Prevalence of Toxoplasma gondii indirect fluorescent antibodies in naturally- and experimentally-infected chickens (Gallus domesticus) in Thailand

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Abstract

Toxoplasma gondii infections in free-range (FR) chickens (*Gallus domesticus*) are potential public health risks. Antibodies for *T. gondii* were found in 194 out of 303 serum samples (64.03%) from FR chickens in Thailand tested by the indirect fluorescent antibody test (IFAT, 1:16). To verify the validity of serologic data in this survey, sera from chickens experimentally infected with the RH strain of *T. gondii* were tested by the IFAT. Antibodies against *T. gondii* were detected as early as 7 days p.i., peaked at 2 weeks, and then declined by 10 weeks p.i.

Keywords: Toxoplasma gondii; toxoplasmosis; free-range chickens; Gallus domesticus ; seroprevalence; serodynamics

Introduction

Free-range (FR) chickens are considered an important source of *Toxoplasma gondii* infection for cats. Infected cats shed millions of environmentally resistant *T. gondii* oocysts (Ruiz and Frenkel 1980). Antibodies to *T. gondii* were reported in FR chickens worldwide and viable *T. gondii* was isolated from infected chickens; these studies were reviewed by Dubey (2009). Most of the surveys in FR chickens were performed using the modified agglutination test (MAT). Although the MAT is easy to perform and commercially available, the reagents used are expensive (Dubey 2009). The Sabin-Feldman dye test, which is the most specific test for *T. gondii* infection in humans, does not work with chicken sera (Frenkel 1981). Little is known of the validity of the indirect fluorescent antibody test (IFAT) for the diagnosis of *T. gondii* infection in chickens.

In the first part of this study we determined seroprevalence of *T. gondii* FR chickens from selected areas of Thailand because prevalence of *T. gondii* in FR chickens from Thailand was unknown. Secondly, we investigated the dynamics of IFAT antibody responses to *T. gondii* in experimentally-infected chickens.

Materials and methods

Seroprevalence study

Source of chickens: Free-range chickens (n = 303) were obtained from 17 family-farms in 2 selected districts, approximately 50 km east of Bangkok. The chickens from these farms were raised in the backyard for meat and egg consumption within families, as well as selling some chickens and eggs in the neighborhood. The size of the farms varied from 20–100 birds. The age of bleeding chickens varied from 1–12 months and most of them were older than 5 months. Blood samples were drawn from the wing vein and sera were kept in the freezer until serological tests were performed.

Serological test for IgG IFAT antibody against *T. gondii*: For initial screening, chicken sera were diluted 1:16 in diluting buffer (0.010 mol/l phosphate buffer, pH 7.2, 1% BSA, 0.15 mol/l NaCl) (Garcia *et al*. 2000). The positive samples were diluted further two-fold 1:16 to 1:64. The RH strain tachyzoites of *T. gondii* were used as antigen fixed on wells of immunofluorescent slides and kept at –20°C until use. Ten microliters of each diluted unknown serum, including positive and negative controls were placed on the well of the slides and incubated in a moisture chamber at 37°C for 1 h. Slides were washed in FA rinse buffer (0.5 mol/l carbonate buffer, pH 9.0, 0.15 mol/l NaCl) twice (3 and 10 min), dried by

pressing blotter to the front surface, and then incubated for another 1 h at 37°C with goat anti-chicken IgG conjugate (KPL Co., USA) at a dilution of 1:100. Slides were washed again (3 and 10 min). Ten microliters of mounting fluid per well were added and each slide was covered with a cover-slip. Finally, the samples were examined under a fluorescent microscope (Zeiss, Axioskop40/HBO 50) at 100–200 times magnification, and confirmed at 400 times magnification.

Serodynamic study

Experimental infections: Seven 8 week-old chickens reared in confinement were used; 5 birds were each injected intramuscularly with 1.5 × 107 RH strain tachyzoites and 2 birds were injected with normal saline as controls. *T. gondii*-inoculated chickens were bled once a week starting at the first week post inoculation (1 wpi) until 12 wpi. The control birds were bled 4 times during the period at 1, 4, 8 and 12 wpi. Sera were tested for IFAT antibodies.

Results

Antibodies to *T. gondii* were found in 194 of 303 (64.03%) serum samples with a 30.8–100% seropositivity in farms tested. The antibody titers were 1:16 in 154 chickens, 1:32 in 37 chickens, and 1:64 in 3 chickens. Results of seroprevalence and antibody titers against *T. gondii* are summarized in Table I.

All experimentally inoculated birds remained healthy and seroconverted from <1:16 to >1:16, starting 1 wpi (Table II) and titers peaked at 2 wpi. After the peak, they showed a rapid

decline of antibody titers within a short period of 8 weeks, after which they declined much more slowly until the end of the experiment. The control birds were negative throughout the experiment.

Discussion

The 64% seropositivity of *T. gondii* infection in the present study is probably related to management. The birds were from small farms with no farm-biosecurity and cats were often observed while the blood was bled. And birds were raised freerange. Under these conditions bird feed is likely to be contaminated with oocysts shed by infected cats.

Results of the present study confirm earlier reports that chickens can develop IFAT antibodies. The onset of antibodies will vary with the dose, route of inoculation, and stage of the parasite used. We used a high dose of tachyzoites intramuscularly because of convenience, availability, and safety issues. Although it would have been ideal to infect birds orally with *T. gondii* oocysts, our objective was to study dynamics of IFAT antibodies. The early detection of antibodies at 1 wpi is probably due to the introduction of tachyzoites parenterally. Sedlák *et al*. (2000) detected antibodies at 2 wpi after oral infection of chickens with 105 oocysts. Earlier, Kaneto *et al*. (1997) stated that chickens inoculated orally with *T. gondii* oocysts developed IFAT antibodies, but did not provide quantitative data.

Toxoplasma gondii has been recovered from naturally-exposed chickens found to have IFAT antibodies. Sreekumar *et al*. (2001) isolated viable *T. gondii* from IFAT-positive chickens in India but did not provide quantitative data. Brandão *et al*. (2006) in Brazil isolated viable *T.*

gondii from 2 of 3 chickens with IFAT titer of <1:16, 1 of 2 chickens with IFAT titer of 1:16, and 8 of 11 chickens with an IFAT titer of 1:64 or higher.

The high seroprevalence of *T. gondii* in the present study stress the need to raise chickens in biosecure environment.

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Table I. Seroprevalence of *T. gondii* infection and antibody titers found from free-range chickens in Thailand

			No. positive in each titer (%)		
Farm no.	No. of chickens	No. positive (%)	1:16	1:32	≥ 1:64
1	10	7 (70)	4 (57.1)	3 (42.9)	0 (0)
2	10	10 (100)	6 (60)	2 (20)	2 (20)
3	23	23 (100)	16 (69.6)	7 (30.4)	0 (0)
4	32	20 (62.5)	17 (85)	3 (15)	0 (0)
5	12	4 (33.3)	4 (100)	0 (0)	0 (0)
6	18	7 (38.9)	5 (71.4)	2 (28.6)	0 (0)
7	19	15 (78.9)	13 (86.7)	2 (13.3)	0 (0)
8	35	18 (51.4)	13 (72.2)	5 (27.8)	0 (0)
9	25	22 (88)	17 (77.3)	4 (18.2)	1 (4.5)
10	13	4 (30.8)	4 (100)	0 (0)	0 (0)
11	10	6 (60)	6 (100)	0 (0)	0 (0)
12	37	12 (32.4)	9 (75)	3 (25)	0 (0)
13	15	14 (93.3)	9 (64.3)	5 (35.7)	0 (0)
14	8	7 (87.5)	7 (100)	0 (0)	0 (0)
15	13	8 (61.5)	8 (100)	0 (0)	0 (0)
16	10	6 (60)	6 (100)	0 (0)	0 (0)
17	13	11 (84.6)	10 (90.9)	1 (9.1)	0 (0)
Total	303	194 (64.03)	154 (79.4)	37 (19.1)	3 (1.5)

Table II. Antibody titers in chickens experimentally inoculated with *T. gondii* tachyzoites

Chickens	Weeks post inoculation												
No.[a]	0	1	2	3	4	5	6	7	8	9	10	11	12
C1	<16[b]	<16	-[c]	-	<16	-	-	-	<16	-	-	-	<16
C2	<16	<16	-	-	<16	-	-	-	<16	-	-	-	<16
T1	<16	4096	8192	8192	4096	1024	1024	256	256	128	64	64	64
T2	<16	4096	8192	8192	4096	2048	2048	512	256	128	64	64	64
T3	<16	2048	8192	4096	4096	1024	1024	256	256	128	128	128	64
T4	<16	2048	8192	4096	2048	512	512	256	128	128	64	64	64
T5	<16	2048	4096	4096	2048	512	512	128	64	64	32	32	32

[a]C, control; T, treatment

[b]<16, titer less than 1:16

[c]-, not tested