RISK FACTORS FOR INFECTION WITH TOXOPLASMA GONDII FOR RESIDENTS AND WORKERS ON SWINE FARMS IN ILLINOIS

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Abstract. Risk factors for Toxoplasma gondii infection in workers and residents of swine farms were studied on 43 farms in Illinois. Blood samples were collected from 174 adults in 1993. The T. gondii seroprevalence was 31%. An interview was conducted with each participant, obtaining information on demographic characteristics and behaviors suspected to affect the risk of T. gondii infection. Factors associated with increased risk of T. gondii seropositivity were a higher number of seropositive cats trapped on the farm, male sex, rearing pigs on pasture, and gardening. Factors associated with a decreased risk were handling of pig feed and presence of cats inside the pig facilities. Thus, infection of cats with T. gondii increased the risk of human infection, and contact with soil was a likely mechanism for transmission. The increased risk of seropositivity in males is attributed to less attention paid to cleanliness in food preparation and eating.

Human infection with the protozoan parasite Toxoplasma gondii is common worldwide. Serologic surveys in the United States have produced a range of estimates (10–60%) of the prevalence of infection in adults. There can be serious health consequences due to infection with T. gondii. Initial maternal exposure to the parasite during pregnancy can cause fetal or neonatal complications, including mortality, low birth weight, blindness, hearing loss, and the development of mental and motor disabilities during childhood. Immune-compromised persons, such as cancer and acquired immunodeficiency syndrome (AIDS) patients and transplant recipients receiving immunosuppressive drugs, may also suffer serious health consequences due to T. gondii infection, including encephalitis and retinal inflammation. Toxoplasmosis is a major cause of death in AIDS patients.

There are several potential sources of T. gondii infection for humans. Cats are the definitive host for the parasite, excreting oocysts in feces. Transmission of T. gondii to humans has been hypothesized to occur via handling of contaminated cat feces, soil, water, and animal feed. The other suspected mode of transmission is through handling or consumption of raw or undercooked meat infected with T. gondii tissue cysts.

There have been numerous studies of risk factors for human infection with T. gondii. Exposure to cats and cat feces has been considered a major risk factor for acquisition of infection. However, while several studies have concluded that exposure to cats increased the risk of T. gondii seropositivity, other studies found no association, and one study found that possession of cats decreased this risk. Exposure to cats is not sufficient for transmission of T. gondii infection to humans; infection in cats and personal hygiene should also be evaluated. Only one of the studies mentioned above tested cats for possible infection with T. gondii; in study there were data suggesting that infection of cats with T. gondii was associated with an increased risk of human infection.

The role of contact with soil in affecting the risk of T. gondii infection has been rarely studied. Whereas contact with soil and gardening have been implicated as risk factors for T. gondii seropositivity, several studies have found no association between soil contact and seropositivity.

The role of handling or consumption of raw meat in the acquisition of T. gondii infection has not always been clear. Several studies have found no association, although some studies have identified an association between eating raw meat and T. gondii seropositivity, and handling of raw organ meats used for pet food has been identified to increase the risk of T. gondii infection in men. The seroprevalence for T. gondii increases with age, presumably due to accumulated opportunities for exposure. Numerous studies have found no sex differences in T. gondii seroprevalence, although one study found a higher seroprevalence in male farmers in Japan, and attributed this to more consumption of raw meat.

Rural rearing, exposure to farm animals, and rearing of pigs have been identified as increasing the risk of human seropositivity. Two epidemiologic investigations have identified a high seroprevalence of T. gondii infection in cats inhabiting swine farms: 42% and 67%, and infection in cats has been implicated as a risk factor for T. gondii infection in pigs. Thus, residents and workers on swine farms have a high risk of environmental exposure to T. gondii.

The purpose of the study reported here was to conduct a survey of the T. gondii seroprevalence in residents and workers on swine farms, and to identify behavioral, demographic, and farm characteristics that were risk factors associated with T. gondii seropositivity.

METHODS

An epidemiologic study of sources and risk factors for transmission of T. gondii infection to swine had been conducted on 47 swine farms in Illinois in 1992 and 1993. In this field investigation, each farm was visited three times between late March and early October in a given year (either 1992 or 1993). On each visit one night of overhead live trapping was conducted, and blood and fecal samples were obtained from cats (mostly feral) by jugular venipuncture following anesthesia. Cats were classified as adult if they
were adult size, which is usually achieved by 8–9 months, otherwise they were classified as juveniles. Additional details on the procedures of the epidemiologic investigation have been previously reported.39

Farm owners or managers were contacted by telephone to request further participation in a study of human exposure to *T. gondii* on swine farms. Consent was obtained for 43 (91%) of the farms. Each farm was visited once during the summer of 1993 for the purpose of obtaining human data. The potential pool of subjects included all adults (≥ 18 years of age) working or residing on the farm at the time of the farm visit. Written consent for participation was obtained from all subjects. The protocol for this project was reviewed and approved by the Institutional Review Board of the University of Illinois.

There were 174 adult farm workers and residents who participated in the study. An interview was conducted in private with each participant, in which information was obtained on demographic characteristics and behaviors associated with possible acquisition of *T. gondii* infection. In addition, for each subject a registered medical technologist drew approximately 3 ml of blood from a large vein in the forearm. Blood samples were transferred to serum separator tubes, refrigerated, and centrifuged the same day. Samples were shipped by overnight air express to the Parasite Biology Laboratories, refrigerated, and centrifuged the same day. Samples were analyzed for seropositivity within 48 hours. Seropositive samples were drawn approximately 3 ml of blood from a large vein in the forearm. Blood samples were transferred to serum separator tubes, refrigerated, and centrifuged the same day. Samples were shipped by overnight air express to the Parasite Biology Laboratories, refrigerated, and centrifuged the same day. Samples were analyzed for seropositivity within 48 hours. Seropositive samples were analyzed directly by enzyme-linked immunosorbent assay. A positive test result at the 1:25 dilution was the threshold for seropositivity. This was the same test that was performed on cat sera.39

The individual risk factors analyzed for their association with human *T. gondii* seropositivity included several demographic characteristics: resident versus non-resident status, sex and age of the worker/resident, and years working/residing on the farm. Behavioral risk factors considered were whether or not the subject engaged in gardening, handling raw meat, handled cats, cleaned cat litter boxes, handled pig feed, ate food inside the pig facilities, and washed hands after handling raw meat, after leaving the pig facilities, and before eating. In addition, data on several farm characteristics, obtained from the previous epidemiologic investigation of risk factors for *T. gondii* infection in pigs,39,40 were included in the analysis. The risk of exposure to *T. gondii* oocysts shed by cats was represented in several variables: the number of seropositive cats on the farm, the number of seropositive juvenile cats on the farm, the number of seropositive oocysts in cat feces, pig feed, or water samples on the farm, and whether cats were present inside the pig facilities. Infection in juvenile cats was analyzed separately from all cats because these are the cats most likely to have shed oocysts recently, and were the class of cats implicated in increasing the risk of infection for pigs.40 Farm characteristics indicating possible environmental exposure of humans to *T. gondii* oocysts included whether pig feed was stored in closed containers (and was thereby protected from becoming a site for cat defecation), and whether or not pigs were housed entirely on pasture.

Bivariate associations between risk factors and *T. gondii* seropositivity were examined in two ways. For risk factors measured at the categorical level, associations were examined in 2 × 2 contingency tables (trichotomous categorical variables were reduced to dichotomies, as indicated below), with calculation of odds ratios (ORs), 95% confidence intervals, and *P* values using the chi-square test.43 For risk factors measured at the interval level, differences between the groups of subjects that were seropositive and seronegative for *T. gondii* were tested for statistical significance using the Mann-Whitney U test.44 To control for confounding effects, multiple logistic regression analysis was used to identify risk factors having an independent association with human *T. gondii* seropositivity. Risk factors measured at the interval level (e.g., age, number of seropositive cats) were retained as interval level variables in the logistic regression analysis. Variable selection was by initial forced entry of all risk factors, with backward elimination of all variables where *P* ≥ 0.1. An alpha level of 0.05 (for two-tailed *P* values) was selected for statistical significance for all tests.

### Results

The mean age of subjects was 39 years (median = 36, range = 18–83). Most (77%) were male. Approximately half (53%) were residents on the sampled farm. The mean number of years working or residing on the farm was 17 (median = 10, range = 2 months to 83 years). Descriptive statistics on behavioral risk factors for *T. gondii* infection are shown in Table 1. Most subjects handled raw meat (92%), pig feed (87%) and cats (74%); few subjects (14%) cleaned cat litter boxes. Almost two-thirds (64%) engaged in gardening. Most subjects (60%) never ate food inside pig facilities. The majority of subjects (58%) always washed their hands after leaving the pig facilities, after handling raw meat (67%), and before eating (52%).

Among the 43 farms in the study, there were 366 cats sampled (93 were juveniles), with a mean of 8.5 cats of any age (median = 8, range = 0–27) and a mean of 2.2 juvenile cats (median = 1, range = 0–11) sampled per farm, a mean of six seropositive cats of any age (median = 4, range = 0–
The results of the multiple logistic regression analysis for risk factors for T. gondii seropositivity are shown in Table 3. Factors associated with an increased risk were an increase in the number of seropositive cats on the farm (adjusted OR [aOR] = 1.1, range = 0–10), Toxoplasma gondii oocysts were detected in cat feces, feed, soil, or water samples on six farms (14%). Cats were present inside the pig facilities on 29 farms (67%). There was evidence of cat infection with T. gondii by either serology or detection of oocysts on 41 of the 43 farms studied (95%). Pigs were housed entirely on pasture on four farms (9%). Pig feed was stored in closed containers on 25 farms (58%).

The T. gondii seroprevalence for the farm workers and residents in this sample was 31% (54 of 174) (95% confidence interval = 24–38%). The results of the bivariate analysis for the association of the categorical risk factors with T. gondii seropositivity are shown in Table 2. Only one risk factor had a significant association. Farmers who engaged in gardening had a higher T. gondii seroprevalence than those that did not engage in gardening (37% versus 21%, OR = 2.18, P = 0.049). The results of the multiple logistic regression analysis for the quantitative risk factors, there were no significant differences between farmers that were seropositive or seronegative for T. gondii (Table 3).

A post hoc multiple logistic regression analysis was conducted to determine if the sex difference in T. gondii seroprevalence could be attributed to sex differences in the behavioral risk factors (Table 1) for T. gondii infection. After backward elimination of variables (two-tailed $P \geq 0.1$), the remaining model variables indicated that men were more likely than women to handle pig feed ($P < 0.0001$), and eat food inside pig facilities ($P = 0.050$), and less likely to wash hands before eating ($P = 0.045$), after handling raw meat ($P = 0.042$), and after leaving the pig facilities ($P = 0.019$).

### DISCUSSION

The study reported here estimated the T. gondii seroprevalence to be 31% in 1993 in a population of predominantly male swine farmers in Illinois, with an average age of 39 years. This compares to an estimated 9% T. gondii seroprevalence for the east northcentral region of the United States for military recruits (80% between the ages of 17 and 20 years) in 1989, which was also determined using the agglutination test for detection of antibodies. A serologic survey of swine farmers in Finland in 1989 (median age in the 41–50 year range) estimated the T. gondii seroprevalence to be 37%. A survey of farmers in Japan (specification unspecified; mean age = 50 years) between 1982 and 1985 estimated T. gondii seroprevalence to be 20%. Due to differences in the age structure of populations and the sensitivity of the serologic tests used, these seroprevalence estimates are not strictly comparable. However, the trend in

### Table 3

Bivariate associations of quantitative risk factors with Toxoplasma gondii seropositivity in Illinois swine farmers

<table>
<thead>
<tr>
<th>Quantitative risk factors</th>
<th>T. gondii seropositive</th>
<th>T. gondii seronegative</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of seropositive cats on farm</td>
<td>5</td>
<td>7.15</td>
<td>4</td>
</tr>
<tr>
<td>Number of seropositive juvenile cats on farm</td>
<td>0</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>Years working or residing on farm</td>
<td>8</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37</td>
<td>41.5</td>
<td>36</td>
</tr>
</tbody>
</table>
prevalence values among these studies suggests that swine farmers are at increased risk for *T. gondii* infection.

In the analysis of the bivariate associations of risk factors with *T. gondii* seropositivity in swine farmers, only gardening was identified as a risk factor. However, in the multivariate analysis, which controlled for intercorrelation among the risk factors, the contribution of several other risk factors to *T. gondii* seropositivity became apparent. In the multivariate analysis, the factor having the strongest statistical association with an increased risk of human *T. gondii* seropositivity was an increase in the number of cats seropositive for *T. gondii* on the farm. This supports the hypothesis that cats infected with *T. gondii* present a major risk to humans in the acquisition of *T. gondii* infection in the environment of the swine production facility. These results are consistent with previous studies also identifying exposure to cats as a risk factor increasing the risk of human *T. gondii* infection, suggesting that the risk of exposure to *T. gondii* is a primary means of transmission of *T. gondii* to humans in this environment.

One unexpected result obtained was that presence of cats inside the swine housing facilities decreased the risk of human exposure to *T. gondii*. This is not merely a residual effect after cat seropositivity was taken into account in the multivariate analysis. The human *T. gondii* seroprevalence for subjects living on farms where cats were present inside swine housing facilities was 27% compared with 41% when cats were not present (crude OR = 0.53, *P* = 0.09). A comparison of farms where cats did and did not have access to swine housing revealed that the *T. gondii* seroprevalence for cats was lower on farms where cats had access to swine facilities (median = 63%) compared with farms where cats did not have access to swine facilities (median = 96%) (*P* = 0.01, by Mann-Whitney U test). Perhaps when cats are denied access to swine housing units, they spend more time outdoors and therefore are more likely to encounter other cats or be exposed to oocysts in soil, or acquire *T. gondii* infection through increased opportunities for predation on a variety of species of rodents and birds.

Handling of cats or cleaning of litter boxes was not associated with *T. gondii* seropositivity, and handling of pig feed was associated with a decreased risk of *T. gondii* infection. This is probably due to the absence of fully sporulated oocysts, the infective form, in these sources. Oocysts shed in feces require at least 24 hr to mature before they become infective. Pig feed is consumed and cat litter is usually replaced within 24 hr, which could account for the absence of an increased risk of *T. gondii* seropositivity associated with handling these sources. However, the role of handling pig feed in decreasing *T. gondii* exposure is unclear. The simple association is nonsignificant (crude OR = 0.76, *P* = 0.74). There is no correlated variable deleted in the backward elimination procedure that can explain the suppression of the association in the bivariate analysis. Further investigation is needed to clarify the role of handling animal feed in the transmission of *T. gondii* to humans.

Handling of raw meat and washing of hands after possible exposure to *T. gondii* were not associated with the risk of *T. gondii* seropositivity in the bivariate analysis or in subsequent multivariate analyses. However, the true association may have been masked by gender differences in behavior. Men had a higher risk of *T. gondii* seropositivity.
tivity than women, and also were also more likely to handle pig feed and eat food inside pig facilities, and less likely to wash hands before eating, after handling raw meat, and after leaving the pig facilities. Thus, although the individual behaviors were not determined to increase the risk of T. gondii seropositivity, a complex of gender-associated behaviors, particularly related to the handling of food and washing of hands, suggests that the higher risk of T. gondii infection in men may be due to less attention paid to personal cleanliness. Price\textsuperscript{20} found an increased risk of T. gondii seropositivity due to handling of raw organ meats used as cat food in men but not in women, suggesting that women reduced the risk of infection in this context more than men; the increased risk of infection due to handling of pet food occurred in households where kitchen cleanliness was lower.

There was no association of age with seropositivity in the multivariable analysis, nor was there an association apparent for a complex of behaviors associated with lack of personal cleanliness. Price\textsuperscript{20} found an increased risk of T. gondii seropositivity due to handling of raw organ meats used as cat food in men but not in women, suggesting that women reduced the risk of infection in this context more than men; the increased risk of infection due to handling of pet food occurred in households where kitchen cleanliness was lower.

This study has implicated cats infected with T. gondii, whose home range includes swine farms, as the apparent source of infection for humans working or residing on these farms. Direct transmission does not appear to be due to contact with cats, as is indicated by the lack of association of human seropositivity with handling cats or changing their litter boxes. In fact, many cats residing on swine farms are feral and do not interact directly with humans. It was even a common occurrence for the farmers to claim that they had not seen some of the cats prior to trapping. The increased risk of human seropositivity with gardening and presence on a farm where pigs were raised on pasture suggests that the primary mode of T. gondii transmission to humans is through contact with soil. There also appears to be an increased risk for a complex of behaviors associated with lack of personal cleanliness in food handling and prior to eating, which in this study was more characteristic of men than women. Given the risks to health due to T. gondii infection, prophylactic measures such as wearing gloves when handling soil and washing hands after risk of contamination from soil, raw meat, and objects inside pig-rearing facilities are warranted.

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REFERENCES


