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Communication

[*Comunicação*]

Genetic variability of *Mycoplasma synoviae* detected in commercial layers in southeastern Brazil

[*Variabilidade genética de Mycoplasma synoviae detectado em poedeiras comerciais do sudeste do Brasil*]

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Poultry farming is an expressive economic activity within the Brazilian livestock scenario. With an estimated laying flock of more than 114 million hens, Brazil ranks among the world's largest egg producers (Relatório…, 2022). The health of these flocks is essential to assure the quantity and quality of eggs produced. Among the diseases that affect the health of birds, mycoplasmosis is one of the most important because it causes significant economic losses in the poultry industry. These losses are mainly related to the decrease in the quantity and quality of eggs produced and by favoring other respiratory infections, in addition to the need for treatment with the use of antimicrobials (Nascimento *et al.*, 2005). Avian mycoplasmosis is included in the lists of notifiable diseases of the National Poultry Health Program and the World Organization for Animal Health (Brasil, 2013; Terrestrial…, 2022).

Mycoplasma synoviae (MS) and *Mycoplasma gallisepticum* (MG) are the main species of importance for the national poultry industry, both being endemic in Brazilian flocks. MS is considered the most prevalent and responsible for respiratory conditions, joint problems, and decreased eggshell quality (Feberwee *et al.*, 2009; Santos *et al.*, 2014; Silva *et al.*, 2020). The association of MS with other pathogens was already shown with the exacerbation of clinical signs in laying hens (Silva *et al.*, 2021). Knowing the different circulating genotypes of a pathogen is important to understand the epidemiology of the disease as well as to trace the relationship between certain genotypes and the clinical signs they cause with impact on production. Several strains of MS have already been phenotypic and genotypically differentiated and have been observed to have different degrees of pathogenicity, virulence, and immunogenicity. (Nascimento *et al.*, 2005; Santos *et al.*, 2014; Silva *et al.*, 2020). The partial variable lipoprotein hemagglutinin A gene (*vlh*A) has been used for strain classification and epidemiological investigations (Limpavithayakul *et al.*, 2016). This is the only genomic target identified so far for MS molecular typing without the need to perform whole genome sequencing. The *vlh*A gene encodes two variable cell surface proteins, lipoprotein, and hemagglutinin, and the proposed mechanism for the variation is gene conversion between a single expressed gene and a series of pseudogenes. The upstream portion of the *vlh*A gene is present in the genome in a single copy and is the only part of the gene that can be used for targeted sequence typing (El-Gazzar *et al.*, 2012).

There are few studies genotyping MS strains detected in the country by the *vlh*A gene, so this study aimed to characterize the genotypes of *M. synoviae* circulating in commercial layers in southeastern Brazil.

Eleven strains previously identified as MS detected in commercial farms in southeastern Brazil in the years 2017 and 2022 were studied. DNA was isolated using the phenol-chloroform

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method (Sambrook and Russell 2006). The phylogenetic characterization of *M. synoviae* strains was performed by amplification of the *vlh*A gene, with primers *vlh*A-F5'TACTATTAGCAGCTAGTGC3' and *vlh*A-R5'AGTAACCGATCCGCTTAAT3' described by Jeffery *et al.* (2007). The amplification reaction was performed with one cycle at 94°C for 2 min, followed by 35 cycles of 96°C for 15s, 54°C for 15s and 72°C for 20s, and final annealing at 72°C for five minutes. All reactions were performed with GoTaq® G2 Hot Start Taq Polymerase (Promega, Brazil).

Amplicons with expected sizes between 350 and 400 bp were purified using the QIAquick PCR purification kit (Qiagen) and sequenced in the DNA sequencer ABI 3730 (Applied Biosystems, USA) at the Fiocruz Sequencing Platform. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Tamura; Neill, 1993). The tree with the highest log likelihood (-1649.15) is shown. The percentage of trees in which the associated rate clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 12 nucleotide sequences. There was a total of 472 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). In addition, 10 DNA sequences obtained from ten *M. synoviae* strains identified in Brazil in a previous study (Silva *et al.*, 2010) (KC506824; KC506823; KC506801; KC506810; KC506809; KC506811; KC506805; KC506803; KC506821; KC506812) and the MSH vaccine strain were included for phylogenetic analyses. The *vlh*A gene of *M. gallisepticum* was used as outgroup.

Sequences of the 11 *M. synoviae* strains used in this study have been deposited in the Genbank under accession numbers OP279775 to OP279785. Figure 1 presents the phylogenetic tree with the relationship among *M. synoviae* strains from Brazil and Table 1 presents the percentage of similarity among the strains.

MS has a high prevalence in laying flocks in Brazil and vaccination is not routinely performed. The most common manifestation related to MS infection has been the respiratory form, mainly with aerosacculitis, which may or may not be associated with *M. gallisepticum* and other bacterial or viral respiratory agents (Silva *et al.*, 2021). MS can carry a latent form of the infection with the ability to localize and survive intracellularly, presenting pathogenicity in moments of debilitation of the host, bypassing the mechanisms of the immune system. In addition to association with other pathogens, some MS strains have a predilection for the reproductive system, colonizing the ovary and oviduct of laying hens (Santos *et al.*, 2014), and this predilection may cause eggs fragility at the apex of the shell (EAA), increasing the risk of cracks during egg storage. A study from (Catania *et al.*, 2016) by using *vlhA* sequencing detected that only some genotypes of *vlhA* can produce EAA and this could explain the discrepancies between the low incidence (Santos *et al.*, 2014) of this abnormality despite the high prevalence of MS in the Brazilian field (Silva *et al.*, 2021).

The sequencing of the *vlh*A gene has been used in the differentiation and epidemiological study of circulating strains of MS. In this study, it was possible to detect the presence of different strains circulating in Brazil (Fig. 1). However, high similarity (>88%) was observed between the strains from Rio de Janeiro; Poultry farming in this state is restricted to the eastern region and has a lower volume of egg production when compared to other regions of Brazil. Strains from the state of São Paulo are genetically closer to strains originated in Goias, Rio Grande do Sul and Ceará reported previously (Silva *et al.*, 2010). Interestingly the two strains from São Paulo, detected in the present study possess the low percentage (68%) of similarity, demonstrating the variability of strains from this locality. The poultry farms in São Paulo have larger flocks, with a greater density of birds, this being the second egg producer in the country, which provides different challenges by mycoplasmas. Our findings corroborate with the report of (Silva *et al.*, 2010) demonstrating the genetic diversity in MS strains present in industrial poultry production flocks in Brazil by *vlhA* analysis. It is also believed that mycoplasmas, by alternating the composition of

their surface proteins, colonize mucosal surfaces more efficiently and become more virulent. Hemagglutinins, which are encoded by the *vlh*A gene, are among the surface proteins mostly involved in the colonization and virulence of avian mycoplasmas (Bencina, 2002; Gorton; Geary, 2006), so there is a need for rapid adaptation to promote bacterial cell survival.

Table 1. Percentage of Genetic Similarity Based on *vlh*A Loci in 22 *Mycoplasma synoviae* strains

Access Number	KC50 6824	KC50 6823	MSH	OP27 9776	OP27 9777	OP27 9775	OP27 9778	OP27 9779	OP27 9780	OP27 9781	OP27 9782
KC506824	100%	91%	81%	80%	76%	80%	80%	80%	80%	80%	80%
KC506823	90%	100%	83%	84%	79%	84%	84%	83%	84%	80%	80%
MSH	77%	79%	100%	87%	82%	87%	87%	86%	87%	85%	85%
OP279776	81%	86%	94%	100%	94%	100%	99%	99%	99%	93%	93%
OP279777	77%	81%	89%	94%	100%	94%	94%	93%	94%	88%	88%
OP279775	81%	86%	94%	99%	94%	100%	99%	99%	99%	93%	93%
OP279778	81%	85%	94%	99%	94%	99%	100%	98%	99%	92%	92%
OP279779	81%	85%	93%	99%	93%	99%	98%	100%	98%	92%	92%
OP279780	81%	86%	94%	99%	94%	99%	99%	99%	100%	93%	93%
OP279781	84%	85%	96%	96%	91%	97%	96%	96%	96%	100%	100%
OP279782	84%	86%	96%	97%	91%	97%	97%	96%	97%	100%	100%
OP279783	81%	86%	94%	99%	94%	100%	99%	99%	99%	93%	93%
OP279784	76%	75%	92%	82%	78%	82%	82%	82%	82%	81%	81%
OP279785	88%	93%	80%	81%	77%	81%	81%	81%	81%	77%	77%
KC506801	91%	89%	85%	79%	75%	79%	79%	79%	79%	80%	80%
KC506810	100%	93%	83%	82%	78%	82%	82%	81%	82%	81%	81%
KC506809	99%	94%	84%	82%	78%	82%	82%	82%	82%	82%	82%
KC506811	99%	94%	83%	82%	78%	82%	82%	81%	82%	81%	81%
KC506805	96%	94%	86%	84%	80%	84%	84%	84%	84%	84%	84%
KC506803	96%	94%	85%	83%	79%	83%	83%	82%	83%	84%	84%
KC506821	93%	91%	94%	95%	91%	96%	95%	95%	95%	97%	97%
KC506812	95%	93%	85%	83%	79%	83%	83%	83%	83%	84%	84%

Access Number	OP27 9783	OP27 9784	OP27 9785	KC50 6801	KC50 6810	KC50 6809	KC5 06811	KC50 6805	KC50 6803	KC50 6821	KC50 6812
KC506824	80%	82%	83%	94%	97%	97%	96%	93%	93%	80%	93%
KC506823	84%	80%	87%	91%	90%	91%	91%	91%	91%	77%	90%
MSH	87%	93%	71%	82%	76%	77%	76%	79%	78%	76%	78%
OP279776	99%	90%	78%	83%	81%	81%	81%	83%	82%	83%	82%
OP279777	94%	85%	74%	78%	77%	77%	77%	79%	78%	79%	78%
OP279775	99%	90%	78%	83%	81%	81%	81%	83%	82%	83%	82%
OP279778	99%	90%	77%	82%	81%	81%	81%	83%	81%	83%	82%
OP279779	98%	89%	77%	82%	80%	81%	80%	82%	81%	82%	81%
OP279780	99%	90%	78%	82%	81%	81%	81%	83%	82%	83%	82%
OP279781	96%	91%	77%	87%	84%	84%	84%	86%	86%	88%	86%
OP279782	97%	92%	77%	87%	84%	84%	84%	87%	86%	88%	86%
OP279783	100%	90%	78%	83%	81%	81%	81%	83%	82%	83%	82%
OP279784	82%	100%	68%	78%	76%	76%	76%	75%	74%	75%	75%
OP279785	81%	78%	100%	88%	87%	88%	89%	88%	88%	75%	87%
KC506801	79%	81%	81%	100%	91%	92%	91%	93%	93%	76%	92%
KC506810	82%	84%	85%	96%	100%	99%	98%	95%	96%	82%	95%
KC506809	82%	84%	85%	97%	99%	100%	99%	96%	97%	82%	95%
KC506811	82%	84%	86%	96%	98%	99%	100%	95%	97%	82%	95%
KC506805	84%	83%	85%	98%	95%	96%	95%	100%	98%	80%	98%
KC506803	83%	82%	85%	98%	96%	97%	97%	98%	100%	80%	97%
KC506821	96%	94%	82%	91%	93%	93%	93%	91%	91%	100%	91%
KC506812	83%	83%	85%	98%	95%	95%	95%	98%	97%	80%	100%

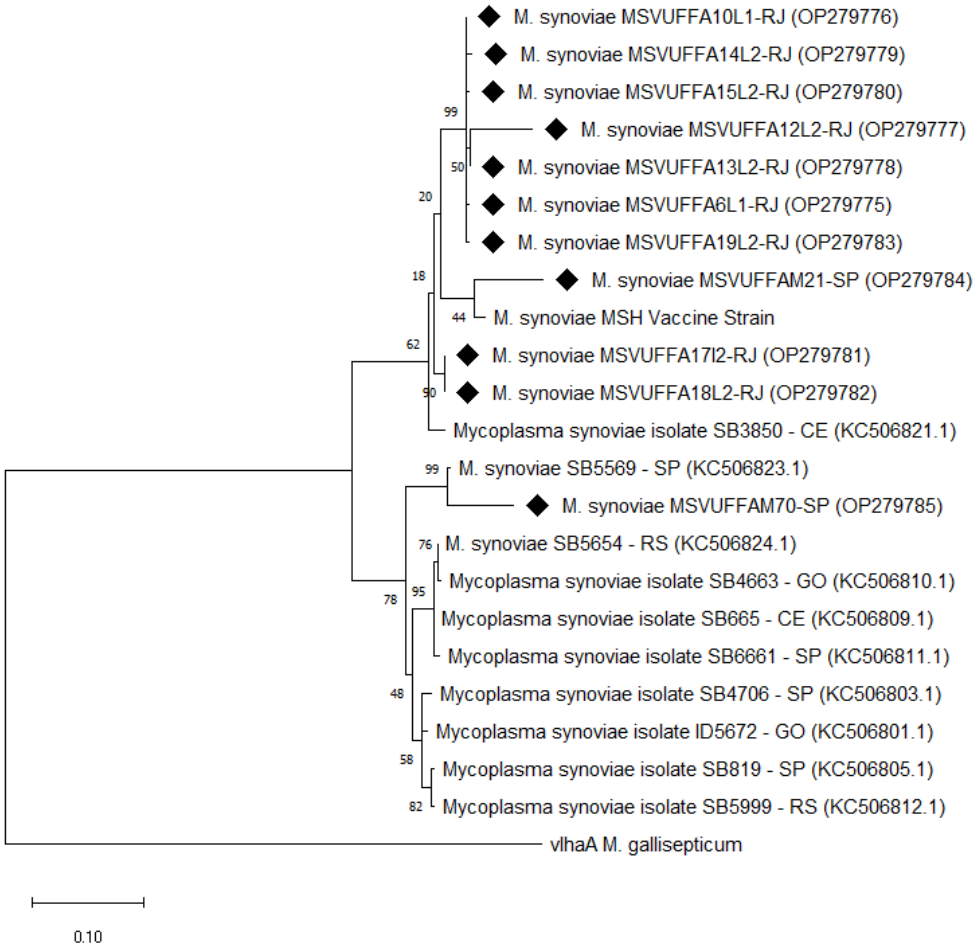

Figure 1. Phylogenetic relationship of *M. synoviae* detected in laying hens from the states of São Paulo and Rio de Janeiro, southeastern Brazil. In parentheses the Genbank accession number of the strains.

The generation of antigenic variants may occur due to the ability of the strains to undergo recombination, due to the availability in the genome of a significant set of pseudogenes, partial sequences related to the *vlh*A gene. Recombination between the single complete *vlh*A gene and one of the multiple copies of the pseudogene guarantees the creation of a new variant of the *vlh*A gene. Although it has been concluded that the potential for variation of *vlh*A genes is considerable, it is not yet known to what extent a *vlh*A gene can diverge without losing its properties (Béjaoui Khiari *et al.*, 2010).

Previous phylogenetic studies by analyzing the 16S rRNA gene region in MS also detected variation in circulating strains in different areas of poultry production in Brazil but recommended the expansion of these studies because their results were not conclusive (Buim *et al*., 2010; Silva *et al*., 2020).

Different strains of MS circulate in laying poultry flocks present in Brazil. The evaluation of the presence of MS and the phylogenetic relationship between the strains supports epidemiological studies and the adoption of more efficient control and prevention measures.

Keywords: Mycoplasmosis, laying hens, *vlhA* gene

RESUMO

Este estudo teve como objetivo caracterizar genótipos de M. synoviae *circulantes em poedeiras comerciais, na Região Sudeste do Brasil. Para estabelecer a relação evolutiva entre as cepas de MS, foi analisada a sequência genética do gene* vlhA *de 11 cepas de MS identificadas no Rio de Janeiro e em São Paulo, de 10 outras cepas de MS identificadas no Brasil e cujas sequências foram depositadas no banco de dados GenBank, e da cepa vacinal MSH. O método da máxima verossimilhança e o modelo de Kimura com dois parâmetros foram utilizados para comparar as cepas. As sequências obtidas foram depositadas no Genbank, sob os números de acesso OP279775 a OP279785. Foi possível verificar a presença de diferentes cepas circulantes no Brasil, com alta similaridade entre as cepas do Rio de Janeiro pela análise do gene* vlhA. *As duas cepas paulistas detectadas no presente estudo possuem o baixo percentual (68%) de similaridade, demonstrando a variabilidade das cepas dessa localidade.*

Palavras-chave: micoplasmose, poedeiras, vlhA

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