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Correlation between endoscopic findings and real-time PCR analysis for *Streptococcus equi* subsp. *equi* DNA of guttural pouches in recovered strangles cases

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Horses that lack obvious clinical signs of respiratory disease can be silent carries of *Streptococcus equi* subsp. *equi (S. equi)* in their guttural pouches. Guttural pouch lavage and repeated nasopharyngeal lavages[(1)] analyzed by real-time PCR[(2)] are sensitive methods for analysis for *S.equi* and are considered to be a standard to identify carrier stage. The carrier screening, identifying and treatment of carriers, in the end of a disease outbreak by sampling guttural pouches is expensive and time consuming. According guidelines carriers that have endoscopic findings of guttural pouch empyema, chondroids or discharging lymph nodules are sampled and treated. The sensitivity of using macroscopic appearance of guttural pouches as an indication of persistent *S. equi* colonization is poorly studied. The aim of this study was to investigate if there is a correlation between endoscopic findings in guttural pouches and positive qPCR analysis for *S.equi*. In total 179 endoscopic findings from upper airways from 91 individual horses after a strangles outbreak were compared with positive qPCR results from nasopharyngeal lavage and guttural pouch lavage samples. Nasopharyngeal lavage was performed with 120 ml saline and guttural pouch lavage with 40 ml saline for each pouch and analyzed separately. The nasopharyngeal lavage was performed with 120 ml saline prior endoscopy and endoscopic guttural pouch lavages using 40 ml saline for each pouch and analyzed separately. The endoscopic findings of guttural pouches, including empyema, chondroids or discharging retropharyngeal lymph nodules were recorded and compared to the qPCR analysis for *S. equi.* There was no statistical correlation between endoscopy findings and qPCR results. However, if the first guttural pouch lavage was positive by qPCR for *S. equi*, it was statistically more likely that the contralateral guttural pouch also was positive, indicating a risk for contamination of the second sample. Horses that lack obvious clinical abnormalities on upper airway endoscopy including the guttural pouches can be silent carriers of *S. equi*. In conclusion, negative endoscopic findings of the guttural pouches of recovered strangles cases cannot rule out silent carrier status. This study was approved by the Swedish Ethical Committee on Animal Experiments and each horse owner had given their informed consent to participate in the study. Financial support was achieved from The Swedish-Norwegian Foundation for Equine Research and Swedish Research Council Formas.

References

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2 Veterinary Microbiology 124 (2007) 219–229.

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Kinetic of biofilm formation by *Streptococcus equi subsp zooepidemicus*

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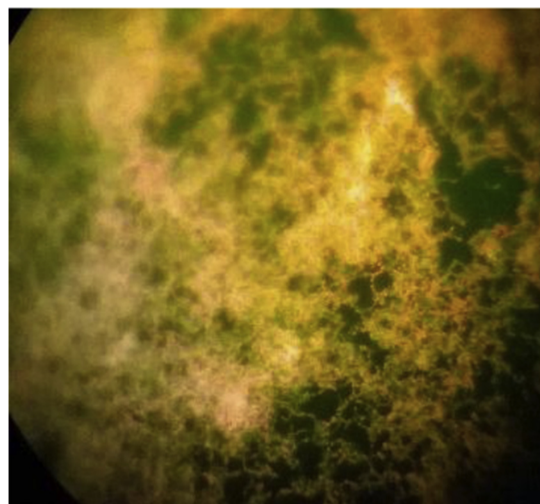

Figure 1. Biofilm stained with Acridine Orange

Streptococcus equi subsp. *zooepidemicus* (*S zooepidemicus*) acts as an opportunistic pathogen in equine reproductive and respiratory tract and it is the most isolated pathogen of the horse. *S zooepidemicus* is associated with several pathologies such as endometritis, placentitis, pneumonia, lymphadenitis, among other. It is also associated with diseases in others animal host, including dogs, monkeys, pigs and humans. A biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface producing matrix of extracellular polymeric substances. Biofilm formation is an important process to colonization and establishment of opportunistic pathogens. The ability to produce biofilm provides a protective environment, in which bacteria can adapt to coexist with the host providing immune protection. Another benefit of biofilm communities is the ability to resist antibiotic treatment. The present study was designed to identify *in vitro* biofilm production by *S zooepidemicus* and evaluated the kinetic of this biofilm. Twenty eight *S zooepidemicus* isolates from nasopharynx and genital mucosa of healthy horses were used. Biofilm formation assays were performed using 96-well plates. Strains were grown in Soy Triptein Broth at 37 °C in CO_2 atmosphere for 18 h. The inoculum was adjusted at $3x10^8$/mL (1 Mac Farland) and 200 µL were dispensed per well into a 96-well plate. Fresh medium was used as negative control. Plates were cultured without shaking at 37 °C in CO_2 atmosphere for 2, 24, 48 and 72 h. The medium of the plates was then poured off, and the wells were washed three times with sterile PBS (Phosphate Buffer Solution

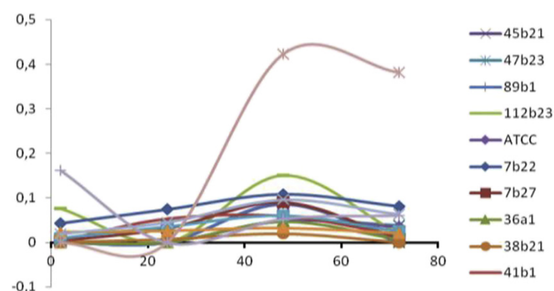

Figure 2. Isolates with highest production between 40-60 h.

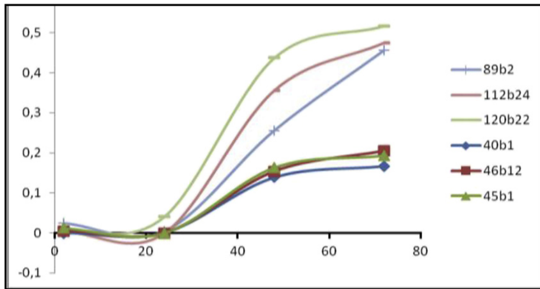

Figure 3. Isolates with highest production at 24 h.

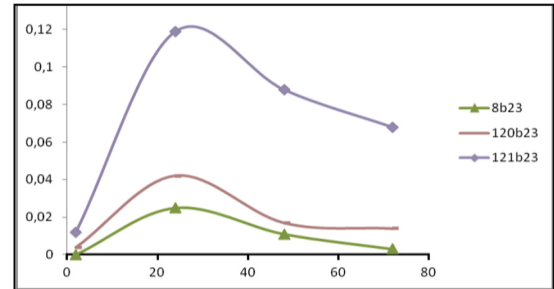

Figure 4. Isolates with highest production at 72 h.

pH 7). After that, plate wells were stained with 200 μL of 1% (w/v) crystal violet for 15 min and washed three times with PBS. The fixed stain was eluted with 200 μL 95% (v/v) ethanol and the absorbance of the supernatant was measured at 570 nm. Furthermore, the 96-well plate incubated for 72h was stained with Acridine Orange (Fig 1) and observed in fluorescence microscope. *In vitro* biofilm production was demonstrated in most of wild isolates (75%) and in ATCC 70400 strain too. Increase production of biofilm was obtained between 40 h and 60 h by 9 isolates and the ATCC (Fig 2). Only three isolates the increase production was demonstrated at 24 h (Fig 3) and six isolates which shown later and higher development of biofilm was observed at 72 h (Fig 4). Different behavior in biofilm production was demonstrated, this suggests that this process may be related with the fact to attach and persist in host tissue. Future studies of biofilm in *S zooepidemicus* and their relation with others phenotypic characteristics will be performed to demonstrated the role of biofilm in the pathogenesis.