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TOXOPLASMA GONDII INFECTIONS IN CHICKENS FROM VENEZUELA: ISOLATION, TISSUE DISTRIBUTION, AND MOLECULAR CHARACTERIZATION

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ABSTRACT: The prevalence of *Toxoplasma gondii*, in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in 46 free-range chickens (*Gallus domesticus*) from Venezuela was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT). Antibodies were found in 16 (32%) chickens with titers of 1:5 in 1, 1:10 in 2, 1:40 in 2, 1:80 in 2, 1:160 in 2, 1:320 in 3, 1:640 in 2, and 1:1,280 or higher in 2. Hearts, pectoral muscles, and brains of 13 chickens with MAT titers of 1:40 or more were bioassayed individually in mice. Tissues of each of 3 chickens with titers of 1:5 or 1:10 were pooled and bioassayed in mice. Tissues from the remaining 30 seronegative chickens were pooled and fed to 1 *T. gondii*-free cat. Feces of the cat were examined for oocysts; it did not shed oocysts. *Toxoplasma gondii* was isolated from 12 of 13 chickens with MAT titers of 1:40 or more. *Toxoplasma gondii* was isolated from pooled tissues of 1 of 2 chickens with titers of 1:10. Eight of these 13 isolates were virulent for mice. Genotyping of 13 of these isolates using the SAG2 locus indicated that 10 were type III, and 3 were type II. Phenotypically and genetically these isolates were different from *T. gondii* isolates from North America and Brazil. This is the first report of isolation of *T. gondii* from chickens from Venezuela.

Toxoplasma gondii infections are widely prevalent in humans and other animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or other factors.

Toxoplasma gondii isolates have been classified into 3 genetic types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997). It has been suggested that type I strains, or recombinants of types I and III, are more likely to result in clinical ocular toxoplasmosis (Howe et al., 1997; Fuentes et al., 2001; Grigg et al., 2001; Boothroyd and Grigg, 2002; Aspinall et al., 2003; Ajzenberg et al., 2004), but genetic characterization has been limited essentially to isolates from patients ill with toxoplasmosis. Unlike these reports, Ajzenberg et al. (2002) found that most (73 of 86) isolates from cases of congenital toxoplasmosis in humans from France were type II. Nothing is known of the genetic diversity of *T. gondii* isolates circulating in the general human population. In animals, most isolates of *T. gondii* were type II or type III, irrespective of clinical status (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002; Dubey, Graham et al., 2004; Dubey, Navarro et al., 2004; Dubey, Parnell et al., 2004). *Toxoplasma gondii* isolates differ markedly in their virulence to out-bred mice. Type I isolates are more virulent to mice than types II and III. Because chickens become infected mostly by feeding from ground contaminated with oocysts, prevalence of *T. gondii* in chickens is a good indicator of the strains prevalent in their environment (Ruiz and Frenkel, 1980).

Recently, we found that 70% of 73 *T. gondii* isolates obtained from asymptomatic free-range chickens from Brazil were type I (Dubey et al., 2002; Dubey, Graham, Silva et al., 2003; Dubey, Navarro et al., 2003), whereas samples from the United States and Egypt were dominated by either type II or type III, but had no type I (Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003). Type II isolates of *T. gondii* have not been found in chickens from Brazil. All 3 types were found in chickens from Argentina (Dubey, Venturini et al., 2003). Nothing is known of the characteristics of isolates of *T. gondii* from animals or humans from Venezuela. In the present paper, we attempted to isolate and genotype *T. gondii* from chickens from Venezuela. Additionally, the distribution of *T. gondii* in the heart, brain, and pectoral muscles of chickens was compared.

MATERIALS AND METHODS

Naturally infected chickens

The chickens (n = 46) were from different households from Sabana Grande de Monay, Trujillo, Venezuela (90°25′18.184″N, 70°18′59.292″W), located in western Venezuela near the foothills of the Andes Mountains. The properties were rural and the chickens from different properties had no physical contact with each other. The chickens, 21 males and 25 females, had not left the property since hatching and were at least 6 mo old at the time they were killed. They were purchased, bled, and then killed by cervical dislocation on 22 April 2004. Serum, heart, pectoral muscle, and brain from each chicken were sent cold by air to Beltsville, Maryland. Five days elapsed between killing the chickens and receiving the samples at Beltsville. Samples were received in excellent condition.

Serological examination

Sera of chickens were tested for *T. gondii* antibodies using 4 serum dilutions, 1:5, 1:10, 1:20, and 1:200, with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). After the completion of the bioassays, all positive chicken sera were rerun using 2-fold dilutions from 1:5 to 1:1,280.

Bioassay of chickens for *T. gondii* infection

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, pectoral muscles, and hearts of 13 chickens with MAT titers of 1:40 or more were each bioassayed individually in out-bred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Each tissue was homogenized individually, digested in acidic pepsin, washed, and homogenate inoculated subcutaneously into 5 mice; in total, 15 mice were inoculated

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TABLE I. Isolation of *Toxoplasma gondii* from seropositive chickens from Venezuela.

Chicken no.	MAT titer	Isolation in mice[a]			Genotype (isolate designation)
		Brain	Heart	Skeletal muscle	
4	1,280	0	5	1	III(TgCkVel)
6	160	4	5	0	III(TgCkVe2)
10	80	0	5 (3)†	0	III(TgCkVe3)
11	160	0	5 (4)	0	III(TgCkVe4)
13	640	5 (5)	5 (4)	0	III(TgCkVe5)
20	640	5 (5)	4 (4)	5 (5)	II(TgCkVe6)
29	80	3	5	0	III(TgCkVe7)
35	320	1	0	0	III(TgCkVe8)
36	40	0	4 (2)	0	III(TgCkVe9)
39	320	2 (2)	5 (5)	0	III(TgCkVe10)
42	1,280	0	5 (4)	5	III(TckVe11)
43	40	4 (2)	0	0	II(TgCkVel2)
24	40	Tissue pool 4 (2)			II(TgChVe13)

* Number of mice infected with *T. gondii* of 5 mice inoculated with each tissue.
† Number of mice dead of acute toxoplasmosis.

with tissues of each chicken. Tissues from each of 3 chickens with titers of 1:5 or 1:10 were pooled and treated as above, but inoculated into 5 mice per chicken.

Tissues from 30 seronegative chickens were pooled and fed to 1 *T. gondii*-free cat (Dubey et al., 2002). Feces of the cat were examined for shedding of *T. gondii* oocysts 3–14 days after ingesting chicken tissues as described previously (Dubey, 1995). Fecal floats were incubated for 1 wk at room temperature to allow sporulation of oocysts and were bioassayed in mice (Dubey and Beattie, 1988).

Tissues of mice that died acutely after inoculation with tissues of chickens 20 and 39 (Table I) were fed to 4 *T. gondii*-free cats to determine whether these mouse virulent strains were capable of producing oocysts; 3 cats were fed mice separately that had been inoculated with brain, heart, and pectoral muscles of chicken 20, and 1 cat was fed tissues of mice inoculated with heart of chicken 39.

Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 39 postinoculation (PI) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 41days PI and brains of all mice were examined 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.

Genetic characterization for *T. gondii*

Toxoplasma gondii DNA was extracted from mouse tissue as described previously (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested PCR on the SAG2 locus according to Howe et al. (1997).

RESULTS

Antibodies to *T. gondii* were found in 16 of 46 chickens with titers of 1:5 in 1, 1:10 in 2, 1:40 in 2, 1:80 in 2, 1:160 in 2, 1:320 in 3, 1:640 in 2, and 1:1,280 or higher in 2 chickens. *Toxoplasma gondii* was isolated from 12 of 13 chickens with MAT titers of 1:40 or more: from the heart, brain, and pectoral muscle of 1, from the hearts and brains of 4, from the hearts and muscles of 2, from the hearts alone of 3, and from the brains alone of 2. *Toxoplasma gondii* was isolated from 1 of 2 chickens with titers of 1:10 (Table I). Eight of these 13 isolates were virulent for mice; 40 of 54 infected mice died of toxoplasmosis.

The cat fed tissues of 30 seronegative chickens did not shed oocysts. The 4 cats that were fed tissues of mice inoculated with tissues from chickens 20 and 39 shed many oocysts, indicating that cats can shed oocysts after ingesting acutely infected tissues.

Genotyping data indicated that 10 isolates were type III, and 3 were type II (Table I).

DISCUSSION

The threshold MAT titer indicative of *T. gondii* infection in chickens has not been determined. Data comparing serology and recovery of viable *T. gondii* from chickens are now accumulating (Dubey et al., 2002; Dubey ,Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003; Dubey, Graham, Silva et al., 2003; Silva et al., 2003; Dubey, Levy et al., 2004; Dubey et al., 2005). Although *T. gondii* was isolated from a few chickens with MAT titers of 1:10 or less, the likelihood of isolation increased with MAT titer. Lack of shedding of oocysts by the cat that consumed entire hearts, brains, and 20–25 g of pectoral muscles of 30 seronegative chickens supports the validity of the MAT.

The mouse virulence data indicate that *T. gondii* isolates from Venezuela are different from isolates from Egypt, India, Mexico, the United States, and Grenada. None of the isolates from Egypt (Dubey, Graham, Dahl, Hilali et al., 2003), India (Sreekumar et al., 2003), Mexico (Dubey, Morales, and Lehmann, 2004), the United States (Dubey, Graham, Dahl, Sreekumar et al., 2003; Lehmann et al., 2003), or Grenada (Dubey et al., 2005) was virulent for mice. The 2 most virulent isolates from chickens from Venezuela were type II or III. Type III isolates had, in general, been considered avirulent for mice (Howe and Sibley, 1995), although type III isolates from Brazil were mouse virulent (Dubey et al., 2002). Moreover, 3 of 13 isolates from Venezuela were type II, whereas type II isolate has not yet been found in chickens from Brazil (Dubey et al., 2002; Dubey, Graham, Silva et al., 2003; Dubey, Navarro et al., 2003).

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