Host and molecular mechanisms behind the persistence of equine strangles

Equine strangles is a widespread and highly infectious disease caused by the bacterium *Streptococcus equi* subspecies *equi* (*S. equi*). Despite the long history of strangles, much is still unknown about *S. equi* and the disease it causes. Understanding the complex interplay between host and bacterial factors that contribute to the carrier state is crucial for effective management of equine strangles. The aims of this project were twofold. Firstly, to evaluate the effectiveness of a strangles screening protocol at a UK welfare centre equids. Secondly, to use nanopore sequencing alongside bioinformatic analysis to investigate structural variants in acute and persistent *S. equi* isolates.

In the first phase of this project, the clinical records of 626 equids admitted to a UK welfare centre between 2017 and 2021 were analysed. Admitted equids were subject to a strangles screening process consisting of paired dual target iELISA serological tests and guttural pouch endoscopy and lavage. Retrospective analysis of the clinical records of admitted equids found that the most effective way to diagnose strangles was through guttural pouch endoscopy and lavage. No host factors or haematological parameters were significantly associated with strangles carriage. The dual target iELISA was found to be unreliable for the purpose of carrier detection.

In the second phase of this project, the genomes of 11 acute and persistent *S. equi* isolates were analysed using nanopore sequencing technologies to investigate structural variants. Deletions and inversions were found in key genomic regions including genes encoding the hyaluronic acid capsule and key sortase-processed cell surface proteins. A prophage was also found, integrated within genes encoding for the hyaluronic acid capsule.

These findings highlight the dynamic nature of persistence within the guttural pouch and reinforce the importance of implementing effective strangles screening protocols to prevent future outbreaks and maintain strangles-free herds. Endoscopically guided guttural pouch lavage and quantitative PCR was confirmed to be the most effective method for carrier detection. *S. zooepidemicus* was found to cause persistent strangles-like disease and should increasingly be considered alongside *S. equi* when dealing with strangles. Evidence of genomic decay was identified, and the inversions observed could lead to the development of subpopulations and represent a molecular mechanism associated with *S. equi* persistence. These structural variants could explain the observed failure of the dual-target iELISA.