SURVEILLANCE FOCUS

Strangles: a pathogenic legacy of the war horse

Strangles, characterised by pyrexia followed by abscessation of the lymph nodes of the head and neck, was first described in 1251 (Rufus 1251) and the causative agent, *Streptococcus equi*, was identified in 1888 (Schutz 1888). However, despite more than a century of research into this disease, strangles remains the most frequently diagnosed infection of horses with over 600 outbreaks being identified in the UK alone each year (Parkinson and others 2011). Here, Andrew Waller reviews some of the recent advances in the understanding of the evolution of *S equi* and puts this into the context of preventing and resolving outbreaks of infection.

RECENTLY, Harris and others (2015) published their analysis of the genomes of a large collection of *Streptococcus equi* strains that were recovered from horses throughout the world and spanned a time period of 55 years. Surprisingly, given that the historical record of strangles suggests that *S equi* should date back to at least the 13th century, strains recovered from horses across the globe were found to share a common ancestor that actually dated to the 19th or early 20th century. This period corresponds to a time when horses were a major mode of transport and played important roles in a number of global conflicts such as World War I, where an estimated eight million horses died on the battlefield. At its peak, 1000 horses per day were imported to the UK from the USA and horses from all around the world were called into action. The mixing of these horses, and their replacement with young animals on an unprecedented scale, through initiatives like the formation of the National Stud, would have provided ideal conditions for the emergence and spread of the fittest strain of *S equi* from which today's global population has evolved.

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A proportion of recovered horses become persistently infected with *S equi*, carrying the organism within their guttural pouches. These healthy 'carrier' animals play a vital role in the recurrence of strangles and the spread of *S equi* to new yards and countries (Waller 2014). Persistent bacteria must survive in the face of a strong immune response, produced by the horse following recovery from strangles, and in an environment that is very different from that experienced by the bacterium during acute disease. These conditions select for changes in the DNA of persistent bacteria, such that genes that are no longer required for survival in the guttural pouch are lost and those that are targeted by the immune response mutate (Fig 1).

Indeed, the rate of DNA mutation and loss was significantly higher in isolates recovered from persistently infected horses when compared to isolates recovered from horses with acute disease (Harris and others 2015). Genes that were lost by persistent strains of *S equi* included those involved in citrate metabolism, production of the hyaluronic acid capsule and the biosynthesis of an iron-binding siderophore, known as equibactin. The loss of at least some of these genes reduces the potential for *S equi* to transmit to other animals (Harris and others 2015). So although the persistent strain survives in the guttural pouch, it may be less able to cause acute strangles.

This decreased virulence potential provides one explanation for outbreaks of 'atypical' strangles in young horses, where naive animals do not display classic clinical signs despite being infected with *S equi* (Prescott and others 1982). The loss of genes that do not affect the ability of *S equi* to cause strangles facilitates streamlining of the genome, whereby the organism loses ancestral capabilities leading to host-restriction, explaining why *S equi* only causes disease in horses.

Loss of genes during persistent infection also highlights potential problems for the detection of infected animals. For example, two horses in Devon tested negative by the *eqbE* qPCR test in a recent study despite being infected with *S equi* (Webb and others 2013). Fortunately, the triplex qPCR described in this study was able to detect *S equi* strains lacking the *eqbE* target and these horses were able to receive treatment before they could transmit the infection to other animals.

Such examples highlight that the various PCR and qPCR tests are not all the same and that single target assays for *S equi* have the potential to generate false negative results.

Interestingly, several genes that encoded proteins located on the cell surface of *S*

FIG 1: Artist's impression of the acute and persistent phases of strangles in the horse. The transition of intact chains of *Streptococcus equi* to the lymph nodes of the horse's head is depicted. An abscess is shown to develop and burst into the guttural pouch where chondroids (dried balls of pus) form, enabling the organism to persist. Within the guttural pouch, the DNA of *S equi* decays, symbolised by the transformation of bacterial chains into an intact and then broken helix, as the bacterium evolves to meet the challenges of its new environment. (Illustration by Alana Woodward, Virology Research Group, Animal Health Trust)

equi were particularly diverse in strains recovered from both persistent and acute infections (Harris and others 2015). The mutation of surface proteins may assist the bacterium to evade the immune response. In the guttural pouch, this may help the mutant variant to be shed and transmit to new animals. Such data have important implications for the design of more effective vaccines, as they suggest that changes in a limited number of proteins could have an unusually large effect on the ability of *S equi* to evade the protection conferred. Unfortunately, the only currently available European vaccine against strangles is based on a strain that rarely infects horses residing in the UK today and differs from the majority of circulating strains (Ivens and others 2011, Parkinson and others 2011) by an evolutionary timespan equivalent to more than 200 years (Harris and others 2015). Indeed, the first identified cases of strangles that were caused by the prevalent ST-151 type of *S equi* (Fig 2) occurred in three horses that had been vaccinated using the current vaccine (Kelly and others 2006, Harris and others 2015). While this finding does not mean that the vaccine will not confer cross-protection against the currently circulating strains of *S equi*, further research is warranted.

Analysis of the genome sequences of isolates recovered from UK horses reveal the complex epidemiology of this disease. For example, a large outbreak of strangles in Lincolnshire was most likely linked to a previous episode of disease and the presence of several persistently infected horses on the affected premises. This highlights the importance of identifying and treating persistently infected animals in order to prevent recurrent outbreaks. Also, two new arrivals at the Lincolnshire premises were shown to be persistently infected with two different strains that were unrelated to the ongoing outbreak, illustrating the prevalence of persistently infected horses in the population of animals entering the premises.

Another outbreak in Essex involved only 10 horses, but yielded three different types of *S equi*. One of the horses in this outbreak was shown to be infected with more than one type of *S equi* at the same time, highlighting the scale of endemic disease within some premises and the limitations of eradication procedures that do not consider all horses on an affected yard.

The ST-151 strains, and variants thereof, are now widespread in the UK and Europe. Their transmission is facilitated through the modern-day international movement of horses and a lack of preimport screening to identify persistently infected carriers. Improved screening of horses and the treatment of carriers before movement is essential to break the life cycle of infection exploited by *S equi*. As with vaccines against equine influenza, updates for strangles vaccines may also prove to be vital in order to ensure that adequate levels of protection against the currently circulating strains of *S equi* can be achieved. The recent advances in knowledge of *S equi* provide an unprecedented opportunity to improve the design of preventative vaccines towards protecting future generations of horses.

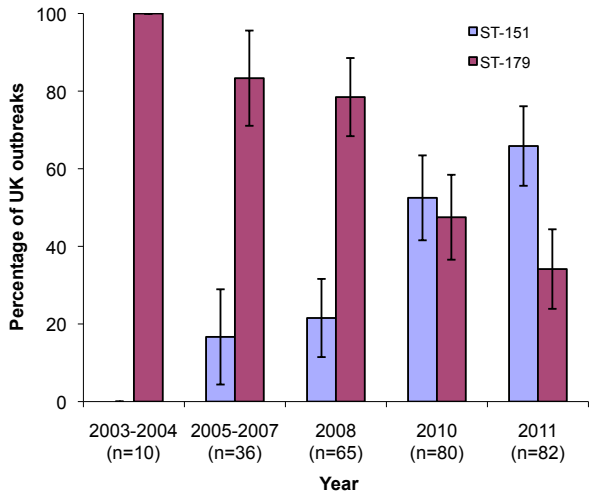

FIG 2: Graph showing the proportion of outbreaks of strangles in the UK from 2003 until 2011 from which ST-179 and ST-151 strains were isolated

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