating pork chops in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Pork Chops	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=649)	270 (41.6)	379 (58.4)		
18-19 (n=115)	43 (37.4)	72 (62.6)	1	
20-24 (n=175)	61 (34.9)	114 (65.1)	1.12 (0.66-1.87)	0.66
25-29 (n=84)	34 (40.0)	50 (60.0)	0.88 (0.47-1.63)	0.66
30-34 (n=80)	38 (47.5)	42 (52.5)	0.66 (0.35-1.23)	0.16
35-39 (n=56)	28 (50.0)	28 (50.0)	0.60 (0.30-1.20)	0.12
40-44 (n=66)	30 (45.5)	36 (54.5)	0.72 (0.37-1.39)	0.29
45-49 (n=49)	26 (53.1)	23 (46.9)	0.53 (0.25-1.10)	0.06
50-53 (n=24)	10 (41.7)	14 (58.3)	0.84 (0.31-2.24)	0.69

OR 0.60<0.77<0.97. Chi Square 4.71, p=0.03

Table B31. Chi-square of frequency of eating pork chops and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork chops at least monthly. No association was found.

Frequency of Eating Pork Chops (n=636)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=263)	23	240
Yearly (n=373)	28	345
Odds Ratio	1.18	p=0.57
Upper 95% confidence limit	2.18	
Lower 95% confidence limit	0.64	

Table B32. Cooking preference of pork chops in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Pork Chops	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=608)	87 (14.3)	521 (85.7)		
18-19 (n=104)	30 (28.8)	74 (71.2)	1	
20-24 (n=165)	28 (17.0)	137 (83.0)	1.98 (1.06-3.72)	0.02*
25-29 (n=76)	9 (11.8)	67 (88.2)	3.02 (1.26-7.42)	0.01*
30-34 (n=78)	8 (10.3)	70 (89.7)	3.55 (1.43-9.06)	<0.01*
35-39 (n=53)	4 (7.5)	49 (92.5)	4.97 (1.53-17.79)	<0.01*
40-44 (n=64)	6 (9.4)	58 (90.6)	3.92 (1.43-11.30)	<0.01*
45-49 (n=46)	0 (0.0)	46 (100.0)	Insufficient data	<0.01
50-53 (n=22)	2 (9.1)	20 (90.9)	4.05 (0.83-26.79)	0.05

OR 2.42<3.52<4.81. Chi Square 54.88, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B33. Chi-square of cooking preference for pork chops and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork chops less than well done. No association was found.

Cooking Preference for Pork Chops (n=596)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=84)	7	77
Well Done (n=512)	42	470
Odds Ratio	1.02	p=0.97
Upper 95% confidence limit	2.47	
Lower 95% confidence limit	0.04	

Table B34. Eating pork roast in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Pork Roast	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=762)	560 (73.5)	202 (26.5)		
18-19 (n=141)	103 (73.0)	38 (27.0)	1	
20-24 (n=221)	151 (68.3)	70 (31.7)	1.26 (0.77-2.06)	0.34
25-29 (n=95)	68 (71.6)	27 (28.4)	1.08 (0.58-2.00)	0.80
30-34 (n=87)	74 (85.1)	13 (14.9(	0.48 (0.22-1.00)	0.05
35-39 (n=59)	46 (78.0)	13 (22.0)	0.77 (0.35-1.66)	0.47
40-44 (n=72)	57 (79.2)	15 (20.8)	0.71 (0.34-1.48)	0.33
45-49 (n=61)	41 (67.2)	20 (32.8)	1.32 (0.65-2.66)	0.40
50-53 (n=26)	20 (77.0)	6 (23.0)	0.81 (0.27-2.36)	0.68

OR 0.73<0.93<1.19, Chi Square 0.28, p=0.60

Table B35. Chi-square of eating pork roast and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork roast. No association was found.

Eat Pork Roast (n=742)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=547)	45	502
No (n=195)	9	186
Odds Ratio	1.85	p=0.10
Upper 95% confidence limit	4.15	
Lower 95% confidence limit	0.85	

Table B36. Frequency of eating pork roast in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Pork Roast	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=559)	171 (30.6)	388 (69.4)		
18-19 (n=103)	34 (33.0)	69 (67.0)	1	
20-24 (n=151)	44 (29.1)	107 (70.9)	1.20 (0.67-2.13)	0.51
25-29 (n=68)	18 (26.5)	50 (73.5)	1.37 (0.66-2.85)	0.36
30-34 (n=74)	24 (32.4)	50 (67.6)	1.03 (0.52-2.04)	0.94
35-39 (n=46)	12 (28.1)	34 (73.9)	1.40 (0.60-3.27)	0.40
40-44 (n=56)	18 (32.1)	38 (67.9)	1.04 (0.49-2.21)	0.91
45-49 (n=41)	13 (31.7)	28 (68.3)	1.06 (0.49-2.48)	0.88
50-53 (n=20)	8 (40.0)	12 (60.0)	0.74 (0.25-2.21)	0.55

OR 0.86<1.13<1.49. Chi Square 0.75, p=0.39

Table B37. Chi square of frequency of eating pork roast and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork roast at least monthly. No association was found.

Frequency of Eating Pork Roast (n=546)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=168)	15	153
Yearly (n=378)	29	349
Odds Ratio	1.18	p=0.62
Upper 95% confidence limit	2.36	
Lower 95% confidence limit	0.58	

Table B38. Cooking preference of pork roast in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Pork Roast	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=523)	82 (15.7)	441 (84.3)		
18-19 (n=90)	26 (28.9)	64 (71.1)	1	
20-24 (n=142)	27 (19.0)	115 (81.0)	1.73 (0.89-3.36)	0.08
25-29 (n=62)	6 (9.7)	56 (90.3)	3.79 (1.36-11.13)	<0.01*
30-34 (n=74)	11 (14.9)	63 (85.1)	2.33 (1.00-5.51)	0.03
35-39 (n=44)	4 (9.1)	40 (90.9)	4.06 (1.22-14.89)	0.01*
40-44 (n=54)	6 (11.3)	48 (88.7)	3.25 (1.15-9.61)	0.01*
45-49 (n=39)	0 (0.0)	39 (100.0)	Insufficient data	<0.01
50-53 (n=18)	2 (11.1)	16 (88.9)	3.25 (0.64-22.05)	0.12

OR 2.06<3.03<4.21, Chi Square 38.77, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B39. Chi-square of cooking preference for pork roast and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork roast less than well done. No association was found.

Cooking Preference for Pork Roast (n=512)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=80)	6	74
Well Done (n=432)	36	396
Odds Ratio	0.89	p=0.80
Upper 95% confidence limit	2.31	
Lower 95% confidence limit	0.33	

Table B40. Eating pork tenderloin in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Pork Tenderloin	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=762)	541 (71.0)	221 (19.0)		
18-19 (n=141)	94 (66.7)	47 (33.3)	1	
20-24 (n=220)	148 (67.3)	72 (32.7)	0.97 (0.61-1.56)	0.91
25-29 (n=95)	63 (66.3)	32 (33.7)	1.02 (0.56-1.83)	0.96
30-34 (n=87)	73 (83.9)	14 (16.1)	0.38 (0.19-0.78)	<0.01*
35-39 (n=60)	47 (78.3)	13 (21.7)	0.55 (0.26-1.18)	0.10
40-44 (n=72)	54 (75.0)	18 (25.0)	0.67 (0.33-1.32)	0.21
45-49 (n=61)	41 (67.2)	20 (32.8)	0.98 (0.49-1.94)	0.94
50-53 (n=26)	21 (80.8)	5 (19.2)	0.48 (0.15-1.14)	0.15

OR 0.59<0.74<0.94, Chi Square 6.01, p=0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B41. Chi-square of eating pork tenderloin and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork tenderloin. No association was found.

Eat Pork Tenderloin (n=742)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=530)	41	489
No (n=212)	13	199
Odds Ratio	1.28	P=0.45
Upper 95% confidence limit	2.58	
Lower 95% confidence limit	0.65	

Table B42. Frequency of eating pork tenderloin in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Pork Tenderloin	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=540)	167 (30.9)	373 (69.1)		
18-19 (n=94)	30 (31.9)	64 (68.1)	1	
20-24 (n=148)	42 (28.4)	106 (71.6)	1.18 (0.65-2.15)	0.56
25-29 (n=63)	21 (33.3)	42 (66.7)	0.94 (0.45-1.96)	0.85
30-34 (n=73)	25 (34.2)	48 (65.8)	0.90 (0.45-1.81)	0.75
35-39 (n=47)	18 (38.3)	29 (61.7)	0.76 (0.34-1.67)	0.45
40-44 (n=53)	13 (24.5)	40 (75.5)	1.44 (0.63-3.32)	0.34
45-49 (n=41)	12 (29.2)	29 (70.8)	1.13 (0.47-2.73)	0.76
50-53 (n=21)	6 (28.6)	15 (71.4)	1.17 (0.38-3.79)	0.77

OR 0.79<1.05<1.38. Chi Square 0.07, p=0.80

Table B43. Chi-square of frequency of eating pork tenderloin and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork tenderloin at least monthly. No association was found.

Frequency of Eating Pork Tenderloin (n=529)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=164)	15	149
Yearly (n=365)	25	340
Odds Ratio	1.37	p=0.36
Upper 95% confidence limit	2.79	
Lower 95% confidence limit	0.67	

Table B44. Cooking preference of pork tenderloin in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Pork Tenderloin	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=506)	84 (16.6)	422 (83.4)		
18-19 (n=84)	23 (27.4)	61 (72.6)	1	
20-24 (n=139)	32 (23.0)	107 (77.0)	1.26 (0.65-2.45)	0.46
25-29 (n=57)	7 (12.1)	50 (87.9)	2.69 (0.99-7.57)	0.03
30-34 (n=72)	10 (13.9)	62 (86.1)	2.34 (0.96-5.78)	0.04
35-39 (n=44)	4 (9.1)	40 (90.9)	3.77 (1.12-13.98)	0.02*
40-44 (n=51)	6 (11.8)	45 (88.2)	2.83 (0.98-8.50)	0.03
45-49 (n=40)	0 (0.0)	40 (100.0)	Insufficient data	<0.01
50-53 (n=19)	2 (10.5)	17 (89.5)	3.20 (0.63-21.81)	0.12

OR 1.73<2.53<3.52, Chi Square 26.79, p<0.01*
*Statistically significant by Chi Square (p<0.01, 1df)

Table B45. Chi-square of cooking preference for pork tenderloin and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat pork tenderloin less than well done. No association was found.

Cooking Preference for Pork Tenderloin (n=496)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=82)	6	76
Well Done (n=414)	33	381
Odds Ratio	0.91	p=0.84
Upper 95% confidence limit	2.38	
Lower 95% confidence limit	0.33	

Table B46. Eating country ham in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Country Ham	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=763)	532 (69.7)	231 (30.3)		
18-19 (n=141)	105 (74.5)	36 (25.5)	1	
20-24 (n=221)	160 (72.4)	61 (27.6)	1.11 (0.67-1.85)	0.66
25-29 (n=95)	66 (69.5)	29 (30.5)	1.28 (0.69-2.38)	0.40
30-34 (n=87)	64 (73.6)	23 (26.4)	1.05 (0.55-2.01)	0.88
35-39 (n=60)	43 (71.7)	17 (28.3)	1.15 (0.55-2.39)	0.68
40-44 (n=72)	43 (59.7)	29 (40.3)	1.97 (1.03-3.76)	0.03*
45-49 (n=61)	31 (50.8)	30 (49.2)	2.82 (1.44-5.56)	<0.01
50-53 (n=26)	20 (76.9)	6 (23.1)	0.88 (0.29-2.55)	0.79

OR 1.09<1.38<1.75, Chi Square 7.07, p=0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B47. Chi-square of eating country ham and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat country ham. No association was found.

Eat Country Ham (n=743)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=517)	44	473
No (n=226)	10	216
Odds Ratio	2.01	p=0.05
Upper 95% confidence limit	4.34	
Lower 95% confidence limit	0.95	

Table B48. Frequency of eating country ham in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Country Ham	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=531)	138(26.0)	393 (74.0)		
18-19 (n=105)	39 (37.1)	66 (62.9)	1	
20-24 (n=160)	44 (27.5)	116 (72.5)	1.56 (0.08-2.73)	0.10
25-29 (n=66)	16 (24.2)	50 (75.8)	1.85 (0.88-3.90)	0.08
30-34 (n=64)	12 (18.8)	52 (81.2)	2.56 (1.15-5.77)	0.01*
35-39 (n=43)	7 (16.3)	36 (83.7)	3.04 (1.15-8.31)	0.01*
40-44 (n=42)	8 (23.5)	34 (76.5)	2.51 (0.99-6.57)	0.03
45-49 (n=31)	9 (29.0)	22 (71.0)	1.44 (0.56-3.79)	0.41
50-53 (n=20)	3 (15.0)	17 (85.0)	3.35 (0.85-15.41)	0.05

OR 1.52<2.05<2.73. Chi Square 22.73, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B49. Chi-square of frequency of eating country ham and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat county ham at least monthly. No association was found.

Frequency of Eating Country Ham (n=516)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=133)	10	123
Yearly (n=383)	33	350
Odds Ratio	0.86	p=0.69
Upper 95% confidence limit	1.86	
Lower 95% confidence limit	0.44	

Table B50. Cooking preference of country ham in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Country Ham	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=499)	66 (13.2)	433 (86.8)		
18-19 (n=94)	19 (20.2)	75 (79.8)	1	
20-24 (n=152)	29 (19.1)	123 (80.9)	1.07 (0.54-2.15)	0.83
25-29 (n=59)	7 (11.9)	52 (88.1)	1.88 (0.68-5.35)	0.18
30-34 (n=63)	6 (9.5)	57 (90.5)	2.41 (0.84-7.24)	0.07
35-39 (n=41)	1 (2.4)	40 (97.6)	10.31 (1.35-210.45)	0.01*
40-44 (n=42)	4 (9.5)	38 (90.5)	2.41 (0.70-9.04)	0.12
45-49 (n=30)	0 (0.0)	30 (100.0)	Insufficient data	0.01
50-53 (n=18)	0 (0.0)	18 (100.0)	Insufficient data	0.04

OR 1.49<2.32<3.29, Chi Square 16.47, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B51. Chi-square of cooking preference for country ham and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat country ham less than well done. No association was found.

Cooking Preference for Country Ham (n=485)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=60)	3	57
Well Done (n=425)	39	386
Odds Ratio	0.52	p=0.28
Upper 95% confidence limit	1.83	
Lower 95% confidence limit	0.12	

Table B52. Eating hamburger in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Hamburger	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=764)	728 (95.3)	37 (4.7)		
18-19 (n=141)	135 (95.7)	6 (4.3)	1	
20-24 (n=221)	203 (91.9)	18 (8.1)	2.00 (0.72-5.78)	0.15
25-29 (n=96)	93 (96.9)	3 (3.1)	0.73 (0.14-3.37)	0.66
30-34 (n=87)	86 (98.9)	1 (1.1)	0.26 (0.01-2.25)	0.19
35-39 (n=60)	60 (100.)	0 (0.0)	0.00 (0.00-2.19)	0.10
40-44 (n=72)	71 (98.6)	1 (1.4)	0.32 (0.01-2.73)	0.27
45-49 (n=61)	54 (88.5)	7 (11.5)	2.92 (0.83-10.36)	0.05
50-53 (n=26)	25 (96.2)	1 (3.8)	Insufficient data	0.92

OR 0.62<1.06<1.80, Chi Square 0.01, p=0.93

Table B53. Chi-square of eating hamburger and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat hamburger. No association was found.

Eat Hamburger (n=744)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=710)	52	658
No (n=34)	2	32
Odds Ratio	1.26	p=0.75
Upper 95% confidence limit	7.85	
Lower 95% confidence limit	0.28	

Table B54. Frequency of eating hamburger in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of eating Hamburger	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=726)	600 (82.6)	126 (17.4)		
18-19 (n=153)	115 (85.2)	20 (14.8)	1	
20-24 (n=203)	164 (80.8)	39 (19.2)	1.37 (0.73-2.57)	0.30
25-29 (n=93)	80 (86.0)	13 (14.))	0.93 (0.41-2.11)	0.86
30-34 (n=86)	69 (80.2)	17 (19.8)	1.42 (0.66-3.06)	0.34
35-39 (n=60)	50 (83.3)	10 (16.7)	1.15 (0.46-2.82)	0.74
40-44 (n=70)	57 (81.4)	13 (18.6)	1.31 (0.57-3.01)	0.49
45-49 (n=54)	47 (87.0)	7 (13.0)	0.86 (0.31-2.33)	0.74
50-53 (n=25)	18 (72.0)	7 (28.)	2.24 (0.74-6.65)	0.11

OR 0.93<1.25<1.69. Chi Square 2.11, p=0.15

Table B55. Chi-square of frequency of eating hamburger and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat hamburger at least monthly. No association was found.

Frequency of Eating Hamburger (n=709)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=585)	40	545
Yearly (n=124)	11	113
Odds Ratio	0.75	p=0.43
Upper 95% confidence limit	1.61	
Lower 95% confidence limit	0.36	

Table B56. Cooking preference of hamburger in women of childbearing age from the Knoxville, Tennessee area stratified by age.

Cooking Preference of Hamburger	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=690)	176 (25.5)	514 (74.5)		
18-19 (n=124)	40 (32.3)	84 (67.7)	1	
20-24 (n=195)	60 (30.8)	135 (69.2)	1.07 (0.64-1.79)	0.78
25-29 (n=86)	21 (24.4)	65 (75.6)	1.47 (0.76-2.87)	0.22
30-34 (n=84)	16 (19.0)	68 (81.0)	2.02 (1.00-4.15)	0.03
35-39 (n=58)	17 (29.3)	41 (70.7)	1.15 (0.55-2.40)	0.69
40-44 (n=69)	15 (21.7)	54 (78.3)	1.71 (0.82-3.61)	0.12
45-49 (n=51)	4 (7.8)	47 (92.2)	5.60 (1.77-19.69)	<0.01*
50-53 (n=23)	3 (13.)	20 (87.0)	3.17 (0.82-14.30)	0.06

OR 0.93<1.25<1.69. Chi Square 2.11, p=0.15
*Statistically significant by Chi Square (p<0.01, 1df)

Table B57. Chi-square of cooking preference of hamburger and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat hamburger less than well done. No association was found.

Cooking Preference for Hamburger (n=674)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=171)	8	163
Well Done (n=503)	42	461
Odds Ratio	0.54	p=0.11
Upper 95% confidence limit	1.22	
Lower 95% confidence limit	0.23	

Table B58. Eating steak in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Steak	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=762)	694 (91.9)	68 (8.9)		
18-19 (n=140)	126 (90.0)	14 (10.0)	1	
20-24 (n=221)	194 (87.8)	27 (12.2)	1.25 (0.06-2.62)	0.52
25-29 (n=96)	87 (90.6)	9 (9.4)	0.93 (0.35-2.42)	0.87
30-34 (n=87)	83 (95.4)	4 (4.6)	0.43 (0.12-1.48)	0.14
35-39 (n=59)	59 (100.0)	0 (0.0)	0.00 (0.00-0.81)	0.01
40-44 (n=72)	69 (95.8)	3 (4.2)	0.39 (0.09-1.52)	0.14
45-49 (n=61)	54 (88.5)	7 (11.5)	1.17 (0.40-3.31)	0.75
50-53 (n=26)	22 (84.6)	4 (15.4)	1.64 (0.41-6.04)	0.42

OR 0.55<0.80<1.17, Chi Square 1.19, p=0.28

Table B59. Chi-square of eating steak and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area stratified by age group. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat steak. No association was found.

Eat Steak (n=742)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=678)	52	626
No (n=64)	2	62
Odds Ratio	2.58	p=0.18
Upper 95% confidence limit	15.66	
Lower 95% confidence limit	0.60	

Table B60. Frequency of eating steak in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of eating Steak	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=694)	480 (69.2)	214 (30.8)		
18-19 (n=127)	85 (66.9(	42 (33.1)	1	
20-24 (n=194)	129 (66.5)	65 (33.5)	1.02 (0.62-1.69)	0.94
25-29 (n=87)	59 (67.8)	28 (32.2)	0.96 (0.51-1.79)	0.89
30-34 (n=83)	61 (73.5)	22 (26.5)	0.73 (0.38-1.40)	0.31
35-39 (n=59)	40 (67.8)	19 (32.2)	0.96 (0.47-1.95)	0.91
40-44 (n=68)	48 (70.6)	20 (29.4)	0.84 (0.42-1.67)	0.60
45-49 (n=54)	42 (77.8)	12 (22.2)	0.58 (0.26-1.28)	0.14
50-53 (n=22)	16 (72.7)	6 (27.3)	0.76 (0.24-2.27)	0.59

OR 0.67<0.86<1.09. Chi Square 1.46, p=0.23

Table B61. Chi-square of frequency of eating steak and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat steak at least monthly. No association was found.

Frequency of Eating Steak (n=678)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Monthly (n=468)	39	429
Yearly (n=210)	12	198
Odds Ratio	1.50	p=0.23
Upper 95% confidence limit	2.73	
Lower 95% confidence limit	0.74	

Table B62. Cooking preference of steak in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Steak	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=656)	329 (50.2)	327 (49.8)		
18-19 (n=115)	60 (52.2)	55 (47.8)	1	
20-24 (n=184)	90 (48.9)	94 (51.1)	1.14 (0.70-1.87)	0.58
25-29 (n=79)	43 (54.4)	36 (45.6)	0.91 (0.49-1.69)	0.76
30-34 (n=81)	41 (50.6)	40 (49.4)	1.06 (0.58-1.96)	0.83
35-39 (n=58)	28 (48.3)	30 (51.7)	1.17 (059-2.31)	0.63
40-44 (n=68)	39 (57.3)	29 (42.7)	0.81 (0.42-1.55)	0.50
45-49 (n=51)	21 (41.2)	30 (58.8)	1.56 (0.76-3.21)	0.19
50-53 (n=20)	7 (35.0)	13 (65.0)	2.03 (0.69-6.11)	0.16

OR 0.88<1.11<1.41, Chi Square 0.77, p=0.38

Table B63. Chi-square of cooking preference of steak and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat steak less than well done. No association was found.

Cooking Preference for Steak (n=641)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=321)	22	299
Well Done (n=320)	27	293
Odds Ratio	0.80	p=0.45
Upper 95% confidence limit	1.49	
Lower 95% confidence limit	0.43	

Table B64. Eating chicken in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Chicken	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=759)	750 (98.8)	9 (1.2)		
18-19 (n=140)	139 (99.3)	1 (0.7)	1	
20-24 (n=221)	220 (99.6)	1 (0.4)	0.63 (0.02-23.29)	0.74
25-29 (n=95)	92 (96.8)	3 (3.2)	4.53 (0.41-1114.85)	0.16
30-34 (n=87)	86 (98.8)	1 (1.2)	1.62 (0.00-59.96)	0.73
35-39 (n=58)	58 (100.0)	0 (0.0)	0.00 (0.00-42.39)	0.52
40-44 (n=72)	72 (100.0)	0 (0.0)	0.00 (0.00-34.04)	0.47
45-49 (n=61)	58 (95.1)	3 (4.9)	7.19 (0.65-183.25)	0.05
50-53 (n=25)	25 (100.0)	0 (0.0)	0.00 (0.00-100.43)	0.67

OR 0.63<1.98<6.45, Chi Square 1.09, p=0.30

Table B65. Chi-square of eating chicken and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chicken. No association was found.

Eat Chicken (n=739)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=730)	54	676
No (n=9)	0	9
Odds Ratio	Insufficient data	p=0.40
Upper 95% confidence limit		
Lower 95% confidence limit		

Table B66. Frequency of eating chicken in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of eating Chicken	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=750)	667 (88.9)	83 (11.1)		
18-19 (n=140)	130 (92.9)	10 (7.1)	1	
20-24 (n=220)	198 (90.0)	22 (10.0)	1.44 (0.63-3.39)	0.35
25-29 (n=92)	82 (89.1)	10 (10.9)	1.59 (0.58-4.34)	0.32
30-34 (n=86)	73 (84.9)	13 (15.1)	2.32 (0.90-6.03)	0.05
35-39 (n=58)	49 (84,5)	9 (15.5)	2.39 (0.83-6.85)	0.07
40-44 (n=71)	64 (90.1)	7 (9.9)	1.42 (0.46-4.30)	0.49
45-49 (n=58)	51 (87.9)	7 (12.1)	1.78 (0.57-5.45)	0.26
50-53 (n=25)	20 (80.0)	5 (20.0)	3.25 (0.86-11.87)	0.04

OR 1.28<1.85<2.73. Chi Square 11.09, p<0.01

Table B67. Chi-square of frequency of eating chicken and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chicken at least monthly. No association was found.

Frequency of Eating Chicken (n=730)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Monthly (n=648)	45	603
Yearly (n=82)	8	74
Odds Ratio	0.69	p=0.36
Upper 95% confidence limit	1.65	
Lower 95% confidence limit	0.30	

Table B68. Cooking preference of chicken in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Chicken	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=706)	73 (10.3)	663 (89.7)		
18-19 (n=125)	22 (17.6)	103 (82.4)	1	
20-24 (n=207)	31 (15.0)	176 (85.0)	1.21 (0.64-2.30)	0.53
25-29 (n=85)	6 7.1)	79 (92.9)	2.81 (1.02-8.16)	0.03*
30-34 (n=84)	4 (4.8)	80 (95.2)	4.27 (1.32-15.29)	0.01*
35-39 (n=58)	3 (5.2)	55 (94.8)	3.92 (1.05-17.24)	0.02*
40-44 (n=70)	4 (5.7)	66 (94.3)	3.52 (1.08-12.68)	0.02*
45-49 (n=54)	2(3.7)	52 (96.3)	5.55 (1.19-35.58)	0.01*
50-53 (n=23)	1 (4.3)	22 (95.7)	4.70 (0.61-98.41)	0.11

OR 1.71<2.58<3.57, Chi Square 25.45, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B69. Chi-square of cooking preference for chicken and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chicken less than well done. No association was found.

Cooking Preference for Chicken (n=688)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=69)	3	66
Well Done (n=619)	48	571
Odds Ratio	0.54	p=0.31
Upper 95% confidence limit	1.87	
Lower 95% confidence limit	0.13	

Table B70. Eating domestic turkey in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Domestic Turkey	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=756)	665 (88.0)	91 (12.0)		
18-19 (n=141)	119 (84.4)	22 (15.6)	1	
20-24 (n=218)	193 (88.5)	25 (11.5)	0.70 (0.36-1.36)	0.26
25-29 (n=95)	81 (85.3)	14 (14.7)	0.93 (0.42-2.04)	0.86
30-34 (n=87)	77 (88.5)	10 (11.5)	0.70 (0.29-1.66)	0.39
35-39 (n=59)	53 (89.8)	6 (10.2)	0.61 (0.21-1.72)	0.31
40-44 (n=70)	65 (92.9)	5 (7.1)	0.42 (0.13-1.23)	0.08
45-49 (n=61)	52 (85.2)	9 (14.8)	0.94 (0.37-2.32)	0.88
50-53 (n=25)	25 (100.0)	0 (0.0)	0.00 (0.00-1.15)	0.03

OR 0.48<0.67<0.93, Chi Square 5.83, p=0.02

Table B71. Chi-square of eating domestic turkey and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat domestic turkey. No association was found.

Eat Domestic Turkey (n=736)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=647)	46	601
No (n=89)	6	83
Odds Ratio	1.06	p=0.90
Upper 95% confidence limit	2.85	
Lower 95% confidence limit	0.42	

Table B72. Frequency of eating domestic turkey in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Domestic Turkey	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=663)	201 (30.3)	462 (69.7)		
18-19 (n=119)	53 (44.5)	66 (55.5)	1	
20-24 (n=193)	83 (43.0)	110 (57.0)	1.06 (0.65-1.73)	0.79
25-29 (n=81)	22 (27.2)	59 (72.8)	2.15 (1.12-4.15)	0.01*
30-34 (n=77)	12 (15.6)	65 (84.4)	4.35 (2.03-9.49)	<0.01*
35-39 (n=52)	6 (11.5)	46 (88.5)	6.16 (2.29-17.42)	<0.01*
40-44 (n=65)	15 (23.1)	50 (76.9)	2.68 (1.29-5.61)	<0.01*
45-49 (n=51)	8 (15.7)	43 (84.3)	4.32 (1.76-10.92)	<0.01*
50-53 (n=25)	2 (8.0)	23 (92.0)	9.23 (1.97-59.44)	<0.01*

OR 1.94<2.52<3.24. Chi Square 52.88, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B73. Chi-square of frequency of eating domestic turkey and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat domestic turkey at least monthly. No association was found.

Frequency of Eating Domestic Turkey (n=645)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=191)	13	178
Yearly (n=454)	33	421
Odds Ratio	0.93	p=0.83
Upper 95% confidence limit	1.89	
Lower 95% confidence limit	0.45	

Table B74. Cooking preference of domestic turkey in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Domestic Turkey	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=608)	(n=75)	(n=533)		
18-19 (n=106)	23 (21.7)	83 (78.3)	1	
20-24 (n=179)	32 (17.9)	147 (82.1)	1.27 (0.67-2.42)	0.43
25-29 (n=71)	5 (7.0)	68 ((93.0)	3.77 (1.27-11.99)	0.01*
30-34 (n=73)	7 (9.6)	66 (90.4)	2.61 (0.99-7.17)	0.03*
35-39 (n=47)	3 (6.4)	44 (93.6)	4.06 (1.07-18.05)	0.02*
40-44 (n=62)	3 (4.8)	59 (95.2)	5.45 (1.46-23.96)	<0.01*
45-49 (n=47)	1 (2.2)	46 (97.8)	12.75 (1.73-261.57)	<0.01*
50-53 (n=23)	1 (4.3)	22 (95.7)	6.10 (0.80-127.73)	<0.01

OR 1.88<2.85<3.92. Chi Square 31.13, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B75. Chi-square of cooking preference of domestic turkey and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat domestic turkey less than well done. No association was found.

Cooking Preference for Domestic Turkey (n=592)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=70)	5	65
Well Done (n=522)	37	485
Odds Ratio	1.01	p=0.99
Upper 95% confidence limit	2.81	
Lower 95% confidence limit	0.34	

Table B76. Eating lamb in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Lamb	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=760)	111 (14.6)	649 (85.4)		
18-19 (n=141)	22 (15.6)	119 (84.4)	1	
20-24 (n=221)	31 (14.0)	190 (86.0)	1.13 (0.60-2.13)	0.68
25-29 (n=94)	14 (14.9)	80 (85.1)	1.06 (0.48-2.33)	0.88
30-34 (n=86)	9 (10.5)	77 (89.5)	1.58 (0.65-3.93)	0.27
35-39 (n=60)	10 (16.7)	50 (83.3)	0.92 (0.38-2.27)	0.85
40-44 (n=71)	14 919.7)	57 (80.3)	0.75 (0.34-1.68)	0.45
45-49 (n=61)	10 (16.4)	51 (83.6)	0.94 (0.39-2.31)	0.89
50-53 (n=26)	2 (7.7)	24 (92.3)	2.22 (0.46-14.63)	0.29

OR 0.80<1.08<1.47, Chi Square 0.20, p=0.65

Table B77. Chi-square of eating lamb and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat steak lamb. No association was found.

Eat Lamb (n=740)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=110)	7	103
No (n=630)	46	584
Odds Ratio	0.86	p=0.72
Upper 95% confidence limit	2.06	
Lower 95% confidence limit	0.35	

Table B78. Frequency of eating lamb in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Lamb	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=112)	10 (8.9)	102 (91.1)		
18-19 (n=22)	3 (13.6)	19 (86.4)	1	
20-24 (n=31)	3 (9.7)	28 (90.3)	1.47 (0.21-10.57)	0.65
25-29 (n=14)	2 (14.3)	12 (85.7)	0.95 (0.10-9.72)	0.96
30-34 (n=9)	0 (0.0)	9 (100.0)	Insufficient data	0.24
35-39 (n=10)	0 (0.0)	10 (100.0)	Insufficient data	0.22
40-44 (n=14)	1 (7.1)	13 (92.9)	2.05 (0.16-57.46)	0.55
45-49 (n=10)	1 (10.0)	9 (90.0)	1.42 (0.10-40.93)	0.77
50-53 (n=2)	0 (0.0)	2 (100.)	Insufficient data	0.58

OR 0.72<2.02<5.62. Chi Square 1.47, p=0.22

Table B79. Chi-square of frequency of eating lamb and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat lamb at least monthly. No association was found.

Frequency of Eating Lamb (n=110)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=10)	1	9
Yearly (n=100)	6	94
Odds Ratio	1.74	p=0.62
Upper 95% confidence limit	Insufficient data	
Lower 95% confidence limit		

Table B80. Cooking preference of lamb in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Lamb	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=101)	32 (31.7)	69 (68.3)		
18-19 (n=20)	6 (30.0)	14 (70.0)	1	
20-24 (n=29)	13 (44.8)	16 (55.2)	0.53 (0.13-2.05)	0.30
25-29 (n=11)	0 (0.0)	11 (100.0)	Insufficient data	0.04
30-34 (n=9)	3 (33.3)	6 (66.7)	0.86 (0.12-6.30)	0.86
35-39 (n=8)	2 (25.0)	6 (75.0)	1.29 (0.15-12.60)	0.79
40-44 (n=14)	5 (35.7)	9 (64.3)	0.77 (0.14-4.15)	0.73
45-49 (n=8)	3 (37.5)	5 (62.5)	0.71 (0.09-5.47)	0.70
50-53 (n=2)	0 (0.0)	2 (100.0)	Insufficient data	0.36

OR 0.52<1.01<1.98, Chi Square 0.02, p=0.90

Table B81. Chi-square of cooking preference of lamb and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat lamb less than well done. No association was found.

Cooking Preference for Lamb (n=99)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Less Than Well Done (n=32)	2	30
Well Done (n=67)	5	62
Odds Ratio	0.83	p=0.83
Upper 95% confidence limit	5.25	
Lower 95% confidence limit	0.10	

Table B82. Eating mutton in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Mutton	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=760)	32 (4.2)	728 (95.8)		
18-19 (n=141)	8 (5.7)	133 (94.3)	1	
20-24 (n=221)	10 (4.5)	211 (95.5)	1.27 (0.44-3.59)	0.62
25-29 (n=94)	3 (3.2)	91 (96.8)	1.82 (0.43-8.94)	0.38
30-34 (n=86)	5 (5.8)	81 (94.2)	0.97 (0.28-3.57)	0.96
35-39 (n=60)	2 (3.3)	58 (96.7)	1.74 (0.33-12.29)	0.48
40-44 (n=71)	3 (4.2)	68 (95.8)	1.36 (0.32-6.72)	0.65
45-49 (n=61)	0 (0.0)	61 (100.0)	Insufficient data	0.06
50-53 (n=26)	1 (3.8)	25 (96.2)	1.50 (0.18-33.45)	0.71

OR 0.90<1.55<2.65, Chi Square 2.39, p=0.12

Table B83. Chi-square of eating mutton and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat mutton. No association was found.

Eat Mutton (n=740)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=31)	2	29
No (n=709)	51	658
Odds Ratio	Insufficient data	p=0.88
Upper 95% confidence limit		
Lower 95% confidence limit		

Table B84. Frequency of eating mutton in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Mutton	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=32)	1 (3.1)	31 (96.9)		
18-19 (n=8)	0 (0.0)	8 (100.0)	1	
20-24 (n=10)	1 (10.0)	9 (90.0)	0.00 (0.00-24.12)	0.36
25-29 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	
30-34 (n=5)	0 (0.0)	5 (100.0)	Insufficient data	
35-39 (n=2)	0 (0.0)	2 (100.0)	Insufficient data	
40-44 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	
45-49 (n=0)	0	0	Insufficient data	
50-53 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	

Table B85. Chi-square of frequency of eating mutton and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat mutton at least monthly. No association was found.

Frequency of Eating Mutton (n=31)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Monthly (n=1)	0	1
Yearly (n=30)	2	28
Odds Ratio	0.00	p=0.79
Upper 95% confidence limit	452.61	
Lower 95% confidence limit	0.00	

Table B86. Cooking preference of mutton in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Mutton	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=31)	10 (32.3)	21 (67.7)		
18-19 (n=7)	4 (57.1)	3 (42.9)	1	
20-24 (n=10)	4 (40.)	6 (60.0)	2.00 (0.19-22.99)	0.49
25-29 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	0.09
30-34 (n=5)	1 (20.0)	4 (80.0)	5.33 (0.24-219.51)	0.20
35-39 (n=2)	1 (50.0)	1 (50.0)	1.33 (Insufficient data)	0.86
40-44 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	0.09
45-49 (n=0)	0	0		
50-53 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	0.28

Table B87. Chi-square of cooking preference of mutton and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat mutton less than well done. No association was found.

Cooking Preference for Mutton (n=30)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=10)	0	10
Well Done (n=20)	2	18
Odds Ratio	0.00	p=0.30
Upper 95% confidence limit	9.02	
Lower 95% confidence limit	0.00	

Table B88. Eating chevon in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Chevon	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=762)	21 (2.8)	741 (97.2)		
18-19 (n=141)	5 (3.5)	136 (96.5)	1	
20-24 (n=221)	7 (3.2)	214 (96.8)	1.12 (0.30-4.04)	0.84
25-29 (n=95)	5 (5.3)	90 (94.7)	0.66 (0.16-2.73)	0.52
30-34 (n=87)	0 (0.0)	87 (100.0)	Insufficient data	0.08
35-39 (n=60)	1 (1.7)	59 (98.3)	2.17 (0.24-50.15)	0.47
40-44 (n=71)	2 (2.8)	69 (97.2)	1.27 (0.21-9.71)	0.78
45-49 (n=61)	0 (0.0)	61 (100.0)	Insufficient data	0.14
50-53 (n=26)	1 (3.8)	25 (96.2)	0.92 (0.10-21.68)	0.94

OR 0.75<1.46<2.85, Chi Square 1.03, p=0.31

Table B89. Chi-square of eating chevon and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chevon. No association was found.

Eat Chevon (n=742)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Yes (n=21)	1	20
No (n=721)	52	669
Odds Ratio	0.64	p=0.67
Upper 95% confidence limit	4.66	
Lower 95% confidence limit	0.03	

Table B90. Frequency of eating chevon in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Chevon	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=112)	10 (8.9)	102 (91.1)		
18-19 (n=5)	0 (0.0)	5 (100.0)	1	
20-24 (n=7)	0 (0.0)	7 (100.0)	Insufficient data	
25-29 (n=5)	1 (20.0)	4 (80.0)	0.00 (0.00-20.66)	0.29
30-34 (n=0)	0	0	Insufficient data	
35-39 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	
40-44 (n=2)	0 (0.0)	2 (100.0)	Insufficient data	
45-49 (n=0)	0	0	Insufficient data	
50-53 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	

OR 0.00. Chi Square 0.99

Table B91. Chi-square of frequency of eating chevon and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chevon at least monthly. No association was found.

Frequency of Eating Chevon (n=21)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Monthly (n=1)	0	1
Yearly (n=20)	1	19
Odds Ratio	0.00	p=0.82
Upper 95% confidence limit	2160.73	
Lower 95% confidence limit	0.00	

Table B92. Cooking preference of chevon in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Chevon	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=20)	4 (20.0)	16 (80.0)		
18-19 (n=4)	3 (75.0)	1 (25.0)	1	
20-24 (n=7)	1 (14.3)	6 (85.7)	18.00 (0.50-3382.38)	0.04
25-29 (n=5)	0 (0.0)	5 (100.0)	Insufficient data	
30-34 (n=0)	0	0	Insufficient data	
35-39 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	
40-44 (n=2)	0 (0.0)	2 (0.0)	Insufficient data	
45-49 (n=0)	0	0	Insufficient data	
50-53 (n=1)	0 (0.0)	1 (100.0)	Insufficient data	

Table B93. Chi-square of cooking preference of chevon and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat chevon less than well done. No association was found.

Cooking Preference for Chevon (n=20)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=4)	0	4
Well Done (n=16)	1	15
Odds Ratio	0.00	p=0.61
Upper 95% confidence limit	88.67	
Lower 95% confidence limit	0.00	

Table B94. Eating wild game in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eat Wild Game	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=764)	105 (13.7)	559 (86.3)		
18-19 (n=141)	16 (11.3)	125 (88.7)	1	
20-24 (n=221)	27 (12.2)	194 (87.8)	0.92 (0.45-1.86)	0.80
25-29 (n=96)	13 (13.5)	83 (86.5)	0.82 (0.35-1.91)	0.61
30-34 (n=87)	15 (17.2)	72 (82.8)	0.61 (0.27-1.40)	0.21
35-39 (n=60)	13 (21.7)	47 (78.3)	0.46 (0.19-1.11)	0.06
40-44 (n=72)	11 (15.3)	61 (84.7)	0.71 (0.29-1.75)	0.42
45-49 (n=61)	7 (11.5)	54 (88.5)	0.99 (0.36-2.83)	0.98
50-53 (n=26)	3 (11.5)	23 (88.5)	0.98 (0.24-4.62)	0.98

OR 0.54<0.75<1.03, Chi Square 3.13, p=0.08

Table B95. Chi-square of eating wild game and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat wild game. No association was found.

Eat Wild Game (n=744)	**Women seropositive for *T. gondii***	**Women seronegative for *T. gondii***
Yes (n=159)	11	148
No (n=585)	43	542
Odds Ratio	0.94	p=0.85
Upper 95% confidence limit	1.94	
Lower 95% confidence limit	0.44	

Table B96. Frequency of eating wild game in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Frequency of Eating Wild Game	At Least Monthly (Percent)	Yearly (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=101)	21 (20.8)	80 (79.2)		
18-19 (n=16)	5 (31.2)	11 (68.8)	1	
20-24 (n=25)	4 (16.0)	21 (84.0)	2.39 (0.43-13.82)	0.25
25-29 (n=12)	2 (16.7)	10 (83.3)	2.27 (0.28-22.03)	0.38
30-34 (n=15)	3 (20.0)	12 (80.0)	1.82 (0.27-12.88)	0.47
35-39 (n=12)	4 (63.3)	8 (66.7)	0.91 (0.14-5.15)	0.91
40-44 (n=10)	1 (10.0)	9 (90.0)	4.09 (0.33-110.94)	0.21
45-49 (n=8)	2 (25.0)	6 (75.0)	1.36 (0.15-14.29)	0.75
50-53 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	0.26

OR 0.93<1.97<2.97. Chi Square 2.97, p=0.09

Table B97. Chi-square of frequency of eating wild game and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat wild game at least monthly. No association was found.

Frequency of Eating Wild Game (n=100)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Monthly (n=20)	2	18
Yearly (n=80)	8	72
Odds Ratio	1.00	p=1.00
Upper 95% confidence limit	5.83	
Lower 95% confidence limit	0.13	

Table B98. Cooking preference of wild game in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cooking Preference of Wild Game	Less Than Well (Percent)	Well (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=94)	15 (16.0)	79 (84.0)		
18-19 (n=14)	4 (28.6)	10 (71.4)	1	
20-24 (n=23)	5 (21.7)	18 (78.3)	1.44 (0.25-8.43)	0.64
25-29 (n=10)	1 (10.0)	9 (90.0)	3.60 (0.27-102.28)	0.27
30-34 (n=14)	2 (14.3)	12 (85.7)	2.40 (0.28-24.38)	0.36
35-39 (n=12)	1 (8.3)	11 (91.7)	4.40 (0.34-122.93)	0.19
40-44 (n=10)	2 (20.0)	8 (80.0)	1.60 (0.17-17.10)	0.63
45-49 (n=8)	0 (0.0)	8 (100.0)	Insufficient data	0.09
50-53 (n=3)	0 (0.0)	3 (100.0)	Insufficient data	0.29

OR 1.15<2.80<6.59, Chi Square 5.16, p=0.02

Table B99. Chi-square of cooking preference of wild game and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat wild game of less than well done. No association was found.

Cooking Preference for Wild Game (n=94)	Women seropositive for *T. gondii*	Women seronegative for *T. gondii*
Less Than Well Done (n=15)	2	13
Well Done (n=79)	8	71
Odds Ratio	1.37	p=0.71
Upper 95% confidence limit	8.31	
Lower 95% confidence limit	0.18	

Table B100. Wash cutting board with soap and/or bleach after cutting raw meat in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Cutting Board with Soap and/or Bleach After Cutting Raw Meat	No (Percent)	Yes (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=672)	28 (4.2)	644 (95.8)		
18-19 (n=123)	9 (7.3)	114 (92.7)	1	
20-24 (n=208)	6 (2.9)	202(97.1)	2.66 (0.84-8.65)	0.06
25-29 (n=84)	1 (1.2)	83 (98.8)	6.65 (0.82-140.74)	0.04
30-34 (n=77)	2 (2.6)	75 (97.4)	2.96 (0.57-20.44)	0.15
35-39 (n=52)	3 (5.8)	49 (94.2)	1.29 (0.30-6.30)	0.71
40-44 (n=60)	3 (5.0)	57 (95.0)	1.50 (0.35-7.30)	0.55
45-49 (n=49)	2 (4.1)	47 (95.9)	1.86 (0.35-2.95)	0.43
50-53 (n=19)	2 (10.5)	17 (89.5)	0.67 (0.12-4.93)	0.63

OR 1.18<2.03<3.76, X^2=6.56, p=0.01

Table B101. Chi-square of washing cutting board with soap and/or bleach after cutting raw meat and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do always wash cutting board properly after contact with uncooked meat. No association was found.

Wash Cutting Board Properly (n=653)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
No (n=27)	2	25
Yes (n=626)	48	578
Odds Ratio	0.96	p=0.96
Upper 95% confidence limit	Invalid	
Lower 95% confidence limit		

Table B102. Wash knife with soap and/or bleach after cutting raw meat in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Knife with Soap and/or Bleach After Cutting Raw Meat	No (Percent)	Yes (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=730)	39 (5.3)	691 (94.7)		
18-19 (n=136)	12 (8.8)	124 (91.2)	1	
20-24 (n=215)	10 (4.7)	205(95.3)	1.98 (0.77-5.13)	0.12
25-29 (n=90)	2 (2.2)	88 (97.8)	4.26 (0.87-28.28)	0.04
30-34 (n=85)	3 (3.5)	82 (96.5)	2.65 (0.67-12.21)	0.13
35-39 (n=55)	4 (7.3)	51 (92.7)	1.23 (0.35-4.78)	0.73
40-44 (n=69)	4 (5.8)	65 (94.2)	1.57 (0.45-6.04)	0.44
45-49 (n=56)	3 (5.4)	53 (94.6)	1.71 (0.42-7.98)	0.42
50-53 (n=24)	1 (4.2)	23 (95.8)	2.23 (0.28-48.00)	0.44

OR1.24<2.01<3.30, X^2=8.40, p<0.01

Table B103. Chi-square of washing knife with soap and/or bleach after cutting raw meat and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do always wash knife properly after contact with uncooked meat. No association was found.

Wash Knife Properly (n=653)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
No (n=36)	2	34
Yes (n=674)	51	623
Odds Ratio	0.72	p=0.65
Upper 95% confidence limit	3.19	
Lower 95% confidence limit	0.12	

Table B104. Wash hands with soap and/or bleach after handling raw meat in women of childbearing age from the Knoxville, Tennessee area.

Wash Hands Properly After Handling Raw Meat	No (Percent)	Yes (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=743)	8 (1.1)	735 (98.9)		
18-19 (n=133)	1 (0.75)	132 (99.3)	1	
20-24 (n=218)	1 (0.50)	217 (99.5)	1.64 (0.00-60.62)	0.72
25-29 (n=93)	2 (2.2)	91 (97.8)	0.34 (0.01-4.93)	0.37
30-34 (n=86)	2 (2.3)	84 (97.7)	0.32 (0.01-4.56)	0.33
35-39 (n=58)	0 (0.0)	58 (100.0)	Insufficient data	
40-44 (n=70)	1 (1.4)	70 (98.6)	0.53 (0.01-19.73)	0.65
45-49 (n=60)	1 (1.7)	59 (98.3)	0.45 (0.01-16.67)	0.56
50-53 (n=24)	0 (0.0)	24 (100.0)	Insufficient data	

OR $0.18<0.62<2.04$, $X^2=0.37$, $p=0.54$

Table B105. Chi-square of washing hands with soap and/or bleach after cutting raw meat and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Hands Properly (n=724)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
No (n=8)	0	8
Yes (n=716)	54	663
Odds Ratio	0.00	p=0.42
Upper 95% confidence limit	8.70	
Lower 95% confidence limit	0.00	

Table B106. Washing vegetables before eating it in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Vegetables Before Eating	Frequently, Occasionally or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=813)	225 (27.7)	588 (72.3)		
18-19 (n=157)	57 (36.3)	100 (63.7)	1	
20-24 (n=242)	68 (28.1)	174(71.9)	1.46 (0.93-2.29)	0.08
25-29 (n=102)	31 (30.4)	71 (69.6)	1.31 (0.74-2.30)	0.33
30-34 (n=87)	15 (17.2)	72 (82.8)	2.74 (1.38-5.50)	<0.01*
35-39 (n=60)	17 (28.3)	43 (71.7)	1.44 (0.72-2.91)	0.27
40-44 (n=75)	18 (24.0)	57 (76.0)	1.81 (0.93-3.53)	0.06
45-49 (n=63)	13 (20.6)	50 (79.4)	2.19 (1.05-4.65)	0.02*
50-53 (n=27)	6 (22.2)	21 (77.8)	2.00 0.71-5.89)	0.15

OR 1.35<1.70<2.14, X^2=21.11, p<0.01
*Statistically significant by Chi Square (p=0.1, 1df)

Table B107. Chi-square of washing vegetables before eating and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wash vegetables before its consumption. No association was found.

Wash vegetables before eating (n=790)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Frequently, occasionally, or never (n=220)	9	211
Always (n=570)	44	526
Odds Ratio	0.51	p=0.07
Upper 95% confidence limit	1.11	
Lower 95% confidence limit	0.23	

Table B108. Washing fruit before eating it in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Fruit Before Eating	Frequently, Occasionally or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=813)	287 (35.3)	526 (64.7)		
18-19 (n=156)	67 (42.9)	89 (57.1)	1	
20-24 (n=243)	88 (36.2)	155 (63.8)	1.33 (0.86-2.04)	0.18
25-29 (n=102)	39 (38.2)	63 (61.7)	1.22 (0.71-2.09)	0.45
30-34 (n=87)	21 (24.1)	66 (75.9)	2.37 (1.27-4.43)	<0.01*
35-39 (n=60)	25 (41.7)	35 (58.3)	1.05 (0.55-2.01)	0.86
40-44 (n=75)	22 (29.3)	53 (70.7)	1.81 (0.97-3.42)	0.05
45-49 (n=63)	15 (23.8)	48 (76.2)	2.41 (1.19-4.93)	0.01*
50-53 (n=27)	10 (37.0)	17 (63.0)	1.28 (0.51-3.23)	0.57

Odds Ratio $1.23<1.53<1.90$, $X^2=15.07$, $p<0.01$
*Statistically significant by Chi Square (p=0.1, 1df)

Table B109. Chi-square of washing fruit before eating and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wash fruit before its consumption. No association was found.

Wash fruit before eating (n=790)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Frequently, occasionally, or never (n=281)	18	263
Always (n=509)	35	474
Odds Ratio	0.93	p=0.80
Upper 95% confidence limit	1.73	
Lower 95% confidence limit	0.49	

Table B110. Washing hands before handling food in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Hands Before Handling Food	Frequently, Occasionally or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=815)	241 (29.6)	574 (70.4)		
18-19 (n=157)	57 (36.3)	100 (63.7)	1	
20-24 (n=244)	82 (33.6)	162 (66.4)	1.13 (0.72-1.75)	0.58
25-29 (n=102)	33 (32.4)	69 (67.6)	1.19 (0.68-2.09)	0.51
30-34 (n=87)	25 (28.7)	62 (71.3)	1.41 (0.77-2.59)	0.23
35-39 (n=60)	15 (25.0)	45 (75.0)	1.71 (0.84-3.53)	0.11
40-44 (n=75)	15 (20.0)	60 (80.0)	2.28 (1.14-4.62)	0.01*
45-49 (n=63)	10 (15.9)	53 (84.1)	3.02 (1.36-6.88)	<0.01*
50-53 (n=27)	4 (14.8)	23 (85.2)	3.28 (1.01-11.82)	0.03*

OR 1.25<1.58<1.97, X^2=15.58, p<0.01
* Statistically significant difference (p<0.1, 1df)

Table B111. Chi-square of washing hands before handling food and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wash hands before handling food. No association was found.

Wash hands before handling food (n=792)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Frequently, occasionally, or never (n=231)	13	218
Always (n=561)	40	521
Odds Ratio	0.78	p=0.44
Upper 95% confidence limit	1.54	
Lower 95% confidence limit	0.39	

Table B112. Washing hands before eating in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Hands Before Eating	Frequently, Occasionally or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=813)	472 (58.1)	342 (41.9)		
18-19 (n=157)	92 (58.6))	65 (41.4)	1	
20-24 (n=243)	115 (47.3)	128 (52.7)	1.58 (1.03-2.41)	0.03*
25-29 (n=102)	44 (43.1)	58 (56.9)	1.87 (1.09-3.19)	0.01*
30-34 (n=87)	27 (31.0)	60 (69.0)	3.15 (1.74-5.69)	<0.01*
35-39 (n=60)	17 (28.3)	43 (71.7)	3.58 (1.80-7.19)	<0.01*
40-44 (n=74)	26 (35.1)	48 (64.9)	2.61 (1.42-4.83)	<0.01*
45-49 (n=63)	17 (27.0)	46 (73.0)	3.83 (1.93-7.66)	<0.01*
50-53 (n=27)	3 (11.1)	24 (88.9)	11.32 (3.06-49.42)	<0.01*

OR 2.06<2.55<3.18, X^2=77.47, p<0.01
* Statistically significant difference (p<0.1, 1df)

Table B113. Chi-square of washing hands before eating and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wash hands before eating. No association was found.

Wash hands before eating (n=790)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Frequently, occasionally, or never (n=331)	18	313
Always (n=459)	35	424
Odds Ratio	0.70	p=0.23
Upper 95% confidence limit	1.30	
Lower 95% confidence limit	0.37	

Table B114. Wash hands after handling animals in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wash Hands After Handling Animals	Frequently, Occasionally or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=811)	372 (45.9)	439 (54.1)		
18-19 (n=156)	78 (50.0)	78 (50.0)	1	
20-24 (n=244)	124 (50.8)	120(49.2)	0.97 (0.63-1.48)	0.87
25-29 (n=102)	54 (52.9)	48 (47.1)	0.89 (0.52-1.51)	0.64
30-34 (n=86)	33 (38.4)	53 (61.6)	1.61 (0.91-2.85)	0.08
35-39 (n=60)	22 (36.7)	38 (63.3)	1.73 (0.90-3.34)	0.08
40-44 (n=75)	27 (36.0)	48 (64.0)	1.78 (0.97-3.26)	0.05
45-49 (n=63)	26 (41.3)	37 (58.7)	1.42 (0.76-2.68)	0.24
50-53 (n=25)	8 (32.0)	17 (68.0)	2.13 (0.81-5.74)	0.09

OR 1.06<1.30<1.61, X^2=6.19, p=0.01

Table B115. Chi-square of washing hands after handling animals and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wash hands after handling animals. No association was found.

Wash hands after handling animals (n=788)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Frequently, occasionally, or never (n=362)	22	340
Always (n=426)	31	395
Odds Ratio	0.82	p=0.05
Upper 95% confidence limit	1.50	
Lower 95% confidence limit	0.45	

Table B116. Drinking unpasteurized milk in women of childbearing age from the Knoxville, Tennessee area stratified by age.

Drink Unpasteurized Milk	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% C.I.)	p-value
Age Group (years) (n=809)	47 (5.8)	762 (94.2)		
18-19 (n=157)	6 (3.8)	151 (96.2)	1	
20-24 (n=244)	13 (5.3)	231 (94.7)	0.71 (0.23-2.05)	0.49
25-29 (n=101)	6 (5.9)	95 (94.1)	0.63 (0.17-2.28)	0.43
30-34 (n=85)	8 (9.4)	77 (90.6)	0.38 (0.11-1.27)	0.08
35-39 (n=60)	3 (5.0)	57 (95.0)	0.75 (0.16-3.96)	0.70
40-44 (n=75)	7 (9.3)	68 (90.7)	0.39 (0.11-1.34)	0.09
45-49 (n=62)	4 (6.5)	58 (93.5)	0.58 (0.14-2.54)	0.40
50-53 (n=25)	0 (0.0)	25 (100.0)	Insufficient data	

OR 0.36<0.58<0.95, Chi Square 4.70, p=0.03

Table B117. Chi-square of drinking unpasteurized milk and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that drink unpasteurized milk. No association was found.

Drink Unpasteurized Milk (n=786)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Always, Frequently, or occasionally (n=47)	4	43
Never (n=739)	47	692
Odds Ratio	1.37	p=0.56
Upper 95% confidence limit	4.21	
Lower 95% confidence limit	0.04	

Table B118. Drinking unpasteurized goat milk in women of childbearing age from the Knoxville, Tennessee area stratified by age.

Drink Unpasteurized Goat Milk	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=811)	10 (1.2)	801 (98.8)		
18-19 (n=157)	3 (1.9)	154 (98.1)	1	
20-24 (n=244)	1 (0.4)	243 (99.6)	4.73 (0.44-119.14)	0.14
25-29 (n=101)	2 (2.0)	99 (98.0)	0.96 (0.13-8.40)	0.97
30-34 (n=86)	1 (1.2)	85 (98.8)	1.66 (0.15-41.96)	0.66
35-39 (n=59)	1 (1.7)	58 (98.3)	1.13 (0.10-28.77)	0.92
40-44 (n=75)	1 (1.3)	74 (98.7)	1.44 (0.13-36.59)	0.75
45-49 (n=63)	1 (1.6)	62 (98.4)	1.21 (0.11-30.73)	0.87
50-53 (n=26)	0 (0.0)	26 (100.0)	Insufficient data	

OR 0.65<1.64<4.51, Chi Square =0.88, p=0.35

Table B119. Chi-square of drinking unpasteurized goat milk and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that drink unpasteurized goat milk. No association was found.

Drink Unpasteurized Goat Milk (n=788)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Always, Frequently, or occasionally (n=10)	0	10
Never (n=778)	53	725
Odds Ratio	0.00	p=0.39
Upper 95% confidence limit	7.42	
Lower 95% confidence limit	0.00	

Table B120. Eating raw eggs in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Eating Raw Eggs	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=813)	405 (49.8)	408 (50.2)		
18-19 (n=157)	83 (52.9)	74 (47.1)	1	
20-24 (n=243)	140 (57.6)	103 (42.4)	0.83 (0.54-1.26)	0.35
25-29 (n=102)	54 (52.9)	48 (47.1)	1.00 (0.59-1.69)	0.99
30-34 (n=87)	40 (46.0)	47 (54.0)	1.32 (0.75-2.31)	0.30
35-39 (n=60)	26 (43.3)	34 (56.7)	1.47 (0.77-2.79)	0.21
40-44 (n=75)	27 (36.0)	48 (64.0)	1.99 (1.09-3.36)	0.02*
45-49 (n=63)	28 (44.4)	35 (55.6)	1.40 (0.75-2.63)	0.26
50-53 (n=26)	7 (26.9)	19 (73.1)	3.04 (1.13-8.50)	0.01*

OR 1.02<1.26<1.56, Chi Square 4.74, p=0.03
*Statistically significant by Chi Square (p<0.01, 1df)

Table B121. Chi-square of eating raw eggs and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that eat raw eggs which may be found in raw cookie dough. No association was found.

Eat Raw Eggs (n=790)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Always, Frequently, or occasionally (n=392)	24	368
Never (n=398)	29	369
Odds Ratio	0.83	p=0.51
Upper 95% confidence limit	1.50	
Lower 95% confidence limit	0.46	

Table B122. Vegetarianism in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Vegetarian	No (Percent)	Yes (Percent)	Odds Ratio (95% C.I.)	p-value
Age Group (years) (n=819)	766 (93.5)	53 (6.5)		
18-19 (n=158)	150 (94.9)	8 (5.1)	1	
20-24 (n=245)	223 (91.0)	22 (9.0)	1.85 (0.76-4.65)	0.14
25-29 (n=103)	94 (91.3)	9 (8.7)	1.80 (0.61-5.32)	0.24
30-34 (n=87)	87 (100.0)	0 (0.0)	0.00 (0.00-1.19)	0.03
35-39 (n=61)	60 (98.4)	1 (1.6)	0.31 (0.01-2.55)	0.25
40-44 (n=74)	68 (91.9)	6 (8.1)	1.65 (0.49-5.52)	0.36
45-49 (n=63)	58 (92.1)	5 (7.9)	1.62 (0.44-5.75)	0.41
50-53 (n=28)	26 (92.9)	2 (7.1)	1.44 (0.00-8.00)	0.65

OR 0.81<1.28<1.99, Chi Square 1.00, p=0.32

Table B123. Chi-square of vegetarianism and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that are not vegetarians. No association was found.

Vegetarian (n=797)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
No (n=747)	54	693
Yes (n=50)	1	49
Odds Ratio	3.82	p=0.16
Upper 95% confidence limit	75.82	
Lower 95% confidence limit	0.55	

Table B124. Risky occupation or hobby in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Risky Occupation or Hobby	Yes (Percent)	No (Percent)	Odd Ratio (95% CI)	p-value
Age Group (years) (n=803)	204 (25.4)	599 (74.6)		
18-19 (n=156)	31 (19.9)	125 (80.1)	1	
20-24 (n=241)	48 (19.9)	193 (80.1)	1.00 (0.58-1.70)	0.99
25-29 (n=101)	33 (32.7)	68 (67.3)	0.51 (0.28-0.94)	0.02*
30-34 (n=84)	28 (33.3)	56 (66.7)	0.50 (0.26-0.91)	0.02*
35-39 (n=61)	14 (23.0)	47 (77.0)	0.83 (0.39-1.81)	0.62
40-44 (n=73)	21 (28.8)	52 (71.2)	0.61 (0.31-1.23)	0.13
45-49 (n=61)	21 (34.4)	40 (65.6)	0.47 (0.23-0.96)	0.02*
50-53 (n=26)	8 (30.8)	18 (69.2)	0.56 (0.20-1.55)	0.21

* Statistically significant difference ($p<0.1$, 1df)
OR $0.50<0.64<0.81$, $X^2=13.31$, $p<0.01$

Table B125. Chi-square of having a risky occupation or hobby and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that have a risky occupation or hobby. No association was found.

Risky Occupation or Hobby (n=780)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Yes (n=200)	14	186
Never (n=580)	38	542
Odds Ratio	1.07	p=0.83
Upper 95% confidence limit	2.10	
Lower 95% confidence limit	0.54	

Table B126. Keeping a flower or vegetable garden in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Keep a Garden	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=812)	461 (56.8)	351 (43.2)		
18-19 (n=157)	68 (43.3)	89 (56.7)	1	
20-24 (n=245)	107 (43.7)	138 (56.3)	0.99 (0.64-1.51)	0.94
25-29 (n=102)	49 (48.0)	53 (52.0)	0.83 (0.49-1.41)	0.41
30-34 (n=86)	60 (69.8)	26 (30.2)	0.33 (0.18-0.60)	<0.01*
35-39 (n=60)	45 (75.0)	15 (25.0)	0.25 (0.12-0.52)	<0.01*
40-44 (n=75)	63 (84.0)	12 (16.0)	0.15 (0.07-0.30)	<0.01*
45-49 (n=62)	50 (80.6)	12 (19.4)	0.18 (0.08-0.39)	<0.01*
50-53 (n=25)	19 (76.0)	6 (24.0)	0.24 (0.08-0.69)	<0.01*

* Statistically significant difference ($p<0.1$, 1df)
OR $0.35<0.44<0.53$, $X^2=61.47$, $p<0.01$

Table B127. Chi-square of gardening and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that garden. No association was found.

Garden (n=789)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Always, frequently, or occasionally (n=455)	33	422
Never (n=334)	18	316
Odds Ratio	1.37	p=0.29
Upper 95% confidence limit	2.59	
Lower 95% confidence limit	0.73	

Table B128. Wearing gloves while gardening in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wear Gloves While Gardening	Frequently, Occasionally, or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=461)	387 (83.7)	75 (16.3)		
18-19 (n=68)	62 (91.2)	6 (8.8)	1	
20-24 (n=107)	96 (89.7)	11 (10.3)	1.18 (0.38-3.82)	0.75
25-29 (n=49)	44 (89.8)	5 (10.2)	1.17 (0.29-4.72)	0.80
30-34 (n=60)	45 (75.0)	15 (25.0)	3.44 (1.13-10.88)	0.01*
35-39 (n=45)	35 (77.8)	10 (22.2)	2.95 (0.89-10.12)	0.05
40-44 (n=63)	50 (74.9)	13 (25.1)	2.69 (0.87-8.62)	0.06
45-49 (n=50)	40 (80.0)	10 (20.0)	2.58 (0.78-8.78)	0.08
50-53 (n=19)	14 (73.7)	5 (26.3)	3.69 (0.82-16.55)	0.04

* Statistically significant difference ($p<0.1$, 1df)
OR $1.52<2.31<3.69$, $X^2=15.64$, $p<0.01$

Table B129. Chi-square of wearing gloves while gardening and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wear gloves while gardening. No association was found.

Wear Gloves While Gardening (n=455)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Frequently, occasionally, or never (n=380)	30	350
Always (n=75)	4	71
Odds Ratio	1.52	P=0.44
Upper 95% confidence limit	5.26	
Lower 95% confidence limit	0.49	

Table B130. Wearing a mask while gardening in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Wear Mask While Gardening	Frequently, Occasionally, or Never (Percent)	Always (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=460)	459 (99.8)	1 (0.2)		
18-19 (n=68)	68 (100.0)	0 (0.0)	1	
20-24 (n=107)	106 (99.1)	1 (0.9)	Insufficient data	
25-29 (n=48)	48 (100.0)	0 (0.0)	Insufficient data	
30-34 (n=60)	60 (100.0)	0 (0.0)	Insufficient data	
35-39 (n=45)	45 (100.0)	0 (0.0)	Insufficient data	
40-44 (n=63)	63 (100.0)	0 (0.0)	Insufficient data	
45-49 (n=50)	50 (100.0)	0 (0.0)	Insufficient data	
50-53 (n=19)	19 (100.0)	0 (0.0)	Insufficient data	

Table B131. Chi-square of wearing a mask while gardening and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that do not always wear a mask while gardening. No association was found.

Wear Mask While Gardening (n=454)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Frequently, occasionally, or never (n=453)	34	419
Always (n=1)	0	1
Odds Ratio	Insufficient data	
Upper 95% confidence limit		
Lower 95% confidence limit		

Table B132. Owning a cat in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Own a Cat	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=808)	364 (45.0)	444 (55.0)		
18-19 (n=156)	68 (43.6)	88 (56.4)	1	
20-24 (n=245)	93 (38.0)	152 (62.0)	1.26 (0.82-1.94)	0.26
25-29 (n=100)	52 (52.0)	48 (48.0)	0.71 (0.42-1.22)	0.19
30-34 (n=86)	42 (48.8)	44 (51.2)	0.81 (0.46-1.42)	0.43
35-39 (n=61)	35 (57.4)	26 (42.6)	0.57 (0.30-1.09)	0.07
40-44 (n=73)	37 (50.7)	36 (49.3)	0.75 (0.41-1.36)	0.32
45-49 (n=61)	30 (49.2)	31 (50.8)	0.80 (0.42-1.51)	0.46
50-53 (n=26)	7 (26.9)	19 (73.1)	2.10 (0.78-5.86)	0.11

OR $0.72<0.89<1.09$, $X^2=1.20$, $p=0.27$

Table B133. Chi-square of owning a cat and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that own a cat. No association was found.

Own a Cat (n=785)	**Women seropositive for *T. gondii***	**Women seronegative for *T gondii***
Yes (n=355)	22	333
No (n=431)	27	403
Odds Ratio	0.99	p=0.96
Upper 95% confidence limit	1.83	
Lower 95% confidence limit	0.53	

Table 134. Cat uses litter box and women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Cat Uses Litter Box	No (Percent)	Yes (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=284)	5 (1.8)	279 (98.2)		
18-19 (n=44)	0 (0.0)	44 (100.0)	1	
20-24 (n=75)	1 (1.3)	74 (98.7)	Insufficient data	
25-29 (n=46)	0 (0.0)	46 (100.0)	Insufficient data	
30-34 (n=37)	1 (2.7)	36 (97.3)	Insufficient data	
35-39 (n=24)	2 (8.3)	22 (91.7)	Insufficient data	
40-44 (n=27)	1 (3.7)	26 (96.3)	Insufficient data	
45-49 (n=27)	0 (0.0)	27 (100.0)	Insufficient data	
50-53 (n=4)	0 (0.0)	4 (100.0)	Insufficient data	

Table B135. Chi-square of having cat used litter box and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that don't have a cat that always uses the litter box. Women who had a cat that did not always use a litter box were 12.19 times more likely to be infected than those whose cats did.

Cat Uses Litter Box (n=275)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
No (n=5)	2*	3
Yes (n=270)	14	256
Odds Ratio	12.19	<0.01
Upper 95% confidence limit	101.53	
Lower 95% confidence limit	1.29	

*Statistically significant by Chi Square (p< .01, 1df)

Table B136. Scooping the cat's litter box by women of childbearing age from the Knoxville, Tennessee area stratified by age group.

I Scoop Cat's Litter Box	Yes (Percent)	No (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=272)	204 (75.0)	77 (25.0)		
18-19 (n=43)	20 (46.5)	23 (53.5)	1	
20-24 (n=74)	44 (59.5)	30 (40.5)	0.59 (0.26-1.35)	0.17
25-29 (n=47)	42 (89.4)	5 (10.6)	0.10 (0.03-0.35)	<0.01*
30-34 (n=36)	30 (83.3)	6 (16.7)	0.17 (0.05-0.56)	<0.01*
35-39 (n=23)	18 (78.3)	5 (21.7)	0.24 (0.06-0.87)	0.01*
40-44 (n=26)	20 (76.9)	6 (23.1)	0.26 (0.08-0.87)	0.01*
45-49 (n=28)	26 (92.9)	2 (7.1)	0.07 (0.01-0.35)	<0.01*
50-53 (n=4)	4 (100.0)	0 (0.0)	0.00 (0.00-1.54)	0.04

* Statistically significant difference (p<0.1, 1df)
OR0.1`4<0.23<0.34), X^2=53.69, p<0.01

Table B137. Chi-square of scooping the cat's litter box and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that scoop the litter box. No association was found.

I Scoop the Cat's Litter Box (n=272)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Yes (n=199)	11	188
No (n=73)	3	70
Odds Ratio	1.37	p=0.64
Upper 95% confidence limit	6.37	
Lower 95% confidence limit	0.34	

Table B138. Chewing fingernails in women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Chew Fingernails	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=809)	452 (55.9)	357 (44.1)		
18-19 (n=156)	91 (58.3)	65 (41.7)	1	
20-24 (n=243)	157 (64.6)	86 (35.4)	0.77 (0.50-1.18)	0.21
25-29 (n=101)	63 (62.4)	38 (37.6)	0.84 (0.49-1.46)	0.52
30-34 (n=87)	39 (44.8)	48 (55.2)	1.72 (0.98-3.03)	0.04
35-39 (n=60)	33 (55.0)	27 (45.0)	1.15 (0.60-2.18)	0.66
40-44 (n=74)	29 (39.2)	45 (60.8)	2.17 (1.19-3.98)	0.01*
45-49 (n=63)	29 (46.0)	34 (54.0)	1.64 (0.87-3.09)	0.10
50-53 (n=25)	11 (44.0)	14 (56.0)	1.78 (0.71-4.53)	0.18

* Statistically significant difference ($p<0.1$, 1df)
OR $1.00<1.23<1.52$, $X^2=3.67$, $p=0.06$

Table B139. Chi-square of chewing fingernails and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that chew fingernails. No association was found.

Chew Fingernails(n=786)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Always, Frequently, or Occasionally (n=440)	27	413
Never (n=346)	26	320
Odds Ratio	0.80	P=0.44
Upper 95% confidence limit	1.46	
Lower 95% confidence limit	0.44	

Table B140. Let pets sleep in their bed of women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Let Pets Sleep in Bed	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=732)	361 (49.3)	371 (50.7)		
18-19 (n=145)	59 (40.7)	86 (59.3)	1	
20-24 (n=223)	108 (48.4)	115 (51.6)	0.73 (0.47-1.14)	0.15
25-29 (n=95)	62 (65.3)	33 (34.7)	0.37 (0.21-0.65)	<0.01*
30-34 (n=79)	39 (49.4)	40 (50.6)	0.70 (0.39-1.27)	0.21
35-39 (n=57)	32 (56.1)	25 (43.9)	0.54 (0.28-1.04)	0.05
40-44 (n=65)	26 (40.0)	39 (60.0)	1.03 (0.54-1.95)	0.92
45-49 (n=50)	28 (56.0)	22 (44.0)	0.54 (0.27-1.08)	0.06
50-53 (n=18)	7 (38.9)	11 (61.1)	1.08 (0.36-3.30)	0.88

OR 0.51<0.64<0.80, X^2=15.83, p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B141. Chi-square of let pets sleep in bed and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those let pets sleep in bed. No association was found.

Let Pet's Sleep in Bed (n=709)	Women with *Toxoplasma gondii* infection	Women without *Toxoplasma gondii* infection
Always, Frequently, or Occasionally (n=351)	20	331
Never (n=358)	29	329
Odds Ratio	0.69	p=0.21
Upper 95% confidence limit	1.28	
Lower 95% confidence limit	0.36	

Table B142. Play in children's sandbox by women of childbearing age from the Knoxville, Tennessee area stratified by age group.

Play in Children's Sandbox	Always, Frequently, or Occasionally (Percent)	Never (Percent)	Odds Ratio (95% CI)	p-value
Age Group (years) (n=803)	305 (38.0)	498 (62.0)		
18-19 (n=155)	87 (56.1)	68 (43.9)	1	
20-24 (n=239)	93 (38.9)	146 (61.1)	2.01 (1.31-3.09)	<0.01*
25-29 (n=101)	40 (39.6)	61 (60.4)	1.95 (1.14-3.36)	<0.01*
30-34 (n=87)	28 (32.2)	59 (67.8)	2.70 (1.50-4.86)	<0.01*
35-39 (n=59)	26 (44.1)	33 (55.9)	1.62 (0.85-3.11)	0.11
40-44 (n=74)	17 (23.0)	57 (77.0)	4.29 (2.20-8.45)	<0.01*
45-49 (n=63)	12 (19.0)	51 (81.0)	5.44 (2.56-11.73)	<0.01*
50-53 (n=25)	2 (8.0)	23 (92.0)	14.71 (3.19-3.67)	<0.01*

OR 1.52<1.87<2.40, X^2=32.94 p<0.01
*Statistically significant by Chi Square (p<0.01, 1df)

Table B143. Chi-square of play in children's sandbox and seroprevalence of *T. gondii* in women of childbearing age from the Knoxville, Tennessee area. Odds ratio and 95% confidence limits test the association of *T. gondii* infection in those that play in a child's sandbox. No association was found.

Play in Children's Sandbox(n=780)	Women seropositive for *T. gondii*	Women seronegative for *T gondii*
Always, Frequently, or Occasionally (n=299)	17	282
Never (n=481)	36	445
Odds Ratio	0.75	P=0.33
Upper 95% confidence limit	1.40	
Lower 95% confidence limit	0.39	

APPENDIX C

PROJECT BENCHMARKS AND PUBLICATIONS

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PROJECT BENCHMARKS

Publications:

1996 Seroprevalence of *Toxoplasma gondii* in Hogs in the National Animal Health Monitoring System (NAHMS). Sharon Patton, Jeff Zimmerman, Tanya Roberts, Charles Faulkner, **Vina Diderrich**, Amir Assadi-Rad, Peter Davies, and James Kliebenstien. *Journal of Eukaryotic Microbiology* 43:121S.

1996 Serologic survey of *Toxoplasma gondii* antibodies in free-ranging wild hogs (*Sus scrofa*) from the Great Smoky Mountains National Park and from sites in South Carolina. **Vina Diderrich**, John C. New, Gayle P. Noblet, and Sharon Patton. *Journal of Eukaryotic Microbiology* 43:122S

2001 Swine Production Strategies that reduce the risk of *Toxoplasma gondii* infection as reflected in the 1990 and 1995 National Animal Health Monitoring System (NAHMS) surveys. **Vina R. Diderrich**, Tracy Wang, Xianfeng Hu, Charles T. Faulkenr, Raymond McCord, Eric Bush, Arne Hallum, Jeff Zimmerman, James Kliebenstein, and Sharon Patton. Manuscript submitted to *Veterinary Parasitology*.

Published Reports:

1998 *Toxoplasma gondii* in Swine Operations in the United States: Seroprevalence in Sows and Market-Weight Pigs in the National Animal Health Monitoring System, 1995 and an Assessment of Management Factors. Report for the National Pork Producers Council, Project Number 1724. Sharon Patton, **Vina Diderrich**, Charles Faulkner, Raymond McCord, James Kliebenstein, Tracy Wang. University of Tennessee, Oak Ridge National Laboratories, and Iowa State University.

Research Grants:

1995-96 "Seroepidemiologic survey of human serum for *Toxoplasma gondii* antibodies from a population in Knox County, TN." Venture Grant, UTCVM, $1500.00 (Co-investigator – Dr. Sharon Patton).

Presentations, Abstracts, and Proceedings:

2001 Risk of *Toxoplasma gondii* exposure among women of childbearing age from the Knoxville, Tennessee area. **Vina R. Diderrich**, John C. New and Sharon Patton. Program and Abstracts of the 2001 Meeting of the Southeastern Society of Parasitologists. Berry College, Mount Berry, Georgia.

2000 Serologic prevalence of *Toxoplasma gondii* antibodies in women of childbearing age from East Tennessee. **Vina R. Diderrich** and Sharon Patton, University of Tennessee. Abstract No. 22. Program and Abstracts of the 2000 Joint Meeting of the Southeastern Society of Parasitologists and the Association of Southeastern Biologists. University of Tennessee at Chattanooga, Chattanooga, Tennessee.

Serologic prevalence of *Toxoplasma gondii* antibodies in women of childbearing age from East Tennessee. **Vina R. Diderrich** and Sharon Patton, University of Tennessee. Program and Abstracts of the 2000 Meeting of the American Society of Parasitologists. San Juan, Puerto Rico.

Figure C1. Project Benchmarks.

National seroprevalence of *Toxoplasma gondii* and swine production management in the 1995 NAHMS swineherds. **Diderrich, V. R.**, Wang, T., Faulkner, C. T., McCord, R., Bush, E., Hallum, A., Zimmerman, J., Kliebenstein, J., & Patton, S. University of Tennessee, Oak Ridge National Laboratories, Iowa State University, and United States Department Of Agriculture. Abstract No. 151. International Symposium on Veterinary Epidemiology and Economics. Colorado State University, Breckenridge, Colorado.

1999 Seroprevalence of *Toxoplasma gondii* infection and farm management practices in swineherds in the 1995 National Animal Health Monitoring System (NAHMS). Sharon Patton, Raymond McCord, **Vina Diderrich**, Charles Faulkner, Tracy Wang, Jeff Zimmerman, and Jim Kliebenstein, University of Tennessee, Oak Ridge National Laboratories, and Iowa State University. Abstract No. 25. Program and Abstracts of the 1999 Meeting of the Southeastern Society of Parasitologists. Alabama 4H Development Center, Auburn University. Columbiana, Alabama.

Seroprevalence of *Toxoplasma gondii* in sows and finisher pigs in the 1995 National Animal Health Monitoring System (NAHMS). **Vina Diderrich**, Charles Faulkner, Raymond McCord, Jim Kliebenstein, Jeff Zimmerman, and Sharon Patton. University of Tennessee, Oak Ridge National Laboratories, and Iowa State University. Abstract No. 26. Program and Abstracts of the 1999 Meeting of the Southeastern Society of Parasitologists. Alabama 4H Development Center, Auburn University. Columbiana, Alabama.

National seroprevalence of *Toxoplasma gondii* and farm management practices in swineherds in 1995. Sharon Patton, **Vina Diderrich**, Charles Faulkner, Raymond McCord, Tracy Wang, Jeff Zimmerman, and Jim Kliebenstein, University of Tennessee, Oak Ridge National Laboratories, and Iowa State University. Abstract No. 71. Program and Abstracts of the 1999 Joint Meeting of The American Society of Parasitologists and The Society of Nematologists. University of California-Davis. Monterey, California.

Farm management and national seroprevalence of *Toxoplasma gondii* in swineherds in 1995. Sharon Patton, **Vina Diderrich**, Charles Faulkner, Raymond McCord, Tracy Wang, Jeff Zimmerman, and Jim Kliebenstein, University of Tennessee, Oak Ridge National Laboratories, and Iowa State University. Abstract No. 102. Program and Abstracts of the 1999 Meeting of the American Association of Veterinary Parasitologists. New Orleans, Louisiana.

1998 Survey of serum from pregnant women in Knox County, Tennessee for *Toxoplasma gondii* antibodies. **Vina R. Diderrich** and Dr. Sharon Patton, University of Tennessee. Abstract No. 23. Program and Abstracts of the 1998 Meeting of the Southeastern Society of Parasitologists. Outdoor Laboratory, Clemson University. Clemson, South Carolina.

1997 Epidemiology of *Toxoplasma gondii* in hogs in the United States. Sharon Patton, Jeff J. Zimmerman, Tanya Roberts, C. T. Faulkner, **Vina R. Diderrich**, Amir M. Assadi-Rad, Peter R. Davies, and James B. Kliebenstein, University of Tennessee, Iowa State University, and North Carolina State University. Abstract No. 9. Program and Abstracts of the 1997 Joint Meeting of the Southeastern Society of Parasitologists and the Association of Southeastern Biologists. Furman University. Greenville, South Carolina.

Figure C1. (continued).

1996 Serologic survey of *Toxoplasma gondii* antibodies in free-ranging wild hogs (*Sus scrofa*) from the Great Smoky Mountains National Park, Tennessee and North Carolina, and from South Carolina. **Vina R. Diderrich**, John C. New, Gayle P. Noblet, and Sharon Patton, University of Tennessee and Clemson University. Abstract No. 9. Program and Abstracts of the 1996 Meeting of the Southeastern Society of Parasitologists. Hancock Biological Station, Murray State University. Murray, Kentucky.

Serologic survey of *Toxoplasma gondii* antibodies in free-ranging wild hogs (*Sus scrofa*) from the Great Smoky Mountains National Park, Tennessee and North Carolina, and from South Carolina. **Vina R. Diderrich**, John C. New, Gayle P. Noblet, and Sharon Patton, University of Tennessee and Clemson University. Abstract 148. Program and Abstracts of the 1996 Joint Meeting of the American Society of Parasitologists and the Society of Protozoologists. University of Arizona, Tucson, Arizona.

Seroprevalence of *Toxoplasma gondii* in the National Animal Health Monitoring System (NAHMS). Sharon Patton, Jeff J. Zimmerman, Tanya Roberts, Charles T. Faulkner, **Vina R. Diderrich**, Amir M. Assadi-Rad, Peter R. Davies, James B. Kliebenstien. University of Tennessee, Iowa State University, and USDA Economic Research Service. Abstract No. 151. Program and Abstracts of the 1996 Joint Meeting of the American Society of Parasitologists and The Society of Protozoologists. University of Arizona. Tucson, Arizona.

Seroepidemiology of *Toxoplasma gondii* in the National Animal Health Monitoring System (NAHMS). Sharon Patton, Jeff J. Zimmerman, Charles T. Faulkner, **Vina R. Diderrich**, Amir M. Assadi-Rad, Peter R. Davies, James B. Kliebenstien, University of Tennessee, Iowa State University , and North Carolina State University. Abstract No. 21. Program and Abstracts of the 1996 Annual Meeting of the American Association of Veterinary Parasitologists. Louisville, Kentucky.

Special Training and Workshops:

1998 National Workshop on Toxoplasmosis: Preventing Congenital Toxoplasmosis (NWTPCT). Centers for Disease Control and Prevention, Atlanta, GA. September 9-10.

Awards and Honors:

1995 Marc Dresden Student Travel Award, Joint Meeting of the American Society of Parasitologists and the American Association of Veterinary Parasitologists, Pittsburgh, PA.

1996 Elon E. Byrd-Mary C. Dunn Award for best student presentation at the Annual Meeting of the Southeastern Society of Parasitologists, Murray, KY.

Meritorious Paper Award, Joint Meeting of the American Society of Parasitologists and Protozoologists, Tucson, AZ.

Marc Dresden Student Travel Award, Joint Meeting of the American Society of Parasitologists and Protozoologists, Tucson, AZ.

2000 Marc Dresden Student Travel Award, Meeting of the American Society of Parasitologists, San Juan, Puerto Rico.

Figure C1. (continued).

Seroprevalence of *Toxoplasma gondii* in Hogs in the National Animal Health Monitoring System (NAHMS)

SHARON PATTON,[1*] JEFF ZIMMERMAN,[2] TANYA ROBERTS,[3] CHARLES FAULKNER,[1] VINA DIDERRICH,[1] AMIR ASSADI-RAD,[1] PETER DAVIES,[4] AND JAMES KLIEBENSTEIN[2]

[1]*University of TN, Knoxville,TN,* [2]*Iowa State University, Ames, IA.* [3]*Economic Research Service, USDA, Washington, D.C.,* [4]*North Carolina State University, Raleigh, NC*

The objectives of this study were to assess the seroprevalence of *Toxoplasma gondii* in swine herds in the United States and to compare the prevalence of *T. gondii* in sows to that in market weight pigs.

MATERIALS AND METHODS. We used sera collected in 1990 as a part of the National Animal Health Monitoring System (NAHMS) survey of 412 randomly selected swine herds in 17 states (Alabama, California, Colorado, Illinois, Indiana, Iowa, Maryland, Michigan, Minnesota, North Carolina, Nebraska, Ohio, Oregon, Pennsylvania, Tennessee, Virginia, Wisconsin). Blood samples from up to 10 sows and/or gilts were collected from each herd. For comparison, serum was also collected from market weight pigs in Tennessee in 1991-92 and North Carolina in 1994-95. Samples were stored frozen until analyzed for *T. gondii* antibodies by the modified agglutination test (MAT) using formalin fixed tachyzoites as antigen. Titers of 32 or greater were considered positive.

RESULTS AND DISCUSSION. Twenty percent (679/3479) of the sows tested were positive. Positive hogs were present in each state except Colorado. Prevalence ranged from 12% positive in Illinois, Minnesota, and Nebraska to 34% positive in Alabama and Tennessee and 36% positive in Wisconsin. The Chi-square Goodness of Fit Test ($p<.05$) indicated that Alabama, Ohio, Tennessee, and Wisconsin each had more positive hogs than one would expect by random chance and that Colorado, Illinois, Minnesota and Nebraska had fewer. Using farms as the unit of analysis, 47% (194/412) of the farms had a least one positive sow in the herd. Percentage of positive farms ranged from 22% and 23% in California and Nebraska to 73% and 74% in Alabama and Wisconsin, and 89% in Tennessee. The Chi-square Goodness of Fit Test ($p<.05$) indicated that farms in Colorado, California, and Nebraska were less likely to have an infected hog living on the farm while farms in Ohio, Tennessee, and Wisconsin were more likely to have infected animals in the herd. These differences are probably more related to the number of small farms, or the number of pigs raised outdoors in a state rather than to geographic location. In a previous investigation in Tennessee [1], sows associated with cats were 2.6 times more likely to be seropositive than sows not associated with cats; sows on small farms were 4.5 times more likely to be seropositive than sows on large farms, and sows kept outdoors were 23 times more likely to be seropositive than sows kept indoors. Further investigations of the differences in swine husbandry practices between states and their role in the epidemiology of *T. gondii* in swine herds are warranted.

A previous study [3] reported that 42% of the breeding hogs tested in a national seroprevalence survey in 1983-84 were seropostive for *T. gondii*. This compares to 20% seropositivity in this study. Both surveys employed the MAT. Perhaps the prevalence of *T. gondii* is decreasing in hogs thoroughout the country or possibly this difference is a function of the sampling technique. The samples in the 1983-84 study were collected from swine that were commercially slaughtered: 613 animals that were perhaps past their prime as breeding stock. The animals sampled by NAHMS were the present breeding stock and were probably younger with less time to acquire a *T. gondii* infection.

In North Carolina, serum samples were collected on 29 farms from finishing pigs that were within one month of slaughter; 13/2312 were seropositive for *T. gondii*. In contrast, the seroprevalence of sows in North Carolina in the NAHMS study was 18% , and 39% of the farms had at least one positive sow. In Tennessee, samples were collected from 437 market-weight pigs. All of these animals were on UT experiment stations when sampled, although 297 were purchased as weanlings from farms throughout the state. The seroprevalence was 3% (12/437). This compares to 34% seropositivity in the sows in the NAHMS study where 89% of the farms had at least one positive sow. These numbers are also lower than the 23% prevalence reported in 11,229 market-weight pigs tested at slaughter and found seropositive in 1983-84 [3] with the MAT. Probably the main reasons for these differences are the age of the animals and the husbandry practices employed on the farms. The sows were older and had more time to acquire an infection. Many of the market weight pigs had minimal contact with the outdoors and cats and cat's feces.

The seropositive breeding animals probably do not play a significant role in the transmission of *T. gondii* to humans. These animals are older when sold and are made into processed meats which are usually highly seasoned, smoked, heated, or frozen. They are not sold as cuts of meat and, therefore, are only a danger to individuals in the processing plant handling the raw meat. The market-weight animals are sold as commercial cuts of pork and *T. gondii* has been isolated from pork tissues used for human consumption [4]. However, the prevalence in the finishing swine in our study was only between 1-3%. Another study [7] also found 3% of finishing swine in Illinois positive for *T. gondii* antibodies. Tissue cysts and bradyzoites are killed by heating meat thoroughly to 66 degrees C (150 degrees F) or by having it smoked or cured [2,6]. Also, freezing meat at -20 degrees C for 24 hours will destroy most cysts [5]. [This work was supported in part by a grant from The National Pork Producers Council.]

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Figure C2. Seroprevalence of *Toxoplasma gondii* in Hogs in the National Animal Health Monitoring System (NAHMS).

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Serologic Survey of *Toxoplasma gondii* Antibodies in Free-ranging Wild Hogs (*Sus scrofa*) from the Great Smoky Mountains National Park and From Sites in South Carolina

VINA DIDERRICH,[1] JOHN C. NEW,[1] GAYLE P. NOBLET,[2] AND SHARON PATTON[1]*
[1]*University of TN, Knoxville, TN, and* [2]*Clemson University, Clemson, SC*

The objectives of this study were to assess the seroprevalence of *Toxoplasma gondii* in feral swine populations in the Great Smoky Mountains National Park (GSMNP) and South Carolina and to compare these seroprevalences to those of other feral and domestic swine.

The tissue cyst of *Toxoplasma gondii* containing bradyzoites persists for the lifetime of the infected host. Consequently, domestic and wild animals are important reservoirs in the transmission of *T. gondii* to humans who may ingest cysts in poorly cooked meat. Some feral swine populations are hunted or incorporated into domestic swine herds and used for food. In the GSMNP (located in eastern Tennessee and western North Carolina), feral swine are a non-native species that have upset the ecological balance and aesthetic value of the park, causing large areas of destruction and erosion, greatly reducing wildflowers, and destroying nests and eggs of ground-nesting birds. Such destruction also occurs in SC where feral swine destroy man-made dikes in wildlife management areas.

The European wild hog was brought to the southern Appalachian Mountains in 1912 to stock a private game reserve in North Carolina [4]. In 1920, approximately 100 of these hogs escaped, dispersed throughout the surrounding area, and interbred with feral domestic swine [2]. In 1940, their descendants entered the park [4], and today approximately 800 to 1000 wild hogs live there. These animals are usually black with long cartilaginous snouts resembling the European ancestors. SC feral swine originated from the same southern Appalachian stock. Through the years the feral swine in SC have interbred with domestic swine, creating hybrid crosses with characteristics similar to domestic swine such as patterned hair coats and shorter snouts.

The National Park Service is attempting to eradicate feral swine from the GSMNP. Hogs are killed and buried or trapped and transported to wildlife management areas and released for hunting. In SC, feral swine are hunted for sport, although they are also being depopulated.

MATERIALS AND METHODS. We used sera collected in 1990 from 108 European wild hogs that were shot or captured in the GSMNP [7], and 149 samples that were collected from feral swine in SC in 1993. Age, sex, and site of capture were available for the GSMNP samples; only capture location was available for the SC samples. Samples were stored frozen until analyzed for *T. gondii* antibodies by the modified agglutination test (MAT) using formalin fixed tachyzoites as antigen. Titers of 32 or greater were considered positive. Odds ratios were calculated to identify risk factors associated with *T. gondii* seroprevalence. Variables examined were age class (adult or juvenile), sex, and capture location.

RESULTS AND DISCUSSION. Of the 108 samples collected from hogs in the GSMNP, 46 were from lower elevation sites along creek beds identified as Cades Cove and Little River on the TN side, and Oconaluftee, Deep Creek, and Twentymile on the NC side; 62 were from higher elevations along the Appalachian Trail that follows the state boundary line between TN and NC. There were 49 males, 59 females, 88 adults, and 20 juveniles. In SC, samples were collected from hogs at 4 sites: Starr, North Augusta, Savannah River, and Jacksonboro. Both Starr and North Augusta have breeding stocks of feral swine used for hunting, and the hogs sampled were being tested for pseudorabies and brucellosis. Samples from Savannah River and Jacksonboro were collected from hogs killed in depopulation programs or by hunters.

The prevalence of hogs positive for *T. gondii* antibodies in the GSMNP was 31% (33/108). Seropositivity was not associated with sex, age, or capture site. In SC the prevalence was 37% (55/149). When feral hogs from farms were compared to hunter-killed hogs, no significant difference in antibody prevalence was found. Feral swine are infected during rooting and wallowing behavior when they ingest *T. gondii* sporulated oocysts shed into the environment by infected felids, or tissue cysts in small animals, birds, or infected raw meat in garbage. Feral hogs generally live and behave in similar ways, regardless of sex or age class, so each is at equal risk of infection from these sources. Feral hogs do not stay in one locality. The hog seen in the valley in the morning may be the same hog captured on a ridge in the afternoon.

Previous studies reported a 3% seroprevalence of *T. gondii* in feral pigs in Florida, 13% in California, 33% in West Germany, and 94 % in Argentina [3]. This compares to 31 and 37% in the GSMNP and SC. The earlier investigations did not use the MAT and can not be directly compared to each other or to this study. Using the MAT, the seroprevalence of *T. gondii* antibodies in sows on TN hog farms was 36% [1] and on SC hog farms, 26% (Schmidhauser C, MS Thesis, Clemson Univ). On some of these farms, sows were exposed to many of the same environmental factors as the GSMNP and SC feral swine: living outside on pastures potentially contaminated with oocysts and opportunities to eat small mammals and birds, or raw garbage containing meat scraps.

The potential role of wild game in the transmission of *T. gondii* to humans was shown in 3 recent studies. In 1983, 3 hunters from AL and SC developed toxoplasmosis after eating raw or nearly raw venison [8]. In an epidemiologic study of *T. gondii* infections in farmers in Japan in 1987, antibody prevalence was correlated with consumption of raw meat including wild boar, venison, beef, chicken, horseflesh, and whale meat [5]. In an investigation of an outbreak of toxoplasmosis in 4 pregnant women in Quebec in 1990, risk factors for seroconversion were consumption of dried seal meat, seal liver, and raw caribou meat [6].

The potential role of wild game, including feral hogs, in the transmission of *T. gondii* to humans is supported by the results of this investigation. Common practices that put people at risk for infection are butchering and handling infected carcasses, ingesting improperly cooked traditional cuts of pork, and using feral hog meat to make venison sausage. [We thank the Div of Resource Management and Sci, GSMNP, T. Eleazer, J. Bryan (CU Livestock/Poultry Hlth) and M. Duffy, USDA, APHIS, Columbia, SC for providing samples.]

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Figure C3. Serologic survey of *Toxoplasma gondii* antibodies in free-ranging wild hogs (*Sus scrofa*) from the Great Smoky Mountains National Park and from sites in South Carolina.

VITA

Vina Rachel Diderrich (Faulkner) was born in Oconomowoc, Wisconsin on October 25, 1962. She was raised in Monterey, a small unincorporated community outside of Oconomowoc. She attended the public school system of Waukesha County, and graduated from Oconomowoc Senior High School in June, 1980. She enrolled at Mount Senario College, Ladysmith, Wisconsin the following fall. She received a Bachelor of Science degree in Biology, with a minor in Chemistry in 1984. She continued her education at the University of Wisconsin-Eau Claire under the direction of Dr. Darwin Wittrock and conducted her Master's thesis research using light and electron microscopy to study the morphological changes of a trematode parasite as it matured from the metacercarial stage in fishes to the adult stage in birds. She received her Master of Science degree in Biology in December 1989. Vina returned to Mount Senario College as an Assistant Professor of Biology and Natural Science in 1990 where she taught for three semesters. She was accepted into the Comparative and Experimental Medicine program at the University of Tennessee and began working toward the Doctor of Philosophy Degree in 1992. Her education during this period was focused on diagnostic veterinary parasitology, zoonotic parasites, and epidemiology. The Doctor of Philosophy Degree was awarded in May 2001.

Dr. Diderrich joined the staff of the Clinical Virology Service Laboratory at the University of Tennessee College of Veterinary Medicine as a Laboratory Technologist in 1992. Her duties include management of the Clinical Virology Service Laboratory and training of graduate students, veterinary students, and visiting scholars in the methods of diagnostic virology.

Dr. Diderrich is a member of the American Society of Parasitologists, the Southeastern Society of Parasitologists, the American Association of Veterinary Parasitologists, Helminthological Society of Washington and *Sigma Xi*, the Scientific Research Society.