Endemic avian toxoplasmosis on a farm in Illinois: Clinical disease, diagnosis, biologic and genetic characteristics of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*), and a goose (*Anser anser*)


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Abstract

Clinical toxoplasmosis in chickens (*Gallus domesticus*) has been rarely reported in literature. Here we report that three chickens on a farm in Illinois developed neurological signs. One of these chickens was examined postmortem and it had non-suppurative encephalitis with numerous *Toxoplasma gondii* tachyzoites and tissue cysts. The identity of the protozoa was confirmed immunohistochemically by staining with *T. gondii* specific antibodies, and by transmission electron microscopy. The owner of the 3 chickens donated all 11 remaining chickens and a goose on his property for the present study. All 11 chickens and a goose were euthanized, and blood, heart, brain, and 1 leg were obtained for *T. gondii* examination. Antibodies to *T. gondii* were found in sera of all chickens with titers of 1:40 in one, 1:320 in three, and 1:640 or higher in seven chickens tested by the modified agglutination test (MAT). The goose had a MAT titer of 1:320. For isolation of *T. gondii*, whole heart and brain and 50 g of leg muscles were digested in an acid–pepsin solution and bioassayed in four mice for each tissue. Viable *T. gondii* was isolated from tissues of all 11 chickens and the goose. Genotyping of these 12 *T. gondii* isolates using polymorphism at the genetic loci SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and Apico revealed that all isolates had Type II alleles at all loci, indicating these *T. gondii* isolates belong to the predominant clonal Type II lineages. This is the first report of isolation of viable *T. gondii* from a domestic goose (*Anser anser*).

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Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; USA; Genotype; Goose; *Anser anser*

1. Introduction

*Toxoplasma gondii* infections are widely prevalent in human beings and other warm-blooded animals world-
clinical toxoplasmosis in chickens (Hepding, 1939; Fankhauser, 1951; Erichsen and Harboe, 1953; Biering-Sorensen, 1956; Beauregard et al., 1965; Geißler, 1952; Nobrega et al., 1967). Clinical signs reported were anorexia, jerking of neck and head, paralysis, and loss of eye sight. Biering-Sorensen (1956) regarded necrosis of the optic chiasma as pathognomonic of toxoplasmosis in chickens. Clinical toxoplasmosis with these characteristics has not been reported in literature in the last 40 years. Whether these reports were primary toxoplasmosis or were complicated by unrecognized viral infections is a speculation because to our knowledge there is no archived material for a retrospective study.

There are two reports of clinical toxoplasmosis in chickens from the USA (Ostendorf and Henderson, 1962; Goodwin et al., 1994). Ostendorf and Henderson (1962) reported in a Conference that chickens with toxoplasmic encephalitis and chorioretinitis died on chicken farms in Indiana. Goodwin et al. (1994) reported peripheral neuritis in three chickens from Georgia and the diagnosis was made immunohistochemically, however, herpes virus infection (Marek’s disease) could not be ruled out.

In the present paper, we documented clinical toxoplasmosis in chickens from a farm in west-central Illinois. Viable \textit{T. gondii} isolates from chickens commingled with the clinically ill chickens were identified, and the biologic and genetic characteristics of these \textit{T. gondii} isolates were described. Additionally, we report the isolation of \textit{T. gondii} from a goose (\textit{Anser anser}), first from this host.

2. Materials and methods

2.1. Naturally infected chickens

A group of 14 chickens and a goose were housed in a coop consisting of a 2.5 m × 3 m building with an attached 4.5 m × 6 m flight cage made from chicken wire. The birds were less than 1-year-old and used for egg production. Rats and mice had been seen in the chicken house (14 rats and an indeterminate number of mice have been killed using rodenticides since the chickens were removed from the coop) and there are 10–12 cats on the farm with access to the chicken house. During the summer of 2006, over a period of weeks, three of the chickens became ill, exhibiting torticollis and inability to stand. The third chicken to become ill (chicken no. 1) was submitted alive to the Illinois Department of Agriculture Animal Disease Laboratory (ADL), Galesburg, IL. Torticollis, an inability to stand, and lateral recumbancy were the only clinical signs. Initially \textit{Baylisascaris procyonis} larval migrans was suspected because of neurological signs and history of raccoons in the area. The bird was euthanized at ADL.

2.2. Histopathological, immunohistochemical, and transmission electron microscopical examination of chicken no. 1

Brain, heart, crop, esophagus, trachea, liver, kidney, spleen, duodenum, jejunum, ileum, ceca, colon, pancreas, cloaca, and bursa of Fabricius were fixed in 10% neutral-buffered formalin, processed routinely for sectioning and stained with hematoxylin and eosin (H and E).

Sections of brain were reacted with antibodies to \textit{T. gondii} and \textit{Neospora caninum} polyclonal rabbit antibodies as described (Lindsay and Dubey, 1989; Dubey et al., 2001). In addition sections were reacted with bradyzoite-specific antibodies (McAllister et al., 1996). The polyclonal \textit{T. gondii} antibodies react with both tachyzoites and bradyzoites of \textit{T. gondii} and not \textit{N. caninum} whereas the BAG 1 antibody reacts with bradyzoites and not tachyzoites but is not specific for \textit{T. gondii} or \textit{N. caninum}.

Retrospectively, a piece of paraffin-embedded brain was deparaffinized, post fixed in osmium, and processed for routine transmission electron microscopy.

2.3. Epidemiological investigation

After the diagnosis of toxoplasmosis in the index case (chicken no.1) the owner donated 11 chickens and the goose for the present study. All birds were submitted alive to the ADL. The birds were euthanized and specimens of blood, heart, brain, and one leg were removed from each bird and shipped cold to the Animal Parasitic Diseases Laboratory (APDL), United States Department of Agriculture, Beltsville, MD for \textit{T. gondii} evaluation.

2.4. Serological examination

Sera of birds were tested for \textit{T. gondii} antibodies using eight dilutions, from 1:5 to 1: 640 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Positive and negative sera were included in each test and the MAT is the most specific for the detection of \textit{T. gondii} antibodies in sera of chickens (Dubey et al., 1993).
2.5. Bioassay of chickens for *T. gondii* infection

Tissues of the 11 chickens and the goose were bioassayed for *T. gondii* infection. Whole brain, whole heart, and 50 g of leg muscles of each bird were bioassayed individually in out-bred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Each tissue was homogenized, digested in an acidic pepsin solution, neutralized, centrifuged, the sediment suspended in 4–5 ml antibiotic saline, and most of the homogenate was inoculated subcutaneously into four mice (Dubey, 1998).

All 144 mice that were inoculated with avian tissues were examined for *T. gondii* infection. Lungs and brains of 2 mice that died on day 26 and 43 were examined for *T. gondii* parasites. The remaining 142 mice were bled on day 45 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 49 days p.i. and brains of all mice were examined microscopically for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.6. Genetic characterization for *T. gondii*

*T. gondii* DNA was extracted from the tissues of all infected mice from each chicken (Table 1) and strain typing was performed using PCR-RLFP genetic markers SAG1, SAG2, SAG3, BTUB, GRA6 c22-8, c29-2, L358, PK1, a new SAG2 and Apico (Dubey et al., 2006b, 2007; Su et al., 2006).

### Table 1
Isolation of *Toxoplasma gondii* from chickens and goose in Illinois

<table>
<thead>
<tr>
<th>Chicken number</th>
<th>MAT Isolation in mice</th>
<th><em>Toxoplasma gondii</em> designations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2560 0 4 1</td>
<td>TgCkUsII1</td>
</tr>
<tr>
<td>3</td>
<td>320 2 4 3</td>
<td>TgCkUsII2</td>
</tr>
<tr>
<td>4</td>
<td>320 2 4 4</td>
<td>TgCkUsII3</td>
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<td>TgCkUsII4</td>
</tr>
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<td>640 3 4 2</td>
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<td>TgCkUsII6</td>
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<td>8</td>
<td>2560 1 4 3</td>
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</tr>
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<td>40 0 4 0</td>
<td>TgCkUsII8</td>
</tr>
<tr>
<td>10</td>
<td>640 0 4 0</td>
<td>TgCkUsII9</td>
</tr>
<tr>
<td>11</td>
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</tr>
<tr>
<td>12</td>
<td>320 0 4 4</td>
<td>TgCkUsII11</td>
</tr>
<tr>
<td>Goose</td>
<td>320 3 0 0</td>
<td>TgGoUs 1</td>
</tr>
</tbody>
</table>

*a* Number of mice positive for *T. gondii* of four mice inoculated with each tissue.

3. Results

3.1. Index chicken

Gross postmortem examination revealed a thin chicken with serous atrophy of body fat stores, but no other significant gross lesions. Marked lesions were limited to the brain which had multiple areas of necrosis, perivascular lymphocytic cuffs and gliosis. Numerous protozoa were present in one area. In 5-μm thick H and E stained sections these protozoa were not visible at 100× magnification but were evident at 400× magnification (Fig. 1); most of these groups of protozoa were surrounded by a vacuole. In 1 μm sections these protozoal groups contained 2–80 organisms, often surrounded by a vacuole (Fig. 2).

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Fig. 1. Lesions and *T. gondii* in sections of the cerebrum of chicken no.1 showing perivascular infiltration of mononuclear cells, glial infiltration and numerous protozoa (arrow). H and E stain.

Fig. 2. One micrometer section of cerebrum of chicken no.1 showing several *T. gondii* tissue cyst-like groups. Toluidine blue stain.
The protozoa in the brain reacted positively with *T. gondii* antibodies and unlike sections stained with H and E, numerous organisms were visible at 100× illumination. More *T. gondii* were found in sections reacted with polyclonal antibodies than those reacted with BAG 1 antibodies, suggesting that both tachyzoites and bradyzoites were present. In BAG 1 reacted sections, individual, groups of two, and those with numerous organisms were seen.

By transmission electron microscopy, six tissue cysts were studied. Although the host tissue and protozoa were degenerated tissue cyst wall and certain organelles were recognizable in enclosed bradyzoites including a conoid, numerous amylopectin granules, and a terminal nucleus.

### 3.2. Serological findings

Antibodies to *T. gondii* were found in all 11 chickens with titers of 1:40 in one, 1:320 in three, and 1:640 or higher in seven. The goose had a MAT titer of 1:320.

### 3.3. Isolation of *T. gondii* from chickens

*T. gondii* was isolated by bioassay in mice from all 11 chickens; from the hearts of all 11, brains of five, and legs of eight (Table 1). The number of tissue cysts in most infected mice was very few. Only two of the infected mice (one inoculated with heart of chicken 11 and one inoculated with leg muscle of chicken 12) died of toxoplasmosis.

The number of mice that became infected with *T. gondii* was higher when inoculated with heart tissue versus the brain and leg muscles (Table 2).

The *T. gondii* isolates were designated TgCkUsII 1–11.

### 3.4. Isolation of *T. gondii* from the goose

Viable *T. gondii* was isolated from its brain and not from the heart and leg muscles of the goose. The goose isolate was designated TgGoUs 1.

### 3.5. Genotyping

Genotyping of these 12 isolates using multilocus PCR-RFLP markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and Apico revealed Type II alleles at all loci, indicating these *T. gondii* isolates belong to the clonal Type II lineages, the predominant genotype in North America and Europe.

### 4. Discussion

In the present study, three chickens died with similar clinical signs but only one was examined at necropsy. The diagnosis of toxoplasmosis in the index case was made histologically and confirmed immunohistochemically and by electron microscopy. An unusual histological finding in the present case was the presence of numerous *T. gondii*, especially numerous tissue cysts. The presence of tachyzoites and young tissue cysts indicates acute toxoplasmosis. The tissue cyst walls were very thin and barely recognizable at the light microscopic level. Tissue cyst walls were not recognizable around individual or small groups of bradyzoites.

Experimentally, chickens are relatively resistant to *T. gondii* infection (Bickford, 1966; Biancifiori et al., 1986; Dubey et al., 1993). Four week old chickens fed as many as 100,000 oocysts of the Me 49, a Type II strain became infected but did not develop clinical signs. Chickens fed 100,000 oocysts of the GT1, a type I strain, only developed mild illness.

In the present study, *T. gondii* was isolated from all 11 chickens and the goose from the farm, suggesting that the two chickens that died might have been infected with *T. gondii*. It is noteworthy that *T. gondii* was isolated from the hearts of all 11, brains of five, and leg muscles of eight chickens. Tissues bioassayed were in excellent state of preservation and thus provided an opportunity to compare tissue tropism. These results are in agreement with our previous studies that the heart, and not the brain, is the tissue most often parasitized in chickens chronically infected with *T. gondii*. Although pectoral muscles were not bioassayed in the present study, evaluation of our previous studies (Dubey, 1981; Dubey et al., 2004a, 2005a) and the results of the present study suggest that leg muscles are more heavily parasitized than the pectoral muscles. It is clear from these studies that chicken hearts should always be included among tissues from chickens in attempts to isolate *T. gondii*.

*T. gondii* infections are widely prevalent in human beings and other warm-blooded animals worldwide.
(Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. *T. gondii* infection in chickens and other birds, and small mammals is of epidemiological significance because they serve as source of infection for cats. Free-range chickens have also been used as indicators of environmental contamination by *T. gondii* oocysts because they feed from the ground (Ruiz and Frenkel, 1980).

Recently, we have studied biologic and genetic characteristics of *T. gondii* isolates from free-range chickens from many countries, including the USA (Dubey et al., 2003, 2006a, 2007; Lehmann et al., 2006) and found that *T. gondii* isolates from chickens from Brazil and Colombia, South America were more virulent in mice and the clonal Type II lineage was absent. In contrast, isolates from the USA were not pathogenic for mice and most isolates were clonal Type II strains.

Phenotypically, clonal Type I strains were considered more pathogenic than that of Type II and III in mice (Sibley and Boothroyd, 1992). However, *T. gondii* isolates from asymptomatic chickens from Colombia and Brazil were more virulent for mice, irrespective of the genotype (Dubey et al., 2002, 2005b). These data indicate that parasite genotype may not determine pathogenicity in chickens. As an example here, the *T. gondii* isolates from chickens on the farm belong to Type II lineage but virulent in chickens. There are no reports in the literature linking clinical status with the genotype of the parasite in chickens. In the only outbreak of avian toxoplasmosis and genotype determined fatal toxoplasmosis in black-winged lory (*Eos cyanogenia*) was associated with genotype III (Dubey et al., 2004b). Therefore, further studies are needed to determine if severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors.

Dubey et al. (2004b) isolated viable *T. gondii* from a Canadian goose (*Branta canadensis*), which belongs to a genus different from *Anser anser*. The isolation of *T. gondii* from the goose (*Anser anser*) from the farm in Illinois is, to our knowledge, the first from this host. Fatal toxoplasmosis was diagnosed histologically in two captive Magpie geese (*Anseranas semipalmata*) from Texas (Dubey et al., 2001).

**References**


