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Genetic diversity of *Mycoplasma hyopneumoniae* in finishing pigs in Minas Gerais[1]

Mariana R. Andrade[2], Amanda G.S. Daniel[2], Javier B. Zarate[2], José P.H. Sato[2], Lucas F. Santos[3] and Roberto M.C. Guedes[2]*

ABSTRACT.- Andrade M.R., Daniel A.G.S., Zarate J.B., Sato J.P.H., Santos L.F. & Guedes R.M.C. 2023. **Genetic diversity of *Mycoplasma hyopneumoniae* in finishing pigs in Minas Gerais.** *Pesquisa Veterinária Brasileira 43:e07155, 2023*. Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, Campus Pampulha, Belo Horizonte, MG 30161-970, Brazil. E-mail: guedesufmg@gmail.com

Mycoplasma hyopneumoniae is one of the most challenging respiratory pathogens involved with swine pneumonia worldwide, responsible for a chronic infection with high morbidity, which predisposes secondary bacterial infections in growing and finishing pigs. Advances in diagnostic techniques allowed identification of genetic characteristics associated with high antigenic and proteomic variability among bacterial strains. This study aimed to evaluate the genetic diversity of *M. hyopneumoniae* strains in lungs with pneumonic lesions obtained from 52 pig farms located in Minas Gerais, one of the largest swine production states in Brazil. Genotyping was performed using multilocus variable number of tandem repeat (VNTR) analysis (MLVA), targeting two *loci* encoding P97 and P146 adhesins VNTR. The results showed that this agent is widely disseminated in pig farms and there is a high polymorphism of *M. hyopneumoniae* variants circulating in the state of Minas Gerais. Different *M. hyopneumoniae* genotypes are randomly distributed in several regions of the state, with no specific geographic population structure pattern. *M. hyopneumoniae* association with viral agents was sporadic (3.17% with Influenza A and 1.9% with PCV2).

INDEX TERMS: Enzootic pneumonia, MLVA, genotyping, swine respiratory complex, *Mycoplasma hyopneumoniae*, pigs.

RESUMO.- [Diversidade de cepas de *Mycoplasma hyopneumoniae* em suínos em terminação brasileiros.] *Mycoplasma hyopneumoniae* é um dos patógenos respiratórios mais desafiadores envolvidos com pneumonia suína em todo o mundo. É responsável por uma infecção crônica de alta morbidade, que predispõe a infecções bacterianas secundárias nas fases de crescimento e terminação. Avanços nas técnicas diagnósticas permitiram a identificação de características genéticas do agente, associadas à alta variabilidade antigênica e proteômica entre cepas. Este estudo teve como objetivo examinar a ocorrência e diversidade genética de cepas de *M. hyopneumoniae* em pulmões com lesões pneumônicas em 52 granjas de suínos no estado de Minas Gerais, um dos maiores estados produtores de suínos do Brasil. A genotipagem foi realizada utilizando a técnica de "multilocus variable number of tandem repeat analysis" (VTNR/MLVA), usando dois *loci* que codificam VNTR das adesinas P97 e P146. Os resultados mostraram que esse agente está amplamente disseminado em granjas de suínos e que existe alto polimorfismo das variantes de *M. hyopneumoniae* circulando no estado de Minas Gerais. Diferentes genótipos de *M. hyopneumoniae* estão distribuídos aleatoriamente em várias regiões do estado, sem um padrão de estrutura populacional e geográfica específico. Associação de *M. hyopneumoniae* e agentes virais foi esporádica (3,17% com Influenza A e 1,9% com PCV2).

TERMOS DE INDEXAÇÃO: Pneumonia enzoótica, MLVA, genotipagem, complexo respiratório dos suínos, *Mycoplasma hyopneumoniae*, suínos.

INTRODUCTION

Respiratory diseases represent a large part of the health problems observed in growing and finishing pigs, which generate

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[2] Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, Campus Pampulha, Belo Horizonte, MG 30161-970, Brazil. *Corresponding author: guedesufmg@gmail.com
[3] Microvet Microbiologia Veterinária Especial, Av. Joaquim Lopes de Faria 730, Santo Antonio, Viçosa, MG 36576-001, Brazil.

major economic losses in the swine industry (Simionato et al. 2013). Evidence of this problem is the high prevalence of lung lesions reported in many studies in the most important pig producing countries (Marois et al. 2008, Fraile et al. 2010, Meyns et al. 2011, Fablet et al. 2012, Merialdi et al. 2012, Arsenakis et al. 2019, Ferraz et al. 2020).

Mycoplasma hyopneumoniae is one of the most economically impacting respiratory pathogens in the world, detected in almost all swine farms with intensive production countries (Thacker 2006, Silva et al. 2009, Maes et al. 2018). The bacterium is considered a primary agent of swine enzootic pneumonia and respiratory complex, characterized by coughs, reduced growth rate and high feed conversion rate (Maes et al. 1996). The spread of the agent in the herd is slow (Meyns et al. 2004) and the incubation period range from 14 to 42 days (Fano et al. 2005). Direct contact is considered the main form of transmission. On the other hand, some studies have demonstrated the ability of the bacteria to be spread by the wind, remaining viable after traveling 9.2km (Fano et al. 2005, Otake et al. 2010).

With the advances in diagnostic techniques and the growing demand to control *M. hyopneumoniae*, one of the trends have been to know the different circulating variants within and among herds through various genotyping techniques (Dos Santos et al. 2015). This approach has yielded positive results in understanding transmission chain, preventing future infections, and comparing field and vaccine variants (Foxman et al. 2005). The fact that the species has lability in repetitive DNA sequences known as active recombination sites, possibly involved in the pathogenesis of infection, has made the variable number of tandem repeats (VNTR) assay an efficient method for the analysis of genetic diversity of *M. hyopneumoniae*. Multiple loci analysis containing VNTR, or multilocus VNTR analysis (MLVA), has shown high discriminatory power in previous studies, which has made this one of the most widely used methods for epidemiological characterization of the *M. hyopneumoniae* (Mayor et al. 2007, Vranckx et al. 2011). Molecular characterization of the genes coding for two *M. hyopneumoniae* adhesins P97 (Mhp183) and P146 (Mhp684) (Bogema et al. 2012), by identifying the number of tanden repeats showed to be an important tool to investigate *M. hyopneumoniae* genetic diversity (Dos Santos et al. 2015). Ciliary adhesins are critical components in the pathogenesis of *M. hyopneumoniae*. Gene plasticity is an interesting strategy for altering its surface topography to avoid detection and elimination by the host immune system, which also determines variability among circulating strains (Djordjevic et al. 2004, Browning et al. 2011, Reolon et al. 2014).

Thus, this study considered the importance of *M. hyopneumoniae* as a pathogen in pig production and the relevance of Minas Gerais state in pig production as the fourth largest pig producer in the country (ABCS 2016). The aim of this study was to evaluate the genetic diversity and occurrence of *M. hyopneumoniae* in pneumonic finishing pigs at slaughter originated from different geographic mesoregions of Minas Gerais state.

MATERIALS AND METHODS

Samples. Lungs from 349 pigs from 52 different herds located in nine geographical mesoregions of Minas Gerais state (Fig.1) were collected for analysis during 11 slaughterhouses sample collection from October 2016 to September 2017. The number of collected samples by mesoregion was determined based on number of herds within each mesoregion (Fig.2). Five to six lungs with macroscopic lesions suggestive of *Mycoplasma hyopneumoniae* involvement and one lung without lesions were collected by farm. All collected lungs were directed to the "Departamento de Clínica e Cirurgia Veterinárias" (Veterinary Pathology Department) of the "Universidade Federal de Minas Gerais" (UFMG), where they were submitted to the analyzes described below.

Macroscopic and microscopic evaluation. All lungs were macroscopically evaluated, and an individual lesion score was assigned, according to Halbur et al. (1995). *M. hyopneumoniae* suggestive lesions were defined by consolidation areas usually located in the cranioventral portion with lobular distribution. All lungs were also evaluated for pleurisy, defined as fibrinous or fibrous adhesions areas in the parietal and visceral pleural membranes, as well as the presence of purulent or necrotic nodules, according to Piffer & Brito (1991). A fragment from each affected lobes in the normal transition area was fixed in 10% buffered formalin and processed with routine hematoxylin and eosin (HE) staining for histopathological examination. The microscopic lesion determination score was performed according to Hansen et al. (2010).

Bacterial isolation and immunohistochemistry. To identify secondary bacterial infections, pneumonic affected areas from all lungs were subjected to routine isolation of porcine secondary respiratory pathogens and biochemical characterization through the methodology described by Quinn et al. (1994). If there were macroscopic differences among pneumonic affected areas of the same lung, each area was sampled separately for bacteriology. Samples were seeded on 5% sheep blood agar and MacConkey Agar and incubated at 37°C for 24 to 48 hours in aerobic conditions. A colony of *Staphylococcus aureus* was also used as a factor V source, incubated at 10% CO2 at 37°C for 24 to 72 hours.

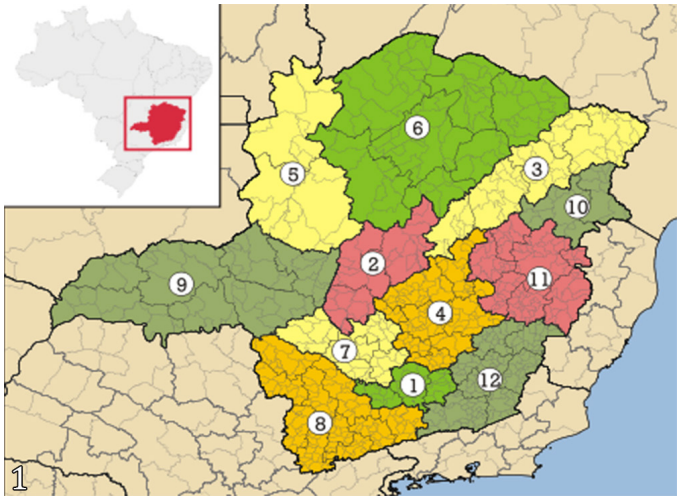

Fig.1. The 12 geographical mesoregions of the state of Minas Gerais according to "Instituto Brasileiro de Geografia e Estatística" (Brazilian Institute of Geography and Statistics - IBGE): Campo das Vertentes (1), Central Mineira (2), Jequitinhonha (3), Zona Metropolitana de Belo Horizonte (ZMBH) (4), Noroeste de Minas (5), Norte de Minas (6), Oeste de Minas (7), Sul e Sudoeste de Minas (8), Triângulo Mineiro e Alto Paranaíba (TMAP) (9), Vale do Mucuri (10), Vale do Rio Doce (11), Zona da Mata (12). Fonte: IBGE (2018).

Formalin-fixed lung fragments were processed for preparation of histological slides and also evaluated for the presence of Influenza A virus and Porcine circovirus type 2 (PCV2) antigen by immunohistochemistry (IHC), according to Paladino et al. (2017).

DNA extraction and nested-PCR. Clinical samples from the bronchial surfaces of pneumonic affected areas were obtained using a sterile rayon swab (Global Swab, Monte Alto, SP). Access to the bronchi closest to the injured lung areas was performed and the contents of this region were collected with a sterile rayon swab. Bronchial swabs from the injured region of all lungs were eluted in 300µL PBS and kept refrigerated at -20ºC. DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Belgium) according to the manufacturer's instructions. Confirmation of *M. hyopneumoniae* presence was detected with a nested PCR reaction (nPCR) according to Calsamiglia et al. (1999) and the products were analyzed by electrophoresis, stained with ethidium bromide, and visualized in UV light. The *M. hyopneumoniae* (USA) vaccine strain 232 was used as a positive control in all reactions. Ultrapure water (Milliq, Millipore, USA) was used as negative control in all reactions, presenting consistent results in gels.

Multiple locus variable repeat analysis (MLVA). The two adhesin VNTR loci, P97 RR1 (mhp138) and P146 RR3 (mhp684) were tested on all nested PCR (nPCR) positive samples. The mix and reaction conditions have been previously described by Vranckx et al. (2011) and optimized for the conditions of our laboratory thermal cycler (Veriti, Applied Biosystems®), in two separate PCR reactions, one for each locus. PCR amplicons were also analyzed by ethidium bromide-stained agarose gel electrophoresis by visualization in UV light. About three agarose gel-positive samples per farm were selected for MLVA. The 232 *M. hyopneumoniae* strain was used as a positive control in all reactions.

DNA sequencing. Three samples per farm containing strong UV bands in the nested-PCR for *M. hyopneumoniae* were selected for sequencing, performed at the UFMG Aquacen laboratory. PCR products were purified using Agencourt AMPure XP (Beckman Coulter Company, Beverly, Massachusetts, USA) according to the manufacturer's instructions. Each product was sequenced in both directions using the P97RR1 F/P97RR1 R and P146RR3 F/P146RR3 R primers and the Big Dye V3.1 Terminator Kit (Applied Biosystems, California, USA) on the ABI 3500 DNA analyzer sequencer (Applied Biosystems, California, USA).

Data analysis. Sequencing data files from both loci (ab1) were organized and contigated and evaluated for sequence quality using the Bioedit software (Hall 2005). The tandem repetition counts of AAKPV (E) amino acids from the P97 R1 locus and polysin from the p146R3 locus was performed manually. Categorical data containing the concatenated genotypes of both loci were imported into file for Bionumerics 7.0 (Applied Maths, East Flanders, Belgium). A dendrogram was constructed using unweighted pair group method with arithmetic means (UPGMA), based on the categorical values of the number of repetitions. To estimate discriminatory power, Simpson's diversity index was calculated according to Hunter & Gaston (1988). Discriminatory indices were calculated for each region and for all samples taken together.

RESULTS

Macroscopic and microscopic evaluation

All affected lungs had typically cranioventral consolidation lesions, with predominantly cranial, cardiac, and intermediate lobes lesioned. Affected areas varied from reddish to grayish, firm and there was a reduction in volume in some cases. Catarrhal exudate to purulent catarrhal was frequently observed within the airways. Regarding the extension of pulmonary hepatization lesions in all lungs, there was a predominance of lungs (79% of the lungs) with lesions of up to 25% of the parenchyma affected. In a smaller scale, 11% of the lungs had macroscopic lesion comprising between 26 and 50% of the organ and only 1% with a score between 50 and 76%. Pneumonic lesions greater than 76% of the pulmonary parenchyma were not observed.

Microscopically, bronchopneumonia was present in 58% of the evaluated lungs. Bronchial associated lymphoid tissue (BALT) hyperplasia, one of the most relevant microscopic features associated with *Mycoplasma hyopneumoniae* infection, was identified in 80% of the lung fragments referred to histopathology. Of these, 32% of the samples had mild hyperplasia, 34%

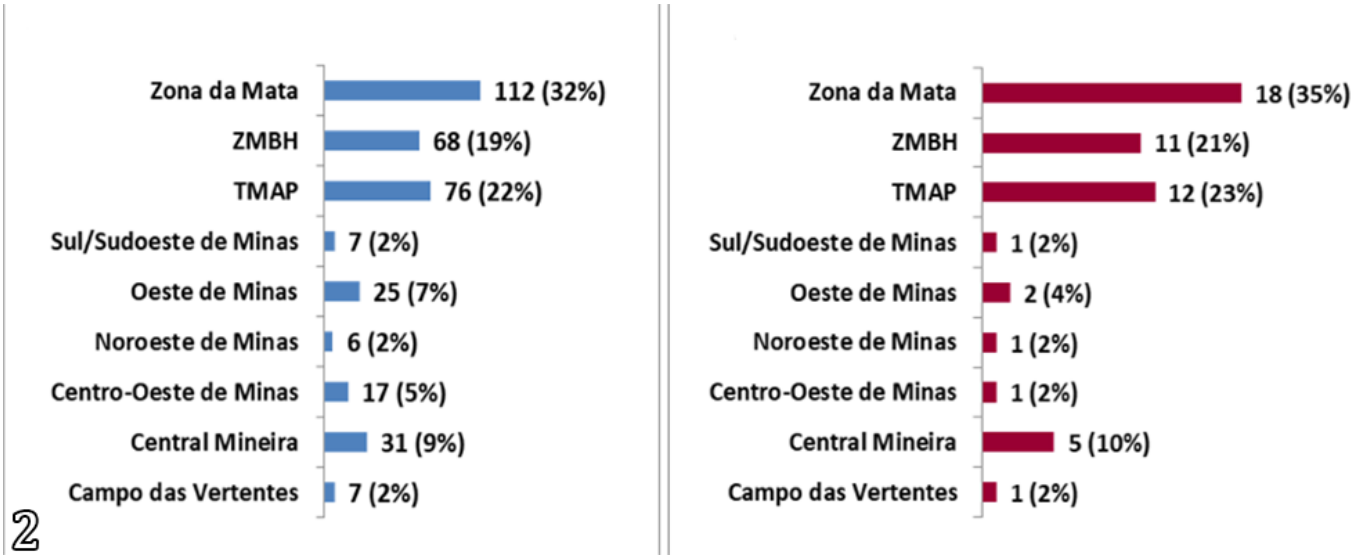

Fig.2. Mesoregions of origin of the samples. Number and percentage of samples collected by mesoregion (blue). Number and percentage of sampled hog farms in each mesoregion (red).

moderate-grade hyperplasia, 11% severe hyperplasia, and only 3% of fragments had severe hyperplasia associated with total airway lumen compression. Lynphoplasmocitic infiltration in alveolar septa, interstitial pneumonia, concomitant with bronchopneumonia lesions and BALT hyperplasia was identified in 25% of the evaluated fragments.

Bacterial isolation and immunohistochemistry

Additionally, all lungs were evaluated for the presence of associated bacterial and viral agents. Regarding bacterial agents, *Pasteurella multocida* was the main microorganism identified, with isolation in the injured parenchyma in 24.13% (76/315) of the lungs. The other agents (*Actinobacillus pleuropneumoniae*, *Glaesserella parasuis*, *Staphylococcus aureus* and *Streptococcus suis*) were detected in 2% or less of the evaluated lungs. Detection of viral agents was sporadic, with positive immunolabeling only in 3.17% (10/315) and 1.9% (6/315) of the total lungs for Influenza A and porcine circovirus type 2 (PCV2), respectively. Either secondary bacterial or viral infections were detected in *M. hyopneumoniae* nested-PCR positive lungs.

Nested-PCR

All bronchial swab specimens (n=349) were submitted to nested-PCR to detect *M. hyopneumoniae* DNA. From all evaluated samples, the DNA of the agent was detected in 91% of bronchial swabs. At least one sample from each farm was positive in nested-PCR, which means that there was positivity for *M. hyopneumoniae* in 100% of the farms evaluated. As a result of the sampling criteria, 9.74% (34/349 samples) of lungs had no macroscopic lesions, however not all herd were represented as all the examined lungs of a few herds had gross lesions. Considering only these lungs with no macroscopic lesions (n=34), 66% of them were nested-PCR positive for *M. hyopneumoniae*, while lungs with macroscopic lesions (n=315) were 94% positive.

Genotyping

MLVA analysis in this study evaluated two loci named P97 RR1 and P146 RR3, which encode two adhesins whose DNA sequences have VNTR. Sequential repeat numbers at each locus were concatenated and used to define the MLVA genotype of each sample, separated by a hyphen (P97 RR1 locus repeating number – P146 RR3 locus repeats number). It was not possible to obtain sequence types (STs) of samples from four farms due to poor quality of the obtained sequences not allowing to get a consensus, one from each of the following mesoregions: TMAP, ZMBH, Central Mineira and Midwest Minas Gerais regions. Therefore, out of the 52 farms, the sequencing data of 144 samples from 48 farms were available, which are described below. Of these, 82% of the samples represent the three largest producing regions of Minas Gerais (38% of the samples from the Zona da Mata region, 23% from the TMAP region and 21% from the ZMBH), while 19% correspond to the other smaller pig production mesoregions of the state.

Simpson's diversity index was calculated for each mesoregion, for all mesoregions together, for the loci separately and together. The diversity index was D=0.92 for the Triângulo Mineiro and Alto Paranaíba mesoregion (TMAP) (n=32), D=0.90 for Zona da Mata (n=55), D=0.92 for Belo Horizonte Metropolitan Zone (ZMBH) (n=28) and D=0.94 for the other producing mesoregions (n=27). The combination of discriminatory power for all samples (n=144) was D=0.94. The discriminatory index for locus p97 was D=0.78 and for locus p146 was D=0.84.

The MLVA analysis of the two loci in this study identified 43 STs for all samples collected from Minas Gerais. The most frequent STs were 12-14 (19%), 7-24 (9.1%), 7-23 (8.4%), 10-16 (7.7%), 8-14 (6.3 %), and 10-14 (4.9%) (Table 1). Less frequently, 15 STs had a distribution greater than 1%, namely: 12-20, 4-21, 7-21 and 7-25 (4%); 12-15, 10-23 and 7-27 (2.1%); 12-37, 12-35, 9-14, 11-20, 14-20, 10-17, 7-22 and 8-23 (1.4%). The remaining STs (22 genotypes) presented a distribution frequency of 0.7%: 12-23, 12-53, 15-51, 12-45, 12-39, 7-14, 11-14, 8-20, 9 -20, 7-20, 8-17, 10-39, 10-48, 10-15, 10-22, 11-21, 8-21, 4-51, 5-23, 5-34, 5-24 and 5-22. All these STs, including the most common ones, were distributed in several mesoregions of the state, without formation of a specific population structure of a given geographic region (Fig.3 and 4-5). Most of the herds (60%) had more than one ST, 21% of the farms had three STs, the maximum number of STs found in a single rearing system.

Table 1. Main genotypes of *Mycoplasma hyopneumoniae* circulating in the state of Minas Gerais according to the distribution frequency analyzed by MLVA

Genotype	Minas Gerais*	Mesoregion**	
	No. of samples (%)	Source	No. of samples (%)
12-14	27 (19.0)	Zona da Mata	16 (11.2)
		ZMBH	6 (4.2)
		TMAP	3 (2.1)
		Central Mineira	1 (0.7)
		Oeste de Minas	1 (0.7)
7-24	13 (9.1)	Zona da Mata	5 (3.5)
		Central Mineira	4 (2.8)
		ZMBH	3 (2.1)
		TMAP	1 (0.7)
7-23	12 (8.4)	TMAP	6 (4.2)
		Central Mineira	4 (2.8)
		ZMBH	2 (1.4)
10-16	11 (7.7)	TMAP	5 (3.5)
		ZMBH	4 (2.8)
		Zona da Mata	2 (1.4)
8-14	9 (6.3)	ZMBH	3 (2.1)
		Zona da Mata	3 (2.1)
		TMAP	3 (2.1)
10-14	7 (4.9)	Zona da Mata	3 (2.1)
		ZMBH	1 (0.7)
		Oeste de Minas	3 (2.1)

MLVA = multilocus variable number of tandem repeat analysis, ZMBH = zona metropolitana de Belo Horizonte, TMAP = Triângulo Mineiro e Alto Paranaíba; * Genotype frequency in MG: number of samples and percentage of samples, ** mesoregion of origin of animals and frequency of genotype in their mesoregions in relation to total samples.

The P97 locus presented nine types, ranging from 4 to 14 repetitions, while 19 types were detected in the P146 locus, ranging from 14 to 53 repetitions.

Minimal spanning tree of MLVA results per mesoregion is shown in Figure 6-9. DNA from 32 swine bronchial swab specimens from 11 TMAP mesoregion farms were evaluated. MLVA analysis identified 13 different STs in this region. The most frequent ST was 7-23 (18.7%), present in three distinct farms, followed by STs 10-16 (15.6%) and 4-21 (12.5%), both also present in more than one farm. In this mesoregion, four farms had more than one genotype. At the Zona da Mata region, 23 different STs were detected in 55 animals from 18 farms. The most frequent ST was 12-14 (29% of the samples from the region), distributed in five farms. A considerable number of farms (14 farms, 77%) had more than one circulating genotype in the herd. Thirteen STs were identified in ZMBH. In 28 animals from 10 farms in the region, ST 12-14 had the highest distribution frequency (21%), followed by 10-16 (14%), both present in more than one commercial herd. In this mesoregion, half of the farms had more than one genotype of *M. hyopneumoniae*. The other mesoregions evaluated in this study were represented by samples of 27 animals from nine farms, and comprise the South/Southwest of Minas, West of Minas, Central Mineira, Midwest of Minas and Campo das Vertentes. MLVA analysis identified 14 genotypes, and the most frequently STs were 7-23 and 7-24 (15%), and 7-25 and 10-14 (11%). Similar to the other mesoregions, most farms (6 farms, 66%) had more than one genotypic pattern present on the farm.

DISCUSSION

The aim of this study was to evaluate the genetic diversity and occurrence of *Mycoplasma hyopneumoniae* in pneumonic finishing pigs at slaughter originated from different geographic mesoregions of Minas Gerais State. The sampling included the largest swine production regions in Minas Gerais (TMAP, Zona da Mata and ZMBH), as well as other mesoregions where there is a smaller relative number of hog farms. The study design was to perform a lung sampling proportional to the number of hog farms in each mesoregion of the state, as described by Gonçalves (2016). Even though sample collection was dependent on batches of animals available during slaughter and the permission of different slaughterhouses to perform the sampling, it represented the proportionality originally designed.

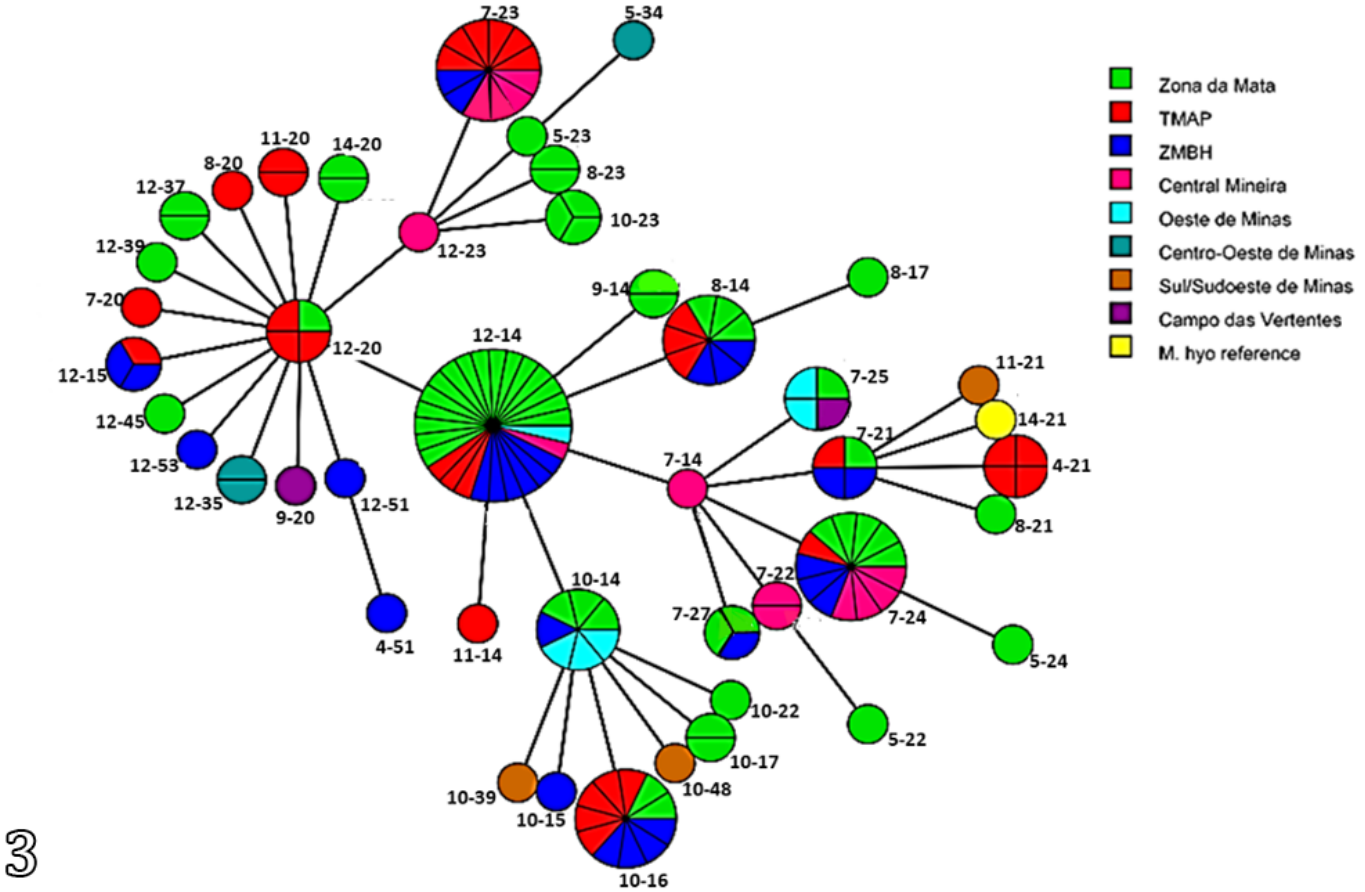

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Fig.3. Minimum spanning tree showing the diversity and distribution of 142 MLVA genotyped *Mycoplasma hyopneumoniae* samples. The mesoregions of origin of the samples are represented by different colors. Reference strain 232 is represented in yellow. The circles represent the genotypes. The size of the circles represents the number of clonal variants in each genotype. A total of 43 genotypes were detected, 23 of them in Zona da Mata, 13 genotypes in Belo Horizonte Metropolitan Zone, 13 genotypes in Triângulo Mineiro and Alto Paranaíba and 14 genotypes in other mesoregions.

Our results identified a high occurrence of *M. hyopneumoniae* through nested-PCR in the state of Minas Gerais, Brazil. The detection method used is known to be highly sensitive and specific. Regarding the best lung location to obtain the ideal clinical sample, upper tract samples have lower sensitivity than those obtained in the lower respiratory tract (Calsamiglia et al. 1999). Recently, Pieters et al. (2017) reported that laryngeal swabs had the highest sensitivity in early detection of *M. hyopneumoniae* in live experimentally inoculated pigs. Nevertheless, Fablet et al. (2010) reported that bronchial swabs were the best sample type in naturally infected finishing pigs. As our samples were collected at slaughter, bronchial swabs were used as clinical material for the nested-PCR.

Our study also detected positive *M. hyopneumoniae* DNA even in animals with lungs with no macroscopic lesions. Several studies have shown that the agent is able to last for a long period in the swine respiratory tract, even after lung lesions have resolved (Pieters et al. 2009). In addition, the occurrence of the disease is dependent on doses, strain type and management-related factors (Vicca et al. 2002). For Thacker (2001), it is possible that *M. hyopneumoniae* is never completely eliminated from the respiratory tract. Thus, infected animals often act as asymptomatic carriers for longer periods, capable of infecting susceptible pigs. Regarding the 6% of the lungs with macroscopic lesions that were negative for *M. hyopneumoniae* by the nested-PCR, it could be explained the possible involvement of the infectious agent, not corroborated by the testing for secondary bacterial or viral infection, or as false negative based on the that the test is not 100% sensitive.

It is noteworthy that despite the high frequency of detection of *M. hyopneumoniae*, the sampling was directed to organs with lesions, performed in a small number of lungs, in a single batch of farms, and without considering the size of the herd, information that was not available. Therefore, this data is subjective only to infer about the occurrence in each farm and cannot be used as a prevalence data for *M. hyopneumoniae* in the state of Minas Gerais. However, it should not be ruled out that the presence of positive results through nested-PCR in lungs samples from all farms represents relevant data that reveals the ubiquitous character of the *M. hyopneumoniae* in Minas Gerais. Pneumonia is one of the most commonly used

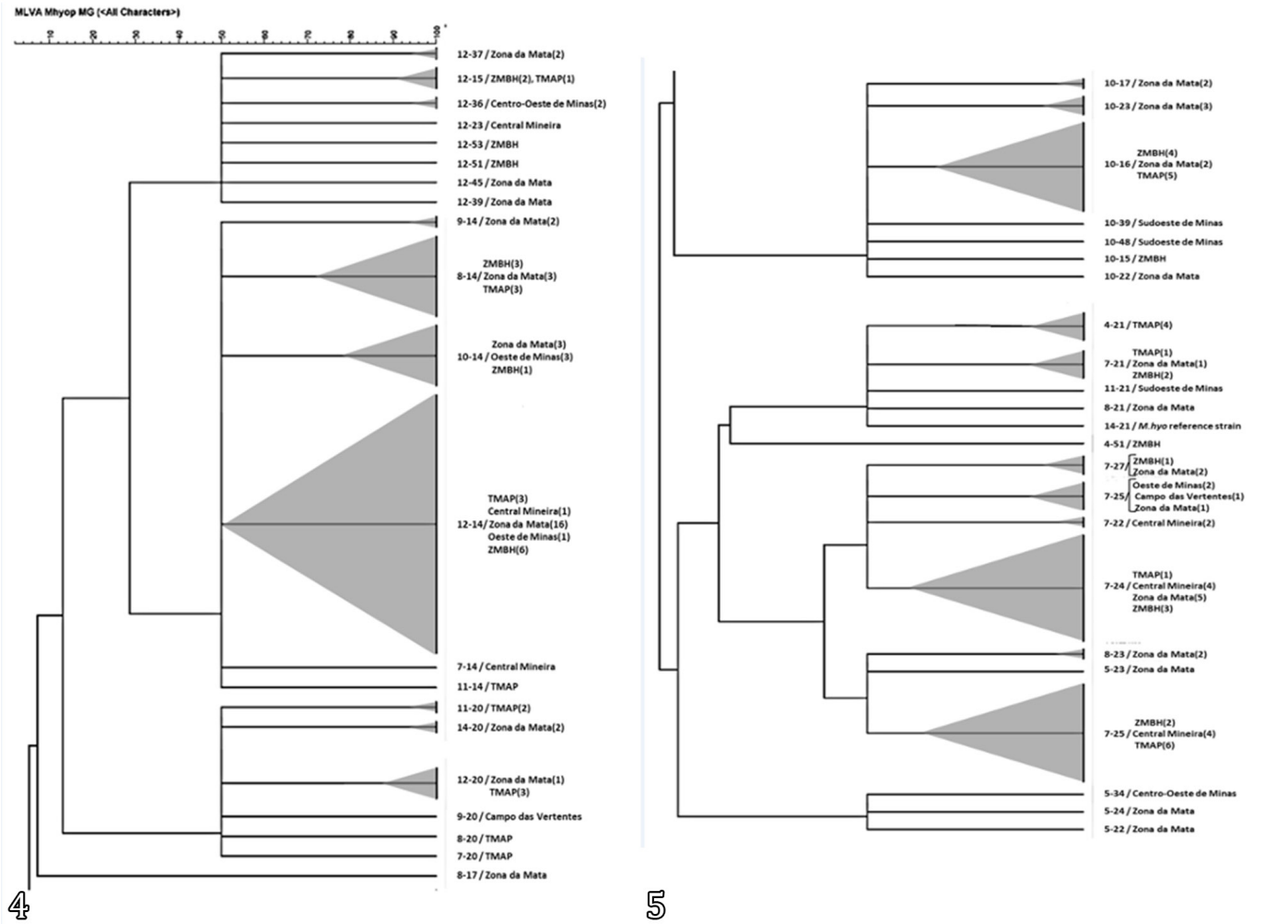

Fig.4-5. Dendrogram representing the distribution of *Mycoplasma hyopneumoniae* MLVA genotypes in the state of Minas Gerais. MLVA type is indicated by the number of repetitions at each of the loci, followed by the mesoregions of origin and number of samples in each mesoregion. Eg genotype/mesoregion (number of samples in mesoregion). The dendrogram was generated through UPGMA in Bionumerics 7.6 software and adjusted in two pages.

indicators in health monitoring to diagnose the impact of infection on herds. Previous national studies show that the prevalence of pneumonia in slaughter pigs is high, with values around 72 to 75% (Silva et al. 2001, Gabardo et al. 2013, De Conti et al. 2021). *M. hyopneumoniae* is known to be widely distributed in countries where there is commercial pig farming and is considered the main primary bacterial agent in this process. In this context, the involvement of this pathogen in pneumonia in another Brazilian study was reported to be around 74% based on immunohistochemistry (Morés et al. 2015) and 79.3% based on qPCR (De Conti et al. 2021). However, these studies on the pathogen have been carried out predominantly in the southern region, which has most of the national slaughter pigs. However, epidemiological conditions in Minas Gerais are, in general, quite distinct from the southern states of the country, in particular the production systems which are individually owned farrow to finish farms in Minas Gerais compared to large integrator companies with multisite units, and cooler weather conditions in the south (Roppa 2014, ABCS 2016).

The evaluation of finishing pig lungs showed that the high occurrence of *M. hyopneumoniae* associated with lung lesions is related to high circulating variants diversity in Minas Gerais, with 43 STs (genotypes) in 144 studied animals (48 farms), and a predominance of farms with more than one genotype of the bacteria. According to Mayor et al. (2008), this high diversity of variants within a herd is possibly related to frequent recombination events, associated with polymorphic sites located in ciliary adhesin genes. The genetic diversity of these adhesins is also a result of the selection pressure

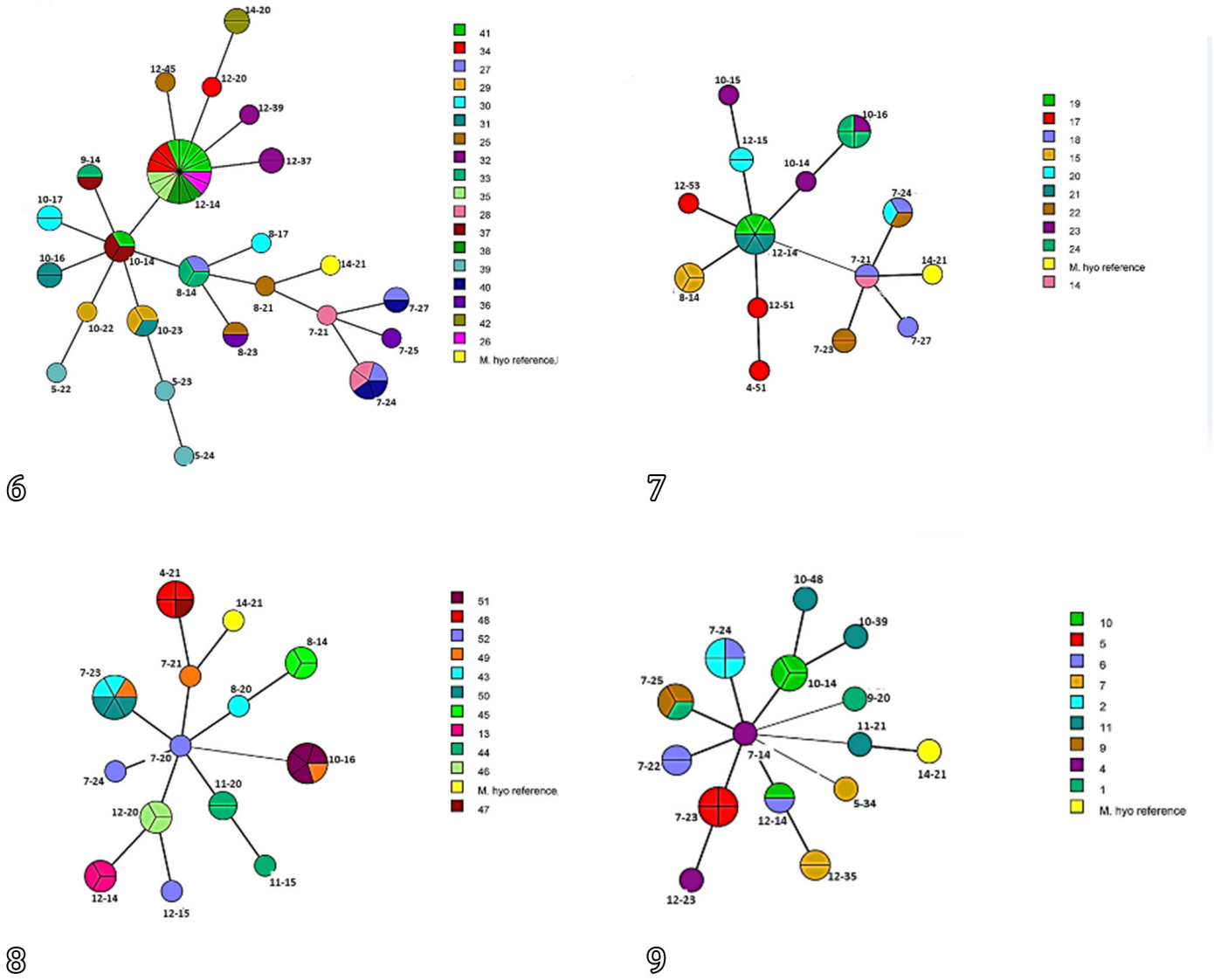

Fig.6-9. Minimum Spanning Tree showing the distribution of genotypes in each mesoregion of Minas Gerais state. (**6**) Zona da Mata, (**7**) Belo Horizonte Metropolitan Area (ZMBH), (**8**) Triângulo Mineiro and Alto Paranaíba (TMAP), (**9**) other mesoregions. MLVA type is indicated by the number of repetitions at each of the loci. The circles represent the genotypes. The size of the circles represents the number of clonal variants in each genotype. The source farms of the samples are represented by different colors. The MST were generated through UPGMA in Bionumerics 7.6 software and distributed on two pages.

triggered by the host immune system, which is variable for each gene locus (Zimmerman 2014). A high polymorphism VNTR locus is probably associated with a faster evolution rate than a low polymorphism locus. Therefore, VNTRs are among the bacterial genetic segments with the highest diversity patterns. However, eventually, certain VNTR loci may evolve very rapidly, which compromises epidemiological agreement and makes them unsuitable for phylogenetic relationships (Van Belkum et al. 2007, Li et al. 2009).

The above considerations may be associated with the fact that it was possible to note in the present study the absence of a specific genotypic pattern (ST) of large distribution in one geographical location, that was otherwise characterized by several genotypes circulating in more than one mesoregion. Sorting samples correctly is of fundamental importance in epidemiological studies, and the use of MLVA with the evaluated loci showed high discriminatory power, as also demonstrated by the Simpson diversity index. However, visualizing the distribution of genotypes in MLVA does not demonstrate clearly separated clusters. This suggests that the identification of genetic markers with less variability is necessary to demonstrate greater geographic coherence among samples within the state, which can be performed in further studies with other genes containing VNTRs. These new markers may provide further refinement and higher resolution of the method, so it can be applied in future epidemiological assessments that enable precision in identifying specific transmission chains. Assao et al. (2019) evaluating 266 lung samples from Alto do Paranaíba and Zona da Mata regions by profiling 16 different pathogenic genes of *M. hyopneumoniae* also demonstrated high variants diversity.

The Zona da Mata mesoregion showed the highest genetic diversity of *M. hyopneumoniae*, both in number of genotypes found, 15 STs, and in number of herds with more than one genotype, while TMAP farms had greater uniformity. The higher number of genotypes in Zona da Mata may have been influenced by the number of samples obtained, but also by other factors associated with biosecurity or because of the higher density of farms present in this mesoregion. Genotype 12-14 had the highest frequency of distribution in Minas Gerais (19% of the total), with predominance of this ST in Zona da Mata (11% of the total). The high frequency of this genotype corroborates Dos Santos et al. (2015) findings, who also identified it in seven other states in Brazil, with a frequency of distribution of 35.8% in Brazil. In this study, the authors detected, in 95 samples from different Brazilian states, 39 different types of MLVA genotypes, twelve of them were from Minas Gerais. Our study detected all STs previously reported by Dos Santos et al. (2015) in Minas Gerais (7-22, 7-24, 7-25, 7-27, 7-32, 10-14, 10-17, 10-18, 12-14, 12-15, 12-32, 12-48), in addition to ST 10-17, reported by the authors in a sample from Rio Grande do Sul.

The lung samples evaluated in the present study predominantly reflect commercial farms, except for one multiplier herd. In this herd (farm 13), only one ST was detected (12-14) in three evaluated lung samples. Recently, the diversity of *M. hyopneumoniae* variants was observed in three multiplier hog farms in Brazil (Takeuti et al. 2017), with a total of 17 distinct STs, showing that the high strain diversity can also be found in herds source of replacement animals. In a multiplier herd located in Minas Gerais, Takeuti et al. (2017) identified 6 distinct STs (3-14, 3-17, 3-24, 10-14, 12-14, 12-15), three them were also detected in our study (10-14, 12-14, 12-15), and only 1 common ST (11-21) in a multiplier herd located in Rio Grande do Sul. Comparative analysis of the genotypes observed in our study and from this previous study evaluating samples from Minas Gerais revealed genotypes that are not common among farms. On the other hand, the presence of the same clonal types in different farms was also identified, suggesting the presence of the same variant in different rearing systems. This similarity may be associated with *M. hyopneumoniae* chain of transmission among farms. According to Oliveira et al. (2013), Minas Gerais pig farming involves intense animal traffic and, consequently, represents a complex flow network, having already registered 9,548 different pig routes. Part of this transit is interstate and involves the movement of 53% of the pigs produced in Minas Gerais for breeding purposes, which are sent to numerous states in the country (Oliveira et al. 2013). This may imply the dissemination of these variants nationwide, originated from Minas Gerais herds.

In the present study, most herds had more than one circulating genotype, with the largest number of genotypes proportionally to the number of evaluated samples. Thus, it is very likely that more genotypic variants exist beyond these findings. The number of variants present in a farm seems to be a risk factor for lung problem in the herd. Michiels et al. (2017) observed that batches of pigs at slaughter infected with more genotypes of *M. hyopneumoniae* had higher prevalence of pneumonia and lesion severity. The diversity of circulating strains can be influenced by management practices such as all in/all out system, the type of system (farrow-to-finish or other), replacement rate and proximity to other farms (Maes et al. 2008, Charlebois et al. 2014). In our study, the unavailability of this information from the evaluated farms limits the possibility of an epidemiological investigation.

One of the objectives of the genetic evaluation of pathogens is the association of the evaluated markers with the pathogenicity of the agent. Our study identified 86% of samples with eight or more repeats on P97 adhesin. It has been suggested in the case of P97 that at least eight repetitions in the R1 region are required for adherence to porcine cilia (Hsu & Minion 1998, Minion et al. 2000). However, our results are in line with other studies (Vasconcelos et al. 2005, Castro et al. 2006), since we verified the presence of pigs with severe pneumonia and detection of P97 adhesin with a smaller number of repetitions, which reinforces the perception that there are other determinants involved in pathogenicity.

Perivascular and peribronchial lymphoproliferation were changes that were identified in most of the lungs in this study. This lesion of lymphoid tissue hyperplasia associated with the bronchi is caused by the continued interaction of the agent with the immune system as infection progresses, resulting in chronic infection and macroscopic hepatization lesions (Razin et al. 1998, Thacker & Minion 2012). The extension and severity of lesions may be associated with the strain or strains infecting animals, as there are reports of difference in virulence among *M. hyopneumoniae* strains (Vicca et al. 2002, Meyns et al. 2007). Villarreal et al. (2009, 2011) have shown that infection with a low virulence strain does not protect pigs from subsequent infection with a high virulence strain, and in fact increases the severity of subsequent infection with a

highly virulent isolate. Therefore, this has motivated research on virulence and genotype association.

Although Charlebois et al. (2014) have suggested the association between *M. hyopneumoniae* virulence and absence of locus 1, which amplifies a gene that encodes a hypothetical protein, the authors also found that strains were distributed among all clusters regardless of lesions' severities. Although not specific tested in the present study, no suggestive pattern between genotype and lung lesion score was observed. Anyhow, the recognition of the different *M. hyopneumoniae* variants that circulants in specific regions demonstrates that there are substantial differences among strains, despite the fact of pathogenicity not being directly related to the MLVA type, shows that a positive herd can be contaminated by different strains that could be more pathogenic. A result, reducing the number of sources of replacement animals associated with the reduction of origins in nurseries and finishing farms are strongly recommended to minimize the chances of entrance of new *M. hyopneumoniae* strains, and consequently, the severity of the disease.

Another important point to discuss is whether there are genotypes that may be associated with co-infections. The identification of *Pasteurella multocida* as the main bacterial agent associated with *M. hyopneumoniae* infection, and the lower frequency of detection of other pathogens in this study corroborate previous reports (Fablet et al. 2012, Morés et al. 2015). Often, *M. hyopneumoniae* infection predisposes bacterial and viral invasion, aggravating clinical signs and lesions (Thacker & Minion 2012). However, no association between these pathogens and specific genotypes was observed in the present study. This lack of association may be due to the great diversity of *M. hyopneumoniae* obtained with the evaluated loci.

CONCLUSIONS

Postmortem bronchial swabs proved to be a sensitive technique for the detection and genotyping of *Mycoplasma hyopneumoniae*.

All farms tested were positive, and 43 different genotypes were detected in the animals. In 60% of the farms, more than one genotype was detected involved in the infection of pigs in the finishing phase, which is considered a risk factor for the development of severe pneumonia and leading pigs to death.

The new data on diversity of *M. hyopneumoniae* obtained in this study once again warn of the need for research on antigenicity and virulence in this important respiratory pathogen, with the aim of developing more current and effective vaccines to control the disease.

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REFERENCES

ABCS 2016. Mapeamento da suinocultura brasileira. Associação Brasileira de Criadores de Suínos. Brasília, DF. Available at <http://www.abcs.org.br/images/-01_mapeamento_completo_bloq.pdf/> Accessed on Jan. 2018.

Arsenakis I., Michiels A., Schagemann G., Gomez-Duran C.O., Boyen F., Haesebrouck F. & Maes D.G.D. 2019. Effects of pre-farrowing sow vaccination against *Mycoplasma hyopneumoniae* on offspring colonisation and lung lesions. Vet. Rec. 16:1-10. <https://dx.doi.org/10.1136/vr.104972> <PMid:30630875>

Assao V.S., Scatamburlo T.M., Araujo E.N., Santos M.R., Pereira C.E.R., Guedes R.M.C., Bressan G.C., Fietto J.L.R., Chang Y.-F., Moreira M.A.S. & Silva-Júnior A. 2019. Genetic variation of *Mycoplasma hyopneumoniae* from Brazilian field samples. BMC Microbiol. 19:234. <https://dx.doi.org/10.1186/s12866-019-1603-7>

Bogema D.R., Deutscher A.T., Woolley L.K., Seymour L.M., Raymond B.A., Tacchi J.L., Padula M.P., Dixon N.E., Minion F.C., Jenkins C., Walker M.J. & Djordjevic S.P. 2012. Characterization of cleavage events in the multifunctional cilium adhesin Mhp684 (P146) reveals a mechanism by which *Mycoplasma hyopneumoniae* regulates surface topography. Mol. Biol. 3(2): e00282-11. <https://dx.doi.org/10.1128/mBio.00282-11> <PMid:22493032>

Browning G.F., Marenda M.S., Noormohammadi A.H. & Markham P.F. 2011. The central role of lipoproteins in the pathogenesis of mycoplasmoses. Vet. Microbiol. 153(1/2):44-50. <https://dx.doi.org/10.1016/j.vetmic.2011.05.031> <PMid:21684094>

Calsamiglia M., Pijoan C. & Trigo A. 1999. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. J. Vet. Diag. Investig. 11(3):246-251. <https://dx.doi.org/10.1177/104063879901100307> <PMid:10353356>

Castro L.A., Pedroso T.R., Kuchiishi S.S., Ramenzoni M., Kich J.D., Zaha A., Vainstein M.H. & Ferreira H.B. 2006. Variable number of tandem aminoacid repeats in adhesion-related CDS products in *Mycoplasma hyopneumoniae* strains. Vet. Microbiol. 116(4):258-269. <https://dx.doi.org/10.1016/j.vetmic.2006.04.022> <PMid:16730926>

Charlebois A., Marois-Crehan C., Helie P., Gagnon C.A., Gottschalk M. & Archambault M. 2014. Genetic diversity of *Mycoplasma hyopneumoniae* isolates of abattoir pigs. Vet. Microbiol. 168(2/4):348-356. <https://dx.doi.org/10.1016/j.vetmic.2013.11.006> <PMid:24345410>

De Conti E., Takeuti K.L., Schwertz C.I., Bianchi R.M., Driemeier D. & Barcellos D.E.S.N. 2021. Agents of pneumonia in slaughtered pigs in southern Brazil. Pesq. Vet. Bras. 41:e06669. <https://dx.doi.org/10.1590/1678-5150-PVB-6669>

Djordjevic S.P., Cordwell S.J., Djordjveic M.A., Wilton J. & Minion F.C. 2004. Proteolytic processing of the *Mycoplasma hyopneumoniae* cilium adhesin. Infect. Immun. 72(2):2791-2802. <https://dx.doi.org/10.1128/IAI.72.5.2791-2802.2004> <PMid:15102789>

Dos Santos L.F., Sreevatsan S., Torremorell M., Moreira M.A.S., Sibila M. & Pieters M. 2015.Genotype distribution of *Mycoplasma hyopneumoniae* in swine herds from different geographical regions. Vet. Microbiol. 175(2/4):374-381. <https://dx.doi.org/10.1016/j.vetmic.2014.11.018> <PMid:25497236>

Fablet C., Marois C., Kobisch M., Madec F. & Rose N. 2010. Estimation of the sensitivity of four sampling methods for *Mycoplasma hyopneumoniae* detection in live pigs using a Bayesian approach. Vet. Microbiol. 143(2/4):238-245. <https://dx.doi.org/10.1016/j.vetmic.2009.12.001> <PMid:20036079>

Fablet C., Marois-Crehan C., Simon G., Grasland B., Jestin A., Kobisch M., Madec F. & Rose N. 2012. Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: a crosssectional study. Vet. Microbiol. 157(1/2): 152-263. <https://dx.doi.org/10.1016/j.vetmic.2011.12.015> <PMid:22226820>

Fano E., Pijoan C. & Dee S. 2005. Dynamics and persistence of *Mycoplasma hyopneumoniae* infection in pigs. Can. J. Vet. Res. 69(3):223-228.

Ferraz M.E.S., Almeida H.M.S., Storino G.Y., Sonálio K., Souza M.R., Moura C.A.A., Costa W.M.T., Lunardi L., Linhares D.C.L. & De Oliveira L.G. 2020. Lung consolidation caused by *Mycoplasma hyopneumoniae* has a negative effect on productive performance and economic revenue in finishing pigs. Prev. Vet. Med. 182:105091. <https://dx.doi.org/10.1016/j.prevetmed.2020.105091> <PMid:32683190>

Foxman B., Zhang L., Koopman J.S., Manning S.D. & Marrs C.F. 2005. Choosing an appropriate bacterial typing technique for epidemiologic studies. Epidemiol. Perspect. Innov. 2:10. <https://dx.doi.org/10.1186/1742-5573-2-10> <PMid:16309556>

Fraile L., Alegre A., López-Jiménez R., Nofrarías M. & Segalés J. 2010. Risk factors associated with pleuritis and cranioventral pulmonary consolidation in slaughter-aged pigs. Vet. J. 184(3):326-333. <https://dx.doi.org/10.1016/j.tvjl.2009.03.02> <PMid:19527939>

Gabardo M.P., Zandonai A.D., Camargo M.C., Gava A., Cristani J. & Traverso S.D. 2013. Caracterização sanitária de suínos criados em sistema "wean-to-finish". Med. Vet. 7(3):23-31.

Gonçalves J.P.M. 2016. Caracterização de fatores de risco epidemiológico na suinocultura de Minas Gerais, Brasil. Tese de Doutorado, Escola de Veterinaria, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

Halbur P.G., Paul P.S., Frey M.L., Landgraf J., Eernisse K., Meng X.-J., Lum M.A., Andrews J.J. & Rathje J.A. 1995. Comparison of the pathogenicity of two US porcine reproductive e respiratory syndrome virus isolates with that of the Lelystad virus. Vet. Pathol. 32(6):648-660. <https://dx.doi.org/10.1177/030098589503200606> <PMid:8592800>

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium 41:95-98. Available at <www.mbio.ncsu.edu/BioEdit/bioedit.html/> Accessed on Mar. 2018.

Hansen M.S., Pors S.E., Jensen H.E., Bille-Hansen V., Bisgaard M., Flachs E.M. & Nielsen O.L. 2010. An investigation of the pathology e pathogens associated with porcine respiratory disease complex in Denmark. J. Comp. Pathol. 143(2/3):120-131. <https://dx.doi.org/10.1016/j.jcpa.2010.01.012> <PMid:20181357>

Hsu T. & Minion F.C. 1998. Identification of the cilium binding epitope of the *Mycoplasma hyopneumoniae* P97 adhesin. Infect. Immun. 66(10):4762-4766. <https://dx.doi.org/10.1128/IAI.66.10.4762-4766.1998> <PMid:9746576>

Hunter P.R. & Gaston M.A. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. 26(11):2465-2466. <https://dx.doi.org/10.1128/JCM.26.11.2465-2466.1988> <PMid:3069867>

IBGE 2018. Instituto Brasileiro de Geografia e Estatística. Censo 2010. Available at <https://ww2.ibge.gov.br/home/estatistica/populacao/censo2010/caracteristicas_da_populacao/caracteristicas_da_populacao_tab_municipios_zip_xls.shtm/> Accessed on Jan. 2018.

Li W., Raoult D. & Fournier P.-E. 2009. Bacterial strain typing in the genomic era. FEMS Microbiol. Rev. 33(5):892-916. <https://dx.doi.org/10.1111/j.1574-6976.2009.00182.x> <PMid:19453749>

Maes D., Segales J., Meyns T., Sibila M., Pieters M. & Haesebrouck F. 2008. Control of *Mycoplasma hyopneumoniae* infections in pigs. Vet. Microbiol. 126(4):297-309. <https://dx.doi.org/10.1016/j.vetmic.2007.09.008> <PMid:17964089>

Maes D., Sibila M., Kuhnert P., Segalés J., Haesebrouck F. & Pieters M. 2018. Update on *Mycoplasma hyopneumoniae* infections in pigs: knowledge gaps for improved disease control. Transbound. Emerg. Dis. 65(Supl.1):110-124. <https://dx.doi.org/10.1111/tbed.12677> <PMid:28834294>

Maes D., Verdonck M., Deluyker H. & De Kruif A. 1996. Enzootic pneumonia in pigs. Vet. Quart. 18(3):104-109. <https://dx.doi.org/10.1080/01652176.1996.9694628>

Marois C., Cariolet R., Morvan H. & Kobisch M. 2008. Transmission of pathogenic respiratory bacteria to specific pathogen free pigs at slaughter. Vet. Microbiol. 129(3/4):325-332. <https://dx.doi.org/10.1016/j.vetmic.2007.11.030> <PMid:18242013>

Mayor D., Jores J., Korczak B.M. & Kuhnert P. 2008. Multilocus sequence typing (MLST) of *Mycoplasma hyopneumoniae*: a diverse pathogen with limited clonality. Vet. Microbiol. 127(1/2):63-72. <https://dx.doi.org/10.1016/j.vetmic.2007.08.010> <PMid:17884308>

Mayor D., Zeeh F., Frey J. & Kuhnert P. 2007. Diversity of *Mycoplasma hyopneumoniae* in pig farms revealed by direct molecular typing of clínical material. Vet. Res. 38(3):391-398. <https://dx.doi.org/10.1051/vetres:2007006> <PMid:17506969>

Merialdi G., Dottori M., Bonilauri P., Luppi A., Gozio S., Pozzi P., Spaqqiari B. & Martelli P. 2012. Survey of pleuritis and pulmonary lesions in pigs at abattoir with a focus on the extent of the condition and herd risk factors. Vet. J. 193(1):234-239. <https://dx.doi.org/10.1016/j.tvjl.2011.11.009> <PMid:22182431>

Meyns T., Maes D., Calus D., Ribbens S., Dewulf J., Chiers K., De Kruif A., Cox E., Decostere A. & Haesebrouck F. 2007. Interactions of highly and low virulent *Mycoplasma hyopneumoniae* isolates with the respiratory tract of pigs. Vet. Microbiol. 120(1/2):87-95. <https://dx.doi.org/10.1016/j.vetmic.2006.10.010> <PMid:17123752>

Meyns T., Maes D., Dewulf J., Vicc J., Haesebrouck F. & Kruif A. 2004. Quantification of the spread of *Mycoplasma hyopneumoniae* in nursery pigs using transmission experiments. Prev. Vet. Med. 66(1/4):265-275. <https://dx.doi.org/10.1016/j.prevetmed.2004.10.001> <PMid:15579347>

Meyns T., Van Steelant J., Rolly E., Dewulf J., Haesebrouck F. & Maes D. 2011. A cross sectional study of risk factors associated with pulmonar lesions in pigs at slaughter. Vet. J. 187(3):388-392. <https://dx.doi.org/10.1016/j.tvjl.2009.12.027> <PMid:20122861>

Michiels A., Vranckx K., Piepers S., Sacristán R.D.P., Arsenakis I., Boyen F., Haesebrouck F. & Maes D. 2017. Impact of diversity of *Mycoplasma hyopneumoniae* strains on lung lesions in slaughter pigs. Vet. Res. 48:2. <https://dx.doi.org/10.1186/s13567-016-0408-z>

Minion F.C., Adams C. & Hsu T. 2000. R1 region of P97 mediates adherence of *Mycoplasma hyopneumoniae* to swine cilia. Infect. Immun. 68(5):3056-3060. <https://dx.doi.org/10.1128/IAI.68.5.3056-3060.2000> <PMid:10769015>

Morés M.A.Z., Oliveira Filho J.X., Rebelatto R., Klein C.S., Barcellos D.E.N., Coldebella A. & Morés N. 2015. Aspectos patológicos e microbiológicos das doenças respiratórias em suínos de terminação no Brasil. Pesq. Vet. Bras. 35(8):725-733. <https://dx.doi.org/10.1590/S0100-736X2015000800004>

Oliveira C.S.F., Gonçalves J.P.M., Utsch J.B., Nicolino R.R., Silva M.X. & Haddad J.P.A. 2013. Trânsito de suídeos em Minas Gerais, Brasil. Arq. Bras. Med. Vet. Zootec. 65(5):1313-1320. <https://dx.doi.org/10.1590/S0102-09352013000500008>

Otake S., Dee S., Corzo C., Oliveira S. & Deen J. 2010. Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. Vet. Microbiol. 145(3/4):198-208. <https://dx.doi.org/10.1016/j.vetmic.2010.03.028> <PMid:20418029>

Paladino E.S., Gabardo M.P., Lunardi P.N., Morés N. & Guedes R.M.C. 2017. Anatomopathological pneumonic aspects associated with highly pathogenic *Pasteurella multocida* in finishing pigs. Pesq. Vet. Bras. 37(10):1091-1100. <https://dx.doi.org/10.1590/s0100-736x2017001000009>

Pieters M., Daniels J. & Rovira A. 2017. Comparison of sample types and diagnostic methods for in vivo detection of *Mycoplasma hyopneumoniae* during early stages of infection. Vet. Microbiol. 203:103-109. <https://dx.doi.org/10.1016/j.vetmic.2017.02.014> <PMid:28619131>

Pieters M., Pijoan C., Fano E. & Dee S. 2009. An assessment of the duration of *Mycoplasma hyopneumoniae* infection in an experimentally infected population of pigs. Vet. Microbiol. 134(3/4):261-266. <https://dx.doi.org/10.1016/j.vetmic.2008.08.016> <PMid:18835112>

Piffer I.A. & Brito J.R.F. 1991. Descrição de um modelo para avaliação e quantificação de lesões pulmonares de suínos e formulação de um índice para classificação de rebanhos. Embrapa Suínos e Aves, Concórdia. 16p. (Comunicado Técnico n.23).

Quinn P.J., Carter M.E. & Markey B. 1994. Clinical Veterinary Microbiology. Wolfe, London, p.237-242.

Razin S., Yogev D. & Naot Y. 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62(4):1094-1156. <https://dx.doi.org/10.1128/MMBR.62.4.1094-1156.1998> <PMid:9841667>

Reolon L.A., Martello C.L., Schrank I.S. & Ferreira H.B. 2014. Survey of surface proteins from the pathogenic *Mycoplasma hyopneumoniae* strain 7448 using a biotin cell surface labeling approach. Plos One 9(11):e112596. <https://dx.doi.org/10.1371/journal.pone.0112596>

Roppa L. 2014. Estatísticas da produção, abate e comercialização brasileira e mundial de suínos, p.30-37. In: ABCS (Eds), Produção de Suínos: teoria e prática. 1ª ed. Associação Brasileira de Criadores de Suínos (ABCS), Brasília.

Silva A.F., Paganini F.J., Acosta J.C., Rocha P.H., Mistura H., Marcon E., Simon V.A., Casagrande H.A. & Floss J.M. 2001. Programa de gerenciamento de doenças respiratórias em suínos. I. Estudo do perfil das doenças respiratórias nas regiões sul, sudeste e centro-oeste do Brasil. Anais do Congresso Brasileiro de Veterinários Especialistas em Suínos, Porto Alegre, RS.

Silva F.M.F., Castro L.A., Silva Júnior A., Moraes M.P., Moreira M.A.S. & Almeida M.R. 2009. Detection of *Mycoplasma hyopneumoniae* in lungs and nasal swabs of pigs by nested PCR. Arq. Bras. Med. Vet. Zootec. 61(1):149-155. <https://dx.doi.org/10.1590/S0102-09352009000100021>

Takeuti K.L., Barcellos D.E.S.N., Andrade C.P., Almeida L.L. & Pieters M. 2017. Infection dynamics and genetic variability of *Mycoplasma hyopneumoniae* in self-replacement gilts. Vet. Microbiol. 208:18-24. <https://dx.doi.org/10.1016/j.vetmic.2017.07.007> <PMid:28888635>

Thacker E.L. 2001. Mycoplasma diagnosis and immunity. Proceedings of AASP Annual Meeting, Nashville, TN, p.467-469.

Thacker E.L. 2006. Mycoplasmal diseases, p.701-720. In: Straw B., Zimmermann W., D'Allaire S. & Taylor D.J. (Eds), Diseases of Swine. 9th ed. Iowa State University Press, Ames.

Thacker E.L. & Minion F.C. 2012. Mycoplasmosis, p.779-797. In: Zimmerman J.J., Karriker L.A., Ramirez A., Schwartz K.J., Stevenson G.W. & Zhang J. (Eds), Diseases of Swine. 10th ed. Iowa State University Press, Ames.

Van Belkum A., Tassios P.T., Dijkshoorn L., Haeggman S., Cookson B., Fry N.K., Fussing V., Green J., Feil E., Gerner-Smidt P., Brisse S. & Struelens M. & European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM). 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. 13(Supl.3):1-46. <https://dx.doi.org/10.1111/j.1469-0691.2007.01786.x> <PMid:17716294>

Vasconcelos A.T.R., Ferreira H.B., Bizarro C.V., Bonatto S.L., Carvalho M.O., Pinto P.M., Almeida D.F., Almeida L.G.P., Almeida R., Alves-Filho L., Assunção E.N., Azevedo V.A.C., Bogo M.R., Brigido M.M., Brocchi M., Burity H.A., Camargo A.A., Camargo S.S., Carepo M.S., Carraro D.M., Mattos Cascardo J.C., Castro L.A., Cavalcanti G., Chemale G., Collevatti R.G., Cunha C.W., Dallagiovanna B., Dambros B.P., Dellagostin O.A., Falcão C., Fantinatti-Garboggini F., Felipe M.S.S., Fiorentin L., Franco G.R., Freitas N.S.A., Frias D., Grangeiro T.B., Grisard E.C., Guimarães C.T., Hungria M., Jardim S.N., Krieger M.A., Laurino J.P., Lima L.F.A., Lopes M.I., Loreto E.L.S., Madeira H.M.F., Manfio G.P., Maranhão A.Q., Martinkovics C.T., Medeiros S.R.B., Moreira M.A.M., Neiva M., Ramalho-Neto C.E., Nicolas M.F., Oliveira S.C., Paixão R.F.C., Pedrosa F.O., Pena S.D.J., Pereira M., Pereira-Ferrari L., Piffer I., Pinto L.S., Potrich D.P., Salim A.C.M., Santos F.R., Schmitt R., Schneider M.P.C., Schrank A., Schrank I.S., Schuck A.F., Seuanez H.N., Silva D.W., Silva R., Silva S.C., Soares C.M.A., Souza K.R.L., Souza R.C., Staats C.C., Steffens M.B.R., Teixeira S.M.R., Urmenyi T.P., Vainstein M.H., Zuccherato L.W., Simpson A.J.G. & Zaha A. 2005. Swine and poultry pathogens: the complete genome sequences of two strains of *Mycoplasma hyopneumoniae* and a strain of *Mycoplasma synoviae*. J. Bacteriol. 187(16):5568-5577. <https://dx.doi.org/10.1128/JB.187.16.5568-5577.2005> <PMid:16077101>

Vicca J., Thermote L., Maes D., D'Hooghe I., Peeters J., Haesebrouck F. & De Kruif A. 2002. Patterns of *Mycoplasma hyopneumoniae* Infections in Belgian Farrow-to-Finish Pig Herds with Diverging Disease-Course. J. Vet. Med. B 49(7):349-353. <https://dx.doi.org/10.1046/j.1439-0450.2002.00579.x> <PMid:12420871>

Villarreal I., Maes D., Meyns T., Gebruers F., Calus D., Pasmans F. & Haesebrouck F. 2009. Infection with a low virulent *Mycoplasma hyopneumoniae* isolate does not protect piglets against subsequent infection with a highly virulent *Mycoplasma hyopneumoniae* isolate. Vaccine 27(12):1875-1879. <https://dx.doi.org/10.1016/j.vaccine.2008.12.005> <PMid:19114074>

Villarreal I., Maes D., Vranckx K., Calus D., Pasmans F. & Haesebrouck F. 2011. Effect of vaccination of pigs against experimental infection with high and low virulence *Mycoplasma hyopneumoniae* strains. Vaccine 29(9):1731-1735. <https://dx.doi.org/10.1016/j.vaccine.2011.01.002> <PMid:21237277>

Vranckx K., Maes D., Calus D., Villarreal I., Pasmans F. & Haesebrouck F. 2011. Multiple-locus variable-number tandem-repeat analysis is a suitable tool for differentiation of *Mycoplasma hyopneumoniae* strains without cultivation. J. Clin. Microbiol. 49(5):2020-2023. <https://dx.doi.org/10.1128/JCM.00125-11> <PMid:21389157>

Zimmerman C.U. 2014. Current insights into phase and antigenic variation in mycoplasmas, p.165-196. In: Browning G.F. & Citti C. (Eds), Mollicutes Molecular Biology and Pathogenesis. Caister Academic Press, Norfolk.