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Zoonosis of public health interest in slaughtered Brazilian equidae

Zoonoses de interesse em saúde pública em equídeos brasileiros abatidos

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Abstract

The objective of this study was to investigate the presence of *Trichinella* spp. in the musculature and anti-*Toxoplasma gondii* and anti-*Leptospira* spp. antibodies in the serum of equidae slaughtered at two abattoir-cold storage facilities licensed by the brazilian Federal Inspection Service. Masseter muscle and blood samples were collected from 398 animals of both sexes and different ages originating from six different Brazilian states. The serum samples were subjected to the Indirect Fluorescence Antibody Test for *T. gondii* (IFAT ≥ 64) obtaining 46 (11.6%) samples reagent. For *Leptospira* spp. antibody quantification, the serum samples were tested by microscopic seroagglutination test (MAT ≥ 100), and 123 (30.9%) reagent results were obtained. It was possible to identify the most likely infecting serovar in 95 samples (77.2%): Hardjo (26.3%) and Autumnalis (12.6%) were the ones most prevalent. None of the 398 masseter muscle samples analyzed revealed any presence of *Trichinella* spp. larvae. The slaughtered equidae that were investigated were probably exposed to the etiologic agents of toxoplasmosis and leptospirosis, but they did not present *Trichinella* infection.
Key words: Slaughterhouse, equidae, *Toxoplasma gondii, Leptospira, Trichinella*

Resumo

O objetivo deste estudo foi investigar a presença de *Trichinella* spp. na musculatura e anticorpos anti-*Toxoplasma gondii* e anti-*Leptospira* spp. em soro de equídeos abatidos em dois matadouros-frigoríficos com serviço de Inspeção Federal. Amostras de músculo masséter e sangue foram coletados de 398 animais de ambos os sexos e várias idades, provenientes de seis diferentes estados brasileiros. As amostras de soro foram submetidas à Reação de Imunofluorescência Indireta para *T. gondii* (IFI ≥ 64) obtendo 46 (11,6%) amostras reagentes. Para *Leptospira* spp. as amostras de soro foram testadas pelo teste de soroaglutinação microscópica (MAT ≥ 100) e 123 (30,9%) amostras foram reagentes. Foi possível identificar o sorovar mais provável em 95 amostras (77,2%) sendo Hardjo (26,3%) e Autumnalis (12,6%) os mais encontrados. Nenhuma das 398 amostras de músculo masséter analisados revelou qualquer presença de larvas de *Trichinella* spp. Os equídeos destinados ao abate que foram investigados, provavelmente foram expostos aos agentes da toxoplasmose e leptospirose, porém não apresentaram infecção por *Trichinella*.
Palavras-chave: Matadouro-frigorífico, equídeos, *Toxoplasma gondii, Leptospira, Trichinella*

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Introduction

In Brazil, rearing of equines is not an economic activity aimed towards horse meat production. This kind of meat comes from animals that are old or discarded ones which were taken away from work or reproduction, and is destined solely for exportation, since the domestic Brazilian trade is insignificant. Brazilian social culture considers horses mainly as pet animals (TORRES; JARDIM, 1987; JUNQUEIRA, et al., 2005).

According to Inacio (2010), Brazil is the fourth largest supplier of this kind of meat in the world, accounting for 10.9%, following behind Argentina (28.7%), Canada (24.6%) and Poland (14.6%). Brazil is the third largest horse meat supplier to the European Union, which is the main destination of Brazilian meat, after Canada and Argentina. These exports decreased by 10% in 2009, partly because of restrictions imposed by the European Union.

This decrease in exports was due to rigorous new rules imposed on imports of this kind of meat by the European Union (the destination of 70% of Brazilian exports of horse meat) in 2009. Projects aimed at controlling residues and the use of medicine and other substances were demanded (INACIO, 2010).

Unlike Brazil, horse meat is commonly used for human consumption in some countries in Europe and Asia. This has raised concern in relation to the consumption of raw or undercooked meat, when this meat originates from potentially infected animals. This kind of consumption can be an important oral infection route for etiological agents such as *Toxoplasma gondii* and *Trichinella* spp.

Outbreaks of trichinellosis in Italy and France at the end of 1975 were caused by consumption of horse meat. Until then, equines had not been considered to present risks regarding this disease (MAILLOT, 1998). Outbreaks of human trichinellosis in European countries (MAILLOT, 1998) and in South America (ECHAZARRETA et al., 2010), and one reported a case of congenital toxoplasmosis in France, where a pregnant woman, reagent for toxoplasmosis before pregnancy, became reinfected after consuming raw horse meat imported. Was isolated from a newborn, strain of *Toxoplasma gondii* genotype atypical for Europe but has been described in South America (ELBEZ-RUBINSTEIN et al., 2009), indicate the importance of assessing the epidemiology of these agents in horses intended for export.

Trichinella infection has not been reported in animals or in humans in Brazil, even though it is endemic in Argentina, a neighboring country (BJORLAND et al., 1993; VENTURIELLO et al., 1998).

The prevalence of *T. gondii* infection in Brazil for equines varies according to the region, the diagnosis and the cutoff point chosen. Using indirect fluorescent antibody test, Garcia et al. (1999) and Vidotto et al. (1997) found prevalences of 12% (IFAT $\geq$ 64) and 31.55% (IFAT $\geq$ 16) respectively, in Paraná state. In Minas Gerais state, Naves et al. (2005) found that 12.82% (IFAT $\geq$ 64) were positive.

The incidence of human leptospirosis is high in countries with a tropical and/or subtropical climate (SOUZA, 1988; LEVETT, 2000). In Brazil, from 2007 to 2010, about 12.000 human cases were notified, of which around 10% were lethal (SINAN, 2011). Among animals, pigs and cattle are more susceptible than horses, goats and sheep (MORIKAWA, 2011). The aim of the present study was to research some of the zoonoses of public health interest in equines for slaughter.

Material and Methods

Sampling location

Samples were obtained from equines slaughtered at two refrigerated meat-exporting cold storage facilities licensed by the Federal Inspection Service in the state of Paraná, taken from randomly chosen batches. The target population was around 4000 animals and the sample size was calculated using the Epi Info 6.04 software (DEAN et al., 1994), with an estimated prevalence of 50%, error of 5%

and significance level of 5%, totaling 384 animals. We defined the prevalence of 50% to calculate the sample size to determine the prevalence of antibodies against various diseases. The study was registered and approved by the Ethics Committee on Animal Experimentation of the State University of Londrina, Paraná (Nº 60/09).

Blood and masseter sample collection

Between July 2009 and January 2010, adult equines of various ages and both sexes were studied, originating from the following states and cities in Brazil:

Paraná (n = 152) – Apucarana (4), Araruna (4), Borrazopolis (4), Cafezal do Sul (15), Campina Grande do Sul (27), Imbituva (13), Londrina (3), Lobato (33), Marquinho (12), Novo Itacolomi (7), Prudentopolis (12), Ribeirao Claro (9), Rio Bom (2), São João do Ivai (3) and Santa Fe (4);

Minas Gerais (n = 53) – Frutal (33) and Itapagipe (20);

Rio de Janeiro (n = 39) – Italva (39);

Goias (n = 6) – Caiaponia (6);

Mato Grosso do Sul (n = 99) – Coronel Sapucaia (10), Deodapolis (21), Paranaiba (22) and Rio Verde de Mato Grosso (46);

Mato Grosso (n = 49) – Caceres (20), Canarana (12) and Cuiaba (17).

398 samples of 10 mL of animal blood were collected after the animals had been stunned and bled. After clot retraction, the serum was placed in polyethylene tubes of capacity 1.5 mL, and was stored at –15°C until serological tests were performed in the Leptospirosis, Zoonosis and Public Health Laboratories of the State University of Londrina. We collected 50 g of masseter muscle and sent to laboratories for *Trichinella* spp. in the Federal Inspection Service located in the slaughterhouse.

Indirect Fluorescent Antibody Test – IFAT

The serological analyses to investigate the presence of anti-*T. gondii* antibodies were accomplished using the indirect fluorescent antibody test (IFAT) test, as described by Camargo (1974). Tachyzoites of RH samples were used as the antigen, along with conjugated anti-IgG (Sigma Chemical) and positive and negative controls for equine serum. Dilutions ≥ 1:64 for equines were considered to be positive (GARCIA et. al., 1999). The positive controls for *T. gondii* were obtained from naturally infected animals.

Microscopic seroagglutination Test (MAT)

MAT was used to detect the presence of anti-*Leptospira* spp. antibodies (MYERS, 1985). The serum samples were tested for 24 serovars of *Leptospira*: Australis, Bratislava, Autumnalis, Fortbragg, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Copenhageni, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarasovi, Sentot, Ballum and Andamana. The readings were performed using a dark-field microscope, at a magnification of 200x, and titers ≥ 100 were considered to be positive.

Enzymatic digestion for diagnosing Trichinella

10g of masseter muscle was collected from each of the 398 selected animals and 5g was subjected to the artificial digestion method for combined samples, with porcine pepsin (1:10,000), hydrochloric acid and a magnetic stirrer. This process is considered satisfactory when no more than 5% of the initial weight of the sample remains on the sieve after digestion. The samples were examined under a stereomicroscope, at a magnification of 15 to 20x. In cases of doubt or if the result was positive, the remaining 5g was used for a counter-assay. If the parasite was found, it had to be kept in ethyl alcohol 90%, for conservation and identification of the species (OIE, 2004).

Statistics

The prevalence was calculated for each etiological agent. Associations between the results obtained were investigated using the chi-square test and the Epi Info 6.04 statistical package (DEAN et al., 1994). The significance level of 5% was used.

Results

All of the 398 samples (100%) were negative for *Trichinella* spp. larvae. Forty-six (11.6%) of equines had antibodies against *T. gondii*, according to IFAT (Table 1). There was a significant difference (p = 0.0026) regarding the region of origin of the sampled animals: the lowest prevalence was registered in the central-western region (Table 2).

Table 1. Positive results in the IFAT serological test for *Toxoplasma gondii* among 398 serum samples from equines slaughtered at abattoir-cold storage facilities in Paraná state, in 2009-2010.

REGION	STATE	MUNICIPALITY	IFAT*
SOUTH	PR	Araruna	1
	PR	Borrazopolis	1
	PR	Cafezal do Sul	1
	PR	Campina Grande do Sul	3
	PR	Imbituva	5
	PR	Lobato	4
	PR	Londrina	2
	PR	Marquinho	4
	PR	Novo Itacolomi	1
	PR	Prudentopolis	1
	PR	Ribeirao Claro	2
	PR	Rio Bom	2
SOUTHEAST	MG	Itapagipe	2
	MG	Frutal	5
	RJ	Italva	4
CENTER- WEST	MS	Coronel Sapucaia	2
	MS	Deodapolis	1
	MT	Caceres	1
	MT	Cuiaba	4
TOTAL			**46 (11.6%)**

IFAT* Indirect fluorescence antibody test, cut-off ≥ 64 Positive results
RJ = Rio de Janeiro; MS = Mato Grosso do Sul; MT = Mato Grosso
MS = Mato Grosso do Sul;
Source: Elaboration of the authors.

Table 2. Positive results for *Toxoplasma gondii* through IFAT and *Leptospira* spp. through MAT, for slaughtered equines in abattoir-cold storage facilities in Paraná state, according to the region of origin, 2009-2010.

		Toxoplasma gondii*	***Leptospira* spp.****
REGION	**STATES SAMPLED**	Positive / Total (%)	
South	PR	27 / 152 (17.7)	53 / 152 (34.9)
Southeast	MG, RJ	11 / 92 (11.9)	23 / 92 (25)
Center-West	GO, MS, MT	8 / 154 (5.2)	47 / 154 (30.5)
	TOTAL	**46 / 398 (11.6)**	**123 / 398 (30.9)**
	Value of *p*	* 0.0026	** 0.2683

Source: Elaboration of the authors.

Out of the 398 serum samples that were tested for *Leptospira* spp., 123 (30.9%; 95% CI = 26.4-35.7) were reactive to MAT, with titers of between 100 and 1600. Of these, 73 (59.4%) reacted to a single serovar and 50 (40.7%) reacted to more than one serovar: 28 (22.8%) with the same titer for different serovars and 22 (17.9%) with a larger titer for one serovar, thus indicating the most likely serovar. In 95 reactive samples (77.2%), it was possible to identify the most likely infecting serovar (Table 3). There was no significant difference (p = 0.2683) regarding positive serological findings and Brazilian regions (Table 2).

The percentage of the samples that was positive to two of the etiological agents evaluated was 0,03% (14/398) (Table 4).

Table 3. Most likely *Leptospira* spp. serovars, detected through MST, among 398 serum samples from equines slaughtered at abattoir-cold storage facilities in Paraná state, 2009-2010.

SEROGROUP	SEROVAR	POSITIVE
Serjroe	Hardjo	25
Autumnalis	Autumnalis	12
Pyrogenes	Pyrogenes	8
Canicola	Canicola	8
Grippotyphosa	Grippotyphosa	8
Shermani	Shermani	8
Icterohaemorrhagiae	Copenhageni	5
Australis	Bratislava	2
Ballum	Castellonis	2
Cynopteri	Cynopteri	3
Icterohaemorrhagiae	Icterohaemorrhagiae	3
Australis	Australis	2
Autumnalis	Butembo	2
Pomona	Pomona	2
Tarasovi	Tarasovi	2
Autumnalis	Fortbragg	1
Serjroe	Wolffi	1
Djasiman	Sentot	1
	TOTAL	**95**

Source: Elaboration of the authors.

Table 4. Numbers of animals that were serologically positive for *Toxoplasma gondii*[1] and *Leptospira* spp.[2], among 398 equines slaughtered at abattoir-cold storage facilities in Paraná state, according to state and region of origin, 2009-2010.

REGION	STATE	*T.g.+L*	TOTAL (%)
South	PR	11	11/152
Southeast	RJ	0	0/39
	MG	1	1/53
Central-West	GO	0	0/6
	MT	1	1/49
	MS	1	1/99
	TOTAL	14	14/398 (0,03)

T.g. = Toxoplasma gondii; L = Leptospira spp.
[1] IFAT = Indirect Fluorescent Antibody Test ,
[2] MAT = Microscopic Seroagglutination.
Source: Elaboration of the authors.

Discussion

The negative results from searches for *Trichinella* spp. larvae among the equines of this study are compatible with other studies developed in Brazil, on rats and pigs. Catão et al. (1975) used the official digestion method on 6452 samples of diaphragms of adult pigs in the states of Paraná (75.69%), Minas Gerais (23.12%), Goiás (0.91%) and São Paulo (0.26%) and did not observe the larvae of this parasite.

Paim and Cortes (1979) used a trichinoscope and did not detect the larvae of this nematode in 594 diaphragms of *Rattus norvegicus* from the port area of the municipality of Santos, SP, even though this is a favorable place for entry of rodents from other regions. Daguer, Geniz and Santos (2005) also used the official method and analyzed the musculature of 3774 adult pigs from 68 municipalities in the three states of the southern region of Brazil between 2002 and 2004, obtaining negative results regarding the presence of *Trichinella* spp. larvae. The results relating to *Trichinella* spp., both in the present study and in others developed in Brazil, confirm the country's status of being free from trichinellosis, as published in June 2010 (OIE, 2011).

Pozio et al. (2002) analyzed 2502 serum samples from equines, using the ELISA method, and circulating antibodies were detected only four to five months after experimental infection with *Trichinella* spp., even though infecting larvae were present, which indicates that this technique is inefficient for diagnosing or determining the prevalence of infection in equines. Artificial digestion of 5g of preferential muscle tissue continues to be the preferred method for prevention of human trichinellosis in horse meat (OIE, 2004).

After detecting *Trichinella spiralis* infection in a horse in Serbia, Murrell et al. (2004) investigated how it acquired the disease, given that horses are herbivores. The infected horse's history was traced and it was found that the animal had been provided with food scraps containing meat with the aim of improve its condition before sale. In the same study, investigations revealed that this was a common practice among breeders, thus demonstrating that intentional exposure of horses to meat residues represents a risk factor for trichinellosis.

In relation to toxoplasmosis, in the present study, 11.6% of the samples were positive for *Toxoplasma gondii,* thus agreeing with Vidotto et al. (1997), who obtained positive results from 14.3%, using IFAT, in a study on 561 serum samples from equines originating from the states of Paraná, São Paulo,

Mato Grosso and Mato Grosso do Sul, which were also slaughtered in one of the abattoir-cold storage facilities of the present study. These results are similar even though there was an interval of 15 years. Garcia et al. (1999) registered prevalence of 12.1% among 173 serum samples from equines on farms in Jaguapitã, Paraná state.

Lower percentages than in the results from the present study were demonstrated by Mendonça et al. (2001), who obtained positive results from 1.5% of 343 samples tested using IFAT, from equines originating from two regions of Bahia. Naves et al. (2005), found prevalence of 5.1% when evaluating 117 serum samples from Mangalarga horses on three stud farms in Uberlândia, Minas Gerais state. Camossi, Silva and Langoni (2010) tested 253 serum samples from horses in the region of Botucatu, São Paulo state, and found seroprevalence of 0.4% for *T. gondii*. However, Larangeira, Ishizuka and Hyakutake (1985) found prevalence of 27.6% using IFAT, in an analysis on 750 equines on farms in Mato Grosso do Sul state.

These differences can be attributed to various factors such as: resistance of the equine species to infection, environmental conditions, type of strain, diet provided, water supply and the density of cats or wild felines in the environment (VIDOTTO et al., 1997; TENTER; HECKEROTH; WEISS, 2000; MENDONÇA et al., 2001; NAVES et al., 2005; KIJLSTRA; JONGERT, 2009; KOUAM et al., 2010). According to Kouam et al. (2010), the kind of activity and the location of the equines have a significant effect on the presence of infection by *T. gondii*.

In relation to Leptospirosis, the present study demonstrated that out of the 398 serum samples tested, 30.9% were positive and in 77.2% of these it was possible to identify the most likely serovar: Hardjo (26.3%) and Autumnalis (12.6%) were the most present ones. The Hardjo serovar occurs in cattle and its presence in equines is probably due to rearing these animals together. The presence of the Autumnalis serovar suggests that the equines had been in contact with wild animals.

Similar prevalence results were obtained by Favero et al. (2002) in an analysis on 2903 equines from the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Minas Gerais, Mato Grosso, Paraíba and Piauí, between 1984 and 1997, using MAT to investigate 24 serovars, in which positive results were obtained from 29%. The most frequent serovars were Icterohaemorrhagiae in Paraná, São Paulo, Santa Catarina, Rio de Janeiro and Minas Gerais; Grippotyphosa in Mato Grosso; Pirogenes in Paraíba and Patoc in Rio Grande do Sul; and no positive results were observed from Piauí.

Linhares et al. (2005) evaluated 182 equines in Goiania and of these, 45.05% were reactive to one or more of the 16 serovars tested. The most frequent serovar was Icterohaemorrhagiae. Hashimoto et al. (2007) studied 320 equines that were used to pull wagons in Londrina, Paraná, between 1996 and 2005, using MAT to investigate 22 serovars, and obtained positive results from 66.88%. Icterohaemorrhagiae was the most likely serovar. Lilenbaum et al. (2010) tested 140 female Campolina horses with low reproductive performance, using MAT to investigate 21 serovars of *Leptospira* spp., and obtained positive results from 58.6%, of which 87.8% were the Bratislava serovar. This reinforced indications that this serovar had adapted to horses and was provoking mild clinical signs associated with reproductive failure.

In a study on 150 employees at a cold storage facility who were tested using MAT to investigate 22 serovars. Gonçalves et al. (2006) obtained positive results for *Leptospira* spp. from 4%, with predominance of the Hardjo serovar. In the rendering sector, three employees were positive, thus indicating that this sector presented a high risk of infection because of the great exposure to organs and entrails of animals that might be infected, and demonstrating that the infections among the employees occurred occupationally.

Rodents seem to be the primary hosts for the Icterohaemorrhagiae serovar, cattle for the Hardjo, Hebdomadis and Castellonis serovars, equines for Bratislava, dogs for Canicola, pigs for Pomona and wild animals for Grippotyphosa (SANTA ROSA et al., 1975; FAINE, 1982; LINS;LOPES, 1984; ELLIS, 1984; FAVERO et al., 2002; LILENBAUM et al., 2010).

Factors relating to movement of equines, environmental factors, rodent sustainment conditions and direct contact with urine contaminated with *Leptospira* spp. seem to be the main risk factors for infection (ROBERTS, 1969; FAINE, 1982; HASHIMOTO et al., 2007).

Kouam et al. (2010) studied serum from 773 equines in four regions of Greece, using ELISA to investigate the presence of anti-IgG for *Toxoplasma, Leishmania, Echinococcus* and *Trichinella*. They found that anti-*T. gondii* antibodies were present in all the regions, with a general prevalence of 1.8%. The prevalence of infection with *Leishmania* spp. was 0.3% and with *Echinococcus* and *Trichinella*, 0.1%.

These last two were detected only in racehorses in the Attica region. Only one animal was reactive to all three etiological agents: *T. gondii, Leishmania* spp. and *Trichinella* spp. These researchers suggested that the risk of contracting these infections through equines was low. On the other hand, in the present study, the percentage of positive results for two etiological agents was 0,03% (Table 4), thus showing a lower greater prevalence of interaction than was reported from Greece, which may decrease the risk of infection among humans.

The equines in the present study were in contact with agents for toxoplasmosis and leptospirosis, but were not exposed to the agent for *Trichinella* infection. They shared the same environment that humans used and may serve as a source of infection for *Leptospira* spp.

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