



High frequency and molecular characterization of porcine hemotropic mycoplasmas in Brazil



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ABSTRACT

Mycoplasma suis and *Mycoplasma parvum* are the two hemotropic mycoplasmas species described in pigs. *M. suis* is involved in infectious anemia, while *M. parvum* infection is commonly subclinical. The objectives of this study were twofold: (i) to investigate the prevalence of porcine hemotropic mycoplasmas in sows from the southern region of Brazil by quantitative real-time PCR (qPCR) and (ii) to genetically characterize a subset of the samples based on the 16S rRNA gene. A total of 429 blood samples were evaluated from 53 different farm sites. Porcine hemoplasmas was detected at all the 53 tested sites and in 79.72% of the samples (342/429). Two sequences were obtained for *Mycoplasma* spp. The phylogenetic analysis based on the 16S rRNA gene (900 bp) showed that the *Mycoplasma* sequences were closely related to the *M. suis* cluster and that one sequence was positioned in the *M. parvum* cluster. In conclusion, porcine hemoplasmas have a high rate of prevalence in sows from commercial farms in the southern region of Brazil. This study demonstrated the first molecular detection and characterization of partial 16S rRNA gene of *M. parvum* in Brazil.

1. Introduction

Hemotropic mycoplasmas (HMs) (class *Mollicutes*) are pleomorphic uncultivable bacteria with small genomes that lack cell walls and have a unique tropism for red blood cells (RBCs). So far, only two HMs that infect swine have been described, namely, *Mycoplasma suis* and *Mycoplasma parvum*. These hemoplasmas have been shown to cause single or coinfection in pigs (Fu et al., 2017). In phylogenetic analyses based on the 16S rRNA gene, these species clustered into different genetic groups (Watanabe et al., 2011). While *M. suis* is considered the main etiological agent of infectious anemia in pigs around the world, *M. parvum* infection is usually characterized by the absence of clinical signs. These agents adhere to RBCs, triggering the cell's death and dodging the immune system (Hoelzle, 2008; Groebel et al., 2009). These organisms are also known for their ability to maintain a persistent infection, despite antimicrobial treatment (Smith, 1992).

M. suis, the most characterized hemoplasmas species affecting pigs, has been detected in four mammalian species: pigs (Guimaraes et al., 2009), humans (Yuan et al., 2009), dogs (Mascarelli et al., 2016) and wild boars (*Sus scrofa scrofa*) (Hornok et al., 2018). Although the zoonotic potential of *M. suis* has been reported in China (Yuan et al., 2009),

the clinical signs associated with infection in humans remain unknown.

M. suis, the primary agent of infectious anemia in pigs (IAP) (Stadler et al., 2014), can lead to economic losses in swine production (Ritzmann et al., 2009). The process of adhesion to and invasion of RBCs damages the cell membrane, inducing eryptosis and anemia (Felder et al., 2011). Furthermore, disease severity is correlated with strain virulence (Felder et al., 2011; Stadler et al., 2014). While the acute phase of swine hemoplasmosis is characterized by hemolytic anemia, high fever, icterus and hemorrhagic diathesis, the chronic phase usually is characterized by the absence of anemia, retarded growth in piglets and decreased reproductive efficiency in sows (Henry, 1979; Messick, 2004; Hoelzle, 2008). Recently, a study showed that chronic infection interferes with the transcription of the host pathways frequently used by biomedical researchers and affects the scientific results of studies using porcine models (do Nascimento et al., 2018).

The detection of *M. suis* in domestic pigs of different ages has been reported around the world. The molecular prevalence in growing pigs and sows ranges from 5% to 80% and has been reported in several countries (Table 1). Nevertheless, some authors have assessed the phylogenetic positioning of the different strains of *M. suis* to establish the relationship among the other hemoplasmas species and *M. suis*,

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Table 1
Summarized studies that assessed the molecular prevalence of porcine hemoplasmas around the world.

Country	Molecular test	Category	Prevalence (%)	Reference
Brazil	qPCR [†]	Wild boar	50 (7/14)	Dias et al., 2019
Hungria		Wild boar	47.1 (8/17)	Hornok et al., 2018
China		Growing pigs	42.4 (101/238)	Fu et al., 2017
China		Sows	70.9% (61/86)	Fu et al., 2017
Brazil		Non-technified pigs	76.2 (112/147)	Toledo et al., 2016
Japan		Growing pigs	5 (6/120)	Watanabe et al., 2012
Japan		Growing pigs	50 (6/12)	Watanabe et al., 2011
Brazil		Sows	80 (32/40)	Guimaraes et al., 2011
United States		Pigs	7.5 (3/40)	Guimaraes et al., 2011
Germany		Wild boar	10.03 (36/359)	Hoelzle et al., 2010
Germany		Growing pigs	13.9 (164/1176)	Ritzmann et al., 2009
Switzerland		Sows	19 (19/100)	Hoelzle et al., 2007
Germany		Growing pigs	10.6 (17/160)	Hoelzle et al., 2007
Argentina	cPCR [‡]	Dog	2.9 (2/70)	Mascarelli et al., 2016
Argentina		Growing pigs	65.1 (155/284)	Pereyra et al., 2010
China		Growing pigs	86 (148/172)	Yuan et al., 2009
China		Humans	49 (32/65)	Yuan et al., 2009
Brazil		Sows	18.2 (22/121)	Guimaraes et al., 2007
Brazil		Piglets	0.61(1/61)	Guimaraes et al., 2007
Brazil		Boars	25 (1/4)	Guimaraes et al., 2007

[†] quantitative real time PCR (qPCR).

[‡] Conventional PCR (cPCR).

which also allows the description of novel hemoplasmas (Watanabe et al., 2011; Fu et al., 2017; Gonçalves et al., 2016).

The information regarding the prevalence of *M. suis* infection within sow herds in Brazil is scarce (Guimaraes et al., 2007; 2011). Since Brazil is the fourth largest pork producer in the world and has a great impact on pork meat exports, it is necessary to maintain the health status of the pig herds in the country. Accordingly, the objective of this study was twofold: (i) to investigate the prevalence of porcine hemotropic mycoplasmas in sows from the southern region of Brazil with a quantitative real-time PCR (qPCR) assay and (ii) to genetically characterize a subset of the samples based on the 16S rRNA gene.

2. Materials and methods

2.1. Study design

This work was designed as a cross-sectional study to assess the molecular prevalence of porcine hemoplasmas in blood samples from sows. The sows originated from 53 commercial farm sites and 29 municipalities from three different states in Brazil (Paraná, Santa Catarina and Rio Grande do Sul States), which comprise the major pig-production region (Fig. 1). Three slaughterhouses, located in the state of Santa Catarina, were conveniently selected to collect samples for this study. All the pigs from the sampled farms were slaughtered between March and September, 2017. During the sampling period, blood samples from all the slaughtered sows were collected.

2.2. Eligibility criteria

The sampled animals consisted of sows. The animals' origin data were collected from the mandatory Animal Transit Permits issued by the local official veterinary service offices.

2.3. Samples and DNA extraction

A total of 429 blood samples were obtained from the three different states located in South Brazil. The blood samples were collected by jugular vein puncture, at the time of slaughter. EDTA-whole blood was stored at -80°C as duplicates in 2.0 mL microtubes, until shipment to São Paulo State University (Unesp), Jaboticabal, São Paulo, where molecular analyses were performed. Each sample represented an

individual sow, and individuals were not resampled. This research was submitted to and approved by the CONCEA (National Council of Animal Experimentation Control) and the institution's CEUA (Ethics Commission for Animal Experimentation) under protocol number 001217/18.

DNA extraction was performed from whole blood samples using the protocol previously described by Kuramae-Izioka, 1997. The DNA concentration and quality (260/280 nm ratio) were measured using a Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific®, Wilmington, Delaware, USA).

2.4. Conventional PCR for the endogenous *gapdh* gene

To avoid false-negative results in the qPCR assay for porcine hemoplasmas, all DNA samples were tested for the *gapdh* gene, using the primers *gapdh*-F (5'-CCTTCATTGACCTCAACTACAT-3') and *gapdh*-R (5'-CCAAAGTTGTCATGGATGACC-3'), which flank a fragment of 437 base pairs (bp) (Birkenheuer et al., 2003). The conventional PCR (cPCR) was performed as previously described by Birkenheuer et al. (2003), with some modifications (Toledo et al., 2016).

2.5. qPCR for porcine hemoplasmas based on 16S rRNA gene

The DNA samples exhibiting positive results in the conventional PCR for the *gapdh* gene underwent qPCR for porcine hemoplasmas based on the 16S rRNA gene using the primers F (5'-CCCTGATTGTAC TAATTGAATAAG-3') and R (5'- GCGAACACTTGTTAAGCAAG-3') and the probe TaqMan (5'FAM- TGRATACACAYTTCAGMGBNFQ3') (Thermo Fisher Scientific, Waltham, MA, USA) (Guimaraes et al., 2011; do Nascimento et al., 2014). All samples were tested in duplicate. When the difference of Cq values obtained for the duplicates of the same sample was higher than 0.5, the sample was retested in triplicates. The amplification reaction was performed in a Thermal Cycler Model 96 CFX (BioRad®, Hercules, California, USA) using a final volume of 10 μL containing a mixture of 1 μL of DNA sample, 0.2 μM of each primer and probe, PCR buffer (GoTaq® Probe qPCR Master Mix, Madison, WI, USA) and 10 μL of sterilized ultrapure water (Nuclease-Free Water, Promega®, Madison, Wisconsin, USA) q.s.p. The thermal conditions were 95°C for 10 min followed by 40 cycles at 95°C for 15 s, 58.5°C for 45 s and 72°C for 30 s. The qPCR assays followed the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin

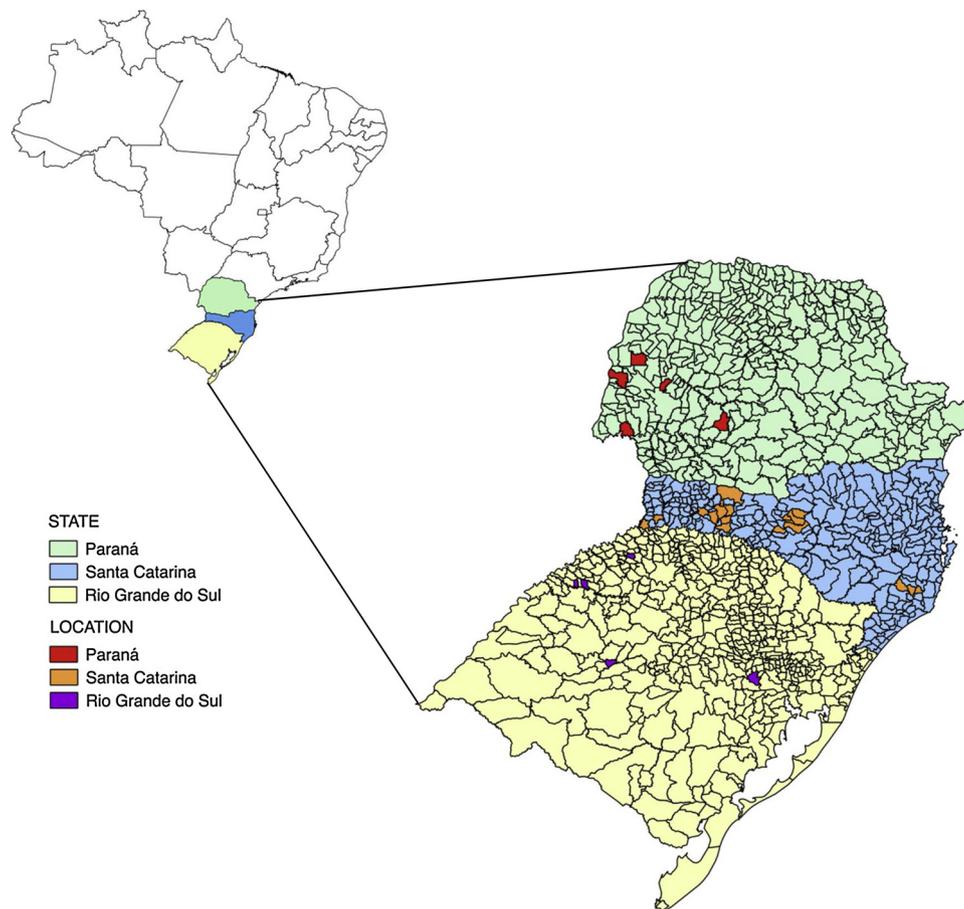


Fig. 1. Locations where sows were sampled in South Brazil.

et al., 2009). The sensitivity of the qPCR was determined using 10-fold serial dilutions of pIDT SMART plasmids (Integrated DNA Technologies, Coralville, Iowa, USA) encoding the 16S rRNA *M. suis* sequence (insert containing 156 bp). The number of the plasmid copies was determined according to the formula $(Xg/\mu L/DNA [plasmid\ size\ (bp) \times 660]) \times 6.022 \times plasmid\ copy \times 10^{23}/\mu L$. The plasmids and ultrapure water were used as positive and negative controls, respectively. The amplification efficiency (E) was calculated from the slope of the standard curve of each run using the following formula: $E = 10^{-1/slope}$. To determine the limit of detection and quantification of the TaqMan assay, the standard curves were generated by serial dilution, with the standards containing from 10^7 to 10^0 copy numbers of the plasmids.

2.6. Conventional PCR for the hemoplasmas based on the 16S rRNA gene

To amplify the *Mycoplasma* spp. DNA, the qPCR-positive samples showing quantification values ranging from 10^4 to 10^6 copies/ μ l were selected. The two cPCR assays based on the 16S rRNA gene were performed using two sets of primers, namely, HemMycop16S-41 s (5'-GYATGCMTAAAYACATGCAAGTCGARGC-3') and HemMyco16S-938as (5' - CTCACCACTTGTTCAGGTCCCGTC - 3') (fragment I of ~800 bp), HemMycop16S-322 s (5' - GCCCATATTCCTACGGGAAGCAG CAGT - 3') and HemMycop16S-1420 as (5' - GTTTGACGGGCGGTGT GTACAAGACC - 3') (fragment II of ~800 bp) (Maggi et al., 2013). Five microliters of DNA was used as a template in the 25 μ L reaction mixtures containing 10 \times PCR buffer, 1.0 mM MgCl₂, 0.8 mM deoxynucleotide triphosphate mixture, 1.5 U Taq Platinum DNA Polymerase (Life Technologies™, Carlsbad, California, USA), and 0.3 μ M each primer. The *Mycoplasma* spp. PCR amplifications were performed at 94 °C for 2 min followed by 55 repetitive cycles of 94 °C for 15 s, 68 °C

for 15 s, and 72 °C for 18 s, followed by a final extension at 72 °C for 1 min (Maggi et al., 2013). The DNA obtained from naturally infected cattle and pigs and ultrapure sterile water (Nuclease-Free Water, Promega™, Madison, Wisconsin, USA) were used as positive and negative controls, respectively. The products obtained from all the cPCR assays were separated by electrophoresis on a 1% agarose gel stained with ethidium bromide (Life Technologies™, Carlsbad, California, USA).

2.7. Sequence analyses

The amplified products were purified using the Silica Bead DNA Gel Extraction Kit (Thermo Fisher Scientific™, Waltham, Massachusetts, USA) and submitted for sequencing (Sanger et al., 1977), which was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific™) and the ABI PRISM 310DNA Analyzer (Applied Biosystems™, Foster City, California, USA).

2.8. Phylogenetic analysis

The sequences obtained from the positive samples were first submitted to a screening test using Phred-Phrap software version 23 (Ewing et al., 1998; Ewing and Green, 1998) to evaluate the electropherogram quality and to obtain the consensus sequences from the alignment of the sense and antisense sequences. The BLAST program (Altschul et al., 1990) was used to analyze the sequences of the nucleotides (BLASTn) in addition to browse and compare the results with the sequences housed in an international database (GenBank) (Benson et al., 2013). The consensus sequences obtained in the present study and those retrieved from GenBank were aligned using Clustal/W software (Thompson et al., 1994) via BioEdit version 7.0.5.3 (Hall, 1999).

Table 2
Parameters of porcine hemoplasmas-qPCR assays.

Plate	(E)	Slope	r ²	Cq values	Quantification of porcine hemoplasmas-16S rRNA gene (number of copies/μL)
1	94.4	-3.465	0.997	18.25 to 37.46	1.32×10^1 to 3.88×10^6
2	102.0	-3.275	0.998	25.19 to 37.14	8.27×10^7 to 3.58×10^4
3	101.3	-3.292	0.995	24.72 to 37.92	1.98×10^8 to 4.05×10^4
4	91.5	-3.545	0.990	21.63 to 38.76	7.44×10^{-1} to 1.17×10^6
5	102.1	-3.273	0.993	20.88 to 38.36	5.33×10^8 to 1.17×10^6
6	103.9	-3.233	0.992	22.42 to 36.59	6.06×10^7 to 2.06×10^5
7	97.7	-3.377	0.995	22.46 to 38.28	1.12×10^7 to 2.68×10^5
8	96.8	-3.402	0.993	24.68 to 37.48	1.18×10^1 to 6.80×10^4
9	97.2	-3.392	0.995	23.29 to 37.66	8.33×10^7 to 1.42×10^5
10	97.3	-3.387	0.992	24.03 to 38.13	4.99×10^8 to 7.22×10^4
11	94.1	-3.471	0.986	23.37 to 38.21	9.87×10^8 to 1.20×10^5

r²: correlation coefficient.

The phylogenetic inferences made were based on the Bayesian inference (BI) method. The BI analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) via CIPRES Science Gateway (Miller et al., 2010). The Markov chain Monte Carlo (MCMC) simulations were run for 10^6 generations with a sampling frequency of every 100 generations and a burn-in rate of 25%. The best evolutionary model was selected by the program jModelTest2 (version 2.1.6) on XSEDE (Darriba and Posada, 2012), under the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Posada and Buckley, 2004). The tree was examined in TreeGraph 2:0:56–381 β (Stöver and Müller, 2010).

3. Results

All DNA samples amplified the predicted product for the *gapdh* gene, which indicates a successful DNA extraction. Samples were processed in duplicates in eleven different plates with reaction efficiencies (E) ranging from 94.1 to 103.9. The assay analytical sensitivity was 10^1 numbers of copies/μL, associated slopes ranged from -3.545 to -3.233, the r² values from 0.986 to 0.998, the Cq values from 18.25 to 38.76 and the quantification values from 7.44×10^{-1} to 3.88×10^6 (Table 2, Fig. 2). A summary of the qPCR results is presented by the sample set

ID, state and location in Table 3. The detailed information regarding the qPCR results by individual samples can be found in Table S1. Samples were distributed into seven groups (10^{-1} to 10^6) according to the number of copies/μL. Most of the samples were distributed between the four groups ranging from 10^1 to 10^4 copies of porcine hemoplasmas/μL of naturally infected sow blood, with 24.2% (77/318), 24.5% (78/318), 25.1% (80/318) and 14.2% (45/318) of the samples falling into these groups, respectively.

Briefly, porcine hemoplasmas DNA was detected in all 53 tested farm sites and in 79.72% of the samples (342/429). The positivity for porcine hemoplasmas was 77.03% (57/74) in Paraná state, 79.94% (251/314) in Santa Catarina state, and 82.93% (34/41) in Rio Grande do Sul state. Twenty-four samples demonstrated inconsistent quantification results (difference in replicate's Cq values higher than 0.5) even after retested in triplicate (Table S2). This finding was most likely due to the Monte Carlo effect (Bustin et al., 2009), which represents an inherent limitation of the technique, particularly in samples with a low number of porcine hemoplasmas DNA copies. In the present study, the assay sensitivity threshold was 10 copies/μL.

Two positive samples from two different cPCR protocols based on the 16S rRNA gene were selected and submitted to sequencing (Table 4). Based on the BLAST analysis, one of the *Mycoplasma* 16S

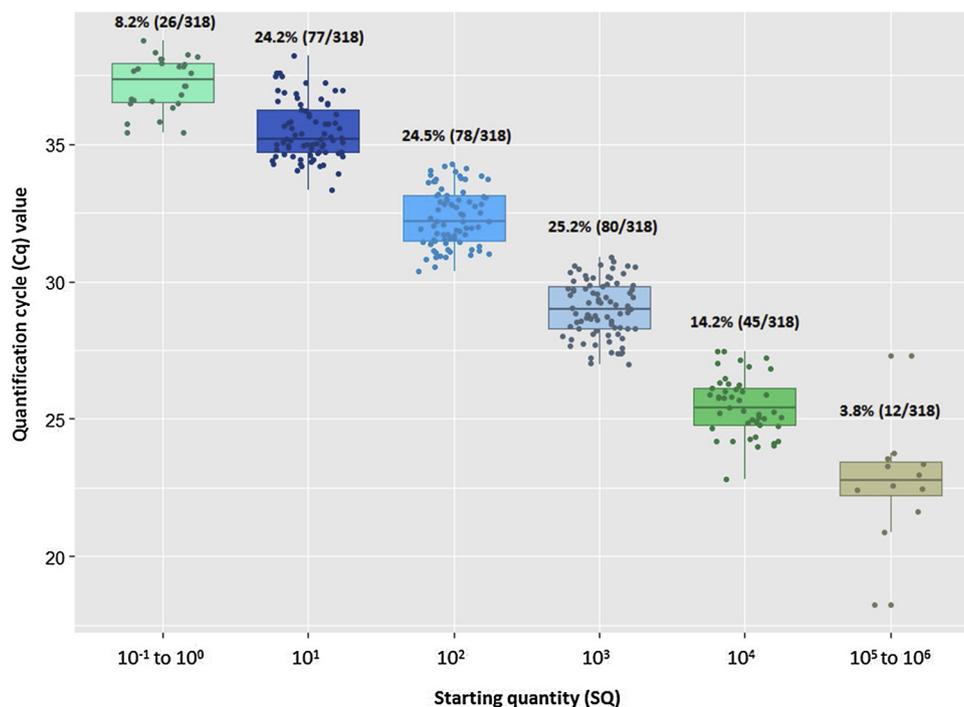


Fig. 2. Box plot showing the qPCR quantification cycle (Cq) results and distribution of starting quantity (SQ) degree of dispersion by each sample.

Table 3
qPCR per cent positive by sample set ID and location.

Sample set ID	State	Location	qPCR per cent positive
1	Paraná	A	100.00 (5/5)
2	Paraná		77.78 (7/9)
3	Paraná	B	100.00 (2/2)
4	Paraná	C	95.00 (19/20)
5	Paraná	D	66.67 (12/18)
6	Paraná	E	60.00 (12/20)
7	Santa Catarina	F	100.00 (5/5)
8	Santa Catarina		85.71 (6/7)
9	Santa Catarina		71.43 (5/7)
10	Santa Catarina		66.67 (2/3)
11	Santa Catarina		66.67 (2/3)
12	Santa Catarina		50.00 (3/6)
13	Santa Catarina		20.00 (1/5)
14	Santa Catarina	G	100.00 (7/7)
15	Santa Catarina		100.00 (4/4)
16	Santa Catarina		100.00 (8/8)
17	Santa Catarina		80.00 (16/20)
18	Santa Catarina		66.67 (6/9)
19	Santa Catarina		60.00 (3/5)
20	Santa Catarina	H	88.89 (8/9)
21	Santa Catarina		87.50 (7/8)
22	Santa Catarina		75.00 (6/8)
23	Santa Catarina		71.43 (5/7)
24	Santa Catarina		50.00 (4/8)
25	Santa Catarina		37.50 (3/8)
26	Santa Catarina	I	100.00 (10/10)
27	Santa Catarina		88.24 (15/17)
28	Santa Catarina		63.64 (7/11)
29	Santa Catarina		54.55 (6/11)
30	Santa Catarina	J	100.00 (6/6)
31	Santa Catarina		100.00 (6/6)
32	Santa Catarina	K	100.00 (7/7)
33	Santa Catarina		85.71 (6/7)
34	Santa Catarina	L	100.00 (5/5)
35	Santa Catarina		62.50 (5/8)
36	Santa Catarina	M	66.67 (4/6)
37	Santa Catarina		66.67 (4/6)
38	Santa Catarina	N	100.00 (4/4)
39	Santa Catarina	O	100.00 (7/7)
40	Santa Catarina	P	100.00 (4/4)
41	Santa Catarina	Q	100.00 (7/7)
42	Santa Catarina	R	100.00 (6/6)
43	Santa Catarina	S	100.00 (6/6)
44	Santa Catarina	T	90.00 (9/10)
45	Santa Catarina	U	87.50 (7/8)
46	Santa Catarina	V	80.00 (8/10)
47	Santa Catarina	W	75.00 (6/8)
48	Santa Catarina	X	71.43 (5/7)
49	Rio Grande do Sul	Y	90.00 (9/10)
50	Rio Grande do Sul	Z	85.71 (6/7)
51	Rio Grande do Sul	Aa	85.71 (6/7)
52	Rio Grande do Sul	Bb	80.00 (8/10)
53	Rio Grande do Sul	Cc	71.43 (5/7)
TOTAL	–	–	77.79 (342/429)

rRNA gene sequences (GenBank accession number [MK287839](#)) was 99% identical to the complete genome of the *M. suis* strain Illinois described in the United States (GenBank accession number [CP002525](#)) while the other sequence (GenBank accession number [MK287838](#)) was 99% identical to the *M. parvum* strain Morioka4 sampled in Japan (GenBank accession number [AB610846](#)).

The phylogenetic analysis inferred from the Bayesian method and the TIM + I + G4 evolutionary model for the 16S rRNA gene of the *Mycoplasma* spp. showed the formation of two distinct clusters. One sequence of the *Mycoplasma* spp. (GenBank accession number [MK287839](#)) were found to be closely related to the *M. suis* clade (clade support of 63%), and the other sequence (GenBank accession number [MK287838](#)) was positioned in the *M. parvum* clade (clade support of 100%). In addition, the two clusters represented by *M. suis* and *M. parvum* were closely related, which is supported by the phylogenetic

Table 4

Positive samples for porcine hemoplasmas in two different protocols of cPCR based on 16S rRNA gene and submitted to sequencing.

Samples	Cq [†]	SQ [‡]	16SrRNA gene (fragment I)	16SrRNA gene (fragment II)	Sequencing of 16SrRNA gene (fragment I and II)
65	25.19	3.58 × 10 ⁴	+		
86	24.72	4.05 × 10 ⁴	+	+	
99	25.01	3.55 × 10 ⁴	+		
135	24.17	6.21 × 10 ⁴	+		
138	24.19	6.38 × 10 ⁴	+		
156	21.63	2.74 × 10 ⁵	+	+	
186	20.88	1.33 × 10 ⁶	+	+	
198	23.75	1.36 × 10 ⁵	+		
219	24.33	5.76 × 10 ⁴	+		
224	22.57	1.27 × 10 ⁵	+	+	
246	22.42	1.90 × 10 ⁵	+	+	
259	24.27	1.28 × 10 ⁵	+	+	+
271	22.46	2.59 × 10 ⁵	+	+	
288	23.98	8.17 × 10 ⁴	+	+	
310	25.04	4.73 × 10 ⁴	+		
312	24.68	6.97 × 10 ⁴	+		
354	23.29	1.33 × 10 ⁵	+	+	+
408	24.03	5.68 × 10 ⁴	+		
411	24.11	6.35 × 10 ⁴	+		
428	23.57	1.22 × 10 ⁵	+		
431	23.37	1.27 × 10 ⁵	+		

[†] Cq = quantification cycle value.

[‡] SQ = starting quantity (copies/μL).

analysis (Bayesian method) with 100% clade support (Fig. 3).

4. Discussion

This is the first report of the detection and molecular characterization of *M. parvum* in sows from Brazil. Overall, porcine hemoplasmas were detected by qPCR in 342 of the 429 blood samples (79.7%) collected from 53 sites located in three different states in southern Brazil, it is important to highlight that the sampling procedure used herein does not represent the whole pig population for each state. Indeed, these samples are only representative of the slaughterhouse from which sows were sampled. Our study showed a porcine hemoplasmas prevalence greater than 79%, corroborating studies by [Guimaraes et al. \(2011\)](#) in Brazil and [Fu et al. \(2017\)](#) in China, that detected prevalence rates of 80% and 70.9% in sows, respectively. On the other hand, our study showed a higher prevalence than that detected in sows by [Hoelzle et al. \(2007\)](#) in Switzerland (19%). According to the literature, the prevalence of *M. suis* ranges from 0.61% in piglets to 86% in growing pigs. Even though the prevalence seems to be higher in older pigs, the association between occurrence of hemoplasmas and pigs age has not been deeply accessed yet (Table 1). Thus, the differences in the prevalence of *M. suis* may be explained by the chronic stage of infection which is characterized by low parasitemia, making it undetectable by low-sensitivity techniques such as cPCR.

[Yuan et al. \(2009\)](#) pointed out that the ingestion of food contaminated with blood, the reuse of needles and the presence of arthropod vectors may play a role in the transmission of *M. suis* in commercial swine farms exhibiting poor sanitary conditions in China. [Smith \(1992\)](#) and [Messick \(2004\)](#) also highlighted that the presence of mosquitoes and lice (*Haematopinus suis*) may act as a possible risk factor for the occurrence of *M. suis* in swine herds. Although the present study detected porcine hemoplasmas infection in 100% of the sampled farms, all the farms followed standard sanitary conditions. However, the authors did not rule out the possibility of transmission occurring through the reuse of needles or other materials commonly used in animal management (e.g., restrainer) as well as the potential for the active participation of arthropod vectors since the region sampled in this study

demonstrated by Yuan et al. (2009) in China. Therefore, more studies should be conducted to determine if these hypotheses are true and how they could impact pig production.

5. Conclusion

Porcine hemoplasmas have a high rate of prevalence (79.72%) in sows from commercial farms in the southern region of Brazil. Our results report the first molecular detection and characterization of partial 16S rRNA gene of *M. parvum* in Brazil.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetmic.2019.02.024>.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GeneBank. *Nucleic Acids Res.* 41, D36–D42.
- Birkenheuer, A.J., Levy, M.G., Breitschwerdt, E.B., 2003. Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *J. Clin. Microbiol.* 41, 4172–4177. <https://doi.org/10.1128/JCM.41.9.4172-4177.2003>.
- Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55, 611–622. <https://doi.org/10.1373/clinchem.2008.112797>.
- Darriba, D., Posada, D., 2012. jModelTest 2.0 Manual. *Nat. Methods* 9, 772. <https://doi.org/10.1093/molbev/msn083>.
- Dias, G.B., do Amaral, R.B., Gatto, I.R.H., Lopera, I.M., de Oliveira, L.G., Lux Hoppe, E.G., Machado, R.Z., André, M.R., 2019. Molecular detection of *Mycoplasma suis* in captive white-lipped peccaries (*Tayassu pecari*) and wild boars (*Sus scrofa*) in Brazil. *Comp. Immunol. Microbiol. Infect. Dis.* 63, 94–96. <https://doi.org/10.1016/j.cimid.2019.01.013>.
- do Nascimento, N.C., dos Santos, A.P., Chu, Y., Guimaraes, A.M.S., Baird, A.N., Weil, A.B., Messick, J.B., 2014. Microscopy and genomic analysis of *Mycoplasma parvum* strain Indiana. *Vet. Res.* 45, 86. <https://doi.org/10.1186/s13567-014-0086-7>.
- do Nascimento, N.C., Guimaraes, A.M.S., dos Santos, A.P., Chu, Y., Marques, L.M., Messick, J.B., 2018. RNA-Seq based transcriptome of whole blood from immunocompetent pigs (*Sus scrofa*) experimentally infected with *Mycoplasma suis* strain Illinois. *Vet. Res.* 49, 49. <https://doi.org/10.1186/s13567-018-0546-6>.
- Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* 8, 186–194. <https://doi.org/10.1101/gr.8.3.186>.
- Ewing, B., Hillier, L.D., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* 8, 175–185. <https://doi.org/10.1101/gr.8.3.175>.
- Felder, K.M., Hoelzle, K., Ritzmann, M., Kilchling, T., Schiele, D., Heinritz, K., Groebel, K., Hoelzle, L.E., 2011. Hemotropic mycoplasmas induce programmed cell death in red blood cells. *Cell. Physiol. Biochem.* 27, 557–564. <https://doi.org/10.1159/000329957>.
- Fu, Y., Shi, T., Xu, L., Wei, W., Lu, F., Zhang, X., Yuan, X., Li, J., LV, J., Fang, W., 2017. Identification of a novel *Hemoplasma* species from pigs in Zhejiang province. *China J. Vet. Med. Sci.* 79, 864–870. <https://doi.org/10.1292/jvms.16-0545>.
- Gonçalves, L.R., Favacho, A.R., de, M., Roque, A.L.R., Mendes, N.S., Fidelis, O.L., Benevenuto, J.L., Herrera, H.M., D'Andrea, P.S., de Lemos, E.R.S., Machado, R.Z., André, M.R., 2016. Association of *Bartonella* species with wild and synanthropic rodents in different Brazilian biomes. *Appl. Environ. Microbiol.* 82, 7154–7164. <https://doi.org/10.1128/AEM.02447-16>.
- Groebel, K., Hoelzle, K., Wittenbrink, M.M., Ziegler, U., Hoelzle, L.E., 2009. *Mycoplasma suis* invades porcine erythrocytes. *Infect. Immun.* 77, 576–584. <https://doi.org/10.1128/IAI.00773-08>.
- Guimaraes, A.M.S., Biondo, A.W., Lara, A.C., Messick, J.B., 2007. Exploratory study of *Mycoplasma suis* (*Eperythrozoon suis*) on four commercial pig farms in southern Brazil. *Vet. Rec.* 160, 50–53. <https://doi.org/10.1136/vr.160.2.50>.
- Guimaraes, A.M., Vieira, R.F., Poletto, R., Vemulapalli, R., Santos, A.P., Moraes, W., Cubas, Z.S., Santos, L.C., Marchant-Forde, J.N., Timenetsky, J., Biondo, A.W., Messick, J.B., 2011. A quantitative TaqMan PCR assay for the detection of *Mycoplasma suis*. *J. Appl. Microbiol.* 111. <https://doi.org/10.1111/j.1365-2672.2011.05053.x>.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser. (Oxf) doi:icteulike-article-id:691774*.
- Henry, S.C., 1979. Clinical observations on eperythrozoonosis. *J. Am. Vet. Med. Assoc.* 174, 601–603.
- Hoelzle, L.E., 2008. Haemotropic mycoplasmas: recent advances in *Mycoplasma suis*. *Vet. Microbiol.* <https://doi.org/10.1016/j.vetmic.2007.12.023>.
- Hoelzle, L.E., Helbling, M., Hoelzle, K., Ritzmann, M., Heinritz, K., Wittenbrink, M.M., 2007. First LightCycler real-time PCR assay for the quantitative detection of *Mycoplasma suis* in clinical samples. *J. Microbiol. Methods* 70, 346–354. <https://doi.org/10.1016/j.mimet.2007.05.009>.
- Hoelzle, K., Engels, M., Kramer, M.M., Wittenbrink, M.M., Dieckmann, S.M., Hoelzle, L.E., 2010. Occurrence of *Mycoplasma suis* in wild boars (*Sus scrofa* L.). *Vet. Microbiol.* 143, 405–409. <https://doi.org/10.1016/j.vetmic.2009.11.015>.
- Hornok, S., Sugár, I., Fernández de Mera, I.G., de la Fuente, J., Horváth, G., Kovács, T., Micsutka, A., Gónczi, E., Flaisz, B., Takács, N., Farkas, R., Meli, M.L., Hofmann-Lehmann, R., 2018. Tick- and fly-borne bacteria in ungulates: The prevalence of *Anaplasma phagocytophilum*, haemoplasmas and rickettsiae in water buffalo and deer species in Central Europe, Hungary. *BMC Vet. Res.* 14. <https://doi.org/10.1186/s12917-018-1403-6>.
- Kuramae-Izioka, E.E., 1997. A rapid, easy and high yield protocol for total genomic DNA isolation of *Colletotrichum gloeosporioides* and *Fusarium oxysporum*. *Unimar* 19, 683–689. <https://doi.org/10.1088/0031-9155/57/21/6903>.
- Maggi, R.G., Chitwood, M.C., Kennedy-Stoskopf, S., DePerno, C.S., 2013. Novel hemotropic *Mycoplasma* species in white-tailed deer (*Odocoileus virginianus*). *Comp. Immunol. Microbiol. Infect. Dis.* 36, 607–611. <https://doi.org/10.1016/j.cimid.2013.08.001>.
- Mascarelli, P.E., Tartara, G.P., Pereyra, N.B., Maggi, R.G., 2016. Detection of *Mycoplasma haemocanis*, *Mycoplasma haematoparvum*, *Mycoplasma suis* and other vector-borne pathogens in dogs from Córdoba and Santa Fé, Argentina. *Parasit. Vectors* 9, 1–5. <https://doi.org/10.1186/s13071-016-1920-8>.
- Messick, J.B., 2004. Hemotropic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. *Vet. Clin. Pathol.* <https://doi.org/10.1111/j.1939-165X.2004.tb00342.x>.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop, GCE 2010. <https://doi.org/10.1109/GCE.2010.5676129>.
- Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike. *Syst. Biol.* 53, 793–808. <https://doi.org/10.1080/10635150490522304>.
- Ritzmann, M., Grimm, J., Heinritz, K., Hoelzle, K., Hoelzle, L.E., 2009. Prevalence of *Mycoplasma suis* in slaughter pigs, with correlation of PCR results to hematological findings. *Vet. Microbiol.* 133, 84–91. <https://doi.org/10.1016/j.vetmic.2008.06.015>.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>.
- Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* 74, 5463–5467. <https://doi.org/10.1073/pnas.74.12.5463>.
- Smith, A.R., 1992. Eperythrozoonosis. In: Straw, B., Mengeling, W.L., D'Allaire, S.D., Taylor, D.J. (Eds.), *In Diseases of Swine*, 7th edn. Iowa State University Press, Ames, pp. 470–474.
- Stadler, J., Jannasch, C., Mack, S.L., Dietz, S., Zöls, S., Ritzmann, M., Hoelzle, K., Hoelzle, L.E., 2014. Clinical and haematological characterisation of *Mycoplasma suis* infections in splenectomised and non-splenectomised pigs. *Vet. Microbiol.* 172, 294–300. <https://doi.org/10.1016/j.vetmic.2014.05.012>.
- Stöver, B.C., Müller, K.F., 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinform.* 11. <https://doi.org/10.1186/1471-2105-11-7>.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>.
- Toledo, M.A., Leite, A.L., Gonçalves, L.R., de Sousa, K.C.M., de Amaral, R.B., da Silva, G.C.P., Machado, R.Z., André, M.R., 2016. High occurrence of *Mycoplasma suis* infection in swine herds from non-technified farms in Mossoró, state of Rio Grande do Norte, Northeastern Brazil. *Rev. Bras. Parasitol. Vet.* 25, 414–417. <https://doi.org/10.1590/s1984-29612016084>.
- Watanabe, Y., Fujihara, M., Obara, H., Nagai, K., Harasawa, R., 2011. Two genetic clusters in swine hemoplasmas revealed by analyses of the 16S rRNA and RNase P RNA genes. *J. Vet. Med. Sci.* 73, 1657–1661. <https://doi.org/10.1292/jvms.11-0293>.
- Watanabe, Y., Fujihara, M., Suzuki, J., Sasaoka, F., Nagai, K., Harasawa, R., 2012. Prevalence of swine hemoplasmas revealed by real-time PCR using 16S rRNA gene primers. *J. Vet. Med. Sci.* 74, 1315–1318. <https://doi.org/10.1292/jvms.12-0096>.
- Yuan, C.L., Liang, A.B., Yao, C.B., Yang, Z.B., Zhu, J.G., Cui, L., Yu, F., Zhu, N.Y., Yang, X.W., Hua, X.G., 2009. Prevalence of *Mycoplasma suis* (*Eperythrozoon suis*) infection in swine and swine-farm workers in Shanghai, China. *Am. J. Vet. Res.* 70, 890–894. <https://doi.org/10.2460/ajvr.70.7.890>.