2012

Seroprevalence and isolation of Toxoplasma gondii from free-range chickens from Espirito Santo state, southeastern Brazil

VETERINARY PARASITOLOGY, AMSTERDAM, v. 188, n. 3-4, pp. 225-230, SEP 10, 2012
http://www.producao.usp.br/handle/BDPI/42332

Veterinary Parasitology 188 (2012) 225–230

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens from Espírito Santo state, southeastern Brazil

M.A.V. Beltrame[a], H.F.J. Pena[b], N.C. Ton[a], A.J.B. Lino[a], S.M. Gennari[b], J.P. Dubey[c], F.E.L. Pereira[d,*]

[a] *Curso de Medicina Veterinária, Universidade de Vila Velha, Rua Viana, s/n, Bairro Boa Vista, Vila Velha, CEP 29102-690, ES, Brazil*
[b] *Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo, São Paulo, SP, CEP 05508-270, Brazil*
[c] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[d] *Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Av. Marechal Campos, 1468, CEP 29040-091, Vitória, ES, Brazil*

ARTICLE INFO

Article history:
Received 5 January 2012
Received in revised form 26 March 2012
Accepted 29 March 2012

Keywords:
Toxoplasma gondii
Chickens
Gallus domesticus
Seroprevalence
Isolation
Espírito Santo state
Brazil

ABSTRACT

Prevalence of *Toxoplasma gondii* infection in 510 free-range (FR) chickens (380 from 33 small farms, and 130 from a slaughter house for FR chickens) from Espírito Santo state, southeastern Brazil, was investigated. Antibodies to *T. gondii* were sought using commercial indirect haemagglutination (IHAT, Imuno-HAI Toxo®, Wama Diagnóstica, São Paulo, Brazil, cut-off 1:16) and the modified agglutination test (MAT, cut-off 1:25) tests. Attempts were made to isolate viable *T. gondii* from seropositive chickens by bioassay in mice. Pooled samples of brain, heart and quadriceps muscle of one thigh (total 40 g) from 64 chickens with IHAT titers of ≥1:16 were minced, digested in pepsin and bioassayed in mice. Antibodies to *T. gondii* were found in 40.4% (206/510) FR chickens by IHAT (titer ≥ 1:16) and 38.8% (198/510) by MAT (titer ≥ 1:25); concordance between IHAT and MAT was 81.6% (kappa index = 0.614). Viable *T. gondii* was isolated (designated TgCkBr234-281) from 48 of 64 (75%) seropositive (IHAT titers ≥ 1:32) FR chickens. Most isolates of *T. gondii* were virulent for mice; 100% of mice inoculated with 44 of 48 isolates died of toxoplasmosis within 30 days post inoculation (p.i). An epidemiological investigation revealed that people living in rural areas have little knowledge about the parasite and about the risk of acquiring it from raw meat. Results indicated that the locally available IHAT was useful for screening of chicken sera for *T. gondii* antibodies.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammals and birds (Dubey, 2010a). The seroprevalence of *T. gondii* in humans and the burden of disease in congenitally infected children in Brazil are highest are among the countries in the world (Gilbert et al., 2008; Dubey, 2010a). Espírito Santo (ES) state is located in southeastern part of Brazil. Little is known of the prevalence of *T. gondii* in animals and humans from this state. In a recent survey, antibodies to *T. gondii* were reported in 73.5% of 1135 pregnant women attending an antenatal clinic in Vitória (ES) (Areal and Miranda, 2008).

The high prevalence of *T. gondii* in Brazil is probably related to high contamination of the environment with oocysts. Direct detection of oocysts in soil is technically difficult and only 1% of cats, the definitive hosts, are found shedding *T. gondii* oocysts at any time. *T. gondii* prevalence in free-range (FR) chickens has been used as an indicator

* Corresponding author. Tel.: +55 27 3335 7210; fax: +55 27 3335 7206.
E-mail address: felp@ndi.ufes.br (F.E.L. Pereira).

0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2012.03.053

of the strains prevalent in the environment, as FR chickens become infected mostly by feeding from soil contaminated with oocysts (Ruiz and Frenkel, 1980). In the last 12 years we and others have determined prevalence of *T. gondii* in FR chickens from different regions of Brazil (Peixoto and Lopes, 1990; Garcia et al., 2000; Dubey et al., 2002; da Silva et al., 2003; Dubey et al., 2003a,b; Brandão et al., 2006; Dubey et al., 2006, 2007; Aigner et al., 2010; de Oliveira et al., 2009; Soares et al., 2011).

Seroprevalence and genetic diversity of *T. gondii* isolates obtained from FR chickens from different regions of Brazil was summarized (Dubey et al., 2008; Dubey, 2010b; Soares et al., 2011).

In the present study we investigated the prevalence of *T. gondii* in FR chickens from different rural areas of ES state.

Information on serological diagnosis of *T. gondii* in chickens is limited (reviewed in Dubey, 2010b). The Sabin Feldman dye test, the most specific test for diagnosis of toxoplasmosis in humans, does not detect antibodies to *T. gondii* in naturally or experimentally infected chickens (Dubey et al., 1993). The modified agglutination test (MAT) is currently considered the specific and sensitive test for the detection of *T. gondii* antibodies in chickens (Dubey, 2010b). However, the commercial kit for MAT is made in France (bioMérieux) and very expensive to import in Brazil. An indirect haemagglutination antibody test (IHAT) kit is marketed locally in Brazil (Imuno-HAI Toxo®, Wama Diagnóstica, São Paulo, Brazil). In the present study we studied the usefulness of this IHAT kit for the diagnosis of toxoplasmosis in chickens. For this, sera from FR chickens were tested by both MAT and IHAT. In MAT, whole killed *T. gondii* tachyzoites are used as the antigen, which is treated with 2-mercaptoethanol to remove cross reacting IgM. In IHAT, soluble antigen from tachyzoites is coated on tanned red blood cells which are then agglutinated by immune serum (Dubey and Beattie, 1988). Details of antigen used and the epitopes recognized are unknown.

Bioassays in mice were performed to attempt to isolate *T. gondii* from seropositive chickens by IHAT and biologically characterize these isolates. We also conducted an epidemiological investigation to gather information concerning the knowledge of the people living in farms about the parasite, toxoplasmosis, and the possibility of chicken to have the parasite.

2. Material and methods

2.1. Free-range chickens

In total, 510 free-range chickens from Espírito Santo (ES) state (20°00′S and 40°45′W) were sampled from December 2007 through January 2009. Of these, 380 adult chickens were from 33 small farms (2–7 km apart) from seven municipalities (50–110 km apart) and 130 were from a

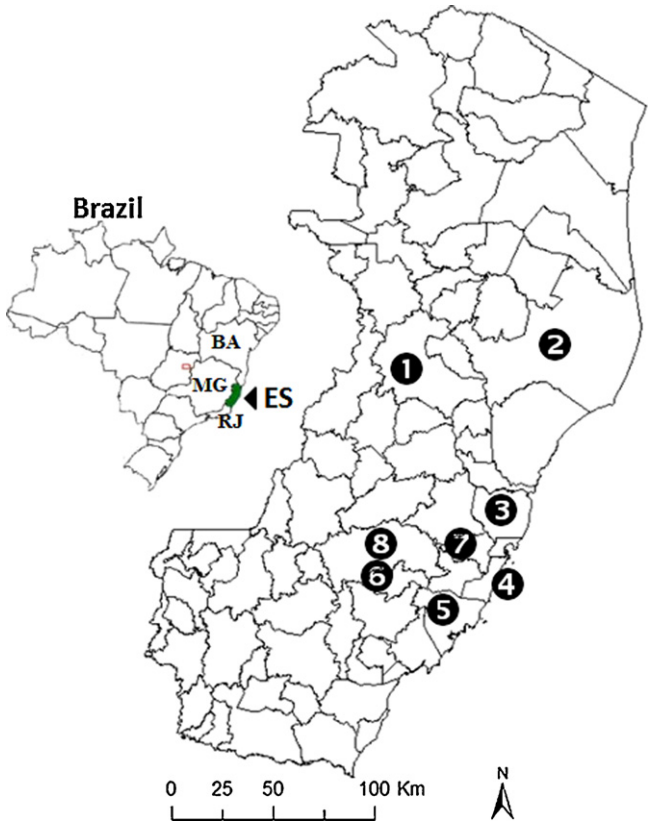

Fig. 1. Map of Espírito Santo (ES) state showing municipality locations and origin of sampled free-range chickens. 1: Colatina; 2: Linhares; 3: Serra; 4: Vila Velha; 5: Guarapari; 6: Marechal Floriano; 7: Cariacica; 8: Domingos Martins. States bordering ES are indicated in Brazil map. BA: Bahia; MG: Minas Gerais; RJ: Rio de Janeiro.

Table 1
Frequency of anti-*Toxoplasma* antibodies investigated by IHAT and MAT, and isolation of the parasite in free-range chickens from Espírito Santo state, southeastern Brazil.

Municipality	No. examined	IHAT[c] No. positive (%)	MAT[d] No. positive (%)	*T. gondii* isolated/bioassayed	Strain designation
Colatina	99	63 (63.6)	73 (73.7)	23/27	TgCkBr234-256
Cariacica	10	0 (0.0)	2 (20.0)	Ni[a]	Na
Domingos Martins	10	0 (0.0)	0 (0.0)	Na[b]	Na
Guarapari	53	10 (18.8)	13 (24.5)	4/4	TgCkBr257-260
Linhares	60	18 (30.0)	24 (40.0)	6/7	TgCkBr261-266
Marechal Floriano	41	10 (24.3)	13 (31.7)	9/10	TgCkBr267-275
Serra	107	16 (14.9)	17 (15.9)	2/11	TgCkBr276-277
Vila Velha	130	89 (68.5)	56 (43.1)	4/5	TgCkBr278-281
Total	510	206 (40.4)	198 (38.8)	48/64	TgCkBr234-281

[a] Not investigated.
[b] Not applicable.
[c] Indirect haemagglutination test, positive: titers ≥ 1:16.
[d] Modified agglutination test, positive: titers ≥ 1:25.

slaughter house for FR chickens at the municipality of Vila Velha (Fig. 1). Sera from 80 chickens collected in a slaughter for industrial chickens were used as negative control of the commercial IHAT kit used. Blood was collected by puncture of a brachial or jugular vein. Sera were stored in at −20 °C.

2.2. Serologic examination of chickens

Sera of chickens were examined for anti-*T. gondii* antibodies by the IHAT, according to the manufacturer's instructions. Initially, sera were screened at 1:16 dilution and then end titrated using a 2-fold serial dilution. Later, chicken sera were also tested at a 1:25 dilution for antibodies to *T. gondii* using the MAT as described previously by Dubey and Desmonts (1987). The antigen was prepared at the Laboratory of Parasitology, National Reference Centre on Toxoplasmosis (Reims, France), as described (Dubey et al., 2011) and mailed by air to Universidade de São Paulo, Brazil, from the Animal Parasitic Diseases Laboratory, USA. Positive and negative controls were used in both tests.

2.3. Bioassay of chicken tissues in mice

Bioassay was performed as described by Dubey (2010a). Only chickens which owners agreed to sell the bird for euthanasia were used for bioassay. This sample may be considered a random and representative sample of all seropositive chickens (64/206; 31%) because it contains animals from all the six municipalities where seropositive chickens were identified by IHAT.

Briefly, brain, heart and quadriceps muscle of one thigh (total 40 g of tissue) from each of 64 chickens with IHAT titers ≥ 16 were pooled for each chicken, minced and digested in acid pepsin at 37 °C for 1 h. After filtration, neutralization with sodium bicarbonate and centrifugation, the sediment was diluted in 10 ml of saline and inoculated intraperitoneally into five outbred Swiss, female, two-month old mice. Five ml of the leftover inocula was stored at 4 °C, and inoculated in the same mice next day (Navarro et al., 1992). Mice were observed until 42 days post inoculation (p.i.). Peritoneal exudate from each mouse that died was examined microscopically for tachyzoites using both fresh preparations and after staining the smears with Giemsa stain. All survivors were killed with a lethal dose of pentobarbital and blood was collected by cardiac puncture. Mouse sera were examined for *T. gondii* antibodies using 1:16 dilution in IHAT.

2.4. Epidemiological questionnaire

Owners of 31 of 33 farms were interviewed personally and asked about their education, property conditions and knowledge about the parasite and the disease it causes.

2.5. Statistical analyses

Levels of agreement between serological tests for detection of antibodies to *T. gondii* (IHAT and MAT) were evaluated using the kappa test (Landis and Koch, 1977).

3. Results

Frequency of *T. gondii* seropositive free-range chickens was 40.4% (206/510) by IHAT (titer ≥ 1:16) and 38.8% (198/510) by MAT (titer ≥ 1:25) (Table 1). All 80 chickens from the slaughter for industrial chickens were seronegative for *T. gondii* antibodies by IHAT.

The frequency of seropositive FR chickens varied in samples from different regions (Table 1). Higher frequencies (MAT results) were observed in samples from North (Linhares and Colatina: 44.6%; 95% CI: 37.3–51.9) than in samples from Metropolitan Vitória (Vila Velha, Serra, Guarapari and Cariacica: 19.6%; 95% CI: 24.5–35.7) or Serrana Region (Domingos Martins and Marechal Floriano: 19.6%; 95% CI: 7.3–31.3).

Two hundred and sixty-two chickens were negative both by IHAT and MAT; 52 serum samples that were positive by IHAT were negative by MAT and 42 that were negative for IHAT were positive for MAT. The kappa value of agreement between the two serological tests was 0.614, with a concordance value of 81.6%. The sensitivity and specificity of IHAT in relation to MAT were 78.5% and 83.4%, respectively (Table 2).

T. gondii was isolated from 48 of 64 seropositive chickens with titers ≥ 1:32 by IHAT. The percentage of isolation increased with the antibody titer (Table 3). All isolates were from seropositive chickens both by IHAT and MAT. Sixteen

Table 2
Comparison between indirect haemagglutination test (IHAT) and modified agglutination test (MAT) performed in 510 serums of free range chicken from Espirito Santo State Brazil.

IHAT	MAT		Total
	Positive	Negative	
Positive	154	52	206
Negative	42	262	304
Total	196	314	510

Concordance = 81.6%; κ index = 0.614. Sensitivity 78.5% and specificity 83.4%, of IHAT in relationship to MAT.

samples from which the parasite was not isolated were negative by MAT, but had titers from 16 to 256 by IHAT.

Most isolates were lethal to mice; for 44 of 48 isolates 100% of infected mice died whereas for the remaining four isolates only 20–50% of infected mice died (Table 4). All infected mice died of acute toxoplasmosis between 10 and 30 days p.i. (Table 4) and tachyzoites were found in their peritoneal exudates during this period.

The questionnaire answered by the owners revealed that 64.5% had only elementary school education and 42% had no knowledge about *T. gondii* or the disease it could induce. None of the interviewed, despite the education level, knew about the presence of *T. gondii* in chickens and that cats are responsible by soil contamination with oocysts of the parasite. In the same way, the transmission of the parasite by manipulating meat from mammals or birds, by eating raw meat or vegetables was unknown by interviewed people.

4. Discussion

This is the largest survey of *T. gondii* infection in FR chickens from any state in Brazil. Results indicated that chickens from 31 of 33 farms in ES state were seropositive. Only in 1 farm all 10 chickens sampled were seronegative in both MAT and IHAT. Seropositivity in other farms varied from 20 to 73.7% using a cut-off of 25 in the MAT and this variability is probably related to access of cats to chickens. It is likely that seroprevalence could have been higher if the cut-off value in MAT was lower because in previous studies from Brazil, viable parasite has been occasionally isolated from chickens with a MAT titer of 1:10 or even 1:5 (Dubey et al., 2002; Dubey, 2010b).

In the present study, we used the commercial IHAT to detect antibodies to *T. gondii* in chickens for the first time and results were encouraging. The IHAT and MAT had a concordance of 81.6% (kappa index = 0.614), considered a substantial agreement between the two methods. In addition, the IHAT presented 83% of specificity and 78% sensitivity in relation to MAT. There was a high frequency of isolation of *T. gondii* (75%) among seropositive chickens by IHAT, however it would be higher (90%) if only seropositive chickens by MAT were used. It is noteworthy that IHAT is easy to perform and kits are available commercially in Brazil. Results here presented show that IHAT can be used in epidemiological studies to detect antibodies to *T. gondii* in chickens. However, preparation of *T. gondii* antigen for IHAT has not been standardized. The quality and species of erythrocytes used have also not been standardized. Therefore, it is strongly advised that each IHAT kit should be evaluated according to a gold standard test for detection of anti-*T. gondii* antibodies in chickens, as MAT (Dubey, 2010b), before use in a survey. The sensitivity of IHAT is dependent on the quality of *T. gondii* antigen, stability of sheep erthrocytes used, and the need for higher salt concentration while using the test for chicken sera (Frenkel, 1981). In experimentally infected chickens and using the procedures of Lunde and Jacobs (1967), IHAT was only 46% sensitive (Frenkel, 1981). Ghorbani et al. (1990) isolated *T. gondii* from six of 109 (5.4%) chickens from Iran; 1 isolate was from a chicken with IHAT (in-house test) titer < 1:20. Other reports using IHAT to detect antibodies to *T. gondii* in naturally infected chickens were summarized by Dubey (2010b), but there were no sensitivity and specificity data.

In the present study it was observed that the percentage of isolation increased with the antibody titers as found previously with chickens, cats, and sheep (Dubey et al., 2002; Pena et al., 2006; Ragozo et al., 2008; de Oliveira et al., 2009).

Mortality ratio was high among inoculated mice. Forty four isolates killed all *T. gondii* infected mice between 10 and 30 days p.i., corroborating other studies that show that most isolates from the mainland Brazil are mouse virulent

Table 3
Frequency of isolation of viable *Toxoplasma gondii* in 64 free-range chickens from Espírito Santo state, southeastern Brazil, according to IHAT titer.

Antibody titer	No. of seropositive chickens	No. of chickens		
		Bioassayed[a]	*T. gondii* isolated[b]	%
16	40	2 (2)	0	0.0
32	66	8 (5)	3	37.5
64	69	36 (8)	28	77.7
128	13	5	5	100.0
256	7	5 (1)	4	80.0
512	6	4	4	100.0
1.024	2	2	2	100.0
2.048	3	2	2	100.0
Total	206	64	48	75.0

[a] Numbers in parenthesis represent number of bioassayed chicken seronegative by MAT (titer < 1:25).
[b] All isolates were obtained from seropositive chickens by MAT.

Table 4
Isolation of *Toxoplasma gondii* from free-range chickens from Espírito Santo state, southeastern Brazil.

Municipality (no. of isolates)	Chicken no. (property[a])	Isolate designation	Antibody titer by IHAT	Mouse bioassay		
				No. of mice per group	No. died/no. of infected	Day of death (p.i.)
Colatina (23)	42 (A)	TgCkBr234	512	5	5/5	12,13,13,14,14
	28 (A)	TgCkBr235	64	5	3/3	14,14,15
	65 (A)	TgCkBr236	128	5	5/5	12,12,13,13,17
	61 (A)	TgCkBr237	64	5	5/5	14,14,14,15,16
	63 (A)	TgCkBr238	128	5	5/5	13,14,14,15,16
	58 (A)	TgCkBr239	64	4	4/4	13,13,14,14
	66 (A)	TgCkBr240	256	5	5/5	10,11,12,13,14
	43 (A)	TgCkBr241	64	5	3/3	14,16,17
	53 (A)	TgCkBr242	64	5	5/5	14,16,17,18,18
	34 (A)	TgCkBr243	512	5	5/5	12,12,14,15,16
	38 (B)	TgCkBr244	256	5	5/5	14,15,17,19,22
	36 (B)	TgCkBr245	64	5	5/5	13,19,23,28,31
	64 (B)	TgCkBr246	256	5	1/2	15
	49 (C)	TgCkBr247	64	5	5/5	14,14,15,15,16
	56 (C)	TgCkBr248	512	4	4/4	14,15,17,21
	52 (C)	TgCkBr249	64	5	5/5	13,15,16,17,19
	48 (C)	TgCkBr250	64	5	5/5	16,17,19,21,21
	57 (C)	TgCkBr251	64	5	5/5	15,15,16,17
	35 (C)	TgCkBr252	64	5	2/2	23,24
	50 (C)	TgCkBr253	512	5	4/4	16,19,21,27
	41 (D)	TgCkBr254	64	5	5/5	14,14,15,15,16
	45 (D)	TgCkBr255	64	5	5/5	14,15,15,17,21
	47 (D)	TgCkBr256	64	5	5/5	12,12,12,13,13
Guarapari (4)	1 (E)	TgCkBr257	64	5	5/5	12,12,12,1314
	2 (E)	TgCkBr258	32	5	5/5	11,11,11,11,11
	3 (F)	TgCkBr259	128	5	5/5	10,10,10,10,10
	4 (F)	TgCkBr260	64	5	5/5	12,12,13,14,14
Linhares (6)	60 (G)	TgCkBr261	64	5	5/5	13,13,14,14,15
	40 (H)	TgCkBr262	1.024	5	4/4	14,14,16,17
	62 (H)	TgCkBr263	64	5	5/5	13,13,14,14,15
	44 (H)	TgCkBr264	64	5	1/5	16
	54 (H)	TgCkBr265	256	5	5/5	11,11,13,1315
	59 (H)	TgCkBr266	64	5	1/4	16
Marechal Floriano (9)	5 (I)	TgCkBr267	128	5	5/5	14,14,15,17,21
	6 (I)	TgCkBr268	64	5	5/5	16,16,18,19,21
	7 (I)	TgCkBr269	2.048	5	5/5	12,13,13,14,16
	8 (I)	TgCkBr270	2.048	5	5/5	14,15,16,1922
	9 (I)	TgCkBr271	64	5	5/5	16,18,19,21,23
	10 (J)	TgCkBr272	1.024	4	4/4	14,14,15,15
	11 (J)	TgCkBr273	32	5	5/5	12,12,13,14,16
	14 (J)	TgCkBr274	64	5	5/5	11,11,13,14,14
	15 (J)	TgCkBr275	64	5	1/4	27
Serra (2)	22 (K)	TgCkBr276	32	5	2/2	12,20
	21 (K)	TgCkBr277	64	5	1/1	15
Vila Velha (4)	17 (SH)	TgCkBr278	64	5	5/5	12,13,13,14,14
	7 (SH	TgCkBr279	128	5	5/5	10,10,11,12,13
	6 (SH)	TgCkBr280	64	5	5/5	11,12,12,12,13
	10 (SH)	TgCkBr281	64	5	5/5	11,11,11,13,14

[a] A to K: identification of farms; SH: slaughter house for FR chickens.

(Dubey et al., 2007; Pena et al., 2008; Ragozo et al., 2008). It has to be pointed out that mouse virulence depends on several factors including the stage of the parasite, route, dose, types of mice used, and the strain of the parasite. In the present study, mice were inoculated intraperitoneally, a route that can increase *T. gondii* virulence (Dubey et al., 2002). These results also confirm previous findings that mouse virulent *T. gondii* strains circulate in asymptomatic hosts in Brazil (Pena et al., 2008; de Oliveira et al., 2009).

It was observed during collection of samples among farms that the cohabitation of chickens with cats in the same environment was frequent. In addition it was also frequent the habit of feeding domestic animals (cats and dogs) with raw viscera of chickens, enhancing the chance of spreading *T. gondii* to other hosts. Evaluation of the questionnaire indicated that people living in rural areas have little knowledge about the parasite, the disease it can induce and the risk of acquire it from raw meat. This lack

of knowledge was related to the low level of education in these areas.

The results of the present study reveal *T. gondii* infections are also widely prevalent in ES state, southeastern Brazil.

Acknowledgment

H.F.J. Pena and S.M. Gennari are in receipt of fellowships from Conselho Nacional de Pesquisa, CNPq, Brazil.

References

Aigner, C.P., Silva, A.V., Sandrini, F., Osório P. de, S., Poiares, L., Largura, A., 2010. Real-time PCR-based quantification of *Toxoplasma gondii* in tissue samples of serologically positive outdoor chickens. Mem. Inst. Oswaldo Cruz 105, 935–937.

Areal, K.R., Miranda, A.E., 2008. Soroprevalência de toxoplasmose em gestantes atendidas na rede básica de Saúde de Vitória, ES. NewsLab 87, 122–129.

Brandão, G.P., Ferreira, A.M., Melo, M.N., Vitor, R.W., 2006. Characterization of *Toxoplasma gondii* from domestic animals from Minas Gerais, Brazil. Parasite 13, 143–149.

da Silva, D.S., Bahia-Oliveira, L.M., Shen, S.K., Kwok, O.C., Lehman, T., Dubey, J.P., 2003. Prevalence of *Toxoplasma gondii* in chickens from an area in southern Brazil highly endemic to humans. J. Parasitol. 89, 394–396.

de Oliveira, L.N., Costa-Junior, L.M., de Melo, C.F., Ramos Silva, J.C., Bevilaqua, C.M., Azevedo, S.S., Muradian, V., Araújo, D.A., Dubey, J.P., Gennari, S.M., 2009. *Toxoplasma gondii* isolates from free-range chickens from the northeast region of Brazil. J. Parasitol. 95, 235–237.

Dubey, J.P., 2010a. Toxoplasmosis of Animals and Humans, 2nd ed. CRC Press, Boca Raton, Florida, 313 p.

Dubey, J.P., 2010b. Toxoplasma gondii infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health 57, 60–73.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, 220 p.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Ruff, M.D., Camargo, M.E., Shen, S.K., Wilkins, G.L., Kwok, O.C., Thulliez, P., 1993. Serologic and parasitologic responses of domestic chickens after oral inoculation with *Toxoplasma gondii* oocysts. Am. J. Vet. Res. 54, 1668–1672.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M., Nishi, S.M., Shen, S.K., Kwok, O.C., Hill, D.E., Thulliez, P., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Graham, D.H., da Silva, D.H., Lehmann, T., Bahia-Oliveira, L.M., 2003a. Toxoplasma gondii isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. J. Parasitol. 89, 851–853.

Dubey, J.P., Navarro, I.T., Graham, D.H., Dahl, E., Freire, R.L., Prudencio, L.B., Sreekumar, C., Vianna, M.C., Lehmann, T., 2003b. Characterization of *Toxoplasma gondii* isolates from free range chickens from Paraná, Brazil. Vet. Parasitol. 117, 229–234.

Dubey, J.P., Gennari, S.M., Labruna, M.B., Camargo, L.M., Vianna, M.C., Marcet, P.L., Lehmann, T., 2006. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon, Brazil. J. Parasitol. 92, 36–40.

Dubey, J.P., Sundar, N., Gennari, S.M., Minervino, A.H., Farias, N.A., Ruas, J.L., dos Santos, T.R., Cavalcante, G.T., Kwok, O.C., Su, C., 2007. Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations. Vet. Parasitol. 143, 182–188.

Dubey, J.P., Velmurugan, G.V., Chockalingam, A., Pena, H.F.J., de Oliveira, L.N., Leifer, C.A., Gennari, S.M., Oliveira, L.M.G.B., Su, C., 2008. Genetic diversity of *Toxoplasma gondii* isolates from chickens from Brazil. Vet. Parasitol. 157, 299–305.

Dubey, J.P., Rajendran, C., Ferreira, L.R., Martins, J., Kwok, O.C.H., Hill, D.E., Villena, I., Zhou, H., Su, C., Jones, J.L., 2011. High prevalence and genotypes of *Toxoplasma gondii* isolated from goats, from a retail meat store, destined for human consumption in the USA. Int. J. Parasitol. 41, 827–833.

Frenkel, J.K., 1981. False-negative serologic tests for *Toxoplasma* in birds. J. Parasitol. 67, 952–953.

Garcia, J.L., Navarro, I.T., Ogawa, L., Marana, E.R.M., 2000. Soroprevalência do *Toxoplasma gondii* em galinhas (*Gallus gallus domesticus*) de criações domésticas, oriundas de propriedades rurais do norte do Paraná, Brasil. Ciência Rural 30, 123–127.

Ghorbani, M., Gharavi, M.J., Kahnamoui, A., 1990. Serological and parasitological investigations on *Toxoplasma* infection in domestic fowls in Iran. Iranian J. Public Health 19, 9–17.

Gilbert, R.E., Freeman, K., Lago, E.G., Bahia-Oliveira, L.M., Tan, H.K., Wallon, M., Buffolano, W., Stanford, M.R., Petersen, E., European Multicentre Study on Congenital Toxoplasmosis (EMSCOT), 2008. Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl. Trop. Dis. 2, e277.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159–174.

Lunde, M.N., Jacobs, L., 1967. Evaluation of a latex agglutination test for toxoplasmosis. J. Parasitol. 53, 933–936.

Navarro, I.T., Vidotto, O., Giraldi, N., Mitsuka, R., 1992. Resistance of *Toxoplasma gondii* to sodium chloride and condiments in pork sausage. Bol. Oficina Sanit. Panam. 112, 138–143.

Peixoto, C.M.S., Lopes, C.W.G., 1990. Isolamento do *Toxoplasma gondii* Nicolle e Manceaux, 1909 (Apicomplexa, Toxoplasmatinae) em galinhas naturalmente infectadas. Arq. Univ. Fed. Rur. Rio de J. 13, 105–111.

Pena, H.F.J., Soares, R.M., Amaku, M., Dubey, J.P., Gennari, S.M., 2006. *Toxoplasma gondii* infection in cats from São Paulo state, Brazil: seroprevalence, oocyst shedding, isolation in mice, and biologic and molecular characterization. Res. Vet. Sci. 81, 58–67.

Pena, H.F.J., Gennari, S.M., Dubey, J.P., Su, C., 2008. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. Int. J. Parasitol. 38, 561–569.

Ragozo, A.M., Yai, R.L., de Oliveira, L.N., Dias, R.A., Dubey, J.P., Gennari, S.M., 2008. Seroprevalence and isolation of *Toxoplasma gondii* from sheep from São Paulo state, Brazil. J. Parasitol. 94, 1259–1263.

Ruiz, A., Frenkel, J.K., 1980. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. Am. J. Trop. Med. Hyg. 29, 1161–1166.

Soares, R.M., Silveira, L.H., da Silva, A.V., Ragozo, A., Galli, S., Lopes, E.G., Gennari, S.M., Pena, H.F.J., 2011. Genotyping of *Toxoplasma gondii* isolates from free range chickens in the Pantanal area of Brazil. Vet. Parasitol. 178, 29–34.