Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Short Communication

Cross-sectional survey for toxoplasmosis in free-range chickens (*Gallus gallus domesticus*) from the Atlantic Forest area in Alagoas state, Northeastern Brazil

Estudo transversal para toxoplasmose em galinhas caipiras (*Gallus gallus domesticus*) de área de Mata Atlântica do estado de Alagoas, Nordeste do Brasil

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How to cite: Braz BMA, Feitosa BCO, Romão EA, Silva EM, Pinheiro Júnior JW, Mota RA, et al. Cross-sectional survey for toxoplasmosis in free-range chickens (*Gallus gallus domesticus*) from the Atlantic Forest area in Alagoas state, Northeastern Brazil. *Braz J Vet Parasitol* 2020; 29(4): e014120. https://doi.org/10.1590/S1984-29612020087

Abstract

Toxoplasmosis is a parasitic disease, which is prevalent across the world and has a strong environmental component in its transmission chain. The present study determined the seroprevalence and factors related to *Toxoplasma gondii* infection among free-range chickens from an Atlantic Forest area in Northeastern Brazil. A total of 550 free-range chickens were tested for *T. gondii* antibodies usingthe indirect fluorescent antibody test (IFAT, cut-off point 1:16), and 269 (48.9%; 95% CI = 44.7-53.1%) were positive. Among the 61 properties 57 (93.4%) had at least one seropositive animal. The robust Poisson regression model showed that the variables/categories associated with the seroprevalence of *T. gondii* were: region of origin of the animals/urban area (prevalence ratio [PR] = 2.346; *P*<0.001), management system/use of cages (PR = 1.591; *P* = 0.019), presence of rodents/yes (PR = 1.295; *P* = 0.035), and the type of food/use of food scraps (PR = 1.603; *P* = 0.009). The high prevalence found suggest that this scenario demands careful management, mainly regarding the use of scraps of undercooked or raw food, adequate environmental hygiene and frequent rodentcontrol.

Keywords: *T. gondii*, serology, free-range chickens, epidemiology, prevalence.

Resumo

A toxoplasmose é uma doença parasitária mundial com um forte componente ambiental em sua cadeia de transmissão. Nesta pesquisa, foram determinados a soroprevalência e os fatores associados em galinhas caipiras de uma área de Mata Atlântica no Nordeste do Brasil. Um total de 550 galinhas caipiras foi testado para anticorpos anti-*Toxoplasma gondii* pela reação de imunfluorescência indireta (RIFI) com ponto de corte de 1:16, e 269 (48,9%; IC95% = 44,7- 53,1%) foram positivas. Das 61 propriedades visitadas, 57 (93,4%) apresentaram pelo menos um animal positivo. O modelo de regressão de Poisson robusta mostrou que as variáveis/categorias associadas à soroprevalência de *T. gondii* foram: origem dos animais/área urbana (razão de prevalência - RP = 2,346; *P*<0,001), sistema de manejo/uso de gaiolas (RP = 1,591; *P* = 0,019), presença de roedores/sim (RP = 1,295; *P* = 0,035), e tipo de alimento/uso de sobras de alimentos (RP = 1,603; *P* = 0,009). A alta prevalência sugereque este cenário demanda cuidados de manejo, principalmente no que se refere ao fornecimento de sobras de alimentos mal cozidos ou crus, higiene adequada do meio ambiente e controle frequente de roedores.

Palavras-chave: *T. gondii*, sorologia, galinhas caipiras, epidemiologia, prevalência.

Received June 12, 2020. Accepted august 14, 2020.
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Toxoplasmosis is a zoonotic disease with a worldwide distribution, caused by an intracellular Coccidia, *Toxoplasma gondii*. Domestic cats and other felids are the known definitive hosts of the agent, which can eliminate viable oocysts through their feces (Dubey, 2010). Free-range chicken farming is an income-generating enterprise, which provides a nutritional alternative for low-income families, especially in Northeastern Brazil. In the afore said region, the production scale continues to increase every year and the product is largely consumed by internal consumers. However, these animals can get infected by ingesting *T. gondii* oocysts because they feed from the ground and they are considered as good indicators of environmental contamination (Dubey et al., 2012). In addition, free-range chickens have an epidemiological significance because raw or undercooked chicken meat is a major source of infection for humans and other animals, if consumed or handled without proper hygiene (Casartelli-Alves et al., 2015). Moreover, it is worth mentioning that in previous studies *T. gondii* was isolated from free-range chickens in Northeastern Brazil and in the municipality investigated in this study (Clementino Andrade et al., 2013; Feitosa et al., 2016; Santos Silva et al., 2020).

To our knowledge, serological surveys using planned sampling of free-range chickens in the regions of fragmented Atlantic Forest are very few.In the present study, a cross-sectional survey was carried out to determine the prevalence and the factors associated with *T. gondii* infection in free-range chickens (*Gallus gallus domesticus*) from an area of fragmented Atlantic Forest in the state of Alagoas, Northeastern Brazil, specifically in the county of Viçosa. According to the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), the afore mentioned geographic region has high humidity, the climate is tropical with rains from autumn to winter and a well-defined dry season. The average annual rainfall varies from 1.300mm to1.400mm. The above-mentioned conditions, together with the peculiarities of the predominant vegetation, sub-perennial forests with a rich biodiversity, present epidemiological conditions that need to be analyzed in a different manner compared to other regions.

All procedures were approved by the Committee on Ethics in the Use of Animals (CEUA - Federal University of Alagoas, Protocol No. 43/2015). The formula for simple random sampling was used to calculate the sample size (Thrusfield, 2004) and the following parameters were taken into consideration: expected prevalence of 50% (sample maximization), confidence level of 95% and a statistical error of 5%, which resulted in an estimated minimum sample size of 384 animals. However, 550 animals from 61 farms were included in the study; 251 from urban and 299 from rural areas. Blood samples were collected through a puncture of the brachial vein from March to June 2015.

The indirect fluorescent antibody test (IFAT) was used to detect anti-*T. gondii* antibodies in the serum samples, as described by Camargo (1964). The tachyzoites of *T. gondii* from the RH reference strain, fixed on a slide, were used as antigens. Samples known to be positive and negative were used as controls in each slide, and the anti-chicken IgG conjugate from Sigma-Aldrich (reference F4137). The samples were read in double blind to confirm the result. A cut-off point of 1:16 (Millar et al., 2012) was considered as the indicator of seropositivity.

All the owners involved in the study answered epidemiological questionnaires and the data collected were used to analyze the factors associated with the seroprevalence of *T. gondii*. The variables and respective categories analyzed are presented in Table 1. The data obtained from the epidemiological questionnaires and serological tests were stored in a Microsoft Excel software database.

Initially, association analyses (univariable analysis) between each independent variable and the dependent variable (seropositivity in serological tests) were performed. Variables with *P*-values less than or equal to 0.2 in the chi-squared test were selected for the multivariable analysis using the robust Poisson regression model. Collinearity between independent variables was verified through a correlation analysis (cut-off: correlation coefficient >0.9) (Dohoo et al., 2003). The Omnibus test was used to verify the significance of the model, and the Pearson chi-squared test was applied to verify the goodness-of-fit of the final model. The current study assumed a significance level of 5% in the multiple analyses. The statistical analyses were performed using the SPSS software for Windows, version 20.0.

Among the 550 serum samples evaluated, 269 (48.9%; 95% CI = 44.7-53.1%) were positive for anti-*T. gondii* IgG antibodies and among the 61 properties involved in the survey, 57 (93.4%) had at least one infected animal. The results of the univariable analysis and the variables selected for multivariable analysis are presented in Table 1. The sample size available for analyses of some of the variables was less than the total sample size of the current study (n = 550) on account of the inadequate data collection by the interviewers. The final robust Poisson regression model is shown in Table 2. The variables' categories associated with the seroprevalence of *T. gondii* were as follows: urban area (Prevalence ratio [PR] = 2.346; 95% CI = 1.801-3.056; *P*<0.001), use of cages (PR = 1.591; 95% CI = 1.078-2.350; *P* = 0.019), presence of rodents (PR = 1.295; 95% CI = 1.018-1.647 ; *P* = 0.035) and use of food scraps (PR = 1.603; 95% CI = 1.126-2.282; *P* = 0.009). The final model presented good fit (Pearson chi-squared value = 254.94; degree of freedom (df) = 480; value/df = 0.531).

Table 1. Univariable analysis of factors associated with the seroprevalence of toxoplasmosis infree-range chickens from the Atlantic Forest area of the Alagoas state, Northeastern Brazil.

Variable	Categories	Total number of chickens	Number of positive chickens (%)	*P*-value
Origin of the animals	Rural area	299	92 (30.8%)	
	Urban area	251	177 (70.5%)	< 0.001*
Management system	Extensive	374	165 (44.1%)	
	Cages	47	34 (72.3%)	
	Extensive/cages	120	64 (53.34%)	0.001*
Backyard division	Fence	447	194 (43.4%)	
	Wall	74	57 (77%)	
	Others	20	12 (60%)	< 0.001*
Presence of cats	Yes	460	219 (47.6%)	
	No	81	44 (54.3%)	0.320
Presence of rodents	Yes	74	51 (68.9%)	
	No	467	212 (45.4%)	< 0.001*
Presence of other animals	Yes	453	204 (45%)	
	No	88	59 (67%)	< 0.001*
Type of food	Commercial food	124	38 (30.6%)	
	Food scraps	73	40 (54.8%)	
	Commercial/food scraps	337	182 (54%)	
	Others	7	3 (42.9%)	< 0.001*
Feeding arrangements	Chicken feeders	131	58 (44.3%)	
	On the ground	377	196 (52%)	
	Both	33	9 (27.3%)	0.013*
Cat feces disposal	On the ground	244	133 (54.5%)	
	Near pastures	8	6 (75%)	
	Near water troughs	18	9 (50%)	
	On the ground/near pastures	226	85 (37.6%)	0.001*

*Variables selected for robust Poisson regression; *P*-value = probability of rejecting the null hypothesis when it is actually true.

Table 2. Factors associated with the seroprevalence of toxoplasmosis in free-range chickens from the Atlantic Forest area of the Alagoas state, Northeastern Brazil.

Variable category	Coefficient estimates	Standard error	Wald chi-squared	Prevalence ratio (PR)	95% CI for PR	*P*-value
Urban area	0.853	0.135	39.960	2.346	1.801-3.056	< 0.001
Use of cages	0.465	0.199	5.457	1.591	1.078-2.350	0.019
Presence of rodents	0.258	0.123	4.422	1.295	1.018-1.647	0.035
Use of food scraps	0.472	0.180	6.858	1.603	1.126-2.282	0.009

Pearson chi-squared value = 254.94; degree of freedom (df) = 480; value/df = 0.531; 95% CI = Confidence interval of 95% for prevalence ratio; *P*-value = probability of rejecting the null hypothesis when it is actually true.

According to Casartelli-Alves et al. (2014), the modified agglutination test (MAT) was used for anti-*T. gondii* antibody detection in free-range chickens and proved to be simple, low cost and does not require a specific conjugate, being the first choice when compared to the enzyme-linked immunosorbent assay (ELISA) and IFAT. However, the properly standardized IFAT can produce high sensitivity and specificity, being a reproducible option when available, and very effective using cut-off ≥16 in field studies in chickens. The prevalence rate in the current study was higher compared to most of the recent surveys carried out in Brazil, with the reported rates varying from 17.2% to 31.5% (Feitosa et al., 2016; Millar et al., 2012; Sá et al., 2017), which indicates that, compared to intensive industrial systems, *T. gondii* is more wide-spread in free-range systems and soil is probably the main source of oocysts. Furthermore, the chickens have a shorter life span in the industrial systems and contact with cats and contaminated soil is less likely in this scenario (Liu et al., 2017). The region of fragmented Atlantic Forest in the state of Alagoas is situated in an area with a predominance of hills with altitudes of up to 500 meters, which interferes with the relative humidity of air (about 70%). The temperature in the area varies from 23 °C to 28 °C, which can favor the survival and sporulation of *T. gondii* oocysts, as they can resist high temperatures and conditions of moderate humidity and survive for months in the soil (Torrey & Yolken, 2013).

Toxoplasmosis is an emerging food-borne disease in humans and animals with variable worldwide distribution depending on geographic characteristics and cultural habits of the population. Therefore, the disease is significant from the One Health perspective, as it has a strong environmental component in the transmission chain, which makes it a problem that impacts both human and animal health (Latha & Hareendran, 2018). The high seroprevalence found here indicate that focusing on the sources of infection is fundamental, since *T. gondii* is directly influenced by the environment, which allows its survival in the form of oocysts in the soil. Considering that free-ranging chickens are raised for domestic consumption, both the handler and the consumer are exposed to the risk of infection, either during the processing of the carcass or due to the consumption of raw or semi-cooked meat (Chumpolbanchorn et al., 2013).

The design of cross-sectional survey is one of the most frequently used study designs in observational studies on animal toxoplasmosis and in veterinary epidemiology, probably because they are rapid, in expensive, of moderate difficulty and the odds ratio (OR) is the most common association measure between an exposure and an outcome. However, when the binary outcome is common, usually with a prevalence greater than 10%, the prevalence ratio (PR) can be over estimated by the OR when the PR is greater than one round estimated when the PR is less than one (Martinez et al., 2017). In the current study, a robust Poisson regression model was used to estimate the PR and determine the factors associated with seroprevalence. Free-range chickens reared in the urban areas were 2.346 times more likely to be seropositive for *T. gondii*, i.e., a 134.6% increase in prevalence. This may be related to the improper management system because in this area, most of the breeding is done in small clusters in the backyards of houses, generally in unhygienic and unsanitary conditions with a constant circulation of felines (Millar et al., 2012). Moreover, the presence of sewage and garbage in peri-urban areas can increase the chickens' exposure to sources of infection, as they usually feed on the remains of food supplied directly on the ground (Feng et al., 2016).

The use of cages in chicken rearing increased the seroprevalence of *T. gondii* by 1.591 times (59.1%). Free-range chickens are deemed more susceptible to *T. gondii* infection and are good indicators of environmental contamination by oocysts because of their habit of scratching and are being used as sentinel animals in the regions with a high prevalence of human toxoplasmosis (Dong et al., 2018). However, in backyard rearing systems, in which chickens are kept in cages, access to oocysts in the soil is limited, and these animals are fed food scraps and ingest untreated water, which are important routes of disease transmission (Millar et al., 2012).

The presence of rodents was associated with a 29.5% increase in the prevalence of *T. gondii* infection. Rodents and chickens are considered the main intermediate hosts of *T. gondii* when being hunted by felids (Wang et al., 2013). Nevertheless, the epidemiological importance of rodents in the context of toxoplasmosis is lower, compared to that of chickens, considering that they act as efficient intermediate hosts due to their longevity, are more resistant to the agent and do not develop the clinical disease (Dubey, 2010). It is important to note that the properties involved in the current study had favorable conditions to attract rodents, such as the lack of hygiene and basic sanitation and the accumulation of garbage in the yards.

The use of food scraps for feeding free-range chickens was associated with a 60.3% increase in the prevalence, indicating that the animals that receive mixed feeding are more likely to be seropositive. This fact may be related to the consumption habits of the owners because in Brazil, eating undercooked meat and feeding animals with leftover meat and raw viscera is a common practice (Sá et al., 2017). In these cases, rectification and improvement

of management systems is an alternative method, which can be employed to reduce the frequency of the disease in backyard farms (Feitosa et al., 2016).

In the present study a high seroprevalence of *T. gondii* was found in free-range chickens from a fragmented Atlantic Forest area in Northeastern Brazil, which suggests a widespread dissemination of the agent and a significant impact on public health, since these animals are low-cost sources of protein. Therefore, the scenario demands careful management, mainly regarding the use of scrap from undercooked or raw food, adequate environmental hygiene and frequent rodent control.

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