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PREVALENCE OF VIABLE *TOXOPLASMA GONDII* IN BEEF, CHICKEN, AND PORK FROM RETAIL MEAT STORES IN THE UNITED STATES: RISK ASSESSMENT TO CONSUMERS

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ABSTRACT: The prevalence of viable *Toxoplasma gondii* was determined in 6,282 samples (2,094 each of beef, chicken, and pork) obtained from 698 retail meat stores from 28 major geographic areas of the United States. Each sample consisted of a minimum of 1 kg of meat purchased from the retail meat case. To detect viable *T. gondii*, meat samples were fed to *T. gondii*-free cats and feces of cats were examined for oocyst shedding. Initially, 100 g of meat from 6 individual samples of a given species were pooled (total, 600 g), fed to a cat over a period of 3 days, and feces were examined for oocysts for 14 days; the remaining meat samples were stored at 4 C for 14 days (until results of the initial cat fecal examination were known). When a cat fed pooled samples had shed oocysts, 6 individual meat samples from each pool were bioassayed for *T. gondii* in cats and mice. *Toxoplasma gondii* isolates were then genetically characterized using the SAG2 locus and 5 hypervariable microsatellite loci. In all, 7 cats fed pooled pork samples shed oocysts. *Toxoplasma gondii* oocysts were detected microscopically in the feces of 2 of the cats; 1 isolate was Type II and the second was Type III. Analyzed individually, *T. gondii* was detected by bioassay in 3 of the 12 associated samples with genetic data indicating *T. gondii* isolates present in 2. The remaining 5 pooled pork samples had so few oocysts that they were not initially detected by microscopic examination, but rather by mouse bioassay of cat feces. Two were Type I, 1 was Type II, and 2 were Type III. None of the cats fed chicken or beef samples shed oocysts. Overall, the prevalence of viable *T. gondii* in retail meat was very low. Nevertheless, consumers, especially pregnant women, should be aware that they can acquire *T. gondii* infection from ingestion of undercooked meat, and in particular, pork. Cooking meat to an internal temperature of 66 C kills *T. gondii*.

The protozoan *Toxoplasma gondii* infects virtually all warm-blooded animals, including humans, livestock, and marine mammals (Dubey and Beattie, 1988; Dubey et al., 2003). Infection in humans and livestock occurs worldwide. In the United States, various surveys have found that 10–50% of the adult population has been exposed to this parasite (Dubey and Beattie, 1988; Sever et al., 1988; Smith et al., 1996; Roghmann et al., 1999; Jones, Kruszon-Moran, et al., 2001; Jones et al., 2003). *Toxoplasma gondii* infection causes mental retardation, loss of vision, and other congenital health problems in humans, and it is an important cause of morbidity and mortality in individuals with immunosuppression, including those with acquired immune deficiency syndrome and organ transplant recipients (Desmonts and Couvreur, 1974; Frenkel, 1990; Luft et al., 1993; Rabaud et al., 1994; Bertoli et al., 1995; Smith, 1997, 1998, 1999; Jones, Lopez et al., 2001). It also causes serious health problems in immunocompetent humans (Benenson et al., 1982; McCabe et al., 1987; Bowie et al., 1997; Montoya et al., 1997; Montoya and Liesenfeld, 2004).

It is estimated that approximately 400–4,000 children are born with congenital *T. gondii* infection in the United States each year (MMWR, 2000). The cost to society of congenital toxoplasmosis is believed to be as high as $8.8 billion in the United States annually (Roberts et al., 1994) and there is no evidence that these estimates have decreased during the last decade. Estimated costs do not include maternal suffering and those associated with postnatally acquired infections. *Toxoplasma gondii* is 1 of 3 pathogens (along with *Salmonella* and *Listeria*) that account for >75% of all deaths due to food-borne disease in the United States (Mead et al., 1999).

There are 2 major modes of transmission of *T. gondii*. Infection may occur by ingestion of food or water contaminated with oocysts excreted by infected cats or by ingesting uncooked or undercooked meat containing tissue cysts of *T. gondii*. The proportion of the human population that acquires infection by ingestion of oocysts in the environment or by eating contaminated meat is not known, and there are no tests available that can determine the infection source. However, sero-epidemiologic data suggest that ingesting improperly cooked meat containing *T. gondii* is a major source of infection for humans in the United States (Kimball et al., 1974; Dubey and Beattie, 1988). There are 2 major pieces of data that are used to support this hypothesis. First, the prevalence of *T. gondii* in U.S. children <10 yr of age is very low compared with the prevalence in the adult population. If infection from soil contaminated with *T. gondii* was a major route of infection, then one would expect a higher prevalence in children, when the chances for ingestion of soil are higher. Such is the case in Central and South America, where up to 60% of elementary school children have antibodies to *T. gondii* (Dubey and Beattie, 1988). Second, there is a rapid increase of *T. gondii* infection during the teenage years, which may reflect increased ingestion of uncooked or undercooked meat. Despite the accumulating evidence for the role of meat in human exposure to *T. gondii*, little information is available on risk to the consumer from retail meats.

In the United States, approximately 100 million pigs, 35 million beef cattle, and 8.5 billion chickens are killed for human consumption each year (USDA National Agricultural Statistics Service, 2003). One study in 2003 estimated that Americans consume 23.5 kg of pork, 29.5 kg of beef, and 37.2 kg of poultry annually (USDA Economic Research Service, 2004, http://www.ers.usda.gov/data/foodconsumption/FoodAvailSpreadsheets.htm#mtpcc). About one-third to one-half of the meat consumed in the United States is further processed. Because it has been shown that *T. gondii* is killed by many of the salting, curing, freezing, or heating procedures that are used in meat processing, these products are not a likely source of human exposure. Similarly, frozen imported meat is probably not important in the epidemiology of *T. gondii* because freezing kills the parasite (Kotula et al., 1991).

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Thus, the major risk to consumers is believed to be from products purchased in the form of fresh meat.

There has been no comprehensive survey for viable *T. gondii* in meat sold in retail stores in the United States. In a small study conducted more than 30 yr ago, *T. gondii* was found in 16 of 50 (32%) pork loins, 2 of 50 (4%) lamp chops, and 0 of 50 beef loins in samples from Palo Alto, California (Remington, 1968). It was not stated whether these samples were from 1 or more grocery stores, or the time or duration of sampling.

A relative estimate of the risk to consumers of acquiring *T. gondii* infection from ingestion of contaminated fresh meat might be gathered from information on prevalence of these pathogens in live animals. However, with the exception of pigs, there is little information available on *T. gondii* in live food animal species. In the United States, infection was estimated in 23.9% of pigs in 1983–1984 with higher prevalence in breeders (42%) than in market pigs (23%) (Dubey et al., 1991). When pigs from these same areas were tested in 1992, prevalence had dropped to 20.8% of breeders and 3.1% of finisher pigs (Dubey, Weigel, Siegel et al., 1995). Prevalence of *T. gondii* was 20% in sows tested in the 1990 National Animal Health Monitoring System (NAHMS) swine survey (Patton et al., 1996; S. Patton, pers. comm., 2000). Using sera from the NAHMS swine survey conducted in 1995, sow prevalence had fallen to 15.0% and finisher pigs had a seroprevalence of 3.2% (Patton et al., 1996; S. Patton, pers. comm., 2000). The prevalence of *T. gondii* in pigs is influenced by management systems. Thus, in the northeast, where management is largely via nonconfinement systems, prevalence runs as high as 47.4% (Gamble et al., 1999).

Few studies have been conducted to directly demonstrate the presence of *T. gondii* in pork. Viable *T. gondii* were isolated from 170 of 1,000 sow hearts from a plant in Iowa (Dubey, Thulliez, and Powell, 1995). However, sow meat is generally processed, and *T. gondii* is likely to be killed. Little is known of the prevalence of viable *T. gondii* in finisher pigs. Recently, viable *T. gondii* was isolated from 51 of 55 finisher pigs from a farm in Massachusetts (Dubey, Gamble et al., 2002). It is noteworthy that in both isolation studies, *T. gondii* was recovered from approximately 3% of serologically negative pigs (Dubey, Thulliez, and Powell, 1995; Dubey, Weigel, Andrews et al., 1995; Dubey, Gamble et al., 2002). Therefore, serologic surveys alone do not provide information about the prevalence of viable *T. gondii*.

In limited studies, *T. gondii* has not been isolated from beef obtained from cattle slaughtered in the United States (Jacobs et al., 1960; Jacobs, 1967; Remington, 1968; Dubey and Streitel, 1976; Dubey, 1986). *Toxoplasma gondii* was isolated once from the intestines, but not from any edible tissues of a beef cow (Dubey, 1992). These negative results might also be related to the methods of investigation. Results from experimentally infected cattle indicate that, at least dairy cattle, are resistant to *T. gondii* and eliminate/reduce the number of *T. gondii* from their tissues (Dubey, 1983; Dubey and Thulliez, 1993) so much so that <1 *T. gondii* may be present in 100 g of beef; *T. gondii* was not demonstrable by bioassay in mice inoculated with pepsin digests from 100 g of meat, whereas bioassay in cats using 500-g samples indicated that *T. gondii* was present in the tissues of experimentally infected cattle (Dubey and Thulliez,1993). These cattle were slaughtered 1 yr postinoculation (PI) and they weighed 390–500 kg. Thus, bioassay in cats is more likely to detect *T. gondii* in beef than bioassays in mice because more tissue can be fed to cats. Even a few *T. gondii* can induce oocyst shedding that is easily detected in cat feces (Dubey and Frenkel, 1976; Dubey, 2001). Another problem with surveys for *T. gondii* in cattle is that some of the serologic tests (dye test, indirect hemagglutination test, and latex agglutination test) give erratic results with bovine sera (Dubey et al., 1985). The modified agglutination test (MAT) is considered the most reliable for detecting *T. gondii* infection in cattle and has been used to identify positive cows in a general population (Dubey et al., 1985; Dubey, 1992). There is a need to determine the prevalence of *T. gondii* in beef before a role in the epidemiology of *T. gondii* can be definitively excluded.

To our knowledge, there have been no surveys for *T. gondii* infection in commercially raised poultry in the United States. Although backyard poultry, raised on small farms primarily for eggs, have been found infected with *T. gondii* (Dubey, 1981; Dubey et al., 2003), little is known of the prevalence in battery-raised chickens. Jacobs and Melton (1966) isolated *T. gondii* from tissues of 4 of 108 chickens from a slaughter plant in Baltimore, Maryland. There has been no recent serologic survey for *T. gondii* in chickens.

Based on limited information on the occurrence of *T. gondii* in food animal species and a lack of models to predict risk based on processing methods unique to each animal industry, we conducted a statistically valid study of the prevalence of *T. gondii* in pork, beef, and chicken in retail grocery stores in the United States. This survey provides an accurate picture of the risk of exposure to *T. gondii* in retail meat.

MATERIALS AND METHODS

Sample size

One thousand nine hundred fifty-eight samples per commodity (chicken, beef, pork) was determined to be the minimum number necessary to ensure with a design effect of 2 and with 95% confidence (5% alpha), that the estimate was within $\pm 0.5\%$ nationally and $\pm 2\%$ regionally. It was assumed that *T. gondii* prevalence was 0.5% (Patton et al., 1996; Gamble et al., 1999) and did not vary by region. The number of samples was made proportional to the population of the region from which drawn. For practical purposes, the actual sample size was increased to 2,100 per commodity to produce an equal number of samples from each store (700 stores $\times$ 3 samples = 2,100) (Table I).

Statistical design/sampling procedure

A 3-stage (region, stores, meat), stratified cluster sample was undertaken to collect the samples. Primary sampling units of metropolitan statistical areas (MSAs) and primary metropolitan statistical areas (PMSAs) were selected with probability proportional to size from a randomized U.S. Census Bureau list stratified by geographical region (Midwest, South, Northeast, and West) (Table II). Any ambiguous MSAs/PMSAs whose geographical area encompassed overlapping regions were assigned to the region incorporating the largest percentage of the estimated population. The target population excluded rural areas. Approximately 1 MSA/PMSA (with a required population $>100{,}000$) was sampled for every 8 million population (Table II).

Within each MSA, 25 supermarkets, grocery outlets, or wholesale club stores were randomly selected with probability proportional to weekly sales from a comprehensive list of store profile data compiled by Trade Dimensions, Inc., Wilton, Connecticut. Samples (n = 2,094) of each commodity were randomly selected from each of 698 of 700 stores (6 samples of each type meat could not be collected from 2 stores in Yakima, Washington, due to a scarcity of product brought on by a scare of mad cow disease). Twelve stores from New York City were resampled because of potential contamination in the first set of samples (Table II).

TABLE I. Population and sample size estimates by region, July 1999.*

Region	Population†	% of Total	Required sample size	Areas sampled	Actual sample
Northeast	46.3	21.2	415	6	450
Midwest	46.6	21.3	417	6	450
South	72.6	33.2	650	9	675
West	53.1	24.3	476	7	525
Totals	218.6	100.00	1,958	28	2,100

*Source: http://www.census.gov/population/estimates/metre-city/ma99-06.txt
†Metropolitan area (millions).

All samples were obtained from boneless cuts of meat; namely, chicken breasts; beef eye-of-round roast, rib roast, rib eye, strip loin, and tenderloin; and pork loin, loin chops, center cut loin chops, and tenderloin. Muscle samples were selected to eliminate potential mixture of tissues from multiple carcasses that might result from sampling ground product. A minimum of 1 kg of muscle tissue was selected for each sample, with consideration given to the weight of fat in each package. Samples ranged from 1 kg (if no fat was seen on the sample) to 2 kg (if weight of the fat was indeterminable). If a selected package did not meet the minimum weight requirement for a 1-kg sample, additional packages were chosen from adjacent packages until the summed weight(s) equaled at least 1 kg. No discrimination was made by brand. Samples were packed on ice at the retail outlet and shipped the same day by overnight carrier to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for processing. Information available on the meat packages was recorded (Table III).

Statistical analysis

Confidence intervals for prevalence rates were computed using StatXact version 6.2 (Cytel Software Corporation, Cambridge, Massachusetts). Binomial probabilities were used to assess 1- and 10-yr risks of purchasing contaminated meat based on 2004 consumer meat consumption rates (Fig. 1). Because prevalence was calculated using on average 1.36-kg samples, annual consumption was transformed by dividing the number of kilograms consumed by 1.36 so that the probabilities would reflect the actual sampling unit. Ten-year risk was calculated by computing the joint probability of purchasing 0 contaminated packages every year for 10 yr and subtracting the result from 1. The resulting probability is the chance of purchasing any contaminated meat over the same period. Binomial calculations were performed using SAS version 9.1 (Carey, North Carolina). Alpha was set at .05.

Sample processing and bioassay for viable *T. gondii*

Each sample was trimmed of fat and a 100-g portion was collected for bioassay in cats; the remaining sample was stored at 4 C for at least 14 days. Portions (100 g) from 6 individual same-commodity samples were pooled and fed to 1 cat over a period of 3 days (3 same-commodity samples were collected from each store; therefore, 1 cat was fed the pork samples from 2 stores; Fig. 2). Each cat was housed individually and the feces were collected daily for 14 days and microscopically

TABLE II. Selected metropolitan statistical areas used for the retail meat study.

Region	Date meat sampled	Metropolitan area	Population
Midwest	April 2003	Columbus, Ohio	1,489,487
	July 2004	Chicago, Illinois	8,008,507
	June 2003	Appleton-Oshkosh-Neenah, Wisconsin	348,100
	June 2004	Cleveland-Lorain-Elyria, Ohio	2,221,181
	July 2003	Minneapolis-St. Paul, Minnesota-Wisconsin	2,872,109
	May 2004	Detroit, Michigan	4,474,614
Northeast	October 2003	Bergen-Passaic, New Jersey	1,342,116
	November 2002	Boston, Massachusetts	3,297,201
	March 2004, May 2005	New York, New York	8,712,600
	July 2002	Buffalo-Niagara Falls, New York	1,142,121
	August 2003	Philadelphia, Pennsylvania-New Jersey	4,949,867
	May 2003	Portland, Maine	234,814
South	January 2003	Miami, Florida	2,175,634
	May/June 2002	Atlanta, Georgia	3,857,097
	March 2003	Lexington, Kentucky	455,617
	December 2003	Fort Lauderdale, Florida	1,535,468
	December 2002	Wilmington-Newark, Delaware-Maryland	571,420
	February 2004	Greensboro-Winston-Salem-High Point, North Carolina	1,179,384
	September 2002	Charlotte-Gastonia-Rock Hill, North Carolina-South Carolina	1,417,217
	October 2002	Fort Worth-Arlington, Texas	1,629,213
	November 2003	Greenville-Spartanburg-Anderson, South Carolina	929,565
West	April 2004	Yakima, Washington	220,785
	February 2003	Los Angeles-Long Beach, California	9,329,989
	August 2002	Seattle-Bellevue-Everett, Washington	2,334,934
	September 2003	Fort Collins, Colorado	236,849
	January 2004	San Jose, California	1,647,419
	August 2004	Orange County, California	2,760,948
	September 2004	Salem, Oregon	335,156

TABLE III. Code identification for the meat surveyed.

Code number	Enhancements/additional label information
01	All natural/fresh and natural (chicken)
08	1% retained water (chicken)
09	2% retained water (chicken)
10	3% retained water (chicken)
28	Enhanced up to 15% with chicken broth-sodium phosphates, salt, and flavorings
44	All natural (beef)
54	Naturally raised (pork)
61	Enhanced up to 7% with water and sodium phosphate (pork)
66	Enhanced up to 10% in basting solution (not specified) (pork)
72	Enhanced up to 12% with salts and phosphates (pork)
74	Enhanced up to 12% with water, potassium lactate, sodium phosphate, salt and sodium diacetate (pork)
77	Enhanced up to 12% with pork stock, potassium lactate, sodium phosphate, salt, and flavoring, and sprayed with ascorbic acid to maintain color (pork)
80	Enhanced up to 12% with pork stock, potassium lactate, sodium phosphate, salt, and flavoring (pork)
82	No percentage listed; enhanced with water, salt, and sodium phosphate (pork)
83	No enhancement indicated

examined for oocyst excretion by fecal flotation (Dubey, 1995). When oocysts were observed in the fecal samples of cats fed pooled meat samples (Table IV), 500 g of the individual samples that made up the pool were fed individually to cats to identify the sample from which the infected tissue was derived (Fig. 2; Table V).

The cats used for this study were raised in captivity and were either from the U.S. Department of Agriculture facility in Beltsville, Maryland (Dubey, 1995), or from Liberty Laboratories, Waverley, New York. They were 2 to 4 mo old, and of mixed breed and sex. All cats were bled before feeding meat and tested for antibodies to *T. gondii* by the MAT as described by Dubey and Desmonts (1987); none had MAT antibodies at a 1:25 serum dilution.

Fecal samples were collected daily from each cat and stored at 4 C until processed. Because this study involved a large number of cats and fecal samples, the following schedule was followed for fecal examination. Feces were collected for a total of 14 days starting from day 3 after the first meat meal and continuing for 10 days after the last meal. These intervals are based on earlier observations that the prepatent period for oocyst shedding for cats fed tissue cysts was 3–10 days, irrespective of the number of viable *T. gondii* bradyzoites in the inoculum (Dubey and Frenkel, 1976; Dubey, 2001) and the peak oocyst shedding of *T. gondii* occurred 6–8 days after feeding tissue cysts (Dubey and Frenkel, 1972). For this reason, samples collected at 6, 7, and 8 days were examined separately. Samples for other days were pooled (i.e., 3–5 days, 9 and 10 days, and 11–14 days) for examination. Thus, for each cat, there were at least 6 fecal examinations.

Each fecal sample was kept separate until the microscopic examination had been completed. Feces from each cat (about 30–65 g) were emulsified with a small amount of water and approximately 5–10 g were mixed with a 2 M sucrose solution, filtered through gauze, and centrifuged in 50-ml tubes for 10 min at 1,200 *g* (Dubey and Beattie, 1988). After centrifugation a few drops were removed from the very top of the float and examined microscopically for *T. gondii* oocysts; an additional 1 ml from the top of the float was mixed with 10 ml of 2% sulfuric acid in a 50-ml tube. For pooled samples, approximately 10 g of feces from each day were combined according to the schedule described (3–5 days, 9 and 10 days, 11–14 days), mixed thoroughly, and 10 g were floated in sucrose.

If *T. gondii* oocysts were found microscopically in the feces of the cat fed meat pooled from 6 100-g samples (Table IV), each meat sample was bioassayed separately (Table V). Associated meat samples previously stored for 8 or 12 days (Table V) were removed from storage at 4 C and 50 g from each was processed for bioassay in mice (see below), and an additional 500 g from each sample was fed individually to a cat (Table V). Feces of these cats were examined for *T. gondii* oocysts individually, each day, for 14 days after the cats ate meat.

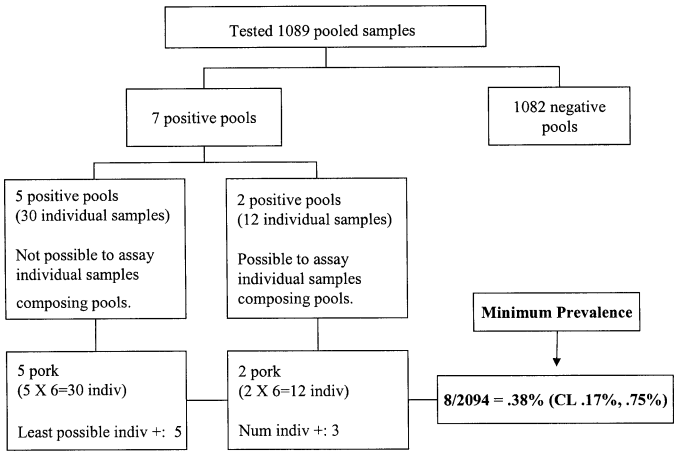

FIGURE 1. Results for viable *Toxoplasma gondii* in 6,282 samples of meat (2,094 each of beef, chicken, and pork) obtained from 698 retail stores in the United States.

Bioassay of cat feces for *T. gondii*

Fecal floats from all cats were incubated in 2% sulfuric acid at room temperature for at least 1 wk, then bioassayed in mice. For this procedure, 5 ml from all fecal floats from each negative cat were pooled, centrifuged, neutralized with 3% NaOH, and inoculated orally into 2–5 Swiss Webster albino female mice (Taconic Farms, Germantown, New York). The mice were examined for *T. gondii* infection as described by Dubey and Beattie (1988). Tissue imprints of mesenteric lymph nodes, brain, and lungs of mice that died were examined for tachyzoites. Survivors were bled 8 wk later and a 1:25 dilution from each mouse serum was examined for MAT antibodies. After serological examination, mice were killed and their brains were examined microscopically for tissue cysts, irrespective of serological status. Mice were considered to be infected when *T. gondii* was demonstrated in their tissues. If *T. gondii*-like parasites were found, a subpassage was made in mice to distinguish it from the related parasite, *Hammondia hammondi* (Dubey and Sreekumar, 2003).

Samples from oocyst-positive cats were also bioassayed in mice. Oocysts were inoculated orally into 2–5 mice. Four to 7 days later, impression smears from mesenteric lymph nodes were examined for tachyzoites. Portions were cryopreserved in liquid nitrogen for retrieval of strains, and frozen at −20 C for DNA extraction. After ascertaining the presence of tachyzoites, homogenized lymph nodes were subinoculated into mice to exclude *H. hammondi* infection.

Bioassay of meat samples in mice

Fifty-gram portions of meat (Table V) were homogenized in 0.85% NaCl (saline), digested in pepsin, centrifuged, neutralized, suspended in antibiotic saline, and inoculated subcutaneously into 10 mice as described in detail by Dubey (1998). Tissue impression smears of mice that died were examined for *T. gondii* tachyzoites or tissue cysts (Dubey, Graham et al., 2002). Survivors were bled at 8 wk post-inoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies by MAT. Mice were killed 8–10 wk PI and brain squashes from all mice were examined microscopically for tissue cysts as described previously (Dubey and Beattie, 1988). Portions of brain with demonstrable *T. gondii* were frozen for DNA extraction. Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were microscopically demonstrable in tissue smears.

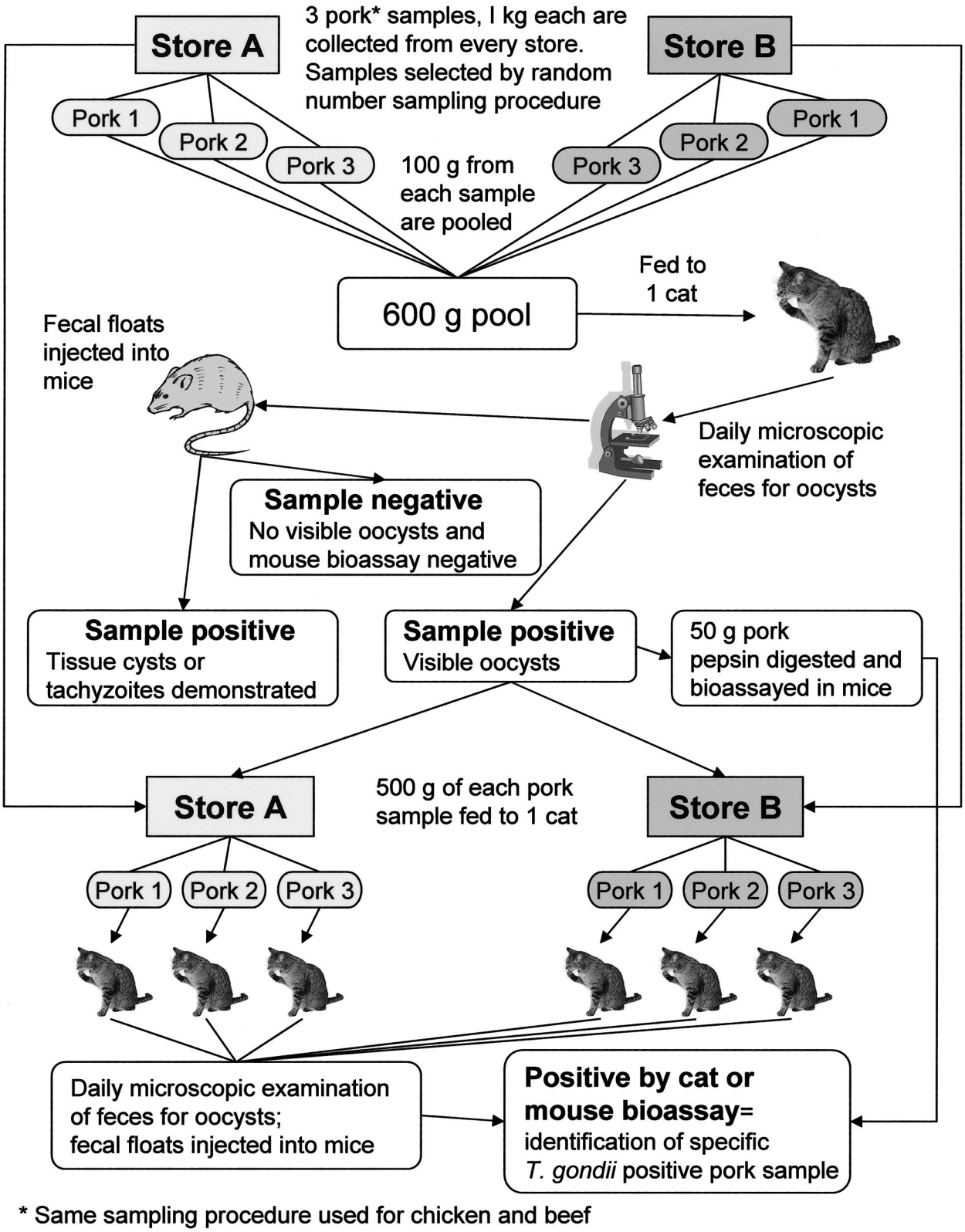

FIGURE 2. Flow chart for pork sampling and assay procedures. Identical sampling procedure used for pork, chicken, and beef.

TABLE IV. Isolation of *Toxoplasma gondii* from pooled pork samples.

Collection location; collection date, sample nos. (enhancement code)*	Store no.	Cat no. (day oocysts shed)	Infectivity of oocysts to mice†	Genotype	
				Sag 2 type	Multilocus genotype‡
Boston, Massachusetts; November 2002, 388–393 (54, 66)	BOS05; BOS17	QDM (6–9)	Positive	II	A
Miami, Florida; January 2003, 544–549 (83)	MIA25; MIA18	INA2	Positive	II	F
Columbus, Ohio; April 2003, 781–786 (83)	COL24; COL21	ISG-1 (9–12)	Positive	III	B
Bergen-Passaic, New Jersey; October 2003, 1243–1248 (77) 1255–1260 (61)	BER11; BER01, BER07, BER16	(16; 19)	Positive	III; I	D; C
Greenville, South Carolina; November 2003, 1336–1341 (82)	GRE14; GRE11	(31)	Positive	II	A
San Jose, California; January 2004, 1498–1500 (83)	JOS28	(21)	Positive	I	C

*See Table III for code.
†Mice were fed fecal floats from cats.
‡Multilocus genotypes: A: microsatellite locus (mi) 6 = 212−, mi 48 = 215−, mi 102 = 175−, mi 163 = 160−, mi 95 = 218; B: mi 6 = 200−, mi 48 = 215−, mi 102 = 191−, mi 163 = 172−, mi 95 = 402; C: mi 6 = 200−, mi 48 = 229−, mi 102 = 175−, mi 163 = 194−, mi 95 = 228; D: mi 6 = 202−, mi 48 = 215−, mi 102 = 191−, mi 163 = no data, mi 95 = 402; E: mi 6 = 184−, mi 48 = 239−, mi 102 = 175−, mi 163 = 194−, mi 95 = 228; G: mi 6 = 184−, mi 48 = 231−, mi 102 = 167−, mi 163 = 164−, mi 95 = 218; F: mi 6 = 200−, mi 48 = 225−, mi 102 = 189−, mi 163 = 158−, mi 95 = 402.

Serology testing of tissue fluids

Tissue fluid, for use in the ELISA test, was collected by freezing individual meat samples from all commodities at −18 C overnight, then thawing at room temperature. Fluids were cleaned of debris by centrifugation. The ELISA was performed using a commercial test kit (*Toxoplasma* Microwell Immunoassay Kit, Safe-Path Laboratories, Carlsbad, California). This kit uses formalin-fixed whole tachyzoites as antigen, and has been validated for use with pork samples (Gamble et al., 2005). For testing beef and chicken tissue fluids, kits were modified by changing the species-specific conjugate. A positive cutoff was established as 5 × the mean ± standard deviation of the mean of all samples tested. Positive cutoff values (5× the population mean) were as follows: pork, 0.060; beef, 0.120; and chicken, 0.165.

Genetic characterization

Toxoplasma gondii DNA was extracted from infected mouse tissues or oocysts (Lehmann et al., 2000). The PCR-RFLP genotypes of the SAG2 locus were used to determine the genetic type (Howe et al., 1997). The PCR products generated from the above reactions were also sequenced. Fifteen microliters of the nested product was electrophoresed in 2% gels. The specific amplicons were purified from agarose gel and directly sequenced in both directions using the Big Dye terminator system, version 3.1 (Applied Biosystems, Foster City, California) using an ABI 373 × 0 capillary sequencer. The sequence chromatograms were edited using SEQUENCHER 4.1 software (Genecodes Corp., Ann Arbor, Michigan). The regions of the chromatograms representing the polymorphic nucleotides at the enzyme recognition sites were examined for the presence or absence of double peaks to identify mixed infection.

All *T. gondii* isolates obtained from cats shedding oocysts after being fed pooled meat samples or individual meat samples were further genotyped in 5 hypervariable microsatellite loci (Blackston et al., 2001). Together, the multilocus genotype provides near fingerprinting resolution (Blackston at al., 2001; Lehmann et al. 2004) and was used here

TABLE V. Isolation of *Toxoplasma gondii* from individual pork samples.

Meat survey location, date, and number (enhancement code)*	Store no.	No. days meat stored at 4 C	Bioassay	
			In mice†	In cats (oocysts shed, days)‡
Boston, Massachusetts (November 2002)		12		
388 Pork (54)	BOS17		0/10	AMAS (+) (day 9)§
389 Pork (54)			0/10	ADL5 (−)
390 Pork (54)			0/10	QDM2 (+) (day 9)§
391 Pork (66)	BOS05		0/10	ING4 (−)
392 Pork (66)			0/10	QDY2 (−)
393 Pork (66)			0/10	ADL6 (−)
Columbus, Ohio (April 2003)		8		
781 Pork (74)	COL21		0/10	QLR7 (−)
782 Pork (74)			0/10	879 (−)
783 Pork (74)			0/10	866 (−)
784 Pork (83)	COL24		6/10	IRY4 (+) (days 6–10)‖
785 Pork (83)			0/10	864 (−)
786 Pork (83)			0/10	877 (−)

*See Table III for code.
†Number of mice infected/number of mice inoculated with digest of 50 g of pork.
‡Cats were fed 300–500 g of meat. (+) Indicates oocysts were shed; (−) indicates oocysts were not shed.
§SAG2 Type II, microsatellite code C (see Table IV).
‖SAG2 Type III, microsatellite code B (see Table IV).

TABLE VI. ELISA data on meat juice from pork samples.

Sample type (no.)	Bioassay result, cat/mouse	ELISA result*	Enhancement code no.†
Atlanta, Georgia (4)	−/−	+	83
Atlanta, Georgia (12)	−/−	++	83
Fort Worth, Texas (354)	−/−	+	72
Boston, Massachusetts (388)	+/−	++	54
Boston, Massachusetts (390)	+/−	++	54
Wilmington, Delaware (470)	−/−	++	83
Miami, Florida (549)	−/+	+	83
Miami, Florida (561)	−/−	+	83
Columbus, Ohio (784)	+/+	+++	83
Bergen-Passaic, New Jersey (1,243–1,248)	−/+	−	77
Bergen-Passaic, New Jersey (1,255–1,260)	−/+	−	61
Greenville, South Carolina (1,336–1,341)	−/+	−	82
San Jose, California (1,498–1,500)	−/+	−	83
New York, New York (1,625)	−/−	+	61
Yakima, Washington (1,653)	−/−	+++	83
Chicago, Illinois (1,890)	−/−	+	83

*ELISA results: <.06 = negative; >.06 = +, >0.1 = ++; >0.2 = +++.
†See Table III for code.

to distinguish between independent versus possibly dependent sources of isolates. The probability of sampling the same multilocus genotype by chance depends on the distance (and time) between infection events and on the genetic diversity in the region. No information is available about the distance between isolate, but based on previous results (cited above) we assumed this probability to be negligible.

RESULTS

Toxoplasma gondii was isolated from the feces of 7 cats fed pooled samples of pork (Table IV). Of these 7 cats, oocysts were microscopically identified in the feces of 2 cats on initial screening; in the remaining 5 cats, oocysts were too few in number to identify on initial screening. Fecal floats from these 5 cats were found to be positive for *T. gondii* by bioassay in mice; the individual meat samples fed to these cats could not be individually bioassayed because they had been discarded by the time results of the oocyst bioassay in mice were known. The 12 pork samples that made up the 2 pools for which oocysts were observed in cat feces were collected, and individually bioassayed in mice and in cats (Table V). *Toxoplasma gondii* oocysts were observed microscopically in the feces of 3 cats that were fed pork samples 388, 390, and 784. It is noteworthy that pork samples 388 and 390 were from the same store; whether they were from the same animal is unknown. *Toxoplasma gondii* was also isolated from pork sample 784 (Table V) by mouse bioassay during this secondary screening.

According to the original packaging, samples 388 and 390 were ''naturally raised pork'' and contained no pumped salt solutions. Among the other positive samples, 549, 784, and the 1498–1500 pool also contained no pumped salt solutions. Half the samples in the 1336–1341 and 1255–1260 pools were pumped with a sodium phosphate solution, and half the samples in the 1243–1248 pool were pumped with a solution containing potassium lactate.

Of 2,094 pork tissue fluid samples tested by ELISA, 12 samples had optical density (O.D.) values greater than 5× the mean for the population (Table VI). Three of these samples were the same as the 3 individual meat samples representing cats that had shed oocysts (pork samples 388 and 390 from Boston, Massachusetts; and pork sample 784 from Columbus, Ohio); a fourth ELISA-positive sample was positive by mouse bioassay (pork sample 549 from Miami, Florida). The remaining 8 ELISA-positive pork samples were not positive by bioassay.

No chicken breast samples were found to be positive by cat or mouse bioassay. However, of the 2,094 chicken samples, 27 (1.3%) were positive by ELISA, with O. D. values ranging as high as 6 times the mean value for the population (Table VII).

All beef samples were negative by both bioassay and ELISA.

The *T. gondii* isolates that were obtained from pooled and individual pork samples were genetically characterized. Of the 2 cats with microscopically demonstrated oocysts obtained by feeding pooled pork samples from Boston, Massachusetts, and Columbus, Ohio, 1 was SAG 2 Type II, and 1 was SAG 2 Type III; these isolates were genetically identical to those that were obtained from cats and mice inoculated with the individual pork samples (Tables IV and V). Of the 5 remaining *T. gondii* isolates that were obtained from mice inoculated with pooled fecal floats, 1 was SAG 2 Type III and 2 each were SAG 2 Types I and II; microsatellite data indicated that they were distinct isolates (Tables IV and V).

Based on the findings of 8 pork samples (5 pools + 3 individual) out of a total of 2,094 assayed containing viable *T. gondii* tissue cysts, the minimum prevalence of *T. gondii* in commercially available pork in the retail meat case was calculated to be 0.38% (confidence limits [CL] .17%, .75%) (Tables VIII–X).

DISCUSSION

Overall, a low prevalence of *T. gondii* was found in meat sold from retail stores in the United States. *Toxoplasma gondii* was identified in 7 pork pools from a total of 2,094 pork samples. These data are based on bioassay of pools made of 6 100-g samples obtained from each of the 2,094 individual samples. Results of mouse bioassay suggest that the number of *T. gondii* in pork is low because in 2 instances *T. gondii* was not isolated

TABLE VII. ELISA data on meat juice from chicken samples.

Sample type (no.)	Bioassay result, cat and mouse	ELISA result*	Enhancement code no.
Atlanta, Georgia (6)	−	++	83
Buffalo, New York (149)	−	+++	83
Seattle, Washington (170)	−	+++	83
Fort Worth, Texas (310)	−	++	83
Wilmington, Delaware (459)	−	++++	83
Wilmington, Delaware (485)	−	++	83
Wilmington, Delaware (492)	−	++	83
Miami, Florida (561)	−	++	83
Miami, Florida (582)	−	+++++	83
Columbus, Ohio (781)	−	+++++	01
Appleton-Oshkosh, Wisconsin (929)	−	+++	01
Philadelphia, Pennsylvania (1,082)	−	++	01, 08
Philadelphia, Pennsylvania (1,092)	−	+++	01
Philadelphia, Pennsylvania (1,102)	−	++	01, 08
Fort Collins, Colorado (1,178)	−	++	83
Greenville, South Carolina (1,304)	−	+	10
Fort Lauderdale, Florida (1,416)	−	++++	83
San Jose, California (1,491)	−	+++++	83
San Jose, California (1,500)	−	++	28
New York, New York (1,596)	−	+	83
New York, New York (1,610)	−	+	83
New York, New York (1,627)	−	+	01, 10
New York, New York (1,636)	−	++++	83
Yakima, Washington (1,680)	−	+	09
Detroit, Michigan (1,764)	−	++	28
Chicago, Illinois (1,918)	−	+++++	83
Orange County, California (2,015)	−	++	01, 09

*ELISA results: <.165 = negative; >.166 = +; >0.2 = ++; >0.3 = +++; >0.4 = ++++; >0.5 = +++++.

in mice inoculated with homogenates from 50-g samples, but cats fed 500-g samples shed oocysts.

In the present study, only a low number of *T. gondii* oocysts were found in the feces of cats that were fed pooled tissues. Oocysts were detected microscopically in the feces of 2 cats and by mouse bioassay for 5 other cats. This was unexpected because in a previous study cats fed approximately 500 g of myocardium and tongue tissue from 51 of 55 naturally infected pigs from a farm in Massachusetts shed 25–810 million oocysts (Dubey, Gamble et al., 2002). These differences in part maybe due to differences in *T. gondii* concentration in different organs of pigs. In a previous study (Dubey et al., 1986) of 6 pigs tested, *T. gondii* was found in the tongue of 5 and the tenderloin of 3. Although data are based on only 2 laboratory-adapted strains of *T. gondii* (VEG and M7741), cats fed even a few bradyzoites shed millions of oocysts (Dubey and Frenkel, 1976; Dubey, 2001). However, the number of *T. gondii* oocysts shed by cats in nature is unknown. Ruiz and Frenkel (1980) found *T. gondii* oocysts in 55 of 237 cats in Costa Rica. Oocysts were detected microscopically in the feces of only 12.7% and were detected by mouse bioassay in the remaining 87.3%. Low oocyst counts in our study may be related to the meat being inadvertently frozen during transport from the slaughterhouse to the store. These findings should be considered in future surveys for *T. gondii* infection in livestock.

The ingestion of infected uncooked pork was believed to be a major meat source of *T. gondii* infection for humans in the United States (Mead et al., 1999). This is paradoxical because most consumers, since childhood, are aware of the danger of eating uncooked pork in the United States due to a fear of acquiring *Trichinella spiralis* infection. Therefore, further studies are needed concerning the dietary habits and other sources of meat consumed in the United States.

Previous studies have indicated that enhancement, or ''pumping,'' of pork products with salt or lactate-based solutions results in a loss of viability of *T. gondii* tissue cysts in pork (Hill et al., 2004). In the present study, of the 8 (388 and 390 were likely from the same animal) positive pork samples identified from individual and pooled meat samples, 2 were naturally raised and not enhanced, 3 others contained no enhancement solutions, and 2 were pumped with water and sodium phosphate, which was shown not to affect *T. gondii* tissue cyst viability in the previous study. The final sample from Bergen-Passaic, New Jersey, consisted of the pooled samples 1243–1248 and was positive by mouse bioassay. Unfortunately, the individual samples were discarded before the results of the mouse bioassay were known and were, therefore, not individually tested. However, 3 of the 6 samples that made up this pool were enhanced with a lactate-based solution and were, therefore, not the likely source of viable tissue cysts, suggesting that samples 1246, 1247, or 1248 (or a combination of these) were the likely source of the infectious tissue cysts.

In a previous report (Gamble et al., 2005), we validated the use of ELISA for testing pigs for *T. gondii* infection. In that study, using serum samples, the ELISA performed comparably to the MAT, with a sensitivity of 88.6% and a specificity of

TABLE VIII. Risk to consumers of purchasing *Toxoplasma gondii* contaminated meat from U.S. retail stores.

Meat	Annual meat consumption (kg)	Minimum likely prevalence (no. positive/no.) (95% CL)	Probability of purchasing *T. gondii* contaminated meat over time*	
			1 yr	10 yr
Pork	23.5	.0038 (8/2094) (.00165, .0075)	.0626	.4765
Beef	29.5	0 (0/2094) (.0000, .0176)	0	0
Chicken	37.2	0 (0/2094) (.0000, .0176)	0	0

*Binomial probability computations assume a mean sample weight of 1.36 kg.

98.0%. When tissue fluids were used in place of serum samples, sensitivity was reduced to 60%. In the present study, 4 of 12 ELISA positives were from pork samples that were also positive by cat or mouse bioassay (samples 388, 390, 784, and 549). Considering the relatively low sensitivity reported for the ELISA using tissue fluids from pork samples, it is likely that the 12 positive values reported here was an underestimate of seropositivity in this population. The 8 positive ELISA values obtained that were not supported by bioassay may be false positive results or may be true positive results from meat that had been treated by one or more methods known to inactivate *T. gondii* (via hard chilling, pumping, etc.). Of the 4 samples that were bioassay-positive and ELISA-negative, at least 3 had been injected with enhancement solutions up to 12% by weight of the meat sample. The low sensitivity of tissue fluid ELISA, coupled with the dilution effect of the injected enhancement solutions on the tissue fluids, probably eliminated the possibility of detection of any anti-*Toxoplasma* antibody that was present.

Toxoplasma gondii has not been isolated from edible beef in the United States. Previous studies are based on a relatively small sample (100 g) from a total weight (500 kg or more). In the current study, we did not detect any bioassay or ELISA-positive samples in any of the 349 pools and 2,094 individual beef samples tested.

The lack of isolation of *T. gondii* from chicken breasts from the meat sold in retail meat stores is noteworthy. *Toxoplasma gondii* has been isolated frequently from the tissues of backyard chickens in the United States (Dubey et al., 2003). Better rates of recovery of *T. gondii* could be obtained by collecting chicken hearts, because *T. gondii* in chickens localizes more in the heart than in skeletal muscle and the brain (Dubey et al., 2004, 2005). However, a surprisingly high number of positive ELISA results were obtained with chicken (1.3% positive). The ELISA test used here in chickens has not been validated as was the test for pork; thus results can be considered only as preliminary. However, the high rate of positives by ELISA, coupled with negative bioassay results, are consistent with the knowledge that most chicken sold in retail meat outlets has been frozen to the extent that *T. gondii* would be inactivated. Further studies on the prevalence of *T. gondii* in domestic chickens, especially those raised in nonbiosecure systems, and the use of ELISA to detect infection in chickens, are warranted.

In total, 10 isolates (7 from initial pooled sample testing and 3 from individual sample testing) were obtained from different meat samples from 6 states. Three isolates were obtained from meat samples of 1 store in Boston, Massachusetts (cats QDM, QDM2, and AMA5 from Massachusetts), as well as 2 other isolates from Columbus, Ohio (ISG1 and IRY4; Tables IV and V). Among the multilocus genotypes of the 10 isolates, 7 were unique. These 7 represented meat samples from different stores. Identical multilocus genotypes were found among the multiple isolates from the same store (in Massachusetts and Ohio; Table IV). These results indicate that isolates from different stores originated from independent sources, and that at a given time only 1 infective pork sample is likely to be found in a given store.

All genotypes based on oocysts and subpassages in mice produced a single allele for each locus, as expected if a single strain (genotype) was infecting each animal. Isolates from subpassages of the oocysts into mice (Table V) produced identical multilocus genotypes as the 1 derived from the oocysts. The lack of recombinant genotypes in these cases further suggests that only 1 genotype of *T. gondii* was present in the original pig. Although these results are consistent with low probability of multiple strain (genotype) infection in the same pig, our small sample size does not allow us to rule out that multiple strain infections do occur.

In the present study, of the 7 *T. gondii* isolates from pork, 3 were Type III, 2 were Type II, and 2 were Type I. To our knowledge the Type I strain has not been reported from pork in the United States. Of the 170 *T. gondii* isolates from sows in Iowa, 43 were selected for SAG2 genotyping; 36 isolates

TABLE IX. Risk to consumers of purchasing *Toxoplasma gondii*-contaminated meat from retail stores in the northeastern United States.

Meat	Annual meat consumption (kg)	Minimum likely prevalence (no. positive/no.) (95% CL)	Probability of purchasing *T. gondii*-contaminated meat over time*	
			1 yr	10 yr
Pork	23.5	.0089 (4/450) (.0024, 0226)	.1409	.7812
Beef	29.5	0 (0/450) (.0000, .0082)	0	0
Chicken	37.2	0 (0/450) (.0000, .0082)	0	0

*Binomial probability computations assume a mean sample weight of 1.36 kg.

TABLE X. Risk to consumers of purchasing *Toxoplasma gondii*-contaminated meat from retail stores in the Midwest, South, and Western United States.

Meat	Annual meat consumption (kg)	Minimum likely prevalence (no. positive/no.) (95% CL)	Probability of purchasing *T. gondii*-contaminated meat over time*	
			1 yr	10 yr
Pork	23.5	.0024 (4/1644) (.0007, .0062)	.0406	.3391
Beef	29.5	0 (0/1644) (0, .0022)	0	0
Chicken	37.2	0 (0/1644) (0, .0022)	0	0

*Binomial probability computations assume a mean sample weight of 1.36 kg.

were Type II, and 7 were Type III (Mondragon et al., 1998). One (P 89) of these 170 isolates was identified as a recombinant of Type I and Type III strain (Howe and Sibley, 1995). Of the 25 isolates from finisher pigs (Dubey et al., 2002) selected for SAG2 typing, 20 were Type III and 5 were Type II (Lehmann et al., 2003).

Aspinall et al. (2002) detected *T. gondii* DNA by PCR in 19 of 57 (33%) pork, 6 of 9 (66%) lamb, and 1 of 4 beef samples from grocery stores in the United Kingdom. These prevalences are much higher than those found in our study, which was based on the detection of viable *T. gondii* in a population-based sample of whole fresh meats.

In our survey, the overall risk of a person acquiring meat contaminated with *T. gondii* was less than 50% per decade (Tables VIII–X). The risk of a person acquiring infection from contaminated meat would be considerably lower because meat (especially pork) is often well cooked, which kills *T. gondii* tissue cysts. *Toxoplasma gondii* antibody testing in a representative sample of human sera from the National Health and Nutrition Examination Survey (NHANES) (Jones, Kruszon-Moran et al., 2001; Jones et al., 2003) indicated that 22.5% of the U.S. population ≥12 yr old is infected with *T. gondii*, and that 15% of those between the ages of 20 and 29 yr are infected. The risk of acquiring contaminated meat as determined from our meat-case survey seems too low to explain the source of most of the *T. gondii* infections in the United States. Therefore, other factors must be responsible for many of the human infections. These include other types and cuts of meat, exposure to soil contaminated with cat feces (e.g., soil on fruits and vegetables, from gardening, and from soil-related occupations), and unfiltered water. In addition, a portion of persons infected with *T. gondii* in the United States may acquire it when living or traveling outside the United States.

Pork was the only meat we found to contain viable *T. gondii* tissue cysts, but it is possible that the other meats evaluated were infected at levels too low to detect with the laboratory methods utilized, or that postharvest processing, such as hard chilling and pumping, may render *T. gondii* tissue cysts nonviable. ELISA results using tissue fluids from the chicken samples suggest that >1% of battery-raised chickens may have been exposed to *T. gondii*, and that postharvest technologies are effective in killing tissue cysts that are present. Increasing consumption of organically raised and free-range chickens may indicate that a significant risk exists for meat from these types of chickens, and further study is essential on *T. gondii* prevalence in these types of management systems and on postharvest technologies that are effective in killing *T. gondii* tissue cysts in chicken meat.

It is important to note that these results cannot be generalized to other types of meat not tested in this study (e.g., lamb or game meats) or ground meats that are prepared from many animals.

In our study, meat sampled from the northeastern United States had a higher prevalence for *T. gondii*, which is consistent with higher seroprevalence found in the northeastern U.S. in the NHANES survey (Jones, Kruszon-Moran et al., 2001). Because of the potential for *T. gondii* to lead to severe disease, particularly in pregnant women and persons with immunosuppression (many of whom are infected with the human immunodeficiency virus), it is essential to cook all meat thoroughly to prevent infection with *T. gondii* (Dubey et al., 1990). In a retrospective study of 131 mothers who had given birth to children infected with *T. gondii*, 50% recalled having eaten uncooked meat (Boyer et al., 2005).

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