Parasite Epidemiology and Control 3 (2018) e00080

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Prevalence of *Toxoplasma gondii* infections in swine of non-tecnified rearing farms of the northeastern region of the state of São Paulo, Brazil and associated risk factors

Gabriela Capriogli Oliveira [a], Henrique Meiroz de Souza Almeida [b], Raissa Saran Sartori [a], Gabriel Augusto Marques Rossi [b,*], Luis Guilherme de Oliveira [b], Helio Langoni [a]

[a] *Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade Estadual Paulista (Unesp), Câmpus de Botucatu, Distrito de Rubião Junior s/n, Botucatu, SP 18618-970, Brazil*

[b] *Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista – UNESP, Câmpus de Jaboticabal, Via de Acesso Prof. Paulo Donato Castellane s/n, São Paulo 14884-900, Brazil*

ARTICLE INFO

Article history:
Received 11 November 2018
Received in revised form 9 December 2018
Accepted 9 December 2018

Keywords:
Pigs
Toxoplasmosis
MAT
IFA

ABSTRACT

Toxoplasmosis is a zoonosis present worldwide. Its protozoal aethiological agent, *Toxoplasma gondii*, has the ability to infect several homeothermic animals and mainly human beings. The consumption of raw or undercooked meat products containing *T. gondii* cysts, consumption of vegetables without washing and using non-treated water are risk factors associated to the occurrence of human toxoplasmosis. Furthermore, raw or undercooked pork is an important infection source of *T. gondii* to humans. Due to the importance of toxoplamosis in public health, this study focused on establish the prevalence of the disease in non-technified swine herds in the northeastern region of the State of São Paulo, Brazil, using Modified Agglutination Test (MAT) and the Indirect Immunofluorescence Assay (IFA) and the risk factors for its occurrence. In addition, the agreement among both diagnostic tests was evaluated. A low prevalence of toxoplasmosis was found at animal level (7.02%). The Fisher's exact test detected correlation between positive cases with the presence of food garden in the farm ($p = 0.01$) and the use of non-treated water to irrigate the food garden ($p = 0.005$). The agreement among tests was considered moderate (*Kappa index* = 0.5). The results show that toxoplasmosis is a risk for humans who consume under cooked pork meat in this region and an acceptable agreement between MAT and IFA tests.

1. Introduction

Toxoplasmosis is a zoonosis distributed worldwide that occurs in several warm-blooded species including humans and is caused by *Toxoplasma gondii*. The clinical presentation is mild in healthy adults, however when it comes to children or immunocompromised patients the disease can cause blindness, mental retardation (congenital infection) or even death (Silveira et al., 2003; Hill et al., 2006). Almost one third of the world population is supposed to have been exposed to this parasite (Millar et al., 2008). A study reported prevalence values ranging from 50% to 80% of humans infected in South and Central America, respectively (Hill and Dubey, 2002).

* Corresponding author.
E-mail address: gabrielrossiveterinario@gmail.com. (G.A.M. Rossi).

https://doi.org/10.1016/j.parepi.2018.e00080

Felines are the definitive hosts producing oocysts which are eliminated in feces. Depending on environmental temperature and other conditions, those oocysts might turn into spores and contaminate water, pasture and vegetables. All other mammals such as humans, pigs, ewes, goats and cattle are intermediate hosts and transmit toxoplasmosis through congenital infection or when preyed and the parasite's cysts in the muscles are ingested by carnivores (Tenter et al., 2000).

The main risk factors associated with toxoplasmosis in humans are the consumption of raw/undercooked meat of infected animals, ingestion of greens and water contaminated with oocysts, or even, the accidental ingestion of free oocysts in the environment (Hill et al., 2006; Dubey and Jones, 2008). Pigs are an important infection source for humans and the first report of toxoplasmosis in Brazilian pig herds was done by Silva (Silva, 1959), who reported the histological diagnosis of a disease's case in the state of Minas Gerais.

The presence of domestic felines in swine rearing farms is highly frequent in Brazil; in addition, several papers have been reporting high prevalence values in swine herds of extensive and intensive rearing systems (Dubey et al., 1995). In Brazil, the consumption of artisanal meat products is very common, being an important transmission route of toxoplasmosis for consumers and even for the manufacturers (Spalding et al., 2005). This data is concerning since the cysts of *T. gondii* remain infective in swine muscles for long periods and are not detected during inspection of carcasses (Dubey and Beattie, 1988; Koski, 1990).

The Indirect Immunofluorescence Assay (IFA) and the Modified Agglutination Test (MAT) are serological tests based on the recognition of parasite's surface antigens and used for toxoplasmosis diagnostic (Silva et al., 2002; OIE, 2008). Serological tests are very used in toxoplasmosis epidemiological investigations to estimate regional and herd level prevalence (Gamble et al., 2005).

It is a well-known fact that swine from non-technified farms are reared and slaughtered under poor health conditions. Considering that the meat from those animals are illegally sold and consumed allied with the impact toxoplasmosis causes in public health as a foodborne pathogen (including pork), this study focused on assessing the prevalence of the disease, identifying risk factors for such herds and comparing two toxoplasmosis diagnostic tests.

2. Material and methods

2.1. Sample's eligibility criteria, design and collection

Only herds with <150 animals were included in the sampling. All sampled farms had to have low adoption of techniques in production, low scale production being mostly for subsistence or locally sold. The animals had to be reared extensively most of the times in the dirt and with interspecies interaction. The sampling sites were in the vast majority small family owned farms from peasant's settlement in the northeastern region of the São Paulo state, Brazil.

In order to establish a representative sample size, the following equation was used:

$$n = \frac{z^2 \cdot p \cdot q}{d^2}$$

In which: n = sample size, Z = normal standard deviation, p = disease's expected prevalence, q = 1 – p; and d = maximum admitted error value. The pig population in this region was 4100 animals (São Paulo – São Paulo state Agriculture and Food Supply office, 2008), the expected disease's prevalence of 1%, based on a 0.8% prevalence value found in a previous study performed in the state of São Paulo (Caporali et al., 2005) and a maximum admitted error value of 2.5%. The obtained value (n) was corrected (nc) to the population size using the equation:

$$Na = \frac{N \times n}{N + n}$$

As a result, in order to have a sample size representative of the regional population, at least 246 blood samples had to be collected. In total, a set of 356 swine serum samples of 46 extensive rearing farms in the region of Jaboticabal municipality were collected. The samples were from animals of both sexes, all age groups and breeds. The selection of the sampled unit was by voluntary acceptance of the farmer (convenience sample). The number of animals sampled in the herd was approximately 10% of the total and the animals were randomly chosen.

2.2. Risk factor questionnaire

In order to obtain specific epidemiological data of the herds, an epidemiological questionnaire was applied in each sampled farm. The questions were all related to the main risk factors associated with the presence of toxoplasmosis in swine herds, such as: presence of cats, presence of rats in the farm, presence of sewage system, direction of rainwater flow, use of treated water, presence of food garden in the farm and occurrence of reproductive disorders in sows (abortion, and stillborns). Unfortunately, in six of them epidemiological information was not given by the owners. Consequently, the risk factor analysis was performed using data of only 37 herds.

2.3. Diagnostic tests

In the laboratory, the blood samples were centrifuged at 1500 rpm for 10 min. A 1.5 ml aliquot of the serum was separated and placed in Eppendorf® plastic microtubes that had identification with sample and farm number and were kept at −20 °C freezers until use.

Even though the gold standard technique for diagnosing toxoplasmosis is the "Dye Test" (OIE, 2008), the MAT has been widely used in researches with swine samples presenting high specificity and sensitivity (Dubey et al., 1995). This is why this research considered the MAT as the reference test in the diagnosis of toxoplasmosis in swine. The MAT antigen production was performed according to Desmonts & Remington (Desmonts and Remington, 1980), using the RH strain genotype type I (Sabin, 1941).

The swine serum samples were diluted in flat bottom microplates, adding 150 μl of SST 0.01 M pH 7.2 in all cavities. In the first column, it was added 10 μl serum (dilution of 1:16) and afterwards homogenized. In the following step, 50 μl was transferred to the next cavity, resulting in the 1:64 dilution. This process was repeated until a dilution of 1:4096 was obtained. Afterwards, 25 μl of each serum dilution was transferred to another V shaped bottom microplate and added 25 μl of 2-mercaptoetanol 0.2 M, diluted in SST 0.01 M pH 7.2, and 50 μl of the antigen diluted in a 8.7 pH borate buffer solution. Finally, all plaques were sealed with plastic film, homogenized by 1 min and incubated at room temperature overnight. The positive samples were those which formed a thin membrane covering at least half of the bottom of the cavity and in a dilution higher than 1:64 [13 OIE 2008]. Negatives were those in which there was no membrane formation in the bottom of the cavity.

The IFA was performed according to Camargo (Camargo, 1984), using commercial anti-swine IgG for the detection of anti-toxoplasma antibodies. The serum was diluted in SST 0.01 M pH 7.2, starting a two-fold serial dilution at 1:16. The final antibody titer was the highest dilution in which complete fluorescence was noted in at least 50% of tachyzoites. The cutoff point used in the test was 64 UI (OIE, 2008).

2.4. Data analysis

To detect association between the variables and the presence of the disease the Fisher's exact test (SAS Institute, 2011) was performed using a 95% confidence interval. The kappa coefficient was calculated to estimate the agreement between IFA and MAT results. For all prevalence values 95% confidence interval was calculated using Wilson's method (Thrusfield, 2010).

3. Results

Out of the 356 tested serum samples, 25 (7.02%; CI 95% 4.37%–9.67%) had positive serological reaction in the MAT. The titers frequencies observed were 64 UI in 64% (CI 95% 45.18%–82.81%) of positive samples, 256 UI in 16% (CI 95% 1.63%–30.37%), and 1024 UI in 20% (CI 95% 4.32%–36.68%). According to the 95% CI, the 64 IU titer was significantly more frequent than the others were.

Regarding to the IFA results, 48 (13.48%; CI 95% 9.93%–17.03%) animals were positive. The titers observed were 64 UI in 75% (CI 95% 62.75%–87.25%) of animals, 256 UI in 18.75% (CI 95% 7.71%–29.79%) and 1024 UI in 6.25% (CI 95% 2.15%–16.84%) of the samples. According to the 95%CI the frequency of the 64 IU titer was significantly higher than the others. Out of the 331 negative samples in the MAT, 303 were also negative in the IFA, and out of the 25 positive samples at MAT, 20 were positive as well when tested by the IFA (Table 1). Regarding to the agreement between both tests, the kappa coefficient was 0.5, showing a moderate agreement.

Furthermore, a significant association was found between the presence of toxoplasmosis and the presence of food garden in the farm ($p = 0.010$) and using non-treated water in the food garden ($p = 0.004$) (Table 2).

4. Discussion

This present research detected a 7.02% (25/356, IC95 4.8–10.16%) seroprevalence of toxoplasmosis among the studied swine through the MAT test. This value differs from the 24.6% previously found by another study (Santos et al., 1978) which sampled the same region and used the Direct Agglutination Test. During the last 30 years, the health status of the Brazilian swine herd has improved what consequently had an impact in prevalence rates of several diseases, like toxoplasmosis and resulted in the difference between both studies. Still, the different diagnostic technique used could also interfere in the results.

Table 1
Comparison among serological results of the Modified Agglutination Test (MAT) and the Indirect Immunofluorescence Assay in swine serum samples.

IFA	MAT				Total	
	Positive		Negative			
	N	%	N	%	N	%
Positive	20	5,61	28	7,86	48	13,48
Negative	5	1,4	303	85,11	308	86,51>
Total	25	7,02	331	92,98	356	100

Kappa agreement index (κ): 0.5.

Table 2
Association between the occurrences of toxoplasmosis and possible risk factors in the sampled farms using the MAT as the diagnostic test according to the Fisher's Exact Test.

Variables	N	Positive herds N (%)	*p* value
Presence of other animal species	32	8(21.62)	0.27
Dogs	33	8 (21.62)	0.35
Cats	23	7 (18.92)	0.08
Cattle	19	4 (10.81)	0.30
Horses	18	3(8.11)	0.24
Others	6	3 (15.00)	0.18
Presence of rats	26	6 (16.22)	0.32
Feed Storage			
Direct contact with the floor	16	6 (16.67)	0.03
Open bags	14	4 (11.11)	0.12
Absence of sewage system	5	0	0.27
Direct contact with Rainwater flow	26	5 (13.51)	0.28
Use of treated water for animals	10	0	0.05
Use of commercial feed for the animals	12	1 (2.70)	0.14
Use of raw vegetables in the swine feed	27	6 (16.22)	0.34
Use of other animal viscera in the swine feed	12	1 (2.70)	0.14
Presence of food garden in the farm	17	7 (18.92)	0.01
Use of non-treated water to water the food garden	16	15 (34.88)	0.004
Direct contact between cats and the pigs	20	7 (18.92)	0.02
Direct contact of cats with the pigs food	20	7 (18.92)	0.02
Dog/cats usually eat the pigs placenta	3	1 (2.70)	0.20
Reproductive disorder in sows	10	2 (5.41)	0.34
Uses natural breeding in the swine production	26	5 (13.51)	0.28
Free range system	26	6 (16.22)	0.32

P value at 95% Confidence Level in Fisher's Exact Test.

Correa et al. (Correa et al., 1978) and Vasconcelos et al. (Vasconcelos et al., 1979) respectively found 19.1% and 47% of toxoplasmosis infected swine in the State of São Paulo. However, this study's data agrees with Suaréz-Aranda et al. (2000) (Suaréz-Aranda et al., 2000) who found 9.6% of prevalence out of 300 slaughtered pigs in the same region.

Dos Santos et al. (Santos et al., 2005) using the MAT as diagnostic test reported 17% prevalence in animals from extensive farms in the Midwestern region of the state, suggesting that it is more common to have higher prevalence values in such farms due to the poor sanitary condition in which the animals are reared. Thus, also using the MAT another research found a prevalence of 20.18% of positive serum samples among 550 swine serum from non-technified farms' herds in Registro, state of São Paulo, Brazil (Oliveira et al., 2007). The data obtained in this study was similar to reported in developed countries, as North of Portugal (9.8%) (Lopes et al., 2013).

Association between risk factors and the presence of the disease were detected in this study. A significant association was observed between variables “presence of food garden in the farm” and the occurrence of toxoplamosis. Another association noted was between the use of non-treated water in the food garden and the presence of the disease. Another research (Bonna et al., 2006) reported a prevalence value of 65.8% and association between the disease and practices such as consumption of untreated water and greens by the animals. Contaminated water ends up contaminating vegetables when used to water food gardens, and since the use of raw vegetables from the own food garden to feed animals is a common practice in these non-technified farms, the association between those risk factors and the occurrence of the disease is quite clear.

Animals reared in free-range system have a higher risk of acquiring *T. gondii* (*Wallander* et al., *2016*). Since the oocysts are commonly found contaminating the pasture, due to the use of non-treated water in irrigation or the rainwater flow that can carry the oocyst from one place to another. A very high prevalence of toxoplasmosis in pigs reared in free-range oranic system was recently reported in Italy (Bacci et al., 2015).

Even though these animals are reared under poor health and biosecurity standards, their meat and meat products are consumed by the farmers or illegally sold to the local population. The presence of anti-*Toxoplasma gondii* in swine serum of extensive farms serves as an alert to public health authorities, since if the meat of such animals is consumed raw or undercooked it could transmit toxoplamosis to the population, posing a risk to public welfare and increased costs with treatment of the disease by the public health service.

The authors suggest that sanitary education measures should be applied to the regional farms in order to aware the health risks of toxoplasmosis and enhance basic health surveillance actions in extensive herds. Also, other control practices should be improved in these farms, such as avoid contact with contaminated food and water infected with oocysts controlling the presence of cats and rodents and vaccinations against tissue cyst formation, avoid informal slaughter and the use of heat-treatments (freezing and cooking) to consume pork (Kijlstra and Jongert, 2008). Rodents control appears to be the most effective sanitary practice to avoid toxoplasmosis infections in swines (Kijlstra et al., 2008). Also, to avoid the presence of cats near areas where swine are reared or food is produced is necessary hue the high occurrence of this disease in this specie in some regions in Brazil (Munhoz et al., 2017).

Regarding the titer values found, when using either of the test the most frequent antibody titer was 64 IU (MAT 64% and IFA 75%), that was also significantly different than the other titers frequency. Low antibody titer is indicative of either chronic or recent infections, enabling us to state only that the tested animals were infected by *Toxoplasma gondii*. The presence of higher titers found (such as 1024 IU) is related to active or recent infections, consequently meaning that the agent is present in the region and potentially infecting and cause disease in pigs.

The IFA is the most commonly used assay for serological toxoplasma diagnosis in several animal species, however, in this study when compared to the MAT, both tests presented only fair agreement (*Kappa* coefficient: 0.5). Two other studies reported a high agreement between both MAT and the IFA (*Kappa* = 0.86) (Cavalcante et al., 2006; Minho et al., 2004). Dubey et al. (Dubey et al., 1995) presented a 82.9% sensitivity and 90.29% specificity for the MAT when compared to direct isolation of *T. gondii* from swine meat/tissues. On the other hand, the IFA presented a good performance when it comes to toxoplasmosis diagnosis in swine serum. When using the MAT as reference the IFA presented 80% de sensitivity and 91.54% specificity.

The same tests when used with other species' samples presented a fair agreement. When comparing the IFA and the MAT using mice serum a *Kappa* coefficient of 0.55, was found when adopting a cutoff point of 50 IU (Cola et al., 2010); Silva et al. (Silva et al., 2002) found *Kappa* ranging from 0.59 to 0.84 when testing the IFA and the MAT with 16 IU, 64 IU and 256 IU titers in the serum of ewe, goats, cats and dogs. A sensitivity of 85% and specificity of 100% of IFA was reported (Franco et al., 2003) when comparing to MAT results in dog serum of the state of Roraima. Thus, another report showed 92% agreement between tests when examining 93 cats' serum samples (Abate et al., 1989).

In this research, the MAT detected significantly higher titers than the IFAT for the same sample, like previous studies have shown. The highest titer obtained by the MAT was 1024 IU in five different samples, while only three samples of those samples had the same titer in the IFA, a fact explained by the different type of immunoglobulin detected by each test.

5. Conclusions

Even though low toxoplasmosis prevalence was found at animal level (7.02%), this study associated common practices of non-technified farming with presence of the disease in swine herds. Both tests (IFA and MAT) presented satisfying performances in diagnosing toxoplasmosis with a moderate agreement of results. This paper results suggests that health education regarding toxoplasmosis prophylaxis are required to improve animal health and consequently food safety in this region.

Conflict of interest statement

The authors hereby declare that there is no conflict of interest in the study.

References

Abate, O., Gasbarra, S., Dotta, U., 1989. Toxoplasmosi: indagine sul titolo anticorpale in cani e in gatti sani e portatori di patologie. Veterinaria 3 (2), 19–24.

Bacci, C., Vismarra, A., Mangia, C., Bonardi, S., Bruini, I., Genchi, M., et al., 2015. Detection of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods. Int. J. Food Microbiol. 202 (2), 54–56.

Bonna, I.C.F., da Costa, F.B. Figueiredo T., Vicente, R.T., Santiago, C.A.D., Nicolau, J.L., et al., 2006. Estudo soroepidemiológico da infecção por *Toxoplasma gondii* em suínos e frangos, para abate, em região rural do Rio de Janeiro. Rev. Bras. Ciênc. Vet. 13 (3), 186–189.

Camargo, M.E., 1984. Introduction to immunofluorescence techniques. Rev. Bras. Patol. Clín. 10, 43–169.

Caporali, E.H.G., Silva, A.V., Mendonça, A.O., Langoni, H., 2005. Comparison between diagnostic methods to determine the antibody prevalence of anti-*Toxoplasma gondii* in swines of the states of São Paulo and Pernambuco-Brazil. Arq. Ciên. Vet. Zool. 8 (1), 19–24.

Cavalcante, G.T., Aguiar, D.M., Chiebao, D., Dubey, J.P., Ruiz, V.L.A., Dias, R.A., et al., 2006. Seroprevalence of *Toxoplasma gondii* antibodies in cats and pigs from rural western Amazon, Brazil. J. Parasitol. 92 (4), 863–864.

Cola, G.A., Garcia, J.L., Costa, L., Ruffolo, B., Navarro, I.T., Freire, R.L., 2010. R, Comparação da reação de imunofluorescência indireta e do teste de aglutinação modificado na detecção de anticorpos anti-*Toxoplasma gondii* em ratos. Semina. Semina Cienc. Agrar. 31 (3), 717–722.

Correa, F.M.A., Salata, E., Oliveira, M.R., 1978. *Toxoplasma gondii*: diagnosis using indirect immunofluorescence assay in swine of the state of São Paulo. Brazil. Arq. Inst. Biol. 45 (4), 209–212.

Desmonts, G., Remington, J.S., 1980. Direct agglutination test for diagnosis of toxoplasma infection: method for increasing sensitivity and specificity. J. Clin. Microbiol. 11 (6), 562–568.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC, Boca Raton.

Dubey, J.P., Jones, J.L., 2008. *Toxoplasma gondii* infection in humans and animals in the United States. Int. J. Parasitol. 38 (11), 1257–1278.

Dubey, J.P., Thulliez, P., Weigel, R.M., Andrews, C.D., Lind, P., Powell, E.C., 1995. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am. J. Vet. Res. 56 (8), 1030–1036.

Franco, C., Alberto, W., Bergamaschi, D.P., Richtzenhain, L.J., Nogueira, Y., Camargo, L.M.A., Souza, S.L.P.D., Gennari, S.M., 2003. Evaluation of the performance of the modified direct agglutination test (MAT) for detection of *Toxoplasma gondii* antibodies in dogs. Braz. J. Vet. Res. Anim. Sci. 40 (6), 452–456.

Gamble, H.R., Dubey, J.P., Lambillotte, D.N., 2005. Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma* infection in the domestic pig. Vet. Parasitol. 128 (3–4), 177–181.

Hill, D., Dubey, J.P., 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbiol. Infect. 8 (10), 634–640.

Hill, D.E., Chirukandoth, S., Dubey, J.P., Lunney, J.K., Gamble, H.R., 2006. Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine. Vet. Parasitol. 141 (1–2), 9–17.

Kijlstra, A., Jongert, E., 2008. Control of the risk of human toxoplasmosis transmitted by meat. Int. J. Parasitol. 38 (12), 1359–1370.

Kijlstra, A., Meerburg, B., Cornelissen, J., de Craeye, S., Vereijken, P., Jongert, E., 2008. The role of rodents and shrews in the transmission of *Toxoplasma gondii* to pigs. Vet. Parasitol. 156 (3–4), 183–190.

Koski, V.H., 1990. Evaluation of ELISA for the detection of *Toxoplasma* antibodies in swine sera. Acta Vet. Scand. 31 (4), 413–422.

Lopes, A.P., Dubey, J.P., Neto, F., Rodrigues, A., Martins, T., Rodrigues, M., Cardoso, L., 2013. Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the north of Portugal for human consumption. Vet. Parasitol. 193 (1–3), 266–269.

Millar, P.R., Daguer, H., Vicente, R.T., da Costa, T., Sobreiro, L.G., Amendoeira, M.R.R., 2008. *Toxoplasma gondii*: serological and epidemiological research in the south-western region of the state of Paraná. Pesq. Vet. Brassica 28 (1), 15–18.

Minho, A.P., Freire, R.L., Vidotto, O., Gennari, S.M., Marana, E.M., Garcia, J.L., Navarro, L.T., 2004. Evaluation of the indirect fluorescent antibody test and modified agglutination test for detection of antibodies against *Toxoplasma gondii* in experimentally infected pigs. Pesqui. Vet. Bras. 24 (4), 199–202.
Munhoz, A.D., Hage, S.B., Cruz, R.D.S., Calazans, A.P.F., Silva, F.L., Albuquerque, G.R., Lacerda, L.C., 2017. Toxoplasmosis in cats in northeastern Brazil: frequency, associated factors and coinfection with *Neospora caninum*, feline immunodeficiency virus and feline leukemia virus. Vet. Par. Reg. Stud. Rep. 8, 35–38.
OIE, 2008. Toxoplasmosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.
Oliveira, K.R., Domingues, P.F., Langoni, H., Silva, R.C., Gottschalk, S., 2007. Detection of antibodies for *Toxoplasma gondii* in swine serum reared in non tehcnified conditions in the microregion of Registro, São Paulo using the Modified Agglutination Test (MAT). Vet. Zootec. 14, 169–175.
Sabin, A.B., 1941. Toxoplasmic encephalitis in children. JAMA 116 (9), 801–807.
Santos, S.M., Amaral, V., Rebouças, M.M., 1978. Prevalence of antitoxoplasma antibodies using indirect hemagglutination test in swine serum of several municipalities in the state of São Paulo. Biológico 44, 149–153.
Santos, C.B.A., de Carvalho, A.C.F.B., Ragozo, A.M.A., Soares, R.M., Amaku, M., Yai, L.E.O., Dubey, J.P., et al., 2005. First isolation and molecular characterization of *Toxoplasma gondii* from finishing pigs from São Paulo State, Brazil. Vet. Parasitol. 131 (3–4), 207–211.
São Paulo – São Paulo state Agriculture and Food Supply office, 2008. Censo agropecuário e de unidades produtivas do Estado de São Paulo [Agricultural census of productive units in the São Paulo state]. Integral Technical Assistance Coordination, Jaboticabal, São Paulo, Brazil.
SAS Institute, 2011. SAS/STAT User's Guide. Version 9.3. SAS Institute Inc, Cary.
Silva, I.M.L., 1959. A case reports of instantaneous toxoplasmosis in 8 swines. Arq. Esc. Sup. Vet. E. M. G. 12, 425–428.
Silva, A.V., Cutolo, A.A., Langoni, H., 2002. Comparison of indirect fluorescent antibody test and modified agglutination test to detect antibodies to *Toxoplasma gondii* in sera from sheep, goat, dogs and cats. Arq. Inst. Biol. 69 (1), 7–11.
Silveira, C., Ferreira, R., Muccioli, C., Nussenblatt, R., Belfort, R., 2003. Toxoplamosis transmitted to a newborn from the mother infected 20 years earlier. Am J. Ophthalmol. 136 (2), 370–371.
Spalding, S.M., Amendoeira, M.R.R., Klein, C.H., Ribeiro, L.C., 2005. Serological screening and toxoplasmosis exposure factors among pregnant women in south of Brazil. Rev. Soc. Bras. Med. Trop. 38 (2), 173–177.
Suaréz-Aranda, F., Galisteo, A.J., Hiramoto, R.M., Cardoso, R.P.A., Meireles, L.R., Miguel, O., Andrade, H.F., 2000. The prevalence and avidity of *Toxoplasma gondii* IgG antibodies in pigs from Brazil and Peru. Vet. Parasitol. 91 (1–2), 23–32.
Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30 (12—13), 1217–1258.
Thrusfield, M.V., 2010. Veterinary Epidemiology. 3rd edition. Rocca (556p).
Vasconcelos, O.T., Costa, A.J., Ávila, F.A., 1979. Epidemiological aspects of *Toxoplasma gondii* infection in swines. Científica 7, 83–87.
Wallander, C., Frössling, J., Dórea, F.C., Uggla, A., Vågsholm, I., Lundén, A., 2016. Pasture is a risk factor for *Toxoplasma gondii* infection in fattening pigs. Vet. Par. 224 (15), 27–32.