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Sheep toxoplasmosis diagnose by ELISA and IFAT

Diagnose de Toxoplasmose em carneiros por ELISA e RIFI

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ABSTRACT
Toxoplasmosis in sheep herds is a major cause of abortion, causes great economic impacts and sheep are a potential transmitters of the disease, either by meat consumption or contaminated dairy products. The diagnosis of toxoplasmosis is done by serological and molecular techniques. The reaction of indirect immunofluorescence assay (IFA) and enzyme immunoassay (ELISA) are most commonly used diagnostic methods. The seroprevalence of toxoplasmosis in sheep herd IZ was 16.86% (ELISA) and 21.51% (IFAT), the results between the two tests showed sensitivity of 77.70%, specificity of 99.25%, positive predictive value of 96.55%, negative predictive value of 94.36% and Kappa 0.83 (CI 1.0 to 0.796). Kappa index showed almost perfect agreement, reinforcing the choice of ELISA as a screening method. There was no significant statistical difference (P>0.05) of 95% in the Fisher test between races and ages of the sheep.

Keywords: ELISA, IFA, *Toxoplasma gondii*, sheep

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RESUMO
A toxoplasmose em rebanhos ovinos representa a maior causa de abortos, causando grandes prejuízos econômicos além do fato de ovinos serem transmissores da doença, seja pelo consumo de carne ou leite contaminados. O diagnóstico da toxoplasmose e realizada por sorologia e técnicas moleculares. A Reação de imunofluorescência indireta (RIFI) e o teste imunoenzimatico ELISA são os mais comuns dos métodos utilizados no diagnóstico. A soroprevalencia de toxoplasmoses no rebanho avaliado no Instituto de Zootecnia foi 16,86% por ELISA e 21,51 % por RIFI. Os resultados de ambos os testes mostraram sensibilidade de77,7%; especificidades de 99,25%, valores positivos presumidos de 96,55; valores presumidos negativos de 94,36% e índice Kappa 0,83 (coeficiente entre 1,0 e 0,796). O índice Kappa mostrou concordância perfeita, reforçando o uso de ELISA como método de varredura inicial. Não houve diferença estatística entre as raças e idades dos animais (P < 0,05) avaliados a 95% no teste exato de Fisher.

Palavras-chave: ELISA, Imunofluorescência, *Toxoplasma gondii*, ovinocultura.

1 INTRODUCTION

Ovine toxoplasmosis is caused by the parasite *Toxoplasma gondii* and its transmission to animals occurs as congenital form, by the flow of traquizoits by the placentary membrane or it is acquired by the consumption of sporulated oocites in the pastures, water or food as well as it can be transmitted by sex.

Sheep herd are considered potential transmitters of toxoplasmosis, where the cists of the parasite are found into tissues for long periods of time. The ingestion of milk, raw meat can be the major causes of human infections of this disease from ovine herds.

Several risk factors can be associated to ovine toxoplasmosis in literature. Moraes et al. (2011) suggest the sanitary conditions during management, climate, air humidity, oocyte viability in the environment can influence the spread of it.

The diagnosis of the presence of the anti - *T. gondii* antibody is necessary so that there is no economic loss for sheep farmers, since the disease causes abortion, birth of stillborn animals, early retinue of estrus and public health problems.

Different immunoassays are used for the detection of anti-*T. gondii* antibodies, such as modified agglutination (MAT), latex agglutination (LA), indirect hemagglutination, Imunoblot, Sadin-Feldman (DT) dye test, Immunofluorescent Antibody Test (IFAT), considered as gold-standard technique with high specificity and the ELISA, with high sensitivity and possible epidemiological analysis (GARCIA et al., 2006; GARCIA et al., 2008) In addition, the results obtained in this study are similar to those reported in the present study (Gutierrez et al., 2008). The ELISA technique or enzyme linked immunoenzymatic assay is an enzyme-labeled antigen-antibody reaction, usually phosphatase or peroxidase, which allows visual color matching to more complex methods such as spectrophotometry, amperometry and differential pulse voltammetry (TIBOLA et

al., 2013). This technique has high sensitivity and specificity, is a fast and easy to perform method (Meirelles et al., 2006). In addition, it can be used in the field, is capable of detecting trace concentrations of a compound (ng or pg) and has little or no need for cleaning and concentration of the sample to be analyzed The greatest advantage of the test is the amount of samples that can be analyzed in a reduced time and with relatively low cost (MEIRELLES et al., 2006). Immunofluorescent Antibody Test (IFAT) is based on the antigen-antibody reaction and has a fluorescent label. Its major advantage, in addition to high sensitivity, is the possibility of visualizing the antigen in a tissue or cell, which occurs with the use of the fluorescence microscope. The principle of the test is based on the emission of a light source with high intensity, which through excitation filters produce a wavelength capable of activating the fluorochromes. Fluorochromes are dyes that absorb and excite radiation by emitting visible light.

North of USA and Canada consume cervides, from hunting practices (deer, mooses, elks, etc) and this way of life has leading to meat contamination with toxoplasmosis and subsequently, human contamination (GAULLIN et al., 2020) The diagnosis of the presence of the anti - *T. gondii* antibody is necessary so that there is no economic loss for sheep farmers, since the disease causes abortion, birth of stillborn animals, early retinue of estrus and public health problems. Different immunoassays are used for the detection of anti-*T. gondii* antibodies, such as modified agglutination (MAT), latex agglutination (LA), indirect hemagglutination, Imunoblot, Sadin-Feldman (DT) dye test, Immunofluorescent Antibody Test (IFAT), considered as gold-standard technique with high specificity and the ELISA, with high sensitivity and possible epidemiological analysis (GARCIA et al, 2006 e 2008). In addition, the results obtained in this study are similar to those reported in the present study *(*GUIMARAES et al., 2013) The ELISA technique or enzyme linked immunoenzymatic assay is an enzyme-labeled antigen-antibody reaction, usually phosphatase or peroxidase, which allows visual color matching to more complex methods such as spectrophotometry, amperometry and differential pulse voltammetry (SOUSA et al., 2009). This technique has high sensitivity and specificity, is a fast and easy to perform method (LUPTAKOVA et al., 2015).

The objective of this work was to perform a screening of 172 sheep plasma samples collected from the Santa Inês and Morada Nova breeds of the IZ (Institute of Animal Science) through a commercial ELISA kit with optical density reading (OD) to 450nm in comparison to results obtained with the IFAT technique, in order to determine the health status of the herd in relation to *T. gondii* infestation.

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2 MATERIAL AND METHODS

This study was approved by the Instituto de Zootecnia's Ethics Committee (Protocol CEUA/IZ No.200/2014). The experiment was conducted at the Center for Research and Development of Diversified Animal Husbandry (Sheep Unit) at the Institute of Zootechny (IZ), APTA/SAA, Nova Odessa, SP, Brazil. The analyzes were conducted in the Laboratory of Antibody Production and Immunoassays of this same Institute. According to data from Nova Odessa municipality, the climate of the city is tropical and semi-humid, presenting dry winter and southeast wind, with temperatures oscillating between minimum 10ºC and maximum 35ºC, average 26ºC and humidity 76%. The rainfall is 1,317.1mm / year and the soils are: dark red latosol, clayey, clayey-sand and gently undulating relief, with weak slopes and long slopes. The average altitude is 540m from sea level, latitude 47º19'51"west and longitude 22º47'20"th. The samples were taken on Winter season at the Sheep Unit. A 24-gauge needle was used to collect blood by puncturing the internal jugular vein, blood samples were harvested in tubes containing EDTA (Ethylenediamine tetra acetic acid) from 172 ewes from Morada Nova and Santa Inês breeds. The number of samples collected for each of the breeds was representative according to the size of the herd, such as 65 samples from Morada Nova and 107 samples from Santa Inês breed type. The ewes were from 2 to 8 years old and were raised in a semi-extensive system, with supplementation, with access to water (from a water box) and mineral mixing. The samples were centrifuged at 5000rpm (rotation per minute) for 15 minutes to obtain the plasma which was aliquot into 1.5mL eppendorf- type tubes and frozen in -80 ° C freezer until the time of analysis (MACHADO, 2016)

For the detection of anti-*Toxoplasma gondii* antibody, commercial ELISA kit (IDEXX Toxotest, reference TXT113ST, lot SN A281 - Switzerland) was used. IDEXX Toxotest consists of the detection of anti-*T. gondii* antibodies which, when present in the sample, bind to the specific antigen pre-impregnated in the microtiter plates, forming the antigen-antibody complex, which is flagged by the action of the labeled peroxidase enzyme to the anti-IgG conjugate (Class G immunoglobulin). The appearance of color occurs by the binding of the TMB Substrate (Tetramethylbenzidine) to the enzyme, which is gradual and directly proportional to the number of antibodies present in the sample. Samples were thawed at room temperature and diluted 1: 400 with wash solution in individual Eppendorf -type tubes. Test was prepared and done according to fabricants slide. Absorbance reading of the samples was performed on a Bio-Tek Instruments Microplate Reader with 450 nm wavelength using the X-Chek software. The interpretation of the results was done according to the test protocol, where the average of the absorbance of the samples and controls obtained by the spectrophotometer was calculated. A O.D. of the samples were corrected,

calculating the value of the sample subtracted by the negative control, divided by the positive control subtracted from the negative control, according to the formula: O.D. corrected= [(sample O.D.- negative control O.D.)/ (positive control O.D-negative control O.D.).

When the test result presented interpretation with correction of O.D. higher than or equal to 20% and less than 30%, ie suspect, the manufacturer recommended that a further analysis be carried out with the same sample. The low positive results are not repeated, because they present the presence of the anti - *T. gondii* antibody, according to manufacturer, even in a lower concentration.

For confirmation of positive, low positive and suspect results for anti-*T. gondii* antibodies obtained in the ELISA technique, it was used the commercial Kit of IFAT (Immunoteste - Toxoplasma - Imunodot Diagnósticos, Brazil). The test consisted in the detection of the anti-*T. gondii* antibody, which when present in the sample binds to the antigens fixed on the microscopy slide. A conjugate was subsequently used to evidence the reaction.

The reading and preparation of the slides were carried out in the NAP - Nucleus of Researchs Support of Electronic Microscopy, ESALQ / USP. Samples were thawed at room temperature and diluted 1:40 with 1X concentrated PBS. Then, 10 µL of each sample and the negative and positive controls were added to the wells of the slides.

The slides were incubated in the wet chamber for 30 minutes and washed 5 minutes, three times using washing solution. After, the Conjugate was diluted with Evans Blue solution. Incubation and previous washing were repeated, with buffered Glycerin, slides were mounted with coverslots and readings were performed on a ZEISS Axioskop 2 fluorescence microscope, with AXIO CAM MRC-ZEISS camera and AXIO VISION VS 40 RELEASE 4.0 program. Light incidence of excitation wavelength at 495 nm and emission at 519 nm, showed green fluorescence.

Both sensitivity and specificity are parameters determined by the manufacturer, but can be confirmed and compared after the tests have been performed. Both should be close to the values stipulated by the test . To calculate the sensitivity of the ELISA technique, the following formula was used, where the total of positive and low positive samples were divided by the total of positive samples plus the low positive samples and suspected, and the result multiplied by 100.

[positive samples/(positive samples+low positive+suspect)]X100

To calculate the specificity of the ELISA test was used to the formula below, where the total of negative samples were divided by the total of samples analyzed and the result was multiplied by 100.

[negative samples/total samples analyzed)X100]

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Statistical Analysis

To determine the sensitivity, specificity, negative and positive predictive values, the results obtained by ELISA and IFR were classified according to fabricants procedure and application of the formulas below, according to Karen et al., (2011):

• Sensitivity: A/(A+C); • Specificity: D/(D+B); • Positive predictive value: A/(A+B) and • Negative predictive value: D/(D+C)

The results obtained by the ELISA and IFAT were submitted to concordance analysis by the Kappa test and the statistical relationship between the ages and races of the animals was established by the Fisher exact test. Both through the GraphPad Prism 5.0 program (GraphPad Software, San Diego, California, USA) (Rizzo et al., 2014). The Kappa index was interpreted according to Landis & Koch (1977).

3 RESULTS AND DISCUSSION

The ideal diagnosis should have specificity close to 100%, for many years the Sabin-Feldam (DT) dye test was used as a gold standard technique for the diagnosis of ovine toxoplasmosis, however the use of live parasites for its execution generates risk to health. Currently IFAT is established by the literature as gold standard test (RIZZO et al., 2014).

The IFAT requires the use of an immunofluorescence microscope for diagnosis, making the technique not feasible in some situations. In the present study, the use of ELISA as a method for screening the samples by different authors (GAMBLE et al., 2005; ZHU et al., 2012) was proposed as an alternative method, as it presented high sensitivity and specificity. reduced execution time (ROSSI et al., 2011). In the present work the commercial ELISA (Toxotest Ab Test - Idexx - Switzerland) presented negative, positive, low positive and suspicious results, obtained by reading the OD of each sample and interpreting according to the manufacturer 's protocol. The low positive results were maintained as positive because, despite the low OD, they showed antibodies against T. *gondii*. The suspicious result was reevaluated and maintained as suspect, according to table 1.

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Table 1 - Seroprevalence of Anti-*T. gondii* antibodies in sheep of the Institute of Zootechny, using ELISA techniques (IDEXX - Switzerland) and IFAT (Imunodot - Brazil)

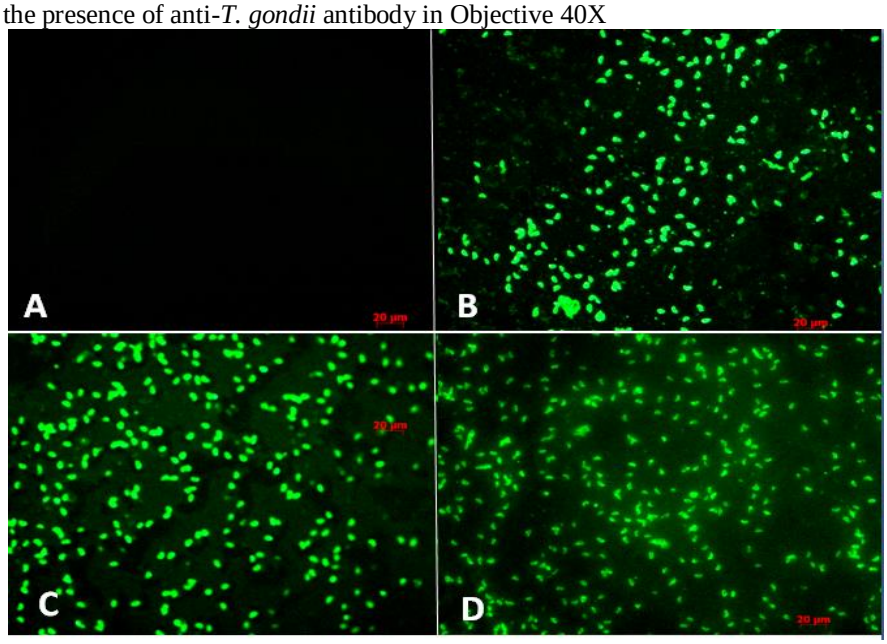
	ELISA		IFAT	
	n	%	n	%
Positive	29	16.86%	37	21.51%
Negative	142	82.56%	135	78.49%
Suspect	1	0.58%	-	
	172	100%	172	100%

- = absent

Thus, the prevalence of ovine toxoplasmosis in the herd of the Institute of Zootechny through the ELISA diagnosis was 16.86%. The seroprevalence found is below the results obtained in the literature using other commercial ELISA kits, ranging from 22.10% in Rio Grande do Norte (ANDRADE et al., 2013) and 75% in Minas Gerais (ROSSI et al., 2011).

The commercial IFAT kit (Imunoteste - Toxoplasma - Imunodot - Brazil) was used for comparison, knowing that this is the gold standard method. The results were obtained through the visual reading of the green light emission, through the use of immunofluorescence microscope, according to figure1.

Figure 1 - Indirect immunofluorescence of ovine toxoplasmosis (IFAT), where A - negative control sample, with absence of antibodies to *T. gondii*; B: positive control sample with the presence of antibody and; C and D samples from sheep sera analyzed showing the presence of anti-*T. gondii* antibody in Objective 40X



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The seroprevalence was obtained through IFAT for the IZ sheep herd of 21.51%, according to table 4. Results varied for the literature for the different forms of the Brazilian states, through IFAT, varying from 7% in Paraná (MOURA et al., 2007) and 80% in Santa Catarina (SAKATA et al., 2012). This comparison of results obtained by IFAT may depend on different factors such as the number of animals used in each analysis; management; age; sex; breed of each animal; presence or absence of cats close to the herd; source of water and sanitary conditions.

The literature suggests the age of animal can influence on the incidence of toxoplasmosis and it can be explained due to the wide time of exposition of the animal with the parasite (MODOLO et al., 2008; LUCIANO et al., 2011; GUIMARÃES et al., 2013). In this work, despite the prevalence of anti- *T. gondii* were mathematically higher for animals from 4-5 and > 6 years old, there were no statistic difference (P <0.001) by Fisher's exact test (CORREIA et al, 2015).

These data are consistent with Mendonça et al., (2013), Pereira et al., (2012); Sakata et al. (2012), who found no relationship between age and seroprevalence to increase toxoplasmosis. Sakata et al. (2012) suggest changes in the incidence of toxoplasmosis depending on the breed of the animal and explain that this fact may be related to the management of the animals. However, in this work no significant difference was found for the breeds analyzed Table 8 when compared through the result of IFAT and ELISA, table 2.

Table 2 - Presence of toxoplasmosis in Morada Nova and Santa Inês sheep through IFAT

	Animals Tested		Presence of toxoplasmosis by IFAT(gold standard)		By ELISA
	n	%	n	%	%
Morada Nova	65	37.42 [a]	12	6.97 [a]	4.06[b]
Santa Inês	107	62.58 [a]	25	14.53 [a]	12.8[b]
Total	172	100.00	37	21.5	16.86

*similar letters in the same column means no statistical difference for different breed analyzed, P < 0,05.

The kappa index found in this study was 0.83 (CI = 1.0 - 0.796), indicating almost perfect agreement between ELISA and IFAT. This value indicates that both tests are effective for the detection of *T. gondii* antibodies in sheep. Rossi et al., (2011), compared the same tests in Minas Gerais, and found substantial agreement (k = 0.69). In the present study, the results obtained in the present study were similar to those reported in the literature for the diagnosis of ovine toxoplasmosis.

The sensitivity of the ELISA and IFAT kits of the present study was 77.70%, the specificity was 99.25%. These values suggest the ability of the test to diagnose anti-*T. gondii* antibodies, these being close to 100%, desirable for a 100% effective test. The positive predictive value was 96.55%

and the negative predictive value was 94.36%, but the values were close to 100%, minimizing the chance of false positive or false negative results. Cost and time dispending for the comercial kits and IFAT, used in this study are listed in table 3.

Table 3 – Cost and time dispending to get the disgnostic of sheep toxoplasmosis by EIA and IFTA.

Technique	Cost of Kit	Price per Sample	Sera amount	Execution time	Time per sample
ELISA (IDEXX)	U$526.00	U$2.85	1 µL	2h 20min	1m45s/96 samples
IFAT (Imunodot)	U$143.00	U$1.43	1 µL	2h30min	1m56s/10 samples

Comparing the execution time of the techniques the ELISA is more vantage, since 96 samples are analyzed in 2h20min, whereas by the IFAT it is possible to analyze 10 samples in 2h30min. These data suggest the use of ELISA as a method for screening samples and epidemiological results.

4 CONCLUSION

The prevalence of anti-IgG antibodies to *T. gondii* in the sheep herd at Institute of de Zootechny and Animal Husbandry was 16.86% for ELISA and 21.51% for IFAT.

The agreement, by Fisher test was almost perfect 0.8; the sensitivity of 77.70%; specificity of 99.25%; positive predictive value 96.55% and negative predictive value 94.36%.

The Idexx ELISA kit can be used as screening or for epidemiological evaluations and the IFAT as confirmation of results. No statistical differences were found for the races and ages of the evaluated animals.

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REFERENCES

ANDRADE, M.M.C.; CARNEIRO, M.; MEDEIROS, A.D.; ANDRADE NETO, V.; VITOR, R.W.A. Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. Parasite, v.20, p.1 –5, 2013.

CORREIA, E.L.B.; FEITOSA, T.F.; SANTOS, F.A.S.; AZEVEDO, S.S.; PENA, H.F.J.; GENNARI, S.M.; MOTA, R.A.; ALVES, C.J. Prevalence And Risk Factors For *Toxoplasma Gondii* In Sheep In The State Of Paraíba, Northeastern Brazil. Revista Brasileira De Parasitologia, v.24; n.3, p. 383 – 386, 2015.

DUARTE, K.M.R.; PASCHOAL, J.A.R.; GOMES, L., H. Imunoensaios e zootecnia: aplicações práticas. Boletim da Indústria Animal. v.63, n.2, p.121-130, 2006.

GAMBLE, H.R.; DUBEY, J.P.; LAMBILOTTE, D.N. Comparision of a commercial ELISA with the modified agglutination test for detections of Toxoplasma infection in the domestic pigs. Veterinary Parasitology, v. 31 , n. 128, p. 177 – 181, 2005.

GARCIA, J.L.; NAVARRO, I.T.; VIDOTTO, O.; GENNARI, S.M.; MACHADO, R.Z.; PEREIRA, A.B.L.; SINHORINI, I.L. *Toxoplasma gondii*: Comparision of a rhoptry- ELISA with IFAT and MAT for antibody detection in será of experimentally infected pigs. Experimental Parasitology. v. 113, p. 100- 105, 2006.

GARCIA, J.L.; GENNARI, S.M.; NAVARRO, I.T.; MACHADO, R.Z.; HEADLEY, S.A.; VIDOTTO, O.; JUNIOR GUIMARÃES, J.S.; BUGNI, F.M.; IGARASHI, M. Evaluation of IFAT, MAT, ELISAs and immunoblotting for the detection of anti- *Toxoplasma gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs. Veterinary Science. v. 84, p. 237 – 242, 2008.

GAULLIN, C, RAMSAY D, THIVIERGE K, TATARYN J, COURVILLE A, MARTIN C, CURNINGHAM P, DESILETS J, MORIN, D, DION R Acute Toxoplasmosis among Canadian Deer Hunters Associated with Consumption of Undercooked Deer Meat Hunted in the United States. Emerging Infectious Diseases, v.26; n.2, p.199-205, 2020.

GUIMARÃES, L.A.; BEZERRA, R.A.; ROCHA, D.D.; ALBUQUERQUE, G.R. Prevalence and risk factors associated with anti- *Toxoplasma gondii* antibodies in sheep from Bahia State, Brazil. Revista Brasileira de Parasitologia. v. 22, n. 2, p. 220 – 224, 2013.

KAREN, A.M.; DARWISH, S.; RAMOUN, A.; TAWFEEK, K.; HANH, N.V.; SOUSA, N.M.; SULON, J.; SZENCI, O.; BECKERS, J-F. Accuracy of transrectal palpation for early pregnancy diagnosis in Egyptian buffaloes. Tropical Animal Health Production. V. 43, p. 5– 7, 2011.

LANDIS, J.R.; KOCH, G.G. The measurement of observer agreement for categorical data. Biometrics. v. 33, n. 1, p. 159 – 174, 1977.

LUCIANO, D.M.; MENEZES, R.C.; FERREIRA, L.C.; NICOLAU, J.L.; NEVES, L.B.; LUCIANO, R.M.; DAHROUG, M.A.A.; AMENDOEIRA, M.R. Seroepidemiologia da toxoplasmose em caprinos e ovinos de três municípios do estado do Rio de Janeiro. Pesquisa Veterinária Brasileira. v. 31, n.7, p. 569- 574, 2011.

LUPTAKOVA, K.; BENOVA, K.; RENCHO, A.; PETROVOVA, E. DNA Detection Of Toxoplasma *Gondii In* Sheep Milk And Blood Samples In Relation To Phase Of Infection.Veterinary Parasitology, v. 208; n. 3-4, p. 250-53, 2015.

MACHADO, D.C. Diagnóstico de toxoplasmose ovina através de imunoensaios. Dissertação (mestrado, Nova Odessa, SP). 2016, 76p.

MENDONÇA, C.E.A.; BARROS, S.L.B.; GUIMARÃES, V.A.A.; FERRAUDO, A.S.; MUNHOZ, A.D. Prevalence and risk factors associated to ovine toxoplasmosis in northeastern Brazil. Revista Brasileira de Parasitologia. v. 22, n.2, p. 230 – 234, 2013.

MEIRELES, L.R.; GALISTEO JUNIOR, A.J.; ANDRADE JUNIOR, H.F. Serological survey of antibodies to *Toxoplasma gondii* in food animals from São Paulo state, Brazil. Brazilian Journal of Veterinary Research and Animal Science. v.40, p. 267- 271, 2006.

MODOLO, J.R.; LANGONI, H.; PADOVANI, C.R.; BARROZO, L.V.; LEITE, B.L.S.; GENNARI, S.M.; STACHISSINI, A.V.M. Aavaliação da ocorrência de anticorpos anti- *Toxoplasma gondii*, em soros de caprinos do estado de São Paulo, e associação com variáveis epidemiológicas, problemas reprodutivos e risco à saúde pública. Pesquisa Veterinária Brasileira. v. 28, n. 12, p. 606-610, 2008.

MORAES, L.M.B.; RAIMUNDO, J.M.; GUIMARÃES, A.; SANTOS, H.A.; JUNIOR MACEDO, G.L.; MASSARD, C.L.; MACHADO, R.Z.; BALDANI, C.D. Occurence of anti– *Neospora caninum* and anti – *Toxoplasma gonii* IgG antibodies in goats and sheep in western Maranhão, Brazil. Revista Brasileira de Parasitologia Veterinária. v. 20, n. 4., p. 312 – 317, 2011.

MOURA, A.B.O.; OSAKI, S.C.; ZULPO, D.L.; MARANA, E.R.M. Ocorrência de anticorpos contra *Toxoplasma gondii* em suínos e ovinos abatidos no município de Guarapuava, PR, Brasil. Revista Brasileira de Parasitologia. v. 16, n. 1, p. 54 – 56, 2007.

PEREIRA, M.F.; PEIXOTO, R.M.; LANGONI, L.; GRECA JUNIOR, H.; AZEVEDO, S.S.;

ROSSI, G.F.; CABRAL, D.D.; RIBEIRO, D.P.; PAJUABA, A.C.A.M.; CORRÊA, R.R.; MOREIRA, R.Q.; MINEO, T.W.P.; MINEO, J.R.; SILVA, D.A.O. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infection in sheep from Uberlândia, Minas Gerais State, Brazil, by different serological methods. Veterinary Parasitology. v. 175, p. 252 – 259, 2011.

RIZZO, H.; CARVALHO, J.S.; HORA, J.H.; RODRIGUES, F.M.; FRAGA, G.J.M.; VILLA LOBOS, E.M.C. Comparação do ensaio imunoenzimático (ELISA- teste) com a reação da imunofluorescência indireta na detecção de anticorpos IgG anti- *Toxoplasma gondii* em ovinos naturalmente infectados. Ciência Veterinária nos Trópicos, v. 17, n. ½, p. 16- 20, 2014.

SAKATA, F.B.L.S.; BELLATO, V.; SARTOR, A.A.; MOURA, A.B.; SOUZA, A.P.; FARIAS, J.A. *Toxoplasma gondii* antibodies sheep in Lages, Santa Catarina, Brazil, and comparison using IFA and ELISA. Revista Brasileira de Parasitologia Veterinária, v. 21, n. 3, p. 196 – 200, 2012.

SOUSA, S.; THOMPSON, G.; SILVA, E.; FREIRE, E.; LOPES, D.; COSTA, J.M.C.; CASTRO, A.; CARVALHEIRA, J.; Canada, N. Determination Of The More Adequate Modified Agglutination Test Cut-Off For Serodiagnosis Of *Toxoplasma gondii* infection in sheep. Zoonoses Public Health, v.56, p.252-56, 2009.

ZHU, C.H.; CUI, L.L.; ZHANG, L.S. Comparision of a commercial ELISA with the Modified Agglutination Test for detection of *Toxoplasma gondii* antibodies in será of naturally infected dogs and cats. Iranian Journal Parasitology, v.7, p. 89 – 95, 2012.