Research Note

Braz. J. Vet. Parasitol., Jaboticabal, v. 24, n. 3, p. 383-386, jul.-set. 2015
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612015043

Prevalence and risk factors for *Toxoplasma gondii* in sheep in the State of Paraíba, Northeastern Brazil

Prevalência de rebanhos positivos e de animais soropositivos e fatores de risco associados com a infecção por *Toxoplasma gondii* em ovinos no Estado da Paraíba, Nordeste do Brasil

Érico Luís de Barros Correia[1]; Thais Ferreira Feitosa[1]; Fabrine Alexandre dos Santos[1]; Sérgio Santos de Azevedo[1]; Hilda Fátima de Jesus Pena[2]; Solange Maria Gennari[2]; Rinaldo Aparecido Mota[3]; Clebert José Alves[1]*

[1]Laboratório de Doenças Transmissíveis, Programa de Pós-graduação em Medicina Veterinária, Universidade Federal de Campina Grande - UFCG, Patos, PB, Brasil

[2]Laboratório de Doenças Parasitárias, Programa de Pós-graduação em Epidemiologia Experimental Aplicada às Zoonoses, Universidade de São Paulo - USP, São Paulo, SP, Brasil

[3]Laboratório de Bacterioses, Programa de Pós-graduação em Biociência Animal, Universidade Federal Rural de Pernambuco - UFRPE, Recife, PE, Brasil

Received February 8, 2015
Accepted March 12, 2015

Abstract

The aims of this survey were to determine the flock-level and animal-level prevalences of anti-*Toxoplasma gondii* antibodies in sheep in the State of Paraíba, Northeastern Brazil, and to identify risk factors. Blood samples were collected from 540 sheep in 63 flocks in 14 municipalities in the Sertão mesoregion. To serologically diagnose *T. gondii* infection, the indirect immunofluorescent antibody test (IFAT) was used. Flocks with at least one seropositive animal were considered positive. Among the 63 flocks, 28 (44.44%) were positive, and 60 (11.11%) of the 540 animals were seropositive. Variable flock size > 25 animals was identified as a risk factor (odds ratio = 3.2; 95% CI = 1.09 – 9.34; $P = 0.033$). The results from this survey demonstrate that *T. gondii* is spread among sheep in the State of Paraíba.

Keywords: Toxoplasmosis, small ruminants, serology, epidemiology, control.

Resumo

Os objetivos deste trabalho foram determinar a prevalência de rebanhos positivos e de animais soropositivos para *Toxoplasma gondii* em ovinos do Estado da Paraíba, Nordeste do Brasil, bem como identificar fatores de risco. Foram colhidas amostras de sangue de 540 ovinos de 63 rebanhos em 14 municípios da mesorregião do Sertão. Para o diagnóstico sorológico da infecção por *T. gondii*, foi utilizado o teste de imunofluorescência indireta (RIFI). Rebanhos com pelo menos um animal soropositivo foram considerados positivos. Dos 63 rebanhos, 28 (44,44%) foram positivos, e 60 (11,11%) dos 540 animais foram soropositivos. A variável tamanho do rebanho > 25 animais foi identificada como fator de risco (*odds ratio* = 3,2; IC 95% = 1,09 – 9,34; $P = 0,033$). Os resultados deste trabalho sugerem a disseminação de *T. gondii* em ovinos no Estado da Paraíba.

Palavras-chave: Toxoplasmose, pequenos ruminantes, sorologia, epidemiologia, controle.

Introduction

The Brazilian national sheep flock is approximately 17 million head, and 56.7% of this effective are concentrated in the Northeastern region. In the State of Paraiba, sheep farming has economic importance, and this state has a headcount of 433,032 animals distributed in 19,826 farms (IBGE, 2010). In this context, epidemiological investigations of infectious and parasitic diseases are of relevance. Among these diseases, toxoplasmosis can be highlighted, given that this is an important cause of abortions among sheep (DUBEY, 2010). Furthermore, the fact that this disease is a widespread zoonosis has to be taken into account.

Toxoplasma gondii is a protozoan parasite with worldwide distribution that can infect a wide range of animal species, including sheep. It has an indirect life cycle with felids as the definitive hosts (DUBEY, 2010). In sheep, *T. gondii* has been recognized as one of the main causes of infectious ovine abortion in New Zealand, Australia, the United Kingdom,

***Corresponding author:** Clebert José Alves. Laboratório de Doenças Transmissíveis, Unidade Acadêmica de Medicina Veterinária, Universidade Federal de Campina Grande – UFCG, Av. Universitária, s/n, Santa Cecília, CEP 58700-970, Patos, PB, Brasil.
e-mail: clebertja@uol.com.br

Norway and the United States (DUBEY & BEATTIE, 1988; DUBEY, 2010). This agent is estimated to infect between 4% and 77% of the human population (TENTER et al., 2000). Although not normally a significant problem for healthy individuals, *T. gondii* infection can be life-threatening to infants infected congenitally and to immunosuppressed patients (CHINTANA et al., 1998). In animals, *T. gondii* infection not only results in significant reproduction losses and, hence, economic losses, but also has implications for public health, since consumption of infected meat or milk can lead to zoonotic transmission (DUBEY, 2010).

To date, there have not been any reports of epidemiological investigations relating to toxoplasmosis in sheep in the State of Paraiba, Northeastern Brazil. Therefore, the aims of this survey were to determine the flock-level and animal-level prevalences and to identify the risk factors for *T. gondii* infection among sheep in the State of Paraiba, using a planned sampling.

Materials and Methods

Sampling

The sampling was designed for determining the flock-level and animal-level prevalences. The number of flocks to be sampledwas calculated using the formula for simple random sampling (THRUSFIELD, 2007), taking the following parameters into account: expected prevalence of 88.9%, found in the State of Maranhão (BRANDÃO, 2009); 95% confidence level; and absolute error of 8%. This error was chosen to guarantee the operational capacity of the fieldwork. The number of sheep to be sampledin each flock was determined individually per flock using the formula for detecting the presence of infection (THRUSFIELD, 2007). The probability of detecting at least one seropositive animal in the flock was determined with a 95% confidence level ($P = 0.95$) and the number of seropositive animals per flock was calculated assuming an intra-flock prevalence of 56.94% (SAKATA et al., 2012). In total, 540 animals from 63 flocks in 14 municipalities in the Sertão mesoregion were used. Blood sample collection was performed between December 2012 and December 2013.

Serological diagnosis of T. gondii infection

The indirect fluorescent antibody test (IFAT) was used to detect anti-*T. gondii* antibodies, taking the dilution of 1:64 as the cutoff point (GARCIA et al., 1999), in accordance with the method described by Camargo (1974), using as antigens RH strain tachyzoites grown and maintained in Swiss mice. Positive and negative control sheep serum samples were provided by the Laboratory of Parasitic Diseases of the Faculty of Veterinary Medicine, University of São Paulo (USP). Anti-sheep IgG (whole molecule; Sigma, St. Louis, MO, USA) was used as the conjugate at a dilution of 1:400 in sterile PBS (0.105 M Na_2HPO_4, 0.018 M KH_2PO_4, 1.37 M NaCl and 0.027 M KCl), at pH 7.6.

Statistical analysis

Flocks with at least one seropositive animal were considered positive. For the risk factor analysis, data gathered through epidemiological questionnaires that had been applied to each farm owner were used. The analysis was performed in two steps: univariable and multivariable analyses. Univariable analysis was performed using the chi-square test or Fisher's exact test (ZAR, 1999), and variables that presented $P \leq 0.20$ were used for multivariable logistic regression. Multivariable analysis was then performed, using the stepwise forward method (HOSMER & LEMESHOW, 2000). The significance level in the multivariable analysis was 5%. The collinearity among independent variables was assessed using correlation analysis, and when two variables were highly collinear (correlation coefficient > 0.90), only one variable was likely to enter the multivariable analysis. In such situations, selection of which collinear variable to put into the model was guided by biological plausibility (DOHOO et al., 1997). The tests were performed using the SPSS software package, version 13.0 for Windows.

Results and Discussion

Among the 63 flocks, 28 (44.4%) presented at least one seropositive sheep, and among the 540 sheep used, 60 were seropositive for *T. gondii*, thus resulting in a prevalence of 11.1% with antibody titers ranging from 64 to 2,048. Sakata et al. (2012) found a seroprevalence of 56.94% among sheep in the municipality of Lages, State of Santa Catarina. This high prevalence, in comparison to that obtained in the present study, may be explained by the high humidity of the Lajes region, with greater and more regular rainfall, which may contribute towards higher viability among *T. gondii* oocysts in the environment. On the other hand, Pereira et al. (2012) and Andrade et al. (2013) found prevalence rates of 16.9% and 26.29% among sheep in the States of Pernambuco and Rio Grande do Norte, respectively, which are both located in the semiarid region of Brazil.

Even though the prevalence of seropositive animals was low, there was at least one seropositive animal in 44.4% of the flocks. This demonstrates that this agent is spread in the region. In this context, it should be noted that the sheep-rearing profile in this region consists of extensive and semi-intensive management systems, in which the animals are set free during the day and are gathered into rustic pens at night, lacking basic infrastructure, with inadequate hygiene practices. This demonstrates that there is a need to apply appropriate technologies to the realities of sheep farming in the semiarid region. Absence of technification, such as practicing regular cleaning of the installations and reproductive management during the postpartum period, may facilitate the contact with *T. gondii* oocysts present in the environment (TENTER et al., 2000).

In the risk factor analysis, the variables of purpose of rearing ($P = 0.164$), technified rearing ($P = 0.066$), flock size ($P = 0.066$) and occurrences of abortions ($P = 0.081$) were selected from the univariable analysis (Table 1). In the multivariable analysis, the variable of flock size > 25 animals was identified as a risk factor

Table 1. Univariable analysis on risk factors associated with flock-level prevalence of *Toxoplasma gondii* infection in sheep, in the State of Paraiba, Northeastern Brazil, from December 2012 to December 2013.

Variable	Total number of flocks	Number of positive flocks (%)	*P*
Management system			
Intensive	1	1 (100)	
Extensive	43	17 (39.5)	
Semi-intensive	19	11 (57.9)	0.225
Purpose of rearing			
Breeding	18	7 (38.9)	
Raising/fattening	30	15 (50)	
Reproduction	4	3 (75)	
Subsistence	11	2 (18.2)	0.164*
Type of exploitation			
Meat	54	25 (46.3)	
Milk	2	1 (50)	
Others	7	3 (42.9)	0.979
Technified rearing			
No	54	21 (38.9)	
Yes	9	7 (77.8)	0.066*
Major activity on the farm			
No	48	20 (41.7)	
Yes	15	9 (60)	0.344
Flock size			
Up to 25 animals	35	12 (34.3)	
> 25 animals	28	17 (60.7)	0.066*
Contact with other animals			
No	26	10 (38.5)	
Yes	37	19 (51.4)	0.451
Native pasture			
No	1	1 (100)	
Yes	62	28 (45.2)	0.460
Mineral supplementation			
No	34	13 (38.2)	
Yes	29	13(44.8)	0.785
Animal purchases			
No	30	12 (40)	
Yes	33	16 (48.5)	0.672
Participation in animal agglomerations			
No	60	24 (40)	
Yes	3	2 (66.7)	0.564
Occurrences of abortions			
No	41	22 (53.7)	
Yes	22	6 (27.3)	0.081*
Occurrences of stillbirths			
No	40	18 (45)	
Yes	23	7 (30.4)	0.384
Occurrences of death at weaning			
No	47	20 (42.5)	
Yes	18	6 (33.3)	0.692

*Variables selected for the multivariable analysis ($P \leq 0.20$).

(odds ratio = 3.2; 95% CI = 1.09 – 9.34; $P = 0.033$). This indicator reinforces the argument that the risk of introduction and spreading of diseases and, consequently, occurrence of economic losses in sheep production is greater when the flock size is larger, however, variables related to herd size are not liable of correction, and in this case, an adequate sanitary planning assumes an important role as a control measure.

Regarding occurrences of abortions, although this variable was not associated with *T. gondii* prevalence in the multivariable analysis, the frequency of positivity was greater in flocks with no reports of abortions (53.7%) than in flocks with history of abortions (27.3%) (Table 1), with an odds ratio of 3.09 (95% CI = 1.01 – 9.48) (data not shown). This situation raises the awareness that is a need of analysis in which the matter of sub-notification of abortions can be taken into consideration, particularly with regard to extensive rearing systems. The predominance of extensive systems under semiarid conditions, in association with precarious management practices and absence of a zootechnical register, contribute towards ineffective monitoring of events relating to reproductive problems. This may make it difficult to evaluate the productive and economic performance.

Conclusion

We concluded that *T. gondii* infection is spread among sheep in the State of Paraíba, Northeastern Brazil, and our findings suggest that the implementation of adequate sanitary planning is necessary to avoid the spreading of the infection and, consequently, occurrence of economic losses in sheep production.

References

Andrade MMC, Carneiro M, Medeiros AD, Andrade Neto V, Vitor RWA. Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. *Parasite* 2013; 20(20). PMid:23707895.

Brandão VM. *Avaliação soroepidemiológica de anticorpos anti - Toxoplasma gondii em rebanhos caprinos e ovinos na ilha de São Luís - MA* [Dissertação]. São Luís: Universidade Estadual do Maranhão; 2009.

Camargo ME. Introdução às técnicas de imunofluorescência. *Rev Bras Patol Clín* 1974; 10(3): 143-171.

Chintana T, Sukthana Y, Bunyakai B, Lekkla A. *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. *Southeast Asian J Trop Med Public Health* 1998; 29(2): 383-386. PMid:9886133.

Dohoo IR, Ducrot C, Fourichon C, Donald A, Hurnik D. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Prev Vet Med* 1997; 29(3): 221-239. http://dx.doi.org/10.1016/S0167-5877(96)01074-4. PMid:9234406.

Dubey JP, Beattie CP. *Toxoplasmosis of animals and man*. Boca Raton: CRC Press; 1988.

Dubey JP. *Toxoplasmosis of animals and humans*. 2nd ed. Boca Raton: CRC Press; 2010.

Garcia JL, Navarro IT, Ogawa L, Oliveira RC. Soroepidemiologia da toxoplasmose em gatos e cães de propriedades rurais do município de Jaguapitã, Estado do Paraná, Brasil. *Cienc Rural* 1999; 29(1): 99-104. http://dx.doi.org/10.1590/S0103-84781999000100018.

Hosmer DW, Lemeshow S. *Applied logistic regression*. New York: John Wiley & Sons; 2000. http://dx.doi.org/10.1002/0471722146.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Sistema IBGE de Recuperação Automática – SIDRA* [online]. Brasília; 2010 [cited 2014 Sep 9]. Available from: http://www.sidra.ibge.gov.br/bda

Pereira MF, Peixoto RM, Langoni H, Greca H Jr, Azevedo SS, Porto WJN, et al. Fatores de risco associados à infecção por *Toxoplasma gondii* em ovinos e caprinos no estado de Pernambuco. *Pesqui Vet Bras* 2012; 32(2): 140-146. http://dx.doi.org/10.1590/S0100-736X2012000200009.

Sakata FBLS, Bellato V, Sartor AA, Moura AB, Souza AP, Farias JA. *Toxoplasma gondii* antibodies sheep in Lages, Santa Catarina, Brazil, and comparison using IFA and ELISA. *Rev Bras Parasitol Vet* 2012; 21(3): 196-200. http://dx.doi.org/10.1590/S1984-29612012000300004. PMid:23070426.

Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 2000; 30(12-13): 1217-1258. http://dx.doi.org/10.1016/S0020-7519(00)00124-7. PMid:11113252.

Thrusfield M. *Veterinary epidemiology*. 3rd ed. Oxford: Blackwell Science; 2007.

Zar JH. *Biostatistical analysis*. 4th ed. Upper Saddle River: Prentice Hall; 1999.