Expression of capsule and biofilm formation by *Streptococcus equi* subsp. *equi*

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*Streptococcus equi* subsp. *equi* (S. equi) of Lancefield group C and beta haemolytic streptococci causes Strangles, a contagious infectious disease of young horses1,2. Bacterial adhesion, colonization and immune evasion are required to pathogenesis. *S. equi* is a host-restricted pathogen derived from *Streptococcus equi* subsp. *zoopneumoniae*3. *S. equi* has many virulence factors as capsule, SeM protein, streptolysin S and superantigens4,5,6. The hyaluronic acid capsule reduces the phagocytic function of neutrophils and it is also required for the activity of other proteins1,4. In addition, the capsule protects the bacterium from immune recognition because of mimicry with host tissue components. Other bacteria are able to produce biofilm that is sessile microbial community in which cells are attached to a surface and enveloped within an extracellular polymeric matrix7. Bacterial biofilms are more resistant than planktonic cells to antimicrobial agents and host immune defense systems8,9. *S. equi* produces high levels of capsule and that may be related with the low adhesion to the mucosal surface9. The aim of this study was determined capsule and biofilm formation by *S. equi*. Ninety isolates of *S. equi* were obtained from horses with clinical Strangles and healthy carriers in Buenos Aires, Argentina. The isolates were culture 24 h (to capsule) and over night (to biofilm) at 37°C in Todd Hewitt broth supplemented with 0.2% yeast extract and 10% adult horse serum. Then, capsule was observed by negative staining with India ink solution and crystal violet at 100X. The inoculum to biofilm was diluted 1/10 in fresh medium and biofilm formation was performed on glass slides at 37°C and CO2 enriched atmosphere for 18 h. After washing and fixing, the biofilm was staining with Alcian Blue 4X and crystal violet and then biofilm was observed at 40X and 100X. Results were elaborated by *X*² test and the level of significance was established at P<0.05 by statistical analysis. Staining of capsule showed 85% of encapsulated isolates and different levels of capsule expression: high (49%) and low (51%). Staining of biofilm on glass slides showed 95.56% of adherent isolates which were grouped in high agglomerate (40%), low agglomerate (27%) and chain of coccus (33%) and 66% of them formed extracellular polysaccharide (PSE) (figure 1). There was a significant correlation between agglomerate and PSE formation (p<0.0001). Expression and level of capsule were not correlated neither with agglomerate nor PSE formation (p>0.05). Our results of capsule are agree with others studies which described that virulent isolates of *S. equi* are usually highly encapsulated1,4,5. Biofilm formation in *S. equi* could be demonstrated and agglomerate and PSE formation were observed in a high percentage of isolates. Several reports of *Streptococcus pneumoniae* have demonstrated that nonencapsulated pneumococcal mutants show increased adhesion properties and biofilm in vitro formation but we could not demonstrated correlation between capsule and biofilm formation1. Biofilm may be associated with persistent infections in carriers as with others pathogens2,3. We propose to continue studying this virulence factor, their relation with bacterial adhesion, resistance and persistence in healthy animals.

References


083 “Strangles-like” disease and outbreaks caused by *Streptococcus equi* subspecies *zoopneumoniae*: Case cluster description and diagnostics by a real-time PCR strangles screen

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“Strangles” has been traditionally associated with *Streptococcus equi* subspecies *equi* (S.equi). Other streptococcus bacteria have been suspected to cause strangles-like clinical presentation and outbreaks in horses; 1. *Streptococcus equi* subspecies *zoopneumoniae* (S. zoo), is considered an opportunist pathogen in equine upper airways, a common cause of abscess formation in horses, and an important pathogen in equine respiratory disease and metritis. 2. *Streptococcus dysgalactiae* subspecies *equisimilis* (S. equisimilis), an infrequent cause of placentitis and lymphangitis in the horse. IDEXX® developed a real-time PCR-based screens for all three strains of beta hemolytic streptococci. The outbreak reviewed for this report started with the arrival of a new horse with heavy nasal discharge. Two additional horses subsequently developed similar nasal discharge and lymphadenopathy. Common equine respiratory viral pathogens and *S. equi* were ruled out by a respiratory PCR panel on nasal swabs. Consequently, samples were submitted to be screened for the 3 beta hemolytic streptococci; they were all positive for *S. zoo*. The results were confirmed by an aerobic culture that grew pure heavy growth of *S.zoo.* The correct identification of pathogens causing equine pyrexia and respiratory disease is essential for the identification of potentially significant equine biosecurity pathogens such as EHV1, EHV4, EIV, and *S. equi*. Occasionally, other streptococcal bacteria cause significant upper airway morbility in individual horses and outbreaks. A beta hemolytic streptococci real-time PCR panel is an improvement over current diagnostic tools and provides a more accurate identification in cases with suspected strangles clinical presentations and negative culture results.

Figure 1. Biofilm formation of *S. equi* in glass slides. A. High agglomerate. B. Low agglomerate. C. Chain of coccus.