Horserace Betting Levy Board
Parnell House
25 Wilton Road
London, SW1V 1LW

Tel: 020 7333 0043
Fax: 020 7333 0041
Web: www.hblb.org.uk
Email: equine.grants@hblb.org.uk

The functional and immunological consequences of SeM variation on the pathogenesis of strangles

Andrew Waller
Animal Health Trust

Completed Project - 1st January 2007 to 31st October 2010
HBLB VAC reporter: CM Marr

PRJ: 733

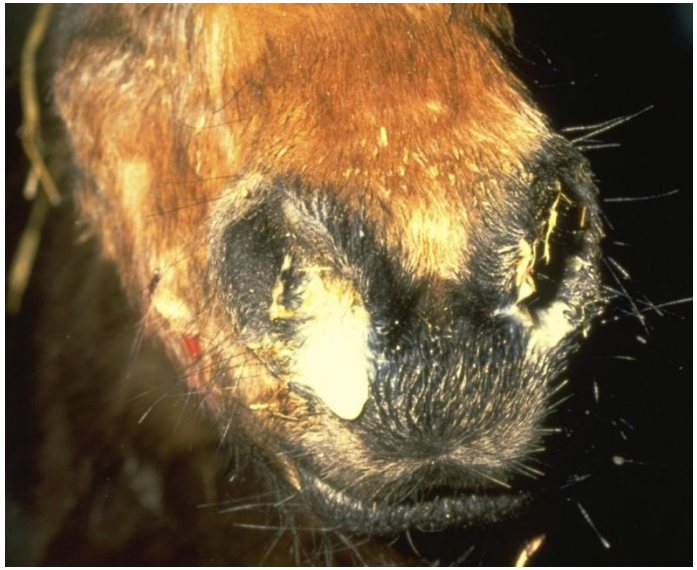
Immunity: what does that mean?

- Immunity is the biological defence system that fights off infection.
- When challenged by an invading bacteria, the equine immune system first mounts a non-specific response activating a variety of cells which can engulf and kill invaders. This is known as innate immunity and can be regarded as the first line of defence.
- Very quickly, immune cells begin to produce antibodies that are targeted specifically at engulfing the invading bacterium and this represents the adaptive response to infection.
- Micro-organisms that cause disease can evade their host's immune defence through variability in proteins found on their surfaces that are the target of the host's protective antibodies.

Better vaccines for horses

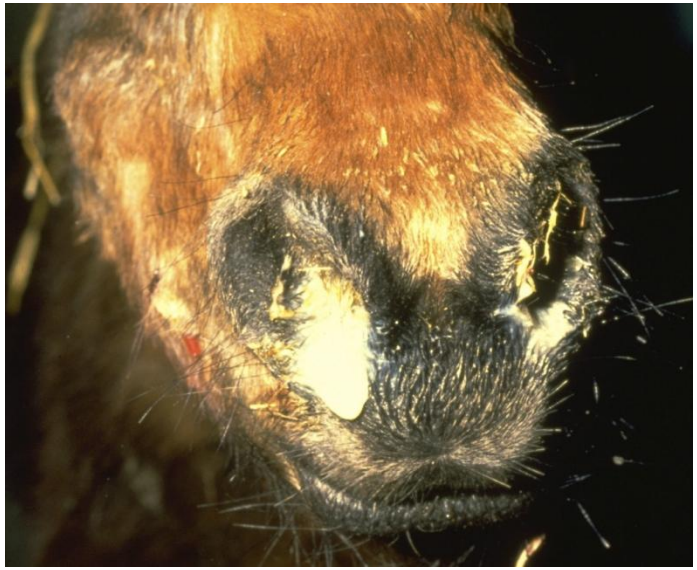
- The SeM protein of the bacteria *Streptococcus equi (S.equi),* that causes the equine disease 'Strangles', is one such surface protein that inhibits host defences and therefore might be important in allowing some strains of this bacteria to be more aggressive than others.

- SeM proteins have been found to vary between strains of *S. equi* and 128 distinct sub-types of *S. equi* have been identified.



Better vaccines for horses

- We need to find out why specific strains of this bacteria are able to evade the antibodies that infected horses produce in order to fight off infection and the SeM protein might be one key to this phenomenon.



Summary of this study

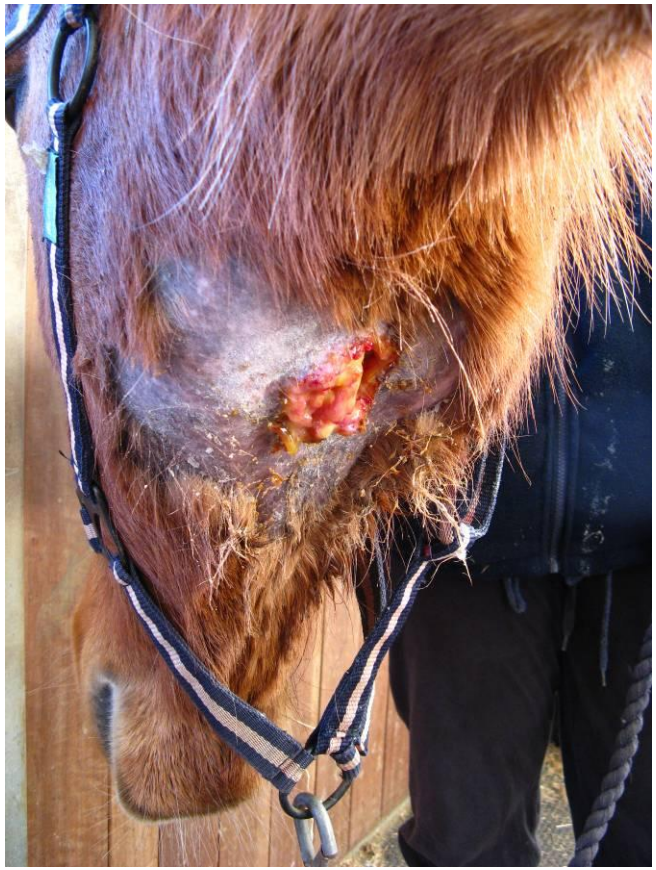
- Genetic analysis of bacteria isolated from disease outbreaks used to test hypothesis that:
  - variation in SeM protein of *S. equi* resulted in differences in binding to the neutralising antibodies of horses.
  - due to the importance of SeM in conferring a selective advantage to the bacteria, SeM function is largely maintained despite this variation.

- The data demonstrated that although variation of SeM has a significant functional effect, depressing the response to non-specific host defence mechanisms, it does not affect the specific immune response (involving binding to neutralising antibodies).

- More work is needed to find out how this important bacteria evades the horses' immune responses.

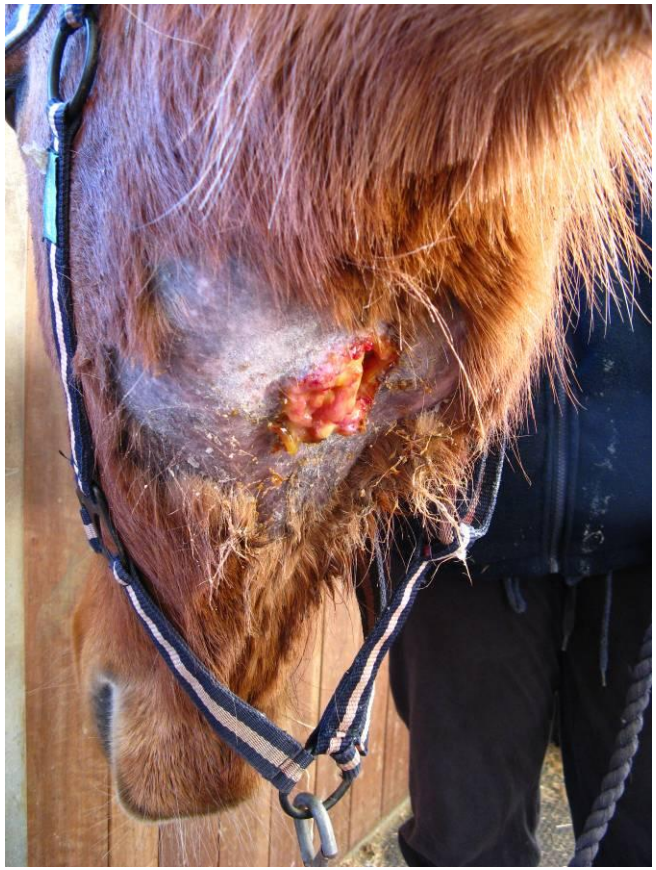
Strangles in horses

- Strangles is the most important infectious disease of horses worldwide.
- Caused by the bacteria *Strep equi.*
- Is of major welfare and economic importance
- Affects all types of horse including racehorses, and is particularly life-threatening in young foals.
- We know that after infection, recovered horses are immune for several years therefore it should be possible to develop an effective vaccine

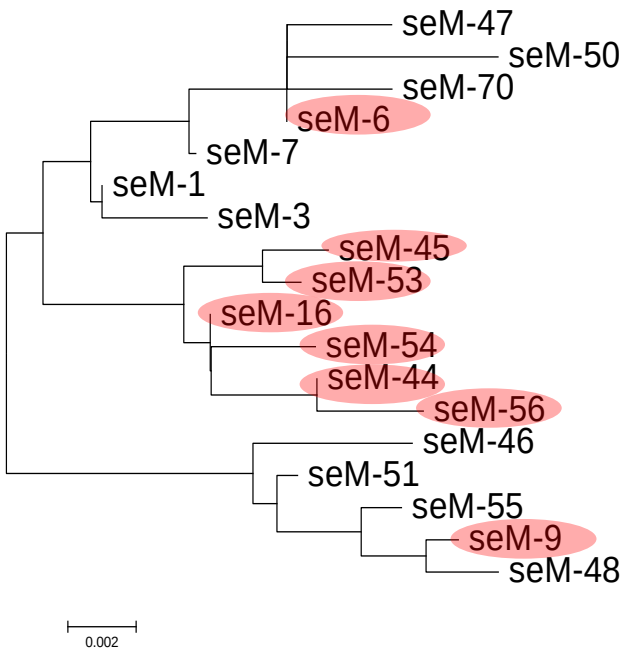


Aim of this project

- Bacteria infects lymph glands and creates abscesses, usually around the throat but sometimes affecting internal organs.
- Previous research has shown that there are a number of strains, with differing capacity to create disease and induce immunity.
- Some horses become "carriers" i.e. permanently infected, and are an important source of infection to others.
- Genetic differences amongst strains may be important.
- This research sought to answer the question:
  - Why do *Strep equi* strains differ in the sequence of their SeM gene?



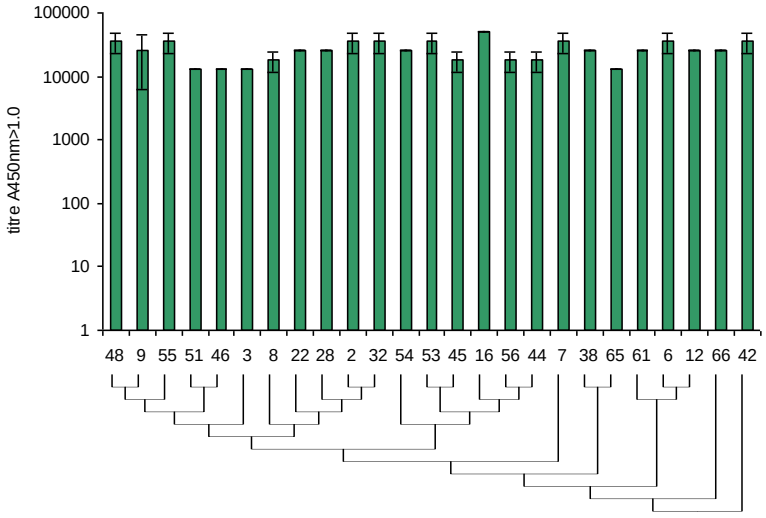
Creating genetic maps to study variation of SeM genes in a number of outbreaks:



- The SeM gene of 259 isolates from 23 outbreaks was sequenced
  - 16 different alleles (ie. groups of genes).
- Identified pre-existing carriers.
- Highlights the complexity of many strangles outbreaks.

The strains highlighted in red were all recovered from outbreak 5, where >200 horses were infected over a 2 year period. This outbreak nicely demonstrates the evolution of SeM over time (from SeM-16 to 44, 45, 53, 54 and 56), and the presence of pre-existing carriers (SeM-6 and 9), which was confirmed on genome sequencing.

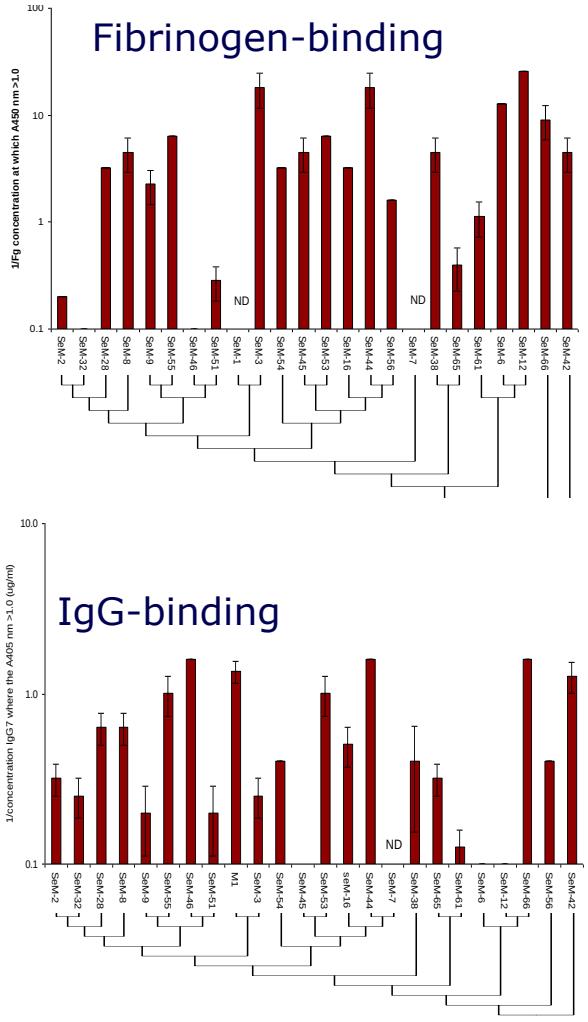
Variation of SeM has little antigenic effect



This is a representative graph showing minimal variation in the ability to induce immunity of different SeM variants when tested against serum from a horse infected with one strain of *S. equi*.

- Little difference was found between the antibody responses to different SeM variants
- This suggests SeM variation does not assist *S. equi* to influence antibody production

However, variation of SeM has functional consequences



- Different *Strep equi* strains have different effects on components of the immune system that affect the horse's ability to bind to, and to destroy, invading bacteria

Conclusions

- SeM genetic variation affects the function of the immune system, but this is not a result of how much antibody is produced.

- Further studies are needed to determine why the SeM gene varies between strains
  - Could this be an evolutionary adaptation that the strangles bacteria undergo while living in a carrier host in the guttural pouch?
  - Might this be an important strategy that the bacteria use to evade the horses' developing herd immunity during outbreaks?

Find more information on respiratory diseases that affect Thoroughbreds:

- Facing the threat of equine influenza, D Elton and N Bryant, http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2010.00357.x/full

- Lower airway disease, now and in the future, KC Smith, http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2011.00375.x/full

Scientist's Summary

- Many pathogenic micro organisms evade host immunity through variability in surface proteins targeted by protective antibodies.

- The SeM protein of *Streptococcus equi* is an important virulence factor capable of binding fibrinogen, a protein that is produced in response to inflammation, and inhibiting the deposition of complement on the bacterial surface.

- In this study, we found that the N-terminal region of SeM is subjected to high selective pressure. The resulting variability was exploited to identify 43 distinct sub-types of *S. equi*. However, the functional and immunological consequences of this variability had not been determined.

Scientist's Summary

- We hypothesised that SeM antigenic variability serves to confer a selective advantage in the presence of neutralising antibodies and that due to the importance of SeM as a virulence determinant, its function is largely maintained despite this variation.

- However, our data demonstrated that variation of SeM has a significant functional, but not antigenic effect.

- We extended our genetic analysis of *S. equi* to reveal a dynamic and flexible genome that continues to decay, acquire and exchange new genetic elements. Examples of multiple incursions of *Streptococcus equi* and pre-existing carriers in individual outbreaks shed new light on the epidemiology of this global equine disease.

To find out more about HBLB's research go to:

"HBLB's Advances in equine veterinary science and practice"

- http://onlinelibrary.wiley.com/journal/10.1001/%28ISSN%292042-3306/homepage/hblb_virtual_issue.htm