

Occurrence of *Toxoplasma gondii* and risk factors for infection in pigs raised and slaughtered in the Triângulo Mineiro region, Minas Gerais, Brazil¹

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ABSTRACT.- Marques-Santos F, Amendoeira M.R.R., Carrijo K.F, Santos J.P.A.F, Arruda I.F, Sudré A.P., Brener B. & Millar P.R. 2017. **Occurrence of *Toxoplasma gondii* and risk factors for infection in pigs raised and slaughtered in the Triângulo Mineiro region, Minas Gerais, Brazil.** *Pesquisa Veterinária Brasileira* 37(6):570-576. Laboratório de Parasitologia, Departamento de Microbiologia e Parasitologia, Universidade Federal Fluminense, Rua Vital Brazil Filho 64, Santa Rosa, Niterói, RJ 24230-340, Brazil. E-mail: patriciariddell@vm.uff.br

The Triângulo Mineiro region from Minas Gerais state, is an important meat-exporting region of Brazil and data about *Toxoplasma gondii* infection in pigs raised and slaughtered in this area are scarce. Therefore, the aim of this study was to evaluate the occurrence of *T. gondii* in swine and establish the risk factors associated with the infection. Samples were collected from 600 pigs raised under intensive system in farms located at three different counties (Carmo do Paranaíba, Patrocínio and Perdizes). The samples were submitted to indirect hemagglutination antibody test with dilution of 1:32 and to indirect immunofluorescence antibody test with a cutoff of 1:64. The occurrence of positive pig was 3.3% (n=20) and 51.8% (n=311) respectively. A significant difference was observed between toxoplasmatic infection and factors such as lineage, animal origin, size of the farm, collective raising with others species, presence of rodents and type of water offered (p≤0.05). There was no difference between gender and the farm goals. The results demonstrated an occurrence of anti-*T. gondii* antibodies higher than expected for intensive pig raising system on the studied area, which could indicate a possible sanitary management problem on the studied properties. Improvements on the raising techniques are necessary to reduce *T. gondii* infection sources.

INDEX TERMS: Toxoplasmosis, *Toxoplasma gondii*, serology, IHA, IFAT, swine.

RESUMO.- [Ocorrência e fatores de risco da infecção por *Toxoplasma gondii* em suínos criados e abatidos na região do Triângulo Mineiro, Minas Gerais, Brasil.]

A região do Triângulo Mineiro, no estado de Minas Gerais, é uma importante região exportadora de carne do Brasil e pesquisas sobre a infecção por *Toxoplasma gondii* em suínos criados e abatidos nesta região são escassos. Portanto, o objetivo deste estudo foi avaliar a ocorrência de *T. gondii* nesses animais e estabelecer os fatores de risco associados com a infecção. Foram coletadas amostras de 600 suínos criados sob sistema intensivo, em fazendas localizadas em três municípios diferentes (Carmo do Paranaíba, Patrocínio e Perdizes). As amostras foram submetidas à Hemaglutinação Indireta com diluição de 1:32 e à Reação de Imunofluorescência Indireta com ponto de corte 1:64. A ocorrência de suínos positivos foi de 3,3% (n=20) e 51,8% (n=311), respectivamente. Foi observada diferença significativa entre a infecção toxoplásmica e fatores como linhagem, procedência dos animais, tamanho das propriedades, criação

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em conjunto com outras espécies, presença de roedores e tipo de água consumida ($p \leq 0,05$). Não houve diferenças estatísticas entre o sexo e finalidade de produção em relação à infecção por *T. gondii*. Os resultados demonstraram uma ocorrência de anticorpos anti-*T. gondii* superior à esperada em criações intensivas de suínos na região estudada, o que poderia indicar uma possível falha no manejo sanitário das propriedades estudadas. Melhorias nas técnicas de criação são necessárias para redução das fontes de infecção por *T. gondii* nos rebanhos.

TERMOS DE INDEXAÇÃO: Toxoplasmose, *Toxoplasma gondii*, sorologia, HAI, RIFI, suíno.

INTRODUCTION

Toxoplasma gondii is an obligatory intracellular parasite and is the etiological agent of toxoplasmosis, a zoonosis present worldwide that affects human and other warm-blooded animals (mammals and birds) (Elmore et al. 2010). The infection by *T. gondii* in farm animals is important because infected animals can act as direct or indirect source of infection to human. Also, toxoplasmosis can cause reproductive disorders such as abortion, neonatal death, and congenital defects leading to great economic losses (Sawadogo et al. 2005, Yu et al. 2007). The occurrence of *T. gondii* infection in animals can be a good parameter to alert for problems in rural areas since they are in direct contact with the environment (Amendoeira et al. 1999, Jiang et al. 2012).

Among farms animals, the pig is the most frequently infected and in several countries, including Brazil, its meat is considered the main transmission route to humans (Dubey et al. 2005, Dubey & Jones 2008, Dubey 2009). Viable forms of the parasite have been isolated from a great variety of meat products and serological studies in Brazil showed a wide distribution of this infection in pigs (Bonna et al. 2006, Millar et al. 2008, Muraro et al. 2010, Azevedo et al. 2010, Valença et al. 2011, Sousa et al. 2014).

The proportion of the human population infected by ingesting oocysts present in the environment or by raw or undercooked meat is unknown. It is not yet possible to determine the most important infection source for each individual. However, Dubey et al. (2005) suggest that in some regions the ingestion of raw or undercooked pig meat products with *T. gondii* cysts would be the main transmission route to humans. According to Fajardo et al. (2013), although detection of *T. gondii* in animal meat is an important short term measure, control strategies can only be performed with a deeper knowledge about the epidemiology of toxoplasmosis and few studies were conducted to study and identify the risk factors related to *T. gondii* infection in animals used for human consumption. The identification of these risk factors is critical for the development of raising systems that properly promote animal health and consequently avoid the production of meat containing infective forms of this parasite.

Minas Gerais state has a great contribution to pig industry in Brazil, nevertheless studies regarding the epidemiology of toxoplasmosis in pigs are scarce. The few studies on Minas Gerais state are mostly old (Schenk et al. 1977,

Passos et al. 1984, Guimarães et al. 1992, Pezerico et al. 2007), with only one recent study published by Almeida et al. (2015). Although Minas Gerais state has a total of more than five million pigs in raising systems, and the Triângulo-Mineiro region is considered the biggest pig raising area of Brazil, with 85% of the production destined to the export market (IBGE 2012, IBGE 2014), there are no studies about toxoplasmic infection in pigs raised and slaughtered in this area. Thus, the aim of this research is to evaluate the occurrence of *Toxoplasma gondii* in these animals and to establish risk factors associated with infection

MATERIALS AND METHODS

The studied animals were raised and slaughtered in Triângulo Mineiro, an important region in Brazilian pig industry. Geographical location of this region is as follows: 18°18' and 20°27' South and longitude 47°28' and 51°30' West. All pigs, 311 females and 289 males, were about 160 days-old and were feed exclusively with ration and raised under an intensive system. Serum samples were collected between May and July 2014 in three properties, each one located in a different county of Triângulo Mineiro region (Carmo do Paranaíba, Patrocínio, and Perdizes). The animals of all properties were slaughtered in abattoirs with official sanitary inspection service and therefore authorized to commercialize the products throughout national territory.

The number of samples was determined by Epi-info software based on the total population of pigs in the studied region calculated by Statistic and Geographic Brazilian Institute (IBGE 2012). The reference point was an expected prevalence of 50%, confidence interval of 95%, and absolute error of 5%. The minimum number of animals considered representative of the studied population by sample calculation (Epi Info 6) was 385. However, a total of 600 samples were randomly collected, 445 from Patrocínio, 120 from Carmo do Paranaíba, and 35 from Perdizes, and the animals lineages were respectively ADB, Agroceres PIC and unknown.

Blood samples were collected from animals approved in *ante-mortem* inspection by the official sanitary inspection. The samples (10 mL) were collected at exsanguination area of the abattoir in proper identified flasks without anticoagulant. The samples were rest at room temperature until clot retraction and then were centrifuged (1000g for 10 minutes). Serum was separated, kept in sterile flask at -20°C and later forwarded to the Parasitological Immunodiagnosis Laboratory of the Microbiology and Parasitology Department of Universidade Federal Fluminense and to the Toxoplasmosis and other protozoan diseases Laboratory of IOC, Fiocruz. The samples arrived frozen at these laboratories, where the indirect hemagglutination antibody test (IHA) and indirect immunofluorescence antibody test (IFAT) for anti-*T. gondii* antibody detection were respectively performed. The IHA test was performed using a commercial kit (TOXO-HAI®, WAMA) with dilution of 1:32, the protocol was performed according to manufacture instructions. The IFAT was performed according to Camargo (1964). Taquizoits from RH strain of *T. gondii* (Sabin 1941) were used as antigen. Serum samples previously tested by IFAT were used as positive and negative control. Animals were considered positive with antibody titration ≥ 64 .

The farm owners and/or abattoir technical manager answered an epidemiological questionnaire regarding the follow information about the animals raising system: age, gender, lineage, diet, contact with cats and rodents, purpose of the animals, type of raising system, type of water offered to the animals, and dewor-

Table 1. Occurrence of anti-*Toxoplasma gondii* antibodies in serum samples of pigs slaughtered in Triângulo Mineiro region-MG during May-July 2014, according to their origin

Farm	Location	Samples (n)	IHA		IFAT	
			Reactives n(%)	Non-Reactives n(%)	Reactives n(%)	Non-Reactives n(%)
I	Carmo do Paranaíba, MG	120	11 (9.2)	109 (90.8)	50 (41.7)	70 (58.3)
II	Patrocínio, MG	445	9 (2.0)	436 (98.0)	238 (53.5)	207 (46.5)
III	Perdizes, MG	35	0 (0.0)	35 (100.0)	23 (65.7)	12 (34.3)
Total		600	20 (3.3)	580 (96.7)	311 (51.8)	289 (48.2)

ming program. This study was approved by the Ethical Committee for Animal Use/Laboratory Animal Nucleus (CEUA/NAL) of Federal Fluminense University (register #320/13).

The serological results and epidemiological variables were analyzed using EPI-info software. The agreement level between the serological tests was evaluated by agreement index Kappa (K). The association between two categorical variables was determined by Pearson chi-square test (χ^2). Tables formed by two rows and two columns were evaluated by Fischer test with significance level of 5%. In order to evaluate the impact among the variables, *odds ratio* (OR) values were described with their respective confidence interval (CI) of 95%.

RESULTS

Occurrence

All samples were submitted to IHA test and IFAT for detection of antibodies anti-*T. gondii*. These antibodies were detected in 3.3% (20/600) of the samples using IHA test. Seropositivity of 51.8% (311/600) was detected by IFAT (cutoff titer of 1:64). Considering the IHA test results, only in one farm the presence of antibodies anti-*T. gondii* was not detected. However, the results from IFAT showed that

all farms housed positive animals (Table 1). Among the positive samples by IFAT, 96.8% (301/311) had antibody titer of 1:64 and only 3.2% (10/311) had antibody titer of 1:256.

The agreement index Kappa (K) between the two serological tests used in this study was 0.03 (Table 2). According to Landis & Koch (1977) this value indicates a weak agreement between these two techniques for the detection of anti-*T. gondii* antibodies. Therefore, for data analysis only the samples positive in IFAT test were used since this test is considered the gold standard to detect the serological occurrence in pigs. The use of IFAT as a gold standard me-

Table 2. Comparison between serological techniques (IHA and IFAT) for anti-*Toxoplasma gondii* antibody detection in serum samples from pigs slaughtered in Triângulo Mineiro region-MG during May-July 2014

IHA	IFAT		Total
	Positive	Negative	
Positive	15	5	20
Negative	296	284	580
Total	311	289	600

Table 3. Occurrence of anti-*T. gondii* antibodies in serum samples of pigs slaughtered in Triângulo Mineiro-MG during May-July 2014, according to the risk factors for *Toxoplasma gondii* infection

Variable	Reactive n (%)	Non-Reactive n (%)	Total n (%)	OR	CI 95%	P value
Sex						
Male	142 (49.1)	147 (5.9)	289 (100.0)	0.81	(0.59 - 1.12)	0.202
Female	169 (54.3)	142 (45.7)	311 (100.0)	1.23	(0.89 - 1.70)	
Total	311 (51.8)	289 (48.2)	600 (100.0)			
Lineage						
ADB*	238 (53.5)	207 (46.5)	445 (100.0)	-	-	0.017
Agroceres PIC	50 (41.7)	70 (58.3)	120 (100.0)	0.62	(0.41 - 0.93)	
No information	23 (65.7)	12 (34.3)	35 (100.0)	1.67	(0.81 - 3.43)	
Total	311 (51.8)	289 (48.2)	600 (100.0)			
Collective raising						
Yes (Bovines)	50 (41.7)	70 (58.3)	120 (100.0)	0.6	0.40 - 0.90	0.013
No	261 (54.4)	219 (45.6)	480 (100.0)			
Total	311 (51.8)	289 (48.2)	600 (100.0)			
Farm size						
Medium	50 (41.7)	70 (58.3)	120 (100.0)	0.6	0.40 - 0.90	0.013
Large	261 (54.4)	219 (45.6)	480 (100.0)	1.67	1.11 - 2.50	
Total	311 (51.8)	289 (48.2)	600 (100.0)			
Presence of Rodents						
Yes	50 (41.7)	70 (58.3)	120 (100.0)	0.6	0.40 - 0.90	0.013
No	261 (54.4)	219 (45.6)	480 (100.0)			
Total	311 (51.8)	289 (48.2)	600 (100.0)			
Type of Water						
Treated	261 (54.4)	219 (45.6)	480 (100.0)	1.67	1.11 - 2.50	0.013
Well or Creek	50 (41.7)	70 (58.3)	120 (100.0)	0.6	0.40 - 0.90	
Total	311 (51.8)	289 (48.2)	600 (100.0)			

*Reference category for OR calculation.

thod to detect toxoplasmic infection in pigs was validated by Minho et al. (2004) by detecting anti-*T. gondii* antibodies in 46 experimentally infected pig serum with a sensitivity of 95.7% and specificity of 97.8%. Fialho & Araújo (2002) compared the IFAT and HAI techniques and concluded that for diagnosis purposes the IFAT is superior to HAI, and thus more adequate for epidemiological surveys.

Herd and management characteristics

All pig farms were located in rural areas of Triângulo-Mineiro region and used an intensive raising system. The farms located at Patrocínio and Perdizes were considered large in size while the other farm located at Carmo do Paranaíba was considered medium. The presence of cats was not detected in any of the farms studied. However, the presence of rodents was reported in the farm located at Carmo do Paranaíba. In this same farm the collective raising with bovines was also reported. The occurrence of positive animals for anti-*T.gondii* antibodies was higher in the farm located at Perdizes (65.7%, $p=0.017$).

Although the percentage of infected female by *T. gondii* (54.3%) was, in absolute numbers, higher than the percentage of infected male (49.1%), there was no difference between the mean number of infected males and females ($p=0.202$).

Regarding the lineage of the pigs, it was observed that 76.5% (238/311) of the positive pigs were from ADB lineage, 16.1% (50/311) from Agrocères PIC lineage, and 7.4% (23/311) were from unknown lineage. The association between infection by *T. gondii* and lineage of the animals was significant ($p=0.017$). However, the frequency of infection was directly proportional in relation to the total number of samples per lineage.

The evaluation of the risk factors related to *T. gondii* transmission to pigs, showed that among positive pigs 83.9% (261/311) were not raised with other animal species and the presence of rodents was not reported in the farm of origin ($p=0.013$). Among reactive samples, 83.9% (261/311) were from farm of large size that offered treated water (water from public supply companies) to animals and 16.1% (50/311) were from farm of medium size that offered well or creek water to the animals. The odds of seropositivity among pigs raised in large farms that offered treated water were 1.67 higher than those raised in medium farms that offered well or creek water to the animals. All the variables observed had statistical significance ($p=0.013$) (Table 3).

DISCUSSION

In spite of the fact that it is not possible to determine the exact importance of production animals in toxoplasmosis transmission to humans, it is known that pig meat represents the major infection source in many countries. To our knowledge there are no studies about *Toxoplasma gondii* infection in pigs raised in Triângulo Mineiro region. In Brazil, although the consumption of pig meat is lower than other kind of meat (bovine and poultry) there is a risk of *T. gondii* infection, especially if sanitary care were not taken during pig farming. The habits of the consumers also put

them at risk of infection (Schlindwein & Kassouf 2006, Silva et al. 2010).

The Kappa value found in this study (0.003) demonstrated a weak association between IHA test and IFAT to detect antibodies anti-*T. gondii* in pigs. This result differs from a previous study performed at Rio de Janeiro that found 100% agreement in cats (Barros et al. 2015). Therefore, the IHA test kit for human toxoplasmosis diagnosis must be standardized for other animal species before its use in epidemiological studies.

Our occurrence data corroborates with similar studies performed in other Brazilian states such as Pará (50.0%; Freitas et al. 2009) and São Paulo (48.0%; Villalobos et al. 2011). However, the occurrence of toxoplasmosis in pigs found in this study was higher than those observed in previous studies: 36.2% in Paraíba (Azevedo et al. 2010); 26.9% in Alagoas (Valença et al. 2011); 18.2% in Bahia (Bezerra et al. 2009); 12.8% in Mato Grosso (Muraro et al. 2010); 12.6% in Paraná (Santos et al. 2015) and 12.5% in Pernambuco (Fernandes et al. 2012). But was lower than observed in Rio de Janeiro (65.8%; Bonna et al. 2006) and Rio Grande do Sul (86.0%; Silva et al. 2003). The comparison between the present study and the results obtained by other authors should be done with caution. Brazil is a country of continental dimensions, with different climate and environmental conditions, sometimes very specific, a factor that could explain the observed differences when comparing our results with those published by others. Moreover, other relevant points that should be considered are the type of raising system (intensive, semi-intensive or extensive), the different serological methods used for diagnosis or the different cut off points employed, and thus differences on sensitivity of each technique should be considered.

The occurrence of *T. gondii* infection in pigs observed on the present study calls into question the health of consumers, especially those who eat raw or undercooked meat. The absence of serological exams on slaughtered animals allows the products to be freely marketed, without inspection, since it is not possible to detect the presence of the parasite on the slaughter line. This scenario points to the need for animal health planning on the production chain origin, and the awareness of producers for ways to control this disease (adequate management practices, control of rodents and felines on properties, health education) to transform the product in a positive factor for animal and collective health, and also to meet the health requirements.

Regarding the correlation between positivity for *T. gondii* infection and animal origin, despite the greater number of animals from Patrocínio, the positive animal rate for toxoplasmosis was higher in Perdizes. This association was significant ($p=0.017$) and the odds for seropositivity in this farm was 2.68 higher than observed in other farms. However, this information should be observed with caution, due to the small number of properties, which could have influenced in some statistical associations, and also the sampling varied between properties. Variation of positive frequency among farms (41.7 to 65.7%) corroborate with a previous study performed in another state (Goiás) that observed a variation

between 8.33% and 60% in the 40 farms evaluated (Matos et al. 1999). However, this greater variation can be related to the greater number of farms studied. This variation can occur mainly due to the variety of risk factors present among the counties which can have different ecosystems or in different farms from the same county which can have different management measures that can favor *T. gondii* infection (Suárez-Aranda et al. 2000, Caporali et al. 2005).

The animal gender did not affect the rate of positive. This data is similar to other study that observed that both genders were exposed to the same risk factors (Azevedo et al. 2010). According to Garcia et al. (1999) the animal breed or genetics can contribute to variation observed in the occurrence of *T. gondii* infection. In the present study a significant association was observed between seropositivity and the animal lineage, however, the higher percentage observed on ADB lineage could be due to a higher number of these animals on the sample used in this study, and not necessarily due to intrinsic factors of this lineage.

The destination of the pig after slaughter and the infection occurrence did not showed statistical difference ($p=0.171$). However, most of the positive pigs were used to make meat products, which alert us to the great number of infected animals used as human food. In this regard, several researches had detected *T. gondii* presence in pig meat products. The presence of *T. gondii* was confirmed in 8.72% (13/149) of the swine sausage samples in Londrina-PR (Dias et al. 2005). In pigs slaughtered in small and medium slaughterhouses in Erechim/RS (southern Brazil), 34% (17/50) of the diaphragm samples and 66% (33/50) of the tongue samples were positive for *T. gondii* (Belfort-Neto et al. 2007).

In the present study, pig raising collectively with other animal species (bovine) did not represent risk of infection by *T. gondii*, actually it represented a protective factor (OR=0.6, CI 95%, 0.40-0.90). This was probably due to the management measures adopted in the farm and not due to the collective raising *per se*.

The chance of infection by *T. gondii* was 1.67 times higher in large farms than in medium ones (OR=1.67, CI 95%, 1.11-2.50). The use of intensive raising systems (confined animals) reduced pig exposition to *T. gondii* when compared to semi-confined animals (AFSSA 2005). In our study, however, the great occurrence found in pigs raised in feedlot system and the greater risk to acquire *T. gondii* infection in large farm could be explained by the greater animal density in the larger farms. Management factors such as water in the piggery, groove drinking fountain and flooded areas in the farm were also associated with a higher prevalence of toxoplasmosis (Tsutsui et al. 2003).

All animals used in the present study were fed with ration. This feeding system can be directly related to a high occurrence of *T. gondii* infection in pigs (Vidotto et al. 1990). In the intensive raising systems, the ration is stored for a longer period, increasing the risk of contamination of food by *T. gondii* oocysts from cats. In the present study the presence of cats was not reported in the farms evaluated, however, we must consider that some bias could have occurred during questionnaire answer.

The water is an important source of infection by *T. gondii* oocysts (Jones & Dubey 2010). In the present study, two farms offered water from wells or creeks to their animals. Unexpectedly, the use of treated water (water from public supply companies) increased the chances for *T. gondii* infection (OR = 1.67, CI 95%, 1.11-2.50). This result indicated an event that was not detected by the present study and points out the need for further investigation. However, a justification for such an event would be a probable inadequacy or fail on treatment of water that is destined to animals or the contamination of water from drinkers and reservoirs by parasite sporulated oocysts.

The presence of rodents on pig farms was described by Valença et al. (2011) as a risk factor not only because rats attract cats, which could eliminate oocysts in their feces and contaminate the environment, but also because these animals could be infected by ingesting rodents with tissue cysts. Although the presence of rodents did not represent risk for pig infection in the present study (OR=0.6, CI 95%, 0.40-0.90), the epidemiological questionnaire revealed that 41.7% of the animals from the farm with rodent presence were positive for *T. gondii*, which suggests a participation of the rodents in pig infection. In addition, a bias regarding the presence of rodents could have happened during questionnaire answer. Moreover, the observation of rodents only on the medium size propriety could be related to the fact that, in larger proprieties, could be more difficult to identify the presence of these animals, because of an extensive area making it difficult for the owners to observe the rodents.

Although the presence of cats was not reported, this did not exclude the unnoticed presence of stray cats that could have contaminated the ration or the environment with *T. gondii* oocysts.

CONCLUSIONS

The prevalence of *Toxoplasma gondii* infection was 51.8% in pigs by IFAT, which demonstrates the importance of these animals as infection sources for humans by ingestion of raw or undercooked meat or meat products.

Positive association ($p>0.05$) of epidemiological variables (farm size, type of drinking water and presence of rodents) were important factors for *T. gondii* transmission in the studied area. Thus improvements on raising techniques could reduce *T. gondii* infection sources on pig herds.

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