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

Abhishek Wadhawan,

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Saint Elizabeths Hospital, Psychiatry Residency Training Program, Washington, DC 20032, USA

Dolores E. Hill,

U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Beltsville, MD 20705, USA

Aline Dagdag,

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Hira Mohyuddin,

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Patrick Donnelly,

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Amish Research Clinic, University of Maryland School of Medicine, Lancaster, PA 17602, USA

Jeffrey L. Jones,

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Teodor T. Postolache*

Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA; Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Veterans Integrated Service Network (VISN) 19, Military and Veteran Microbiome Consortium for Research and Education (MVM-CoRE), Denver, CO 80220, USA; Mental Illness Research, Education and Clinical Center (MIRECC), Veterans Integrated Service Network (VISN) 5, VA Capitol Health Care Network, Baltimore, MD 21201, USA, tpostola@som.umaryland.edu

Abstract

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*Corresponding author: Teodor T. Postolache.

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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

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 12–49 years, continued to decline from nearly 14% (from 1988–1994) [3], to 9% (from 1999–2004) [4], to 6.7% (from 2009 to 2010) [5]. In comparison, Old Order Amish (OOA) from Lancaster County, Pennsylvania, a primarily agrarian community, have a much higher *T. gondii* seroprevalence rates (46–65%) [6–8] (Postolache et al., unpublished data). *T. gondii* has three infectious stages, with all stages having the capability of infecting the definitive as well as the intermediate hosts, including tachyzoites, sporozoites (present in the oocysts), and bradyzoites (present in the tissue cysts) [1, 9]. Though tissue cysts enclosing bradyzoites are found in the skeletal muscles, cardiac muscles, and neuronal tissues like the brain and eyes, they also occur in other organs of the body, such as the kidneys, lungs and the liver [10]. Tissue cysts that remain intact may remain viable in host tissues throughout the life-span of the host. However, tissue cysts may rupture intermittently to release bradyzoites that may convert to tachyzoites within immunosuppressed hosts (e.g., patients with Acquired Immunodeficiency

Syndrome), resulting in acute infection and formation of new tissue cysts [11]. When cats consume tissue cysts, bradyzoites are released in the gut, which results in the production of oocysts that are released into the soil/surroundings along with cat feces [9]. Oocysts can withstand extreme environmental conditions [12]. Invertebrates like cockroaches, flies, earthworms and dung beetles can carry and transmit oocysts contained in the soil to animal fodder and food destined for human consumption [1, 9].

The various routes by which humans are infected with *T. gondii* are: 1) ingestion of infectious oocysts by mouth through food, hands, and water contaminated with oocysts; 2) ingestion of tissue cysts via eating infected raw or undercooked meat from intermediate hosts; 3) congenital transmission of tachyzoites vertically from the mother to the fetus; and 4) transplantation of an organ or blood transfusion from an infected individual [1, 13]. Congenital transmission occurs when a seronegative mother becomes infected during pregnancy [14, 15] or has a reactivation of a dormant infection during pregnancy. Women with prior infection may be protected from transmitting *T. gondii* to the fetus, unless infection occurred shortly before conception, or if women who are immunocompromised and chronically infected have a reactivation of infection [14]. Tachyzoites colonize placental tissue and gain access to the fetal compartment in about 30% of cases [16]. Abortion, stillbirth and premature births may occur [14]. The risk of vertical transmission increases with gestational age at maternal infection: 15% at 13 weeks, 44% at 26 weeks, and 71% at 36 weeks of gestation [14, 15]. 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 airconditioning 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 [62]. 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.

Results

As expected, the vacuumed air-borne 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.

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 a 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).

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List of abbreviations

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

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