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Review

Pathobiology of *Mycoplasma suis*

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ABSTRACT

Mycoplasma suis is an uncultivable bacterium lacking a cell wall that attaches to and may invade the red blood cells of pigs. *M. suis* infections occur worldwide and cause the pig industry serious economic losses due to the disease known as infectious anaemia of pigs or, historically, porcine eperythrozoonosis. Infectious anaemia of pigs is characterised predominantly by acute haemolytic or chronic anaemia, along with non-specific manifestations, such as growth retardation in feeder pigs and poor reproductive performance in sows. The fastidious nature of *M. suis*, as well as the lack of an in vitro cultivation system, has hampered the understanding of the biology and pathogenicity of this organism. Pathogenetic mechanisms of *M. suis* include direct destruction of red blood cells by adhesion, invasion, nutrient scavenging, immune-mediated lysis and eryptosis, as well as endothelial targeting. Recently published genome sequences, in combination with proteome analyses, have generated new insights into the pathogenicity of *M. suis*. The present review combines these data with the knowledge provided by experimental *M. suis* infections.

Introduction

Mycoplasma suis belongs to the highly specialised and unique group of uncultivable haemotrophic mycoplasmas (HMs) that target the red blood cells (RBCs) of a wide range of mammalian species (Neimark et al., 2001; Hoelzle, 2008). The resulting disease is known as infectious anaemia of pigs (IAP) or, historically, porcine eperythrozoonosis. *M. suis* is an important cause of anaemia in pigs worldwide and is responsible for economic losses to the pig industry (Ritzmann et al., 2009). The acute form of IAP is characterised by high fever, haemolytic anaemia and hypoglycaemia (Hoelzle et al., 2003; Groebel et al., 2009). Chronic, low grade, *M. suis* infections vary from subclinical infections to conditions such as anaemia in neonates, growth retardation in feeder pigs, and poor reproductive performance and dysgalactia in sows (Henry, 1979; Hoelzle et al., 2003; Strait et al., 2012). In addition, *M. suis* and other HMs appear to be zoonotic agents, with strains of *M. suis*, *Mycoplasma haemofelis* and *Mycoplasma ovis* being detected in human beings (dos Santos et al., 2008; Yuan et al., 2009; Sykes et al., 2010; Maggi et al., 2013).

The implementation of PCR and serology using recombinant antigens has improved our knowledge of the distribution and prevalence of *M. suis* infections (Hoelzle et al., 2003, 2007a and b; Guimaraes et al., 2011b). Infection has been identified in domestic pigs in Europe, the USA, South America and Asia, and in wild boars in Europe (Guimaraes et al., 2007; Ritzmann et al., 2009; Yuan et al., 2009; Hoelzle et al., 2010). The prevalence of *M. suis* infection, as determined by qPCR, ranges from 13.9% in Germany to 18.2% in South America (Guimaraes et al., 2007; Ritzmann et al., 2009).

Although first described in the early 1930s in the USA, our knowledge of the pathobiology of *M. suis* is still limited, mainly due to the inability to cultivate *M. suis* in vitro. Substantial insights into the pathogenesis of *M. suis* have been obtained from experimental infections of pigs after splenectomy (Zachary and Basgall, 1985; Zachary and Smith, 1985; Heinritzi et al., 1990a and b; Groebel et al., 2009). In addition, new insights into the pathobiology of *M. suis*, specifically RBC adhesion and invasion, cell tropism and immunopathology, have been gained in the last few years by applying molecular technology, including genomics and proteomics (Hoelzle et al., 2007c and d; Felder et al., 2012; Sokoli et al., 2013).

Despite sequencing and comparative analysis of two strains of *M. suis*, no genes have been identified that might encode virulence factors involved in the direct lysis of RBCs (Guimaraes et al., 2011a). Furthermore, genes encoding primary virulence factors, such as adhesins, invasins, haemolysins, haemagglutinins, toxins or known persistence factors, have not been identified in *M. suis* (Guimaraes et al., 2011a; Oehlerking et al., 2011). The mechanisms of pathogenicity of *M. suis* appear to be complex and genetically inapparent ('hidden'), as has been described for other mycoplasmas, such as *Mycoplasma mycoides* subspecies *mycoides* (Pilo et al., 2007). The present review summarises and evaluates laboratory and animal experimental data relating to the pathobiology of *M. suis*.

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Taxonomy

The taxonomic classification of HMs is still in progress. Previously, HMs were classified in the genera *Eperythrozoon* and *Haemobartonella* within the Family *Anaplasmataceae*, Order *Rickettsiales*. Phylogenetic analyses of 16S rRNA genes led to their reclassification as members of the Genus *Mycoplasma*, Family *Mycoplasmataceae*, Order *Mycoplasmatales*, Class *Mollicutes* (Rikihisa et al., 1997; Neimark et al., 2001, 2002; Messick et al., 2002). Analysis of RNase P RNA genes has further validated the 16S rDNA-based phylogeny (Peters et al., 2008). All HMs form a single cluster within the 'pneumoniae' group, with *Mycoplasma fastidiosum* as the closest relative (Rikihisa et al., 1997; Neimark et al., 2001; Peters et al., 2008). However, the unique biological characteristics of HMs (mainly tropism for RBCs), in conjunction with the lack of an in vitro culture system and the insufficient level of sequence similarity of 77–83% when compared to the closest related *Mycoplasma* spp. (Uilenberg et al., 2004), have led to uncertainties in their classification. As a consequence, the current Bergey's Manual of Systematic Bacteriology classifies HMs within the Family *Mycoplasmatales*, incertae sedis, and the taxonomic position of these organisms remains uncertain.

Cultivation

M. suis, as well as all other known HM species, cannot be cultured and, thus far, all attempts to propagate these fastidious organisms in the laboratory have failed. HM research has relied on the propagation of these organisms in splenectomised or immunocompromised animals. Cultivation of *M. suis* in porcine RBC cultures in a Petri dish system cell (Nonaka et al., 1996) and in cell-free *Mycoplasma* media (Schreiner et al., 2012a) resulted in persistence and maintenance, but not propagation, of the organism. Ultrastructurally, *M. suis* cells cultivated in cell-free media transformed into nanoforms as an adaptive response to adverse growth conditions (Schreiner et al., 2012a). We hypothesise that the evolution of the use of RBCs as a niche has resulted in the development of special adaptations in many *M. suis* metabolic pathways.

Anaemia

Anaemia is the cardinal clinical sign of *M. suis* infection in pigs. Anaemia can occur in association with both massive and minimal bacteraemia (Zachary and Basgall, 1985; Heinritzi et al., 1990a and b). Therefore, several mechanisms must exist for the destruction and elimination of RBCs from the blood of *M. suis* infected pigs. Direct interaction (adhesion and invasion) between the pathogen and the RBC leads to direct destruction and eryptosis, as well as extravascular haemolysis due to phagocytosis by macrophages in the spleen and liver, including splenic sequestration of infected and, therefore, deformed or modified RBCs. RBC modifications may be due to the binding of *M. suis* specific or autoreactive antibodies (immunoglobulin G, IgG), or *M. suis*-induced abnormalities that restrict RBC deformability. *M. suis* also evokes indirect immune-mediated intravascular lysis of RBCs due to autoreactive antibodies (Zachary and Smith, 1985; Hoelzle et al., 2006; Groebel et al., 2009; Felder et al., 2010, 2011).

Adhesion

Adhesion to host cells is a major virulence factor for animal mycoplasmas; adhesion-deficient mycoplasma mutants are not virulent (Rottem, 2003). Models of *M. suis*-induced anaemia have identified adhesion, and subsequent mechanical and/or osmotic damage, as a major trigger for extravascular haemolysis in the spleen, liver, lungs and bone marrow (Zachary and Basgall, 1985; Heinritzi and Plank, 1992; Groebel et al., 2009; Guimaraes et al., 2011a). Electron microscopy has demonstrated that *M. suis* is in intimate contact with RBCs, resulting in membrane deformation and damage (Fig. 1A).

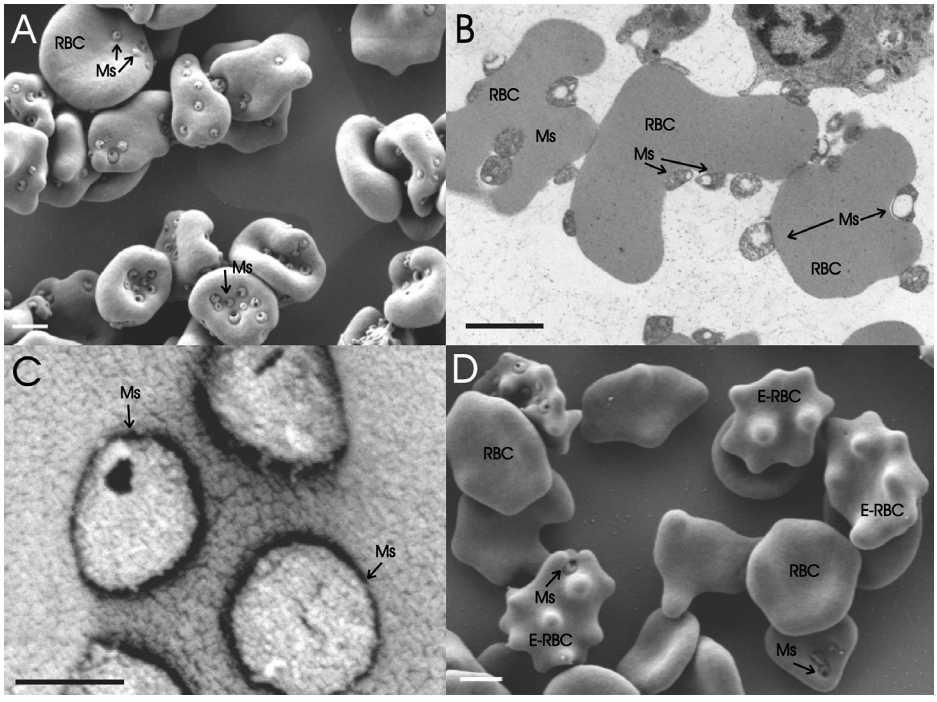

Fig. 1. Scanning electron microscope (A, C, D) and transmission electron microscope (B) images of *Mycoplasma suis* (Ms) infected red blood cells (RBCs). During the acute phase of infection, numerous *M. suis* are visible on the surface of the RBCs (A; scale bar = 2 μm). The RBCs form invaginations upon the adhesion of *M. suis* (B; scale bar = 2 μm). The contact between *M. suis* and the RBC surface is intimate and appears to be mediated by fibrils (C; scale bar = 200 nm). During the course of *M. suis* infection, eryptotic RBCs (E-RBCs) are observed (D; scale bar = 2 μm).