

Risk factors associated with *Toxoplasma gondii* infection in free-range chickens in the semiarid region of Brazil

Fatores de riscos associados com a infecção de *Toxoplasma gondii* em galinhas de vida livre na região do semiárido do Brasil

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Abstract

This study aimed to investigate the frequency of anti-*Toxoplasma gondii* antibodies in serum from 629 chickens on 39 family farms in seven municipalities in the semiarid region, Pernambuco, Brazil, and to identify risk factors associated with *T. gondii* infection. The risk factors were studied in 421 samples from 29 farms. Anti-*T. gondii* antibodies were investigated by indirect fluorescent antibody test with a 1:16 cutoff. The frequency of positive chickens was 27.9% (176/629) and 94.8% of the farms studied had chickens infected by *T. gondii*. Multivariate analysis showed variables significantly associated with anti-*T. gondii* antibodies in serum: slaughter of animals on the farm, reproductive disorders in sheep, consumption of fetal adnexa and placentas by chickens, presence of sheep in the property and birth of sheep the property. The results suggest that there is a complex relationship between general management practices for different animal species raised on the same farm and the prevalence of *T. gondii* infection in chickens. In addition, the results draw attention to the risk of human infection by *T. gondii* via consumption of infected chicken meat, because the farming conditions and the low human development indices observed in the region studied result in inappropriate meat preparation practices.

Keywords: Toxoplasmosis, chickens, family poultry, risk factors.

Resumo

Objetivou-se investigar a prevalência de anticorpos anti-*Toxoplasma gondii* em 629 soros de galinha em 39 propriedades da agricultura familiar em sete municípios de Pernambuco, Brasil e identificar os fatores de risco associados à infecção. Para a pesquisa de anticorpos anti-*T. gondii* empregou-se a reação de imunofluorescência indireta com ponto-de-corte 1:16. O estudo dos fatores de risco foi realizado em 29 propriedades, totalizando 421 amostras. A prevalência de aves positivas foi de 27,9% (176/629) e 94,8% das propriedades tinham galinhas infectadas por *T. gondii*. Na análise multivariada, obteve-se como variáveis significativas associadas com anticorpos anti-*T. gondii* a ocorrência de abate de animais na propriedade, relato de distúrbios reprodutivos em ovinos, ingestão de anexos fetais e placentas pelas galinhas, presença de ovinos na propriedade e nascimento de ovinos na propriedade. Os resultados sugerem relações complexas entre o manejo das espécies animais criados nas propriedades e a prevalência da infecção nas galinhas. Em adicional, chama-se atenção para o risco de infecção humana por *T. gondii* via consumo de carne de galinha infectada, uma vez que as condições de criação e os baixos índices de desenvolvimento observados na região resultam em inapropriada preparação da carne para consumo.

Palavras-chave: Toxoplasmose, galinhas, criação familiar, fatores de risco.

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Introduction

Toxoplasma gondii is found worldwide and can infect an exceptionally broad range of hosts such as humans, livestock, pets and wildlife. All felids are considered to be definitive hosts of *T. gondii* (MONTROYA & LIESENFELD, 2004).

Chickens are one of the most important intermediate hosts in the epidemiology of toxoplasmosis because they are an efficient source of infection for cats and humans through consumption of undercooked infected chicken meat (DUBEY, 2010). Studies have showed that chickens are an important source of *T. gondii* infection for humans (DUBEY, 2010; TENTER et al., 2000). In addition, chickens are good indicators of environmental contamination by *T. gondii* oocysts because they feed from the soil and thus become exposed to oocysts (DUBEY, 2010; HILL & DUBEY, 2013). Therefore, poultry is used as a sentinel in regions with high prevalence of *T. gondii* infection in humans (DUBEY et al., 2002).

In Brazil, studies have showed high prevalence of *T. gondii* infection in poultry in the southern region (GARCIA et al., 2000) and southeastern region (CASARTELLI-ALVES et al., 2012), thus suggesting that high environmental contamination is present, with a high risk of human and animal infection. However, few studies on occurrences of *T. gondii* infection in free-range chickens have been conducted, especially in the northeastern region of the country.

In this context, the present study aimed to estimate the frequency of anti-*T. gondii* antibodies in free-range chickens and to identify risk factors associated with infection by this protozoon on family farms in the semiarid region of Brazil.

Material and Methods

Area of study and sampling

A cross-sectional epidemiological study was conducted on family farms located in seven municipalities in the semiarid region of the state of Pernambuco, Brazil: Jupí, Lajedo, Paratama, Itaíba, Iati, Sertânia and Tuparetama.

The sample size was calculated considering a population of 336,221 chickens in the state of Pernambuco (IBGE, 2010) and an expected prevalence of toxoplasmosis of 50%. A prevalence of 50% was chosen to maximize the sample size, thereby ensuring a minimum 95% confidence interval (CI) with a statistical error of 5% (THRUSFIELD, 2004). Therefore, according to the calculation, the minimum sample size to be analyzed was 384 animals. We collected blood serum samples from 629 chickens on 39 farms.

Collection of blood samples

The chickens were individually restrained and their general clinical condition was evaluated. Afterwards, approximately 2 ml of blood was collected from adult birds of both sexes through puncture of the brachial vein. The blood samples were then centrifuged to obtain serum.

Serological examination

The serum samples were evaluated by an in house indirect fluorescent antibody test (in house IFAT) to detect any presence of anti-*T. gondii* antibodies, in accordance with the method proposed by Camargo (1974). A serum dilution of 1:16 was adopted as cut-off (MILLAR et al., 2012). Known positive and negative controls were included in all reactions. *T. gondii* tachyzoites (RH strain) were used as antigens in the slide preparation.

Sera were diluted in Phosphate Buffered Solution (PBS, pH 7.2) at room temperature and were placed in wells on slides and incubated at 37 °C for 30 minutes in humid chamber. The slides were washed in PBS, three times during ten minutes, followed by an incubation (37 °C for 30 minutes in humid chamber) with anti-chicken IgG antibody conjugated for fluorescein isothiocyanate (Sigma Chemical®, USA), diluted 1:400 in PBS containing 0.05% of Evans Blue (Sigma Chemical®, USA). The slides were washed again, covered with buffered glycerin (pH 8.0) and a cover slip and then examined under a fluorescent microscope (Nikon Eclipse; objective: 40x) by one trained person.

Investigation of risk factors and statistical analysis

To study risk factors associated with *T. gondii* infection, questionnaires with multiple choice questions relating to production and sanitary management of poultry and the epidemiology of toxoplasmosis were applied on 29 of the 39 farms on which blood samples were collected, totaling 421 samples. These farms were located in the municipalities of Jupí, Lajedo, Paratama and Itaíba. The same person applied the questionnaires on all the farms. The number of farms was chosen according to convenience, but it fulfilled the minimum sample size that had previously been calculated.

The variables studied were: presence of cats on the property; occurrence of birth of cats on the property; presence of sheep in the property; birth of sheep in the property; intake of fetal adnexa, fetal fluids and placentas by chickens; slaughter of animals in the property; reproductive disorders (abortion, return to estrus, stillbirths) in sheep.

The variables of the questionnaires were evaluated by means of univariate analysis using Pearson's chi-square test or Fisher's exact test ($p < 0.05$), when necessary, adopting a 95% confidence interval. The logistic regression model took the serological results (positive or negative) as the dependent variable for use in the multivariate analysis ($p < 0.05$). The statistical analyses were performed in the Epi Info 3.5.1 software (Centers for Disease Control and Prevention).

Ethical aspects

All procedures had previously been approved by the Ethics Committee for Animal Use (CEUA-UFAL; License no. 54/2014).

Results

In the present study, 27.9% (176/629) of the blood samples from the chickens were positive for anti-*T. gondii* antibodies (CI: 24.5% -31.7%). Itaíba (46%) and Lajedo (14.1%) were the municipalities with the highest and lowest percentages of positive samples, respectively (Table 1). A total of 94.8% (37/39) of the farms had at least one positive animal. According to the univariate analysis, all seven variables were associated with *T. gondii* infection in chickens (Table 2).

Logistic regression (Table 2) confirmed the following variables as risk factors for *T. gondii* infection in poultry in the semiarid: slaughter of animals on the farm (OR 1.66); reproductive disorders

in sheep (OR 2.29); intake of fetal adnexa, fetal fluids and placentas by chickens (OR 2.58); presence of sheep in the property (OR 1.96) and birth of sheep the property (OR 1.72).

Discussion

In our study, all the farms had similar social and economic characteristics and were categorized as family-based farms (UN-PNDU, 2013). On these farms, the average frequency of chickens positive for anti-*T. gondii* antibodies was 27.9%, ranging from 14.1% to 46.0%.

Itaíba presented the highest prevalence of chickens positive for *T. gondii*. The farms in this municipality belong to a rural

Table 1. Absolute and relative frequencies of free-range chickens exposure to *T. gondii* accessed by indirect immunofluorescence test (cutoff at dilution of 1:16).

Municipalities	Positive farms /Total farms	Animals	
		N total	Positives (%)
Jupi	2/4	62	12 (19.4%)
Lajedo	5/5	64	9 (14.1%)
Paranatama	12/12	208	49 (23.6%)
Itaíba	9/9	100	46 (46.0%)
Iatí	4/4	36	9 (25.0%)
Sertânia	1/1	67	17 (25.4%)
Tuparetama	4/4	92	34 (37.0%)
Total	37/39	629	176 (27.9%)

N - number.

Table 2. Univariate and multivariate analysis of risks factors associated with *Toxoplasma gondii* infection in free-range chicken from semiarid region, Brazil.

Variables	RIFI and Univariate analysis			Logistic regression		
	Positive ^a	Negative	p-value	OR ^b	p-value	C.I. 95% ^c
Presence of cats on the property						
Yes	91 (29.4%)	219 (70.6%)	0.032	1.68	0.053	0.99-2.84
No	22 (19.8%)	89 (80.2%)				
Occurrence of birth of cats on the property						
Yes	32 (34.8%)	60 (65.2%)	0.036	1.63	0.053	0.99-2.68
No	81 (24.6%)	248 (75.4%)				
Presence of sheep in the property						
Yes	80 (32.0%)	170 (68.0%)	0.002	1.96	0.004	1.23-3.12
No	33 (19.3%)	138 (80.7%)				
Birth of sheep in the property						
Yes	58 (33.1%)	117 (66.9%)	0.009	1.72	0.014	1.11-2.65
No	55 (22.4%)	191 (77.6%)				
Slaughter of animals in the property						
Yes	77 (30.8%)	173 (69.2%)	0.017	1.66	0.027	1.05-2.63
No	36 (21.1%)	135 (78.9%)				
Reproductive disorders (abortion, return to estrus, stillbirths) in sheep						
Yes	27 (42.2%)	37 (57.8%)	0.002	2.29	0.003	1.32-3.99
No	86 (24.1%)	271 (75.9%)				
Intake of fetal adnexa, fetal fluids and placentas by chickens						
Yes	18 (46.2%)	21 (53.8%)	0.005	2.58	0.005	1.32-5.06
No	95 (24.9%)	287 (75.1%)				

^a Absolute frequency (Relative frequency); ^b Odds ratio; ^c Confidence interval.

settlement project in which the houses are in a village system and are approximately 25 meters apart from each other. The close proximity of the houses and cohabitation of chickens of different farmers in the same contaminated environment may explain the high frequency of positive chickens found in this municipality. The other farms evaluated in the present study were hundreds of meters apart from each other and had lower prevalence of chickens positive for anti-*T. gondii* antibodies. Only the municipality of Jupí had farms (two) with all chickens negative for anti-*T. gondii* antibodies. In these two farms, there is no breeding other domestic animals that could be protective factor for chickens because lack probable sources of infection.

Studies on the prevalence of anti-*T. gondii* antibodies in free-range chickens have been conducted worldwide using different techniques. In this regard, Casartelli-Alves et al. (2012) observed that most studies conducted in Brazil on chickens on family farms aimed to genetically characterize *T. gondii* isolates instead of determining the prevalence of this protozoon. In Brazil, the frequency of positive chickens has ranged from 10.3% (GARCIA et al., 2000) to 80% in different studies (COSTA et al., 2012).

The presence of cats eliminating oocysts may contaminate the soil from which the chickens feed, thus increasing the frequency of positive chickens. Overall, the present study no found significant association between the presence of cats on the farms and the frequency of positive chickens. This result is similar to that of Marques et al. (2009), who also did not detect any statistical association between the presence of cats and *T. gondii* infection in chicken, even after observing that 57% (8/14) of the cats were positive for anti-*T. gondii* antibodies. On the other hand, the participation of cats in environmental contamination by oocysts is well known (MONTROYA & LIESENFELD, 2004) and Millar et al. (2012) described an association between high prevalence of positive chickens and the presence of cats. According to Beltrame et al. (2012), variations in the seropositivity of chickens for *T. gondii* may be related to the presence of cats on farms.

However, other sources of infection should not be ruled out. Reproductive disorders in sheep were considered a risk factor for infection of chickens (p 0.003; OR 2.29), in addition, observed that chickens had access to and consumed fetal adnexa, fetal fluids and placentas of sheep (p 0.005; OR 2.58) suggesting that there is some relationship between these two species in the transmission of toxoplasmosis. It is known that *T. gondii* cysts may be present in placentas and fetal adnexa from aborted sheep fetuses (BUXTON, 1998; OWEN et al., 1998).

Consumption of undercooked or raw meat from free-range chickens can be a source of *T. gondii* infection for humans and other animals because chickens can host viable *T. gondii* cysts in their tissues (DUBEY, 2010). Moreover, lack of hygiene during handling of poultry meat may contribute towards human infection and inadequate disposal of viscera from infected chickens can facilitate transmission of *T. gondii* to other hosts such as cats. On the farms studied here, we observed that dogs, cats, pigs and chickens routinely consumed viscera from chickens. The multivariate analysis showed that slaughter of animals on the farm (OR 1.66) is a risk factor for *T. gondii* infection in chickens. This result corroborates the findings of Kaneto et al. (1997), who showed that *T. gondii* cysts were present in viscera from experimentally infected chicken,

such as the intestines and proventriculus, which are organs disposed of in the environment after chickens are slaughtered on the farms studied and could be source of infection to definitive host.

This is the first study conducted in the semi-arid region of the state of Pernambuco, Brazil, that focused on the frequency of anti-*T. gondii* antibodies in chickens. The results suggest that there is a complex relationship between general management practices for different animal species raised (sheep) on the same farm and the prevalence of *T. gondii* infection in chickens. In addition, the results draw attention to the risk of human infection *T. gondii* by consumption and manipulation of infected chicken meat. It is also important to perform a serological survey in humans in this region that presents low human development indices to verify if there is relation between the positivity to *T. gondii* and the manipulation or consumption of chickens meat infected with *T. gondii*.

Conflict of interest statement

There was no conflict of interest for any of the authors involved in this study.

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