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Low prevalence of viable *Toxoplasma gondii* in fresh, unfrozen American pasture-raised pork and lamb from retail meat stores in the United States

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Abstract

In a national survey of fresh, unfrozen, American pasture-raised lamb and pork, the prevalence of viable *Toxoplasma gondii* was determined in 1500 samples selected by random multistage sampling (750 pork, 750 lamb) obtained from 250 retail meat stores from 10 major geographic areas in the USA. Each sample consisted of a minimum of 500g of meat purchased from the retail meat case. To detect viable *T. gondii*, 50g meat samples of each of 1500 samples were bioassayed in mice. Viable *T. gondii* was isolated from 2 of 750 lamb samples (unweighted: 0.19%, 0.00–0.46%; weighted: 0.04%, 0.00–0.11%) and 1 of 750 pork samples (unweighted: 0.12%, 0.00–0.37%; weighted: 0.18%, 0.00–0.53%) samples. Overall, the prevalence of viable *T. gondii* in these retail meats was very low. Nevertheless, consumers, especially pregnant women, should be aware that they can acquire *T. gondii* infection from ingestion of undercooked meat. Cooking meat to an internal temperature of 66°C kills *T. gondii*.

Keywords

Toxoplasma gondii; pork; lamb; retail meats; bioassay; isolation; genetic characterization

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Conflict of interest
None.

1. Introduction

The protozoan *Toxoplasma gondii* infects virtually all warm-blooded animals, including humans, livestock, and marine mammals (Dubey, 2010).

The ingestion of food or water contaminated with oocysts excreted by infected cats or ingestion of uncooked or undercooked meat containing tissue cysts of *T. gondii* are the 2 major modes of transmission of *T. gondii*. In a national survey of 6,282 samples of meats from grocery stores conducted from April 2002 to September 2005, viable *T. gondii* was isolated from 7 of 2094 pork samples but not from any of 2094 beef or 2094 chicken samples purchased from 698 retail meat stores from 28 major geographic areas of the USA (Dubey et al., 2005). Although *T. gondii* has been isolated from slaughtered lambs in the USA (Dubey et al., 2008), there is no information on the risk of eating lamb sold in grocery stores.

About one-third to one-half of the meat consumed in the USA is further processed. Since 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, such processed products are not likely sources of human exposure (Hill et al., 2006b; Hill et al., 2018). Similarly, frozen imported meat is probably not important in the epidemiology of *T. gondii* because freezing kills the parasite (Kotula et al., 1991). Thus, the major risk to consumers is thought to derive from products in the fresh meat case.

Serological surveys have indicated that up to 30% of domestic pigs have been exposed to *T. gondii* worldwide (Limon et al., 2017; Rostami et al., 2017) and viable *T. gondii* has been isolated from lamb and pork tissues obtained at slaughter (Dubey, 2010). A recent study in Brazil detected *T. gondii* DNA in processed pork (sausages and salami) in 39% of 118 samples from different producers (Costa et al., 2018). However, detection of *T. gondii* DNA does not distinguish between dead or live *T. gondii*.

Therefore, to better understand the risks that pastured pork may pose to consumers in the form of viable toxoplasmosis, we undertook a national survey of *T. gondii* infection in fresh, unfrozen, pasture-raised American (FUPA) pork and lamb.

2. Materials and methods

2.1. Sampling design

Original plans for the first stage of the design sampling called for a sampling frame of all metropolitan statistical areas (MSAs) in the United States with selection into the study by probability proportional to the MSA's population size. Challenges arose immediately as small MSAs contained a paucity of stores selling (FUPA)-raised lamb and pork. Thus, the sampling frame was limited to metropolitan statistical areas (MSAs) with a 2012 U.S. Census Bureau estimated population size of 600,000 or greater. The remaining 77 MSAs were then stratified by US Census Bureau regions (Northeast, South, Midwest, and West) (Supplementary Table 1) and randomly sampled proportional to the estimated 2012

population. The Louisville–Jefferson County MSA was considered as part of the Midwest region for these analyses.

Originally, a total of 10 supermarkets, grocery outlets, or wholesale club stores per MSA were to be randomly selected proportional to weekly total meat sales from a comprehensive list of store profile data compiled by InfoGroup, Inc., Papillion, NE, USA. Unfortunately, many stores did not carry (FUPA) lamb or pork. Of stores contacted, 37.6% (range=5.5–100%) of stores across all MSAs sold (FUPA) lamb. The percentage and range was the same for (FUPA) pork. Given the large percentage of stores without (FUPA) lamb or pork, we chose to treat the second stage as a simple random sample of stores.

Three samples of each commodity were randomly selected from each of 250 stores, for a total of 1,500 (FUPA) meats, 750 each of pork and lamb. Each sample weighed 0.5 kg. The samples were collected March 2013–October 2015.

2.2. Sampled MSAs

All samples were obtained from boneless or bone-in cuts of meat; namely, pork loin, pork loin chop, center cut loin chop, and pork tenderloin; and lamb leg, lamb chop (blade, arm, or loin), lamb shank, or loin roast. Whole muscle cuts were selected to eliminate potential mixture of tissues from multiple carcasses that might result from sampling ground product. A minimum of 0.5 kg of muscle tissue was selected for each sample, with consideration given to the weight of fat and bone in each package. Samples ranged from 0.5 kg (if no bone was present and no fat was seen on the sample) to 2 kg (if weight of the fat and bone was indeterminable). If a selected package did not meet the minimum weight requirement for a 0.5 kg sample, additional packages were chosen from adjacent packages until the summed weight (s) equaled at least 0.5 kg. No discrimination was made by brand. Only samples not previously frozen were sampled, and only American pork and lamb was sampled. Inquiries were made at each meat counter to identify the (FUPA) nature of each product; when available, original packaging and labeling was checked. Most samples were prepackaged in the meat case. Inquiries were made about the samples in the meat case that were not prepackaged and were identified in the meat case and by store personnel as FUPA. All meat samples were collected, packed on ice at the retail outlet, and shipped the same day by overnight carrier to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland, for processing.

At APDL, information available on the meat packages was recorded digitally (Table 1). In some cases, the information was missing or illegible.

2.3. Sample processing and bioassay for viable *T. gondii*

From each sample, several small pieces of meat were cut to make a total 50 g portion for bioassay in mice. Fifty-gram portions of meat were homogenized in 0.85% NaCl (saline), digested in acidic pepsin, centrifuged, neutralized with sodium bicarbonate, suspended in 5 ml of antibiotic saline, and approximately half of the final digested suspension was inoculated subcutaneously into 5 Swiss Webster (SW) mice as described in detail by Dubey (2010). The SW mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA).

The mice inoculated with meat homogenates were observed for 7–9 weeks. Tissue impression smears of mice that died were examined for *T. gondii* tachyzoites or tissue cysts (Dubey, 2010). Survivors were bled after 46 days post inoculation (p.i.), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies by the modified agglutination test (MAT) as described previously (Dubey, 2010). Mice were euthanized after serological testing 8–10 week p.i. Brain squashes from all mice were examined microscopically for tissue cysts, irrespective of serological tests, as described previously (Dubey, 2010). Mice were considered infected when tachyzoites or tissue cysts of *T. gondii* were microscopically demonstrable in tissue smears. Brain homogenates from *T. gondii* infected SW mice were cryopreserved as described (Dubey, 2010).

2.4. Genotyping of *T. gondii*

The number of *T. gondii* in the brains of SW mice is often too low for obtaining sufficient yield of DNA for genotyping. Therefore, *T. gondii* strains from infected lambs were expanded in interferon-gamma gene knockout mice (KO; Jackson Labs) as previously described (Dubey, 2010). These KO mice are highly susceptible to toxoplasmosis and usually die of acute toxoplasmosis pneumonia within 4 weeks p.i. and tachyzoites can be found easily in their lungs.

For further propagation of *T. gondii*, attempts were made to revive parasites. For this, the cryopreserved vial of brain homogenate was thawed, and contents inoculated subcutaneously into KO mice (Table 1) as described (Dubey, 2010). When the KO mice became ill, they were euthanized, and tachyzoites in lung homogenates were seeded onto Vero cells. DNA extracted from cell-cultured derived tachyzoites was characterized genetically using PCR amplification and restriction fragment length polymorphism (RFLP) using the genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22–8, c29–2, L358, PK1 and Apico (Su et al., 2010).

2.5. Statistical analyses

Sampling probabilities for the MSAs were capped at one for very large MSAs. Due to the large proportion of stores without the commodities studied here, a generalized linear mixed model (Venables and Ripley, 2002) was fit in R version 3.5.1 (R Core Team, 2018) to assess the relationship between sales volume of all meat and organic lamb or pork availability. This was then used to predict the number of stores which would carry (FUPA) lamb or pork from the entire list of stores. Sampling weights were then created by inverting the product of the MSA and store selection probabilities. For stores with multiple commodities, the weight was divided by the number of commodities included in the analysis.

Further analyses were performed in SAS Software version 9.4 (SAS Institute, Inc., Cary, NC). Data were analyzed as a multistage survey. Analyses are presented as both unweighted and weighted due to the changes in sampling design. Both analyses account for clustering by store and MSA and stratification by region. Confidence intervals use Taylor series linearization (Woodruff, 1971). All analyses use the 5% level of significance.

2.6. Ethics

Infected mice were housed at the USDA's Beltsville Agricultural Research Center and cared for in accordance with the Animal Welfare Act, Guide for the Care and Use of Laboratory Animals (<https://www.nap.edu/search/?term=Guide+for+the+Care+and+Use+of+Laboratory+Animals+>) and with the approval of the USDA/ARS Beltsville Area Institutional Animal Care and Use Committee (BAACUC Approval # 16-014).

3. Results and discussion

Viable *T. gondii* was isolated from 2 of 750 samples of lamb (unweighted: 0.19%, CI: 0.00–0.46%; weighted: 0.04%, CI: 0.00–0.11%) and 1 of 750 pork samples (unweighted: 0.12%, CI: 0.00–0.37%; weighted: 0.18%, CI: 0.00–0.53%; Table 1). All SW mice inoculated with pork and lamb tissues remained asymptomatic. Tissue cysts were found in the brains of infected mice, and *T. gondii* antibodies were demonstrable in the positive mice by MAT.

The different sell dates marked on the packages (May 11, and May 15, 2015) (Supplementary Table 2) and the different *T. gondii* genotypes (Supplementary Table 3) indicate that these two positive meat samples came from different lambs. Whether both lambs originated from the same producer could not be ascertained.

Attempts to revive *T. gondii* from cryopreserved bradyzoites from the *T. gondii* strain isolated from pork failed; genotyping was performed on DNA extracted from cryopreserved SW mouse brain homogenate. The 2 *T. gondii* strains from lamb were revived successfully in KO mice and in cell culture (Table 1). Genotyping revealed that the strains were Toxo DB type #2 and 3 (Table 1); details of genotyping for each of 10 markers are shown in Supplementary Table 3.

This was the first national survey of *T. gondii* in FUPA lamb and pork. In the present study, viable *T. gondii* was estimated to be isolated from less than 0.2% of products sampled, with upper confidence limits no higher than 0.53%. Previous studies have revealed that the density of *T. gondii* in commercially available pork and other meats is low (Dubey, 2010). The findings for pork (unweighted: 0.12%, CI: 0.00–0.37%; weighted: 0.18%, CI: 0.00–0.53%) had a lower prevalence estimate than a prior survey of general meat performed 15 years ago (0.38%, CI: 0.17–0.75%; Dubey et al., 2005) but with overlapping confidence intervals. The reasons for low prevalence detected in the present study are unknown.

Results of the present study demonstrate that FUPA labeled pork and lamb can be infected with *T. gondii* and precautions should be taken while handling meat to minimize transmission of *T. gondii* to humans. Ground meats should be cooked to an internal temperature of 160° F (71° C). If freezing natural lamb or pork, meat should be frozen for at least 2 days at or below 0° F before cooking (Kotula et al., 1991; Jones and Dubey, 2012; Opsteegh et al., 2015)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.Isolation of viable *T. gondii* from organic lamb and pork.

Origin	Sample	Bioassay (SW) ^a	Subpassage (KO)	Cell culture	ToxoDB genotype
New Mexico	Lamb S9L3	1/5 ^b	Yes	Yes	#3
New Mexico	Lamb S8L2	1/5	Yes	Yes	#2
Ohio	Pork S5P3	5/5	No	No	#2-like

^aSW= Swiss Webster albino mouse; KO= IFN- γ gene knockout mouse.^bNo. of mice infected with *T. gondii*/No. of mice inoculated.