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REGULAR ARTICLES

# Cross-sectional study of *Leptospira* spp. in commercial pig farms in the state of Goiás, Brazil

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## Abstract

Leptospirosis is an infectious, contagious disease highly important to the world pig industry, which causes reproductive loss in breeding herds. Endemic infections in a herd may produce little evidence of clinical disease despite resulting in economic losses. However, some epidemiological features of leptospirosis in midwestern Brazil, such as risk factors and prevalence of the disease, remain unclear. Therefore, this study focused on assessing the prevalence of the *Leptospira* spp. in intensive pig herds and associating its risk factors. A set of 900 blood samples, equally distributed between nursery, growing, and finishing pigs of 30 intensive farrow-to-finish farms, were analyzed using the microagglutination test (MAT), in order to detect anti-*Leptospira* spp. antibodies for 24 different *Leptospira* spp. serovars. An occurrence of 4.67% (55/342) seropositive samples were detected in fattening pigs. The variables associated with the disease occurrence were animals per square meter at fattening (OR 0.006, CI 95% 0.004–0.42, $p = 0.0105$) and pen division between growing and fattening pigs (OR 3.56, CI 95% 0.563–22.541, $p = 0.185$). Thus, the variables semi-hollow floor in the maternity (OR 16.66; CI 95%: 2.17–128.2 and $p = 0.006$) and animals per trough at fattening (OR: 0.08, CI 95% 0.009–0.87 and $p = 0.025$), observed in this study, highlight the importance of the fattening phase in the epidemiology of the disease, bringing information on risk factors involved in the occurrence and dissemination of leptospirosis in intensive pig herds.

**Keywords** Antibodies · Epidemiology · *Leptospira* spp. · risk factors · Swine

## Introduction

Leptospirosis is an infectious disease caused by a gram-negative spirochete of the genus *Leptospira*. It is a globally widespread life-threatening, yet, neglected zoonotic disease (Levett, 2001) that has a significant impact on pig production. Endemic infection in swine herds may not result in clinical disease (Faine et al. 1999), and it may be introduced into a susceptible breeding herd during periods of low herd immunity (Soto et al. 2008). Besides, the efficacy of the swine leptospirosis vaccines is highly questionable once they induce low production of protective antibodies, and these antibodies may not be specific for the circulating *Leptospira* spp. (Balakrishnan and Roy, 2014).

The disease causes important losses in pig herds, especially through abortion, stillborn or weak pigs of reduced viability (Ellis 2006), fetal mummification (Borges et al. 2005), as well as temporary infertility or permanent sterility in sows (Boqvist et al. 2002). Still, indirect losses involving treatment costs, vaccines, and veterinary assistance are commonly associated with the infection (Ellis 2015; Adler and de La Penã Moctezuma 2010). In the intensive rearing system, the occurrence of reproductive disorders and lack of biosecurity in farms were positively associated with disease occurrence in swine herds in Brazil (Boqvist et al. 2002; Delbem et al. 2004; Valença et al. 2012). So, controlling and reducing the transmission of pathogens and preventing disease incidence are the main health goals in the swine industry nowadays.

Considering herd immunization, several commercial vaccines containing five to ten inactivated serovars of *Leptospira* spp. with variable efficacy are available for swine (Faine et al.

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2000). In Brazil, commercial polyvalent vaccines against leptospirosis include the serovars Icterohaemorrhagiae, Canicola, Hardjo, Grippotyphosa, Pomona, Bratislava, and Tarassovi, as presented in Table 1 (Figueiredo et al. 2013; MAPA 2014). In general, these vaccines have demonstrated partial protection due to a lack of cross-immunity to serovars not found in the composition and the presence of other potentials endemic serovars in the herd (Sonada et al. 2018). Likewise, even vaccinated animals are not fully protected against the infection, renal colonization, and urinary excretion by *Leptospira* spp. (Dib et al. 2014; Zuerner et al. 2011), which also represents a risk of exposure to humans (Ramos et al. 2006; Bharti et al. 2003).

In Brazil, the disease occurs at multiple frequencies and varies according to the studied region, as reported by Delbem et al. (2004). Several serovars of *Leptospira interrogans* have been linked to infections in swine. Favero et al. (2002) reported a seroprevalence of 33.4%, 50%, 62.5%, and 66.6% for the serovars Grippotyphosa, Autumnalis, Icterohaemorrhagiae, and Pomona, respectively, in ten different Brazilian states (Bahia, Maranhão, Minas Gerais, Rio de Janeiro, Paraná, Goiás, Santa Catarina, São Paulo, Ceará, Rio Grande do Sul, Pernambuco). Similarly, Ramos et al. (2006) reported the infection with serovars Pomona, Copenhageni, Tarassovi, Hardjo, Bratislava, and Wolffi in 18 technified pig farms with reproductive problems in Rio de Janeiro, Brazil. Freitas et al. (2004) isolated the Canicola serovar in two liver samples obtained from 36 sows at a slaughterhouse in Southern Brazil.

Knowledge about pathogens that can potentially affect animal welfare and cause economic losses in swine production is critical. Also, diseases of the reproductive system in pigs may affect the animal industry with financial losses related to piglet death, abortions, stillbirths, mummified fetuses (Ramos et al. 2006), and drug costs (Adler and de La Penã Moctezuma 2010). Besides, if we consider that Brazil is the fourth biggest pork producer in the world and that the Goiás state, in recent years, has been growing in the export of pork meat and derived products, corresponding to 2.61% of the demand (ABPA 2019), it is essential to know the main problems that can lead to economic losses. Therefore, measuring the occurrence of *Leptospira* spp. in pig production systems in Brazil and its impact on the sanitary and economic sphere is of extreme importance.

In fact, biosecurity encompasses sets of practices that are standardized to reduce the risk of pathogen introduction into farms and prevent the spread of endemic agents within or among production sites, as well as to control the presence of rodents (Goarant 2016) and other animals (Bharti et al. 2003; Adler and de la Pena Moctezuma, 2010). In addition, the lack of effective sanitary measures contributes to the spread of pathogens in hog farms (Barragan et al. 2017; Delbem et al. 2004). Until now, a few studies have investigated the risk factors associated with the occurrence of antibodies anti-*Leptospira* spp. in pigs from commercial farms in Brazil (Delbem et al. 2004; Valença et al. 2012; Azevedo et al. 2006; Aguiar et al. 2006). Therefore, this study aimed to investigate the occurrence and risk factors associated with *Leptospira* spp. infections in pigs from technified farms in the state of Goiás, Brazil.

## Materials and methods

### Sample design and study area

Sampling was performed in 2016 and 2017 in the state of Goiás, Brazil. For this, 30 farrow-to-finish intensive pig farms were conveniently selected. The criteria for inclusion was based on the register of the Goiás Agency of Agricultural Defense—AGRODEFESA, taking into consideration farms of one site and multiple sites, categorizing them by the number of the sow herd, as follows: large farms (> 1000 sows), medium farms (500–999 sows), and small farms (< 499 sows). Goiás state contains approximately 100.000 sows, representing 5% of Brazil sow herd (ABCS 2016). Out of the 30 herds, 24 had nursery and breeding sites at the same location while on the other six herds, the nursery was located in the same farm as the fattening animals. All sampling sites are within the microregion of Rio Verde, Goiás, Brazil (Table 2).

**Table 1** Composition of polyvalent bacterin vaccines commonly used for the immunization of swine or Bovidae in Brazil

| Commercial polyvalent vaccine | Serovars of *Leptospira* spp. included |
|---|---|
| Vaccine 1 | Pomona, Grippotyphosa, Canicola, Icterohaemorrhagiae, Wolffi, and Hardjo |
| Vaccine 2 | Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, and Wolffi |
| Vaccine 3 | Canicola, Hardjo, Icterohaemorrhagiae, Grippotyphosa, and Pomona |
| Vaccine 4—allows immunization against parvoviruses, erysipelas, and leptospirosis | Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona |
| Vaccine 5—allows immunization against parvoviruses, erysipelas, and leptospirosis | Pomona, Grippotyphosa, Canicola, Icterohaemorrhagiae, Bratislava, and Hardjo |

**Table 2** Sampled farms, number of sows per farm and its respective municipality

| Farm ID | No. of sows | Location |
|---|---|---|
| 01 | 1300 | Aparecida do Rio Doce |
| 02 | 858 | Montividiu |
| 03 | 713 | Rio Verde |
| 04 | 511 | Rio Verde |
| 05 | 556 | Rio Verde |
| 06 | 1200 | Santo Antônio da Barra |
| 07 | 2340 | Montividiu |
| 08 | 1200 | Rio Verde |
| 09 | 412 | Rio Verde |
| 10 | 1200 | Rio Verde |
| 11 | 2300 | Rio Verde |
| 12 | 2300 | Rio Verde |
| 13 | 2600 | Santa Helena de Goiás |
| 14 | 4000 | Cachoeira Alta |
| 15 | 2360 | Rio Verde |
| 16 | 1160 | Rio Verde |
| 17 | 1160 | Rio Verde |
| 18 | 1170 | Rio Verde |
| 19 | 2600 | Aparecida do Rio Doce |
| 20 | 1271 | Rio Verde |
| 21 | 1196 | Montividiu |
| 22 | 2500 | Cachoeira Alta |
| 23 | 2333 | Bom Jesus de Goiás |
| 24 | 2380 | Rio Verde |
| 25 | 1296 | Rio Verde |
| 26 | 2611 | Rio Verde |
| 27 | 2800 | Rio Verde |
| 28 | 2369 | Rio Verde |
| 29 | 1170 | Rio Verde |
| 30 | 1190 | Rio Verde |

It is important to mention that no animals from these farms were exposed to any leptospirosis vaccination protocol because in this region, it is not a common practice, and it is not mandatory (Brazil 2002, 2004). Instead, the reproductive vaccination protocol for sows, adopted by the sampled farms, contemplated only the use of bivalent commercial vaccines (erysipelas and parvoviruses), with a dose at 40 and 20 days before artificial insemination, with reinforcement in lactation up to 10 days after farrowing.

The nursery pigs were sampled at 50 days old, the growing pigs at 94 days old, and fattening pigs at 130 days old, from which a fixed number of 10 blood samples were collected in each phase. So, 30 blood samples were collected from each farm, totalizing 900 samples. Considering that this was a cross-sectional study, blood samples from each farm were collected on the same day, by jugular puncture using sterilized disposable syringes and needles, deposited in commercial tubes (BD® Franklin Lakes, NJ, USA) with clot activator. Blood serum was separated by centrifugation at 1500×*g* for 10 min, aliquoted, and stored in plastic graduated microtubes at − 20 °C until processing.

## Questionnaire

To obtain epidemiologic data about the herd, the farm owner or its technical team had to fulfill a questionnaire (composed of 65 questions), mostly related to biosecurity measures, zootechnical indexes, management practices, vaccination protocols, structure of facilities, welfare practices, environmental data, and feeding system used in the farm (Online Resource 1). The questionnaire was based on Loeffen et al. (2009), composed mostly of dichotomous questions where the possible answers were only "yes" or "no." Continuous data were obtained by questions that allowed any type of numeric answer. The variable "herd size" was included in the model. For this purpose, the interviewer was trained not to influence the responses.

## Microscopic agglutination test

A serological investigation was carried out with the microscopic agglutination test (MAT). The test was used to demonstrate *Leptospira* spp. antibodies in pig sera as described by Hartskeerl et al. (2001) in the manual for International Course on Laboratory Methods for the Diagnosis of Leptospirosis, also recommended by the Leptospirosis animal health programs in Brazil, according to Santa Rosa (1970) and Brazil (2009). A collection of live antigens previously isolated and composed of 22 pathogenic serovars of *Leptospira* spp. and two saprophytes serovars was used (Favero et al. 2002). Thus, the following serovars were used as live antigens: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Batavie, Canicola, Whitcombi, Cinoptery, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Sentot, Andamana, and Patoc. All serum samples were tested for each serovar, previously standardized and performed by the same person.

Since there was limited information on the circulating serovars in the region, the serovars included in the test panel were based on the occurrence in the worldwide pig scenario, including some Brazilian herds (Bertelloni et al. 2019; Ngugi et al. 2019; Osava et al. 2010; Ramos et al. 2006; Lilenbaum and Souza 2003).

For screening and titration, the strains were peeled from the Fletcher medium to the modified Ellinghausen McCullough-Johnson-Harris (EMJH) (Difco®, USA) supplemented with plus fluorouracil, chloramphenicol, vancomycin, nalidixic acid, and neomycin (Freitas et al. 2004) and then enriched with

rabbit serum. *Leptospira* spp. culture was maintained in Leptospira Medium Base EMJH, sub-cultured every 7–10 days, and checked for purity, mobility, and agglutination power until the use. Known positive samples, used in the routine of the Laboratory of Brucellosis and Leptospirosis (Unesp/Jaboticabal), served as positive control.

### Antibody detection

In summary, a mixture of 980 μl of solution with PBS + 20 μl of blood serum was prepared. In a 96-well sterilized microplate, with a multichannel pipettor, a volume of 0.025 ml (25 μl) of the prepared solution was placed in each well of all lines (A, B, C, D, E, F, G, and H) of the plate, where a total of eight serum samples were tested per plate. In each column (from 1 to 12), an aliquot of 0.02 ml (20 μl) of the *Leptospira* spp. serovar (Ag) in culture in the EMJH medium was added for reaction with the tested serum.

The same procedure was performed for the remaining 12 serovars, totalizing 24 serovars tested for each blood serum. Homogenization was carried out, and the plate was taken into the oven for 40 min prior to its reading. For the screening of seroreactive animals, 1:100 dilution was used. Reactive samples were then examined with increasing dilutions from 1:100 to 1:3200, considering the highest positive dilution to be the titer of the serum (Faine et al. 1999). The final titer considered was the reciprocal of the highest dilution when at least 50% agglutination of leptospires occurred at a magnification of 100 times, under dark-field microscopic visualization (Santa Rosa 1970).

The test was considered reactive when there was an increase of at least 4-fold in the microagglutination of paired serum samples. The serovar that showed a 50% agglutination titer at the highest dilution was considered the likely predominant serotype. Besides, the test was deemed to be non-reactive if there was no agglutination in any of the listed serovars.

### Data analysis

The seroprevalence values found for each of the serovars had a 95% confidence interval calculated by the Wilson method. To verify possible associations between the investigated variables in the questionnaire and the occurrence of animals that reacted for *Leptospira* spp., Fisher's exact test ($p < 0.05$) was used, where each herd was used as the sample unit. If significant differences were found in the frequency of exposure between cases and non-cases of the disease, the relative risk estimate (odds ratio) was calculated. Variables with $p < 0.2$ in the univariate analysis were submitted to multivariate logistic regression. Then, placed in a final model, a significance of $p < 0.05$ was adopted. The analyses were performed using the EpiInfo® software (EpiInfo version 7.2.2.6-CDC, Atlanta, USA).

## Results

### Occurrence of antibodies anti-*Leptospira* spp.

Overall, 17 of the 900 serum samples were considered reagents for *Leptospira* spp., with a titer of 1:100. The occurrence of anti-*Leptospira* spp. antibodies in all sampled farms was 1.89% (17/900; 95% CI 0.99–2.78%). Out of the 30 tested farms, eight had at least one reagent sample, and the occurrence ranged from 0.2 to 10.9%. The number of reagent samples found in the different stages of production and the corresponding 95% CI is shown in Table 3.

The production phase with the highest occurrence of anti-*Leptospira* spp. was the fattening phase, which presented 4.67% (14/300; 95% CI 2.28–7.05%) of reagent samples. Only three samples from the nursery were reagent, characterizing an occurrence of 1% (3/300; 0.35–2.9% CI 95%), as presented in Table 4.

The most common serovars observed in this study were Patoc (0.78%; CI 95% 0.38–1.60%), Icterohaemorrhagiae (0.67%; CI 95% 0.31–1.45), Copenhageni (0.56; CI 95% 0.24–1.29%), and Canicola (0.22%; CI 95% 0.06–0.81%). Moreover, it can be observed that nursery pigs also had a titer that can be considered reagent by the MAT test. These animals were reagent for more than one serovar, as shown in Table 4. The fattening pigs were reagents for the following serovars: Patoc, Icterohaemorrhagiae, Copenhageni, and Canicola, as demonstrated in Table 5.

As some samples were reagent for more than one *Leptospira* spp., these samples were tested separately for the different serovars. The results of antigen-antibody reactions with serovars and their respective titers are shown in Table 6.

### Risk factor association with *Leptospira* spp. infection

Regarding the data obtained from the epidemiological questionnaires, after conducting the statistical analysis, the significant variables were the following: animals per trough at growing, animals per trough at fattening, semi-hollow floor in the maternity, divisions in the growing and finishing, animals per square meter in the growing, animals per square meter in the fattening, ventilation/fogging in the barn, mortality in the growing, and number of sows.

The possible protective factors, in this circumstance, were the variables "Animals/$m^2$ at growing" ($p = 0.012$, OR 0.052, CI 95% 0.005–0.57) and "ventilation/nebulization in the barn" ($p = 0.002$, OR 0.06, CI 95% 0.0095–0.47).

The variables "semi-hollow floor in the maternity" and "animals per trough at fattening", were identified as possible

**Table 3** Detailed information in positive technified farms that presented at least one reagent sample in the microscopic agglutination test (MAT) for the detection of anti-*Leptospira* spp. antibodies, in the state of Goiás, Brazil

| Farm identification | Age group | No. of sampled animals | No. of reagent samples | Occurrence (%) | 95% CI | Municipality |
|---|---|---|---|---|---|---|
| Farm 03 | Nursery | 10 | 2 | 6.67 | 1.85–21.32 | Rio Verde |
| | Growing | 10 | 0 | 0.0 | – | |
| | Fattening | 10 | 1 | 3.33 | 0.59–16.67 | |
| | Total | 30 | 3 | 3.33 | 1.14–9.35 | |
| Farm 04 | Nursery | 10 | 0 | 0 | – | Rio Verde |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 2 | 6.67 | 1.85–21.32 | |
| | Total | 30 | 2 | 2.22 | 0.61–7.74 | |
| Farm 05 | Nursery | 10 | 0 | 0 | – | Rio Verde |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 1 | 3.33 | 0.59–16.67 | |
| | Total | 30 | 1 | 1.11 | 0.2–6.03 | |
| Farm 06 | Nursery | 10 | 1 | 3.3 | 0.59–16.67 | Santo Antônio da Barra |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 3 | 10 | 3.46–25.62 | |
| | Total | 30 | 4 | 4.44 | 1.74–10.88 | |
| Farm 09 | Nursery | 10 | 0 | 0 | – | Rio Verde |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 2 | 6.67 | 1.85–21.32 | |
| | Total | 30 | 2 | 2.2 | 0.61–7.74 | |
| Farm 20 | Nursery | 10 | 0 | 0 | – | Rio Verde |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 3 | 10 | 3.46–25.62 | |
| | Total | 30 | 3 | 3.33 | 1.14–9.35 | |
| Farm 28 | Nursery | 10 | 0 | 0 | – | Rio Verde |
| | Growing | 10 | 0 | 0 | – | |
| | Fattening | 10 | 2 | 6.67 | 1.85–21.32 | |
| | Total | 30 | 2 | 2.22 | 0.61–7.74 | |

risk factors for the presence of reagent animals to anti-*Leptospira* spp. antibodies in the univariate analysis, as presented in Table 7.

The variables associated with the presence of anti-*Leptospira* spp. antibodies in the analysis of risk factors ($p < 0.2$) were "animals per square meter at fattening" (OR 0.2, CI 95% 0.029–1.374, $p = 0.068$) and "division between growing and fattening" (OR 3.56, CI 95% 0.563–22.541, $p = 0.185$) and were used in a logistic regression model. In this scenario, only the first variable, "animals per $m^2$ at fattening" was significant ($p = 0.0105$, with OR 0.006), also becoming a protective factor for the exposed animals by presenting anti-*Leptospira* spp. antibodies. All risk factors and protective factors are grouped in Table 7.

A linear regression analysis was performed with the two variables, "fattening mortality" and "number of sows," showing significant values ($p = 0.03$, $R^2 = 0.75$) as represented in Fig. 1. Then, we tested the strength between these variables through the Pearson's test (coefficient = − 0.39, $p = 0.03$), which indicated a weak correlation.

**Table 4** Occurrence of anti-*Leptospira* spp. antibodies in intensive pig farming according to each production stage, Goiás State, Brazil

| Age group | Analyzed samples | Reagent samples | Occurrence (%) | 95% CI value |
|---|---|---|---|---|
| Nursery | 300 | 3 | 1 | 0.34–2.90 |
| Growing | 300 | 0 | 0 | 0 |
| Fattening | 300 | 14 | 4.67 | 2.28–7.05 |
| TOTAL | 900 | 17 | 1.89 | 1.00–2.78 |

**Table 5** Serological results of pigs in different phases with their respective *Leptospira* spp. serovars

| Order | Age group | Farm | Microscopic agglutination test (MAT) | |
|---|---|---|---|---|
| | | | Titer | Serovar |
| 1 | Nursery | 3 | 200/200/200 | Canicola/Pomona/Grippotyphosa |
| 2 | Nursery | 3 | 100/100 | Butembo/Sentot |
| 7 | Nursery | 6 | 100 | Patoc |
| 3 | Fattening | 3 | 100 | Patoc |
| 4 | Fattening | 4 | 100 | Patoc |
| 5 | Fattening | 4 | 200 | Patoc |
| 6 | Fattening | 5 | 100 | Icterohaemorrhagiae |
| 8 | Fattening | 6 | 100 | Patoc |
| 9 | Fattening | 6 | 100 | Patoc |
| 10 | Fattening | 6 | 100/100 | Icterohaemorrhagiae/Patoc |
| 11 | Fattening | 9 | 100 | Copenhageni |
| 12 | Fattening | 9 | 100 | Copenhageni |
| 13 | Fattening | 20 | 100/100 | Icterohaemorrhagiae/Copenhageni |
| 14 | Fattening | 20 | 200/100 | Icterohaemorrhagiae/Copenhageni |
| 15 | Fattening | 20 | 100 | Icterohaemorrhagiae |
| 16 | Fattening | 28 | 100 | Canicola |
| 17 | Fattening | 28 | 200/400 | Icterohaemorrhagiae/Copenhageni |

## Discussion

Samples considered reagents were found in a more significant proportion in the fattening phase (14/300–4.67%; CI 95% 2.28–7.05) and in a smaller proportion in the nursery phase (3/300–1%; CI 95% 0.34–2.90). Once the immunization protocol of the sows in the sampled farms does not include commercial polyvalent vaccines for Leptospirosis, including the serovars Icterohaemorrhagiae, Canicola, Hardjo, Grippotyphosa, Pomona, Bratislava, and Tarassovi (Table 1), it is likely that the presence of antibodies anti-*Leptospira* spp. resulted from the exposure to the etiological agent.

According to Soto et al. (2007), the main pathogenic serovars reported in swine from Brazil by MAT were the following: Pomona, Icterohaemorrhagiae, Grippotyphosa, Pyrogenes, Canicola, Autumnalis, and Javanica. However, in our study, the serovars Icterohaemorrhagiae, Copenhageni, Canicola, Pomona, Grippotyphosa, Butembo, and Sentot were found. Moreover, animals in the nursery phase with antibodies against the *Leptospira*, even in low quantity, suggest that these animals had earlier contact with the infectious agent. Also, the infection may have been reduced by the immune system of these animals due to the humoral response (Chattha et al. 2013). Therefore, since the epitheliochorial characteristic of swine placenta does not allow the direct passage of immunoglobulins (Ig) to fetuses during pregnancy (Chattha et al. 2013), it is necessary to vaccinate the sows. A two-dose protocol of the reproductive vaccine may be used as follows: a pre-parity dose for providing

**Table 6** *Leptospira* spp. Reagent samples in the MAT tests, with respective titers and occurrence

| Serovars | Titer | | | Total of reagent samples | Occurrence (%) | 95% CI |
|---|---|---|---|---|---|---|
| | 100 | 200 | 400 | | | |
| Patoc | 6 | 1 | 0 | 7 | 0.78 | 0.38–1.60 |
| Icterohaemorrhagiae | 4 | 2 | 0 | 6 | 0.67 | 0.31–1.45 |
| Copenhageni | 4 | 0 | 1 | 5 | 0.56 | 0.24–1.29 |
| Canicola | 1 | 1 | 0 | 2 | 0.22 | 0.06–0.81 |
| Pomona | 0 | 1 | 0 | 1 | 0.11 | 0.02–0.63 |
| Grippotyphosa | 0 | 1 | 0 | 1 | 0.11 | 0.02–0.63 |
| Butembo | 1 | 0 | 0 | 1 | 0.11 | 0.02–0.63 |
| Sentot | 1 | 0 | 0 | 1 | 0.11 | 0.02–0.63 |

**Table 7** Univariate analysis of risk factors associated with the presence of pigs reactive for *Leptospira* spp. protective factors identified after univariate and multivariate analysis of the variables obtained by applying epidemiological questionnaires

| | OR | CI OR (95%) | *p* |
|---|---|---|---|
| Risk factor | | | |
| Semi-hollow floor in the maternity | 16.67 | 2.17–128.2 | 0.0066 |
| Animals per trough at fattening | 0.08 | 0.009–0.87 | 0.0253 |
| Protective factors | | | |
| Animals per square meter at growing | 0.052 | 0.005–0.57 | 0.0118 |
| Animals per trough at growing | 0.07 | 0.009–0.65 | 0.0083 |
| Animals per trough at fattening | 0.08 | 0.009–0.87 | 0.0253 |
| Ventilation/fogging in the barn | 0.067 | 0.009–0.47 | 0.0024 |
| Animals per square meter in the fattening | 0.006 | 0.004–0.42 | 0.0105 |

immunoglobulins IgA and IgG against Leptospirosis for newborns piglets through colostrum (Butler and Wertz 2012) and a second dose at 10 days postpartum in the sow.

A larger number of samples reactive to any *Leptospira* spp. in the finishing phase are expected due to the exposure to a greater amount of pathogenic microorganisms, as shown by Fablet et al. (2018). It happens either because of the hygienic-sanitary conditions of the facilities, the high density in the housing of these animals, or by the decreasing of antibodies against the disease throughout life. However, this could become a public health concern once these animals may be excreting *Leptospira* spp. through slaughter; they are increasing the risk of infection for the people at the slaughterhouse. Besides that, the fattening phase is the production phase with a great cost involved, reaching 65–75% of the total cost, becoming the most expensive stage in the pig production cycle (Pomar and Remus 2019) and, therefore, deserves special attention regarding sanitary conditions.

Serovar Pomona and Icterohaemorrhagiae, commonly cited as the predominant serovars all over the world with substantial economic losses in seropositive pigs (Ramos et al. 2006; Boqvist et al. 2002; Nagy, 1993) like abortion, stillborn, and deaths in pigs, considered serological reagent. In our study, the seroprevalence of this serovar was very low (0.11%, CI 95% 0.02–0.63) followed by the serovar Icterohaemorrhagiae (0.67%, CI 95% 0.31–1.45), corroborating the results of Delbem et al. (2002) and Favero et al. (2002).

The detection of serovars Icterohaemorrhagiae and Copenhageni has been reported worldwide; however, few isolations of the agent have been done in pigs, as reported by Bertelloni et al. (2019). Similarly, Miraglia et al. (2015) performed a study of characterization of serovar Pomona in swine herds from Brazilian states (São Paulo and Minas Gerais) and concluded that this serovar might have a different antibiotic susceptibility profile than previously reported for *Leptospira* spp. isolates. Moreover, studies performed in different regions of Brazil (Paraná, Alagoas and Minas Gerais) reported an occurrence of 98.16% for the Icterohaemorrhagiae serovar (Delbem et al. 2004), and 65.71% (Hashimoto et al. 2008), 67.10% (Osava et al. 2010), and 41.80% (Valença et al. 2012) were reported in slaughter pigs and sows of technified farms.

An important source of infection for pigs of all stages of production in commercial farms are synanthropic rodents. Pigs can become infected by contact with the urine of these

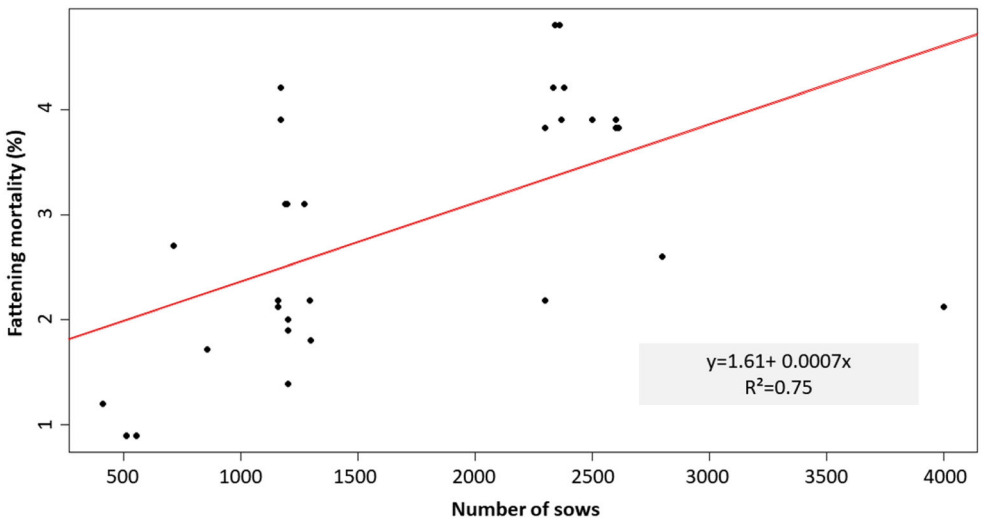


**Fig. 1** Graphical representation of linear regression with the variables "fattening mortality" and "number of sows"

animals, contaminated environments, and food. In this context, rats (*Rattus norvegicus*) are important in the epidemiological chain of the disease and are also the main reservoir of the serovar icterohaemorrhagiae (Goarant 2016; Shimabucuro et al. 2003). Likewise, Langoni et al. (2004) suggested that this positivity occurs in consequence to the exposure to rodents, which also corroborates the study of Chiareli et al. (2008). Therefore, the high frequency of this serovar suggests the presence of rodents (Shimabucuro et al. 2003).

In addition to leptospirosis, other diseases, such as toxoplasmosis, can also be a problem in farms that do not perform control of synanthropic rodents (Smith et al. 1992). A study about risk factors for *Leptospira* spp. in pig farms, conducted by Valença et al. (2012), showed that the lack of rodent control on the property was the probable cause of infection for serovar Icterohaemorrhagiae in animal with clinical signs as increased frequency of estrus recurrence and the increased weaning-to-estrus interval. As reported by Boqvist and Hothi (2005), farms that do not adequately control these synanthropic animals showed 7.8 times more susceptible to swine infection.

On the other hand, serovar Canicola is maintained by dogs and, until now, has no other known maintenance host. It can be found naturally in the urine of dogs or due to vaccination programs (Ellis 2010). In this study, it was found in pigs with an occurrence of 0.22% (95% CI 0.06–0.81%) of antibodies anti-*Leptospira* spp. This serovar may not cause acute infection or the clinical form of the disease in pigs. Still, the animal can become a disseminator, eliminating the agent through urine for up to 90 days (Michna and Campbell 1969). Even though pigs are not susceptible to the serovars Pomona, Bratislava, and Tarassovi in serological tests (Azevedo et al. 2008; Favero et al. 2002), in this study, one animal was seroreagent for the first one. This information suggests that there was a cross-reaction among serovars and the failure of infected animals to seroconvert (Miraglia et al. 2015).

In addition, only three sampled animals were reagent in the nursery phase in the MAT test, being positive for the Canicola, Grippotyphosa, Pomona, Butembo, and Sentot serovars. These animals had a 200 titer after the test, which indicates a mild-to-moderate antibody response. In this study, most titers were low (100), but it is not known whether these were rising or declining. It should be noted that MAT is an indirect test for the diagnosis of leptospiral infection and that cross-reactions between serovars are a common feature (Faine et al. 1999). Since no sow or piglet was exposed to the disease agent through vaccination, it is possible that these animals had contact with the bacteria in the maternity and/or nursery environment.

Considering the protective factors in this context, "animals per square meter in growing" and "ventilation/fogging in the barn," for the first, a maximum of 35 animals per pen is recommended in the growing. Therefore, they may be protected from presenting anti-*Leptospira* spp. antibodies, once there are fewer animals per pen than the maximum recommended, decreasing the direct contact between the pigs. Secondly, the presence of ventilation/fogging in the farm barns offers some thermal comfort to the animals, who seek pools of water and excrement less frequently to cool themselves and are therefore not as exposed to the agent. Also, the variable "animals per square meter at fattening" is recommended to be less than or equal to 1 animal per square meter and less than 35 animals per trough. In the present study, it became a protective factor when associated with the variable "divisions between growing and finishing," inferring that there is no mixing or contact between animals from different phases within the same barn.

Risk factors suggest that the facilities are important for the presence or absence of animals with anti-*Leptospira* spp. antibodies. The variable "semi-hollow maternity floor," used in the univariate analysis, indicated that animals in this type of facility were 16-fold more likely to be exposed to the etiological agent. This finding can be explained by the fact that *Leptospira* is excreted in the urine of the infected sow (Adler and de La Penã Moctezuma 2010), and the piglets came into contact in the first days of life. Even though in this work there was no statistical association between wild animals, cats, dogs or other species with leptospirosis, there are many significant studies. Delbem et al. (2004) reported the main risk factors for swine breeding stock are related to the lack of biosecurity, such as the presence of dogs, cats, and rodents on the farms and also failure to clean feeders and facilities.

In addition to the facilities and synanthropic animals, the presence of fields where pigs are raised along with other animal species may lead to a high number of seropositive animals (Boqvist et al. 2002). Also, properties located near other farms may facilitate the transmission of infectious agents (Pierozan et al. 2016). Likewise, a study on leptospirosis in cattle, conducted in Brazil (Lilenbaum and Souza 2003), showed that raising more than one animal species on the same property, especially pigs, was associated with the seropositivity of leptospirosis in cattle.

It is known that vaccination is a safe and effective way to fight bacteriological infections, as they allow the prevention and control of diseases through the response of the host's immune system. The occurrence of diseases in swine farms can be very variable, and the vaccination period can be adjusted according to the time in which the infection is occurring (Oliveira 1999). Additionally, once commercial leptospirosis vaccines sold in Brazil are produced abroad, there is a chance that those strains are different from the ones found in the field and, thus, are incapable of promoting an effective immune response.

Nevertheless, in the new scenario of world pig farming, the removal of antimicrobials as preventive methods should induce producers to invest more in improvements in the

environmental and in the facilities of pig farms, to decrease the infection pressure of agents such as *Leptospira*. Therefore, knowing risk factors, implementing adequate day-to-day management, intensive control of rodents and using alternatives to antimicrobial protocols may be necessary to maintain a good sanitary status for pig production.

## Conclusion

The presence of samples reacting to *Leptospira* spp. in commercial pig farms suggests that this pathogen circulates in herds from Brazil. However, the occurrence of seroreagent animals for anti-*Leptospira* spp. antibodies is low, even without the vaccination of the animals against the disease, which indicates that biosecurity measures may be useful in the sampled properties in the state Goiás, Brazil. The risk factors identified in this study may play an important role regarding agent dissemination and, therefore, should be adjusted to control the disease in the studied herds.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Statement of animal rights** The institution's ethics committee approved this research and the certificate registered under protocol no. 001216/18 on Feb. 8, 2018.

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