Globetrotting strangles: the unbridled national and international transmission of Streptococcus equi between horses

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Methods: Brain Heart Infusion (BHI) containing *R. equi* was mixed with BHI-AC, horse plasma collected with different AC, or control. CFUs were counted at different timepoints. *R. equi* was opsonised with plasma using each AC and RAW264.7 macrophages were infected. Infected cells and CFU were counted at different times post-inoculation. Normality was assessed using Shapiro-Wilks test, variance using Levene's test. Data were analysed using repeated measures two-factor ANOVA, significance was set at $p<0.05$.

Results: There was no direct effect of ACD, Li Heparin or Na Citrate on *R. equi* growth. These three products significantly ($p < 0.001$) delayed growth for 12h post-opsonisation but there was no AC effect. Intracellular *R. equi* growth was significantly lower in Na Citrate ($p = 0.02$) 72 h post-infection. In contrast, direct contact with K_2EDTA completely inhibited the formation of *R. equi* colony forming units by 12 h as well as the intracellular growth at all the time points evaluated.

Main limitations: Only one strain was used for this study (*R. equi* #ATCC 103+).

Conclusions: K_2EDTA had a pronounced direct effect and resulted in intracellular growth inhibition whereas Na Citrate delayed intracellular *R. equi* growth. The use of ACD and Li Heparin appear to be more appropriate choices for the selected *in vitro* assays.

Ethical animal research: Plasma used for this study was obtained from horses from the WSU research herd.

Informed consent: Not applicable.

Competing interests: None declared.

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Streptococcus equi subsp. *equi*

Oral Presentations

17 | Globetrotting strangles: the unbridled national and international transmission of *Streptococcus equi* between horses

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Background: *Streptococcus equi* subspecies *equi (S.equi)* is the cause of the highly contagious equine respiratory disease 'strangles'. Approximately 10% of recovered animals can persistently carry the bacteria and transmit it to naïve animals. The global movement of horses is an ideal mechanism for widespread transmission to geographically distant locations.

Objectives: Utilise whole-genome sequence data to disentangle the transmission of *S. equi* and subsequent outbreaks of strangles.

Study design: *In vitro* analysis of micro-organisms.

Methods: Isolates (n = 670) of *S. equi* were recovered from clinical samples submitted to multiple collaborating clinics and institutions globally. Following species confirmation, isolates underwent whole-genome sequencing using Illumina technology. Sequence reads passing quality control measures were assembled and uploaded to Pathogenwatch, which assigned a phylogeny based upon sequences of core genome alleles. Population structure was inferred using the population mixture analysis in BAPS.

Results: BAPS clustered the isolates into six different clusters (BAPS 1-6) and showed dominant lineages in different geographical areas but also global transmission within the clusters. Sub-groups within the clusters highlighted multiple outbreaks at local, national and international scales and highlighted population structures and transmission dynamics within single locations. For example, four different strains collected over just seven months were identified in a single location. Sequence data also identified a statistically significant decline in BAPS-5 since 2010.

Main limitations: Pathogenwatch has shown its utility in investigating *S. equi* transmission and population structure. However, it is based upon a curated set of 1286 core genome loci. Further investigations will need to be conducted using the full spectrum of data available from whole-genome sequencing.

Conclusions: Pathogenwatch was used as a tool to rapidly identify and visualise the whole-genome sequence data of a large *S. equi* dataset. The data demonstrate widespread transmission of multiple *S. equi* lineages and provide strong evidence that asymptomatic carrier horses are perpetuating this dissemination.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Explicit owner consent was not stated but isolates were archived for further research with permission from submitting veterinarians.

Competing interests: A. Waller is employed by Intervacc AB.

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18 | Conservation of antigen sequences across a global population of *Streptococcus equi*

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Background: *Streptococcus equi* (*S. equi*) can be differentiated into six Bayesian-analysis-of-population-structure (BAPS) groups using core genome polymorphisms. However, conservation of *S. equi* genomes coding for antigens in the Strangvac protein-subunit vaccine has not been determined.

Objective: To define the diversity of Strangvac vaccine antigens in a diverse *S. equi* population.

Study design: *In vitro* analysis of micro-organisms.

Methods: Genomic antigen sequences of 1026 *S. equi* isolates from 19 countries between 1955 and 2019 were analysed. Predicted amino acid sequences of SEQ0256(Eq5), SEQ0402(Eq8), SEQ0721(EAG), SEQ0855(SclF), SEQ0935(CNE), SEQ0999(IdeE), SEQ1817(SclB), SEQ2101(SclC) (in Strangvac) and SeM were extracted from 1026 assembled genomes and compared.

Results: The predicted amino acid sequences of SclF, SclI and IdeE were identical across all 1026 genomes. CNE was truncated in the genomes of six (0.6%) isolates. SclC was absent from one genome and another encoded a single P^{85} to L substitution. EAG was truncated in two genomes. Eq5 was truncated in four genomes and 137 genomes encoded a single I^{201} to L substitution. Eq8 was truncated in three genomes, one genome encoded four amino acid substitutions (E^{212} to G, E^{214} to G, A^{218} to D and L^{223} to I) and 726 genomes encoded a single H^{225} to Y substitution at the final amino acid. Therefore, at least 1579 (99.9%) of 1580 amino acids in Strangvac were identical in 1009 (98%) genomes, and all genomes had identical amino acid sequences for at least six of the eight Strangvac antigens. For comparison, 86 different amino acid changes were identified within the N-terminal 107 amino acids of SeM encoded by this collection and 26 (2.5%) isolates encoded truncated forms of SeM.

Main limitations: The majority (655, 64%) of isolates in this study were recovered from horses in the UK.

Conclusions: The predicted amino acid sequences of antigens in Strangvac, but not SeM, were highly conserved across this collection of *S. equi*.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not stated.

Competing interests: J.-I. Flock and A.S. Waller are employed by Intervacc AB. B. Guss is a member of the board of Intervacc AB.

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