

FOODBORNE TOXOPLASMOSIS

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ABSTRACT

Toxoplasmosis, a disease of mammals and birds, is caused by the obligate intracellular protozoan parasite, Toxoplasma gondii. It is believed that approximately half of the human population of the U.S. is infected and that 500 million of the world population demonstrate a positive serological reaction to the parasite. Only a small percentage of the infected individuals show symptoms; however, in immunocompromised persons, the disease can be quite severe and even fatal. The infectious agent is generally foodborne and is due to the ingestion of raw or undercooked meats derived from infected animals. In this review, several aspects of the T. gondii organism, including its survival, its distribution in the environment and animals, its presence and survival in foods, role of virulence factors, and its transmission to humans, are reviewed. In addition, human toxoplasmosis, its treatment and prevention, and the economic aspects of the disease are discussed.

INTRODUCTION

Toxoplasma gondii, an obligate intracellular protozoan which is ubiquitous in nature, can infect all warm-blooded animals and birds. In humans, clinical toxoplasmosis presents as: (1) self-limiting febrile lymphadenopathy, (2) highly lethal infection of immunocompromised individuals, and (3) congenital disease of infants. However, in healthy adults, infection is generally asymptomatic and toxoplasmosis is not recognized (Plorde 1984).

Human toxoplasmosis can generally be traced to man's close association with animals as pets or food. Approximately one half of the human population of the U.S. are infected with *T. gondii* as evidenced by positive serological reactions (August and Chase 1987), and nearly one half of the British population aged 50 and over have antibodies against the organism (Galbraith and Barrett 1986). It

has been estimated that about 500 million of the world population demonstrate a positive serological reaction to *T. gondii* (August and Chase 1987). The organism, usually ingested from rare or raw meats, eventually encysts in skeletal muscle, heart muscle, brain tissue and other organs of the host; the encysted organism is inactive and is held at bay by the immune system. The use of immunosuppressing drugs, the advent of AIDS and the growing numbers of an older population has led to increased numbers of immunocompromised individuals. In immunosuppressed persons, the resulting reactivation of latent toxoplasmosis (recrudescent infection) often leads to a fatal disease in the immunocompromised individual. Various aspects of *T. gondii* and toxoplasmosis, as well as the behavior of the organism as a food borne pathogen, will be summarized in this review. Early studies on *T. gondii* and toxoplasmosis have been summarized previously by Weinman (1952), Jacobs (1963, 1967, 1973), and Frenkel (1970).

THE ORGANISM

The taxonomic status of *T. gondii* is as follows (Levine *et al.* 1980):

Subkingdom:	<i>Protozoa</i>
Phylum:	<i>Apicomplexa</i>
Class:	<i>Sporozoa</i>
Subclass:	<i>Coccidia</i>
Order:	<i>Euoccidiiida</i>
Suborder:	<i>Eimeriina</i>
Genus, species:	<i>Toxoplasma gondii</i>

An obligate intracellular parasite, *T. gondii* has three infectious stages: (1) tachyzoites (trophozoites, endozoites) are rapidly multiplying forms, (2) bradyzoites (cystozoites) are present in tissue cysts, and (3) sporozoites are present in oocysts and found only in cat (*Felidae*) feces. The definitive hosts are felines, i.e., the sexual cycle of *T. gondii* is completed only in members of the cat family. The disease is acquired by both cats and intermediate hosts through carnivorousness, ingestion of cat feces, and/or by congenital infection. Generally, the cat (e.g., a domestic cat) acquires the disease by ingestion of cysts present in prey or uncooked meats. The cyst wall is dissolved by enzymes present in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine where a few cycles of asexual generation take place. Following asexual development, male and female gametes are produced. When the female gamete has been fertilized by a male gamete, a protective wall is formed producing the oocyst. The noninfective oocysts are shed in the cat feces; upon exposure to air, the oocysts sporulate in one to five days. Air (oxygen)

stimulates sporulation while it is inhibited by anaerobic conditions, by heat at 45–50°C and by temperatures of 4°C or lower. The infective oocysts contain two sporocysts each with four sporozoites. Production of oocysts has not been demonstrated for any animal species other than members of *Felidae* (Dubey 1986c, 1987; Dubey *et al.* 1970; Frenkel 1970).

In the intermediate host, infection occurs via ingestion of tissue cysts or oocysts. The bradyzoites (released from cysts) or sporozoites (released from oocysts) penetrate intestinal cells and multiply as tachyzoites; eventually, they spread to other parts of the body via lymph and blood. Tachyzoites can multiply in most cells and eventually destroy the invaded cells and then invade new cells. As immunity develops, tissue cysts are formed which do not produce a host tissue reaction. They can be found in brain, muscles, heart and visceral organs, and appear to persist for life (cyst formation occurs in felines, also). Cysts may occasionally rupture, but the released bradyzoites are inactivated by the immunocompetent host; this mechanism may aid in keeping immunity high (August and Chase 1987; Plorde 1984; Markell *et al.* 1986).

Survival of *T. gondii*.

Fecal deposits containing infectious oocysts, or a water suspension of feces containing infectious oocysts or washed oocysts (washed free of fecal material) suspended in water retained infectivity for approximately 150–400 days at temperatures ranging from 4–37°C (Yilmaz and Hopkins 1972). Infective samples stored in sunlight generally lose infectivity sooner than those stored in shade. Frenkel *et al.* (1975) found that oocysts present in feces covered by soil retain infectivity for at least one year in Costa Rica and for at least 18 months in Kansas; infectivity persists longer in moist soils than in dry areas. Exposure of cat feces to heat over 77°C for at least 5 min inactivates unsporulated oocysts (Frenkel 1975); presumably, a longer period is necessary if the oocysts have sporulated.

Sporulated oocysts in feces lose infectivity during drying: oocysts are infective for at least 30 days at 100% relative humidity (RH) and they are infective at 7 days but not at 30 days when the RH was 58–80%. Oocysts lose infectivity at 3 days when RH was 0–37% (Dubey *et al.* 1970). Sporulated oocysts are not affected by constant or intermittent freezing at –6°C (alternating with 24 h at room temperature) but some killing is observed when oocysts are subjected to –21°C (Frenkel and Dubey 1973). Unsporulated oocysts are killed within 1–7 days by freezing. Thus, freezing weather alone will not necessarily eliminate toxoplasma from soils.

Oocysts are killed (lose infectivity) by 28% ammonia after 10 min. An iodine solution (7% I₂ + 5% KI) is effective after 30 min (Frenkel and Dubey 1972). Exposure of infectious oocysts present in feces to 1–10% formaldehyde for 24

h is effective in eliminating infectivity. Exposure to 5% ammonia for 30 min or to 10% ammonia for 10 min is also effective (Dubey *et al.* 1970).

Sporulated oocysts are resistant to proteolytic enzymes. Bradyzoites survive in pepsin solution (0.5%) for 120 min but not 180 min. Survival in trypsin (1%) is much longer; there was only a 10^2 reduction in numbers after 180 min (Sharma and Dubey 1981). Tachozoites are more susceptible to proteolytic activity; they survive 15 min but not 60 min in 1% trypsin solution (Sharma and Dubey 1981) and are completely inactivated in 1 h by 0.25% pepsin solution (Jacobs *et al.* 1960b). Bradyzoites are not infectious after 1 h at 50°C or 10 min at 56°C (Jacobs *et al.* 1960b). Washed trophozoites receiving 70 J m^{-2} of ultraviolet (254 nm) radiation are not infective for mice (Grimwood 1980). However, the irradiated trophozoites can attach and enter cultured monolayer cells but are unable to proliferate.

Distribution of *T. gondii* in Nature

Water. Murrell *et al.* (1986) stated that toxoplasmosis in grazing or grain-fed animals may be due in part to drinking water from sources contaminated with infected cat feces. Epidemiological evidence obtained by Benenson *et al.* (1982) indicated that a toxoplasmosis epidemic in soldiers was due to drinking contaminated jungle water. The authors postulated that jungle cats deposited oocyst-containing feces in the water. The drinking of oocyst contaminated water may be important in transmitting *T. gondii* to both animals and humans.

Soil. *T. gondii* oocysts have been isolated in the infective state from a variety of soils (Ruiz *et al.* 1973; Frenkel *et al.* 1975; Coutinho *et al.* 1982). An infected house cat (or other feline) sheds oocysts in feces which are generally deposited in soil and lightly covered (Frenkel and Ruiz 1981). Mice, rats, and small birds can be infected with *T. gondii* when they come in contact with oocyst-containing soils (ingestion of contaminated soil during the process of grooming or by eating coprophagous insects or earthworms). The organism encysts in these animals and thus persists throughout life. These infected small animals probably serve as foci of infection for foraging, noninfected cats. Frankel and Ruiz (1981) suggested that children become infected with toxoplasma when they play in dirt and sand in which cats have defecated, especially if the children are at the age where they put their fingers in their mouths in an indiscriminate manner.

Coprophagous Invertebrates. Dubey *et al.* (1970) demonstrated that earthworms transfer toxoplasma oocysts from deeper to uppermost layers of soil; thus, upper soil layers may become heavily contaminated with oocysts. Earthworms have been found to be infectious, also (Frenkel *et al.* 1975). It is probable that oocysts-containing earthworms transmit toxoplasmosis to birds or other animals that may ingest them.

Flies and other insects are attracted to feces, and cat feces is no exception.

The common house fly and the oriental blow fly, coming in contact with infected cat feces, were able to contaminate milk with oocysts 1–2 days after contact with the feces (Wallace 1971a). The location of the oocysts in the flies was not determined. Oocysts can be isolated from fly larvae and pupae reared in infected cat feces but not from newly emerging adult flies (Wallace 1971a). Frenkel *et al.* (1975) demonstrated that flies, depositing their eggs in oocyst-containing cat feces, may pick up oocysts and transfer them to foods. Oocysts remain viable in the gut of the cockroach and are eliminated in cockroach feces; the oocysts remain infectious in cockroach feces for several weeks (Wallace 1972; Chinchilla and Ruiz 1976). Thus, food can be contaminated by flies or cockroaches that have contacted oocyst-containing feces. Also, contaminated cockroaches may serve as food for birds and small rodents, which in turn can serve as prey for cats.

Cattle. Recently, Dubey (1986a) reviewed the literature on toxoplasmosis in cattle. Older serological tests to detect toxoplasma antibodies in cattle greatly overestimated its incidence; natural toxoplasmosis actually appears to be rare in cattle (Dubey 1986a). Dubey (1986a, 1990a) indicated that there is really no good evidence that *T. gondii* causes a clinical disease in cattle under natural conditions nor are there well-documented natural cases of abortion in cattle induced by toxoplasma.

T. gondii can multiply in bovine tissue, but it appears to be eliminated from tissues rapidly; thus, the significance of beef in epidemiology of toxoplasmosis is uncertain, but at present, beef is considered to be a minor source of infection to man (Dubey 1986d). Similarly, milk from infected cows is not an important factor in the transmission of the disease because *T. gondii* is rarely found in cows's milk (Dubey 1986a, 1990a).

Swine. Dubey (1986b, 1990b) has recently reviewed the literature on toxoplasmosis in pigs. Ingestion of *Toxoplasma* infected pork containing bradyzoites (tissue cysts) is considered to be the major meat source of toxoplasmosis in the human population, especially if the meat is undercooked (Dubey 1986b). It is uncertain how pigs acquire *T. gondii* in nature, but the omnivorous habits of pigs continually expose them to the organism. They may be infected via cannibalism; ingestion of small intermediate hosts found in the barnyard such as rats, mice, birds, or fowl; or by feeding on uncooked garbage (Weinman and Chandler 1956). Since pigs are rooters, ingestion of oocyst-containing soil is a potential route of infection, also.

Clinical toxoplasmosis sometimes ending in fatality occurs mostly in young pigs. Typically, older pigs exposed to *T. gondii* have a subclinical infection and appear asymptomatic. Oocyst ingestion induces a more severe infection than ingestion of tissue cysts (Dubey 1986b). Durfee *et al.* (1974) show that ingestion of as few as four oocysts induces infection in pigs; regardless of the number of

oocysts ingested (up to 150), infection occurred without evidence of clinical disease.

A number of studies concerning the seropositivity of pigs in the U.S., and summarized by Dubey (1986b), indicate that 32% are positive (3707 pigs were assayed) for *Toxoplasma* antibodies; the range varies from less than 1 to 69%. Of 73,717 pigs examined worldwide, the seropositive incidence for *T. gondii* is 22% (Dubey 1986b) with ranges from 0 to 97%. Since a large number of the world's swine population are infected with *Toxoplasma*, consumers eating undercooked pork are at risk.

Sheep and Goats. Combining data from several surveys (Vanderwagen *et al.* 1974; Waldeland 1976; Cross *et al.* 1976; Riemann *et al.* 1977; Ruppanner *et al.* 1978; McColm and Hutchison 1981; Dubey 1985b; Chhabra *et al.* 1985; Dubey and Livingston 1986; O'Donoghue *et al.* 1987), the prevalence of *T. gondii* seropositivity in sheep is approximately 21% of 5862 animals tested while that of goats is approximately 25% of 2795 tested. In a survey reported by Dubey (1990c), the incidence of *T. gondii* seropositivity in sheep was approximately 37% of 5936 sheep tested and that of goats was approximately 23 of 2449 tested. It would appear that most cases of toxoplasma in sheep and goats are subclinical. Death, however, can occur in goats if large numbers of oocysts are consumed or if the animals are in poor health (Dubey *et al.* 1980b; Mehdi *et al.* 1983).

The major clinical effect of *Toxoplasma* infection of sheep and goats is abortion. Examination of aborted fetuses and dead lambs revealed positive titers for *T. gondii* antibodies of 16.2% in 2164 samples (Seefeldt *et al.* 1989; Dubey and Kirkbride 1990; Dubey *et al.* 1990b). Thus, *Toxoplasma*-induced abortions can lead to large economic losses in sheep raising. Abortion occurs when animals are infected during pregnancy, while infection before pregnancy protects the fetus from toxoplasmosis. *T. gondii* infections in pregnant goats and sheep can lead to early embryonic death and resorption, fetal death, abortion, stillbirth, neonatal death or weak lambs or kids, depending on stage of pregnancy at the time of infection (Dubey 1981c; Dubey *et al.* 1981; Dubey and Schmitz 1981; Huffman *et al.* 1981; Dubey and Kirkbride 1984; Behymer *et al.* 1985; Dubey *et al.* 1986b; Dubey *et al.* 1986d; Dubey and Welcome 1988). Sheep that are seropositive or have previously aborted (due to *Toxoplasma* infection) generally have normal pregnancies (Dubey and Welcome 1988).

It is not known how sheep or goats acquire *T. gondii* infection. While it is not passed from sheep to sheep, possible sources of infection are cat feces present in hay (Behymer *et al.* 1985) or in straw bedding spread onto pasture land (Faull *et al.* 1986). Sheep graze close to the ground and oocysts present on pasture could be ingested (Waldeland 1976). There is a report of finding *T. gondii* in semen of goats, but it is not known whether these organisms can be transmitted venereally (Dubey and Sharma 1980b).

Sheep and goats infected with *T. gondii* show encysted *Toxoplasma* in various tissues of the body including edible tissues (Waldeland 1976; Dubey *et al.* 1980b); thus, the meat from infected animals is a potential source of *T. gondii* to consumers.

Fowl. In surveys of native (U.S.) wild birds (Franti *et al.* 1975, 1976; Burrige *et al.* 1979), only 28 out of 1800 birds examined were positive for *Toxoplasma* antibody (1.6% positive). None of the positives were birds that are used for human consumption but could serve as food for carnivorous animals. However, the incidence of *Toxoplasma* in birds is probably much higher since infected birds generally have low or nonexistent titers (Hubbard *et al.* 1986). Zoo birds, canaries, and other cage birds have been found to be susceptible to *T. gondii* also (Parenti *et al.* 1986; Hubbard *et al.* 1986). *T. gondii* has been found in tissues of seronegative chickens, including edible tissues (Dubey 1981a, 1986d). Biancifiori *et al.* (1986) experimentally inoculated laying hens with either 5000 or 50,000 oocysts (via the crop). Neither group showed signs of clinical illness. *T. gondii* could be isolated from brain, heart and liver at 2 weeks after inoculation with 5000 oocysts, whereas the spleen and lung also showed the organism at 2 weeks after inoculation with 50,000 oocysts. At six weeks after inoculation, only the brain and heart contained infective *T. gondii* regardless of inoculum size. In hens inoculated with 50,000 oocysts, there was a decrease in egg production as well as extensive mortality of embryonated eggs. *T. gondii* could not be isolated from 720 eggs laid by these hens (Biancifiori *et al.* 1986).

Pigeons, infected with 500–5000 oocysts, developed clinical signs of toxoplasmosis ending in death (Biancifiori *et al.* 1986); those pigeons receiving only 50 oocysts showed no sign of clinical illness. Regardless of the size of the infective dose, the parasite can be isolated from several organs as well as from muscles of pigeons.

Fowl is probably not an important cause of human toxoplasmosis because it is normally well-cooked before consumption (Dubey 1986d). However, wild fowl and barnyard fowl infected with *T. gondii* probably contribute to the prevalence of the parasite in felines.

Horses. Data from a number of surveys (Vanderwagen *et al.* 1974; Riemann *et al.* 1975b; Tizard *et al.* 1978; Al-Khalidi and Dubey 1979; Chhabra *et al.* 1985) suggest that the incidence of *Toxoplasma* seropositivity in horses is 14.7% (2747 animals). Older animals are more likely to be positive than younger animals. Al-Khalidi and Dubey (1979) obtained infectious *T. gondii* from the tissues of both seropositive and seronegative horses. Equids (horses, ponies, and a mule), experimentally infected with 10,000–100,000 oocysts via the oral route remained clinically normal except for fever in some of the ponies (Al-Khalidi *et al.* 1980; Dubey 1985a). Thus, clinical cases of toxoplasmosis in equids appear to be rare; however, *T. gondii* can remain for several months after infection in various tissues including skeletal muscle.

Wild and Zoo Animals. The prevalence of toxoplasmosis in American wild game animals has been poorly studied. Columbia black-tailed deer (382 tested) in northern California were found to be 20% seropositive for *T. gondii* (Franti *et al.* 1975), whereas only 3% of white-tailed deer (30 tested) in Florida are positive (Burrige *et al.* 1979). Dubey (1985b) reported that 3.1% of bison (of 93) were seropositive but none of 56 elk were positive. Clinical toxoplasmosis has been induced in pronghorn and mule deer by intraruminal inoculation of oocysts (Dubey *et al.* 1982) but not in elk (Dubey *et al.* 1980a). Bradyzoites could be found in tissues and muscles of all of the inoculated animals, both deer and elk. Encysted *T. gondii* were found in the musculature of naturally infected moose, pronghorn, white-tailed deer and mule deer (Dubey 1981b, 1982b; Lindsay *et al.* 1991). It is probable that most wild ruminants that are hunted in the U.S. are susceptible to *T. gondii* and their meats may possibly serve as parasite reservoirs.

Toxoplasma antibodies are present in other wild animals (Miller *et al.* 1972; Franti *et al.* 1975, 1976; Tizard *et al.* 1978; Burrige *et al.* 1979; Frenkel and Sousa 1983; Childs and Seegar 1986; Jackson *et al.* 1986). Of particular interest is the presence of toxoplasma antibodies in small wild animals that are in or close to the human habitat. In the U.S., these include house and other mice, both the Norway and black rat, raccoons, skunks, squirrels, and opossum.

Captive and zoo animals are seropositive for *T. gondii*, also. Riemann *et al.* (1974) tested the sera of 109 zoo animals for evidence of *Toxoplasma* exposure. They found that 32% of the animals were positive with the highest prevalence in felines, marsupials, and canines. Fifty-three percent of the carnivores were positive for *T. gondii* antibodies, whereas only 12% of ungulates were positive. Dubey *et al.* (1985) recommend that all meats fed to zoo animals should be cooked at 66°C (internal temperature) for 30 min, felines should be spatially separated from other zoo animals, and that animal caretakers should be trained to prevent contamination of cages by material potentially containing oocysts. Access to zoos by feral cats (*Felis domestica*) should be prevented since they can easily contaminate animal bedding and feeds with oocysts, but it is probably impossible to eliminate feral cats from zoos.

Reptiles. *Toxoplasma* antibodies and/or parasites cannot be demonstrated in lizards (Miller *et al.* 1972) or in tortoises, turtles, alligators, or snakes (Burrige *et al.* 1979).

Dogs. Dubey (1985c) has recently reviewed the incidence of toxoplasmosis in dogs (*Canis familiaris*). All dog breeds appear to be susceptible to *T. gondii*. Combining the data presented by Dubey (Table 1 in Dubey 1985c, including only those dogs with no clinical signs) with that of Childs and Seegar (1986), the seropositivity for *T. gondii* in U.S. dogs is 17% (4532 animals tested) with ranges of 0 to 80%. Worldwide, the prevalence is 29% (19,613 tested) with ranges from 0 to 94%. In most dogs, infection is subclinical with clinical cases

TABLE 1.
PREVENTION OF *T. GONDII* INFECTION

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- A. Home environment
1. All meats should be cooked to 70°C internal temperature (a distinct color change); meats should not be tasted during preparation or cooking.
 2. Hands, cutting boards, countertops, utensils should be thoroughly washed after contact with raw or undercooked meats. Do not touch mucous membranes of nose, eyes, or mouth until after hands are washed.
 3. Avoid raw eggs and goat milk; thoroughly wash fruits and vegetables, particularly if eaten raw.
 4. Do not let cockroaches, flies, or other insects come in contact with foods.
 5. Pet cats should not be allowed to hunt in order to prevent infection from rodents or birds.
 6. Do not feed pets raw or undercooked meats; feed only dry, canned, or thoroughly cooked foods.
 7. Cat litter should be removed daily by a family member *not at risk* and sealed in a plastic bag for disposal or the litter can be incinerated (wear gloves, and hands should be washed thoroughly afterwards).
 8. Cat litter boxes should be disinfected regularly with hot, boiling water poured repeatedly over the litter for a 5 min period.
 9. Individuals at *high risk* should wash hands thoroughly after handling cats.
- B. Outdoor environment
1. Gloves should be worn during gardening, since cats like to defecate in such soils.
 2. Geophagy (pica) in children should be prevented.
 3. Sandboxes should be covered when not in use; if the sand is contaminated with cat feces it should be discarded, since children have poor hygienic practices during play.
 4. Do not drink untreated waters from lakes or streams.
- C. Farm environment
1. Pregnant women should not be involved in lambing.
 2. During lambing, remove all placentas and aborted materials from access to cats or rodents.
 3. Keep ewes that are seropositive to *T. gondii*.
 4. Use rodenticides and traps instead of cats to control rodents.
 5. If cats are present, keep them out of feed-storage facilities particularly for meat animals; do not allow cats to defecate in bedding materials.
 6. Provide farm cats with easily accessible source of dry or canned food; do not give cats uncooked meats.
 7. Do not give pigs uncooked foods; prevent cannibalism in pigs.
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These suggestions for preventing *T. gondii* infections are a composite of ideas from Jones (1973), Hall (1986), August and Chase (1987), and Lautenslager (1987).

usually appearing in dogs under one year of age. Fatal toxoplasmosis has been reported in young dogs and in dogs concurrently infected with distemper virus; this probably occurred because distemper causes immunosuppression (Dubey 1985c). Other canines (*Canidae*) such as fox, coyote, and wolf are also susceptible to toxoplasma (Miller *et al.* 1972; Riemann *et al.* 1974; Franti *et al.* 1976; Burrige *et al.* 1979; Dubey 1982a). Since dogs do not produce oocysts, and it has never been shown unequivocally that dogs contain *T. gondii* in their saliva, they are not considered to be a source of *Toxoplasma* infection to their owners (Dubey 1985c). Thus, there is no direct infection to humans via dogs.

Cats. Members of the cat family (*Felidae*) are the only animals known to shed *T. gondii* oocysts; however, in terms of human disease, the common house cat, both domestic and feral, is far more important. In the U.S., out of 3957 cats (*Felis familiaris*) tested for *T. gondii* antibodies, 30% are positive. Worldwide, 37% of 12,688 cats are positive (Dubey 1986c; Childs and Seegar 1986). Cats over 6 months of age are 3–4 times more likely to have antibodies for *T. gondii* than cats under 6 months of age (Wallace 1971b). Other felines have also been shown to have antibodies against *T. gondii* including the bobcat (Franti *et al.* 1975, 1976; Burrige *et al.* 1979; Oertley and Walls 1980), the African lion (Feldman 1982) as well as other wild *Felidae* (Riemann *et al.* 1974). While the shedding of oocysts by the domestic cat is well documented, the shedding of oocysts by other felines has not been well studied. *T. gondii* oocysts are shed by felines such as bobcat, mountain lion, tigers, leopards as well as other members of the cat family (Miller *et al.* 1972; Dubey 1987). It is probable that all *Felidae* acts as the definitive host for *T. gondii* and will shed oocysts.

Occurrence of *T. gondii* in Foods

In industrialized countries, meat containing *Toxoplasma* cysts probably serves as the major source of human toxoplasmosis. While a limited number of people prefer raw or undercooked meats, changes in eating habits—foods are eaten away from home more often—may expose more consumers to undercooked meats which might not occur if the meals were prepared at home. Modern cookery methods, such as the use of microwave ovens, may not expose meats to proper time-temperature inactivation which would ensure the destruction of *Toxoplasma* tissue cysts. As the U.S. population becomes more sophisticated in their food preferences, there is more willingness to try new food combinations which may include raw or undercooked meats with resultant exposure to *T. gondii*.

Beef and Veal. Beef and veal seem to be less often contaminated with *T. gondii* than other meats; however, it should not be assumed that eating raw beef poses no danger from toxoplasmosis. *T. gondii* was found in one or more tissues of 201 cattle (5%) out of a total of 4302 examined (Dubey 1986a). While the sample size was small, the results indicate that the presence of the parasite in tissues of cattle is low. *T. gondii* could not be demonstrated in milk or colostrum of experimentally infected cows (Stalheim *et al.* 1980). Dubey (1986a, 1990a) concluded that although *T. gondii* can be found to persist in bovine tissues, the epidemiologic significance of this fact is uncertain since so few animals have been tested. Beef could become contaminated with *T. gondii* via another route. *T. gondii* cysts present in pork, mutton, or lamb may be ruptured during grinding or cutting and contaminate the equipment with bradyzoites. If thorough cleaning is not done, these bradyzoites can cross-contaminate other meats that are further processed using the same equipment. Thus, use of a contaminated grinder or

knife could introduce *T. gondii* into ground beef or other beef cuts (Dubey 1986d). Many people consume undercooked beef and consumption of beef contaminated by other (infected) meats could lead to toxoplasmosis.

Small Game Animals. Gray squirrels have been shown to develop toxoplasmosis and the parasite has been isolated from their tissues (Roher *et al.* 1981). Rabbits, too, can become infected with *T. gondii*; thus, rabbit meat may be an important source of tissue cysts (August and Chase 1987). Caution should always be used in the preparation of small game meats to ensure that cysts are not ruptured and bradyzoites accidentally enter a cut on the hand or contaminate knives. Of course, thorough cooking is a must.

Fowl. *T. gondii* has been isolated from tissues and organs of chickens and pigeons (Dubey 1981a; Biancifiori *et al.* 1986). However, hen eggs do not appear to contain the parasite (Biancifiori *et al.* 1986). Poultry is probably not an important source of toxoplasmosis in humans because poultry is normally well cooked before it is served.

Horse Meat. Cysts persist in horse tissues for long periods (Al-Khalidi and Dubey 1979; Dubey 1985a). In the U.S., eating horse meat is rare. Horse meat is a common ingredient in pet foods in the U.S. and pets should be fed thoroughly cooked horse meat (internal temperature of 70°C). In those countries where horse meat is consumed, eating of raw or undercooked horse meat may pose the risk of toxoplasmosis.

Deer and Elk Meat. *T. gondii* was found in tissues of naturally infected moose, pronghorn, and mule deer (Dubey 1981b, 1982b). Pronghorns, mule deer, and elk experimentally infected with *T. gondii* showed evidence of tissue cysts in various tissues (Dubey *et al.* 1980a; Dubey *et al.* 1982). Wild animals can serve as a source of toxoplasmosis during eviscerating and handling of meats by hunters. If the meat is served undercooked, the parasites, if present, will be infectious. Toxoplasmosis has been associated with the eating of undercooked venison (Sacks *et al.* 1983). Viscera from infected animals that have been field dressed may serve as sources of *T. gondii* to *Felidae* and other wild carnivores.

Mutton, Lamb and Goat Meat. The prevalence of *T. gondii* in market lambs is unknown but has been estimated to be more than 5% (Dubey 1986d); Waldeland (1976) estimated that 10–15% of the lamb carcasses in Norway contained encysted *T. gondii*. Viable *T. gondii* cysts were isolated from edible lamb tissues (Dubey and Kirkbride 1989), and tissues from mature sheep were shown to contain cysts (Jacobs *et al.* 1960a; Work 1967; Waldeland 1976; Dubey and Sharma 1980a). Lamb is not a commonly consumed meat in the U.S. and mutton is even less so. However, mutton is used in pet foods and should be thoroughly cooked before use.

T. gondii were present in the tissues of goats and kids naturally and experimentally infected with the parasite (Dubey 1980, 1981c; Dubey *et al.* 1980b). The parasite was also present in the milk produced by goats infected experi-

mentally with *Toxoplasma* oocysts (Dubey 1980; Dubey *et al.* 1980b), but not in the milk of naturally infected goats (Dubey 1980). Infants may be more susceptible to tachyzoites in goat milk than adults because the concentration of proteolytic enzymes in the infant gastrointestinal tract may be insufficient to kill the parasites (Dubey 1986d). Obviously, pasteurization of goat milk would kill any *T. gondii* present.

Pork. Eating of undercooked pork is believed to be the major cause of toxoplasmosis in humans. The distribution of *T. gondii* in swine tissue has been documented by Dubey (1986b; Table 2); out of 7313 pigs tested, 724 (10%) had tissue cysts in one or more tissues or organs. However, only a limited number of the samples represented edible tissue.

T. gondii is present in edible tissue (skeletal muscle, heart, liver, brain) of experimentally infected pigs (Durfee and Chien 1971; Prickett *et al.* 1985; Dubey *et al.* 1984). Dubey *et al.* (1984) found that *T. gondii* persisted in edible tissue of live pigs for at least 171 days; in a later study, Dubey (1988) found viable *T. gondii* in edible tissue of live pigs more than 800 days after infection. Tissue cysts of the parasite are found in commercial cuts of pork prepared from both naturally and experimentally infected pigs (Dubey *et al.* 1986c; Dubey 1988). Heart and tongue contained more tissue cysts than other organs or tissue (Dubey 1988). Since viable *T. gondii* may be found in pork, knives and other implements used in preparation of pork should be cleaned thoroughly as possible before using for other purposes. Cuts on the hands should be covered to avoid penetration of bradyzoites from ruptured cysts. During the preparation of pork sausages, tasting of the raw meats must be avoided. Generally, undercooked pork is not consumed in the U.S., and thorough cooking of pork will obviate any toxoplasmosis risk. However, in some countries, raw pork is consumed which offers some risk of *Toxoplasma* infection. Raw pork should not be given to pets. Dubey (1988) recommends cooking all pork meats (including brains) to an internal temperature of 70°C before consumption by humans or pets.

Thus, *T. gondii* has been found in various tissues, including edible tissue, in most, if not all warm-blooded meat animals. And the parasite appears to persist in the animal for long periods, probably for its lifetime. However, there is a need for surveys of market meats to determine the incidence of *T. gondii* in the marketplace. It is probable that a goodly part of the raw meats being sold today contain *Toxoplasma*. The limited amount of screening done for presence of viable *T. gondii* in meats may represent the difficulty of the bioassay which consists of feeding of tissue to cats and observing for oocyst excretion or by intraperitoneal inoculation of tissue digest into mice and then searching for tissue cysts.

Nonmeat Foods. Cats often defecate on soft ground such as gardens and usually bury their feces (Dubey 1986d). It is plausible, therefore, that certain garden produce, in particular root crops, may be contaminated with *T. gondii* oocysts. If oocysts are present in the soil, then some oocysts could easily be

present on the vegetables. Washing, peeling or cooking should remove or kill the oocysts from the vegetables. However, children may obtain root vegetables "straight out of the garden" and give them only a perfunctory cleansing before eating.

Elimination of *T. gondii* from Foods

Salting, curing or heating meats generally renders *T. gondii*, if present, non-infectious. However, little is known about the effect of commercial meat curing conditions on the viability of *T. gondii* cysts. *T. gondii* appears to be more sensitive to meat curing conditions and processes than *Trichinella spiralis* (Kotula 1990). Since cured meats are processed to ensure inactivation of *T. spiralis*, it is assumed that *Toxoplasma* is inactivated also, but whether inactivation of *T. gondii* really occurs has not always been determined. Research is urgently needed to determine the effects of meat curing technology (curing salts, water activity, fermentation, drying, etc.) on the viability of *T. gondii* cysts.

T. gondii cysts survived for at least 10 days in pork muscle and 30 days in pork brain at refrigerated temperatures of 2–5°C (Weinman and Chandler 1956). Hearts from sheep naturally infected with *T. gondii* were infectious to cats fed the tissue that had been stored at room temperature for 6 h, 4 days at 4°C, or at –20°C for 2–3 h (Dubey 1974), but were not infectious to cats when the heart muscle tissue was stored for 11 days at –20°C. Heart, tongue, and limb muscle from *T. gondii* infected pigs was ground, mixed and either stored frozen (–12°C) or refrigerated (5°C); cats receiving the meat frozen four days did not shed oocysts, whereas cats receiving the refrigerated meat did shed oocysts (Dubey 1988). Kotula *et al.* (1991) demonstrated that *T. gondii* cysts present in pork were inactivated by freezing more readily than *T. spiralis*; *Toxoplasma* was inactivated instantly by freezing at –12.37°C.

Using meat from naturally infected swine, Work (1971) found that meat products cooked, either by frying, roasting, or cooking in water, to an internal temperature of 80°C and maintained for several min (to reach doneness) do not contain infectious tissue cysts of *T. gondii*. These products include fried meatballs, pork chops, spareribs, and shank. In a report concerning fast foods, Paradisi *et al.* (1985) interpret Work's study to mean that meat must reach an internal temperature of 80°C and must be maintained at that temperature (or higher) for 20 min. Paradisi and his coworkers (1985) report that hamburger cooked 4–5 min only reaches an internal temperature of 58–60°C; hamburgers cooked to higher internal temperatures are less appetizing due to overcooking. Florentine steaks (a T-bone cut) cooked in the traditional way were quite rare and reached an internal temperature of 32–35°C. These conditions probably do not eliminate *Toxoplasma*; therefore, Paradisi *et al.* (1985) suggest that current methods for

food preparation may lead to increased risk of toxoplasmosis. The Food and Drug Administration's draft unicode for pork cooking temperatures indicates that pork products should reach an internal temperature of 73.9°C in order to ensure inactivation of *Toxoplasma* cysts (Anon. 1980). However, Dubey *et al.* (1990a) have shown that *T. gondii* cysts present in pork tissue are generally killed by heating to an internal temperature of 61°C for 3.6 min. Dubey *et al.* (1990a) derived an equation describing heat inactivation of *T. gondii* cysts. Use of the formula should enable food processors to achieve the correct time-temperature relationships to ensure that heated foods do not contain viable *Toxoplasma* cysts.

Muscle from tongue and heart as well as skeletal muscle from pigs orally inoculated with *T. gondii* oocysts were irradiated and then fed to cats (Dubey *et al.* 1989a). Cats fed meat which was irradiated with 25 or more krad (cobalt-60 or cesium-137 sources) do not shed oocysts. Treatment of pork with 16 krad (cesium-137 source) leads to inactivation of *T. spiralis* (Brake *et al.* 1985); thus, radiation conditions that ensure trichinosis-free pork is not sufficient to inactivate *T. gondii* tissue cysts. The Food and Drug Administration has approved radiation doses of 100 Krads for rendering meats noninfectious for *T. gondii*.

Detecting *T. gondii* and/or Its Antibody

The classical method to detect *T. gondii* is to inject, intraperitoneally, tissue or bodily fluids containing bradyzoites or tachyzoites into mice and examining for the presence of brain cysts or presence of the organism in the peritoneal exudate. The test may take more than a month. Derouin *et al.* (1987) inoculated both mice and human embryonic tissue cells with either bradyzoites or tachyzoites. Both tests are equally sensitive; however, the mouse test takes up to 45 days, whereas the tissue culture assay takes less than a week. In AIDS patients with pulmonary toxoplasmosis, inoculation of tissue culture with lung biopsy material or bronchoalveolar lavage fluid could demonstrate the presence of *T. gondii* in 2 days (Derouin *et al.* 1989). Tissue culture would seem a logical replacement for mice since it would be cheaper, demand less space and give faster results. Other workers have used tissue culture to produce relatively pure *T. gondii* with low contamination from foreign material and to produce secreted antigens and whole cells for use in various immunoassays (Hughes *et al.* 1986).

The classical serological test for the presence of *Toxoplasma* antibody is the Sabin Feldman dye test. Live *T. gondii* is incubated with complement (fresh normal human serum), and dilutions of the test serum. Upon addition of methylene blue, the organisms stain blue if the serum is negative for antibodies against *Toxoplasma*; however, if the serum is positive, the organisms are damaged and bind very little stain (August and Chase 1987; Fleck 1989). The test is very sensitive but is costly, hazardous and difficult to do in field situations since fresh normal human serum and living organisms are required. Other tests are avail-

able—indirect fluorescent antibody, complement fixation, indirect hemagglutination, latex agglutination, direct agglutination, immunosorbent agglutination—but nowadays, the enzyme linked immunosorbent assay is used because of its sensitivity and ease of use. A number of enzyme immunoassays for detection of human immunoglobulin (IgM and IgG) anti-*Toxoplasma* antibodies have been developed (Tomasi *et al.* 1986; Herbrink *et al.* 1987; Fuccillo *et al.* 1987; Rotmans *et al.* 1988; Schaefer *et al.* 1989; Sluiters *et al.* 1989). There are also commercial serodiagnostic kits available for detecting *Toxoplasma* antibodies (Moyer *et al.* 1987; Smith and Repetti 1987; Wilson *et al.* 1987). These kits should prove useful for rapid screening and for routine use in clinical laboratories.

By use of the polymerase chain reaction (PCR), Burg *et al.* (1989) were able to amplify a particular segment of DNA (called B1) of *T. gondii* tachyzoites. By amplification of the DNA, they were able to detect 10 *Toxoplasma* organisms in the presence of 100,000 human leukocytes. The combination of PCR with the use of a DNA probe could prove quite useful in clinical laboratories. By amplification of the B1 segment of *T. gondii*, Grover *et al.* (1990) were able to diagnose congenital toxoplasmosis before birth by use of amniotic fluid. Savva (1989) developed a DNA probe for tachyzoites of *T. gondii* by cloning *Toxoplasma* DNA fragments in *E. coli* plasmids.

At the present time, it is difficult to detect the presence of *T. gondii* in foods. A food, usually meat, can be ground, digested and injected into mice or inoculated into tissue culture. A better procedure would be to determine a gene sequence of cyst bradyzoites and use PCR to amplify the DNA. A suspect meat would be ground to rupture the cysts to release the bradyzoites and then subjected to PCR. The amplified bradyzoite DNA could then be detected with the appropriate DNA probe.

Transmission of *T. gondii* to Humans

Ingestion of Oocysts. Meat animals can ingest oocysts from grains or feeds contaminated by *Toxoplasma*-infected cats. Consumption of meats from animals infected by eating such feed may lead to toxoplasmosis. If contaminated grains are ground and mixed, aerosols containing oocysts will be widely disseminated in the farm environment with possible inhalation by animals and humans. Farm personnel handling contaminated grains and feeds may get oocysts on their hands and they may later contaminate human food or food preparation equipment. Faull *et al.* (1986) implicated straw bedding (contaminated with cat feces) which had been spread on pasture land as the source of oocysts leading to toxoplasmosis in a sheep flock. Removal and spreading of oocyst-contaminated animal bedding could create aerosols that may be infectious to farm workers. Similarly, the householder who handles cat litter may get oocysts on his or her hands or if the litter is dry, inhalation of aerosols containing oocysts is possible.

Children playing in areas frequented by cats may get oocysts on their hands and then may infect themselves by putting their fingers in their mouths. Coprophagous insects may introduce oocysts into foods stored under unsanitary conditions.

Miller *et al.* (1972) presented evidence which indicated that laboratory infections by oocysts can occur. Laboratory personnel who handle oocyst-containing material can become seropositive to *T. gondii*.

Ingestion of Tissue Cysts. Meat animals that have been infected with *T. gondii* eventually form tissue cysts containing large numbers of bradyzoites. Upon ingestion of meat from infected animals, digestive juices break down the cysts to release the bradyzoites which are then transformed into the invasive form, tachyzoites, which will eventually form tissue cysts in the new hosts.

Cooking destroys bradyzoites but ingesting raw or rare meats may lead to infection. Since a tissue cyst may be ruptured during cutting or grinding, even the handling of raw meats may be hazardous, particularly if the person has a cut on the hands, or bradyzoites from a ruptured cyst may be transferred by hand or via cutting tools to other foods. Knaus (1975) studied 6106 pregnant women for their immunological reaction to *Toxoplasma*; 5012 of the women admitted to eating raw meat whereas 1094 did not. Those women who ate raw meat were 44% seropositive for *T. gondii* whereas those that did not had only 29% seropositive reactions. Durfee *et al.* (1976) studied a particular population in Indonesia and determined that the percent seropositive individuals was 31% and the seropositivity of goats in that area was approximately 60%. A major food item in the area was lightly cooked goat meat; epidemiological studies indicated that the source of infection was goat meat. In a German study, Braveny *et al.* (1977) found that 42% of 337 patients with acute toxoplasmosis admitted to eating raw meats whereas only 21% of 198 seronegative patients admitted to eating raw meats. In a study involving Japanese farmers, Konishi and Takahashi (1987) found that *T. gondii* seropositivity was greater in males than females and seropositivity was correlated with the eating of raw meat. It would appear, therefore, that there is a relationship between eating raw or rare meats and having toxoplasmosis or being seropositive for *T. gondii*.

Prenatal Transmission. *T. gondii* infection during pregnancy exposes the fetus to risk of intrauterine infection and the risk increases as pregnancy progresses. In approximately half of cases of maternal toxoplasmosis, the fetus will be infected also. If the infection occurs during the first trimester, 17% of the infants will be infected; it is 24% if the mother is infected during the second trimester and 62% for the third (Swartzberg and Remington 1975). Thus, the later the infection during pregnancy, the greater the chance that the infant will be infected with *T. gondii*, also. Severity of the disease in the infant is greatest when maternal infection occurs in first trimester (McCabe and Remington 1988). There does not appear to be a risk if the woman is seropositive before pregnancy.

In the U.S., frequency of congenital infection with *Toxoplasma* ranges from 10–40 per 10,000 live births. Approximately 3000 infants per year will be infected and of these, one-third will either die or show adverse symptoms at birth (Swartzberg and Remington 1975). The remaining asymptomatic babies will develop symptoms either as children or young adults.

Jackson *et al.* (1986) has suggested that congenital toxoplasmosis may occur in successive litters from an infected dam in rodent populations. Thus, a congenital mode of transmission of *T. gondii* in a wild population of rodents means that *Toxoplasma* can be present over long periods without the presence of cats.

Miscellaneous. Recipients of organs or blood from *T. gondii* infected individuals are at risk. Individuals receiving heart transplants (Lautenslager 1987; Wreghitt *et al.* 1989; Sluiters *et al.* 1989), heart and lung transplant (Wreghitt *et al.* 1989), bone marrow transplants (Kusne *et al.* 1987; Heurkens *et al.* 1989), or leukemic patients receiving leucocyte transfusions (Lautenslager 1987), as well a recipient of a liver transplant (Kusne *et al.* 1987), developed toxoplasmosis. The transplants were the probable source of infection. Handling tissues and bodily fluids of infected patients by hospital personnel poses some risk.

Drinking raw goat milk has been implicated as a source of toxoplasmosis (Riemann *et al.* 1975a; Sacks *et al.* 1982) and milk goats inoculated with oocysts secrete tachyzoites in their milk (Dubey 1980; Dubey *et al.* 1980a). The tachyzoites present in goat milk should be inactivated by gastric enzymes; however, in a case involving an infant, the tachyzoites present in the milk may have penetrated the buccal or pharyngeal mucosa, since the child had sores in the area of the mouth (Riemann *et al.* 1975a). A vitamin C deficiency probably accounted for the lesions on the buccal mucosa (Ruppanner *et al.* 1978). Sacks *et al.* (1982) reported a large family outbreak of toxoplasmosis that was probably due to ingestion of raw goat milk. One person was symptomatic but the other family members only showed evidence of seropositivity. The authors suggest that tachyzoites present in the milk may have infected the individuals via penetration of the oropharyngeal mucosa, but it was considered possible that milk could have neutralized gastric enzymes and the tachyzoites escaped destruction and penetrated the more alkaline duodenum mucosa.

Virulence Factors in *T. gondii*

Host-Penetration Factor. Invasion of cells by *T. gondii* has been reviewed by Werk (1985). *T. gondii* invades almost all eukaryotic nucleated cells and penetration is rapid—on the order of a minute or less. Invasion (penetration) is an energy-dependent active process involving both host and parasite. The protozoon attaches to the host cell and the cell invaginates with eventual penetration of the host by the organism (Werk 1985). Phagocytosis may be of importance for infection of true phagocytic cells, but even these cells may be infected via the active process (Lycke *et al.* 1975).

T. gondii produces a penetration-enhancing factor (PEF), a protein of MW 70,000, which behaves as an enzyme (Lycke *et al.* 1975; Werk 1985). PEF is active at 1 ng/mL of tissue culture fluid with optimum pH at 7.6 and optimum temperature at 37°C (Norrby 1971). Calcium and magnesium ions stimulate PEF activity (Lycke *et al.* 1975). PEF stimulates the penetration of *Toxoplasma* into tissue culture cells and enhanced the virulence of the organism in mice (Lycke *et al.* 1968).

Toxins. Weinman (1952) reviewed studies done on toxotoxin, a toxic moiety produced by *T. gondii* in the animal body. Toxotoxin is found in the ascitic fluid of mice intraperitoneally injected with *T. gondii*. When the fluid was injected intravenously into mice, rapid death results. Jacobs (1963; 1967) reported that other workers could not find a toxic factor in mouse body fluids. Jacobs (1967) believes that toxotoxin is not important in the pathogenesis of *T. gondii*.

Immunosuppression. *T. gondii* causes immunosuppression in the infected host, both animals and humans (Chan *et al.* 1986). In mice infected with the protozoan, the primary antibody response (Suzuki *et al.* 1981b) and the initiation of memory cells (Suzuki *et al.* 1981a) are suppressed during the acute phase of toxoplasmosis. It appears that activation of suppressor macrophages in *T. gondii* infected mice causes the suppression of helper T and B cells (Suzuki and Kobayashi 1983).

Chan *et al.* (1986) demonstrated that there is a decreased proliferation of lymphocytes and production of interleukin-2 in *T. gondii* infected mice. Both macrophages and T cells are involved in immunosuppression leading to a decrease in concanavalin A-stimulated lymphocyte production. However, the decrease in lymphocyte synthesis may be due to decreased concentration of interleukin-2 or due to the development of active suppressor cells and/or a soluble suppressor factor. Chan *et al.* (1986) also showed that a virulent strain of *T. gondii* causes a greater degree of immunosuppression in mice than an avirulent strain. Thus, it would appear that immunosuppression is an important virulence factor for *Toxoplasma*.

Resistance to Phagocytosis. It has been proposed by past workers that parasitemia during the acute stage of *T. gondii* infection in man is facilitated by engulfment of the protozoan by phagocytes; the phagocytes protect the organism from antibody and transport them to various parts of the body. However, Wilson and Remington (1979) found that while *T. gondii* can survive and multiply in mouse macrophages, they do not survive within human monocytes or polymorphonuclear leukocytes. It is probable that *T. gondii* are not transported via phagocytes but are rapidly destroyed, instead. Thus, circulating human phagocytes restrict rather than disseminate *T. gondii*. Similarly, Israelski *et al.* (1990) demonstrated that human pelvic macrophages (monolayers contained more than 95% mononuclear phagocytes) allow infection by *T. gondii* but are toxoplas-

macidal; however, mouse peritoneal macrophages allow replication of *T. Gondii*. Thus, *T. gondii* is not resistant to phagocytosis in infected humans.

Phospholipase. Antisera against phospholipase A₂ (PLA₂) inhibit the penetration of fibroblasts by *T. gondii*. The PLA₂ inhibitors, p-bromophenacyl bromide and nordihydroguaiaretic acid, also inhibit penetration without disabling the parasite (Saffer *et al.* 1989). If fibroblasts are preincubated with the chemical inhibitors, penetration by *Toxoplasma* is not affected; thus, the effect of the inhibitors is on the organism. The results suggest that a parasite phospholipase is involved in the penetration of cells by *T. gondii* (Saffer *et al.* 1989).

Inhibition of Fusion of Parasitophorous Vacuoles. After entering phagocytic or nonphagocytic cells, *T. gondii* resides within a vacuole. It would be expected that the parasite-containing vacuole would fuse with endosomes and lysosomes with the end result of parasitic death. However, vacuoles containing the parasite are blocked in their fusion capability. Thus, inhibition of fusion of the vacuoles containing *Toxoplasma* with lysosomes or endosomes appears to be a mechanism allowing survival of *T. gondii* tachyzoites (Joiner *et al.* 1990).

Virulence factors that may account for the pathogenic effects of *T. gondii* infection require more study. The role of toxin in pathogenicity of *Toxoplasma* seems uncertain, but the production of penetration-enhancement factor and immunosuppression and suppression of fusion of vacuoles containing *T. gondii* with other vacuoles such as endosomes and lysosomes appear to be important in the survival of the organism. The role of *T. gondii* phospholipase in virulence of the organism also needs more study. Bloom (1979) has discussed various mechanisms that parasites use in order to avoid the host's immune system: these include antigenic variation and mimicry, survival and growth in macrophages, and immunosuppression. It is not known if *Toxoplasma* uses antigenic variation or mimicry to evade the host's defence mechanisms.

Effects of Geographical Location, Age, and Sex on Infection by *T. gondii*

T. gondii infections occur worldwide and affect both human females and males. There is a definite age bias—older populations have a higher prevalence of seropositivity. Since most infections are asymptomatic, the only way to determine prior contact with *T. gondii* is by the use of serological techniques. A few examples are given below to illustrate differences of geographical location, sex and age on the prevalence of seropositivity to *T. gondii* in humans.

In a sample of 369 people from the Seattle, Washington area, Peterson *et al.* (1972) found that 19% of the men and 19% of the women were seropositive. As the age increased from 15–24 to 35–64 years of age, the prevalence of seropositivity to *T. gondii* increased from 16 to 32%. Beach (1979) tested the sera of 95,929 pregnant women and found a seropositivity of 8.1%. Beach (1979)

also determined that 5/1000 women would be infected by *T. gondii* during pregnancy. Approximately one-third of all pregnant women in the U.S. are seropositive at conception (Hershey and McGregor 1987); thus, their babies are not normally at risk. However, Hershey and McGregor (1987) found that out of 120 pregnant women in the Rocky Mountain area (Colorado), only 4 (3%) were seropositive for *T. gondii*. While the sample size was small, the low infectivity rate for *Toxoplasma* suggests that oocysts may not survive well in the high-altitude and arid environment. These results indicate that pregnant women from a low toxoplasmosis incidence area should exercise care when traveling to a high incidence area.

Alaskan natives were thought to be *Toxoplasma*-free, but Peterson *et al.* (1974) demonstrated that 28% of 1572 individuals were seropositive by indirect fluorescent antibody. Indirect hemagglutination indicated that 16% were seropositive. There were no differences in seropositivity between sexes, and older individuals had a higher percentage of seropositivity.

Stray-Pedersen and Lorentzen-Styr (1980) studied 196 women from the Oslo, Norway area and found 56.6% seropositivity for *T. gondii*. Their survey indicates that the eating of raw meat and frequent international travel correlates with an individual being positive for *Toxoplasma*. Examination of 3413 military recruits from Norway indicated that 21.8% were seropositive (Midtveltdt and Vaage 1989). Interestingly, recruits with blood type B had a prevalence of seropositivity of 28.8% whereas recruits with other blood types (A + AB + O) had a prevalence of 21.4% (range 18.0–22.8). However, Lecolier *et al.* (1990) did not find a relationship between seropositivity and blood grouping in 4000 pregnant women in France.

Papoz *et al.* (1986) studied a group of women of child-bearing age from the Paris area; out of 7605 women (15–44 years of age), 36.5% were seropositive for *Toxoplasma*. The infection rate during pregnancy was approximately 10/1000. Jeannel *et al.* (1988) found the prevalence rate for *T. gondii* infection in the Paris area for pregnant women was approximately 67%. Of 550 native French women, 71% were seropositive, whereas only 48% of 401 pregnant immigrant women were seropositive. The probability of infection during pregnancy was 2.3% for French women and 1.6% for immigrants.

Sousa *et al.* (1988) compared a rural population of 326 individuals in Panama to an urban population of 590 people in Panama City: 57.5% of the rural population and 58.6% of the city population were seropositive for *T. gondii*. The incidence rate per year for infection by the protozoan was approximately 10% for both areas. At 1–5 years of age, the seropositivity for the rural children was approximately 26% and that of city children was 38%. Sousa *et al.* (1988) suggests that the high incidence of *T. gondii* infection in young children in Panama is due to playing in soil contaminated by cat feces.

In a population of 3606 individuals from a farming community in Japan, Konishi and Takahashi (1987) found that 2.9% of the people were seropositive

at 20–29 years of age which increased to 40% at 70–90 years of age. There was a higher incidence of seropositivity in men (29%) than in women (16%). The authors felt that the higher rate in men was due to the fact that more men ate raw meat. Durfee *et al.* (1976), studying 1050 people in small villages in Borneo, found that 31.4% of the population were seropositive for *Toxoplasma*. At ages 1–9, 17% of the children were positive for *T. gondii* which steadily increased to 46% in adults 50 years and older.

Adams *et al.* (1987) studied the *T. gondii* prevalence of 517 Marshall Islanders; 93.8% were seropositive. The prevalence was approximately the same for both men and women. At 10–14 years of age, the prevalence was 80% and at 15–19 years, it had increased to 93%. Thus, these people show a high incidence of toxoplasma infection at an early age.

Using 1661 females from the Netherlands, van der Veen and Polak (1980) found that after one year of age, the percent positive sera in the female population increased steadily to approximately 30% by ages 10–14 and to 50–60% by ages 20–29. The estimated annual infection rate from *T. gondii* increased from 0.5% in early childhood to 3% during adolescence and early adulthood. Bowry *et al.* (1986) noted an absence of congenital toxoplasmosis in Kenya. They found that children 1–3 years of age showed 40% seropositivity and by 10 years of age, the level was 60%. In young children 1–5 years of age, girls had a much higher incidence of *T. gondii* positive sera than did boys, but by age 10, the incidence was the same in both sexes.

Shen *et al.* (1990) found that only 0.7% of 3085 individuals from the Guangdong Province of China were seropositive. These authors suggest that infections by *T. gondii* may be low in mainland China due to the Chinese custom of cooking meats well done and to the rarity of cats in Chinese households.

Depending on the geographical location, the prevalence of seropositivity to *T. gondii* can vary widely. This may be due in part to the type of climate, to the ethnic groups inhabiting that location, and to the type of culture (both culture and ethnicity would contribute to eating habits). In general, both sexes appear to be infected equally and older populations have a higher percentage of seropositivity to *Toxoplasma*.

Human Toxoplasmosis

While *T. gondii* infections are found in normal, healthy individuals, an infection is particularly dangerous to the immunocompromised. Examples of immunocompromising states include (Girdwood 1989): (1) pregnancy, (2) age (very young, very old), (3) congenital immunological defects (agammaglobulinaemia, etc.), (4) chronic infections (HIV, measles, malaria, etc.), (5) malnutrition, (6) neoplasia, (7) therapeutic suppression (tumor therapy, transplant surgery, etc.), (8) collagen-vascular diseases (systemic lupus erythematosus, etc.), and (9) surgery (splenectomy, etc.).

The Disease in "Normal" Individuals. When a human being is infected with *T. gondii*, there are usually no clinical symptoms and the presence of infection can only be detected via serology. When clinical symptoms are present, they reflect the destruction of cells in specific tissues or organs. In acute primary infections, the symptoms of toxoplasmosis may include fever, malaise, skin rash, pneumonia, myocarditis, hepatitis, involvement of the lymph glands, and encephalitis. The disease is usually mild and self-limiting. Fatalities are rare; however, there are reports of severe disseminated infections leading to death, generally from encephalitis, in immunocompetent individuals (Mahmoud and Warren 1977; Feldman 1982; Hall 1986; Murrell *et al.* 1986).

The Disease in Immunocompromised Individuals—Congenital Toxoplasmosis. When a person is initially infected with *T. gondii*, there is parasitemia followed by dissemination and encystment of the organism in various parts of the body. If the individual is pregnant and if the placenta is invaded, organisms may be shed into the fetal circulation with possible fetal infection. Women who are seropositive for toxoplasmosis before pregnancy (approximately 30% of the women of child-bearing age in the U.S. are seropositive) do not transmit the organism to the unborn child (Feldman 1982; August and Chase 1987). If the nonimmune woman is infected during pregnancy, there is a 20–40% chance that the fetus will be infected (Lautenslager 1987).

Estimates of congenital infection (positive fetal IgM antibody) in the U.S. range from 0.2–2.0/1000 live births (Krogstad *et al.* 1972), 1.0–4.0/1000 (Swartzberg and Remington 1975) or 2.0–6.0/1000 (Krick and Remington 1978). However, McCabe and Remington (1988) indicate that there is insufficient data to determine the incidence of infection *in utero*.

Congenital infections may present as: neonatal illness; a disease of variable severity during first few months of life; a disease not previously diagnosed and showing up later in infancy, childhood or adolescence; and subclinical infections (August and Chase 1987). Approximately 3000 babies are infected *in utero* each year with *T. gondii*; 5–10% die, 8–10% have brain and ocular lesions, 10–13% have visual damage. The remainder, who are normal at birth, later develop active infections with complications (August and Chase 1987). Nearly all children born with subclinical toxoplasmosis will develop, at a later time, adverse symptoms which can vary in severity. Approximately 85% of these children develop ocular lesions—retinochoroiditis. Brain infections may occur leading to brain damage with ensuing behavioral problems and/or intellectual deficits as sequelae to subclinical infections. The mortality rate of babies with severe symptoms or neurologic signs is approximately 12% (August and Chase 1987; Lautenslager 1987).

Why cell-mediated immunity is depressed during pregnancy leading to enhancement of apparent virulence of *T. gondii* (as well as other protozoan, fungal, and bacterial pathogens) is not understood. Weinberg (1984) has reviewed the

literature and has suggested that a number of factors may play a role in reducing immunity during pregnancy. These include steroids, glycoproteins, lymphocyte reactivity, thymus involution and shifts in T cell subsets. Weinberg (1984) also suggests that iron overload may intensify the immunodepression found in pregnancy.

McCabe and Remington (1988) suggest the use of an active program to screen women for *Toxoplasma* antibodies as a way to prevent congenital toxoplasmosis. Ideally, such a program would start at marriage and those women who are seronegative can be advised how to avoid *T. gondii* if they become pregnant and what to do if they do become seropositive during pregnancy. However, Thorp *et al.* (1988) feel that such a screening program would be cost-prohibitive for the small gain that would be achieved since the number of infected newborns is approximately 3000 (high estimate) or approximately 500 (low estimate). They estimate that the cost for screening all pregnant women in the U.S. would be \$160 million. If 70% of the women are seronegative, then monthly titer assessment would cost more than \$1 billion. In 1975 dollars, Swartzberg and Remington (1975) estimate the cost for health care, institutionalization and special educational needs of congenitally infected children would be in the neighborhood of \$30–40 million annually; however, in 1985, the cost was estimated to be in the range of 430 million dollars (Roberts 1985).

Hall (1986) suggested that congenital toxoplasmosis can be prevented by educating pregnant women how to avoid being infected by *T. gondii*. Hall's suggestions are incorporated into Table 1. If these suggestions were recommended to every pregnant woman (especially those that are seronegative) by the family physician, the incidence of congenital toxoplasmosis would decrease. Carter *et al.* (1989) have developed an education program directed toward pregnant women demonstrating how to reduce the risk of toxoplasmosis. However, Foulon *et al.* (1988) expressed doubt that such educational programs will work effectively and suggested, instead, that medical treatment of each woman who seroconverts during pregnancy would reduce congenital toxoplasmosis significantly. There has been a strong education program to acquaint consumers about the danger of trichinosis from eating undercooked pork. A reduction in human trichinosis has been observed, but the decrease is related to the decreased incidence of *T. spiralis* in swine. Thus, there has been a reduction in the exposure of people to trichinosis, and the reduction does not appear to be related to education efforts (Leighty 1990). Therefore, while educating the public concerning the danger of *T. gondii* infection is important, it can not be relied on alone and it is necessary to reduce the exposure to humans to *T. gondii* by reducing the presence of the organism in the environment.

The Disease in Immunocompromised Individuals—Recrudescence. The toxoplasmosis seen in immunocompromised individuals usually results from

reactivation of a prior (when the individuals were healthy) latent infection, i.e., recrudescence. Evidently, in immunocompromised people, the bodily immune system can no longer maintain *T. gondii* as benign cysts.

Approximately 25% of acquired immunodeficiency syndrome (AIDS) patients at risk, i.e., those that are seropositive for *T. gondii* prior to the development of AIDS, will develop toxoplasmic encephalitis (Araujo and Remington 1987). Thus, in AIDS, toxoplasmosis usually presents as infections of the central nervous system (Mills 1986; Holliman 1988; Tschirhart and Klatt 1988). Rarely, patients may have extraneural manifestations such as myocarditis (Tschirhart and Klatt 1988), pneumonitis (Catterall *et al.* 1986; Tschirhart and Klatt 1988; Derouin *et al.* 1989), orchitis (Cridler *et al.* 1988; Tschirhart and Klatt 1988; Haskell *et al.* 1989), and nephrotic syndrome (Haskell *et al.* 1989) among other effects.

It is important to serologically evaluate both the recipient and donor in organ transplant cases to determine prior exposure to *Toxoplasma*. The recipient, now immunocompromised, is susceptible to his own *T. gondii* cysts (if present) as well as to the cysts that may be present in the donor transplant. Shepp *et al.* (1985) were able to show the reactivation of latent toxoplasmosis in patients receiving allogeneic bone marrow transplants with death of the patients as the eventual sequela.

Treatment of *T. gondii* Infections

A combination of sulfadiazine and pyrimethamine is used in treatment of toxoplasmosis in humans. These compounds act synergistically against the multiplying form—tachyzoites—by blocking the metabolic pathways involved in p-aminobenzoic acid and folic-folinic acid cycles (Frenkel 1985; Dubey 1987). However, these drugs are not effective in eradicating bradyzoites, the cyst form of *T. gondii*; thus, the infection will persist albeit in a benign form. Pyrimethamine and sulfadiazine have toxic side effects and both are teratogenic, thus their use during pregnancy is contraindicated (Feldman 1982). Pyrimethamine has an adverse effect on the bone marrow, so that red blood cells and platelets must be monitored during therapy (Ruskin and Remington 1976). The antibiotic, spiramycin, is used against toxoplasmosis in Europe but is unavailable in the U.S. (McCabe and Remington 1988). However, spiramycin does not kill the parasite effectively and should be used only when toxicity is unacceptable such as prevention of congenital toxoplasmosis and in treatment of toxoplasmic chorioretinitis (Chang and Pechere 1988a).

AIDS patients who have toxoplasmosis often develop cerebral toxoplasmosis and many patients with AIDS develop intolerance toward pyrimethamine and sulfadiazine, necessitating a different regimen. Spiramycin does not penetrate the blood-brain barrier and thus is not effective (Mills 1986). The combination

of pyrimethamine and clindamycin has been successfully used in AIDS patients with cerebral toxoplasmosis (Leport *et al.* 1989); however, clindamycin has serious side effects including pseudomembranous enterocolitis (Ruskin and Remington 1976). In a recent paper, Israelski *et al.* (1989) demonstrated that AZT (azidothymidine, zidovudine) antagonizes the antitoxoplasmic activity of pyrimethamine in vitro and reverses the synergistic action of pyrimethamine and sulfadiazine in vitro. AZT also antagonizes the therapeutic effects of pyrimethamine in mice infected with *T. gondii*. It may be that AIDS patients under AZT treatment and suffering from toxoplasmosis must be administered drugs other than sulfadiazine and pyrimethamine.

Some compounds have been tested against *T. gondii* using a mouse model. A purine analogue, arpinocid, two macrolide antibiotics, roxithromycin (RU 28965) and azithromycin, and the semisynthetic tetracycline analogue, doxycycline are effective against murine toxoplasmosis when tachyzoites are injected intraperitoneally (Luft 1986; Chan and Luft 1986; Araujo *et al.* 1988; Chang *et al.* 1990). Utilizing a mouse model for toxoplasmic encephalitis, Hofflin and Remington (1987b) and Chang and Pechere (1987) found that roxithromycin and interferon act synergistically to delay death against cerebral toxoplasmosis; similarly, azithromycin acts synergistically with interferon against murine cerebral toxoplasmosis (Araujo *et al.* 1988). Pyrimethamine-clindamycin mixtures have been used to treat human cerebral toxoplasmosis in AIDS patients. Interestingly, Hofflin and Remington (1987a) found that clindamycin when used alone is effective against cerebral toxoplasmosis in mice, thus suggesting that it may be effective in humans when used without pyrimethamine.

Studies are underway to find other drugs effective against toxoplasmosis, using tissue culture infected with *T. gondii* as the model. Derouin *et al.* (1988) studied the effect of various macrolides, synergestines, and lincosamides using *T. gondii*-infected human embryonic fibroblast cultures. They found that the macrolides and clindamycin (a lincosamide) were quite effective in vitro, but the synergestines and lincomycin were only partially inhibitory. Another macrolide, azithromycin, was effective against *Toxoplasma* but showed toxicity against mouse macrophages (Chang and Pechere 1988b). When *T. gondii*-infected rat myocytes were treated with 5-fluorouracil and pyrimethamine, the combination behaved synergistically to inhibit *T. gondii* (Harris *et al.* 1988). Dihydrofolate reductase inhibitors were active against *T. gondii*-infected fibroblasts in culture, and when these inhibitors were combined with sulfonamides, the activity was synergistic (Derouin and Chastang 1989). The studies on *T. gondii* inactivation using mouse or tissue culture models indicate that there are a number of drugs effective against the parasite, and these should be tested clinically—particularly in immunocompromised individuals suffering from toxoplasmosis. McCabe and Oster (1989) have reviewed the current status of treatment for toxoplasmosis.

It appears that a new battery of drugs are needed to combat toxoplasmosis—

drugs that are relatively nontoxic, and importantly, will attack both tachyzoites and bradyzoites. At present, there appears to be no drug that will rid the host of encysted *T. gondii*. In the absence of an antibradyzoite drug, there can be no elimination of *Toxoplasma* from the host; ideally, such a drug should be effective in both humans and animals. Combining chemotherapy with an effective vaccine (which is not yet available) would break the vicious cycle of *Toxoplasma* from cat to food animal to man.

Vaccines against *T. gondii*

Leighty (1990) feels that vaccine development has been neglected as a means to control infections by *T. gondii*. Part of the problem is that the economic incentive for developing anti-*Toxoplasma* vaccines is not very high. However, its potential as a means of eventual elimination of *T. gondii* is great.

Since cats are the only animals known to shed oocysts, vaccination of kittens and *Toxoplasma*-seronegative cats would prevent infection and subsequent deposition of *T. gondii* oocysts. The vaccination of house cats would protect pet owners, but vaccination of nonpet cats such as farm or feral cats will prove to be a formidable task.

A vaccine to protect humans at risk—pregnant seronegative females or immunocompromised individuals—would protect them against infection if meats containing *T. gondii* cysts are ingested or if they are exposed to infectious oocysts. A vaccine against *Toxoplasma* suitable for injection into food animals such as swine, sheep, or cattle will ensure a safer food supply. Research exploring the feasibility of vaccines as tools for the elimination of *T. gondii* should have high priority.

Prevention of *T. gondii* Infections

Suggestions for the prevention of toxoplasmosis are listed in Table 1. If these suggestions are followed by the general population, it would lead, in time, to a decrease in the incidence of infections of *T. gondii*. The procedures listed are simple, easy-to-follow and make good sense hygienically.

Economic Aspects of Toxoplasmosis

Impact on Human Life: Congenital Toxoplasmosis. It has been estimated that in the U.S., 3158 to 3300 babies are born each year that are infected with toxoplasmosis *in utero* (Roberts 1985; Bennett *et al.* 1987). Approximately 15% of the babies die (abortion, still birth or soon after birth), and the survivors have complications of varying severity (eye damage, mental retardation, etc.) which may show up at birth, after birth, or in later childhood. Roberts (1985) estimated that the cost for lifetime care of the affected children would be in the order of 430 million dollars each year.

More recently, Roberts and Frankel (1990) have reexamined the economics of congenital toxoplasmosis in the U.S. They estimate that the cost may range from 369 million to 8756 million dollars/year as reflected by life-time income loss, special education and institutional care, and medical costs. The wide range in estimated costs is due to the uncertainty of the actual number of cases of congenital toxoplasmosis cases that occur each year; Roberts and Frankel's range varied from 407 to 9500 cases/year. The estimated number of deaths was revised downward—from 15% to 2%/year. Roberts and Frankel (1990) felt that for social and economic reasons, the incidence of congenital toxoplasmosis in the U.S. may be declining and the lower estimates that they give may be more accurate.

Impact on Human Life: Noncongenital Toxoplasmosis. Very little information is available on the economic effect of toxoplasmosis in the general population since most infections go unrecognized. Bennett *et al.* (1987) estimated that there are 2,300,000 clinically significant infections per year in the U.S. with a case/fatality ratio of 0.0001%. Todd (1989) estimates that 1,437,500 cases of toxoplasmosis occur annually in the U.S., with a death rate ranging from 0.0001 to 15%. The estimated cost of toxoplasmosis per year is 72 million dollars.

Impact on the Food Industry. There does not appear to be any information on recalls or other economic impacts due to foods containing *T. gondii*. However, with the development of rapid serological tests for *Toxoplasma*, foods could be subject to screening. It is probable that the presence of the protozoan in food could lead to a recall with ensuing economic loss to the producer or processor.

CONCLUSIONS

Toxoplasmosis is a foodborne disease of increasing importance. In view of the fact that the incidence of immunocompromised individuals (mainly due to advances in medicine) is increasing in the U.S. population, it is probable that toxoplasmosis will become more prevalent unless preventive measures are initiated. It is imperative that the ingestion of *T. gondii* in foods is deterred; however, prevention of *Toxoplasma* in foods is a long range goal which will be difficult to achieve with present-day strategies. New chemotherapy regimens are needed to protect immunocompromised individuals. Also needed are drugs which will eliminate the parasite from the body and also are relatively nontoxic. Assay procedures are needed which can detect *T. gondii* cysts in meats and other foods so that immunocompromised individuals who are seronegative can eat *Toxoplasma*-free foods. It is important, even if difficult, that future generations be protected from *T. gondii*. This can only be achieved by producing effective vaccines that will prevent infection in both animal and man. In particular, a feline vaccine would eliminate (or greatly decrease) the contamination of animal feeds with *T. gondii*, thereby, ensuring that meat animals would not be infected with the parasite.

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