To the Graduate Council:

I am submitting herewith a dissertation written by Amir Mohsen Assadi-Rad entitled "An Epidemiologic study of Toxoplasma gondii in Swine in Tennessee." I have examined the final 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 Animal Science.

Sharon Patton

Sharon Patton, Major Professor

Hugo Eiler

Hugo Eiler, Major Professor

We have read this dissertation
and recommend its acceptance:

Michael D. Smith

James K. Miller

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

AN EPIDEMIOLOGIC STUDY OF *TOXOPLASMA GONDII* IN SWINE IN TENNESSEE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Amir Mohsen Assadi-Rad

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To Mari, Mohsen, Ardeshir, Azarm, Arash, and Hadigheh Ashorie, for their love and patience with me over the years.

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Abstract

The reliability of the Modified Agglutination Test (MAT) to detect infection with Toxoplasma gondii was evaluated in a murine model. Thirty-five mice (ICR) were infected with bradyzoites of T. gondii (UT-1 strain) and all acquired the infection. IgM and IgG titers were measured every two days in the twenty-five infected mice. IgM and IgG appeared seven days postinfection (PI). IgM peaked from 11 - 15 days PI and was almost undectable at 17 days. IgG peaked at 11 days PI and remained throughout the experiment (45 days). The presence of T. gondii was confirmed post-mortem in all infected mice by impression smear, hematoxylin and eosin and/or immunoperoxidase staining of brain sections. The MAT was proven a reliable test for seroprevalence studies.

The seroprevalence of T. gondii in finishing pigs at the University of Tennessee (UT) Crossville Experiment Station was studied for a period of two years, 1991-92. The seroprevalence for both years was 0.6%. At another UT experiment station, the Ames Plantation, the seroprevalence was evaluated in swine of all ages raised in three different types of management systems. The pigs of all ages in partial confinement had the highest seropositivity ($P<0.001$).

Of 3,841 sow serum samples received from the TN State Diagnostic Laboratory in 1991-92, 1130 were positive for

T. gondii antibody. The total number of farms sampled was 343. The true prevalence was 36% for both years. A survey was mailed to 303 swine farmers concerning their management practices; 107 responses were returned and analyzed. There was no significant difference between the respondents and nonrespondents. From the analysis of the survey sows associated with cats were 2.6 times more likely to be seropositive for T. gondii than sows that were not associated with cats. Sows kept outdoors at any time were 23 times more likely to be seropositive than sows that were kept indoors. Sows on small farms ($\leq$ 29 sows) were 4.47 times more likely to be seropositive than sows on large farms.

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CHAPTER I

Literature Review

Introduction:

Toxoplasma gondii is an obligatory, intracellular, protozoan parasite that is a member of the phylum Apicomplexa. Members of this phylum characteristically have an "apical complex" of organelles at one end of the organism. This organism is unique among protozoans in its ability to parasitize a wide range of hosts and tissues. Toxoplasma gondii is one of the most ubiquitous of organisms; essentially all homeothermic animals can be infected, and natural infections have been shown to occur in nonhuman primates, rodents, insectivores, herbivores and carnivores, including domestic species and humans. Toxoplasma gondii is the causative agent of toxoplasmosis. Serologic surveys indicate that infection is wide-spread in most species of domestic animals; except for abortions in sheep, goats, and pigs, however, overt disease is sporadic (Dubey and Urban, 1990; Dubey et al., 1990b; Patton et al., 1990).

Toxoplasma gondii was first observed in 1908 by Charles Nicole and James Manceaux, who found this parasite in Ctenodactylu gondi, an African rodent, which lent its species name to this new parasite. The genus name is derived from the Greek word toxon meaning bow. This name

refers to the crescent shape of the organism. The complete life cycle of this parasite was not known until 1970 when Frenkel et al. in the United States determined that domestic cats were the definitive hosts. It was subsequently shown that exotic Felidae can be definitive hosts as well (Patton et al., 1986a).

Toxoplasma gondii in swine was first reported in the United States in 1951 on a farm in Ohio (Farrell et al., 1952). It has been found in the lung, lymph nodes, intestine, and liver of diseased hogs (Farrel et al., 1952). This organism is infectious to adult pigs and young piglets and may cause disease and death (Dubey et al., 1990a). The economic impact to the swine industry of toxoplasmosis has not yet been measured (Roberts and Frenkel, 1990).

The Life Cycle of Toxoplasma gondii:

The life cycle of Toxoplasma gondii has been elucidated by several researchers (Dubey et al., 1970; Frenkel et al., 1970; Miller et al., 1972). The oocysts are passed in the feces of Felidae. It takes approximately 1 to 5 days for the oocysts (result of sexual reproduction) to become sporulated, i.e. infective outside of the cat (Dubey and Frenkel, 1976). There are 2 sporocysts inside each oocyst, and each sporocyst contains 4 sporozoites.

When ingested by a cat, the oocyst's wall breaks open and sporozoites are released into the lumen of the small intestine. Sporozoites penetrate the epithelial cells of the cat's small intestine and undergo 4 to 5 asexual generations before the sexual cycle, gametogony, begins. After fertilization, which occurs in the small intestine of the cat, the newly-formed oocysts will be passed in the cat feces. Asexual stages called tachyzoites may also penetrate other tissues and organs after being spread throughout the body by circulating blood and lymph. When the cat's immune system slows down the multiplication of these tachyzoites in secondary replication sites, they may accumulate within a host cell which becomes surrounded by a tough wall, forming a cyst. The individual organisms within the cyst are then referred to as bradyzoites.

Birds and other mammals, including swine and humans, may act as the intermediate hosts for T. gondii. Sexual reproduction does not occur within intermediate hosts, which become infected by ingestion of sporulated oocysts, bradyzoites in cysts, and\or by tachyzoites. After ingestion, the wall of the oocyst or cyst is dissolved by proteolytic enzymes in the stomach and small intestine of the host. Sporozoites are released from the oocyst and penetrate through the intestinal mucosa to begin development in the lamina propria, mesenteric lymph nodes, other distant organs, and white blood cells (Miller et al.,

1972). Unlike in cats, there is no enteroepithelial stage of development in intermediate hosts. After mucosal penetration and distribution by blood and lymph, the sporozoites enter host cells and begin replication by endodyogeny as tachyzoites in extraintestinal sites (Frenkel, 1974a). When their multiplication slows down, bradyzoite cysts are formed. Therefore, both tachyzoites and bradyzoites are found in various tissues in the intermediate host, as well as in cats (Frenkel, 1974b).

Human beings can acquire toxoplasmosis by ingesting undercooked meat (particularly pork and lamb), unpasteurized goat's milk (Weinman and Chandler, 1956; Frenkel, 1974b), or sporulated oocysts from cat feces. Oocysts in the soil or litter box can become airborne and infect people during gardening or when the litter is transfered (Frenkel, 1990). Oocysts may also adhere to fruits and vegetables. If a mother is infected for the first time during pregnancy, transplacental transmission of this parasite from mother to fetus can occur resulting in congenital toxoplasmosis or abortion (Frenkel, 1990).

As discussed previously, there are 3 methods by which intermediate hosts aquire T. gondii infections:

1) congenital (transplacental), via tachyzoites,

2) carnivorism, via tachyzoites and/or bradyzoites, and,

3) fecal, via sporulated oocysts (Dubey and Beattie, 1988).

To date, the source of transmission of toxoplasmosis in swine has not been determined (Dubey, 1986). Swine possibly acquire this parasite by eating infected rodents (Lubroth et al., 1982) or by ingesting sporulated oocysts via contaminated feed or water. However, in a recent study by Smith et al. (1992), no association was found between seroprevalence of T. gondii in swine and other mammalian intermediate hosts on the farms surveyed. They did suggest, however, that the high prevalence of T. gondii in cats could contribute to infection of swine because cats shed oocysts in the food and water supplies of swine. In addition, several studies across the country have found that pigs in confined facilities have a lower prevalence of toxoplasmosis, possibly due to less exposure to cat feces and rodents (Dreesen and Lubroth, 1981; Zimmerman et al., 1990; Smith et al., 1992).

Morphology and Composition:

Toxoplasma gondii is a eukaryotic cell and a member of the phylum Apicomplexa. The tachyzoites are crescent-shaped, about 4 um wide and 6 um long (Gardiner et al., 1988). The bradyzoite cysts are spherical to elongate and range from 10 to 100 um in length (Gardiner et al., 1988). Oocysts measure 10 um in diameter. All of the Apicomplexa

have the structure that is called an apical complex, which is distinguishable only by electron microscopy. An apical complex consists of polar rings, micronemes, rhoptries, subpellicular tubules, micropores (cytosomes) and a conoid. The members of this phylum have one nucleus and no cilia or flagella, except the microgametes, the "male" gametes, which in some groups of this phylum possess a flagellum.

Each T. gondii organism is surrounded by a pellicle. The pellicle of the tachyzoites consists of 3 unit membranes, of which only the outer membrane is continuous (Pfefferkorn, 1990). The 2 inner, discontinuous membranes end at structures called the polar rings. Polar rings are located at both the anterior and posterior ends of the parasite. The innermost membranes have small circular gaps called micropores along the side of the organism (Schmidt and Roberts, 1989). Micropores are possibly involved in the uptake of nutrients (Pfefferkorn, 1990). The subpellicular cytoskeleton is composed of 22 microtubules that radiate from the polar rings and run posteriorly, parallel to the axis of the parasite (Pfefferkorn, 1990). The conoid is a hollow, truncated cone of spirally wound fibers that are also likely to be microtubules and is located in the anterior end of the tachyzoite just within the polar rings. This structure protrudes during the early stages of entry into a host cell (Dubey and Beattie, 1988).

Two to several, club-shaped, electron-dense bodies, the rhoptries, have narrow ends which terminate in the conoid and polar rings. Packed between the rhoptries are a large number of smaller vesicles called micronemes. The smaller, more convoluted, elongate bodies, the micronemes, also extend posteriorly from the apical complex. The ducts of micronemes apparently run anteriorly into the rhoptries or join a common duct system with the rhoptries to lead to the cell surface at the apex (Schmidt and Roberts, 1989). The possible function of these 2 structures, micronemes and rhoptries, suggests a secretory task that is probably carried out in the early stages of host-cell entry (Dubey and Beattie, 1988; Schmidt and Roberts, 1989; Pfefferkorn, 1990).

Disease Occurrence, Clinical and Pathological Findings:

At various stages in the T. gondii life cycle, the sporozoites or tachyzoites enter host cells in the intestine or secondary replication sites by both phagocytosis and active penetration. The broad range of hosts and tissues infected rules out any specific protein as the cell receptor for T. gondii attachment prior to penetration and forces the consideration of components that are common to the cell membrane of most cells, i.e. lipids and glycosyl residues (Suzuki and Remington, 1990). The rhoptries and micronemes are located in the anterior

portion of the organism and secrete substances that promote penetration of this parasite into the host cells (Pfefferkorn, 1990). After penetrating the host cell, T. gondii surrounds itself with a parasitophorous vacuole, possibly derived from the host cell membrane. The ability of T. gondii to survive in the intracellular environment is apparently due to failure of fusion of lysosomal membranes with the membranes of this vacuole. Within this parasitophorous vacuole, the tachyzoites multiply asexually by a type of binary fission known as endodyogeny. The multipication of tachyzoites ultimately lyses the host cell, freeing the tachyzoites to go on and infect others repeating the cycle of cell destruction. Necrosis and ensuing inflammation are found in organs heavily infected with tachyzoites (Jubb et al., 1985).

The outcome of infection is determined by a number of factors, including the number of organisms and strain of T. gondii in the infective dose, the species, age and immune status of the host (Dubey and Beattie, 1988). Lesions in visceral organs are usually evident within 1 to 2 weeks after oral infection, and variable numbers of tachyzoites are usually found in the vicinity of the necrotic areas (Jubb et al., 1985). Immunocompetent animals develop a chronic or dormant form of T. gondii infection characterized by the formation of bradyzoite cysts, which

are mainly located in the brain, skeletal muscle, and myocardium. Cyst formation may occur as early as 1 to 2 weeks postinfection and coincides with the disappearance of tachyzoites from circulation and visceral organs (Parker et al., 1981). The cysts persist for months to years (Dubey and Beattie, 1988). Inflammation is usually not associated with cysts (Dubey and Frenkel, 1976), though if immunity wanes the cyst can rupture, releasing bradyzoites and inciting a severe inflammatory response that is mainly hypersensitive in character (Sharma, 1990).

Ironically, the cell biology of other stages of T. gondii is not as well known, because they can be studied only in vivo. However, bradyzoites have been shown to form in cell culture and have a cyst wall around them (Lindsay et al., 1991). Sexual stages of T. gondii have not yet been observed in vitro (Sharma, 1990).

The first human case of toxoplasmosis was reported in 1920 (Sharma, 1990). It is estimated that 30% to 60% of people in the United States have been exposed to Toxoplasma gondii (Frenkel, 1990). Most people infected with T. gondii do not develop severe disease; however, many immunocompetent people have flu-like symptoms, fever, muscle aches, and swollen lymph nodes (Frenkel, 1990). These signs will disappear after a few days and often the disease remains undiagnosed because of the host's effective protective immunity. In infants and children, T. gondii

can cause a severe, acute, febrile form of disease with evidence of pneumonia, liver dysfunction or even myocarditis (Frenkel, 1990). Once the organism is established, it remains with the host for life.

Women acquiring T. gondii infection for the first time during pregnancy risk spontaneous abortion or bearing children with severe encephalitis, which can lead to mental retardation (Frenkel, 1989, 1990). During 1985 in the United States, the cost of raising children congenitally infected with toxoplasmosis was $430 million (Dubey, 1986). This figure was recently revised and, including the estimated income loss, the range of total preventable cost estimates is now from $368 million to $8.7 billion for the infants estimated to be born each year with congenital toxoplasmosis (Roberts and Frenkel, 1990). Toxoplasmosis not only strikes pregnant women, but also can infect adult males or nonpregnant females causing retinochoroiditis, i.e. the presence of tachyzoites and bradyzoites in the retina of the eyes causing inflammation, which is manifested by occasional blurred vision, blindness, or glaucoma (Frenkel, 1988).

Toxoplasma gondii is getting more attention recently because it can cause severe problems, such as encephalitis, in immunodeficient patients (Frenkel, 1990). More than a dozen opportunistic infections are considered to be

specific indicators of underlying immunodeficiency and are included in the case definition of Acquired Immune Deficiency Syndrome (AIDS). Some of these opportunistic infections include Pneumocystis carinii, Cryptosporidium parvum, and Toxoplasma gondii (Frenkel, 1990). Frenkel et al. (1975) showed that hamsters which became immunosuppressed through irradiation developed encephalitic T. gondii lesions which were analogous to those found in the brains of immunosuppressed humans. The modified agglutination test (MAT) appears to be a highly sensitive diagnostic tool in these immunosuppressed patients (Suzuki and Remington, 1990).

In animals, as in people, acquired infections are usually subclinical (Boughton, 1970). Toxoplasma gondii has been found in most mammals and birds (Dubey and Beattie, 1988). The zoonotic and epizootic potentials of T. gondii are of great concern.

Toxoplasma gondii has been found in various tissues and organs of pigs, i.e. brain, tongue, skeletal muscle, and heart (Dubey et al., 1984, 1986b). Because toxoplasmosis is a systemic infection, a variety of clinical signs are found including fever, diarrhea, respiratory distress, and central nervous system (CNS) signs. Most infections, however, are subclinical (Leman et al., 1986). Pregnant sows may abort or farrow weak or stillborn piglets. On post-mortem examination of affected

swine, lesions have included pneumonia, intestinal ulceration, hepatomegaly and white necrotic foci in any organ (Leman et al., 1986).

Dubey and Urban (1990) studied lesions in experimentally infected pigs. They fed 1000 T. gondii oocysts to 2 sows during different periods of gestation. One sow was fed oocysts at 60 days of gestation and sacrificed 49 days later. Histological studies discovered lesions of toxoplasmosis in 7 of the 8 fetuses from this sow. The second sow was fed 1,000 oocysts at 45 days of gestation and was sacrificed 62 days later. Only 3 of 11 fetuses from this sow showed lesions of toxoplasmosis. This study suggests that transplacental infection in swine is a likely mode of transmission, and the stage of gestation when infected is crucial to the clinical outcome. Sanger and Cole (1955) showed that experimental infection of sows late in gestation (84 to 108 days) did not result in congenital infection of the piglets.

Felines, the definitive hosts, when clinically affected by T. gondii, most commonly present with dyspnea due to a rapidly progressive pnemonia (Dubey and Frenkel, 1974). Other abnormalities include hepatitis, pancreatitis, enteritis, ophthalmitis, anemia, myocarditis, and disorders of the central nervous system (Dubey and Frenkel, 1974). In experimental studies, toxoplasmosis is

a uniformly fatal infection in week-old kittens, but becomes manageable immunologically by an increasing percentage of cats as they get older (Holzworth, 1987). Acute to subacute toxoplasmosis most commonly affects cat from 1 month to several years of age. As epidemiological studies suggest, primary infection must occur in many or most cats at an early age with illness in older animals most often representing reactivation of latent infection (Dubey and Frenkel, 1976).

In a published case report (Dubey et al., 1990c), the presence of T. gondii proved to be fatal in a cat naturally infected with Feline Leukemia Virus (FeLV), a retrovirus that commonly causes acquired immunodeficiency in felines (Cotter, 1984). Histological examination of the cat's tissues post-mortem confirmed the presence of hepatitis, edema and fibrinous exudate in pulmonary alveoli, encephalitis, and multiple necrotic foci in the spleen, pancreas and intestine (Dubey et al., 1990c). The preliminary diagnosis of toxoplasmosis was based on respiratory problems and lesions in the eyes. In another study, FeLV positive cats which were experimentally infected with T. gondii remained clinically and physically normal (Patton et al., 1991). One might speculate on differences between experimental and natural infection of T. gondii in FeLV positive cats and their clinical consequences. Similar to FeLV, canine distemper in dogs

induces an immunosuppressive state making them susceptible to toxoplasmosis (Jubb et al., 1985).

In another case report (Holzworth, 1987), an elderly cat treated for tapeworms with arecoline-acetarsol (Drocarbil) showed acute pulmonary effusion and respiratory and cardiac arrest. The cat died after a few days showing progressive CNS signs. Microscopic post-mortem examination of the brain revealed T. gondii cysts that had ruptured causing a severe cellular reaction which likely led to the animal's death.

Treatment of Toxoplasma gondii Infection:

Frenkel (1990) reviewed the treatment of toxoplasmosis in human patients. Basically, immunosuppressed patients are treated with sulfadiazine and pyrimethamine, which are inhibitors of the vitamin folic acid. These 2 drugs act synergistically in the patient. They inhibit multiplication of tachyzoites and bradyzoites. He also stated that, in immunosuppressed patients, treatment with these drugs must be continued indefinitely, whereas, in immunocompetent patients, treatment can be ended after clinical signs disappear. These drugs are not free of side effects; thrombocytopenia, leukopenia and purpura occasionally develop. These side effects can be treated with folic acid or leucovorin. Other side effects such as

rash, crystalluria, hematuria, vasculitis, and hemolytic anemia also occur. Clindamycin is another drug of choice which is moderately effective against T. gondii. This drug is used in cases where the patient is incompatible with sulfadiazine-pyrimethamine. Clindamycin also has its own share of side effects including pseudomembranous colitis, which is due to an overgrowth of Clostridium difficile (Harris et al., 1988).

The treatment of other animals is similar to that of humans. As in human therapy, no completely satisfactory treatment for toxoplasmosis is known (Levine, 1985). Available drugs suppress replication of tachyzoites, but are not completely effective in killing the parasite. No drug will effectively eliminate the tissue cysts containing bradyzoites from the infected animal (Dubey et al., 1990d). Clindamycin is effective for treatment of clinical toxoplasmosis in dogs and cats. Although less suitable than clindamycin, the combination of sulfonamides and pyrimethamine can be used. High dosages of these drugs can partially control oocyst shedding in cats. Monensin, an anti-coccidial drug used in cattle and poultry, is effective in suppressing oocyst shedding when placed in dry cat food within 1 to 2 days postinfection. Both drugs also have side effects similar to those observed in humans (Dubey et al., 1990d).

Immunology:

Like most protozoal infections, immunological responses to T. gondii are both specific humoral and cell-mediated (Brinkmann et al., 1987). In the humoral response, IgM antibodies appear as early as 7 days after infection, reach maximum titer within a few weeks, and gradually decline over months (Sharma, 1990). IgG antibodies appear within 1 to 2 weeks after infection, the titer usually declines gradually and eventually reaches a final low value that persists for life (Sharma, 1990). Therefore, a high IgG titer (>1000) or IgM titer may indicate a recent infection (Sharma, 1990).

The role of antibodies in infection of T. gondii is not clear; nevertheless, they seem to have a minor role (Sharma, 1990). Passive transfer of sera from mice with high titers to seronegative mice did not protect the mice from infection (Sharma, 1990). In vitro, in the absence of anti-T. gondii antibodies, tachyzoites are ingested by normal mouse and human monocyte-derived macrophages, and replicate within parasitophorous vacuoles (Sharma, 1990). On the other hand, tachyzoites exposed to specific antibody are endocytosed into vacuoles that fuse with lysosomes forming phagolysosomes and killing the parasite (Sharma, 1990). Thus, antibodies may promote rapid killing of the parasites by normal macrophages. After digestion of the

organism and antigen presentation to receptive T-cells, the cell-mediated immune response probably predominates (Sharma, 1990). How antibody might influence cyst formation remains to be elucidated.

Cell-mediated or delayed-type hypersensitivity responses to T. gondii have been studied as well (Lindberg and Frenkel, 1977). Delayed-type hypersensitivity responses (DTH), Type IV, may appear months to years after infection (Gaines, 1972). Type IV hypersensitivity is probably the least understood of the 4 major types of hypersensitivity reactions and occurs during the course of many infectious diseases (Slauson and Cooper, 1990). In this type of hypersensitivity reaction, neither circulating antibody nor complement are involved. Sensitized cells such as activated macrophages and T lymphocytes are involved (Slauson and Cooper, 1990). Because of the unique intracellular survival skills of T. gondii, the parasites persist over a long period of time in the host. In the mouse, the initial appearance and intensity of the DTH reaction coincides with the development of high titers of antibody. DTH becomes apparent on day 30 of infection and remains positive during chronic infection (McLeod et al., 1984).

The pathogenesis of retinal inflammation during relapses of T. gondii is not very well understood. The antibody levels of patients with toxoplasmal

retinochoroiditis were indistinguishable from the titers of asymptomatic seropositive patients (Wyler et al., 1980). Wyler et al. (1980) found that patients with toxoplasmal retinochoroiditis had circulating lymphocytes which were sensitized to retinal antigens.

Life-threatening toxoplasmosis can occur in individuals with diminished cellular immunity subsequent to adminstration of immunosuppressive agents used in cancer chemotherapy or in association with organ transplantation (Frenkel, 1990). Similarly, patients with AIDS frequently suffer from central nervous system toxoplasmosis (Frenkel, 1990). Defects in cell-mediated immunity perhaps allow transformation of bradyzoites (cyst stage) into proliferating stage tachyzoites, resulting in acute relapse (Sharma, 1990).

The inflammatory response to experimental subcutaneous injection of tachyzoites in normal mice was predominantly mononuclear cells that appeared between 4 to 7 days postinfection. Mice that were treated with Cortisone acetate and cyclophosphamide (anti-immune drugs) showed no bradyzoite formation (Hofflin et al., 1987). Cortisone acetate affects several aspects of cell-mediated immunity (Fauci and Dale, 1974). Cyclophosphamide at a low dosage has an effect on B-lymphocytes and at a higher dosage (>300 mg/kg) can suppress virtually all of the immune responses

(Turk and Parker, 1979). The results of this study indicated that in normal mice necrosis developed around the site of tachyzoite inoculation and intense mononuclear inflammation and cyst formation was observed. Tachyzoites were not detectable after 2 weeks. In general, the immunosuppressed mice (drug treated) had greater tissue destruction and tachyzoites were always present. This might suggest that the immune system of the host stimulates bradyzoite cyst formation (Hofflin et al., 1987).

The overall immunologic response of the cat to T. gondii is similar to that of other hosts (Lin and Bowman, 1991). In cats, antibodies appear toward the end of the primary infection and the end of oocyst shedding, which is taken to be one indicator of developing immunity (Frenkel and Dubey, 1972). In an experimental study, immunity appeared to be age-dependent; about 60% of cats younger than 13 weeks of age shed oocysts again when fed infected mice (Frenkel and Dubey, 1972). On the other hand, immunity in infected cats older than 13 weeks of age was much stronger (Dubey and Frenkel, 1974), and they were unlikely to shed oocysts again after challenge. When immunity was not complete, most kittens, except the youngest, shed fewer oocysts after reinfection, and intervals between infection and shedding were longer than after primary infection (Dubey and Frenkel, 1974).

In an experimental study (Dubey et al., 1990a), 17

sows were fed 1,000 oocysts. In this study, the modified agglutination test (MAT) was shown to be useful as a rapid screening test for transplacental T. gondii infection in pigs (Dubey et al., 1990a). Using MAT, they found T. gondii antibody in the fetal serum and/or body fluid from 7 congenitally infected piglets born from 3 sows. While T. gondii antibodies do not cross the placenta in pigs the T. gondii organism is able to cross the placenta and generate an antibody response in the fetus. This study also showed that the colostral antibody disappeared by 6 months of age in newborn piglets. Therefore, if pigs have positive T. gondii titers at 6 months of age or older, this represents postnatal infection from the environment (Dubey et al., 1990a).

Australian marsupials are notoriously susceptible to T. gondii infection and die acutely with no apparent resistance (Patton et al., 1986b). The nature of the immune defects in Australian marsupials, arboreal neotropical primates, Madagascan lemurs, hyrax, etc. has not been studied (Frenkel, 1989). Patton et al. (1986b) showed the presence of antibody to T. gondii in the red kangaroo, gray kangaroo, and a joey.

Immunohistochemistry:

The similarity of other organisms to the tissue stages

of T. gondii requires a specific immunohistochemical staining procedure to distinguish among them in hematoxylin and eosin stained sections. In a retrospective study of 23 dogs with proven fatal toxoplasmosis-like illness, Dubey used a specific immunohistochemical technique to disprove that T. gondii was the etiologic agent in some of these cases. The specificity of immunohistochemistry led to the discovery of a new protozoan, Neospora caninum (Dubey et al., 1988).

Immunoperoxidase is a histochemical technique that can detect T. gondii tachyzoites and bradyzoites in tissue sections. Immunostaining is a two-step process involving first, the binding of an antibody to the antigen of interest, and second, the detection and visualization of bound antibody by 1 of a variety of enzyme chromogenic systems. There are 4 methods of immunoperoxidase staining, the direct, indirect, Peroxidase-Anti-Peroxidase (PAP), and avidin-biotin-peroxidase complex method. The avidin-biotin method is preferred over other techniques (Hsu et al., 1981a, 1981b). The main drawback of the direct method is that for every antigen to be localized a different conjugated antibody is needed (Naish, 1989). In the indirect method, there is a great chance of nonspecific reactions occurring. Another disadvantage of direct and indirect techniques is that they must be performed on frozen sections to achieve consistent results (Hsu et al.,

1981a, 1981b). The PAP method uses three reagents: primary antibody, secondary or link antibody, and a PAP complex which contains the label. The sensitivity of this method is limited by the affinities of the link and label antibodies for each other. However, the PAP method can be used for paraffin embedded tissues (Falini and Taylor, 1983).

In the avidin-biotin method, tissues embedded in paraffin are cut with a microtome into 4 to 8 micrometer thick sections, which are placed on microscopic slides (Naish, 1989). The tissues are deparaffinized in 100% xylene and then hydrated through a series of 100% ethanol to 95% ethanol solutions. Then, 3% hydrogen peroxide is placed directly on the specimen to irreversibly inhibit endogenous peroxidase; this increases the inhibition of enzyme by 100-fold. Also, hydrogen peroxide is the substrate that is acted upon by the peroxidase enzyme to form a colored end product (Naish, 1989).

Nonspecific background staining is a result of highly charged proteins, such as collagen and connective tissue. To prevent this nonspecific staining, innocuous protein solution is added to the specimen before applying the primary antibody. Goat serum is used to fill the charged sites and block the nonspecific sites (Bourne, 1983). After this, the slide is incubated in rabbit-anti-

Toxoplasma-gondii serum, which is the primary antibody. This step is required for the antigens to be localized. The next step is to add biotinylated antibody (goat anti-rabbit) which is a secondary antibody or link antibody. In this link antibody, biotin is covalently attached to the antibody. Open sites on avidin-biotin complex or enzyme-labelled avidin bind to the biotin on the link antibody. The streptoavidin is preferred over avidin because avidin contains no carbohydrates which can bind nonspecifically to lectin-like substances found in normal tissues such as kidney, liver, brain and mast cells (Kuhlmann and Krischan, 1981). The link antibody serves as a bridge between secondary antibody and biotinylated horseradish peroxidase.

Horseradish peroxidase mediates the transfer of an electron from hydrogen peroxide to 3-amino-9-ethylcarbazole or AEC (Bourne, 1983). Of the several suitable electron donating chromagens, AEC, which forms a red end point product that is alcohol soluble, is used (Naish, 1989). Then, the specimens are counterstained with Mayer's hematoxylin. The purpose of the counterstain is to provide a counter staining effect on chromagen. Consequently, specimens should not be dehydrated by alcohol and xylene, but rather coverslipped with a water-based medium, because AEC is soluble in organic solvents. Toxoplasma gondii picks up the red-brown stain, and the background is blue.

Serology:

Many serologic tests have been reported for detection of antibody to Toxoplasma gondii in the serum. Until recently, most researchers considered the Sabin-Feldman Dye test (DT) as the most sensitive and specific test available for detection of T. gondii antibody (Dubey and Beattie, 1988). This test is based on a complement-mediated, neutralizing type of antigen-antibody reaction. Live tachyzoites, complement from human serum, and the test serum are incubated at 37°C for an hour. Methylene blue is then added which stains the tachyzoites unaffected by antibody of the host. Specific antibody induces complement-mediated cytolysis of tachyzoites and causes the cytoplasm to leak out. Therefore, tachyzoites that are affected by antibody do not incorporate methylene blue and appear as "ghosts". The reported titers are the test serum dilutions at which 50% of the tachyzoites remain unstained. This test is expensive, requires a high degree of technical expertise, and is potentially dangerous because of the use of live tachyzoites.

The first agglutination test to detect T. gondii antibody was reported by Fulton and Turk in 1959. In the agglutination test, Fulton used a pure suspension of dead T. gondii for direct agglutination (Fulton, 1965b). This

test had several advantages over the dye test in that it was macroscopic and used dead organisms, which were safer for laboratory personnel to handle (Fulton, 1965b). The micro-agglutination test used only 1/10 to 1/5 of the amount of antigen used in the macro-agglutination test (Fulton, 1965a) and could be easily and rapidly performed. The results of this micro test were compatible with the macro test and other serological tests (Fulton, 1965a), but it had poor sensitivity and lacked specificity.

The indirect fluorescent antibody test for detection of T. gondii was investigated in 1964 by Fulton and Voller and found to correlate well with the other tests available at that time. In the indirect fluorescent antibody test (IFA), whole killed tachyzoites are incubated with serum and antibody detection is enhanced by adding fluorescein-labeled, e.g. fluorscein isothiocyanate or tetramethyl rhodamine isothiocyanate, antispecies IgG. The results of this test are observed by a fluorescent microscope. The disadvantages of this method are the need for a fluorescent microscope, species specific conjugates, and the possibility of cross-reactions with rheumatoid factor and antinuclear antibodies. The fluorescent method is slower, but its specificity was established by absorption studies and by lack of cross-reaction with sera from cases of trypanosomiasis, malaria, leishmaniasis, sarcocystosis, leptospirosis, syphilis, schistosomiasis and filariasis

(Fulton and Voller, 1964). It also had the advantage that all the reagents were commercially available and that slide antigen can be easily prepared and stored for long periods of time (Fulton and Voller, 1964).

Enzyme-Linked Immunoabsorbent Assay (ELISA) is recently gaining popularity for detection of exposure to many pathogens (Paul, 1984) including T. gondii. While radioisotopes may be readily linked to antigens, widespread and routine use of radioimmunoassays has been inhibited by the requirement for complex and expensive counting equipment (Paul, 1980). Because of this, alternative labels such as enzymes have been developed. In ELISA, antigen is chemically bound to a solid surface such as a microtiter plate. After serum is allowed to react with this antigen, surface-bound immune-complexes are formed, and the microtiter plate is washed to remove uncomplexed antibody. Enzyme-linked antiglobulin is then added. After a second wash, the amount of enzyme left in the plate correlates with the level of specific antibody in the serum. This test requires an ELISA reader to quantitate the color reaction, which is very expensive (Dubey and Beattie, 1988). Also, it requires the development of species specific conjugates in order to test the serum of different species, which is impractical in a veterinary setting.

Indirect hemagglutination test (IHA) is a test that until recently was frequently used in many laboratories for detection of T. gondii antibody. In this test, tachyzoites are adsorbed to the surface of erythrocytes, a process called sensitization. In the presence of the T. gondii antibody, the red blood cells agglutinate, and a positive result is observed (Schwartz, 1980). There are several pitfalls to using this technique including a failure to detect antibody in acute stages of infection as well as a tendency to give negative results in congenital infections (Dubey and Beattie, 1988).

All of these tests were often expensive, time consuming, not readily adaptable to screening programs, or not sensitive enough for early diagnosis of T. gondii infection (Desmonts and Remington, 1980). The agglutination test was simple and inexpensive; however, it had 2 drawbacks. First, the sensitivity of this test was poor. The titer in the agglutination test was usually much lower than that in the dye test or indirect immunofluorescent-antibody test (Desmonts and Remington, 1980). Therefore, many positive serum samples were read incorrectly as negative (false-negative). The second drawback was a lack of specificity; some sera that were negative in the dye test (DT) and immunofluorescent test (IFA) were reported as positive in the agglutination (AG) test (false-positive). The reason for the false-positives

was the binding of "normal" IgM to the surface of the organisms. As a result, Desmonts and Remington in 1980 modified the method of preparing antigen which increased the sensitivity and added 2-mercaptoethanol to the agglutination reaction to improve specificity (Desmonts and Remington, 1980).

Desmonts and Remington (1980) used the RH strain of Toxoplasma gondii tachyzoites cultivated along with mouse TG 180 sarcoma cell in the peritoneal cavities of mice to amplify antigen production. This method caused a 10-fold increase in the number of organisms obtained. The harvested T. gondii tachyzoites were then resuspended in phosphate-buffered saline (PBS) at pH 7.2 containing 0.05% trypsin and incubated in a water bath at 37°C under continuous agitation. After several washes and centrifugations, the parasites were kept in formalin. In short, this method increased the purity of the tachyzoites obtained and assured that the suspension produced lacked cell debris and other antigenic components. These problems had not previously been overcome. Fujita and co-workers (1969) used differential centrifugation methods, but still they could not purify antigen as well. Desmonts and Remington also diluted 2-mercaptoethanol (2-ME) in PBS as a diluent in order to destroy IgM and improved the specificity of the test. This additional step reduced the

false-positive results. After these modifications, the qualitative test results are in excellent agreement (98%) with the Sabin-Feldman dye test. This modified agglutination test (MAT), since it is simple, fast, and does not require live tachyzoites, is an excellent screening test for the surveillance of seronegative patients and for detection of seroconversions.

Future Diagnostic Methods for Toxoplasma gondii:

With advances in molecular biology and biotechnology, the detection of T. gondii can be simpler and faster. Definitive diagnosis of this parasite is very important in AIDS patients and congenitally infected fetuses. In Japan, there have been cases where pregnant women continued to have high levels of IgM antibody without a rise in IgG antibody level until delivery (Konishi, 1991). At present if the pregnant mother is IgM seropositive, no simple prenatal test exists to determine if her fetus is infected with T. gondii (Weiss et al., 1991). Therefore, a more definitive diagnostic test is needed for prenatal diagnosis as well as for HIV positive patients.

Several authors have suggested the use of polymerase chain reaction (PCR) and DNA hybridization (Burg et al., 1989; Weiss et al., 1991). For PCR, T. gondii has to be obtained from the patients suspected of having toxoplasmosis. Toxoplasma gondii tachyzoites can be

obtained from the buffy coat of peripheral blood smears because T. gondii invades monocytes, amniotic fluid of pregnant women (Burg et al., 1989), or cerebral fluid obtained from HIV positive patients. After lysing the tachyzoites and separating the DNA from other cell components, the DNA of T. gondii is amplified by using PCR. Specific genes (e.g. B1) could be detected by Southern hybridization. The B1 gene was found in all different strains of T. gondii, and can be used to indicate the positivity of the origin of the sample.

An assay based on amplification of the B1 target would be faster than xenodiagnosis and offer the potential for enhanced sensitivity by detection of the dead or living parasite. Unfortunately at the present time, the presence of nonspecific DNA's is inhibiting the use of this technique in its full capacity, and further optimization of extraction, amplification, and detection procedures will be needed to increase the sensitivity of PCR (Weiss et al., 1991).

Serological Tests in Swine:

There are approximately 330,000 pork producers in the United States (National pork producers council, 1989). The annual gross sales of the swine industry are about

$10 billion. In 1989, Tennessee produced over 1,724,979 hogs and ranked fifteenth in the nation in swine production; Iowa ranked first (National pork producers council, 1989).

Because histological studies of the entire host organism are costly, time consuming and impractical, an easier and faster way of studying the prevalence of T. gondii infection in a host population is via serology. Serological surveys detect antibodies that are produced specifically for T. gondii, both in acute and chronic infections. The possible serological tests that can be utilized include the following: 1) dye test; 2) indirect hemagglutination test (IHA); 3) modified direct agglutination test (MAT); 4) enzyme linked immunosorbent assay (ELISA); and 5) immunofluoresent assay (IFA) (Dubey and Beattie, 1988). In one study comparing ELISA, IFA, and MAT (Seefeldt et al., 1989), MAT was preferred, mainly because it was less complicated and easier to perform. The modified agglutination test was also found to be more specific than ELISA and IFA (Seefeldt et al., 1989). Desmonts and Remington (1980) were the first to design the modified agglutination test. They demonstrated that not only was this test very sensitive, but that it also can detect recent infection. Patton et al. (1991) demonstrated detection of recent infections by MAT in feline populations.

The modified agglutination test is the most sensitive test for detecting T. gondii antibody, unfortunately, it is very expensive and not easily accessible (Dubey et al., 1990a; Patton et al., 1991). Due to these drawbacks, a variety of serologic tests have been used in seroprevalence surveys with varying results.

The seroprevalence of T. gondii in swine in the U.S. has been estimated as high as 69% (Dubey, 1990a). In a study of the seroprevalence of T. gondii from the Memphis, Tennessee region by Eyles et al (1959), 22% of swine were shown to be seropositive by dye test. Eyles et al. (1959) also demonstrated in this study that house cats had the highest seroprevalence of T. gondii among all the animals surveyed. In a study from Iowa, McCulloch et al. (1964) found only 6.7% of the pigs positive from 66 counties of Iowa using the Sabin-Feldman dye test, suggesting that this may be a less sensitive method of detecting T. gondii in swine. In a study from North Dakota, 43 of 97 porcine sera had titers of 1:40 or higher using the indirect hemmagglutination test (1:32 is considered as positive) (McIlwain, 1969).

Smith et al. (1982) studied the seroprevalence of T. gondii in wild swine in the southeastern areas of the United States. They found that 17% of the swine tested were positive for T. gondii antibody by indirect

hemagglutination test. The seroprevalence of T. gondii from Louisiana in 1,219 slaughtered pigs, showed 19.2% positivity of titers of $\geq$ 1:64 by indirect hemagglutination test (Hugh-Jones et al., 1986). Hugh-Jones et al. (1986) also found that 52 farms had an average seroprevalence of approximately 24.6%, using indirect hemmagglutination test. On an Illinois farm, the MAT detected antibody in 95 of 99 pigs (Dubey, 1986).

In a study from Iowa by Zimmerman (1990), 2,616 swine were tested via the ELISA. The seroprevalence of T. gondii was 5.4% among finishing hogs and 11.4% among breeding sows and gilts. In another recent study by Smith et al. (1991) using MAT, the prevalence of T. gondii from 19 central Iowa counties was found to be 14.3%. In this study, they also concluded that there was a lower seroprevalence of T. gondii antibody in pigs kept in the total confinement type of management system than in those pigs not totally confined. In addition, Smith et al. (1992) suggested that there was a direct relationship between age and prevalence.

The advantages and disadvantages of the type of test used should be considered when interpreting seroprevalence data. Overall, the MAT is considered the most reliable test available for seroprevalence studies (Dubey, 1990b).

Summary:

Control of toxoplasmosis must be based on knowledge of its epidemiology, which unfortunately has received little consideration. In the United States, consumption of pork products is probably the major source of human infection with Toxoplasma gondii (Dubey, 1990). People may become infected by ingestion of undercooked meat, which is becoming increasingly popular (Weinman and Chandler, 1956; Dubey et al., 1984).

This research was designed as an epidemiologic study of T. gondii in swine in Tennessee. The objectives were as follows:

1) to evaluate the reliability of the modified agglutination test (MAT) in a murine model for ultimate application in porcine seroprevalence studies,

2) to evaluate whether swine going to market to be consumed by the general public are infected with T. gondii,

3) to investigate the stage of the production cycle in which swine acquire infection with this parasite,

4) to estimate the seroprevalence of T. gondii in swine in the state of Tennessee, and

5) to study the relationship between the seroprevalence of T. gondii in swine and the type of management practices used on the farm.

CHAPTER II

Evaluation of the Modified Agglutination Test in Mice

Introduction:

The immune response of Toxoplasma gondii is complex and requires both humoral and cellular mechanisms (Lindberg and Frenkel, 1977). Cell-mediated immune responses to T. gondii infection have been demonstrated as delayed type hypersensitivity reactions and as antigen-specific lymphocyte transformations (Gaines et al., 1972; Krahenbuhl et al., 1972).

A major role for humoral immunity in resistance to infection remains questionable. Antibody may not be necessary to control acute infection, but it could be important in controlling long-term toxoplasmosis (Frenkel and Taylor, 1982). The passive transfer of immune rabbit serum to rabbits and mice prior to challenge with the highly virulent RH strain of T. gondii did not protect the animals from infection (Gill and Prakash, 1970). However, passive transfer of monoclonal antibodies protected mice from a lethal dose of T. gondii tachyzoites (Sharma, 1990). In another study, mice infected with T. gondii showed depressed antibody responses to sheep erythrocytes and to killed polio vaccine (Suzuki et al., 1981). Whether or not T. gondii infection depresses the production of antibody to

other agents is a question that needs to be elucidated.

The presence of T. gondii antibody in the host serum confirms previous exposure to the organism. The presence of antibody can vary with the experimental infection procedure, i.e. animals, strain virulence, dose, route of infection, and stage of the parasite (Dubey and Frenkel, 1973). Toxoplasma gondii infection can be maintained in mice by serial passage of bradyzoite cysts. The organism can be transferred from mouse to mouse indefinitely. Tachyzoites that were serially passed in mice for 30 to 35 generations failed to produce oocysts in cats, which were fed these mice (Frenkel et al., 1976). This may suggest that continuous passage of tachyzoites can lead to a genetic alteration of the organism (Frenkel et al., 1976).

On average, mouse brain samples contain at least 10,000 cysts per 100 grams of tissue in comparison to porcine muscles that contain less than 2 cysts per 100 grams of tissue (Dubey et al., 1986a). This fact suggests that the mouse model more so than other species should allow for easier detection of bradyzoite cysts in post-mortem tissue samples and that their brain tissue is a ready source of bradyzoite cysts.

Several serologic tests can be utilized to detect the development of T. gondii antibodies. Even though the MAT is still in the process of being researched, it is one of

the most reliable tests available (Dubey, 1990b; Patton et al., 1991). The MAT technique has been suitable for serologic testing in a wide variety of species including Australian marsupials, human, goats, cattle, pigs, horses, sheep, cats, and dogs (Johnson et al., 1990). We are unaware of serologic studies of T. gondii using the MAT in mice. Serologic studies in mice infected with T. gondii have been performed using the dye test and ELISA (Dubey and Frenkel, 1973; McLeod et al., 1984; Brinkmann et al., 1987 Chardes et al., 1990). In these studies, the initial appearance of anti-toxoplasma (IgG) antibody ranged from 7 to 16 days.

An intraperitoneal route of infection was chosen for our study because of its success in prior studies (Ferguson et al., 1989) with T. gondii as well as practical concerns such as the difficulty of oral administration in the mouse. The bradyzoite stage was used for infection in our study because it has been successfully used by an intraperitoneal route previously and can be readily harvested in adequate quantities from brain. Tachyzoites are only present in large quantities in early stages of infection and may change their genetic characteristics with serial infections. The purpose of this study was to confirm the reliability of the MAT in detecting the development and progression of IgM and IgG titers in a murine model in our laboratory. A murine model was chosen due to its

affordability, convenience, and ease of handling, housing and care. The mice were infected by intraperitoneal injection of bradyzoites obtained primarily from mouse brain but also skeletal muscle. Confirmation of the reliability of the MAT allowed us to use this test with confidence in our serologic surveys of the swine population in the state of Tennessee. The antibody titers were compared to similar experiments performed with mice using other serologic tests, and T. gondii infection was confirmed by post-mortem examination of brain tissues.

Material and Methods:

Experimental Animals - The experimental animals were ICR mice obtained from Harlan Sprague Dawley Incorporated (Indianapolis, Indiana). The mice were housed in the University of Tennessee Laboratory Animal Facility. Five mice per cage were kept in polycarbonate microisolators on wood chip bedding and given mouse chow and water ad libitum. Seventy mice were used in the infection experiments; 35 mice were infected with Toxoplasma gondii bradyzoites, and an additional 35 mice were used as uninfected controls.

Source of Inoculum - The bradyzoite cysts for the experimental infection were obtained from the brain and muscles of other mice that were previously infected with T.

gondii (goat, UT-1) and had high IgG titers (>8192) by modified agglutination test (MAT) (Patton et al., 1990). The tissues were diced into half centimeter pieces. Five equivalent volumes of sterilized saline were added to each tissue, and then the tissues were homogenized (Appendix A). Two hundred and fifty ml of digestive fluid was added to each of the homogenates. The homogenate and digestive fluid mixture was placed on a shaker in an incubator at 37°C. The pepsin immediately destroys the cyst walls and releases organisms that can remain viable for 6 hours in digestive fluid (Dubey and Frenkel, 1973).

After a 30 minute incubation, the mixture was removed from the incubator and filtered through 2 layers of sterilized cheese cloth. The mixture was then centrifuged at 400 X G for 15 minutes. The sediments were washed by resuspension in sterilized saline 3 times. After the last wash, the sediments were resuspended in an equal volume of sterile antibiotic-saline solution. The concentration of this solution was 0.1 ml of antibiotic (Penicillin-streptomycin, prepared with 5,000 units/ml penicillin-G sodium and 5,000 mg/ml streptomycin sulfate, GIBCO Laboratories, Grand Island, New York) to 100 ml of saline.

Experimental Procedure - The mice were anesthetized with methoxyflurane and 1.0 ml of the bradyzoite inoculum was injected intraperitoneally (IP) using a 22g needle and a 3cc syringe (Appendix A). In Experiment 1, 10 mice were

inoculated with the UT-1 strain of T. gondii. Bradyzoites from the skeletal muscle of infected mice were used to infect 5 mice; bradyzoites from brain tissue of infected mice were used to infect the other 5 mice. Ten uninfected mice served as negative controls.

In Experiment 2, 25 mice were inoculated with the UT-1 strain of T. gondii. Bradyzoites were obtained from brain tissue of infected mice. Twenty-five uninfected mice served as negative controls.

Twenty to 30 microliters of blood were taken from the orbital sinus of each mouse used in the experiments and from all control mice. The serum was titered for T. gondii IgM and IgG by the modified agglutination test. All mice were bled prior to infection and at intervals thereafter. In Experiment 1, the mice were bled at 5, 18, and 55 days postinfection (PI). Control mice were bled on the same schedule. In Experiment 2, the mice were bled at 2 day intervals beginning at day 5 postinfection. Control mice were bled on the same schedule.

In Experiment 1, the mice were sacrificed 55 days postinfection. The mice in experiment 2 were sacrificed at 33 to 45 days postinfection. All mice were necropsied and examined for bradyzoites.

Serologic Testing - The modified agglutination test (MAT) was used to test for T. gondii antibodies, IgM and

IgG (Appendix B). The MAT, although the most expensive, also is the most sensitive test available, and has been shown to detect antibodies in the early stages of infection (Patton et al., 1991).

The MAT was performed with 96-well serologic plates (Appendix C) (Plate 1). Twenty-five microliters of phosphate buffered saline (PBS) was added to each well initially (pH 7.2). Next, 25 microliters of serum was added to the first well, mixed, and 2-fold dilutions were made across the 12 wells. Twenty-five microliters of 0.2M 2-mercaptoethanol (2ME) (purchased from Sigma, St. Louis, Missouri) in PBS were added to each well (Desmonts and Remington, 1980) to 1:16, 1:512, and 1:8016 dilutions. The 2ME breaks off the sulfide-bonds of the IgM and blocks its effect on the antigen. Toxoplasma gondii antigen (formalin fixed tachyzoites obtained from bioMerieux in Lyon, France) was diluted 1:5 in 0.1N borate buffered saline (diluent), pH 8.9. A few drops of trypan blue dye was added to the diluent/antigen mixture so agglutination could be more easily visualized (Patton et al., 1990). Fifty microliters of this diluent/antigen mixture was added to the 1:16, 1:512 and 1:8016 dilutions. The purpose of screening at these 3 dilutions was to prevent any prozone effect (Seefeldt et al., 1989). A prozone effect occurs when excess IgG saturates the binding sites on the antigen. Therefore, at lower dilutions a prozone effect can prevent

an antibody-antigen reaction, i.e. no agglutination occurs. A positive and negative serum control was included with each batch of serum tested. The positive and negative controls were human serum obtained from laboratory personnel. The positive serum has been repeatedly tested and demonstrated to maintain a titer of 1:1024. The plates were sealed tightly with an adhesive transparent sheet and incubated at 37°C.

The plates were read 6-12 hours later. The results were stable for at least 72 hours (Seefeldt et al., 1989). All positive samples were titered to the end point, i.e. antigen was added to the intervening wells between the positive and higher negative screening wells to pinpoint the actual titer within this interval (Plate 1). A titer was read as the reciprocal of the last dilution of serum of a titration which gives a measurable effect greater than the negative controls (Benjamini and Leskowitz, 1988).

To standardize the results, the plates were visually read by the same person (the author) throughout the study. The samples were randomized after collection so the author was not aware of which samples were from infected or control mice at the time the titers were determined. Wells were considered positive when agglutination occurred similar to the positive control, i.e. agglutination covers half of the well base. A negative result resembled the

negative control in which only a button of antigen would be present on the well base. A conversion factor was used to standardize the samples. This was derived by dividing the positive control's standardized titer (1024) by its raw titer: Standardizing multiplier = 1024/positive control raw titer. For example, if the positive control's raw titer was 2048, the standardized multiplier would be: 1024/2048 = 1/2. Then, the titers of samples would be multiplied by 1/2 to obtain standardized titers of samples (Thrusfield, 1986). It has been shown that Sarcocystis species antibodies react with the T. gondii antigen up to 1:16 dilution (Seefeldt et al., 1989; Patton et al., 1991). Therefore, the antigen was added beginning on the third well, and a reaction less than or equal to 1:16 was not considered to be specific. Anything greater than or equal to 1:32 was considered positive.

To measure IgM, PBS was added to the wells instead of 2-ME; therefore, IgM was not destroyed (Johnson et al., 1989; Johnson et al., 1990; Patton and Funk, 1992). To calculate the IgM titers, the Log_2 titer of serum treated with 2-ME (IgG) was subtracted from the titer of serum not treated with 2-ME (IgM + IgG) also in Log_2.

Brain Cyst Confirmation - To determine the accuracy of serologic diagnosis, the brains of the mice in Experiments 1 and 2 were examined for cyst formation by brain squash preparations, hematoxylin and eosin (H & E), and/or

immunoperoxidase staining (StrAviGen MultiLink Kit purchased from BioGenex Laboratories, San Ramon, California) (Appendix D). Four brains of infected mice were examined histologically for bradyzoite cysts and any abnormalities by H & E and immunoperoxidase. Brains of 4 control mice were examined for bradyzoites cysts by immunoperoxidase.

Results:

The 10 mice in Experiment 1 were negative for IgM on day 5 postinfection. They developed an IgG titer on day 18 P.I. They were all positive for IgG and sacrificed on day 55 P.I. at which time bradyzoite cysts were observed in brain squash preparations. Attempts at infection prior to Experiment 1 were unsuccessful when a ratio of 1 ml of antibiotic to 1 ml of sterile saline was used in preparation of the infective dose. Experiment 1 was performed to confirm success of infection using our laboratory procedure. There was no difference in antibody production between the 5 mice infected with muscle and those infected with brain.

Experiment 2, using 25 mice, was conducted to record the development and progression of both IgM and IgG. None of the mice died from infection; 1 mouse died from a prolonged period of anesthesia on day 9 P.I. Ten mice were

bled on day 4 and tested for both IgM and IgG. Their titers were zero. Another 9 mice were bled on day 5 and tested for both IgM and IgG. Their titers were zero.

The mice appeared ill with ruffled fur, abdominal distension, huddling behaviour, and sometimes hunched backs from 2 to 9 days after infection. The IgM and IgG titers appeared as early as 7 days P.I. (Table II.1) (Appendix E). IgM had a peak from 11 to 15 days after infection, and almost disappeared at 17 days of infection (Figure 1) (Appendix F). IgG titers (Table II.2) increased on day 11 and remained high until the end of the trial, 33 to 45 days P.I. (Figure 1). Table II.1 lists the IgM titers and Table II.2 lists the IgG titers of Experiment 2 mice. None of the control mice developed titers.

The brain squash preparations of all the infected mice confirmed the presence of bradyzoite cysts (Appendix C) (Plate 2). The brains of 4, infected mice were stained with standard hematoxylin and eosin (Plate 3). The histopathological findings included a lymphocytic meningitis with smaller numbers of plasma cells and perivascular cuffing. The majority of bradyzoite cysts were found in the brain stem and peripheral gray matter of the cerebral cortex. Several glial nodules were observed, however, there were no bradyzoites cysts or tachyzoites associated with these nodules. Focal karyorrhexis of inflammatory cells was observed in the meninges. The

immunoperoxidase findings confirmed the presence of many bradyzoites cysts in the brain stem and gray matter of the cerebrum (Plates 4 and 5). Immunoperoxidase and H&E staining of the brains of the control mice did not demonstrate bradyzoite cysts or any other abnormalities.

Discussion:

In our study using mice and MAT, the IgM titer first appeared at 7 days P.I. The IgG appeared at 7 days P.I. also, but its presence was not well established (low titers) until 9 to 11 days P.I. The IgM peaked from 11 to 15 days P.I. which correlated with the Chardes (1990) study in which IgM peaked on the 14th day P.I. The negative control mice did not develop any anti-toxoplasma antibody indicating the lack of contamination in our animal care facilities.

Detection of anti-toxoplasma antibodies in serum is clinically the most useful evidence of prior infection with Toxoplasma gondii. The presence of IgM anti-toxoplasma antibody is regarded as an indication of recent infection as is a rising IgG titer (Chardes et al., 1990). The earliest serologic diagnosis of toxoplasmosis in mice using the dye test could be made with precision after the 16th day of infection (Dubey and Frenkel, 1973). In another study, the presence of IgG using the dye test and Enzyme-

Linked Immunoabsorbent Assay (ELISA) was detected at 14 days P.I. (Brinkmann et al., 1987). In a more recent study, the earliest serodiagnosis in mice by modified dye test was possible 7 days after infection (McLeod et al., 1989). In a fourth study using mice, the IgM and IgG levels were detected with ELISA after 15 days of infection (Chardes et al., 1990). Serum and milk IgG and IgM production began at the same time after infection as did the IgA response. The IgM titer peaked on day 14 and then decreased progressively. The IgG titer reached its plateau on the 28th day after infection and did not change significantly throughout the rest of the study (Chardes et al., 1990).

In an experimental study with opossums using MAT, IgM titers were detectable from 7 to 42 days postinoculation (Patton and Funk, 1992). In this study, the IgG level was measurable by 21 days post infection and remained high for 210 days at which time the opossums were sacrificed for necropsy. In the same study, mice injected with tissues of seropositive opossums did not seroconvert, and organisms could not be detected post-mortem in either the opossums or the mice.

There are several diagnostic tests available on the market for detection of T. gondii antibody. The evaluation and modification of these tests are the concern of many research laboratories. For instance, in an evaluation of

the dye test McCabe et al. (1987) concluded that the use of 1-day old Toxoplasma tachyzoites resulted in a 5-fold decrease in the detection of antibody in human sera in comparison to 2-day old Toxoplasma harvested from a mouse.

In our study, at necropsy 33 to 45 days postinfection, the majority of the cysts were found in the brain stem and cerebrum as demonstrated by H & E histologic sections and immunoperoxidase. Ferguson et al. (1989) found most of the bradyzoite cysts located in the cerebrum. There was no evidence of tachyzoites observed in any of the above mentioned sites and methods.

Brain cyst formation is a well-known feature in toxoplasmosis (Frenkel, 1990). However, it is not yet known how brain cyst formation is related to protective immune mechanisms since both immune and nonimmune mechanisms have been implicated in brain cyst formation (Ferguson et al., 1989). In one study in mice, 3 days after infection with tachyzoites cyst-like structures containing distinct, PAS-positive granules were seen. Argyrophilic walls surrounding the structures were first seen after 6 days (Dubey and Frenkel, 1976). Mice are considered to be in a chronic stage of T. gondii infection starting 60 days after infection (Pomery et al., 1991) .

A lymphocytic meningitis with fewer plasma cells, perivascular cuffing and occasional glial nodules was also

observed in our study. This progression from acute to chronic stages of infection has been previously associated with a change from a lymphocyte/monocyte, inflammatory cell population to one with a predominance of plasma cells (Ferguson et al., 1991). The microglial or inflammatory nodules which start to appear by day 11 are present throughout the acute and chronic phases of infection and may represent "tombstones" of ruptured cysts (Ferguson et al., 1991). The variability of the meningitis in chronically infected animals may represent the effects of intermittent cyst rupture with restimulation of the immune system (Ferguson et al., 1991).

Although cyst rupture is extremely rare in the mouse, the high density of cysts in the brain increases the likelihood of spontaneous rupture (Ferguson et al., 1991). Ferguson et al. (1989) used an avirulent SRRA strain of T. gondii to infect an albino STR strain of mouse to produce and study ruptured cysts in the brain. They observed that a significant feature of cyst rupture was the rapid inflow of inflammatory cells, mainly macrophages, which surrounded the released cystozoites. In our study, no ruptured cysts were observed.

Prior studies have not been performed to detect the reliability of the MAT for detection of T. gondii in a mouse model such as ours. This test detected the chronic as well as the acute stage of infection. The MAT proved to

be an effective tool in the detection and measurement of anti-*toxoplasma* IgM and IgG titers. It produced titers similar to those found in comparable studies using other tests that may be more expensive or potentially hazardous such as the dye test, or ELISA. The MAT is very reliable and does not require the use of live tachyzoites.

Seroconversion and infection in the mice was confirmed by the observation of *T. gondii* bradyzoites cysts in brain squash preparations and H & E stained brain sections as well as with a highly specific, immunoperoxidase staining procedure. The test did not produce false positive results since infection was confirmed post-mortem. Although MAT is expensive (Dubey, 1990b) and at the present time only performed in 2 laboratories in the United States, the use of MAT in seroprevalence studies is well justified.

Chapter III

Seroprevalence of Finishing Pigs for *Toxoplasma gondii*

Introduction:

Commercial swine producers use varying systems of management for different aged groups of pigs. For instance, feeder pig production operation refers to the production and sale of immature pigs from about 30 to 60 pounds of body weight (Ensminger and Parker, 1984). These pigs will then be shipped to other farms for finishing. Feeder pig finishing refers to production systems where these young pigs are purchased and fed until they reach market weight, about 200 to 240 pounds (Pond et al., 1991). This is the simplest form of hog production in that it involves the least amount of both equipment and management ability; however, it requires large sums of operating capital, especially when financial risk is high. Another type of production system is the farrow-to-finish operation, which produces pigs and carries them to market weight (Ensminger and Parker, 1984).

The main requirements for a successful feeder pig finishing system are skills in buying and selling, adequate housing and available supply of feed and water at all times, control of diseases, especially external and internal parasites, proper treatment of sick pigs, vaccination at the time of arrival, and ability to

withstand periods of financial losses or to ensure against such losses (Ensminger and Parker, 1984). If these requirements are met, the optimum weight gain schedule for a successful operation is as follow:

Initial Weight	Days to 220 lb
40 lb	110 d
50 lb	105 d
70 lb	90 d
100 lb	70 d.

This averages out to be about 1.6 pounds a day. However, this is dependent upon management and the weather conditions (Ensminger and Parker, 1984).

The purpose of this study was to determine if finishing pigs were becoming infected with Toxoplasma gondii during their stay in the finishing house. In order to accomplish this, the presence of T. gondii antibody in the finishing pigs was assessed at the time of arrival and at the time of slaughter. Because finishing pigs go directly to slaughter this study is relevant from a public health point of view as an estimation of T. gondii infection of pork products found in the marketplace.

Materials and Methods:

Immature pigs (30 to 60 pounds) were purchased from various farms within the state of Tennessee. To increase the sample size and, therefore, the reliability of this

study, two separate shipments of pigs were studied. The first shipment was purchased at a hog show in March of 1991 and slaughtered in September of 1991. The second shipment was purchased from farrow-to-finish farms in July of 1992 and slaughtered in December of 1992.

These pigs were finished at the University of Tennessee Plateau Experiment Station, Crossville, Tennessee. This finishing operation was entirely indoors, and the farm manager did not allow cats into the facility. Chemical methods were used for rodent control. The pigs were kept in groups of 6, in 6 X 10 foot pens. They were fed a 16% protein, corn-soybean diet fortified with trace minerals and vitamins. Feed and water were provided ad libitum in top-loaded feeders and by automatic watering spouts, respectively. Temperature-regulated, automatic, water sprinklers were used for cooling purposes.

All pigs were bled and tested for T. gondii via modified agglutination test (MAT) upon arrival and again at slaughter, when they had reached approximately 240 lb body weight. These data, pre and post finishing, were used to estimate the seroprevalence of T. gondii in this group of finishing pigs and to discover if these pigs were acquiring the infection during their stay at the finishing operation.

McNemar's test (Agresti, 1990) was used to interpret the significance of the difference between the seroprevalences of the pre and post finishing samples.

McNemar's test is similar to the Z test and produces a Z value as calculated below:

$$Z = \frac{n12 - n21}{(n12 + n21)^{1/2}}$$

n12 = number of positive results at the first bleeding that became negative at the second bleeding

n21 = number of negative results at the first bleeding that became positive at the second bleeding

If Z exceeds the critical Z-value [Z (X, X, 0.05) = 1.96], then Z is significant.

Results:

In 1991, 150 piglets were sampled initially when they arrived at the Crossville finishing operation (Appendix G). In this group, there were 4 (2.7%) positive piglets, 3 (2%) of which had a titer of 32 and 1 (0.7%) of which had a titer of 64. Serum samples from all pigs were negative at the time of slaughter (Table III.1) (Appendix E).

In 1992, 150 piglets were also sampled (Appendix G). Three of them had died by the time of slaughter. In this group initially, there were also 4 (2.7%) positive piglets, 2 (1.3%) of which had a titer of 32, 1 (0.7%) of which had a titer of 8192 and another (0.7%) which had a titer of 2048. At the time of slaughter, the 2 that had titers of 32 had become seronegative, and the piglet with a titer of

8192 had remained the same. However, the piglet with a titer of 2048 had a titer of 512 at the time of slaughter (Table III.1).

Using McNemar's test (Agresti, 1990), which is similar to the Z test, to interpret the 1991 data in which 4 piglets were initially positive and subsequently seronegative at the time of slaughter:

$$Z = \frac{4 - 0}{(4)^{1/2}} = 2$$

Since 2 is greater than 1.96 which is taken from Z table ($P<0.05$), there is a significant reduction in the percentage of positive animals at the second bleeding in 1991. Using the same formula to interpret the 1992 data,:

$$Z = \frac{2 - 0}{(2)^{1/2}} = 1.4$$

This value is less than 1.96 ($P<0.05$), therefore, there was a reduction in the percentage of positives at the second bleeding, but the diffference was not signifcant.

Discussion:

We sampled 150 piglets in 1991 at 3 months of age, 4 of which were positive. These 4 were seronegative 6 months later when they were slaughtered. The other 146 pigs remained negative at the time of slaughter. In the 1992 study, 2 piglets had high titers initially and remained

positive at slaughter 3 months later; 148 piglets were negative.

The presence of T. gondii antibody in the fetal body fluid (Dubey et al., 1990a) and colostrum (Dubey et al., 1989) has been shown. It is likely that the antibody in the serum of the piglets that were positive initially but negative at slaughter 3 months later was due to passive transfer of maternal antibody via colostrum. As the animals aged, the maternal antibodies were eliminated.

The titers of the 2 piglets that remained positive until slaughter were possibily the result of active infection from the environment which occurred during the 6-month, finishing period. The persistence of maternal antibodies throughout the finishing period also cannot be ruled out, though the titer characteristically should have disappeared by the end of the finishing period as it had in the majority of the intially seropositive piglets. This can be interpreted as evidence of T. gondii infection prior to arrival at the finishing farm. Infection may have occurred transplacentally, via the sow's colostrum (Cole et al., 1954), or from the postnatal environment. The possibility of infection prior to arrival seems unlikely because active T. gondii infection in young piglets is often severe and ultimately fatal (Dubey, 1990a).
It would be a worthwhile study to infect pregnant sows at different stages of gestation and observe the effects of

the T. gondii on the current and subsequent pregnancies.

The results indicated that the majority of pork products from this indoor operation were not infected with T. gondii, because the majority of finished, market weight pigs in our study were seronegative. According to the United States Department of Agriculture (1992), 94.80% of the pork products sold in the marketplace are derived from finishing pig, i.e. six months of age, carcasses. The low seroprevalence of T. gondii in finishing stage pigs relative to that reported in sows (Dubey, 1990a) may be due to their short lifespan and limited opportunities for exposure. At the Crossville finishing operation, factors that may have contributed to the low seroprevalence include the cleanliness and newness of the facility, as well as the management's efforts to keep cats out of the buildings and to control the resident rodent population. These conditions may not be met at other finishing operations in Tennessee or in other parts of the country. If not, the infection rate of T. gondii in the pigs could be higher.

Chapter IV

Seroprevalence of *Toxoplasma gondii* in Different Age Groups of Swine Within Three Management Systems

Introduction:

Swine management is the art of caring for, handling and controlling swine. Proper management can be less labor intensive and more beneficial. Available land, capital, labor, feed and the personal preference of the producer can influence the type of management utilized (Ensminger and Parker, 1984).

There are several different management systems. The total confinement system was started in 1955 and has since become increasingly popular. During the 1960s, about 3 to 5% of the market hogs in the U.S. were produced in confinement (Ensminger and Parker, 1984). During the 1980s, 81% of the U.S. swine producers confined sows at farrowing time, 66.2% provided confinement for nurseries, 70.5% confined growing-finishing pigs, but only 24.3% confined the sow herd year round (Ensminger and Parker, 1984).

Some of the factors that make confinement production more advantageous than pasture production follow:

1) it is less labor intensive because of automatic feeding and watering,

2) it is easier to handle the pigs for vaccination,

castration, worming and loading for market,

3) it frees up land so that corn or soybeans can be grown, which may be more profitable,

4) the hogs reach market weight at an earlier age, and,

5) the hogs have less exposure to infectious diseases.

Conversely, pasture production may be favored because:

1) the cost of building and equipment is lower,

2) the cost of feed is lower,

3) it is more flexible than confinement programs, where renters are involved,

4) there is less stress and boredom for the pigs, leading to fewer cases of tail biting, and

5) it does not require high levels of skill and management.

Many farmers can combine pasture and confinement production, using pasture for breeding purposes and confinement production for the growing-finishing pigs.

The purpose of this study was to compare the seroprevalence of Toxoplasma gondii in different age groups of pigs maintained in three types of management systems at the Ames Plantation, an Agricultural Experiment Station of the University of Tennessee (UT). The Ames Plantation provided an excellent opportunity to study the relationship between management practices and exposure to T. gondii.

The results of the study were used to investigate the stage of the production cycle in which swine acquire T. gondii infection and how the seroprevalence varies among the production systems.

Materials and Methods:

For Experiment 1, the seroprevalence of T. gondii in different age groups of swine within three management systems was evaluated. The tested hypothesis was that the distribution of positive animals in all age groups in the population is independent of the management system in which they were raised.

The swine were all housed at the Ames Plantation, which has three mutually exclusive systems of swine management:

1) total pasturing of swine from farrowing to finishing (Pasture),

2) partial confinement of swine which includes breeding of sows on pasture, farrowing indoors and finishing of pigs to market weight on pasture (Partial Confinement), and

3) total confinement of swine from farrowing to finishing (Total Confinement).

Three hundred-ninety, 470, and 630 total pigs were managed within Pasture, Partial Confinement, and Total Confinement, respectively. The large and varying number of

pigs involved in this seroprevalence study made sampling all pigs unfeasible, so an appropriate sample size was calculated for each age group of pigs within each management system.

The age groups used in these calculations and for seroprevalence testing were defined as:

1) piglets 21-90 days of age,

2) finishing pigs 90-150 days of age (about 90 Kg), and

3) breeding sows.

Knowing the total size of each swine population, a 95% Confidence Interval Table (Appendix H) provided in the Livestock Disease Surveys Manual 1982 (Cannon and Roe, 1982) was used to determine an appropriate sample size and confidence limit for T. gondii antibody testing. For example, Pasture had a total of 390 pigs. A sample of 124 was used to give a 95% confidence of detecting the presence of T. gondii antibody if present at a prevalence of 2% or greater. A formula adopted from the same manual was used to calculate the sample size for each age group of pigs according to its proportion of the total population.

The calculations to determine sample size for seroprevalence testing of each age group in Pasture are as follows:

	Number in herd		Sample size required	Actual No. sampled
Piglets	170	170 X 124: 390 =	54	55
Grow/finish	170	170 X 124: 390 =	54	55
Sows	50	50 X 124: 390 =	16	17
Total	390		124	127

The same principles were applied for Partial Confinement.

	Number in herd		Sample size required	Actual No. sampled
Piglets	200	200 X 129 : 470 =	55	55
Grow/finish	200	200 X 129 : 470 =	55	55
Sows	70	70 X 129 : 470 =	19	32
Total	470		129	142

The same principles were applied for Total Confinement.

	Number in herd		Sample size required	Actual No. sampled
Piglets	255	255 X 134 : 630 =	55	55
Grow/finish	255	255 X 134 : 630 =	55	55
Sows	120	120 X 134 : 630 =	25	52
Total	630		135	162

After the sample sizes were determined, the appropriate number of pigs was bled on November 4th and 5th of 1991. The grow/finish pigs were bled again in February of 1992 at the time of slaughter. The modified agglutination test (MAT) was used to evaluate the T. gondii IgG titers of the serum samples.

For the analysis of the results of the seroprevalence testing, Chi square was used. Chi square is a test that reflects the magnitude of the difference between observed and expected frequencies (Devore and Peck, 1986). The advantage of this test is that it makes so few assumptions about the underlying population (Wonnacott and Wonnacott, 1985). When the Chi square value is large compared to the value referenced in the Chi square Critical Points Table, then the observed frequencies cannot be explained by chance alone (Zar, 1984; Devore and Peck, 1986). The tested hypothesis was that the distribution of positive animals in all age groups in the population is independent of the management system in which they were raised.

A second experiment, Experiment 2, was performed to estimate the seroprevalence of T. gondii antibody in presuckled piglets. The sera of 58 newborn piglets that had not yet suckled, the sera of these piglets' sows, and the sows' colostrum were tested with MAT. These samples were obtained during 1989 from a dissertation project

entitled "Colostrum Supplementation and Intestinal Transmission of Macromolecules in Neonatal Swine" by Jerry A. McClain (August, 1990).

Results:

Table IV.1 (Appendix E) presents the results of seroprevalence testing that took place at Ames Plantation (Appendix I). The Grow/finishII group represents a second attempt at sampling the initial group of piglets prior to slaughter. We were only successful in collecting a little more than one-half of their blood samples at the second bleeding. This was because of death or early shipment of finished pigs to a slaughter house. Using McNemar's test, which is similar to the Z test (Agresti, 1990):

$$Z = \frac{7 - 0}{(7)^{1/2}} = 2.64 \ .$$

Since Z = 2.64 is greater than 1.96 (from Z table), there was a significant reduction ($P<0.05$) in the percentage of positive animals at the second bleeding. Seven of the 17 seropositive piglets were seronegative at the time of second bleeding, and 10 remained positive. From a biological point of view, these 17 piglets that had antibody to T. gondii probably received it from their sows's colostrum.

Using Chi square to compare the seroprevalence of T.

gondii antibody in piglets within each management system, we determined that there was a significant difference between Partial Confinement and the other two systems (Chi square = 39.49, P<0.001) (Table IV.1) (Appendix E). When comparing the sows within each management system, a signficant difference between sows of Partial Confinement and the other systems was also found (Chi square = 14.53, P<0.001). The piglets and sows in Partial Confinement have a higher seroprevalence than those in Pasture or Total Confinement.

In Partial Confinement, cats were observed in farrowing pens by the author at the time of bleeding. Since Partial Confinement is significantly different from the other systems, the age groups within this system were compared to each other. Within Partial Confinement, the piglet and sow groups individually and in combination had a higher seroprevalence than the grow/finish group (31 and 28%, respectively, compared to 0%). We also compared the combination of grow/finishing pigs and sows to piglets and found a significantly higher seroprevalence in the piglets of 31% (Chi square = 9.52, P<0.002)(Table IV.2, Appendix E). The grow/finish pig and sow data were combined because these age groups of pigs are older than 6 months of age and will eventually be sent to market for public consumption. On the other hand, the piglets were from 1 week to a month of age and their serologic status may subsequently change.

No significant difference was found between the age groups within Pasture or Total Confinement. In Experiment 2, T. gondii IgG was demonstrated in the sows' sera and colostrum by MAT.

The sera of 3 (37.5%) sows had a titer of 2048, 2 (25%) had a titer of 1024 and 3 (37.5%) were seronegative. Colostrum of 5 (50.0%) sows had a titer of 32, 4 (40.0%) sows had a titer of 64, and 1 (10.0%) sow had a titer of 16. The newborn piglets of all sows prior to suckling were seronegative.

Discussion:

In Pasture, all of the pigs at all ages were negative for T. gondii antibody. The pigs were confined with electric fencing. Perhaps the reason for the seronegativity was that cats cannot get into the pasture. In Partial Confinement, the seroprevalence of T. gondii was higher. The pigs were associated with free-roaming cats in their barn. This may have been the cause of the higher seroprevalence of the pigs in Partial Confinement compared to Pasture and Total Confinement. Total Confinement was confined by buildings so that no cats could enter, which may have been responsible for the low seroprevalence within this system.

Smith et al. (1992) showed a low seroprevalence in

rodents on Iowa swine farms and could not associate the seroprevalence of the rodents with the seroprevalence of the swine. Although it is unlikely that the electric fencing in Pasture kept rodents off the pasture, this finding suggests that they are unlikely to be involved in transmission.

Within Partial Confinement, the high seroprevalence in the piglet group may be due to the presence of maternal antibodies obtained from colostrum. An alternative cause could be an active immune response to T. gondii transmitted via colostrum (Cole et al., 1954) or the environment. The high seroprevalence in sows was probably the result of active infections acquired from the environment. This age group may live for years in this management system and have many opportunities for exposure. Comparing the piglets and grow/finishII within Partial Confinement by Z test, the result shows a significant reduction in the percentage of positive animals at the second bleeding. The 17 piglets in Partial Confinement that had antibody to T. gondii initially and were seronegative at the second bleeding probably received the antibody from their sows's colostrum. The 10 that remained seropositive may have become infected with T. gondii at an early age either as a piglet or during the grow/finishing stage and developed an active immune response. Their initial titers may have also represented maternal antibody or a developing titer from infection.

Dubey et al. (1990a) showed that experimental sows infected with T. gondii either aborted their piglets or the piglets died shortly after birth. Therefore, the chance that these piglets became infected during gestation is unlikely.

The presence of T. gondii antibody was demonstrated in the sows's colostrum and serum. Newborn presuckling piglets were all negative for T. gondii antibody. Therefore, passive transfer of the antibody across the placental membrane did not occur. The placenta of swine is epitheliochorial. This type of placentation completely prevents the transplacental passage of immunoglobulin molecules (Tizard, 1987).

CHAPTER V

Seroprevalence of *Toxoplasma gondii* in Swine in Tennessee

Introduction:

The seroprevalence of *T. gondii* in swine has been studied in all parts of the world. In the U.S., seroprevalence of *T. gondii* in swine was estimated to be 23.9% nationwide (Dubey et al., 1991). Other researchers throughout the country estimated the seroprevalence of *T. gondii* in swine in their own states and found values ranging from 6% to 67% (McCulloch et al., 1964; Burridge et al., 1979; Garcia et al., 1979; Ferguson and Heidt, 1981; Dubey et al., 1986; Dubey et al., 1992). In the most recent study, Zimmerman and coworkers' in Iowa found that 15.9% of pigs were seropositive in 1990 and 14.5% were seropositive in 1992 (Zimmerman et al., 1990; Smith et al., 1992).

The purpose of this study was to estimate the seroprevalence of *T. gondii* in sows on swine farms in the Tennessee. An additional goal was to compare the seroprevalence in swine in different regions of the state.

Materials and Methods:

The samples for this study were obtained from the Tennessee (Tennessee) State Department of Agriculture located in Nashville, Tennessee. Each year the Tennessee

State Department of Agriculture (TSDA) takes blood samples from swine herds in Tennessee specifically for detection of pseudorabies antibody. These samples were collected between January and April of each year. During 1991 and 1992, a portion of each sample was sent to the University of Tennessee parasitology laboratory to be used for this study. The samples were received on a one day a week basis.

During 1991, the TSDA sampled sows from 3,870 different farms. Samples received for this study were from 254 different farms. The sampling plan used by the TSDA was as follows: herds with 10 or less sows, all sows were sampled; herds with 11-29 sows, 10 sows were sampled; herds with 30 or greater than 30 sows, 30% or 30 sows were sampled, which ever was smaller. All sows were bled when a farm was sampled for the first time.

Each serum sample received included information about the county of origin, herd size, herd owner, and veterinarian in charge of bleeding. The modified agglutination test (MAT) was used (explained in chapter II) to test for T. gondii antibodies (results of each farm are in Appendix J). The MAT, although the most expensive, also is the most sensitive test available and has been shown to detect antibodies in the early stages of infection (Patton et al., 1991). Chi square was used for the analysis of

these data.

The state of Tennessee is generally divided into three geographic regions: East, Middle, and West. The guidelines that have been used to separate the three regions are as follow:

1) the separation between East and Middle is the Cumberland Plateau, and

2) the separation between Middle and West is the Tennessee River.

Immunoperoxidase was used to attempt to confirm the presence of tachyzoites and/or bradyzoites in post-mortem tissues of Tennesse pigs suspected of being infected with T. gondii. Three, dead pigs suspected of being infected with T. gondii were submitted to the necropsy service at the University of Tennessee. Their tissues were subsequently examined by immunoperoxidase. Histologic sections of another suspect pig from the TSDA were also examined for presence of T. gondii by immunoperoxidase technique. The StrAvigen MultiLink kit (purchased from BioGenex Laboratories, San Ramon, California) was used for this procedure.

Results:

During 1991, we received 2000 samples which were collected from 254 farms. Of these 2000 samples, 720 samples (36%) were positive and 1280 samples (64%) were

negative. Of the 254 farms, 188 farms (74%) had at least one positive sample (Table V.1) (Appendix E). The crude seroprevalence in 1991 was 36.0%, and the true seroprevalence in 1991 was 42.19% (Table V.2). Crude prevalence is equal to total number of positive sows divided by all of the sows reported as a percent. True prevalence is equal to the sum of the prevalences of individual farms divided by the total number of farms reported as a percent.

During 1992, we received 1841 samples from 89 farms and 410 samples (22.3%) were positive (Table V.1). Fifty-seven farms (64%) had at least one positive sample. The crude prevalence for 1992 was 22.27%, and the true prevalence was 30.45% (Table V.2). The total crude seroprevalence for the years 1991 and 1992 was 29.42%. The true seroprevalence for the years 1991 and 1992 was 36.32%.

The prevalence of different titers for each year is shown in the Table V.3. For example, in 1991, 7.15% or 143 of the sows sampled had a titer of 1:32, and, in 1992, 7.02% or 133 of the sows sampled had a titer of 1:32.

Of the 95 counties in the State of Tennessee, we have received samples from 66 counties. Eighty-four farms (24%) sampled were from the western counties of Tennessee, 236 farms (69%) sampled were from the central counties, and 23 farms (7%) sampled were from the eastern counties in total

for both years. In 1991, there were 625 samples (31%) from Western, 1253 samples (63%) from Middle, and 122 samples (6%) from Eastern counties. In 1992, there was a total of 1089 samples (59%) from Western, 723 (39%) from Middle, and 29 samples (2%) from Eastern counties. Table V.4 presents the information about these three regions.

Using Chi square, there was a significant difference between the three regions of the State of Tennessee regarding the true prevalence and the crude prevalence ($P<0.04$ and $P<0.003$, respectively). There was no significant difference when comparing two regions at a time except when comparing Central and Eastern counties for true prevalence and for crude prevalence ($P<0.015$ and $P<0.007$, respectively).

There was no significant difference between the years 1991 and 1992 in the seroprevalence of the state of Tennessee overall. Also, comparing the seroprevalence of each region between the two years, there was no significant difference.

The largest number of samples was received from Fayette county which is located in Middle Tennessee. However, the largest number of farms were sampled in Grundy county which is also located in Middle Tennessee. Twenty-three farms provided serum samples in both 1991 and 1992, potentially providing an opportunity for a longitudinal study of the rate of infection in this subset of sows.

Unfortunately, none of the individual sows were sampled in both years.

The results of the immunohistochemistry of the tissues were negative for the presence of T. gondii. Some of the histopathological findings of the three UT cases at necropsy included: pneumonias, peritonitis, polyarthritis, enterocolitis, valvular endocarditis and lymphoid atrophy.

Discussion:

In this study, the immediate objective was to test serum samples from the TSDA for T. gondii antibody to approximate the seroprevalence of this parasite in swine in the state of Tennessee. The true seroprevalence was 42.19% in 1991 and 30.45% in 1992. This parasite was found in all three regions of Tennessee-East, Middle, and West. The regional true seroprevalences in a given year ranged from 27.46% to 50.0%. The only significant difference in seroprevalences was found between the Central and Eastern regions. The prevalence of antibody decreased from 1991 to 1992 based on samples from Western and Middle Tennessee, however, these differences were not significant. The seroprevalence increased from 1991 to 1992 in the Eastern counties, but this difference was also not significant.

Serologic surveys have indicated that the seroprevalence of T. gondii in humans and other animals

varies from place to place (Tizard et al., 1976; Garcia et al., 1979; Nene, 1986). Dubey (1990) estimated the prevalence of T. gondii in Tennessee to be 30% (57 positive out of 178 samples collected). In a national study, he estimated the seroprevalence of T. gondii in selected Southern States (North Carolina, Virginia, Tennessee, Alabama, and Mississippi) combined to be 27.4% (Dubey et al., 1991). The samples for both studies were originated as part of a national survey for pseudorabies. This estimations are consistent with our findings of the current study of a true seroprevalence of 42.19% in 1991 and 30.45% in 1992.

There are more swine farms in the Western and Middle portions of the state than in the Eastern portion of the state (Tennessee Agriculture, 1992). Obion, Weakley, Gibson, Henderson, Fayette and McNairy counties of Western Tennessee each produce over 25,000 head per year. Only Franklin county in Middle Tennessee produces over 25,000 head per year. No counties in Eastern Tennessee have this amount of production most likely because the topography is not conducive to grain farming. Twenty of the 30 counties in Eastern Tennessee produce under 2,000 head per year. These smaller farms in the Eastern counties tended to be unsophisticated. Therefore, the majority of the swine population is located in 21 counties in West and Middle Tennessee (Figure 2) (Appendix F).

In a study from south-eastern Norway, it was estimated that the seroprevalence of T. gondii in swine was higher in coastal land than in the inland zone (Hellesnes et al., 1978). They stated that, in general, the seroprevalence of T. gondii in humans and in animals is higher in areas with a warm and moist climate than those with a cold and dry climate. In a serologic survey of T. gondii in wild animals in Florida, there was no difference between the four geographic regions surveyed (Burridge et al., 1979).

The economic burden of T. gondii infection on swine producers has not been clearly estimated (Roberts et al., 1989). The importance of this parasite and its transmission to humans needs further study. Humans can become infected with toxoplasmosis by ingestion of T. gondii cysts in undercooked meat of intermediate hosts. In a study in China, it was estimated that the seroprevalence of T. gondii in people of mainland China was lower than that of people in Europe and America (Shen et al., 1990). They speculated that this is due to the Chinese habit of cooking meat well before consumption and the rarity of cats in households. A high prevalence of toxoplasmosis in animals indicates an occupational hazard to workers in abattoirs and to consumers at home in their kitchens. In Japan, a rigid surveillance of animals at slaughter houses had reduced the seroprevalence of infection from 19.2% in

1964-65 to 1.9% in 1979 (Nene, 1986). According to the World Health Organization (1976), in developing countries with low standards of hygiene and sanitation, raw, unprocessed meat is a more frequent source of infection than processed meat.

The similarity of other organisms to T. gondii demands a specific immunohistochemical staining procedure or electron microscopy to distinguish among them in histologic sections. In a study in Japan, T. gondii cysts were found in the cerebral cortex of 5 dead piglets (Haritani et al., 1988). The avidin-biotin-complex immunoperoxidase technique was used to definitively identify tachyzoites and bradyzoites in microscopic lesions. A number of tachyzoites were observed in glial nodules. In the lung, many tachyzoites were detected in the epithelial cells of the bronchioles, the alveolar epithelium, and infiltrating macrophages. In our study, tissues were obtained from 4 suspect pigs. None of the tissue samples showed any presence of antigen.

CHAPTER VI

Comparison of the Seroprevalence of *Toxoplasma gondii* in Different Management Systems in Tennessee

Introduction:

Toxoplasmosis is a zoonosis found throughout the world. *Toxoplasma gondii* is found in most mammals, birds, and a few species of reptiles (Dubey, 1986). Serologic surveys to date present the prevalence of toxoplasmosis among different species in different parts of the world (Dubey et al., 1976; Tizard et al., 1976; Hellesnes et al., 1978; Dubey, 1987; Dubey and Beattie, 1988; Dubey, 1990; Shen et al., 1990). There is an enormous amount of literature available regarding seroprevalence surveys of farm animals. However, only a few publications investigate the relationship between seroprevalence and transmission of *T. gondii* related to husbandry (Hellesnes et al., 1978; Zimmerman et al., 1990; Weigel et al., 1992). The only such study from Iowa found that swine herds with less than 100 breeding head were more likely to be infected than herds with more than 100 breeding head (Zimmerman et al., 1990). The same study from Iowa suggested that sows raised in a confined environment had a lower seroprevalence of *T. gondii*.

The purpose of our survey was to better understand the distribution patterns of the seroprevalence of this

parasite in Tennessee, and to correlate the distribution patterns/prevalence with the husbandry on the farms. We specifically examined: the presence of cats, the type of management system, herd size, and the rodent control methods used on the farm.

Materials and Methods:

A survey form was designed and sent to the farmers that participated in blood sampling for pseudorabies as mandated by TSDA. A copy of this survey is attached (Appendix K). The first copy of this survey was sent to the farmers on June 22, 1992, and a second copy was sent to the farmers that did not respond to the first survey on August 1, 1992. Along with the survey, a copy of a consent form confirming voluntary participation in the study was included (Appendices L and M). In the consent form we also stated that the test results and suggestions of how to control this parasite would be mailed to the farmer after completion of the study.

In the analysis of these data, we used Chi-square test and odds-ratio to test the relationship of the presence of cats, different types of management, farm size, and rodent control methods on the seroprevalence of T. gondii. Odds ratio is a measure of the strength of the association between exposure to a risk factor and disease (Fletcher et

al., 1988). A risk factor is a factor that is associated with an increased risk of exposure to an etiologic agent (Fletcher et al., 1988). Odds ratio is calculated from data summarized in a 2 X 2 table as exemplified below:

		Disease: yes	Disease: no	Total
Exposure	yes	A	B	A + B
	no	C	D	C + D
	Total	A + C	B + D	

Odds ratio = AD/BC

Results:

Review and Analysis of the Mail Survey Method - We received 117 responses from 303 surveys that were mailed to the farmers. Of these 117 responses, 10 of them responded that they are no longer swine producers (noneligible). Also, there were 29 surveys that were returned as address not found (nonreachable). Using the following formula (Dillman, 1978), our response rate was 40%.

$$\text{Response rate} = \frac{\text{No. of returned}}{\text{No. in sample - (noneligible + nonreachable)}} \times 100$$

$$\text{Response rate} = \frac{107}{303 - (10 + 29)} \times 100 = 40.00\%$$

There is a tremendous amount of information in the literature about survey responses. Most of this research has been done in the areas of psychology, sociology, marketing and advertising. With this substantial amount of information on the mail questionnaire available, much of it experimental, it seems surprising that more has not been learned. There are even some researchers who believe that more research needs to be done in this area (Hawkins, 1975). Most findings are inconsistent for many of the survey techniques commonly recommended in research texts (Linsky, 1975).

Dillman suggested the theory of Total Design Method (TDM) (Crosby et al., 1989). TDM consists of two parts. The first part is to identify each aspect of the survey process that may affect the quality or quantity of the response based on the format of the survey. The second part is to organize the survey efforts so that the design intention is carried out in complete detail. Using TDM, there are three things that must be done to maximize survey response:

1) minimize the cost of responding,

2) maximize the rewards for doing so, and

3) establish trust that those rewards will be delivered. Putting these principles into practice, in our study we provided stamped envelopes labeled with the return

address. In our cover letter, we told the farmers that we will let them know the results of the survey and give recommendations on how this parasite may be affecting their herds' productivity. The farmers were also promised anonymity and prompt delivery of the results as soon as they were available.

Eichner and Habermehl (1981) suggested the number of variables on a survey has no affect on the response rate. However, Heberlein and Baumgartner (1981) think that the number of variables and length of the questions in a survey has an effect on the response rate. Fox et al. (1988) analysed the effect of ten different factors on the response rate. They concluded that university sponsorship, prenotification by letter, stamped return postage, postcard follow-up, first-class outgoing postage, and questionnaire color all improved the response rate on average. Of the above mentioned factors, university sponsorship, stamped returned postage and first-class outgoing postage were utilized by our study. The most effective factor was university sponsorship in Fox's findings. Heberlein and Baumgartner (1978) found little or no effect regarding the prenotification letter.

It may be difficult to reach some respondents such as farmers, soldiers, or executives, because some may have a low educational level, are often travelling, or are too

busy (Watson, 1965; Kanuk and Berenson, 1975). Within recent years, many researchers have noted a significant rise in the nonrespondent rate for various studies utilizing the survey research method (American Statistical Association Conference, 1974). Brambilla and McKinlay (1975) have suggested that a mixed approach to reaching respondents may be better than a mail-only approach. In one health study, they used a combination of mail questionnaires and follow-up telephone interviews of nonrespondents that seemed to decrease their nonrespondent bias; each survey method had a greater appeal for members of different socioeconomic classes interviewed. In this study, the phone interveiws on average lasted for 15 minutes (Brambilla and McKinlay, 1975). If a similar nonrespondent follow-up procedure had been used with the farmers surveyed throughout the state of Tennessee, the cost would have made the survey not feasible. One should not forget the advantages of a mail-only approach. Mail surveys are more valid than either telephone or personal interviews, because the respondents have more time to check their records or consult with other members of the family. They also permit a more leisurely and thoughtful reply (Nuckols, 1964). Mail surveys are relatively cheaper, geographically flexible, and can reach a widely dispersed sample simultaneously without the attendent problems of interview access or the possible distortions of time lag

(Kanuk and Berenson, 1975). The biggest disadvantage of mail surveys is the low response rate (Kanuk and Berenson, 1975). However, Kanuk and Berenson (1975) suggested that findings from nonrespondents to the questionnaire do not differ in significant ways from the findings of those who do respond. To verify this in our case, the respondent and nonrespondent groups were compared to each other using available parameters.

There were 156 farmers that did not respond (nonrespondents) to the survey. Of these 156 farmers, 47 farms were negative for T. gondii antibody and 109 farms had at least one positive sample in their herd. Using chi-square, there was no significant difference in T. gondii seroprevalence between these farmers and the farms of those who responded to the survey. Table VI.1 represents the results of these data (Appendix E). Also, the region of Tennessee in which the farms were located was compared in regards to whether or not they responded to the survey, and no significant difference was found (Table VI.2).

In conclusion, although response rates have been reported which range from below 20% to 100%, there is still no reliable evidence identifying the factors responsible for this enormous variation. This may be true because of the fact that populations surveyed and the subject matter

of surveys are constantly changing (Kanuk and Berenson, 1975).

Analysis of the Survey Responses - One-hundred and seven surveys were used for the statistical analysis of the farmers' responses to the questions in the survey and their relationship to T. gondii seroprevalence. Fifty-nine farmers responded to the first mailing of the survey; 48 responded to the second mailing.

These 107 surveys represented farms in 49 counties. Thirty-two farms (30%) had no positive samples, and 75 farms had at least 1 positive sample for T. gondii antibody. There was a total of 2001 samples taken from farms that also returned the survey. Of these, 467 were positive samples (22.4%), which we received 1130 positive samples for both years. The overall seroprevalence of the both years was 24.9%.

Table VI.3 indicates that the populations of swine on 107 Tennessee farms surveyed are skewed towards the right, because the medians are smaller than the means (Devore and Peck, 1986). This skewness towards the right indicates that the majority of the swine population on these 107 farms comes from smaller farms.

Table VI.4 summarizes the number and percentage of farms with a given inventory and market class of swine and the number of head of swine within that particular class present on the farm at the time of the survey. For

example, there were only two farms that did not have any sows or gilts (2/107=2% of all farms).

Table VI.5 summarizes the data obtained from the surveyed farms including the type of management used, the presence of cats, breeding practices, and herd size. The risk factors of management practice, presence of cats, and herd size will be further evaluated. There were 92 farms that used continuous breeding while only 15 farms used seasonal breeding. These 15 farms were all small sized farms. In previous surveys (Zimmerman et al., 1990), transmission rates of T. gondii were not affected by season. For the remainder of this analysis, we used sows as our statistical unit and not farms, because there are both positive and negative sows within individual farms. Also, it is the individual sows that are associated with cats, infected rodents and tail biting.

The odds ratio of the presence of cats on the farms in relation to the presence of T. gondii antibody in the sows is 2.6 (Table VI.6). This means that if sows were kept on a farm where they were associated with cats, they were 2.6 times more likely to be seropositive for T. gondii (Metha et al., 1985). Table VI.7 through Table VI.9 show the number of sows associated with cats within different management systems.

There was a significant difference in seropositivity

to T. gondii (P<0.001) between sows on pasture only that were associated with cats and those that were not associated with cats. However, the odds ratio did not show any significant association. Therefore, the presence of cats and the seropositivity of sows on pasture only are dependent upon each other i.e. this finding is not due to random chance, but the strength of this relationship fails to support causality.

There was a significant difference in seropositivity to T. gondii (P<0.001) between sows in partial confinement that were associated with cats and those that were not associated with cats. The odds ratio is 2.3, i.e. sows in partial confinement that are associated with cats have a 2.3 times greater chance of being positive for T. gondii. There was no significant difference in seropositivity to T. gondii between sows in total confinement that are associated with cats and those that are not associated with cats.

Table VI.10 states the odds ratio of T. gondii infection (seropositivity) in sows when comparing types of management systems to one another exclusive of cats. There was a significant relationship (P<0.001) between the different types of management and the number of seropositive animals on the farms. The total confinement system had lower numbers of positive animals than the partial confinement and total pasture systems.

Calculation of the odds ratio allows us to state that sows kept on pasture are 15.21 times more likely to be seropositive for T. gondii than those in total confinement. Also, sows in partial confinement are 29.23 times more likely to be seropositive than those in total confinement. However, there is no significant difference between sows kept on pasture versus those in partial confinement, the odds ratio being 0.5.

Table VI.11 compares seroprevalence of T. gondii in sows in different management systems on farms with or without cats. Sows kept outdoors at any time, i.e. sows on pasture and in partial confinement, compared to sows kept entirely indoors, i.e. total confinement, were 23 times more likely to be seropositive for T. gondii. Sows kept outdoors at anytime with no cats on the premises compared to sows kept entirely indoors with cats on the premises are 17 times more likely to be seropositive for T. gondii.

On the survey, farmers were also questioned about their methods of rodent control because ingestion of rodents by swine can serve as a route of transmission. Methods of rodent control were categorized as active if the farmer used chemicals, traps, or destruction of the rodents' habitat and as passive if the farmer used cats, rodent proof containers and other (non-active) methods. Sows on farms that used passive rodent control methods

compared to sows on farms that used active rodent control methods were 1.95 times more likely to be seropositive (Table VI.12).

Farm size was also considered in the analysis of survey responses. Based on State Diagnostic Laboratory sampling procedures, "large farms" were defined as those with 30 or more sows and "small farms" were defined as those with 29 or less sows. Fifteen farms surveyed were "large farms", and 92 were "small farms". Table VI.13 shows the number of sows that were on "small farms" compared to the number of sows on "large farms" in relation to seropositivity for T. gondii.

There was a significant difference between sows on "small farms" versus sows on "large farms" being seropositive for T. gondii. The odds ratio was 4.47, i.e. sows on "small farms" were 4.47 times more likely to be seropositive than sows on "large farms".

Considering farm size, management practices were compared with T. gondii seropositivity and the following results were obtained. On large farms comparing a combination of outdoor to exclusively indoor management methods, the odds ratio was 38.91, i.e. sows on pasture and partial confinement were 38.91 times more likely to be seropositive than sows in total confinement (Table VI.14) In contrast, making the same comparison for small farms, the odds ratio is only 2.37 (Table VI.15). Table VI.16

compares the seropositivity to T. gondii of sows on large farms that are in total confinement to sows on small farms that are in outdoor types of management. The odds ratio of 0.03 was not significant.

From a public health point of view, we asked farmers about the status of their sows that are no longer used for breeding purposes. The categories of culling methods used to evaluate these data included: home slaughter, selling them with finishing hogs, and other methods. Table VI.17 summarizes these results.

Six percent of the sows that were culled were consumed by the farmers themselves, and 93% entered the food chain when they were sold for meat with the finished hogs.

Discussion:

Epidemiology is the study of the health status of populations and its patterns and distributions (Smith, 1991). In the previous chapter (Chapter V), it was estimated that the true seroprevalence of T. gondii on swine farms in Tennessee in 1991 was 42% and in 1992 was 30% (Appendix I). Therefore, one can conclude that infection is occurring. The next step for this research project was to investigate the causes and pattern of distribution of T. gondii infection on swine farms. To accomplish this, we designed a survey and sent it to all of

the farmers (303) from which we had received samples. The response rate was 40%.

The presence of cats on farms was analyzed, and it was concluded that their presence made a significant difference ($P<0.001$) in T. gondii seroprevalence. Sows exposed to cats were 2.6 times more likely to be seropositive than those that were not (Table VI.6). Comparing different types of management practices, it was concluded that sows exposed to the outdoors at any time during their lives were 23 times more likely to be seropositive (Table VI.11). In turn, animals in total confinement are less likely to be seropositive. The magnitude of the odds ratio was 17 when comparing the outdoor sows not associated with cats to the indoor sows associated with cats. This finding emphasizes the importance of environment over that of exposure to cats in T. gondii transmission (Table VI.11). Sows kept outdoors at any time may have an increased risk of exposure to T. gondii regardless of the recognized presence or absence of cats on the premises. Farmers, who in their surveys claimed that cats were not present on the premises, may not have been aware of stray cats, neighbors' cats, and rodents which could be the source of infection.

Smith et al. (1992) trapped rodents on swine farms in Iowa and sent their serum samples to our laboratory for testing for T. gondii antibody by MAT. It was found that rodents are not a major factor in transmission of T. gondii

in Iowa (Smith et al., 1992). In our study, there were no traps placed, however, when we asked farmers about their rodent control programs, sows on farms that used passive rodent control methods were 1.96 times more likely to be seropositive than sows on farms with active rodent control method. The odds ratios for the presence of cats (2.6) and the use of passive rodent control methods (1.96) on the farms were much less than that of outdoor management practices (23). Therefore, they are less important risk factors than management for T. gondii transmission in swine.

In a study from Iowa, the farms that had a total confinement system and 100 or more head of swine had a lower seroprevalence than smaller farms with access to pasture (Zimmerman et al., 1990). Our findings are consistent with this study. Farms that used total confinement and had 30 or more sows had a lower prevalence than farms that used outdoor types of management and had 29 or less sows (Table VI.16). Sows on small farms were 4.47 times more likely to be seropositive than those on large farms (Table VI.13). This finding is probably because outdoor management practices predominate on small farms.

It has been estimated that the prevalence of T. gondii infection in humans worldwide is about 50%, however, the incidence is unknown. In our survey, 6% of culled sows

were being consumed by farmers and their families. It would be of interest to estimate the seroprevalence of T. gondii among the swine producers themselves since they are more likely than the general public to consume the meat of older, potentially infected sows. The older the animals get, in general, the more likely they are to become infected with T. gondii because of the additive effect of more opportunities for exposure. Ninety-three percent of culled sows were sold to market and could potentially serve as a source of infection for people other than the swine producers. Sow carcasses make up 5% of the pork supplied to the marketplace (USDA, 1992), and they are mostly used to make sausage. Also, beef contaminated with pork and sold as beef is a potential source of infection (Georgi and Georgi, 1990; Schantz et al., 1977). It would be a valuable study to estimate the prevalence of T. gondii by immunoperoxidase in pork products sold at retail stores.

This study confirms that presence of cats, type of management, methods of rodent control and farm size are risk factors for swine being seropositive for T. gondii. Cats' feces can contaminate the feed and water supply of swine resulting in direct transmission of T. gondii. Even though some farmers responded that they do not have cats, neighbors' cats and stray cats could still be present on the farm. This is especially true for the farms that keep their sows outdoors, which increases their risk of being

seropositive for T. gondii. Infected rodents may serve as sources of infection for cats, which in turn shed more oocysts, preserving the life cycle of T. gondii. Insects also may be sources of T. gondii infection by acting as transport hosts (Wallace, 1971, 1972; Ruiz et al., 1973). They may transfer oocysts, which stick to their legs, from soil and feces to feed and water or from farm to farm. The larger farms more frequently use total confinement systems and state of the art technology, i.e. automatic feeding and watering, therefore, decreasing the chance of T. gondii infection. The smaller farms more often keep the sows outdoors or partially confined in dilapidated buildings increasing the chance of infection. More studies could be done to evaluate the effects and the cost burden of this parasite on small farms which have a higher seroprevalence.

CHAPTER VII

Conclusion

The estimated seroprevalence of Toxoplasma gondii, a protozoan parasite, in the United States human population is approximately 30%, while in continental Europe it is estimated from 50 to 80% (Weigel et al., 1992a). The common sequela of infection in humans are as follows: 1) death and encephalitis in immunosuppressed patients from the recrudescence of T. gondii bradyzoite cysts, 2) abortion or neurologic disorders in fetuses of women, who become infected for the first time during their pregnancy, however, the subsequent pregnancies are normal, and 3) flu-like symptoms in immunocompetent adults, who usually recover after a week or retintis which causes the blurred vision.

Besides congenital infection, there are two possible routes by which humans can become infected. One route is via accidental ingestion of sporulated oocysts that originated in cat feces, and the other is by ingestion of farm animal commodities such as unpasteurized goat's milk or undercooked meat. It is speculated that the most common source of infection for humans is via pork (Dubey, 1990). The consequences of infection in swine are similar to those in humans. First time infection in pregnant sows may cause stillbirth or abortion. Also, infection in younger animals

may be fatal. Sows that are seropositive for T. gondii are likely to have aborted at one time or another. This propensity, in turn, can cause a loss in dollars and cents to producers especially at large levels of production. Therefore, the economic importance of T. gondii, as well as its public health importance, need to be assessed.

Within the states of the United States, the seroprevalence of T. gondii in swine varies (Weigel et al., 1992b). Florida and Illinois have a low seroprevalence rate (<5%) while states such as Tennessee, Connecticut, and North Dakota have a high seroprevalence rate (>40%) (Weigel et al., 1992b). The high seroprevalence of T. gondii in Tennessee commanded an epidemiologic study. To fulfill this task, we needed a serologic test that could give us an accurate measure of T. gondii antibody in serum samples.

We used a murine model to test the reliability of the modified agglutination test (MAT) (Remington and Desmonts, 1980). In our model, we could detect both IgM and IgG at 7 days post-infection (P.I.). IgM titers peaked at 11 days P.I. and almost disappeared at 17 days P.I. The IgG titers increased steadily from 9 days until 17 days P.I. and remained high until the end of the study. The pattern of the IgG titers was classical for T. gondii infection. The presence of bradyzoites in the brains of the mice post-mortem confirmed the infection and the

serological results. Therefore, knowing that MAT would give us accurate and reliable results, we used it for estimating the seroprevalence of T. gondii in swine in the state of Tennessee.

We received 3,841 sow serum samples from 343 farms during 1991-92 from the State Diagnostic Laboratory in Nashville, Tennessee (TSDA). These serum samples were obtained from a national, pseudorabies testing project. A total of 1,130 sow serum samples in 1991 and 1992 were positive for T. gondii by MAT (29.41%). In the summer of 1992, we mailed 303 questionnaires to the swine farmers from which we received serum samples. These surveys asked farmers about their management practices, their production, the presence of cats, methods of rodent control, herd health, and method of culling old sows.

We received 107 responses to our survey. From the analysis of these responses, it was concluded that sows in total confinement are less likely to be seropositive for T. gondii. In fact, sows that are kept outdoors at any time during their lives, i.e. on total pasture or in partial confinement systems, are 23 times more likely to be seropositive for T. gondii. Even the sows that were not associated with cats and were kept outside had a 17 times greater chance of becoming infected versus the sows that were associated with cats and were kept in total confinement. Therefore, we are convinced that exposing

sows to the outside is the most important risk factor for becoming infected with T. gondii.

On the other hand, we found that the presence of rodents was a relatively less important, risk factor. In our study, we compared the effects of active and passive methods of rodent control on the seroprevalence of T. gondii. Sows on farms that used passive methods of rodent control were two times more likely to be seropositive for T. gondii.

Sows on small farms (those with less than 30 sows) are four times more likely to be seropositive than sows on large farms (those with 30 or greater sows). In our results, most of the small farms had outdoor operations. Therefore, the two risk factors of farm size and outdoor management practices could be closely related.

From the survey study, the seroprevalence was higher among sows that were kept partially or totally on pasture. On a University of Tennessee farm (Ames Plantation), the sows on total pasture were negative. This is possibly due to electric fencing which keeps out cats and wildlife. In future studies, it would be of interest to ask farmers on a survey whether or not their pastured sows are confined by electric fencing. However, the results from the Ames Plantation are from only one farm and cannot be applied to the results of the survey that were mailed for this

study.

In our study, we tested a total of 300 finishing pigs (Crossville Experiment Station) by MAT in 1991-92 and found a low seroprevalence of 0.6%. The finishing pigs from the Ames Plantation that were exposed to outside had a seroprevalence of 5.8% for T. gondii antibody. According to the United States Department of Agriculture (1992), 94.80% of the pork products sold in the marketplace are derived from finishing pigs, i.e. six months of age carcasses. The low seroprevalence to T. gondii in finishing stage pigs relative to that found in sows may be due to their short life-span and limited opportunities for exposure. The infected animals that enter the food chain and represent a potential public health hazard are the old sows. These old sows could transmit toxoplasmosis to humans supporting Dubey's statement that the number one mode of transmission of T. gondii to humans is via pork (Dubey et al., 1992). Dubey has shown that a heavy load of T. gondii tissue cysts is found in heart, skeletal muscle, brain, diaphragm, and tongue (Dubey et al., 1986a). Infection may occur by ingestion of undercooked cuts of pork derived from any of these tissues or possibly more commonly by the ingestion of undercooked beef products unsuspectingly adulterated with pork (Georgi and Georgi, 1990; Schantz et al., 1977).

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APPENDICES

Appendix A

OBTAINING AND PREPARING TISSUE FROM MICE FOR INTRAPERITONEAL INJECTION INTO OTHER MICE

1. EUTHANATIZE THE MICE WITH CO2.
2. REMOVE THE TISSUES.
3. PLACE THE TISSUE IN A SEALABLE PLASTIC BAG AND ADD 5 EQUIVALENT VOLUMES OF STERILIZED SALINE. PLACE THE TISSUES AND SALINE IN A HOMOGENIZER UNTIL HOMOGENIZED.
4. ADD 250 ML OF DIGESTIVE FLUID TO EACH HOMOGENATE.
5. PLACE THE MIXTURE ON A SHAKER IN AN INCUBATOR AT 37C. FOR 30 MIN.
6. FILTER THROUGH TWO LAYERS OF STERILIZED CHEESECLOTH.
7. CENTRIFUGE THE MIXTURE AT 400XG FOR 15 MIN.
8. REMOVE THE SUPERNATANT AND RESUSPEND THE SEDIMENT IN STERILIZED SALINE FOR THREE TIMES.
9. AFTER THE LAST WASH, RESUSPEND THE SEDIMENTS IN AN EQUAL VOLUME OF STERILE ANTIBIOTIC-SALINE SOLUTION (0.1 ML OF ANTIBIOTIC, PENICILLIN-STREPTOMYCIN, TO 100 ML OF STERILE SALINE) (ANTIBIOTIC PURCHASED FROM GIBCO LABORATORIES, GRAND ISLAND, NEW YORK).
10. ANESTHETIZE THE MICE WITH METHOXYFLURANE AND INJECT 1 ML INTRAPERITONEALLY.

Appendix A Cont'd

DIGESTIVE FLUID:

2.6 gm of pepsin (purchased from Sigma, St. Louis, Missouri)

5.0 gm of NaCl

7.0 milliliter of 37% HCl (purchased from Sigma, St. Louis, Missouri)

Dilute in 500 ml of distilled water.

Appendix B

MODIFIED AGGLUTINATION TEST

1. ADD 25 MICROLITERS OF PBS TO ALL WELLS.
2. ADD 25 MICROLITERS OF SERUM TO 1ST WELL.
3. TITER OUT THE SERUM SAMPLES FROM 1ST WELL TO THE 12TH WELL.
4. ADD 25 MICROLITERS OF 2-ME IN 3RD, 8TH, AND 12TH WELLS.
5. ADD 50 MICROLITERS OF ANTIGEN MIXTURE IN 3RD, 8TH, AND 12TH WELLS.
6. SEAL THE PLATE AND PLACE IT IN A 37°C. INCUBATOR FOR 6 HOURS. MINIMUM AND 12 HRS. MAXIMUM.
7. READ THE PLATE AND THEN ADD ANTIGEN TO FIND THE ENDPOINTS.

bioMerieux TOXO-SCREEN PHOSPHATE BUFFERED SALINE (PBS):

36.0 gm of NaCl

7.40 gm of Na2HPO4 (anhydrous)

2.15 gm of KH2PO4 (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 microorganism contamination. If dissolved in 1 liter of distilled water, it needs to be diluted down when required.

Appendix B Cont'd

(Reference: Handbook of experimental immunology, 3rd ed. D. M. Weir, editor. Blackwell Scientific Publications, p. 20.7.).

ANTIGEN MIXTURE: Add 1 ml of antigen to 4 ml of diluent.

BIOMERIEUX TOXO-SCREEN DILUENT (pH 8.95):

7.012 gm of NaCl

3.092 gm of boric acid

24 ml of 1N NaOH (40 gm/1 L distilled water)

4 gm of bovine serum albumin

1 gm of sodium azide

add distilled water to reach 1000 ml.

Add 1 liter of distilled water to sodium chloride, boric acid, BSA, and sodium azide. Stir on a magnetic stirring plate. Gently heat the solution while stirring. Add 24 ml of 1N sodium hydroxide. Store at 4C. in a sterile container. Before use, add 6-8 drops of diluted (30%) trypan blue to 3.2 ml of the diluent.

2-MERCAPTOETHANOL: Add 0.35 ml concentrated 2-ME to 25 ml of PBS.

Appendix C

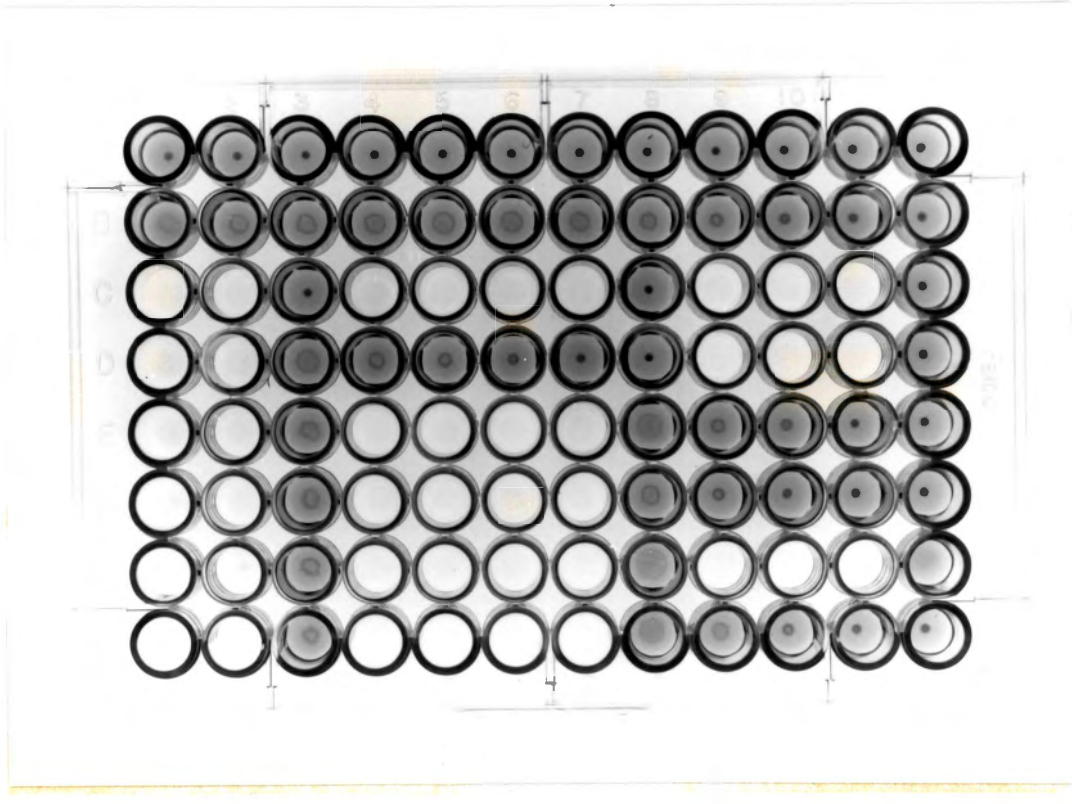

Plate 1. A 96-well microtiter plate demonstrating the modified agglutination test for Toxoplasma gondii antibodies. The first and second rows are the negative and positive control samples, respectively. The negative samples are shown as a dot in a well which indicates lack of agglutination, and the tachyzoites are precipitated at the bottom of the well. In the positive row, agglutination has occurred, and the end point is at the 8th well (dilution 1/512). The remaining rows are sera from pig samples that have been screened at 3rd (dilution 1/16), 8th (dilution 1/512), and 12th (dilution 1/8192) wells. The antigen has been added to find the end point of each sample.

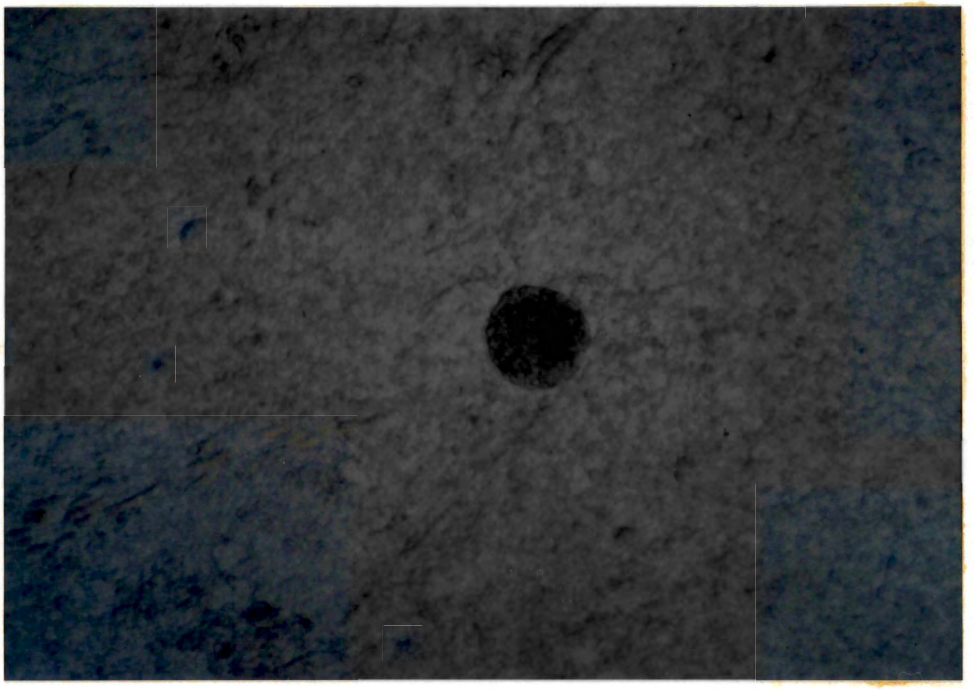

Plate 2. Brain squash preparation showing a tissue cyst containing bradyzoites from the brain of a mouse infected with Toxoplasma gondii. (Magnification X214).

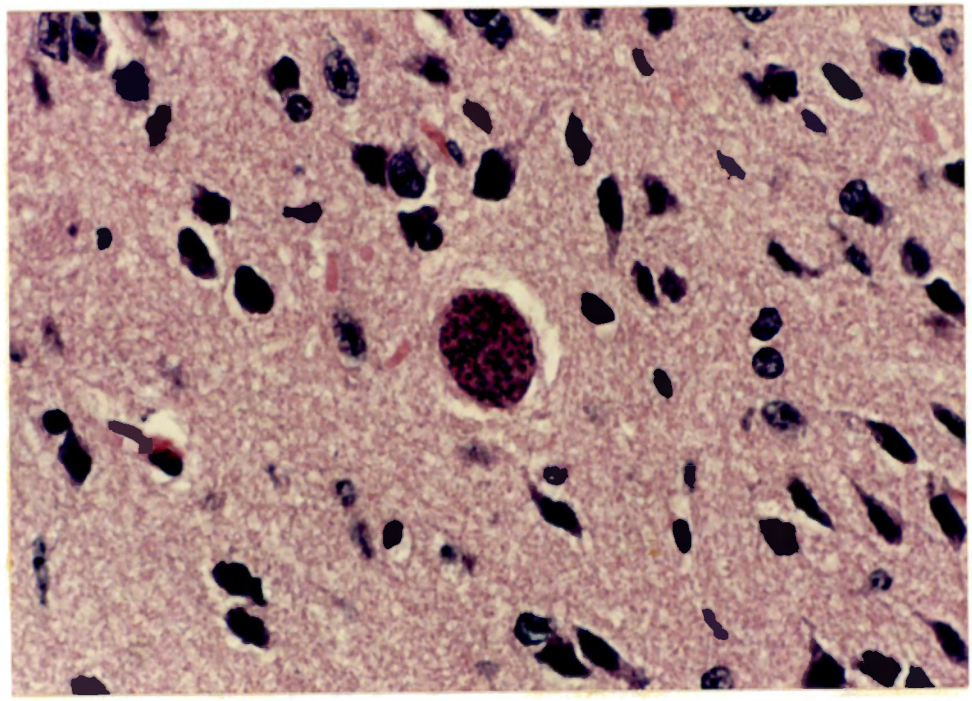

Plate 3. A cross-section showing a tissue cyst containing bradyzoites from the brain of a mouse infected with Toxoplasma gondii. Section was stained with hematoxylin and eosin. (Magnification X198).

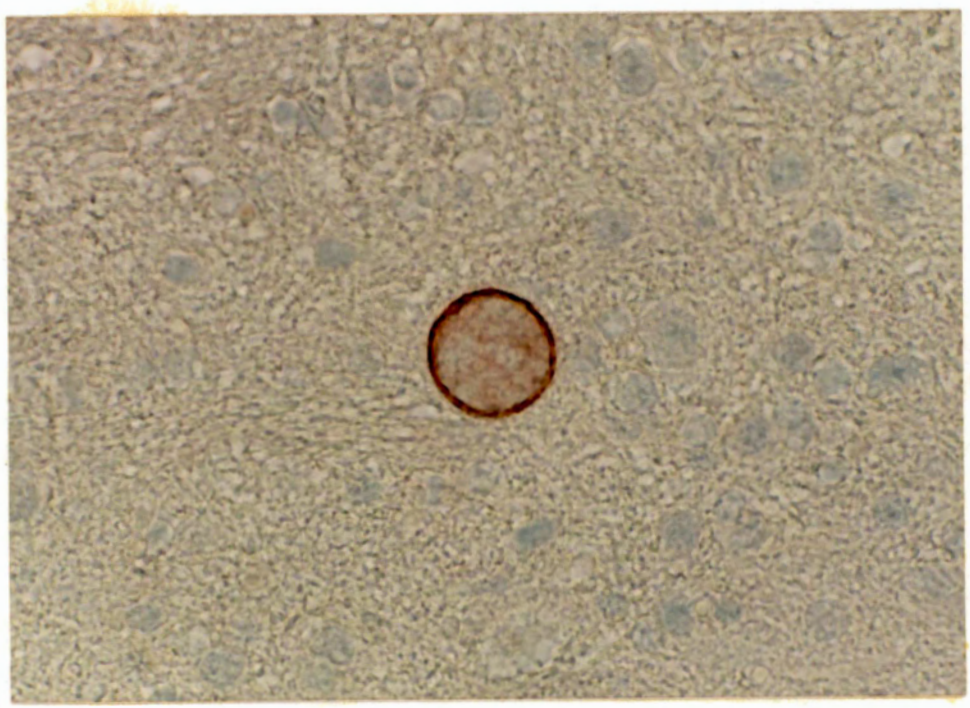

Plate 4. A positive immunoperoxidase test on a Toxoplasma gondii tissue cyst containing bradyzoites from the brain of a mouse. Section was stained using AEC dye and primary antibody. (Magnification X224).

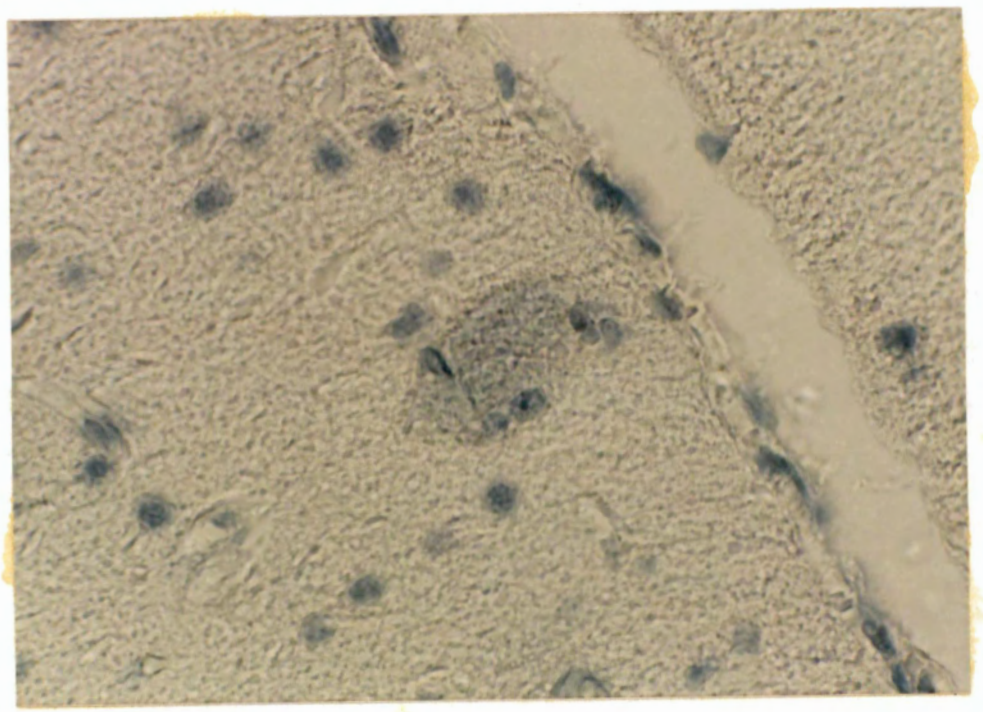

Plate 5. A negative immunoperoxidase test on a Toxoplasma gondii tissue cyst containing bradyzoites from the brain of a mouse. Section was stained using AEC dye but primary antibody was not added. (Magnification X330).

Appendix D

IMMUNOPEROXIDASE

(FROM BIOGENIX MULTIUSE KIT)

(San Ramon California)

1. PLACE SLIDE IN INCUBATOR FOR 30 MIN AT 56C.
2. SUBMERGE SLIDE IN AMERICLEAR HISTOLOGY CLEARING SOLVENT FOR 3 MIN.
3. SUBMERGE SLIDE IN 100% ETOH FOR 3 MIN. TWICE.
4. SUBMERGE SLIDE IN 95% ETOH FOR 3 MIN. TWICE.
5. RINSE SLIDE IN RUNNING TAP WATER FOR 30 SEC.
6. SUBMERGE SLIDE IN DISTILLED WATER 3 MIN.
7. SUBMERGE SLIDE IN PHOSPHATE BUFFERED SALINE (PBS) FOR 5 MIN.
8. PLACE 1-3 DROPS OF 3% HYDROGEN PEROXIDASE ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 5 MIN. 9. RINSE SLIDE WITH PBS FOR 10 MIN.
10. PLACE 1-3 DROPS OF NORMAL GOAT SERUM FROM THE KIT ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 5 MIN. BE SURE NOT TO RINSE WITH PBS.
11. PLACE 1-3 DROPS OF PRIMARY AB ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 30 MIN. USE NEGATIVE ANTIBODY FOR NEGATIVE SLIDES.
12. SUBMERGE SLIDE IN PBS FOR 10 MIN.
13. PLACE 1-3 DROPS OF LINK ANTIBODY ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 20 MIN.

Appendix D Cont'd

14. SUBMERGE SLIDE IN PBS FOR 10 MIN.
15. PLACE 1-3 DROPS OF LABEL ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 20 MIN.
16. SUBMERGE SLIDE IN PBS FOR 10 MIN. DURING THIS TIME MAKE UP YOUR SUBSTRATE SOLUTION (1 ADD OF CHROMAGEN IN 2.5 ML OF SUBSTRATE). SUBSTRATE SOLUTION IS ONLY GOOD FOR ONE TIME USE.
17. PLACE 1-3 DROPS OF SUBSTRATE SOLUTION ON THE SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 10 MIN.
18. SUBMERGE SLIDE IN DEIONIZED WATER FOR 30 SEC.
19. PLACE 1-3 DROPS OF COUNTERSTAIN MAYER'S SOLUTION ON SLIDE UNTIL TISSUE IS COVERED. LEAVE AT ROOM TEMP. FOR 5 MIN. 20. SUBMERGE SLIDE IN TAP WATER.
21. SUBMERGE SLIDE IN AMMONIA WATER FOR 10 SEC.
22. RINSE SLIDE IN RUNNING TAP WATER FOR 30 SEC.
23. MOUNT SLIDE WITH LIQUID AQUEOUS MOUNTING MEDIA.

PHOSPHATE BUFFERED SALINE (PBS, pH 7.6):

1. Weigh out 7.75 gm of sodium chloride
   1.50 gm of potassium phosphate dibasic (K_2HPO_4)
   0.20 gm of potassium phosphate monobasic (KH_2PO_4).
2. Dilute to 1.0 liter with deionized water.

Appendix D cont'd

3. Adjust pH as needed by addition of 3M NaOH or 8.5% phosphoric acid.

AMMONIA WATER: Add 1 milliliter of concentrated NH_4OH per liter of water.

SUBSTRATE SOLUTION: Add 1 ADD of chromagen AEC to 2.5 milliliter of substrate.

PRIMARY ANTIBODY: Add 2 microliter of primary Ab to 998 microliter of PBS.

Appendix E

Table II.1. Anti-*toxoplasma gondii* IgM titers of 25 mice in Experiment 2 that received bradyzoite cysts by intraperitoneal injection. The unit is number of mice with a given titer on days postinfection (P.I.).

Titer	Days P.I. 7	9*	11	13	15	17	33-45
0	7				3	15	20
2					4	4	1
4			1	3	8	5	3
8				3	7		
16	8		4	5	2		
32				6			
64		4	1				
128	3	7	4	2			
256	1	4	12	3			
512		9	2	2			
1024							
2048	6						
4096		1					
Total No. of mice	25	25	24	24	24	24	24

* one mouse died during anesthesia

Table II.2. Anti-*toxoplasma gondii* IgG titers of 25 mice in Experiment 2 that received bradyzoite cysts by intraperitoneal injection. The unit is number of mice with a given titer on days postinfection (P.I.).

Titer	Days P.I. 7	9*	11	13	15	17	33-45
0	23	1					
2							
4							
8	2	13					
16		9	6				
32		1	11	3			
64		1	2	2			
128			2	1			
256				9			
512			3	2	2		
1024				2	4	2	
2048				3	6	2	3
4096				2	3	2	6
8192					8	8	5
16384					1		
32768						5	5
131072						5	5
Total No. of mice	25	25	24	24	24	24	24

* one mouse died during anesthesia

Table III.1. Seroprevalence of Toxoplasma gondii antibody in finishing pigs at Crossville Experiment Station during 1991 and 1992.

Year	Pigs at arrival Neg*	Pos**	total	Pigs at slaughter Neg	Pos	total
1991	146	4	150	150	0	150
1992	146	4	150	145	2	147***

* Neg = seronegative for Toxoplasma gondii antibody
**Pos = seropositive for Toxoplasma gondii antibody
*** 3 pigs died

Table IV.1. Seroprevalence of Toxoplasma gondii in pigs by age group and management system at the Ames Plantation.

		Piglets	Grow/ finish	Sows	Total	Grow/ finishII
Pasture	Neg	55	55	17	127	31
	Pos	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Part. CNF*	Neg	38	55	23	116	20
	Pos	17(31%)	0(0%)	9(28%)	26(18%)	10(33%)
Total CNF**	Neg	55	55	50	160	32
	Pos	0(0%)	0(0%)	2(4%)	2(1%)	0(0%)

*Part. CNF = Partial confinement
**Total CNF = Total confinement

Table IV.2. The seroprevalence of Toxoplasma gondii in the combination of grow/finishing pigs and sows compared to the piglets within system II at the Ames Plantation.

	Grow/finish & Sows	Piglets	Total
Negative	78	38	116
Positive	9(10%)	17(31%)	26
Total	87	55	142

Chi square = 9.53
P value = 0.002

Table V.1. Seroprevalence of Toxoplasma gondii antibodies in sows and on farms in Tennessee, 1991-92.

Year	Number of farms		Number of sows	
	Sampled	Positive*	Sampled	Positive
1991	254	188 (74.0%)	2000	720 (36.0%)
1992	89	57 (64.0%)	1841	410 (22.3%)

* at least one sow positive

Table V.2. Crude and true seroprevalence of Toxoplasma gondii in sows in Tennessee, 1991-92.

Year	Crude seroprevalence* (C.P.)	True seroprevalence** (T.P.)
1991	36.00%	42.19%
1992	22.27%	30.45%

* C.P. = positive sows samples / total # of sows X 100
**T.R. = sum of prevalence of each farm / # of farms X 100

Table V.3. Number of positive sows and their corresponding *Toxoplasma gondii* antibody titers, 1991-92.

Titer	1991 Number of Positive Sows	1992 Number of Positive Sows
32	143 (7.15%)	133 (7.02%)
64	270 (13.50%)	66 (3.53%)
128	33 (1.66%)	15 (0.81%)
256	2 (0.10%)	11 (0.59%)
512	146 (7.30%)	142 (7.65%)
1024	92 (4.60%)	25 (1.41%)
2048	2 (0.10%)	6 (0.32%)
4096	1 (0.05%)	8 (0.43%)
≥ 8192	31 (1.55%)	4 (0.43%)
Total	720	410

Table V.4. Distribution and prevalence of antibody to Toxoplasma gondii in the three different regions of Tennessee, 1991-92.

Counties	1991	1992
Western		
No. of Farms	62	22
No. of + samples	176	205
No. of - samples	449	884
Total of samples	625	1089
True prevalence	28.00%	27.46%
Crude Prevalence	20.16%	18.82%
Central		
No. of Farms	172	64
No. of + samples	548	186
No. of - samples	705	537
Total of samples	1253	723
True prevalence	48.02%	30.56%
Crude Prevalence	43.73%	25.72%
Eastern		
No. of Farms	20	3
No. of + Samples	46	19
No. of - Samples	76	10
Total of Samples	122	29
True prevalence	35.97%	50.00%
Crude Prevalence	37.70%	65.50%

Table VI.1. Toxoplasma gondii serologic status of farms surveyed in Tennessee and their survey responsiveness.

	Nonrespondents*	Respondents**	Total
Neg. Farms	47	32	79
Pos. Farms	109	75	184
Total	156	107	263

* Farmers who did not respond to the survey
**Farmers who did respond to the survey
Chi square = 0.000
P value = 0.96

Table VI.2. Regional location of Tennessee farms surveyed and their survey responsiveness.

	Nonrespondents*	Respondents**	Total
East TN	8	7	15
Middle TN	100	75	175
West TN	48	25	73
Total	156	107	263

* Farmers who did not respond to the survey
**Farmers who did respond to the survey
Chi square = 1.82
P value = 0.402

Table VI.3. Mean, median, and range values of farms' inventory and market classes of swine on 107 Tennessee farms, 1991-92.

	Mean	Median	Range
Annual no. of Sows & gilts	93.55	14	0-2710
Piglets farrowed			
in past 12 mo.	1039.67	122	0-16300
in past 3 mo.	372.56	34	0-4072
Annual no. of feeder pigs	474.09	69	0-16800
Annual no. of finished pigs	469.20	8	0-10800
Stillborn piglets			
in past 12 mo.	53.72	8	0-1650
in past 3 mo.	15.60	2	0-400

Table VI.4 Number and percentage of 107 Tennessee farms surveyed in 1991-92 with a given inventory and market class of swine and the number of head of swine within that class.

Farms with	None	≤ 29 head	≥ 30 head
Sows & gilts	2 (2%)	76 (68%)	29 (30%)
Piglets farrowed			
in past 12 mo.	2 (2%)	11 (10%)	94 (88%)
in past 3 mo.	14 (14%)	27 (25%)	66 (61%)
Feeder pigs	15 (15%)	10 (9%)	82 (76%)
Finished pigs	42 (39%)	12 (11%)	53 (50%)

Table VI.5. Number of 107 Tennessee farms surveyed in 1991-92 and their methods of management, seasonality of breeding, presence of cats, and herd size.

Management	No. (%) farms	Farms with Cats	Cont.	Seas.	Farms >= 30 sows
Pasture	40 (37.4%)	26	31	9	1
Part. CNF	48 (44.8%)	32	43	5	3
Total CNF	19 (17.7%)	8	18	1	11
Total	107	66	92	15	15

Cont. = Continuous breeding
Seas. = Seasonal breeding
Part. CNF = Partial confinement
Total CNF = Total confinement

Table VI.6. The seropositivity to Toxoplasma gondii of sows associated with cats compared to that of sows not known to be associated with cats on 107 Tennessee swine farms in 1991-92.

	Seropositive	Seronegative	Total
Cats present	380	962	1342
Cats absent	87	572	659
Total	467	1534	2001

Odds ratio = 2.60
95% C.I. Lower limit = 2.00, Upper limit = 3.38
Chi square = 56.43
P value = 0.000

Table VI.7. The seropositivity to Toxoplasma gondii of sows kept on pasture only that are associated with cats compared to that of sows not known to be associated with cats on 107 Tennessee swine farms in 1991-92.

	Seropositive	Seronegative	Total
Cats present	42	39	81
Cats absent	163	67	230
Total	205	106	311

Odds ratio = 0.44
95% C.I. Lower limit = 0.25, Upper limit = .77
Chi square = 9.64
P value = 0.001

Table VI.8. The seropositivity to Toxoplasma gondii of sows kept in partial confinement that are associated with cats compared to that of sows not known to be associated with cats on 107 Tennessee swine farms in 1991-92.

	Seropositive	Seronegative	Total
Cats present	71	35	106
Cats absent	258	292	550
Total	329	327	656

Odds ratio = 2.30
95% C.I. Lower limit = 1.45, Upper limit 3.64
Chi square = 14.32
P value = 0.000

Table VI.9. The seropositivity to Toxoplasma gondii of sows kept in total confinement that are associated with cats compared to that of sows not known to be associated with cats on 107 Tennessee swine farms in 1991-92.

	Seropositive	Seronegative	Total
Cats present	13	459	472
Cats absent	21	541	562
Total	34	1000	1034

Odds ratio = 0.73
95% C.I. Lower limit = 0.34, Upper limit = 1.54
Chi square = 0.78
P value = 0.37

Table VI.10. The odds ratio for seropositivity to Toxoplasma gondii of sows on 107 Tennessee swine farms comparing one management system to another with their associated confidence intervals, 1991-92.

Management	Odds ratio	95% C.I. Upper	Lower
Pasture VS Total CNF	15.21	20.62	11.23
Part. CNF VS Total CNF	29.23	38.45	22.26
Pasture VS Part. CNF	0.5	0.69	0.39

Part. CNF = Partial confinement
Total CNF = Total confinement

Table VI.11. The odds ratio of sows on 107 Tennessee swine farms kept outdoors (pasture & partial confinement) at any time vs. sows kept indoors (total confinement) at all times, and the odds ratio of these sows kept outdoors at any time without the presence of cats vs. sows kept indoors at all times with the presence of cats, with their associated confidence intervals, 1991-92.

Managements	Odds ratio	95% C.I. Upper	Lower
Pasture & Part. CNF VS Total CNF	23	35.37	16.48
Pasture & Part. CNF with No Cats VS Total CNF with Cats	17	29.94	9.75

Part. CNF = Partial confinement
Total CNF = Total confinement

Table VI.12. The seropositivity to Toxoplasma gondii of sows on 107 Tennessee swine farms compared to the type of rodent control used on the farm, 1991-92.

	Seropositive	Seronegative	Total
Active*	53	95	148
Passive**	412	1441	1853
Total	465	1536	2001

* Active = farmers used chemicals, traps, or destruction of rodents' habitat
** Passive = farmers used cats, rodents proof containers, and other (non-active) methods
Odds ratio of 1.95
95% C.I. Lower limit = 1.35, Upper limit = 2.82
Chi square = 14.16
Pvalue = 0.000

Table VI.13. A comparison of the seroprevalence of Toxoplasma gondii antibodies in sows on 107 Tennessee swine farms by farm size, 1991-92.

	Seropositive	Seronegative	Total
Small Farms	282	390	672
Large Farms	185	1144	1329
Total	467	1534	2001

Odds ratio = 4.47
95% C.I. Lower limit = 3.57, Upper limit = 5.60
Chi square = 196.19
P value = 0.000

Table VI.14. The seropositivity to Toxoplasma gondii of sows on 15 "large" Tennessee swine farms compared by the management practices, 1991-92.

	Seropositive	Seronegative	Total
Pasture & Part CNF	163	183	346
Total CNF	22	961	983
Total	185	1144	1329

Part. CNF = Partial confinement
Total CNF = Total confinement
Odds ratio = 38.91
95% C.I. Lower limit = 23.75, Upper limit 64.28
Chi square = 430.03
P value = 0.000

Table VI.15. The seropositivity to Toxoplasma gondii of sows on 92 "small" Tennessee swine farms compared by the management practices, 1991-92.

	Seropositive	Seronegative	Total
Pasture & Part. CNF	268	347	615
Total CNF	14	43	57
Total	282	390	672

Part. CNF = Partial confinement
Total CNF = Total confinement
Odds ratio = 2.37
95% C.I. Lower limit = 1.23, Upper limit = 4.65
Chi square = 7.75
P value = 0.005

Table VI.16. The seropositivity to T. gondii of sows in total confinement on "large" Tennessee swine farms compared to the seropositivity to T. gondii of sows on pasture or in partial confinement on "small" Tennessee swine farms, 1991-92.

	Seropositive	Seronegative	Total
Total CNF Farms >= 30 sows	22	961	983
Pasture & Part CNF <= 29	268	347	615
Total	290	1308	1598

Part. CNF = Partial confinement
Total CNF = Total confinement
Odds ratio = 0.03
95% C.I. Lower limit = 0.03, Upper limit = 0.08
Chi square = 435.23
P value = 0.000

Table VI.17. Status of culled sows.

Method of Culling	No.	Percent
Home slaughter	169	6%
Sold with finished hogs	2494	93%
Others	25	1%
Total	2688	100%

Appendix F

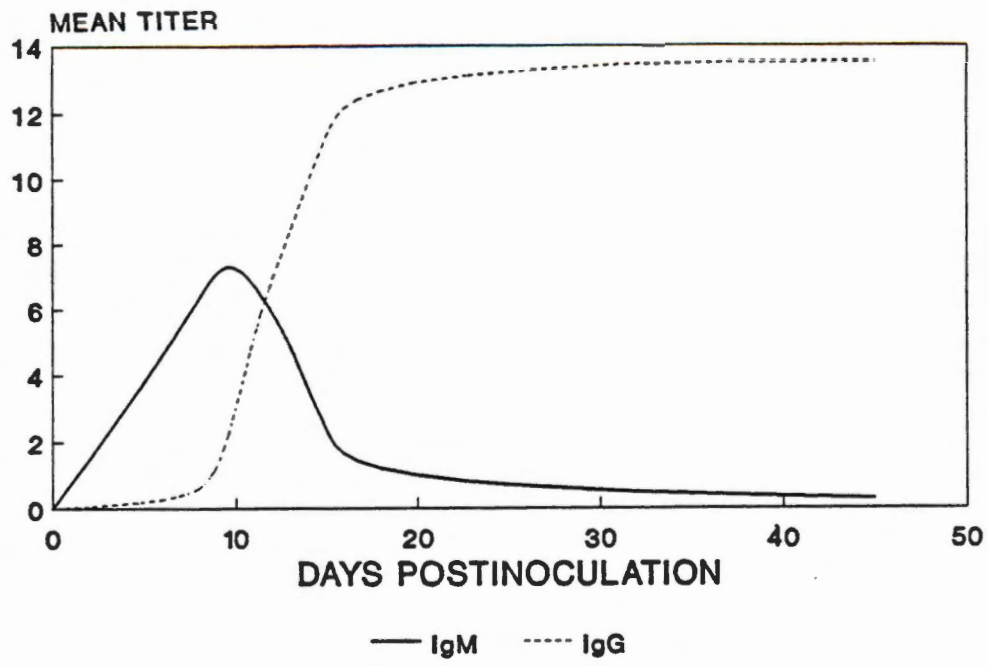

Figure 1. Arithmetic mean of anti-Toxoplasma gondii IgG and IgM titers of 25 mice infected intraperitoneally with bradyzoites of goat, UT-1 strain of T. gondii measured by MAT.

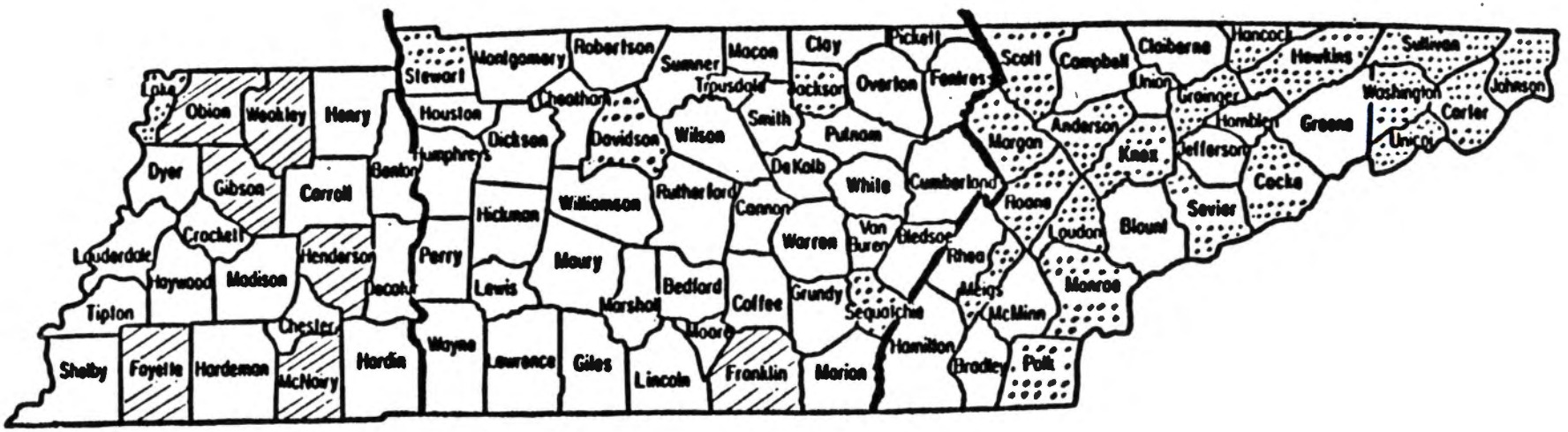

Figure 2. Estimated population of swine in the counties of Tennessee, 1992.

less than 2,000 pigs

More than 2,000 and less than 25,000 pigs

more than 25,000 pigs

Appendix G

Anti-*Toxoplasma gondii* IgG titers of sera obtained from grow/finishing pigs at the Crossville Experiment Station during 1991 determined by MAT.

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative
6	Negative	Negative
7	Negative	Negative
8	Negative	Negative
9	Negative	Negative
10	Negative	Negative
11	Negative	Negative
12	Negative	Negative
13	Negative	Negative
14	32	Negative
15	Negative	Negative
16	Negative	Negative
17	Negative	Negative
18	32	Negative
19	Negative	Negative
20	Negative	Negative
21	Negative	Negative
22	Negative	Negative
23	Negative	Negative
24	Negative	Negative
25	Negative	Negative
26	Negative	Negative
27	Negative	Negative
28	Negative	Negative
29	Negative	Negative
30	Negative	Negative
31	Negative	Negative
32	Negative	Negative
33	Negative	Negative
34	Negative	Negative
35	Negative	Negative
36	Negative	Negative
37	Negative	Negative
38	Negative	Negative
39	Negative	Negative
40	Negative	Negative
41	Negative	Negative
42	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
43	Negative	Negative
44	Negative	Negative
45	Negative	Negative
46	Negative	Negative
47	Negative	Negative
48	Negative	Negative
49	Negative	Negative
50	Negative	Negative
51	Negative	Negative
52	Negative	Negative
53	Negative	Negative
54	Negative	Negative
55	Negative	Negative
56	Negative	Negative
57	Negative	Negative
58	Negative	Negative
59	Negative	Negative
60	Negative	Negative
61	Negative	Negative
62	Negative	Negative
63	Negative	Negative
64	Negative	Negative
65	Negative	Negative
66	64	Negative
67	Negative	Negative
68	Negative	Negative
69	Negative	Negative
70	Negative	Negative
71	Negative	Negative
72	Negative	Negative
73	Negative	Negative
74	Negative	Negative
75	Negative	Negative
76	Negative	Negative
77	Negative	Negative
78	Negative	Negative
79	Negative	Negative
80	Negative	Negative
81	Negative	Negative
82	Negative	Negative
83	Negative	Negative
84	Negative	Negative
85	Negative	Negative
86	Negative	Negative
87	Negative	Negative
88	Negative	Negative
89	Negative	Negative
90	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
91	Negative	Negative
92	Negative	Negative
93	Negative	Negative
94	Negative	Negative
95	Negative	Negative
96	Negative	Negative
97	Negative	Negative
98	Negative	Negative
99	Negative	Negative
100	Negative	Negative
101	Negative	Negative
102	Negative	Negative
103	Negative	Negative
104	Negative	Negative
105	Negative	Negative
106	Negative	Negative
107	Negative	Negative
108	Negative	Negative
109	Negative	Negative
110	Negative	Negative
111	Negative	Negative
112	Negative	Negative
113	Negative	Negative
114	Negative	Negative
115	Negative	Negative
116	Negative	Negative
117	Negative	Negative
118	Negative	Negative
119	Negative	Negative
120	Negative	Negative
121	Negative	Negative
122	Negative	Negative
123	Negative	Negative
124	Negative	Negative
125	Negative	Negative
126	32	Negative
127	Negative	Negative
128	Negative	Negative
129	Negative	Negative
130	Negative	Negative
131	Negative	Negative
132	Negative	Negative
133	Negative	Negative
134	Negative	Negative
135	Negative	Negative
136	Negative	Negative
137	Negative	Negative
138	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
139	Negative	Negative
140	Negative	Negative
141	Negative	Negative
142	Negative	Negative
143	Negative	Negative
144	Negative	Negative
145	Negative	Negative
146	Negative	Negative
147	Negative	Negative
148	Negative	Negative
149	Negative	Negative
150	Negative	Negative

Anti-*Toxoplasma gondii* IgG titers of sera obtained from grow/finishing pigs at the Crossville Experiment Station during 1992 determined by MAT.

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
1	Negative	Negative
2	8192	8192
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative
6	Negative	Negative
7	Negative	Negative
8	Negative	Negative
9	Negative	Negative
10	Negative	Negative
11	Negative	Negative
12	Negative	Negative
13	Negative	Negative
14	Negative	Negative
15	Negative	Negative
16	Negative	Negative
17	Negative	Negative
18	Negative	Negative
19	Negative	Negative
20	Negative	Negative
21	Negative	Negative
22	Negative	Negative
23	Negative	Negative
24	Negative	Negative
25	Negative	Negative
26	Negative	Negative
27	Negative	Negative
28	Negative	Negative
29	Negative	Negative
30	Negative	Negative
31	Negative	Negative
32	Negative	Negative
33	Negative	Negative
34	Negative	Negative
35	Negative	Negative
36	Negative	Negative
37	Negative	Negative
38	Negative	Negative
39	Negative	Negative
40	Negative	Negative
41	Negative	Negative
42	Negative	Negative
43	Negative	Negative
44	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
45	Negative	Negative
46	Negative	Negative
47	Negative	Negative
48	Negative	Negative
49	Negative	Negative
50	Negative	Negative
51	Negative	Negative
52	Negative	Negative
53	Negative	Negative
54	Negative	Negative
55	Negative	Negative
56	Negative	Negative
57	Negative	Negative
58	Negative	Negative
59	Negative	Negative
60	Negative	Negative
61	Negative	Negative
62	Negative	died
63	Negative	Negative
64	Negative	Negative
65	Negative	Negative
66	Negative	Negative
67	Negative	Negative
68	Negative	Negative
69	Negative	Negative
70	Negative	Negative
71	Negative	Negative
72	Negative	Negative
73	Negative	Negative
74	Negative	Negative
75	Negative	Negative
76	Negative	Negative
77	Negative	Negative
78	Negative	Negative
79	Negative	Negative
80	Negative	Negative
81	Negative	Negative
82	Negative	Negative
83	Negative	Negative
84	Negative	Negative
85	Negative	Negative
86	Negative	Negative
87	Negative	Negative
88	Negative	Negative
89	Negative	Negative
90	Negative	Negative
91	Negative	Negative
92	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
93	Negative	Negative
94	Negative	Negative
95	Negative	Negative
96	Negative	Negative
212	Negative	Negative
172	Negative	Negative
229	Negative	Negative
231	Negative	Negative
115	Negative	Negative
202	Negative	Negative
132	Negative	Negative
183	2048	512
111	Negative	Negative
126	Negative	Negative
206	Negative	died
110	Negative	Negative
178	Negative	Negative
151	Negative	Negative
222	Negative	Negative
182	Negative	Negative
130	Negative	Negative
232	Negative	Negative
98	Negative	Negative
176	Negative	Negative
139	Negative	Negative
216	Negative	Negative
134	Negative	Negative
153	Negative	Negative
193	Negative	Negative
167	Negative	Negative
223	Negative	Negative
239	Negative	Negative
163	Negative	Negative
148	Negative	Negative
198	32	Negative
192	Negative	Negative
207	Negative	Negative
220	Negative	Negative
236	Negative	Negative
227	Negative	Negative
122	Negative	Negative
119	Negative	Negative
156	32	Negative
108	Negative	Negative
200	Negative	Negative
173	Negative	Negative
234	Negative	Negative
157	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
107	Negative	Negative
138	Negative	Negative
192	Negative	died
101	Negative	Negative
147	Negative	Negative
160	Negative	Negative
152	Negative	Negative
162	Negative	Negative
100	Negative	Negative
199	Negative	Negative

Appendix H

The sample size (n) required to be 95% certain of including at least one positive in a given population size (N) if the disease is present at the specified level.

Population size (N)	50%	40%	30%	25%	20%	15%	10%	5%	2%	1%	0.5%	0.1%
10	4	5	6	7	8	10	10	10	10	10	10	10
20	4	6	7	9	10	12	16	19	20	20	20	20
30	4	6	8	9	11	14	19	26	30	30	30	30
40	5	6	8	10	12	15	21	31	40	40	40	40
50	5	6	8	19	12	16	22	35	48	50	50	50
60	5	6	8	10	12	16	23	38	55	60	60	60
70	5	6	8	10	13	17	24	40	62	70	70	70
80	5	6	8	10	13	17	24	42	68	79	80	80
90	5	6	8	10	13	17	25	43	73	87	90	90
100	5	6	9	10	13	17	25	45	78	96	100	100
120	5	6	9	10	13	18	26	47	86	111	120	120
140	5	6	9	11	13	18	26	48	92	124	139	140
160	5	6	9	11	13	18	27	49	97	136	157	160
180	5	6	9	11	13	18	27	50	101	146	174	180
200	5	6	9	11	13	18	27	51	105	155	190	200
250	5	6	9	11	14	18	27	53	112	175	228	250
300	5	6	9	11	14	18	28	54	117	189	260	300
350	5	6	9	11	14	18	28	54	121	201	287	350
400	5	6	9	11	14	19	28	55	124	211	311	400
450	5	6	9	11	14	19	28	55	127	218	331	450
500	5	6	9	11	14	19	28	56	129	225	349	500
600	5	6	9	11	14	19	28	56	132	235	379	597
700	5	6	9	11	14	19	28	57	134	243	402	691
800	5	6	9	11	14	19	28	57	136	249	421	782
900	5	6	9	11	14	19	28	57	137	254	437	868
1000	5	6	9	11	14	19	29	57	138	258	450	950
1200	5	6	9	11	14	19	29	57	140	264	471	1102
1400	5	6	9	11	14	19	29	58	141	269	487	1236
1600	5	6	9	11	14	19	29	58	142	275	509	1354
1800	5	6	9	11	14	19	29	58	143	275	509	1459
2000	5	6	9	11	14	19	29	58	143	277	517	1553
3000	5	6	9	11	14	19	29	58	145	284	542	1895
4000	5	6	9	11	14	19	29	58	146	288	556	2108
5000	5	6	9	11	14	19	29	59	147	290	564	2253
6000	5	6	9	11	14	19	29	59	147	291	569	2358
7000	5	6	9	11	14	19	29	59	147	292	573	2437
8000	5	6	9	11	14	19	29	59	147	293	576	2498
9000	5	6	9	11	14	19	29	59	148	294	579	2548
10000	5	6	9	11	14	19	29	59	148	294	581	2588
∞	5	6	9	11	14	19	29	59	149	299	598	2995

Appendix I

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System I piglets at the Ames Plantation in 1991 and in 1992 at the time of slaughter (grow/finishII) determined by MAT.

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
31-5	Negative	Negative
32-4	Negative	Negative
37-2	Negative	Negative
34-10	Negative	Negative
35-6	Negative	N/A*
45-4	Negative	Negative
29-12	Negative	N/A
29-4	Negative	N/A
46-5	Negative	N/A
36-8	Negative	N/A
35-9	Negative	N/A
47-1	Negative	N/A
47-4	Negative	Negative
33-5	Negative	Negative
41-7	Negative	N/A
30-2	Negative	Negative
38-10	Negative	Negative
36-7	Negative	Negative
29-2	Negative	N/A
43-2	Negative	Negative
34-9	Negative	Negative
48-5	Negative	N/A
38-4	Negative	Negative
39-1	Negative	Negative
39-6	Negative	Negative
41-8	Negative	N/A
46-1	Negative	Negative
33-1	Negative	Negative
34-11	Negative	Negative
44-6	Negative	Negative
46-11	Negative	Negative
84-1	Negative	N/A
41-1	Negative	Negative
35-11	Negative	N/A
46-8	Negative	N/A
42-3	Negative	N/A
49-7	Negative	N/A
34-10	Negative	N/A
41-5	Negative	Negative
52-4	Negative	N/A
31-2	Negative	N/A
47-2	Negative	Negative

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
37-5	Negative	N/A
40-10	Negative	N/A
46-4	Negative	Negative
37-6	Negative	N/A
38-11	Negative	Negative
29-8	Negative	Negative
30-4	Negative	N/A
46-2	Negative	Negative
44-4	Negative	Negative
40-8	Negative	Negative
44-9	Negative	Negative
43-10	Negative	N/A
29-3	Negative	Negative

*not available

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System II piglets at the Ames Plantation in 1991 and in 1992 at the time of slaughter (grow/finishII) determined by MAT.

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
96-9	Negative	Negative
90-3	2048	2048
96-1	Negative	N/A*
96-5	Negative	N/A
86-3	Negative	Negative
81-1	64	32
80-4	Negative	Negative
76-9	Negative	N/A
92-8	64	64
83-3	128	Negative
87-7	128	Negative
92-6	1024	Negative
76-13	Negative	N/A
92-2	256	256
83-9	Negative	Negative
85-2	Negative	Negative
84-7	Negative	N/A
84-4	Negative	Negative
81-11	Negative	N/A
91-1	Negative	Negative
86-10	Negative	Negative
87-1	2048	Negative
91-10	Negative	N/A
82-5	Negative	N/A
87-8	2048	2048
91-4	Negative	Negative
91-3	Negative	Negative
88-6	256	256
76-12	Negative	N/A
94-3	Negative	N/A
94-1	Negative	N/A
89-1	Negative	Negative
89-8	Negative	N/A
89-5	Negative	Negative
87-10	1024	1024
79-1	Negative	N/A
80-7	Negative	N/A
92-9	Negative	Negative
81-2	32	32
80-12	Negative	N/A
81-6	32	32
82-8	Negative	N/A

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
79-3	Negative	N/A
76-5	Negative	N/A
79-9	Negative	N/A
78-4	Negative	Negative
77-8	8192	Negative
84-9	Negative	N/A
77-3	2048	Negative
78-8	Negative	N/A
83-10	Negative	N/A
92-4	64	N/A
78-7	Negative	N/A
94-2	Negative	N/A
81-8	64	64

*not available

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System III piglets at the Ames Plantation in 1991 and in 1992 at the time of slaughter (grow/finishII) determined by MAT.

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
143-1	Negative	N/A*
143-6	Negative	Negative
146-1	Negative	Negative
146-3	Negative	Negative
157-4	Negative	N/A
157-2	Negative	Negative
153-9	Negative	Negative
153-3	Negative	Negative
154-10	Negative	Negative
154-4	Negative	N/A
148-7	Negative	N/A
148-11	Negative	Negative
147-4	Negative	Negative
147-2	Negative	Negative
144-2	Negative	N/A
144-11	Negative	N/A
137-2	Negative	N/A
137-5	Negative	Negative
141-4	Negative	Negative
141-10	Negative	Negative
138-8	Negative	Negative
138-1	Negative	Negative
139-3	Negative	N/A
139-1	Negative	Negative
149-5	Negative	Negative
149-9	Negative	N/A
140-3	Negative	Negative
140-10	Negative	Negative
145-7	Negative	N/A
145-2	Negative	N/A
142-4	Negative	N/A
142-8	Negative	Negative
136-6	Negative	Negative
136-4	Negative	Negative
134-10	Negative	N/A
134-7	Negative	N/A
150-9	Negative	N/A
150-6	Negative	Negative
152-2	Negative	Negative
152-5	Negative	N/A
151-11	Negative	N/A

Pig I.D.	MAT titers at arrival	MAT titers at slaughter
151-1	Negative	Negative
156-1	Negative	Negative
156-7	Negative	Negative
135-6	Negative	N/A
135-1	Negative	N/A
155-11	Negative	Negative
155-2	Negative	Negative
5-3	Negative	Negative
5-4	Negative	N/A
5-5	Negative	N/A
5-7	Negative	Negative
3-4	Negative	Negative
3-1	Negative	N/A
3-3	Negative	N/A

*not available

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System I Grow/finish at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers	Pig I.D.	MAT titers
33-1	Negative	4-1	Negative
33-2	Negative	4-4	Negative
33-5	Negative	4-5	Negative
33-6	Negative	4-8	Negative
13-7	Negative	4-6	Negative
13-9	Negative	4-10	Negative
13-6	Negative	1-3	Negative
13-10	Negative	1-5	Negative
13-4	Negative	1-9	Negative
32-3	Negative	1-10	Negative
32-8	Negative		
32-5	Negative		
32-9	Negative		
32-1	Negative		
15-10	Negative		
15-9	Negative		
15-2	Negative		
15-1	Negative		
15-6	Negative		
21-1	Negative		
21-8	Negative		
21-3	Negative		
21-4	Negative		
12-1	Negative		
12-3	Negative		
12-7	Negative		
12-6	Negative		
12-5	Negative		
23-2	Negative		
23-5	Negative		
23-9	Negative		
23-10	Negative		
23-6	Negative		
6-1	Negative		
6-3	Negative		
14-1	Negative		
14-6	Negative		
14-8	Negative		
14-9	Negative		
14-2	Negative		
14-5	Negative		
7-1	Negative		
7-3	Negative		
7-9	Negative		
7-5	Negative		

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System II Grow/finish at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers
48-6	Negative
N/A*	Negative
61-3	Negative
71-6	Negative
65-11	Negative
52-4	Negative
66-3	Negative
60-1	Negative
72-5	Negative
55-6	Negative
55-9	Negative
58-9	Negative
50-3	Negative
64-6	Negative
58-2	Negative
55-1	Negative
54-1	Negative
48-1	Negative
59-3	Negative
68-5	Negative
62-8	Negative
55-10	Negative
61-3	Negative
60-1	Negative
64-9	Negative
61-8	Negative
60-15	Negative
60-5	Negative
52-5	Negative
51-5	Negative
66-3	Negative
51-10	Negative
53-2	Negative
49-2	Negative
55-2	Negative
58-5	Negative
54-2	Negative
48-3	Negative
52-4	Negative
53-11	Negative
58-6	Negative
55-7	Negative
48-5	Negative
49-1	Negative
55-5	Negative
52-8	Negative
60-7	Negative
54-9	Negative
60-2	Negative
53-6	Negative
69-1	Negative
52-2	Negative
58-1	Negative
48-4	Negative
65-4	Negative

* not available

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System III Grow/finish at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers	Pig I.D.	MAT titers
93	Negative	184	Negative
104	Negative	90	Negative
108	Negative	119	Negative
76	Negative	67	Negative
112	Negative	70	Negative
106	Negative	115	Negative
101	Negative	149	Negative
77	Negative	71	Negative
87	Negative	125	Negative
84	Negative	82	Negative
75	Negative		
86	Negative		
65	Negative		
103	Negative		
3	Negative		
4	Negative		
107	Negative		
100	Negative		
113	Negative		
98	Negative		
79	Negative		
105	Negative		
73	Negative		
110	Negative		
5	Negative		
69	Negative		
111	Negative		
74	Negative		
82	Negative		
71	Negative		
89	Negative		
6	Negative		
88	Negative		
68	Negative		
85	Negative		
83	Negative		
72	Negative		
114	Negative		
102	Negative		
95	Negative		
80	Negative		
92	Negative		
94	Negative		
66	Negative		
96	Negative		

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System I sows at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers
33-4	Negative
4-2	Negative
6-6	Negative
15-3	Negative
3-3	Negative
23-4	Negative
6-8	Negative
14-3	Negative
32-2	Negative
7-2	Negative
9-2	Negative
21-2	Negative
1-1	Negative
13-3	Negative
6-9	Negative
8-3	Negative
12-3	Negative

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System II sows at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers
21938-2	4096
21084-4	Negative
21799-3	Negative
21092-5	Negative
218100-4	1024
21099-2	Negative
21855-2	Negative
21753-1	Negative
21038-6	512
21033-5	8192
21991-4	1024
21713-5	8192
21036-3	Negative
21927-3	1024
21099-4	Negative
218108-3	256
21092-7	Negative
21038-7	8192
21119-4	Negative
21137-4	Negative
2-3	Negative
21121-1	Negative
2119-1	Negative
21121-2	Negative
21121-4	Negative
21121-5	Negative
21137-3	Negative
21130-6	Negative
21119-2	Negative
21140-5	Negative
21119-3	Negative
21121-3	Negative

Anti-*Toxoplasma gondii* IgG titers of sera obtained from System III sows at the Ames Plantation in 1991 determined by MAT.

Pig I.D.	MAT titers	Pig I.D.	MAT titers
318114-6	Negative	1-7	Negative
3193-3	Negative	97-11	Negative
57-9	Negative	97-8	Negative
33097-4	Negative	97-6	Negative
31053-5	Negative	168-8	Negative
31914-5	Negative	168-9	Negative
317123-2	Negative	103-2	Negative
31913-8	Negative		
31037-2	Negative		
31033-11	Negative		
317104-5	Negative		
31048-4	Negative		
19-10	Negative		
17-8	Negative		
49-70	Negative		
49-8	Negative		
26-10	Negative		
1-7	Negative		
31041-3	Negative		
31022-2	Negative		
31041-2	Negative		
31048-2	Negative		
31047-2	Negative		
310102-4	Negative		
33081-1	Negative		
33099-1	Negative		
33080-4	Negative		
330100-6	Negative		
330102-1	Negative		
31036-2	Negative		
31047-6	Negative		
89-8	Negative		
89-6	Negative		
89-9	Negative		
52-11	2048		
90-6	Negative		
92-9	4096		
57-2	Negative		
56-3	Negative		
53-8	Negative		
53-7	Negative		
75-4	Negative		
30-9	Negative		
2-8	Negative		
2-7	Negative		

Appendix J

The Counties of Tennessee and their Abbreviations

1.	Anderson	AN	24.	Fayette	FA
2.	Bedford	BE	25.	Fentress	FE
3.	Benton	BN	26.	Franklin	FR
4.	Bledsoe	BL	27.	Gibson	GI
5.	Blount	BO	28.	Giles	GL
6.	Bradley	BR	29.	Grainger	GR
7.	Campbell	CA	30.	Greene	GE
8.	Cannon	CN	31.	Grundy	Gu
9.	Carroll	CR	32.	Hamblen	HA
10.	Carter	CT	33.	Hamilton	HM
11.	Cheatham	CH	34.	Hancock	HN
12.	Chester	CS	35.	Hardeman	HR
13.	Claiborne	CL	36.	Hardin	HD
14.	Clay	CY	37.	Hawkins	HW
15.	Cocke	CC	38.	Haywood	HO
16.	Coffee	CF	39.	Henderson	HE
17.	Crockett	CK	40.	Henry	HY
18.	Cumberland	CM	41.	Hickman	HK
19.	Davidson	DA	42.	Houston	HS
20.	Decatur	DE	43.	Humphreys	HP
21.	Dekalb	DK	44.	Jackson	JA
22.	Dickson	DC	45.	Jefferson	JE
23.	Dyer	DY	46.	Johnson	JO

47.	Knox	KN
48.	Lake	LA
49.	Lauderdale	LU
50.	Lawrence	LW
51.	Lewis	LI
52.	Lincoln	LC
53.	Loudon	LD
54.	McMinn	MM
55.	McNairy	MN
56.	Macon	MA
57.	Madison	MD
58.	Marion	MC
59.	Marshall	MS
60.	Maury	MU
61.	Meigs	ME
62.	Monroe	MO
63.	Montgomery	MT
64.	Moore	MR
65.	Morgan	MG
66.	Obion	OB
67.	Overton	OV
68.	Perry	PE
69.	Pickett	PI
70.	Polk	PO
71.	Putnam	PU
72.	Rhea	RH
75.	Rutherford	RU
76.	Scott	SC
77.	Sequatchie	SQ
78.	Sevier	SE
79.	Shelby	SH
80.	Smith	SI
81.	Stewart	ST
82.	Sullivan	SU
83.	Sumner	SM
84.	Tipton	TI
85.	Trousdale	TR
86.	Unicoi	UC
87.	Union	UN
88.	Van Buren	VB
89.	Warren	WA
90.	Washington	WS
91.	Wayne	WY
92.	Weakley	WE
93.	White	WH
94.	Williamson	WI
95.	Wilson	WL

DATABASE OF SWINE FARMS

EASTERN TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
22	91	SE	3	1	2	33.3333
35	92	RH	18	9	9	50.0000
37	91	HM	6	0	6	0.0000
42	91	GE	25	0	25	0.0000
45	91	SE	3	0	3	0.0000
51	91	HM	1	0	1	0.0000
58	91	KN	4	1	3	25.0000
81	91	CA	3	0	3	0.0000
101	91	HW	11	6	5	54.5455
134	92	CC	1	0	1	0.0000
151	91	KN	1	0	1	0.0000
154	92	HM	10	10	0	100.0000
155	91	MG	2	2	0	100.0000
176	91	SE	6	5	1	83.3333
183	91	BR	1	0	1	0.0000
221	91	GE	1	1	0	100.0000
226	91	MM	7	7	0	100.0000
228	91	MM	6	2	4	33.3333
249	91	MM	7	4	3	57.1429
275	91	CA	7	3	4	42.8571

DATABASE OF SWINE FARMS

EASTERN TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
295	91	SE	4	1	3	25.0000
298	91	SE	4	0	4	0.0000
305	91	HM	20	13	7	65.0000
*** Total ***			151	65	86	

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
2	92	SI	1	0	1	0.0000
3	91	RU	6	2	4	33.3333
5	91	RU	2	0	2	0.0000
6	91	CF	10	0	10	0.0000
6	92	CF	10	2	8	20.0000
8	91	CF	4	4	0	100.0000
9	91	PU	10	9	1	90.0000
10	92	CF	6	3	3	50.0000
11	91	JA	4	3	1	75.0000
12	91	JA	3	0	3	0.0000
13	91	WL	3	1	2	33.3333
14	92	BL	9	7	2	77.7778
15	91	GU	10	8	2	80.0000
15	92	GU	6	3	3	50.0000
17	91	RU	2	2	0	100.0000
19	91	DC	2	0	2	0.0000
21	91	BE	6	4	2	66.6667
23	92	CM	6	6	0	100.0000
24	91	DK	11	10	1	90.9091
25	91	DK	3	1	2	33.3333

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
26	91	SM	8	1	7	12.5000
27	91	CH	10	7	3	70.0000
28	91	GU	10	10	0	100.0000
28	92	GU	3	3	0	100.0000
30	91	RU	10	6	4	60.0000
34	91	DC	10	3	7	30.0000
36	91	CF	5	5	0	100.0000
38	92	SI	10	0	10	0.0000
39	91	CM	4	0	4	0.0000
41	92	FR	10	0	10	0.0000
43	92	BE	10	9	1	90.0000
44	92	BE	40	20	20	50.0000
46	91	WY	5	3	2	60.0000
47	91	CN	6	5	1	83.3333
49	91	CN	10	6	4	60.0000
53	91	JA	6	3	3	50.0000
54	91	LC	2	0	2	0.0000
56	91	DC	8	2	6	25.0000
57	92	SI	5	0	5	0.0000
59	91	WI	7	5	2	71.4286

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
60	91	LC	15	10	5	66.6667
63	91	CY	2	1	1	50.0000
64	91	MT	10	10	0	100.0000
65	91	LC	5	5	0	100.0000
66	91	CY	12	12	0	100.0000
67	91	LC	5	1	4	20.0000
68	91	WY	6	2	4	33.3333
69	91	WY	10	5	5	50.0000
70	92	OV	2	2	0	100.0000
72	91	DC	10	1	9	10.0000
73	92	LC	1	0	1	0.0000
74	91	DC	10	1	9	10.0000
75	92	RU	5	0	5	0.0000
76	91	CY	8	4	4	50.0000
77	91	DC	7	1	6	14.2857
77	92	DC	10	0	10	0.0000
78	91	CN	10	4	6	40.0000
78	92	CN	10	3	7	30.0000
79	91	LC	8	5	3	62.5000
82	92	CM	30	3	27	10.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
83	91	BL	6	2	4	33.3333
84	91	GU	3	3	0	100.0000
87	91	DA	5	3	2	60.0000
88	91	RU	12	1	11	8.3333
89	91	LC	3	1	2	33.3333
91	92	MU	70	0	70	0.0000
92	91	LC	20	2	18	10.0000
93	91	LC	2	0	2	0.0000
94	92	MU	8	1	7	12.5000
96	91	CN	7	3	4	42.8571
99	91	CN	10	1	9	10.0000
100	92	FR	10	2	8	20.0000
102	91	GU	6	5	1	83.3333
108	91	SM	10	6	4	60.0000
108	92	SM	10	8	2	80.0000
109	91	WI	6	2	4	33.3333
111	92	GU	10	8	2	80.0000
112	91	GU	10	9	1	90.0000
114	91	GU	3	3	0	100.0000
115	91	FR	1	0	1	0.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
116	91	TR	6	6	0	100.0000
117	91	HK	2	1	1	50.0000
118	91	MT	1	1	0	100.0000
121	91	JA	4	3	1	75.0000
123	91	WY	10	2	8	20.0000
123	92	WY	7	1	6	14.2857
124	91	WH	3	3	0	100.0000
126	91	FR	9	4	5	44.4444
129	91	WA	10	2	8	20.0000
131	92	FR	35	0	35	0.0000
132	92	BL	2	0	2	0.0000
135	92	LW	3	1	2	33.3333
136	91	LC	2	2	0	100.0000
137	91	DC	5	1	4	20.0000
138	91	WL	4	2	2	50.0000
139	91	RU	6	5	1	83.3333
140	91	MT	3	2	1	66.6667
141	91	RU	2	2	0	100.0000
146	91	RU	2	1	1	50.0000
148	91	WI	7	0	7	0.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
149	91	LC	4	4	0	100.0000
150	91	LC	14	12	2	85.7143
152	91	LW	10	3	7	30.0000
152	92	MU	10	1	9	10.0000
153	91	LW	7	1	6	14.2857
156	91	GU	3	3	0	100.0000
157	91	WI	4	0	4	0.0000
158	92	BE	10	0	10	0.0000
159	91	WA	4	2	2	50.0000
160	91	JA	10	5	5	50.0000
161	91	CM	5	2	3	40.0000
162	91	HS	11	0	11	0.0000
164	91	RU	2	2	0	100.0000
165	91	HS	5	2	3	40.0000
166	91	OV	3	1	2	33.3333
167	91	LC	2	0	2	0.0000
168	92	LW	10	1	9	10.0000
169	91	LW	53	0	53	0.0000
170	91	SM	3	1	2	33.3333
171	91	GU	2	2	0	100.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
172	91	GU	7	7	0	100.0000
173	91	CF	6	0	6	0.0000
174	91	ST	3	0	3	0.0000
175	92	CF	2	2	0	100.0000
177	91	GU	10	7	3	70.0000
179	92	OV	8	1	7	12.5000
180	91	DC	8	2	6	25.0000
181	92	PE	2	1	1	50.0000
182	92	CM	25	0	25	0.0000
186	92	GU	9	5	4	55.0000
187	92	GU	10	3	7	30.0000
191	91	GU	10	2	8	20.0000
192	91	GU	10	9	1	90.0000
195	92	SI	2	0	2	0.0000
196	92	SI	2	0	2	0.0000
197	91	WH	39	7	32	17.9487
197	92	WH	30	27	3	90.0000
198	91	LI	6	0	6	0.0000
199	92	PE	3	2	1	66.6667
200	91	PE	3	0	3	0.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
201	91	HP	15	0	15	0.0000
203	91	SI	12	0	12	0.0000
204	91	CY	6	5	1	83.3333
205	91	GU	7	5	2	71.4286
208	92	CH	10	1	9	10.0000
209	91	CH	10	4	6	40.0000
210	92	GL	5	0	5	0.0000
211	91	RU	13	11	2	84.6154
212	91	RU	3	3	0	100.0000
213	91	JA	10	7	3	70.0000
214	91	CN	10	8	2	80.0000
214	92	WA	10	8	2	80.0000
217	91	WY	3	3	0	100.0000
218	91	MA	14	10	4	71.4286
218	92	MA	18	5	13	27.7778
220	91	LC	18	14	4	77.7778
222	92	WI	20	0	20	0.0000
223	91	CN	2	0	2	0.0000
225	91	RU	10	5	5	50.0000
229	92	CM	10	1	9	10.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
230	92	ST	9	4	5	44.4444
231	91	WI	4	0	4	0.0000
233	91	CN	5	3	2	60.0000
234	91	CN	3	3	0	100.0000
235	91	LC	10	6	4	60.0000
236	91	DC	3	2	1	66.6667
238	91	CN	10	7	3	70.0000
239	91	CF	10	10	0	100.0000
240	91	HK	10	4	6	40.0000
241	91	JA	4	3	1	75.0000
242	91	SM	10	0	10	0.0000
244	91	LI	10	9	1	90.0000
245	92	CN	10	4	6	40.0000
246	91	WL	3	2	1	66.6667
247	91	DC	10	2	8	20.0000
248	91	CY	5	0	5	0.0000
250	91	MS	1	0	1	0.0000
251	91	PE	30	2	28	6.6667
251	92	PE	30	0	30	0.0000
252	92	BL	10	1	9	10.0000

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
253	92	BL	11	2	9	18.1818
254	91	JA	10	5	5	50.0000
255	91	WY	10	1	9	10.0000
255	92	WY	10	0	10	0.0000
256	92	WY	12	6	6	50.0000
257	91	RU	3	2	1	66.6667
258	91	FR	5	4	1	80.0000
259	91	MT	7	1	6	14.2857
260	91	WI	2	0	2	0.0000
261	92	GU	7	7	0	100.0000
262	92	RU	2	0	2	0.0000
264	92	FR	10	4	6	40.0000
265	91	OV	2	2	0	100.0000
266	91	FR	18	1	17	5.5556
267	91	CY	10	7	3	70.0000
268	91	DC	4	1	3	25.0000
269	91	CN	2	1	1	50.0000
270	91	TR	10	4	6	40.0000
271	91	GU	4	3	1	75.0000
272	91	GU	6	5	1	83.3333

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
273	91	GU	10	8	2	80.0000
274	91	WH	7	0	7	0.0000
276	91	OV	10	10	0	100.0000
276	92	OV	11	7	4	63.6364
277	91	GU	10	2	8	20.0000
277	92	MU	2	0	2	0.0000
278	92	FR	10	5	5	50.0000
279	91	WY	10	2	8	20.0000
282	91	RU	10	8	2	80.0000
283	92	RU	8	2	6	25.0000
284	91	PU	1	0	1	0.0000
289	92	PU	11	1	10	10.0000
291	91	LC	2	1	1	50.0000
292	92	CF	30	0	30	0.0000
293	91	CF	26	0	26	0.0000
294	91	MR	11	2	9	18.1818
294	92	MR	10	2	8	20.0000
296	91	WH	8	8	0	100.0000
297	91	WA	9	2	7	22.2222
299	91	HK	6	2	4	33.3333

DATABASE OF SWINE FARMS

MIDDLE TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
301	91	JA	10	6	4	60.0000
302	91	FR	3	1	2	33.3333
306	91	CF	10	5	5	50.0000
307	92	CF	5	1	4	20.0000
310	91	ST	2	1	1	50.0000
311	91	MT	2	0	2	0.0000
312	91	LC	1	1	0	100.0000
313	91	GU	5	0	5	0.0000
315	91	WH	4	0	4	0.0000
317	91	MU	1	0	1	0.0000
318	91	DA	4	1	3	25.0000
319	91	HS	5	2	3	40.0000
320	91	MT	4	2	2	50.0000
321	91	OV	4	2	2	50.0000
322	91	OV	4	2	2	50.0000
323	91	OV	1	1	0	100.0000
*** Total ***			1976	734	1242	

DATABASE OF SWINE FARMS

WESTERN TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
1	91	HD	4	4	0	100.0000
4	91	MD	7	5	2	71.4286
7	91	GI	3	3	0	100.0000
16	91	DE	10	0	10	0.0000
18	91	OB	14	0	14	0.0000
20	91	DE	2	0	2	0.0000
29	91	HO	4	0	4	0.0000
31	91	CR	13	6	7	46.1538
32	91	HO	2	0	2	0.0000
33	91	HO	12	8	4	66.6667
40	91	HD	1	0	1	0.0000
48	91	WE	2	0	2	0.0000
50	91	DE	10	0	10	0.0000
50	92	DE	7	0	7	0.0000
52	92	MN	72	0	72	0.0000
55	92	CK	30	17	13	56.6667
61	91	HE	10	2	8	20.0000
62	91	HO	4	4	0	100.0000
80	92	FA	203	5	198	2.4674
85	91	BN	3	0	3	0.0000

DATABASE OF SWINE FARMS

WESTERN TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
86	91	DE	10	0	10	0.0000
90	91	HE	10	2	8	20.0000
95	91	DY	26	1	25	3.8462
95	92	LU	15	0	15	0.0000
97	91	MN	10	3	7	30.0000
98	91	CR	3	0	3	0.0000
103	91	DE	10	0	10	0.0000
104	91	DE	10	1	9	10.0000
104	92	DE	20	9	11	45.0000
105	91	WE	52	2	50	3.8462
106	92	OB	10	3	7	30.0000
107	91	OB	11	7	4	63.6364
110	92	HD	15	11	4	73.3333
113	92	HO	3	3	0	100.0000
119	91	OB	5	0	5	0.0000
120	91	HD	2	1	1	50.0000
122	91	CR	8	0	8	0.0000
125	91	MD	5	3	2	60.0000
127	92	MN	10	0	10	0.0000
128	92	MN	10	1	9	10.0000

DATABASE OF SWINE FARMS

WESTERN TENNESSEE

FARM	YEAR	COUNTY	TOTAL SOWS	# POSITIVE	# NEGATIVE	% POSITIVE
130	91	HO	2	1	1	50.0000
133	92	DY	8	0	8	0.0000
142	91	MD	10	6	4	60.0000
142	92	MD	10	5	5	50.0000
143	91	HO	6	3	3	50.0000
144	92	OB	10	6	4	60.0000
145	92	FA	247	0	247	0.0000
147	91	FA	9	5	4	55.5556
163	91	HO	5	0	5	0.0000
178	91	HY	10	0	10	0.0000
184	91	GI	4	0	4	0.0000
185	91	FA	15	4	11	26.6667
189	91	HD	5	2	3	40.0000
190	91	MD	7	4	3	57.1429
193	91	HO	10	1	9	10.0000
194	92	HE	5	1	4	20.0000
202	91	DE	10	0	10	0.0000
202	92	DE	10	0	10	0.0000
206	91	WE	30	0	30	0.0000
207	91	DY	6	1	5	16.6667

Appendix K

SURVEY

1. SWINE PRODUCERS MANAGE THEIR HERDS IN A VARIETY OF WAYS. WHICH ONE WAY WOULD BEST DESCRIBE HOW YOU MANAGE YOUR BREEDING SOWS AND REPLACEMENT GILTS

   _____PASTURE ONLY (SHELTER MAY BE PROVIDED BUT THE SWINE ARE NOT CONFINED TO THIS SHELTER)

   _____TOTAL CONFINEMENT (SWINE ARE TOTALLY ENCLOSED WITH NO ACCESS TO THE OUTSIDE)

   _____PARTIAL CONFINEMENT (THE PIGS SPEND PART OF THEIR TIME OUTSIDE AND PART INSIDE, NEITHER ONE OF THE ABOVE)

2. ARE YOU A:

   _____PRIVATE PRODUCER (FAMILY OWNED FARM)

   _____COMMERCIAL PRODUCER (COMPANY OWNED FARM)

3. WHICH CATEGORY BELOW BEST DESCRIBES YOUR SWINE BREEDING SEASON. PLEASE CHECK ONE.

   _____CONTINUOUS (SOWS & GILTS ARE BRED ALL YEAR LONG SO FARROWING IS CONTINUOUS)

   _____SEASONAL (SOWS & GILTS ARE BRED ONLY AT CERTAIN TIMES OF THE YEAR SO FARROWING OCCURS DURING PART OF THE YEAR

   IF YOU CHECKED "SEASONAL" ABOVE, WHAT MONTHS DO YOU BREED SOWS
   CIRCLE AS MANY AS APPLY

   JAN FEB MAR APR MAY JUN

   JUL AUG SEP OCT NOV DEC

4. ON AVERAGE, HOW MANY BREEDING SOWS AND GILTS DID YOU HAVE ON YOUR FARM DURING THE LAST 12 MONTHS? _____

5. ON AVERAGE, HOW MANY PIGLETS DID YOU FARROW IN THE LAST 12 MONTHS? _____

   HOW MANY DID YOU FARROW IN THE LAST 3 MONTHS? _____

6. DURING THE LAST 12 MONTHS, ABOUT HOW MANY FEEDER PIGS

DID YOU SELL? _____

ABOUT HOW MANY FINISHED PIGS DID YOU SELL? _____

7. DO YOU HAVE ANY CATS ON YOUR FARM? YES NO

IF YES, DO THEY HAVE ACCESS TO THE SAME WATER AS THE SWINE? YES NO

DO THEY HAVE ACCESS TO SWINE FEED STORAGE AREA? YES NO

DO THEY HAVE ACCESS TO SWINE PENS OR PASTURES? YES NO

8. ALL FARMS HAVE RODENTS. WHAT METHODS BELOW DO YOU USE TO CONTROL RODENTS? (CHECK AS MANY AS APPLICABLE).

_____FREE-ROAMING CATS: HOW MANY _____

_____TRAPS: HOW MANY _____

_____CHEMICALS

_____KEEPING FEED STORED IN RODENT PROOF CONTAINERS

_____DESTROYING RODENT HABITAT (TRASH)

_____OTHERS (PLEASE DESCRIBE)

9. ABOUT HOW MANY STILLBORN PIGS DID YOU HAVE DURING THE LAST 12 MONTHS? _____

DURING THE LAST 3 MONTHS? _____

10. PLEASE DESCRIBE ANY ILLNESS YOU SAW IN YOUR HERD LAST YEAR?

THE LAST 3 MONTHS?

11. HOW MANY SOWS DID YOU CULL LAST YEAR? _____
HOW MANY WERE:
A) HOME OR CUSTOM SLAUGHTER _____

B) SOLD FOR SLAUGHTER WITH FINISHING HOGS _____

C) OTHERS (METHOD OF DISPOSAL)_____

12. DURING THE PAST YEAR HAS A VETERINARIAN VISITED YOUR FARM? YES NO
IF YES, HOW OFTEN
ONCE_____

2 TO 6 TIMES_____

MORE THAN 6 TIMES_____

Appendix L

CONSENT FORM

Dear Swine producer:

As part of a research project sponsored by Professor Sharon Patton of the Department of Environmental Practice at the University of Tennessee, I am trying to determine the distribution of the Toxoplama gondii (T. gondii) in Tennessee. This organism lives inside hogs, leaving them less productive, and can eventually lead to aborted piglets.

In 1991 and 1992 the State Diagnostic Laboratory in Nashville, Tennessee sampled your swine herd(s) for psuedorabies. We are asking **YOUR PERMISSION** to use part of this blood that was taken from your sows. We will then test these blood samples for the existence of T. gondii **AT NO CHARGE TO YOU.** Upon your request the results of this test will be mailed to you, and if you have positive samples we will also send you information on how to control this organism. Although your participation in this study is voluntary, it's vital to our research.

All the results will be kept STRICTLY confidential. The State Diagnostic Laboratory will not inform our facility about the status of pseudorabies in your herd(s). The information we obtain could help you improve the wellness of your swine herds in the future. Your answers on the attached survey give us permission to run these samples and also help us to understand this organism better. It will take you about 10 minutes to fill out this survey and return in the envelope provided.

Our current aim is to study the distribution of this organism in Tennessee and work to help farmers eradicate this organism. Please respond as soon as possible. Thank you in advance for your time. If you have any questions or concerns, please feel free to contact Dr. Sharon Patton, at (615) 546-9230 Extension 346.

Sincerly yours,

Dr. Sharon Patton
Amir M. Assadi-Rad

Appendix M

July, 1992

Dear Swine Producer:

Last month we wrote you about our research with Toxoplasmosis in Tennessee swine. We included a survey form which we hoped you would complete and mail back to us. We have not yet heard from you. Would you please take a few minutes to complete the form? We belive this information can eventually improve production on the swine farms in TN; however, our information will be incomplete, if the producers do not respond and mail back the survey forms. Please help us, help you.

We are enclosing a copy of the original letter that we mailed to you and a second copy of the survey form. Do not hesitate to call us if you have questions.

Thank for your help.

Sincerely,

Dr. Sharon Patton

Amir M. Assadi-Rad

VITA

The author was born in Tehran, Iran in December of 1963. He traveled with his family to many different cities as he was growing up because of his father's job. He started primary school in the city of Qum, Iran. He remained there until the eighth grade, when he went to Isfahan for freshmen year. He stayed with his sister in the hopes that his sister would be a good influence on him. He returned to Qum for the tenth grade and then came to the United States of America in 1978, when he was only 16 years of age. He stayed with his brother in Grand Forks, North Dakota and finished high school there. In 1979, he started his undergraduate studies in Biology at Winona State University, Winona, Minnesota. He worked for a short period and then attended Murray State University, Murray, Kentucky in 1984. He obtained two masters from Murray State, one in Biology and the other in Agriculture. After his masters, he worked in several research laboratories, where he was inspired by people with higher levels of education. Therefore, in the fall of 1989, he started a Ph.D. program at the University of Tennessee at Knoxville. His future goal in life is to help other people as others have done for him.