

**Title:** Molecular Characterization and Protective/Diagnostic Application of the Capsular Polysaccharide of *Haemophilus parasuis*- **NPB #12-014** revised

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### Scientific Abstract

**Background:** *Haemophilus parasuis* (Hps) is a Gram-negative bacterium responsible for Glässer's Disease in pigs, though little is known regarding its antigenic or virulence factors. Our goals were to isolate and characterize the Hps capsular polysaccharide (CP), determine its role in serotype specificity and virulence, determine if CP can produce an immune response, and develop diagnostic and protective products to help prevent widespread Hps infection within a swine herd.

**Materials and Methods:** CPs were purified from Hps serotypes 4, 5, 9, and 13 using enzyme digestion, phenol extraction, and ultracentrifugation. The CP electrophoretic profiles were visualized by alcian blue/silver-staining and compared to CPs from other Pasteurellaceae. CP glycosylation composition was determined by gas chromatography-mass spectrometry (GC-MS). Rabbits were immunized with CPs to generate antisera for immunofluorescence microscopy (IF), immunoassays, and bactericidal assays. CP was conjugated to the carrier protein Cholera Toxin B (CTB), and used to immunize piglets before challenge with Hps serotype 5 to determine the protective efficacy of the antibody response to Hps CP. CPs from serotypes 4, 5, and 9 were also conjugated to latex particles to create a diagnostic agglutination assay for detection and typing of various Hps serotypes.

**Results:** The electrophoretic profiles of CP from broth-grown Hps resembled those of other Pasteurellaceae, but CP was not isolated from Hps grown on agar medium. The presence of bicarbonate also enhanced production of CP. The composition of CP from the different serotypes examined by GC-MS was similar, but in different ratios and likely different in structure. The CP was visible on broth-grown Hps incubated with homologous CP antiserum by IF and immuno-transmission electron microscopy, but not on agar-grown cells or broth-grown heterologous Hps strains. Antiserum reacted strongly with homologous CPs of Hps grown in broth by enzyme-linked immunosorbent assay, but weaker cross-reactivity occurred with CPs from other Hps serotypes. Western blotting with homologous and heterologous antiserum confirmed that the lipopolysaccharide was highly conserved and not serotype-specific. Agar-grown Hps cells were highly susceptible to killing by normal swine serum, but broth-grown Hps were serum-resistant unless homologous anti-CP serum was present. The CP conjugate produced a robust antibody response to CP, as determined by immunoassays, but because the control animals also survived and contained some antibodies to Hps CP, the vaccine study remains inconclusive. Latex beads sensitized with antibodies against CPs agglutinated when mixed with purified CP or whole cells grown in broth. The latex beads were most reactive with homologous CP and whole cells, but also reacted weakly with heterologous cells. When agar-grown cells were lysed, the lysate produced an agglutination response to homologous sensitized latex particles, whereas unlysed agar-grown cells did not.

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**Conclusions:** Unlike most CPs that are constitutively expressed, expression of the Hps CP was upregulated by environmental factors, such as growth in broth, and the addition of bicarbonate. While CP was identified as the immunodominant antigen, cross-reactivity did occur in highly sensitive assays. Because the Hps serotype-specific antigen is carbohydrate and was not lipopolysaccharide, these results indicate the CP only is serotype-specific. CP was required for bactericidal resistance to normal serum, but antibodies to the CP were bactericidal, indicating the CP protects the bacterium from host immunity, but that antibodies to the CP may be protective. The vaccination study produced inconclusive results, but due to the baseline antibody titers that the