



## Investigation of hemotropic Mycoplasmas in fetuses and sows with reproductive failure

Luiz Carlos Bordin<sup>a,†</sup>, Danielle Gava<sup>a,†,\*</sup>, Karina Sonalio<sup>b</sup>, Marina Lopes Mechler-Dreibi<sup>b</sup>, Janice Reis Ciacci Zanella<sup>a</sup>, Nelson Morés<sup>a</sup>, Luís Guilherme de Oliveira<sup>b</sup>, Eliana Knackfuss Vaz<sup>c</sup>

<sup>a</sup> Embrapa Suínos e Aves, Animal Health Laboratory, Concórdia, SC, Brazil

<sup>b</sup> São Paulo State University, School of Agricultural and Veterinarian Sciences, FCAV/Unesp, Brazil

<sup>c</sup> Santa Catarina State University, Centro de Ciências Agroveterinárias, Lages, SC, Brazil

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### ABSTRACT

Swine eperythrozoonosis or porcine hemoplasmosis is an infectious disease caused mainly by *Mycoplasma suis* and is distributed worldwide. This study investigated the occurrence of porcine hemotropic mycoplasmas (PHMs) in fetuses and sows with reproductive failure. Two hundred and seventy-six samples (80 sows' blood and 196 fetal tissue samples) from 27 farms with reproductive disorders were evaluated. The PHMs DNA was detected in 15 out of 80 (18.7%) sows but it was not detected in the fetuses. The bacterial load ranged from  $1.32 \times 10^2$  to  $2.61 \times 10^5$  copies/ $\mu$ L. From the 27 tested herds, 11 (40.7%) showed at least one positive sow per farm. The majority of the reproductive problems observed in PMHs positive sows were stillborn fetuses (46.7%) and stillborn associated with fetal mummification (26.7%). So, we evidenced that porcine hemoplasmas circulate among sows in Brazilian herds, however, its real impact on reproductive problems remains unknown.

### 1. Introduction

Porcine Hemotropic Mycoplasmas (PHMs), namely *Mycoplasma suis* *Mycoplasma parvum* and '*Candidatus M. haemosuis*' have been described affecting swine red blood cells (Fu et al., 2017; Kinsley, 1932; Splitter, 1950). *Mycoplasma suis* is the most common species affecting pigs, which presents itself under the clinical or sub clinical form (Hoelzle, Zeder, Felder & Hoelzle, 2014; Stadler et al., 2014). The clinical signs include icterus anemia, fever, and decrease of reproductive performance, leading to increased stillbirth rates and dysgalactia (Brissonnier et al., 2020; Henry, 1979; Strait, Hawkins & Wilson, 2012). On the other hand, chronic or sub clinically infected animals do not show specific signs, contributing to the spread of the disease and its underdiagnosis (Ritzmann, Grimm, Heinritzi, Hoelzle & Hoelzle, 2009).

Regarding PHMs diagnosis, these pathogens used to be detected by blood smear and conventional PCR. However, nowadays, the molecular detection of their DNA by quantitative real-time PCR (qPCR) has been commonly applied, due to its high sensitivity and specificity (Guimaraes et al., 2011; Hoelzle et al., 2007). Still, the detection of PHMs through qPCR has shown that a high percentage of positive animals presented

bacterial loads lower than  $10^4$  copies/ $\mu$ L, which could be associated to the chronic infection of the disease (Gatto et al., 2019).

Even though PHMs infections have been described in several countries, some clinical aspects regarding their impact on reproductive performance of sows are still unknown. Thus, the objective of this study was to investigate the occurrence of PHMs in sows with reproductive failure, as well as in their fetuses.

### 2. Material and methods

Twenty-seven farms located in Southern Brazil (22 from Santa Catarina State and five from Paraná State) from nine municipalities were previously selected based on their reproductive parameters. The farms included in this study presented at least one of the following indexes: abortion rate >4%, return to estrus >15%, fetal mummification >15% and stillbirth >10% (Table 1). The sows were vaccinated against porcine parvovirus, erysipelas, leptospirosis and porcine circovirus type 2.

Whole blood samples with EDTA were collected from 80 sows, in addition to the spleen (88 samples) and liver (108 samples) from mummies and/or stillbirths born from these sows. In total, 276 samples

\* Corresponding author.

E-mail address: [danielle.gava@embrapa.br](mailto:danielle.gava@embrapa.br) (D. Gava).

† These authors contributed equally

**Table 1**  
Detailed information on PHMs qPCR positive samples.

Location (Municipality and State)	Farm ID	Sample ID	Cq*	Copies/ $\mu$ L	Reproductive failure
Castro/PR	20	G20 1076	32.1	12,110	Stillbirth
	18	P91850 R	37.9	412.1	Stillbirth
Concórdia/SC	9	G9 1208	34.0	4020	Stillbirth
	1	G1 1518	34.1	3810	Fetal mummification
Erval Velho/SC		G1 1395	39.3	188.9	Return to estrus
	11	G11 s/n	36.0	1240	Stillbirth
	15	3434	32.7	8580	Stillbirth/fetal mummification
Jaborá/SC		2515	38.7	257.8	Stillbirth
	10	G10 6946	39.5	154.5	Stillbirth/fetal mummification
Luzerna/SC	13	2747	38.4	312.2	Stillbirth
	14	2596	37.7	450	Stillbirth/fetal mummification
Ouro/SC		3396	39.8	132.3	Stillbirth
	5	167	26.8	261,700	Abortion
		940	38.0	395.2	Abortion
Seara/SC	7	G7 2066	32.9	7570	Stillbirth/fetal mummification

\*Cq: Cycle of quantification.

were evaluated. DNA extraction from whole blood samples was performed using the Illustra Blood Kit (Cytiva - formerly GE Healthcare, USA) and from tissues was carried out with phenol:chloroform followed by ethanol precipitation protocol.

The quantitative TaqMan real-time PCR (qPCR) was run as previously described (Guimaraes et al., 2011). A known *M. suis*-positive 16S rRNA gene (~1400pb) from a splenectomized piglet was gently provided by Dr. A.M.S. Guimarães and employed as PCRs positive control. For its use in qPCR, the 16S rRNA gene was cloned using a TOPO TA Cloning Kit (Invitrogen). The number of copies of the cloned plasmid was calculated according to Yun et al. (2006). Ten-fold dilutions of *M. suis* plasmid were used to build the standard curve, from  $3.28 \times 10^9$  up to  $3.28 \times 10^0$  copies/ $\mu$ L. Other *Mycoplasmas* (*M. hyopneumoniae*, *M. hyorhinis* e *M. flocculare*) along with a negative sample for *M. suis*, were used as PCRs negative control.

### 3. Results

In the qPCR, 15 out of the 80 sows' blood samples (18.75%) were positive, with bacterial load ranging from  $1.32 \times 10^2$  to  $2.61 \times 10^5$  copies/ $\mu$ L (Table 1). Quantification values (Sq) of the qPCR assay ranged from  $3.28 \times 10^9$  to  $3.28 \times 10^2$  copies/ $\mu$ L, with a linear correlation ( $R^2$ ) of 0.993, slope of -4.14, and average cycle of quantification (Cq) values ranging from 8.33 to 37.60. All fetal samples were negative when tested in the qPCR assay.

Porcine hemoplasmas were detected in 11 out of 27 farms, distributed in seven different municipalities. Curiously out of the 15 positive sows, seven had reproductive problems associated with stillborn fetuses, four with stillborn and fetal mummification, two with abortion, one with fetal mummification and one with return to estrus (Table 1).

### 4. Discussion

In the present study, PHMs were detected in sows showing reproductive failure, mainly stillbirths, in Southern Brazil. Stadler et al. (2019) also reported that *M. suis*-positive farms had significantly more stillborn piglets per litter than negative farms. Similarly, Brissonnier et al. (2020) observed an increase in stillbirths' rate in gilts positive for *M. suis*. However, as stated by Stadler et al. (2019), the occurrence of stillborn piglets in *M. suis* positive farms must be cautiously interpreted

once other infectious and non-infectious factors could be present and were not evaluated on this study.

Regarding PHMs occurrence in Southern Brazil, Gatto et al. (2019) observed that PHMs are widely spread in commercial pig farms, with an overall occurrence of 79.7% in sows and 100% positivity on the tested farms. In addition, 88% of the tested samples presented bacterial loads between  $10^1$  and  $10^4$  copies/ $\mu$ L, suggesting that most animals were chronically infected. Besides, as reported by Stadler et al. (2019), the bacterial loads detected in sows and piglets were considerably higher than the ones found in the sows from the present study, which may have influenced the negative results observed in the fetuses.

Furthermore, in South Germany, Stadler et al. (2019) investigated vertical transmission of *M. suis* in 21 farms and observed 14.3% and 31.2% of blood samples positivity for pre-suckling piglets and sows, respectively. An interesting fact is that the majority of the positive sows delivered negative piglets. As stated by Henderson, O'Hagan, Hawe and Pratt (1997), only a proportion of newborn piglets can be infected from the dam by uterine transmission, which may explain the negative results on fetuses observed in our study.

Considering the aforementioned information, it was not possible to relate the occurrence of reproductive failure in sows with the presence of PHMs in the fetuses, even though 73.4% of the positive sows presented high stillborn rates (>10%). However, PHMs were detected in most of the evaluated farms, which indicates that these pathogens are spread in the Southern Brazilian herds, although its real role in sows and fetuses should be clarified.

### Ethical statement

This study only contains analysis on pig blood and fetal tissues of stillbirths and mummified fetuses. We assured that the care with pigs completely complied with Brazilian Animal Welfare laws, guidelines and policies.

### Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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