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# Seroprevalence of *Brucella ovis*-epididymitis, smooth-*Brucella*, leptospirosis, toxoplasmosis, and Maedi-Visna in sheep slaughtered in Minas Gerais State, Brazil

## Soroprevalência de *Brucella ovis*, *Brucella lisa*, leptospirose, toxoplasmose e Maedi-Visna em ovinos abatidos em Minas Gerais, Brasil

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### ABSTRACT

The present study aimed to estimate the prevalence of antibodies against *Brucella ovis*-epididymitis, smooth-*Brucella*, leptospirosis, toxoplasmosis and Maedi-visna in sheep slaughtered in Minas Gerais, Brazil and to study their simultaneous occurrence, including caseous lymphadenitis, at sheep and flock levels. The study was conducted at a sheep slaughterhouse with Federal Inspection Service. Sera from 594 animals from 21 flocks were collected, in 2007. The agar gel immunodiffusion (AGID) was employed to detect anti-*B. ovis* and anti-Maedi Visna antibodies, whereas Rose Bengal (RB) and the 2-mercaptoethanol test (2ME) were used to test anti-smooth *Brucella* antibodies. For the detection of anti-*Leptospira* antibodies, sera were examined by microscopic agglutination test (MAT), while for the detection of IgG antibodies to *Toxoplasma gondii* ELISA was used. Prevalence of antibodies against smooth *Brucella*, *B. ovis*-epididymitis, *Leptospira* spp., toxoplasmosis and Maedi-Visna found in sheep from Minas Gerais was 0.00%, 24.04%, 25.96%, 10.46% and 3.08%, respectively; whereas the seroprevalence in flocks was 0.00%, 80.95%, 90.48%, 71.43% and 23.81%, respectively. Moreover, when data on antibodies anti-*Corynebacterium pseudotuberculosis*, previously obtained, were included, about 60% of the flocks showed animals that were exposed to four or more of the studied agents. However, only 25.47% of the sheep exhibited simultaneously antibodies against more than one pathogen. Thus, data from the present study on sheep slaughtered in Minas Gerais, Brazil, showed no antibodies to smooth-*Brucella* and a low frequency of antibodies anti-Maedi Visna lentivirus, and a high and widespread seroprevalence of *B. ovis*, *Leptospira* spp., and *T. gondii* among animals and flocks.

**Keywords:** Brucellosis. *C. pseudotuberculosis*. *Leptospira* spp. Maedi-Visna. *T. gondii*. Ovine. Serodiagnosis.

### RESUMO

O presente estudo teve como objetivo estimar a prevalência de anticorpos contra *Brucella ovis* (epididimite ovina), *Brucella lisa*, leptospirose, toxoplasmose e Maedi-visna em ovinos abatidos em Minas Gerais, Brasil, e estudar sua ocorrência simultânea, incluindo linfadenite caseosa, nos ovinos e nos rebanhos. O estudo foi realizado em um abatedouro de ovinos com Serviço de Inspeção Federal. Soros de 594 animais de 21 rebanhos foram coletados, em 2007. A imunodifusão em gel de ágar (IDGA) foi empregada para detectar anticorpos anti-*B. ovis* e anticorpos anti-Maedi Visna, enquanto o teste do antígeno acidificado tamponado (AAT) e o teste de 2-mercaptoetanol (2ME) foram utilizados para testar anticorpos

anti-*Brucella* lisa. Para a detecção de anticorpos anti-*Leptospira*, os soros foram examinados pelo teste de aglutinação microscópica (MAT), enquanto que para a detecção de anticorpos IgG para *Toxoplasma gondii*, foi usado o ELISA. A prevalência de anticorpos anti-*Brucella* lisa, *B. ovis*, *Leptospira* spp., toxoplasmose e Maedi-Visna encontrados em ovinos de Minas Gerais foi de 0,00%, 24,04%, 25,96%, 10,46% e 3,08%, respectivamente; enquanto a soroprevalência em rebanhos foi de 0,00%, 80,95%, 90,48%, 71,43% e 23,81%, respectivamente. Além disso, quando dados de anticorpos anti-*Corynebacterium pseudotuberculosis*, previamente obtidos, foram incluídos, cerca de 60% dos rebanhos apresentaram animais expostos a quatro ou mais dos agentes estudados. No entanto, apenas 25,47% dos ovinos exibiram simultaneamente anticorpos contra mais de um patógeno. Assim, os dados do presente estudo sobre ovinos abatidos em Minas Gerais, Brasil, mostram que ausência de anticorpos anti-*Brucella* lisa e baixa frequência de anticorpos anti-Maedi Visna, e uma soroprevalência alta e generalizada de *B. ovis*, *Leptospira* spp. e *T. gondii* entre animais e rebanhos.

**Palavras-chave:** Brucelose. *C. pseudotuberculosis*. *Leptospira* spp. Maedi-Visna. *T. gondii*. Ovinos. Sorodiagnóstico.

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## Introduction

In Minas Gerais State, Brazil, the number of sheep has increased 93.11% in recent years; from 116,796 in 2000 to 225,549 in 2008 (Instituto Brasileiro de Geografia e Estatística, 2008). This is much greater than the overall Brazilian growth, which recorded a 12.5% increase in sheep herds during the same period (Instituto Brasileiro de Geografia e Estatística, 2008). The rapid growth of commercial sheep herds is due to an expansion of sheep meat markets in all regions of the country. Despite the significant raise in Brazilian production of lamb, this is still insufficient to supply consumer's demands. Consequently, many breeders of beef cattle are now investing in sheep husbandry, with the acquisition of animals from other Brazilian regions. This increase in sheep production has resulted in considerable transit of animals into Minas Gerais State, with acquisition of animals mainly from the Northeast

Region (McManus et al., 2019; Instituto Brasileiro de Geografia e Estatística, 2008).

Information on the sanitary status of sheep slaughtered is a good parameter to evaluate the epidemiological situation of important diseases of economic and public health significance, besides being less expensive than conventional surveys.

Brucellosis is a zoonotic disease caused by *Brucella* spp. It is widespread, and affects different animal species besides humans (Bovine brucellosis..., 2009). In sheep, it is usually caused by *Brucella melitensis*, a smooth *Brucella* species, and the economic importance of ovine brucellosis is based on direct losses and on sanitary barriers to international trade of animals and their products (Corbel et al., 2006). Ovine contagious epididymitis is predominantly associated with *Brucella ovis* infection, a rough *Brucella* species (Burgess, 1982). Infection with *Brucella abortus*, another smooth species, is rare, but both may cause sporadic abortion (Allsup, 1969; Ocholi et al., 2005). In Brazil, serological surveys suggests that *B. melitensis* is exotic in the country (Poester et al., 2002), but infection by *B. abortus* and *B. ovis* are present (Azevedo et al., 2004; Carvalho et al., 2010; Clementino et al., 2007; Marques, 2006; Nozaki et al., 2004; Pinheiro et al., 2008, 2009).

The agent of Leptospirosis, *Leptospira* spp., is worldwide distributed and the seroprevalence in sheep varies according to the region, however serovars Hardjo, Pomona, Grippotyphosa and Bratislava are the most frequently reported in sheep (Faine et al., 2000). In Europe, the disease is prevalent (4.2% to 49.3%), and Pomona and Hardjo are the predominant serovars (Leon-Vizcaino et al., 1987). In a study conducted in New Zealand, 5.7% of the flocks were seropositive, with predominance of serovars Hardjo and Pomona (Dorjee et al., 2008). In a sheep survey conducted in the State of Rio Grande do Sul, Brazil, the most prevalent (30.7%) serovar was Hardjo, followed by serovar Sentot (16.8%) (Herrmann et al., 2004). In Minas Gerais, although leptospirosis was already detected, vaccination against leptospirosis is not frequent among sheep flocks (Guimarães et al., 2009a).

Ovine toxoplasmosis, caused by *Toxoplasma gondii*, is responsible for reproductive losses due to abortion and neonatal death. It causes between 1 and 2% of neonatal losses per year in sheep from United Kingdom (Buxton et al., 2007). The worldwide serum prevalence of ovine toxoplasmosis during 1989-2009 ranged from 3% in Pakistan to 92% in Serbia (Dubey, 2009; Kamani et al., 2010; Zedda et al., 2010). In Brazil, serological surveys in small ruminants show infection rates ranging from 30% to 50% (Carneiro et al., 2009; Romanelli et al., 2007; Ueno et al., 2009).

Maedi-visna virus (MVV) and Caprine arthritis-encephalitis virus (CAEV) initially isolated in sheep and goats, respectively, are retroviruses from the *Lentivirus* genus assigned to a single group, currently referred to as small ruminant lentiviruses (SRLVs) (Caroline et al., 2010). SRLVs are responsible for significant economic losses, such as decrease in milk production (Smith & Cutlip, 1988; Snowden et al., 1990), due to mastitis, decrease in weight gain of lambs (Dungu et al., 2000), reduce in animal lifespan and increase in mortality rates (Arsenault et al., 2003; Sigurdsson et al., 1952). In Brazil, MVV seropositive sheep were detected in 1988 in Rio Grande do Sul from imported animals and in herds with a history of importing animals (Dal Pizzol et al., 1989). Thereafter, there were records of the disease in some Brazilian States (Lombardi et al., 2009; Oliveira et al., 2006; Sotomaior & Milczewski, 1997; Yorinori, 2001; Yorinori et al., 2003).

*Corynebacterium pseudotuberculosis* is the causative agent for caseous lymphadenitis (CLA) in goats and sheep. CLA is also a very important disease of sheep production worldwide, with prevalence varying from 3.6% to 100% of infected animals (Guimarães et al., 2011b). It is distributed in Brazil mainly in the northeast states such as Ceará with 24.6% herd prevalence, Paraíba with 28.9%, Rio Grande do Norte with 33.2% and Piauí with 33.7% (Linfadenite..., 2019). Minas Gerais State its prevalence in sheep was estimated in 70.9%, with 95.9% of flocks infected (Guimarães et al., 2009b). CLA was found in 43.7% of slaughtered sheep in Minas Gerais State (Guimarães et al., 2011a). Although the presence of infection by the diseases is by itself a health problem in the flock, very little is known about the simultaneous occurrence of those diseases in sheep or their flocks. Therefore, the aims of the present study were (i) to determine the seroprevalence of brucellosis, *B. ovis*-epididymitis, leptospirosis, toxoplasmosis and Maedi-visna infection in slaughtered sheep in Minas Gerais State, Brazil and (ii) to study their simultaneous occurrence, including caseous lymphadenitis, at sheep and flock levels.

## Material and Methods

### Sampling

This study was conducted at a sheep slaughterhouse with Federal Inspection Service, localized in the municipality of Patrocínio, Minas Gerais State, Brazil. Visits to the slaughterhouse were made every 30 days, between July and December 2007. Blood samples were collected by jugular vein puncture from animal that were slaughter under humane conditions. Around one animal among each three slaughtered ones was sampled, in order to make possible to inspect the lymph nodes following the slaughter line of the slaughterhouse. Serum was separated by centrifugation and stored at -20°C until tested. Serum samples from 594 animals from 21 flocks were tested.

### Serologic assays

The agar gel immunodiffusion (AGID) employing *B. ovis* soluble antigen (Instituto de Pesquisas Veterinárias Desidério Finamor [IPVDF], Brazil) was performed as previously described (Souza et al., 2002). The Rose Bengal (RB) test (Tecpar, Brazil) was used as screening test according to Ferreira et al. (2003), and the 2-mercaptoethanol test (2ME) (Tecpar, Brazil) as a confirmatory test for the detection of anti-smooth *Brucella* (*B. abortus* and *B. melitensis*) antibodies (Manual técnico..., 2006).

For the detection of anti-*Leptospira* antibodies, sera were examined by microscopic agglutination test (MAT), using 24 serovars representing all the described serogroups (Table 1). Sera with titers  $\geq 100$  were considered reactive and the antigen that presented the highest titer was considered as the infective serovar (Faine et al., 2000).

Sera were assayed in duplicate by an in-house ELISA for the detection of IgG antibodies to *Toxoplasma gondii* (Van der Puije et al., 2000) with slight modifications. Briefly, 96-well-plates (Costar, Corning, USA) were coated with 100  $\mu$ L/well (0.5 $\mu$ g/100 $\mu$ L) of the soluble tachyzoite antigen (STAg) from *T. gondii* (RH strain). All the serum

Table 1 – Serovars of *Leptospira* spp. used in the microscopic agglutination test (MAT) for testing sheep slaughtered in Minas Gerais State, Brazil

Serogroups	Serovar	Strain
Australis	Australis	Ballico
	Bratislava	Jez Bratislava
Autumnalis	Autumnalis	Akiyamy A
Ballum	Castelonis	Castellon 3
Bataviae	Bataviae	Swart
Canicola	Canicola	Hond Utrecht IV
Grippotyphosa	Grippotyphosa	Moskva V
Hebdomadis	Hebdomadis	Hebdomadis
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Mini	Szwajizak	Szwajizak
Pomona	Pomona	Pomona
Pyrogenes	Pyrogenes	Salinem
Sejroe	Hardjo	Hardjoprajitno
	Wolffi	3705
Tarassovi	Tarassovi	Perepelicin

samples were tested at a dilution of 1:400. Peroxidase-labelled donkey IgG anti-sheep IgG A-3415 (Sigma-Aldrich, USA) was used as a secondary antibody at a 1:7500 dilution. Six negative sera and two positive sera, previously tested by an indirect fluorescent antibody test (Andrade et al., 2013), were included as control. The cut-off value for each ELISA plate was calculated as the absorbance mean of six serum samples of sheep tested negative for *T. gondii*, plus three standard deviations tested on each plate.

Antibodies anti-Maedi Visna virus were tested by a commercial AGID kit (Biovetech, Brazil), following manufacturer's instructions.

### Data analysis

Prevalences and confidence intervals were calculated as previously described (Bennett et al., 1991; Noordhuizen et al., 2001) using the packages binGroup (Zhang et al., 2011) and epiR (Stevenson et al., 2012) of R software version 2.14.1 (R Development Core Team, 2015).

Correspondence analysis (Greenacre & Blasius, 2006) was used to study the occurrence of the diseases and its relationship with positivity and negativity of sheep and herd size using the InfoStat version 2015 (Di Rienzo et al., 2015). In the correspondence analyzes, the relationship between the categories was represented in a two-dimensional graph. The relatedness between disease diagnostic results and sheep herd size was demonstrated by evaluating which variables were plotted closely together. Data on antibodies anti-*C. pseudotuberculosis*, previously obtained (Guimarães et al., 2011a), were included in the evaluation of simultaneous infection and in correspondence analysis.

### Results

During the period of the study, 594 serum samples of sheep from 21 flocks were collected at the slaughterhouse. Sampled flocks were located in 16 different municipalities of Minas Gerais State and in two municipalities from nearby states, Vicentinópolis, in the State of Goiás and Ribeirão Preto in the State of São Paulo (Table 2). The mean number of sampled animals per flock was 28.29 (range: 2 to 106).

Seroprevalence of animals and flocks for all investigated diseases are summarized in Table 3. Data of CLA seroprevalence from the same sampling frame previously published (Guimarães et al., 2011a) was included in the analysis of occurrence of simultaneous infection and in correspondence analysis. The distribution of co-infections at animal and flock levels, for all studied diseases, including CLA data (Guimarães et al., 2011a), is shown in Tables 4 and 5, respectively.

Correspondence analysis was performed to evaluate the relationship between the occurrence of the serum antibodies in sheep and the herd size. The representation of the two dimensions and expression of the values of

the third dimension are shown in Figure 1. Those three dimensions explains 48.61% of the total variation, with 16.92% explained by 1st dimension, 16.57% by the 2st dimension and 15.12% by the 3rd dimension.

Table 2 – Number of animals sampled by municipality and statel

Property Number	City and State	Number of animals
32	Bonfinópolis-MG	37
38	Campina Verde-MG	2
20	Frutal-MG	26
43	Frutal-MG	11
58	Itauna-MG	3
1	João Pinheiro-MG	41
4	Oliveira-MG	28
40	Paraopeba-MG	9
42	Patrocínio-MG	35
21	Perdizes-MG	106
46	Porteirinha-MG	10
17	Prata-MG	16
41	Ribeirão Preto -SP	39
45	Teófilo Otoni-MG	53
23	Uberaba-MG	16
31	Uberaba-MG	46
35	Uberaba-MG	15
33	Uberlândia-MG	17
44	Unai-MG	28
13	Várzea da Palma-MG	37
34	Vicentinópolis-GO	19
Total		594

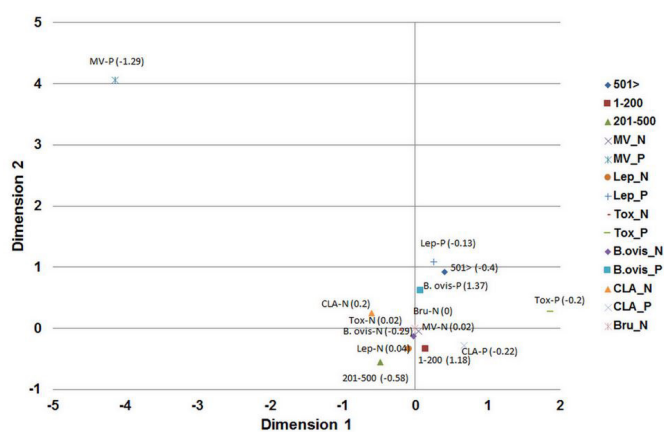


Figure 1. Correspondence analysis of the relationship between the occurrence of seropositivity to brucellosis (Bru\_N= seronegatives), *B. ovis*-epididymitis (*B. ovis*\_N= seronegatives; *B. ovis*\_P= seropositives), leptospirosis (Lep\_N= seronegatives; Lep\_P= seropositives), toxoplasmosis (Tox\_N= seronegatives; Tox\_P= seropositives), Maedi-Visna (MV\_N= seronegatives; MV\_P = seropositives) and caseous lymphadenitis (CLA\_N= seronegatives; CLA\_P= seropositives) in sheep and the herd size (1-200, 201-500 and > 501). This three dimensional representation explains 48.61% of the total variation, with 16.92% explained by 1st dimension, 16.57% by the 2st dimension and 12% by the 3rd dimension. The correspondence analysis dimensional representation is interpreted by considering which categories are plotted closely together.

Table 3 – Sheep and flock seroprevalence of brucellosis, *B. ovis*-epididimitis, leptospirosis, toxoplasmosis and Maedi-Visna in sheep slaughtered in Minas Gerais State, Brazil, 2007

Disease	Animal Prevalence						Flock Prevalence			Parameters of the tests		Ref.
	Apparent Prevalence			True Prevalence			%	95% CI (%)		%		
	%	95% CI (%)*		%	95% CI (%)			Min.	Max.	Sen	Spe	
	Min.	Max.		Min.	Max.							
<i>Brucellosis</i>	0.00	0.00	0.001	0.00	0.00	0.06	0.00	0.00	16.11	98.60	100.00	Ferreira et al. (2003), Nielsen (2002)
<b><i>B. ovis</i></b> infection	16.64	15.17	18.10	24.04	22.67	25.46	80.95	58.09	94.55	69.20	100.00	Xavier et al. (2011)
<b><i>Leptospira spp.</i></b>	28.15	22.49	33.82	25.96	24.74	27.20	90.48	69.62	98.83	98.20	96.40	Bajani et al. (2003)
<i>Hardjo</i>	17.57	14.69	20.44	14.76	13.74	15.82	90.48	69.62	98.83			
<i>Icterohaemorrhagiae</i>	8.22	4.43	12.00	4.88	4.15	5.66	52.38	29.78	74.29			
<i>Hebdomadis</i>	0.00	0.00	0.001	0.00	0.00	0.00	0.00	0.00	16.11			
<i>Bratislava</i>	2.22	0.98	3.45	0.00	0.00	0.00	47.62	25.71	70.22			
<i>Pomona</i>	0.00	0.00	0.001	0.00	0.00	0.00	0.00	0.00	16.11			
<i>Grippotyphosa</i>	2.30	1.59	3.01	0.00	0.00	0.00	47.62	25.71	70.22			
<i>Toxoplasmosis</i>	9.57	5.77	13.38	10.46	9.33	11.66	71.43	47.82	88.72	68.40	97.30	Van der Puije et al. (2000)
<i>Maedi-Visna</i> <i>infection</i>	2.46	1.01	3.91	3.08	2.60	3.63	23.81	8.22	47.17	79.70	100.00	Pinheiro (2001)

\*CI = confidence interval.

Table 4 – Distribution of co-infections in sheep slaughtered in Minas Gerais State, Brazil, 2007

CLA <sup>1</sup>	Leptospirosis	<i>B. ovis</i>	Toxoplasmosis	Maedi-Visna	Sheep	%
Neg <sup>2</sup>	Neg	Neg	Neg	Neg	177	30.05
POS <sup>3</sup>	Neg	Neg	Neg	Neg	156	26.49
Neg	POS	Neg	Neg	Neg	58	9.85
POS	POS	Neg	Neg	Neg	43	7.30
Neg	Neg	POS	Neg	Neg	35	5.94
POS	Neg	POS	Neg	Neg	30	5.09
POS	Neg	Neg	POS	Neg	24	4.07
Neg	POS	POS	Neg	Neg	13	2.21
POS	POS	POS	Neg	Neg	11	1.87
Neg	Neg	Neg	POS	Neg	10	1.70
POS	POS	Neg	POS	Neg	9	1.53
Neg	Neg	POS	POS	Neg	7	1.19
Neg	POS	Neg	POS	Neg	5	0.85
Neg	Neg	Neg	Neg	POS	4	0.68
POS	Neg	POS	POS	Neg	4	0.68
Neg	Neg	POS	Neg	POS	1	0.17
Neg	POS	Neg	Neg	POS	1	0.17
Neg	POS	POS	Neg	POS	1	0.17
POS	POS	POS	POS	Neg	1	0.17

<sup>1</sup>CLA: Caseous lymphadenitis; <sup>2</sup>Neg: negative; <sup>3</sup>POS: positive.

Table 5 – Distribution of co-infections among the 21 flocks supplying sheep to a slaughterhouse in the State of Minas Gerais, Brazil, 2007

CLA <sup>1</sup>	Leptospirosis	<i>B. ovis</i>	Toxoplasmosis	Maedi-Visna	Flocks	%
POS <sup>3</sup>	POS	POS	POS	POS	4	19.05
POS	POS	POS	POS	Neg	8	38.10
POS	POS	POS	Neg	Neg	4	19.05
POS	POS	Neg	POS	POS	1	4.76
POS	POS	Neg	POS	Neg	1	4.76
POS	POS	Neg	Neg	Neg	1	4.76
POS	Neg <sup>2</sup>	POS	POS	Neg	1	4.76
POS	Neg	Neg	Neg	Neg	1	4.76

<sup>1</sup>CLA: Caseous lymphadenitis; <sup>2</sup>Neg: negative; <sup>3</sup>POS: positive.

## Discussion

Results from the present study showed that a large proportion of sheep slaughtered in Minas Gerais State exhibited antibodies against *Leptospira* spp., *B. ovis* or *T. gondii* and that those antibodies were also highly prevalent at flock level. Moreover, evidence of infection or exposure to more than one of the studied agents was very frequent, about 60% of the flocks showed antibodies against four or more of them (Table 5). However, serum antibodies against more than one of the studied pathogens (Table 4) were simultaneously observed only 25.47% of the sheep.

Data from the state agency for animal health (Instituto Mineiro de Agropecuária - IMA) show that, in the last years, there was a large influx of animals from different states of the country (Bahia, Distrito Federal, Espírito Santo, Goiás, Rio de Janeiro, Sergipe, and São Paulo) to Minas Gerais State, mainly for reproduction. This large transit of animals towards Minas Gerais, associated with the absence of specific national health legislation for sheep, could have favored the introduction of and can explain the widespread distribution of the studied diseases into sheep slaughtered in the State (Guimarães et al., 2009b). The observation that only about 30% of the sampled population (Table 3) was free from infection of the infectious agents studied or *C. pseudotuberculosis* (Guimarães et al., 2011a) is of great concern. The diseases evaluated in the present study and CLA, previously studied in the same population (Guimarães et al., 2011a), are associated with major economic losses to sheep production worldwide (Guimarães et al., 2011b) and some of them are also related to important public health issues. Our results indicate an urgent need for implementation of sheep health programs to control those major diseases in Minas Gerais by flock owners and state animal health agents in order to improve the sanity and productivity of sheep in the State.

The high rate of antibodies against *Leptospira* spp., *B. ovis* and *T. gondii* observed in the present study suggests that infection by these microorganisms may play an important role in the etiology of reproductive problems in sheep in the region, since leptospirosis, *B. ovis*-epididymitis and toxoplasmosis are major causes of abortion and reproductive failure in sheep (Burgess, 1982; Carvalho et al., 2010; Innes et al., 2009; Lilenbaum et al., 2009). Corroborating this hypothesis, it is important to take into account that our finding showed that 8.5% of the slaughtered animals showed antibodies against to two or more of those three agents of reproductive diseases (Table 4) and that abortion is frequently reported in sheep flocks from Minas Gerais (23.9%, 51/213) (Guimarães et al., 2009a).

Anti-*Leptospira* antibodies were the most prevalent and widely distributed at both levels, sheep and flock. It was only surpassed by CLA, which was previously shown to have a higher seroprevalence using the same sampling frame (Guimarães et al., 2011a). These high rates of serologic evidence of infection by *Leptospira* spp. in sheep were expected, since previous studies reported similar rates in other countries or in other Brazilian States (Dorjee et al., 2008; Herrmann et al., 2004; Martins et al., 2012). Moreover, in the survey performed by Guimarães et al. (2009b) using 213 sheep flocks in Minas Gerais State, only 2 (1.0%) reported the use of vaccination against leptospirosis, which supports our findings. The most common reactions were directed towards serovar Hardjo, as previously reported in sheep and goat flocks from Brazil (Herrmann et al., 2004; Martins et al., 2012), which could probably be due to close contact between sheep and cattle. The serovar Icterohaemorrhagiae, the second most prevalent in animal and flocks, was also among the most frequently related to infection in Brazilian sheep (Martins et al., 2012; Melo et al., 2010). Analysis of the Table 3 suggests that only infection by serovars Harjo and Icterohaemorrhagiae are epidemiologically significant, as the true prevalence and its confidence interval for infection by the other tested serovars showed null results. Another important aspect associated to the high seroprevalence of leptospirosis is its impact in public health since sheep are carriers of bacteria in kidneys over a long period (Dorjee et al., 2008). This prolonged elimination could constitute a zoonotic risk for people in contact with the sheep, as handlers, farmers and workers in slaughterhouses. The management of reproductive animals or the lack of vaccination, which are more difficult in larger herds, can also contribute to the spread of the disease.

Serological surveys in other States have shown the presence of the *B. ovis* in sheep in different regions of the country with large differences in the frequencies of positive animals. Studies carried out in the state of Paraíba and Bahia showed 8.59% of seropositive herds and 3.27% seropositive animals among 183 sera tested, respectively (Clementino et al., 2007; Silva et al. 2009). Moreover, in 2013, a study carried out by Araújo et al. (2013) also in Bahia, observed 6.94% positive animals among 793 sampled, whereas in Rio Grande do Sul in 2015, 2.89% of 1800 animals analyzed were seropositive (Machado et al., 2015).

Our findings for *B. ovis*-epididymitis suggest that the infection is highly spread among herds and animals slaughtered in Minas Gerais State. Despite comparisons among the present results and others are difficult due differences in experimental designs, our results suggest a great increase

in seroprevalence of *B. ovis* in sheep population in Minas Gerais, as a 2002 study estimated a seroprevalence of 5.3% for animals and of 29.4% for herds in the State (Marques, 2006). This increase in seroprevalence could be explained by the high influx of sheep into flocks of Minas Gerais State, which is associated to its recent establishment and to the introduction of animals from different regions of country, which effectively contributes to the spread of infectious diseases. Furthermore, just a small fraction of animals that move from flock to flock are usually tested for *B. ovis* infection (Marques, 2006) and only a few sheep flock owners (14/213, 11.7%) requires any animal health statement for the introduction of sheep into their flocks (Guimarães et al., 2009a). Those findings indicate that the polices of the Programa Nacional de Sanidade de Caprinos e Ovinos (National Caprine and Ovine Health Program) should be quickly implemented in order to reduce the prevalence and incidence of infection by *B. ovis* among sheep in Minas Gerais State (Ministério da Agricultura, Pecuária e Abastecimento, 2005).

Our findings concerning brucellosis confirm previously unpublished data from our group on Minas Gerais State and also studies in other Brazilian States (Poester et al., 2002), which point toward that Brazil is free from *B. melitensis* infection. The other possibly source of smooth *Brucella* spp. infection to sheep, in the absence of *B. melitensis* infection, is the close contact with cattle, which is the natural hosts of *B. abortus*. The establishment of a compulsory vaccination program in the early 1990's markedly reduced the seroprevalence of bovine brucellosis in Minas Gerais State to levels lower than 1.0% of infected animals reported in 2002 (Gonçalves et al., 2009). The absence of any brucellosis seropositive sheep from our sampling seems to be also influenced by this low rate of *B. abortus* infected cattle in the State.

For *T. gondii*, the data showed high flock seroprevalence (71.43%), although a low proportion of animals (9.57%) present serological evidence of the infection. High sheep flock seroprevalence (66.7%) was also found in a study in Central Ethiopia (Gebremedhin et al., 2013). Similar results for sheep prevalence (7.7%, 7.0%, 11%) were found by others (Moura et al., 2007; Silva & Langoni, 2001) in Rio Grande do Sul, Paraná and São Paulo States. However, higher seroprevalences (38.22% and 43.2% for animals, respectively, and 100% for herds) were observed in Federal District, central region of Brazil (Ueno et al., 2009) and Minas Gerais State (Carneiro et al., 2009), the second largest sheep population in the southern region of Brazil (Instituto Brasileiro de Geografia e Estatística, 2008). The use of different serological tests and cut-off values may have accounted for the difference in these serological studies (Dubey, 2009). The observed

high frequency of positive herds can also be associated to the intense traffic of animals into the State as stated before. Furthermore, the low prevalence of antibodies in studied sheep may be related to the use of appropriate management techniques that could have prevented the contamination of the environment by oocysts of *T. gondii* excreted in cat feces. Nevertheless, this low animal prevalence is still of concern because undercooked meat from infected animals is one of the major sources of human infection by *T. gondii*. Indeed, children under 18 are 2.4 times more likely to become seropositive when there is the consumption of rare meat (Schlundt et al., 2004). Furthermore, toxoplasmosis is of great public health concern in Brazil because there is a higher risk of Brazilian children developing severe toxoplasmosis than children in Europe (Jones & Dubey, 2012).

Antibodies against Maedi-visna virus were the lowest prevalent of all studied diseases at both levels, animal and flock. Similar results were also observed in the State of São Paulo (Lombardi et al., 2009), where the production system resembled that of Minas Gerais. Worldwide, lentivirus infection is associated to intensive production system and to the international movement of European breeds (Manual of diagnostic..., 2008). Similarly to our findings, a previous study in Minas Gerais State (Marques, 2006) also observed a low seroprevalence of Maedi-visna virus, besides an association of the infection with purchase of breeding animals and intensive production. Therefore, the low frequency of antibodies anti- Maedi-visna depicted in the present study is probably associated to the extensive rearing system, common to meat-producing sheep flocks, and used by all sheep owners interviewed (Guimarães et al., 2011a).

In the correspondence analysis, the negativity of sheep for all the studied diseases was plotted close together, suggesting that some herd biosecurity measures to control disease spread may be used in the negative flocks. Considering that the seropositivity for Maedi-Visna and toxoplasmosis were plotted further apart from other variables, it is tempting to speculate that these results may be due to the main form of transmission of these diseases, contaminated milk and colostrum for Maedi-Visna and presence of cats in feeds and barns facilities for *T. gondii*, are not related to the other studied pathogens. This observation is also possible for CLA, *B. ovis* and leptospirosis, since seropositivity for these diseases were associated at some level (Figure 1), which may have occurred because of the proximity of animals and environment contamination that augments the exposure to the infectious agents. Also, the correspondence analysis indicated that the occurrence of leptospirosis was higher in larger flocks (more than 501 animals), while smaller flocks (1-200 and 201-500) tended to be negative for the disease.



## Conclusions

In summary, data from the present study on sheep slaughtered in Minas Gerais, Brazil, showed no antibodies against smooth-*Brucella* and a low frequency of antibodies anti-Maedi Visna, but that seroprevalence of *B. ovis*, *Leptospira* spp., and *T. gondii* are high and widespread among animals and flocks. Moreover, simultaneous occurrence of antibodies of those agents and *C. pseudotuberculosis* were present in the majority of herds, while only a few animals showed seropositivity for more than one agent.

## Conflict of Interest

The authors declare that they do not have any conflict of interest.

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## Ethics Statement

The procedures involving animals were approved by the Ethics committee on the use of animals at the Federal University of Minas Gerais.

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