Short communication

High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming


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Abstract

Little is known of the prevalence of *Toxoplasma gondii* in commercially raised chickens. In the present study, the prevalence of *T. gondii* in 96 free-range chickens (*Gallus domesticus*) from a commercial farm in Israel was assessed. Blood, heart, and brain from each chicken were examined for *T. gondii* infection. Antibodies to *T. gondii*, assayed with the modified agglutination test (MAT ≥ 1:5), were found in 45 of the 96 chickens. Hearts and brains of seropositive (MAT ≥ 1:5) chickens were bioassayed in mice. Additionally, hearts and brains of 51 seronegative (MAT < 1:5) chickens were bioassayed in two *T. gondii*-free cats. *T. gondii* was isolated from 19 of the 45 (42.2%) seropositive chickens by bioassay in mice. Both the cats fed tissues pooled from seronegative chickens shed *T. gondii* oocysts. Tachyzoites and tissue cysts of all 21 isolates of *T. gondii* from chickens were avirulent for mice. Seventeen of the 19 isolates genotyped were found to be type II, and 2 were type III. Understanding of the sources of infection on such farms could be the key to the development of better prevention strategies.

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Keywords: *Toxoplasma gondii*; Toxoplasmosis; Isolation; Chickens; *Gallus domesticus*; Israel; Genotyping
1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally mainly by ingesting tissue cysts from undercooked meat or oocysts from the environment contaminated with infected cat feces. Infected pork and mutton are the major meat source of T. gondii. The ingestion of beef appears to be unimportant in the epidemiology of toxoplasmosis because T. gondii has rarely been found in beef (Dubey and Beattie, 1988). Although T. gondii infection appears to be common in sheep and goats, its prevalence in lambs and goat kids used for meat is largely unknown. The ingestion of infected, uncooked pork is a known risk for toxoplasmosis in humans because T. gondii has been isolated from pork in many countries (Dubey and Beattie, 1988).

Little is known of the prevalence of T. gondii in commercially raised poultry. Nearly 40 years ago, Jacobs and Melton (1966) found viable T. gondii from the ovaries of 3, and leg muscles of 1 of 108 chickens from a slaughterhouse in Maryland, USA. Boch et al. (1968) found T. gondii in brains of 5 and hearts of 1 of 1636 hens in Germany. The objective of the present study was to attempt to isolate and genotype T. gondii from tissues of chickens from a commercial, free-range chicken farm in Israel.

2. Materials and methods

2.1. Source of chickens

Chickens were obtained from a commercial farm, approximately 100 km north of Tel-Aviv. The birds were approximately 2.5-year-old and were kept for egg laying. They were killed at a commercial slaughterhouse in Jerusalem on the 28th of August, 2003. Chickens were bled and killed, and their heads and hearts, along with the respective sera samples, were placed in zipper bags and transported with cold packs to Beltsville, MD. Three days elapsed between the tissue collection and their examination for T. gondii and during this time samples were not refrigerated.

2.2. Serological examination for T. gondii

Sera from chickens were diluted two-fold starting at 1:5 dilution and assayed for T. gondii antibodies with the modified agglutination test (MAT) conducted as previously described (Dubey and Desmonts, 1987).

2.3. Bioassay of chicken tissues for T. gondii infection

Brains and hearts of seropositive (MAT $\geq$ 1:5) chickens were bioassayed individually in mice following procedures described previously (Dubey, 1998; Dubey et al., 2002). The brain and heart of each chicken were pooled, homogenized, digested in pepsin and inoculated subcutaneously into five mice (Dubey, 1998). The mice used were Swiss Webster albino...
females obtained from Taconic Farms (Germantown, New York). Tissue impression smears of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 43 post-inoculation (p.i.), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 48 days p.i. and brain squashes from all mice were examined microscopically for tissue cysts as described previously (Dubey and Beattie, 1988). A portion of the brain with demonstrable *T. gondii* was frozen for DNA extraction. Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were demonstrably microscopically in smears of their tissues.

Hearts and brains from 51 seronegative (MAT < 1:5) chickens were pooled in batches of 25 and 26 chickens and fed to two *T. gondii*-free cats (Dubey, 1995). Feces of the cats were examined for *T. gondii* oocyst shedding as previously described (Dubey, 1995). Oocysts were suspended in 2% H$_2$SO$_4$, allowed to sporulate at room temperature, and bioassayed in mice as described previously (Dubey and Beattie, 1988).

### 2.4. Genetic characterization

*T. gondii* DNA was extracted from infected mouse tissues as described by Lehmann et al. (2000). The PCR-RFLP genotypes of the SAG2 locus were used to determine the genetic type (Howe et al., 1997).

### 3. Results

Antibodies to *T. gondii* were found in 45 of 96 (46.6%) serum samples with titers of 1:5 in 3, 1:10 in 2, 1:40 in 5, 1:80 in 5, 1:160 in 3 and 1:320 or more in 27. Fifty-six of 110 mice inoculated from 22 chickens died of presumed bacterial infections between 1 and 7 days after inoculation with chicken tissues, in spite of additional antibiotic treatment; these mice were discarded and not examined for *T. gondii* infection. None of the remaining mice died after 8 days p.i. At least one mouse survived in each of the 45 groups of mice inoculated with tissues from seropositive chickens. Most (59 of 68) mice that survived from the 19 infected groups had *T. gondii* in their tissues (Table 1).

*T. gondii* was isolated from tissues of 19 chickens by bioassay in mice (Table 1). It was isolated from 13 of 27 chickens with titers of 1:320 or more, from 2 of 3 with titers of 1:160, from 2 of 5 with titers of 1:80, from 1 of 5 with titers of 1:40, and 1 of 3 chickens with titers of 1:5. The low pathogenicity of *T. gondii* isolates from chickens from Israel to mice was remarkable because none of the mice became ill due to confirmed toxoplasmosis, assuming that none of the mice that died between 1 and 7 days p.i. were infected with *T. gondii*. Additionally, very few tissue cysts were found in the brains of mice inoculated with tissues from these chickens. Tissue cysts were so few that one-fourth to one-half of brains of mice had to be examined to find tissue cysts.

Both cats fed tissues of 51 chickens with titers of <1:5 shed *T. gondii* oocysts. The mice fed oocysts from these cats were comatose 7 days later and *T. gondii* tachyzoites were found in their mesenteric lymph nodes; genotypes of these two isolates were types II and III. The mice inoculated with tissues of mice fed oocysts remained asymptomatic and tissue cysts were found in their brains 50 days later. Thus, tachyzoites and tissue cysts of all isolates
Table 1
Isolation by bioassay in mice and genotypes *Toxoplasma gondii* from chickens from a farm in Israel

<table>
<thead>
<tr>
<th>Chicken number</th>
<th>Titer</th>
<th>Number of surviving mice</th>
<th>Number of mice <em>T. gondii</em>-positive</th>
<th>SAG2 typing</th>
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<tbody>
<tr>
<td>1</td>
<td>≥320</td>
<td>2</td>
<td>2</td>
<td>II</td>
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<td>1</td>
<td>II</td>
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<tr>
<td>5</td>
<td>≥320</td>
<td>2</td>
<td>2</td>
<td>Not III</td>
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<tr>
<td>7</td>
<td>≥320</td>
<td>3</td>
<td>3</td>
<td>II</td>
</tr>
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<td>160</td>
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<td>II</td>
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<tr>
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<td>≥320</td>
<td>5</td>
<td>2</td>
<td>II</td>
</tr>
</tbody>
</table>

from chickens were avirulent for mice. The DNA of 17 of 19 isolates obtained by bioassay of chicken tissues directly in to mice was typed genetically; 16 were found to be type II and 1 was type III (Table 1).

**4. Discussion**

The prevalence of *T. gondii* in chickens is largely unknown. Data based on serologic surveys before the development of MAT and ELISA are unreliable because chickens do not develop antibodies to *T. gondii* detectable in the Sabin–Feldman dye test (for reviews see Siim et al., 1963; Dubey and Beattie, 1988; Dubey et al., 1993). *T. gondii* has been isolated many times from tissues of backyard chickens from the US (Gibson and Eyles, 1957; McCulloch, 1968; Foster et al., 1969; Dubey, 1981; Dubey et al., 2003a), Iran (Ghorbani et al., 1990), Czech Republic (Literák and Hejlicek, 1993), Italy (Zardi et al., 1969), Costa Rica (Ruiz and Frenkel, 1980), Brazil (Dubey et al., 2002, 2003b, e), Egypt (Dubey et al., 2003c), Mexico (Dubey et al., 2004), Argentina (Dubey et al., 2003d), and India (Sreekumar et al., 2001, 2003). There are also reports of serologic prevalence of *T. gondii* MAT antibodies in chickens from Egypt (EL-Massry et al., 2000), Brazil (Silva et al., 2003) and India (Devada et al., 1998). There is no recent report of its isolation from commercially raised poultry. In the present study, *T. gondii* was isolated from pools of tissues from seronegative chickens by bioassay in mice confirming an earlier observation (Dubey et al., 2002). However, it is uncertain if the antibodies had decayed and became seronegative because sera from chickens from Israel were not refrigerated and tissues were foul smelling when received in the laboratory at Beltsville.
Jacobs and Melton (1966) examined two batches of tissues from 728 fowl processed at a commercial plant in Maryland. In the first batch, ovaries and oviducts from 620 birds were pooled in 12 batches (10 birds per pool) and in the second batch, brain, leg muscle, ovary, and shelled eggs from each of 108 birds were inoculated individually into mice. They found *T. gondii* in 12 pools of ovaries and oviducts in the first batch and from tissues of 4 chickens from the second batch; ovaries of 3 and leg muscle of 1. Boch et al. (1968) isolated *T. gondii* from the brains of 5 and the heart of 1 of 1636 hens from several farms in Germany. These studies by Jacobs and Melton (1966) and Boch et al. (1968) were performed nearly 40 years ago at a time when the life cycle of *T. gondii* was largely unknown. Jacobs and Melton (1966) had examined ovaries, oviducts and eggs from hens to find out if *T. gondii* can be passed in chicken eggs and serve as a source of infections for humans. Although oviducts and ovaries were found infected with *T. gondii*, there was no evidence for natural *T. gondii* infection in chicken eggs (Jacobs and Melton, 1966). The practice of eating raw eggs by humans is now being discouraged because of the danger of acquiring *Salmonella*.

In the present case, cats did not have access to the chicken housing area but were known to defecate in chicken feed stored in open bins. Raising chickens in confinement in wired cages on feed mixed at a central plant reduces chances of *T. gondii* infection. The recent trend of raising chickens free range in Europe and other countries exposes chickens to *T. gondii* infection. Despite the fact that the birds that we examined were old and probably used for processed food, humans can become infected if proper hygiene is not practiced while dressing the carcasses. Most importantly, the same scenario might be occurring on other free-range organic poultry farms if rodents and cats have access to poultry feed and houses.

Genetically, the isolates of *T. gondii* from free-range chickens from Brazil (Dubey et al., 2002, 2003b,e) were found to be predominantly type I and type II strains have not been isolated from Brazil. Earlier studies on genotyping of chicken isolates of *T. gondii* from Egypt (Dubey et al., 2003c) using the SAG 2 locus indicated that 85% of the isolates were of type II while the rest were type III. In this respect, 17 of 19 isolates genotyped from chickens from Israel were found to be of type II. These findings suggest isolates of *T. gondii* from Brazil are distinct than from the rest of the world. Unfortunately, genotyping using limited markers does not provide clues to sources of *T. gondii* in humans or animals at the present time.

References


