

Seroprevalence and risk factors of sheep toxoplasmosis in Minas Gerais, Brazil

A.C.A.V. CARNEIRO¹, M. CARNEIRO¹, A.M.G. GOUVEIA², L.S. VILAS-BOAS¹, R.W.A. VITOR^{1*}

¹Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, 31270-901, BRAZIL.

²Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, 31270-901, BRAZIL.

*Corresponding author: ricardovitor@icb.ufmg.br

SUMMARY

Serum samples from 711 sheep from 109 farms in Minas Gerais State, Brazil, were investigated for determining the *Toxoplasma gondii* seroprevalence using ELISA (enzyme-linked immunosorbent assay) and IFAT (indirect fluorescence antibody test). The *T. gondii* seroprevalence was 31.1% and 43.2% by ELISA and IFAT, respectively. Among the positive ELISA sheep sera, 19% of samples contained low avidity IgG, suggesting the occurrence of the recent phase of toxoplasmosis in Minas Gerais. Animals' age, sex, breed, geographic origin and others variables associated with *T. gondii* infection were analyzed as risk factors for toxoplasmosis but only the age was significantly associated with the parasite disease, the older animals (age over 36 months) being more frequently infected than young ones (OR = 1.45, 95% CI: 1.20 - 1.74).

Keywords: *Toxoplasma gondii*, sheep, seroprevalence, indirect immunofluorescence test, ELISA, IgG avidity, risk factors, age.

RÉSUMÉ

Séroprévalence et facteurs de risque de la toxoplasmose ovine dans l'état de Minas Gerais, Brésil

Les échantillons sériques de 711 ovins issus de 109 fermes de l'état de Minas Gerais, Brésil, ont été analysés afin de déterminer la séroprévalence de *Toxoplasma gondii* par ELISA (enzyme-linked immunosorbent assay) et immunofluorescence indirecte (IFI). Les séroprévalences obtenues ont été de 31.1 % par ELISA et de 43.2 % par IFI. Parmi les sérums positifs en ELISA, 19 % des prélèvements contenaient des IgG de faible avidité ce qui suggère une phase récente de toxoplasmose dans l'état de Minas Gerais. L'âge, le sexe, la race, l'origine géographique des animaux et d'autres variables liées aux modalités d'infection par *T. gondii* ont été analysés en tant que facteurs de risque de la toxoplasmose mais l'âge seulement est apparu significativement associé à cette maladie parasitaire, les animaux les plus âgés (de plus de 36 mois) étant plus souvent infectés que les plus jeunes (OR = 1.45, IC 95 % : 1.20 - 1.74).

Mots clés : *Toxoplasma gondii*, mouton, séroprévalence, immunofluorescence indirecte, ELISA, avidité des IgG, facteurs de risque, âge.

Introduction

Toxoplasmosis is one of the most common diseases in ewes and results in significant reproductive losses due to abortion and neonatal death [9]. Ovine toxoplasmosis is responsible for 1 to 2% of neonatal losses per year in the UK [1]. Worldwide seroprevalence of ovine toxoplasmosis during 1990-1999 ranged from 3% in Pakistan and Zimbabwe to 92% in France [15]. More recently, prevalence of 84.5% was observed in Serbia [8], 51.3% in Sardinia, Italy [10] and 27.1% in USA [5]. In Brazil, recent serological surveys show infection rates ranging between 38.2% and 76.0% [16].

The aim of this study was to carry out a serum – epidemiological study of ovine toxoplasmosis in the state of Minas Gerais, Brazil, to identify the prevalence of *Toxoplasma gondii* infection in different regions of the state, to identify risk factors associated to toxoplasmosis and to determine anti-*T. gondii* IgG avidity.

Materials and Methods

GEOGRAPHIC AREAS

The state of Minas Gerais comprises 12 regions and is

located in south-eastern Brazil covering a territorial area of 588 383.6 km². The weather of Minas Gerais is characterized predominantly as tropical, with annual mean temperatures below 20°C. Rainfall indices are ranged between 1 000 and 2 000 mm annually. In the present study, sheep are originated from 8 regions: *Central de Minas*, *Metropolitana de Belo Horizonte*, *Oeste de Minas*, *Sul/Sudoeste de Minas*, *Triângulo Mineiro/Alto do Paranaíba*, *Vale do Rio Doce*, *Zona da Mata*, *Campo das Vertentes*. Since a low number of sheep was evaluated in the *Central de Minas* (11 sheep) and *Oeste de Minas* (31 sheep) regions, they were regrouped with the *Metropolitana de Belo Horizonte* region, based on their common characteristics, whereas the sheep from *Campo das Vertentes* (18 sheep) were regrouped into the *Zona da Mata* region.

ANIMALS AND SERUM SAMPLING

The transversal study was carried out in 2002 and the animals (n = 711) were selected from 109 farms. To select the sheep-producing farms, the non-probabilistic sampling method was used. The most representative and largest sheep-producing municipal properties were selected as sampling units, according to the results obtained at the Brazilian government organs: João Pinheiro Foundation and IBGE (Instituto Brasileiro de Geografia e Estatística). Blood (5 mL)

was collected from puncture of the jugular vein into sterile tubes without any anti-coagulant and after clotting at room temperature for 3 hours and centrifugation (1 000 g, 15 minutes, room temperature), serum samples were carefully harvested and stored at -20°C until analysis.

The following information on the farms and herds was collected: water supply, type of water facility, facilities (buildings, pen flooring), land use (extensive/intensive), presence of cats, access of these animals to water supplies and feeding places for ovine, and individual animal data (age, gender, breed).

A total of 711 sheep from 109 farms were investigated in the present study. Females predominated [520 / 708 females (73.4%) and 188 / 708 males (26.6%)]. Three hundred fifty-nine sheep (54.3%) were pure breed (Suffolk, Texel, Merino, and the naturalized Brazilian sheep breeds: Santa Ines, Rabo Largo, Bergamasca, Crioula, Morada Nova), 22.7% (150 / 661) crossbreed (resulting from the crossing of different breeds) and 23.0% (152 / 661) of undetermined breed. Animals were classified into 4 age classes: 6-12 months (215 / 711, i.e. 30.2%), 13-24 months (225 / 711, i.e. 31.6%), 25-36 months (158 / 711, i.e. 22.2%) and > 36 months (113 / 711, i.e. 15.9%).

DETECTION OF ANTI-TOXOPLASMA GONDII ANTIBODIES

Two homemade techniques were used to test all serum samples to detect IgG antibodies against *T. gondii* using RH strain tachyzoites as antigens. This strain was isolated by Sabin (1941) from a case of human acute encephalitis [12]. The indirect immunofluorescence antibody test (IFAT) for *T. gondii* was performed according to the method described previously [17] considering 1:64 as the cut-off dilution. The enzyme-linked immunosorbent assay (ELISA) was carried out as described [18] with some modifications. All serum samples were tested in duplicate at a dilution of 1:400. Peroxidase-labelled donkey IgG anti-sheep IgG (Sigma, A-3415) was used at 1:7500 dilution. The cut-off value for each ELISA plate was calculated as the mean absorbance of serum samples obtained from eight sheep negative for *T. gondii*, plus 3 standard deviations. IgG avidity ELISA was carried out as recommended [4] only for previously positive ELISA sera. IgG avidity (expressed in %) was measured by average absorbance for each urea-treated serum (AU) in relation to

average absorbance of each urea not treated serum (A): AU/A X 100. Avidity values > 50% indicate chronic toxoplasmosis, whereas values < 50% indicate acute toxoplasmosis.

STATISTICAL ANALYSIS

A database was generated using Epidata version 2.1 software, and statistical analyses were performed using the Stata Statistical software version 9.0 and EPI-INFO 3.2 version. Comparative analysis of IFAT and ELISA was estimated using the reliability index (Kappa). Low and high avidity IgG antibodies were correlated with gender, age, breed and regions using the qui-square test.

In order to evaluate the risk factors of the infection, the response variable was defined according to ELISA tests and the factors associated with this variable were analyzed using a random-effects logistic regression model. This model assumed that observations obtained at the same farm were mutually dependent and that observations between sheep were independent. Univariate analysis was performed, and Odds Ratios (OR) and 95% confidence intervals were used to quantify the association between risk factors and *T. gondii* infection. Variables that presented $P < 0.25$ in univariate analysis were applied in the multivariate regression model. Variables that presented co-linearity or low frequency were excluded from the multivariate model, while variables with more than two categories were transformed into indicator (dummy) variables. The models were constructed using forward selection of the variables, and the likelihood-ratio test was used to define the final model.

Results

Among the 711 sheep sera, 221 (31.1%; 95% CI 27.67 - 34.49) were positive with ELISA and 307 (43.2%; 95% CI 39.52 - 46.82) with IFAT. The agreement between IFAT and ELISA tests estimated by kappa was good (kappa index = 0.68).

Seroprevalences in the different studied Minas Gerais regions ranged from 23.3% and 37.7% in the Vale do Rio Doce region to 44.5% and 50.4% in the Sul /Sudoeste de Minas region by ELISA and IFAT tests respectively (Table I).

Regions	Number of sheep	Positive samples	
		IFAT	ELISA
Metropolitana de Belo Horizonte /			
Central de Minas / Oeste de Minas	137	58 (42.3%)	44 (32.1%)
Sul /Sudoeste de Minas**	137	69 (50.4%)	61 (44.5%)
Triângulo Mineiro / Alto do Paranaíba	144	60 (41.7%)	37 (25.7%)
Vale do Rio Doce**	130	49 (37.7%)	30 (23.1%)
Zona da Mata / Campo das Vertentes	163	71 (43.6%)	49 (30.1%)
Total	711	307 (43.2%)	221 (31.1%)

IFAT: indirect immunofluorescence antibody test; ELISA: Enzyme-linked immunosorbent assay. ** $P < 0.01$ between seroprevalences determined with ELISA of the Sul /Sudoeste de Minas and the Vale do Rio Doce regions.

TABLE I: Frequencies of the serum anti-*Toxoplasma gondii* antibodies in sheep as determined with IFAT and ELISA tests according to the 8 different regions of the Minas Gerais, Brazil.

The serum anti-*T.gondii* antibody frequencies significantly varied between these 2 regions ($P < 0.01$).

As shown in Table II, the proportions of positive IFAT and ELISA serum samples were similar in males and females and were closely related between sheep from pure breeds, crossbreed sheep or sheep from undermined breed. Consequently, the sex and the breed did not significantly influence the toxoplasmosis seroprevalence. By contrast, the age was significantly

associated with the *T. gondii* seropositivity measured with the ELISA test ($P < 0.01$) or with the IFAT test ($P < 0.001$): indeed, the older sheep (> 36 months old) were more frequently seropositive than the others (59.3% and 44.2% for IFAT and ELISA respectively). In addition, among the 221 positive ELISA sheep, 179 (81.0%) exhibited high-avidity antibodies and 42 (19.0%) low-avidity antibodies. No differences in IgG avidity were observed according to the geographical region, breed, age or gender (Table III).

Individual variables	Number of sheep	Positive samples	
		IFAT	ELISA
Sex (N = 708)			
Males	188	79 (42.0%)	57 (30.3%)
Females	520	228 (43.8%)	164 (31.5%)
Breed (N = 661)			
Pure	359	160 (44.6%)	113 (31.5%)
Crossbreed	150	66 (44.0%)	52 (34.7%)
Undetermined	152	58 (38.2%)	37 (24.3%)
Age classes (N = 711)			
6 - 12 months	215	75 (34.9%)	58 (27.0%)
13 - 24 months	225	90 (40.0%)	63 (28.0%)
25 - 36 months	158	75 (47.5%)	50 (31.6%)
> 36 months	113	67 (59.3%)*	50 (44.2%)*

IFAT: indirect immunofluorescence antibody test; ELISA: Enzyme-linked immunosorbent assay; N: Number of sheep evaluated for each variable.* $P < 0.01$ and ** $P < 0.001$.

TABLE II : Frequencies of the serum anti-*Toxoplasma gondii* antibodies in sheep as determined with IFAT and ELISA tests according to the individual variables (sex, breed, and age) in the Minas Gerais, Brazil.

	IgG avidity	
	Low	High
ELISA positive samples (N = 221)	42 (19%)	179 (81%)
Regions		
Metropolitana de Belo Horizonte / Central de Minas / Oeste de Minas (N = 44)	8 (18.2%)	36 (81.8%)
Sul /Sudoeste de Minas (N = 61)	10 (16.4%)	51 (83.6%)
Triângulo Mineiro / Alto do Paranaíba (N = 37)	4 (10.8%)	33 (89.2%)
Vale do Rio Doce (N = 30)	12 (40.0%)	18 (60.0%)
Zona da Mata / Campo das Vertentes (N = 49)	8 (16.3%)	41 (83.7%)
Sex		
Males (N = 57)	10 (17.5%)	47 (82.5%)
Females (N = 164)	32 (19.5%)	132 (80.5%)
Breed		
Pure (N = 113)	22 (19.5%)	91 (80.5%)
Crossbreed (N = 52)	9 (17.3%)	43 (82.7%)
Undetermined (N = 37)	7 (18.9%)	30 (81.1%)
Age		
6 - 12 months (N = 58)	12 (20.7%)	46 (79.3%)
12 - 24 months (N = 63)	10 (15.9%)	53 (84.1%)
25 - 36 months (N = 50)	9 (18.0%)	41 (82.0%)
> 36 months (N = 50)	11 (22.0%)	39 (78.0%)

ELISA: Enzyme-linked immunosorbent assay; N: Number of ELISA positive sheep.

TABLE III : Anti-*Toxoplasma gondii* IgG avidity in ELISA positive sheep serum samples according to the geographical region, breed, age or sex.

Results obtained through ELISA to define infection by *T. gondii* were applied to determine risk factors. Variables associated with *T. gondii* infection determined by univariate analysis were: age range, breed, presence of cats, presence of building for food storage, type of water offered to the sheep, type of drinking trough and access of cats to feed and water offered to the sheep. The variable remaining in the final model was age. Older sheep (age over 36 months) were more frequently positive than young ones (OR=1.45; CI 95% 1.20 - 1.74).

Discussion

To our knowledge, this is the first report of seroprevalence of toxoplasmosis in sheep flocks in Minas Gerais State, Brazil. The percentage of seropositivity for *T. gondii* by ELISA (31.1%) or IFAT (43.2%) was higher than the 18.8% detected in ovine from Bahia State, Brazil [11], and lower than the 51.8% obtained by GARCIA *et al.* (1999) in Paraná State, Brazil [7]. Similar results (34.7% and 38.2%) were found in ovine from São Paulo State [6] and in Federal District [16] respectively. This high prevalence may be related with the occurrence of high rates of abortion, indicated as a frequent clinical sign by 23.9% of the farmers interviewed in Minas Gerais State, Brazil [14].

In the Minas Gerais State, the prevalence of antibodies to *T. gondii* ranged from 23.3% to 44.5% by ELISA, and from 37.7% to 50.4% by IFAT, the *Sul/Sudoeste de Minas* region being significantly more affected by *T. gondii* than the *Vale do Rio Doce* region. The difference observed in the prevalence of sheep toxoplasmosis between these 2 regions could be attributed to increasing opportunities of exposure to several sources of *Toxoplasma* infection and mild climatic conditions, more adequate to the longer viability of *T. gondii* oocysts in the high relative humidity pasture of the *Sul/Sudoeste de Minas*. In addition, sample size, age, serological techniques, management system and health practices may account for some of the differences in the reported prevalence of sheep toxoplasmosis in Brazil.

Previous studies have demonstrated that the *T. gondii* IgG avidity ELISA can be used to discriminate between acute and chronic toxoplasmosis in sheep [3, 4, 13]. In this work, 19.0% of the naturally infected sheep presented IgG antibodies with a low avidity which are predominant during the recent phase of toxoplasmosis and were submitted to high risk of abortion or perinatal mortality, if ewe were pregnant. No significant association was found between the anti-*T. gondii* antibody IgG avidity and the geographical region or the individual variables. These results suggest that sheep are exposed to the same risk factors for toxoplasmosis in the state of Minas Gerais, regardless of origin of the flock, breed, age or sex.

ELISA results were used to determine some risk factors because of its high reproducibility, specificity and sensitivity [3, 17]. The alone risk factor identified for sheep toxoplasmosis in the state of Minas Gerais was the age. Animals older than 36 months had a 1.45 (95% CI; 1.20 - 1.74) higher risk of infection than the others which is comparable to the

data obtained by other researchers [2]. Increased risk of toxoplasmosis in sheep older than 36 months is likely due to increasing opportunities of exposure to several sources of *Toxoplasma* infection.

The results of this study confirm that *T. gondii* infection is common in Brazilian sheep from the state of Minas Gerais. The importance of toxoplasmosis to sheep production in this region has yet to be determined, but the high seroprevalence detected in this study suggests that it may have a significant impact on public health: the consumption of their meat certainly presents a risk of transmission of the parasite. The utilization of methods for determining the prevalence of *T. gondii* infection in sheep will allow the adequate management for the control of infection on the sheep herds. Further studies will be needed to determine the impact of toxoplasmosis as a cause of abortions in sheep in Minas Gerais.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento e Pesquisa (CNPq) and Instituto Mineiro de Agropecuária (IMA). We thank Rosalida Estevan Nazar Lopes for technical assistance. RWAV is Research Fellow from the CNPq.

References

1. - BUXTON D., MALEY S.W., WRIGHT S.E., RODGER S., BARTLEY P., INNES E.A.: *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. *Vet. Parasitol.*, 2007, **149**, 25-28.
2. - CABALLERO-ORTEGA H., PALMA J.M., GARCÍA-MÁRQUEZ L.J., GILDO-CÁRDENAS A., CORREA D.: Frequency and risk factors for toxoplasmosis in ovine of various regions of the State of Colima, Mexico. *Parasitology*, 2008a, **135**, 1385-1389.
3. - CABALLERO-ORTEGA H., QUIROZ-ROMERO H., OLAZARÁN-JENKINS S., CORREA D.: Frequency of *Toxoplasma gondii* infection in sheep from a tropical zone of Mexico and temporal analysis of the humoral response changes. *Parasitology*, 2008b, **135**, 897-902.
4. - CLEMENTINO M.M., SOUZA M.F., ANDRADE NETO V.F.: Seroprevalence and *Toxoplasma gondii* - IgG avidity in sheep from Lajes, Brazil. *Vet. Parasitol.*, 2007, **146**, 199-203.
5. - DUBEY J.P., SUNDAR N., HILL D., VELMURUGAN G.V., BANDINI L.A., KWOK O.C., MAJUMDAR D., SU C.: High prevalence and abundant atypical genotypes of *Toxoplasma gondii* isolated from lambs destined for human consumption in the USA. *Int. J. Parasitol.*, 2008, **38**, 999-1006.
6. - FIGLIUOLO L.P.C., KASAI N., RAGOZO A.M.A., De PAULA V.S.O., DIAS R.A., SOUZA S.L.P., GENNARI S.M.: Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in ovine from São Paulo State, Brazil. *Vet. Parasitol.*, 2004, **123**, 29-32.
7. - GARCIA J.L., NAVARRO I.T., OGAWA L., OLIVEIRA R.C.: Soroprevalencia do *Toxoplasma gondii* em suínos, bovinos, ovinos e equinos e sua correlação com humanos, felinos e caninos oriundos de propriedades rurais do norte do Paraná-Brasil. *Cienc. Rural*, 1999, **29**, 91-97.
8. - KLUN I., DJURKOVIC-DJAKOVIC O., KATIC-RADIVOJEVIC S., NIKOLIC A.: Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Vet. Parasitol.*, 2006, **135**, 121-131.
9. - MORLEY E.K., WILLIAMS R.H., HUGHES J.M., THOMASSON D., TERRY R.S., DUNCANSON P., SMITH J.E., HIDE G.: Evidence that primary infection of Charollais sheep with *Toxoplasma gondii* may not prevent foetal infection and abortion in subsequent lambings. *Parasitology*, 2007, **135**, 169-173.

10. - NATALE A., PORQUEDDU M., CAPELLI G., MOCCI G., MARRAS A., SANNA COCCONE G.N., GARIPPA G., SCALA A.: Sero-epidemiological update on sheep toxoplasmosis in Sardinia, Italy. *Parassitologia*, 2007, **49**, 235-238.
11. - PITA-GONDIM L.F., BARBOSA Jr. H.V., RIBEIRO FILHO C.H.A., SAEKI H.: Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle and water buffaloes in Bahia State, Brazil. *Vet. Parasitol.*, 1999, **82**, 273-276.
12. - SABIN A.: Toxoplasmic encephalitis in children. *J. Am. Med. Assoc.*, 1941, **116**, 801-807.
13. - SAGER H., GLOOR M., TENTER A., MALEY M., HASSIG M.M., GOTTSTEIN B.: Immunodiagnosis of primary *Toxoplasma gondii* infection in sheep by the use of a P30 IgG avidity ELISA. *Parasitol. Res.*, 2003, **91**, 171-174.
14. - SEYFFERT N., GUIMARÃES A.S., PACHECO L.G., PORTELA R.W., BASTOS B.L., DORELLA F.A., HEINEMANN M.B., LAGE A.P., GOUVEIA A.M., MEYER R., MIYOSHI A., AZEVEDO V.: High seroprevalence of caseous lymphadenitis in Brazilian goat herds revealed by *Corynebacterium pseudotuberculosis* secreted proteins-based ELISA. *Res. Vet. Sci.*, 2009, in press, doi:10.1016/j.rvsc.2009.07.002.
15. - TENTER A.M., HECKEROTH A.R., WEISS L.M.: *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol.*, 2000, **30**, 1217-1258.
16. - UENO T.E.H., GONÇALVES V.S.P., HEINEMANN M.B., DILLI T.L.B., AKIMOTO B.M., De SOUZA S.L., GENNARI S.M., SOARES R.M.: Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federal District, central region of Brazil. *Trop. Anim. Health Prod.*, 2009, **41**, 547-552.
17. - Van Der PUIJE W.N.A., BOSOMPEM K.M., CANACOO E.A., WASTLING J.M., AKANMORI B.D.: The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Trop.*, 2000, **76**, 21-26.
18. - VITOR R.W.A., FERREIRA A.M., FUX B.: Antibody response in goats experimentally infected with *Toxoplasma gondii*. *Vet. Parasitol.*, 1999, **81**, 259-263.