Research Article

Abhishek Wadhawan, Dolores E. Hill, Aline Dagdag, Hira Mohyuddin, Patrick Donnelly, Jeffrey L. Jones, Teodor T. Postolache*

No evidence for airborne transmission of *Toxoplasma gondii* in a very high prevalence area in Lancaster County

https://doi.org/10.1515/pteridines-2018-0015
received November 9, 2018; accepted November 21, 2018.

**Abstract:** **Background:** *Toxoplasma gondii* (*T. gondii*) has been associated with acute food-borne illness, chronic low-grade inflammation, neuropsychiatric conditions and reactivation of chronic latent infection in immunocompetent hosts. Primary infection with *T. gondii* in pregnant women can lead to congenital toxoplasmosis. In addition to well-known oral tissue-cyst or oocyst ingestion, we hypothesized that the very high prevalence of *T. gondii* in certain populations exposed to agricultural dust could be, in part, a consequence of airborne infection with oocysts.

**Methods:** We collected environmental dust samples from an area with a reportedly high *T. gondii* seroprevalence in the Old Order Amish population, in Lancaster, Pennsylvania. Samples included: a) air filters from air-conditioning units; b) swabs of settled dust; and c) vacuum filters containing airborne field dust. Pools of the swabs and shredded sub-samples of the air filters were fed to pigs, with inoculation into mice of heart tissue from seroconverted pigs. We also investigated the presence of *T. gondii* DNA using PCR amplification.

**Results:** Only one pig seroconverted. However, bioassay of pig heart tissue further inoculated into mice showed no evidence of *T. gondii* infection. Consistently, no evidence of *T. gondii* DNA was revealed in any sample.

**Conclusions:** No evidence of airborne transmission was found in the environmental samples that were examined.

**Keywords:** *Toxoplasma gondii*; airborne transmission; dust; air filters.

**List of abbreviations**

CFM: Cubic feet per minute
OOA: Old Order Amish
PA: Pennsylvania
*T. gondii*: *Toxoplasma gondii*
U.S.: United States

**Introduction**

The neurotropic protozoan parasite, *Toxoplasma gondii* (*T. gondii*) is highly prevalent worldwide and infects almost all homeothermic animals, like humans and cattle, which serve as intermediate hosts for *T. gondii*, while its definitive hosts are from the Felidae, including domestic cats [1]. The seroprevalence rates reflecting *T. gondii* infection in the human population have been reported to vary widely from 0.8 to 77% [2], with an average global prevalence rate of about 30% [1]. According to a series of cross-sectional studies conducted by the Centers for Disease Control and Prevention, the age-adjusted seroprevalence rate for *T. gondii* in the United States (U.S.-born population, aged
The severity of congenital infection, however, is inversely related with gestational age at maternal infection [15]. An untreated fetus infected at the first or second trimester has a higher risk of severe congenital infection, while an untreated fetus infected at the third trimester is more likely to have a subclinical presentation [14]. Major clinical signs of congenital toxoplasmosis include chorioretinitis, cerebral calcifications or hydrocephalus, presenting either alone or in combination [17]. Additional signs of congenital toxoplasmosis include microcephaly, seizures, intellectual disability, strabismus, maculopapular rash, jaundice, decreased intelligence quotient, reduced scholastic development, diarrhea, hypothermia, anemia, hepatosplenomegaly and lymphadenopathy [14, 17-20]. Since toxoplasmosis in pregnant women most often goes unrecognized, systematic education and screening of pregnant women, especially in high prevalence areas, are critical in diagnosing infection and starting early treatment in fetuses and infants [15].

Neurotropic microorganisms, including T. gondii, are of interest since they contribute to low-grade immune-activation [21], which in turn has been reported to be associated with obesity and diabetes mellitus [22-28]. Furthermore, subjects with T. gondii infection have been reported to have twice the odds of being obese as compared to the non-infected subjects [29], thereby indicating that T. gondii infection may be associated with obesity. Additionally, low-grade immune-activation impacts other components of metabolic syndrome [30-36], as well as psychiatric disorders like schizophrenia [37-40], bipolar disorder [41-44] and depression [45-48], all of which have been reported to have high circulating levels of neopterin [48-57], a marker for increased inflammation [58]. Interestingly, it has been speculated that oocysts may become airborne, after being stirred-up in the dust, and can be subsequently inhaled and swallowed (after entering the pharynx via mucociliary transport) [59], or may contaminate food, water, or hands, leading to T. gondii infection [60]. Moreover, seasonal changes in farming conditions, including airborne dust containing T. gondii oocysts could also contribute to the seasonal changes in blood neopterin levels in the OOA, as reported previously by our group [61]. A preliminary investigation was undertaken to determine whether the airborne route of transmission of oocysts could be detected using pig and mouse bioassays, coupled with PCR detection of airborne oocysts captured on air filters or household swabs of settled dust. Identification of household transmission routes, involving oocysts, may lead to important changes to recommendations for preventing T. gondii exposure, in particular in pregnant women.
Methods

Lancaster County is located in the south-central part of the Commonwealth of Pennsylvania (PA) and its largest city is Lancaster. The geographic coordinates of Lancaster, PA are 40°2′N, 76°18′W. It is approximately 368 feet (112 m) above sea level and has a mostly humid continental climate. The yearly average high temperature in Lancaster, PA is 62.8°F (17.1°C) and its yearly average low temperature is 42.8°F (6.0°C). Lancaster, PA receives an average annual rainfall of about 42.8 inches (108.7 cm) and an average annual snowfall of about 18.3 inches (46.5 cm). Lancaster County, PA was chosen for the study due to the high documented seroprevalence for T. gondii infection in the OOA community in the area [6-8] (Postolache et al., unpublished data), as compared to the general U.S. population [5]. Many cats freely roam in the Amish farms and feed by hunting rodents. The predation cycle involving cats and rodents and the high seroprevalence rate in the human population is suggestive of a high level of contamination by T. gondii oocysts in the soil environment. The predominant occupation of the Lancaster County Amish community is farming, which may increase exposure to agricultural dust potentially containing T. gondii oocysts, during plowing or other soil-disturbing farm activities.

Sample collection

University of Maryland Baltimore Institutional Review Board approved the protocol for protection of human subjects and for the collection of all samples in our study. We tested three different sources of airborne dust for T. gondii oocysts: 1) air filters from window air-conditioning units, 2) vacuum residue from airborne dust from crop fields, and 3) swabs of settled dust from inside and outside homes and farm structures. All samples were collected between August 24, 2017, to November 1, 2017. We collected a total of 41 air filters from air-conditioning units in 6 different locations surrounded by OOA farms: a) one Mennonite house, b) two churches, c) one local thrift shop, d) one sports complex, and e) one corn field. Precautions were taken by wearing gloves to prevent contamination of filters. At each location, the air filters were immediately placed together in one to three plastic bags, with the placement of a paper towel wet with purified water in each bag to maintain the viability of T. gondii oocysts by maintaining appropriate humidity in the air inside the plastic bags. All plastic bags were labeled with the global positioning system coordinates of the locations from where they were collected, and photographs of the areas surrounding these locations were taken (available upon request). If known, the dates when every filter was installed were also noted, providing information about the duration for which the filter had been in use. On an average, these air filters had been in use for a duration ranging from 2 months to 4 years. However, the air filters in the corn field were from an air-scrubber that was placed there for approximately 8 hours only. The average air-flow through most of these filters was estimated to be between 1200-2000 cubic feet per minute (CFM), except the filters extracted from the sports complex, where the average airflow was estimated to be between 5000-10,000 CFM.

Dust stirred-up in the fields by the horses and the machines pulled by them was collected onto 2 round air filters of a portable vacuum in 4 different cultivated plots of land. A team member, while utilizing respiratory and eye precautions by wearing a disposable particulate respirator and a face shield, held a portable vacuum in his hand that was directed at the airborne dust stirred-up by the Amish horse-driven machines, which were being used to plow the fields or cut crops. The airborne dust was vacuumed for about 20-30 minutes in each of the fields, while the team member followed the horse-driven machines on foot with the vacuum in hand. Abundant dust was observed with the naked eye and the vacuuming occurred directly in the cloud of the dust.

Swabs of settled dust were taken from the houses and outbuildings in the area, with the primary sources of settled dust being the window frames and the top of the door frames, both outside and inside the houses, barns and other farm-related structures. A total of 191 swab tubes were collected (each of them containing 1-2 swabs) and swabs from these tubes were fed to the pigs. An additional 92 swab tubes were collected and used for T. gondii DNA analysis.

Analyses

Parts of the air-filter samples and 92 swab tubes (collected additionally as described above) were sent to the Dr. Noah Fierer’s laboratory at the University of Colorado, Boulder, CO, USA for T. gondii DNA analysis. At his facility, DNA was extracted from the air filters, vacuumed airborne dust and swab samples and a hypervariable region of the 18S rRNA gene was PCR-amplified using barcoded PCR primers (to permit multiplexing), with the PCR primers designed and validated to cover the entire eukaryotic domain. The amplicons were pooled and sequenced on an Illumina MiSeq run yielding >10,000 reads per sample after quality filtering.
The taxonomic identity of each read was determined by matching against the Silva database. As Dr. Fierer’s team has successfully used this same pipeline to detect Apicomplexan parasites (including *T. gondii*) and other eukaryotic microorganisms in other sample types, such as soil [63] and geothermal spring water [62], we know that these methods could also detect *T. gondii* in our samples.

Additionally, bioassays were conducted in pigs by using the air filter and swab samples. Each filter sample was individually minced, and 100 grams of the minced filter was fed directly to 1 pig at a time. For swab samples, the cotton tip was removed and 28-54 such tips were fed to each pig. A total of 37 pigs were used in the study for the analyses of the air filters and the swabs of settled dust. Pigs (weighing about 50 kg) were derived from the United States Department of Agriculture’s Beltsville herd (proprietary stock), and were serologically tested by ELISA (IVD Research, CA) using the manufacturer’s instructions for the presence of antibody to *T. gondii* before feeding, and at biweekly intervals during the study to monitor for seroconversion [64]. The positive cut-off value of the ELISA was set at 0.30 by the manufacturer. To further validate infection with *T. gondii* in pigs post-feeding, the pigs that seroconverted were sacrificed 60 days post-infection, and 50 grams of heart tissue was digested from each seroconverted pig and inoculated into 10 mice. After 60 days, brain smears from all mice were examined microscopically for the presence of *T. gondii* tissue cysts.

**Discussion**

We found no evidence of the occurrence of *T. gondii* in our samples of air filters, settled dust and vacuumed airborne dust. Previous studies have used this methodology to successfully detect *T. gondii* in other types of samples (e.g., cat feces) [65-67]. Viable *T. gondii* oocysts were either not present in these samples or they were present in insufficient abundance to be detected.

Though our study was conducted in a geographical region of the U.S. that is known to have a higher *T. gondii* seroprevalence rate in people than is typical for the U.S. population, the negative finding in this study should be interpreted with caution. Previous studies have demonstrated the difficulty in detecting *T. gondii* oocysts in the farm, rural and urban environments, where high seroprevalence rates were detected in resident farm animals or humans [68-72]. Though animal bioassay is a highly sensitive method for detecting *T. gondii*, extant naturally occurring environmental factors may negatively impact the viability of oocysts present in the dust (e.g., temperature and humidity), and anthropogenic impacts may also play a role in reducing oocysts numbers in surface soils (e.g., plowing, turning under and removal of cats). Previous serological examination of this OOA community have demonstrated 37.2% seroreactivity to a *T. gondii* oocyst-specific antigen, named *T. gondii* embryogenesis-related protein (TgERP) [6, 73] (Wadhawan et al., unpublished data), thereby strongly implicating *T. gondii* oocysts as the source of infection in these individuals. Since the current study failed to identify viable oocysts in environmental samples collected by us, the source of these oocysts remains enigmatic. Future studies will involve collecting soil samples from the crop fields in parallel to air-borne agricultural dust samples in townships of Lancaster County that have the highest reported *T. gondii* seroprevalence (suggesting a higher occurrence of viable *T. gondii* oocysts in the environment).

**Results**

As expected, the vacuumed airborne dust samples were dominated by the DNA from plants, animals and fungi (including Alternaria and Cladosporium - known allergens). However, no *T. gondii* was detected (though we did pick up some unclassified Apicomplexan sequences). *T. gondii* was either not present in these samples or was in insufficient abundance to be detectable.

ELISA results from the pigs indicated that only one pig had a low-positive seroconversion (0.365; positive cut-off: 0.30) that was initially detected at 6 weeks post-feeding of 100 grams of filter material from a round filter used to collect dust during horse/plow operation in agricultural fields. In fact, this low seropositive status persisted for the remainder of the experiment. However, all mice inoculated with heart tissue digest from this seroconverted pig were negative for the presence of *T. gondii*, based on the microscopic observation of brain smears from each inoculated mouse.

**Acknowledgments:** This work was supported by the U.S. Food and Drug Administration through the cooperative agreement FDU.001418 (TTP). Additional funding for this study was provided by the Mid-Atlantic Nutrition Obesity Research Center (NORC) Pilot & Feasibility Project (Postolache, PI), a sub-award of the parent grant P30DK072488 (Simeon I. Taylor, Program Director). The writing of this manuscript, as well as the sample analysis, was supported by the U.S. Department of Agriculture, Beltsville, MD. We thank Dr. Noah Fierer for his help with the DNA analysis by PCR amplification at his laboratory in the University of Colorado, Boulder, CO.
USA. We express our gratitude to Dr. Hina Makkar from Mood and Anxiety Program and the staff of the Amish Research Clinic in Lancaster, PA, particularly our Amish liaison Mrs. Hanna King, for their overall support in the collection of the samples for this study. The authors thank Alexandra Dagdag for her help in proofreading of this manuscript. We also thank Smucker Mechanical LLC. for providing their professional help in collecting the air filter samples for our study from air-conditioning units across the Lancaster County. The views, opinions, and findings contained in this article belong to the authors and should not be construed as an official position of the US FDA or US Department of Agriculture.

Conflicts of interest: The authors declare that they have no conflict of interest related to the publication of this paper.

References

27. Grace C, Goldrick R. Fibrinolysis and body build: interrelationships between blood fibrinolysis, body


