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Brief Communication

Mycoplasma hyopharyngis Isolation From Swine

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Mycoplasma hyopharyngis is a seldom isolated porcine species. It was found, adequately described, and named by *Erickson et al.* (1986). In that study 7 strains were isolated from the upper respiratory tract of pigs in 2 different herds, one of which was an institutional herd. All the strains fulfilled the usual criteria put up for characterization of mycoplasmas. They could degrade the amino acid arginine, but pathogenic capabilities were not reported. They were found antigenically distinct from all other mycoplasmas, inclusive the recognised porcine species. A later report on the isolation of *M. hyopharyngis* was done by *Bradbury et al.* (1994) who in a single swine herd on 2 occasions found the microorganism in inflamed joints and adjacent subcutaneous abcesses of some animals. In a later phylogenetic study *Pettersson et al.* (2001) found that the mycoplasma belongs to the *Mycoplasma lipophilum* cluster within the *Mollicutes.*

The mycoplasma flora of swine in Denmark has been studied during many years, and in 1995, in a group of approximately 4-month-old pigs brought together from different herds and litters, some hitherto unknown mycoplasma-like isolates were obtained from tonsillar surface scrapings of several animals on one occasion. The isolates were cultivated using liquid and solid media as described by *Kobisch & Friis* (1996). Initially, the growth in liquid medium was indicated after a few days by a slight alkaline colourshift as observed from visual inspection. After adaptation to artificial medium, colonies of the typical fried-egg morphology developed on solid medium.

Six original isolates were compared serologically with the species type strain of *M. hyopharyngis* (strain H3-6B F, ATCC 51909) and with *Mycoplasma hyosynoviae* (type strain S16, ATCC 25591; Danish reference strain M60). Corresponding polyclonal rabbit hyperimmune antisera were used in the conventional Disc Growth Inhibition test (DGI) performed with antiserum-impregnated discs on cultures on solid medium, and the Indirect Epi-immunofluorescence test (IF) performed on colonies on solid medium. The results of the serological comparison (Table 1) have evidenced that the isolated microorganisms belong to the species *Mycoplasma hyopharyngis.*

Further, the 16S rRNA genes of the isolates were amplified by PCR using universal primers (*Weissburg et al*. 1991) and subsequently analysed by sequencing. The 16S sequences of the field strains were mutually identical, and except for 5 single-base mutations they were found identical to the sequence of the species type strain H3-6B F. It was also found that the sequence of H3-6B F was identical to the earlier published strain 538-N partial sequence (*Pettersson et al.* 1994; accession number:

Table 1. Serologic identification of 6 swine pharyngeal mycoplasma isolates by the DGI test and the IF test using type strain antisera for *M. hyopharyngis* and *M. hyosynoviae.*

	Antiserum					
	M. hyopharyngis		*M. hyosynoviae*			
	type strain H3-B6 F		type strain S16		Danish reference M60	
Mycoplasma strain	DGI[a]	IF[b]	DGI[a]	IF[b]	DGI[a]	IF[b]
M. hyopharyngis H3-B6 F	5	+	0	0	0	0
Field strain Mp877/95	5	+	0	0	0	0
Field strain Mp879/95	5	+	0	0	0	0
Field strain Mp881/95	5	+	0	0	0	0
Field strain Mp882 /95	5	+	0	0	0	0
Field strain Mp883/95	5	+	0	0	0	0
Field strain Mp887/95	5	+	0	0	0	0
M. hyosynoviae S16	0	0	5	+	5	+
M. hyosynoviae M60	0	0	5	+	5	+

[a] zone of inhibition in mm. [b] distinct FITC colour of stained colonies

mhu04652) but deviating by 10 bases from another *M. hyopharyngis* 16S rDNA sequence published in GenBank (*Blank et al.* 1996, accession number: mhu58997). All the sequences were clearly different from the 16S rDNA sequences of other *Mycoplasma* species present in GenBank.

Except for the demonstrated single-base substitutions, the 16S rDNA sequences of the field strains were identical to that of the species type strain and clearly different from the 16S rDNA sequence of other *Mycoplasma* species. This confirmed the serological identification of the organisms as *M. hyopharyngis.*

The prevalence of *M. hyopharyngis* among Danish swine appears to be very low, a notion underlined by the fact that this mycoplasma was not observed earlier than the described case, and never since. However, the registration of growth may be difficult because of apparent low titres in the cultures involving a weak alkaline colour change, which is actually little visible, especially as long as the cultures remain alive; maybe just about 2-3 days. No evidence of disease has accompanied the case.

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