Protein and Antigenic Variability among *Mycoplasma hyopneumoniae* Strains by SDS-PAGE and Immunoblot

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ABSTRACT

Porcine enzootic pneumonia (PEP), with *Mycoplasma hyopneumoniae* as the primary agent, is a chronic respiratory disease that causes major economic losses to the pig industry worldwide. The aim of this work was to analyse 18 field strains of *M. hyopneumoniae* isolated in Gran Canaria (Spain) and the reference *M. hyopneumoniae* strain by SDS-PAGE and immunoblot. A monoclonal antibody (MAb) against the membrane protein p46 reacted with all the strains in this study. In contrast, a purified polyclonal antibody (PAb) against the cytoplasmic protein p36 reacted with this protein in only 10 strains. A MAb against the adhesin protein p97 stained multiple proteins of different sizes and with different intensities. Different antigenic patterns in the same *M. hyopneumoniae* strains were also observed after different numbers of passages in culture medium. Furthermore, variability in the staining of the 36 kDa protein was observed, depending on whether the p36 PAb or the antiserum against *M. hyopneumoniae* reference strain was used. It is concluded that local *M. hyopneumoniae* field isolates in Gran Canaria are characterized by protein diversity.

**Keywords:** antigenic variability, immunoblot, *Mycoplasma hyopneumoniae*, SDS-PAGE

**Abbreviations:** LDH, lactate dehydrogenase; MAb, monoclonal antibody; PAb, polyclonal antibody; PBS, phosphate-buffered saline; PEP, porcine enzootic pneumonia; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

INTRODUCTION

*Mycoplasma hyopneumoniae*, the aetiological agent of porcine enzootic pneumonia (PEP), is present in the vast majority of swine farms worldwide, causing respiratory distress resulting from pneumonia in growing pigs. It causes considerable economic losses through retarded growth, poor food conversion and increased susceptibility of pigs to infections by other organisms (Kobisch, 2000; Kobisch and Friis, 1996).

It has been demonstrated that *M. hyopneumoniae* strains are characterized by protein, antigenic and genetic heterogeneity. Ro and Ross (1983) observed differences regarding identification of *M. hyopneumoniae* by serological methods, since the same
antisera gave different inhibition results when tested against different field strains of *M. hyopneumoniae*. It has also been shown that different numbers of passages in culture medium could affect the pathogenicity of *M. hyopneumoniae* in pigs (Zielinsky and Ross, 1990). Genetic heterogeneity between *M. hyopneumoniae* isolates was demonstrated by field-inversion gel electrophoresis (Frey et al., 1992) and by randomly amplified polymorphic DNA analysis (Artiushin and Minion, 1996; Vicca et al., 2003), and Lin (2001) found genetic and protein variation among *M. hyopneumoniae* field strains isolated in the United States.

Regarding species-specific *M. hyopneumoniae* proteins, there are some discrepancies in the literature. Young and Ross (1987) reported the species specificity of proteins with molecular weight of 110, 64, 50, 41 and 36 kDa, while Mori and colleagues (1988) described proteins of 96, 70, 46 and 38 kDa as specific. Later, Strasser and colleagues (1992) described proteins of 110, 78, 64, 41 and 36 kDa and Scarman and colleagues (1997) proteins of 200, 114, 94, 76, 43 and 36 kDa as being specific. Caron and colleagues (2000a,b) claimed that proteins with 97, 74, 65, 46 and 36 kDa were immunodominant and specific for *M. hyopneumoniae*.

The aim of this study was to characterized *M. hyopneumoniae* field strains isolated in Gran Canaria (Spain) by SDS-PAGE and immunoblot techniques, and to investigate whether there was protein and antigenic variability among the local *M. hyopneumoniae* field strains.

MATERIALS AND METHODS

Strains and growth conditions

A total of 19 strains were used for SDS-PAGE and immunoblot characterization. These comprised 18 field strains (I, F, O, 3, 11, 13, 34, 42, 43, 47, 50, 52, 60, 68, 72, 75, 78, 79) isolated from pneumatic lungs of 20- to 24-week-old pigs, collected in an abattoir from Gran Canaria (Spain), and the *M. hyopneumoniae* reference strain (J strain, NCTC 10110). Some of the field strains were also analysed after different numbers of passages in culture medium: I strain after 5, 22 and 35 passages; F strain after 15 and 25 passages; and O strain after 5, 12 and 23 passages. Strains were identified by their cultural and biochemical characteristics (Friis, 1971; Kobisch and Friis, 1996) and confirmed by PCR (Mattsson et al., 1995). The absence of *M. flocculare* and *M. hyorhinis* was confirmed by species-specific PCR (Stemke et al., 1994; Caron et al., 2000a). *M. hyopneumoniae* cells were grown in a modified Friis broth medium (Friis, 1975) containing exclusively horse serum (20%) and ampicillin (0.5 mg/ml), at 37°C with constant gyrorotatory agitation. Cells were harvested at log-phase (72 h) by centrifugation at 14600g for 30 min at 4°C, followed by three washes in phosphate-buffered saline (PBS). The washed cells were resuspended in the same PBS buffer (1/200 of the original volume) and stored at –80°C.