Review

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Streptococcus equi: a pathogen restricted to one host

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Strangles caused by the host adapted Lancefield group C *Streptococcus equi* subspecies *equi* (*S. equi*) is a frequently diagnosed infectious disease of horses worldwide. Critical to the global success of *S. equi* is its ability to establish persistent infections within the guttural pouches of recovered apparently healthy horses that can result in transmission to in-contact animals. Recent research has identified key events in the *S. equi* genome, which occurred during its evolution from an ancestral strain of *S. equi* subspecies *zooepidemicus*, that may enhance its ability to evade host innate immune responses and rapidly multiply in the tonsillar complex and draining lymph nodes. This review discusses the role of these genetic events on the evolution and emergence of this important host-restricted pathogen.

Introduction

Strangles caused by the bacterium *Streptococcus equi* subspecies *equi* (*S. equi*) continues to be the most frequently diagnosed infectious disease of horses worldwide. This highly contagious disease is characterized by abscessation of lymph nodes in the head and neck, and leads to high morbidity and occasional mortality (Timoney, 1993). Extrapolation based on figures in the Equine Quarterly Disease Surveillance Reports (http://www.aht.org.uk/equine_disease.html) and data from a recent study of UK strangles outbreaks (Ivens *et al.*, 2011) suggests there were over 700 outbreaks of strangles in the UK during 2008. Some of these outbreaks involved over 200 horses, and led to significant economic and welfare costs.

The worldwide incidence of strangles is unexpectedly high given that the acute infection should be readily identified, contained and resolved using standard biosecurity measures. However, a proportion of recovered horses fail to drain pus from their guttural pouches following rupture of abscesses formed in adjacent retropharyngeal lymph nodes. Over time this pus becomes inspissated to form 'chondroids', enabling *S. equi* to persist for up to several years and transmit to naïve horses (Newton *et al.*, 2000). The global success of *S. equi* has likely stemmed from its ability to rapidly proliferate in tonsil tissue and draining lymph nodes, subsequently leading to abscesses that drain into the guttural pouches where infection persists. The selective requirements of these very different host environments have likely influenced the events of gene loss and gain that have shaped the *S. equi* genome.

S. equi is believed to have evolved from an ancestral strain of *S. equi* subspecies *zooepidemicus* (*S. zooepidemicus*)

(Jorm *et al.*, 1994; Webb *et al.*, 2008), which is associated with a wide variety of diseases in horses and other animals including humans. Both of these organisms share over 80 % DNA sequence identity with the important human pathogen *Streptococcus pyogenes*. *S. equi* in particular has much in common with *S. pyogenes*; both are host-restricted pathogens, share near identical surface proteins, phospholipase A₂ and superantigens (sAgs), and have acquired the genes encoding these toxins as cargo on related prophage suggesting that they share a common phage pool enabling cross-species gene transfer (Holden *et al.*, 2009). Both pathogens also cause diseases that share similarities with one another, strangles and tonsillitis, and so the study of *S. equi* may shed new light on *S. pyogenes* infections of humans.

S. equi, a poor colonizer

S. zooepidemicus usually infects young horses by entering the nose or mouth and colonizes the mucosal surface and tonsillar tissues of the nasopharynx. *S. equi* also infects via the nose or mouth, most likely through contaminated drinking water, but does not colonize the nasopharynx and is often not detected by nasopharyngeal swabs or washes taken 24 h post-infection. Interestingly, unlike *S. zooepidemicus*, *S. equi* does not persist in the tonsils and only rarely infects other animal species (Ladlow *et al.*, 2006) including humans (Breiman & Silverblatt, 1986).

Multilocus sequence typing of 253 isolates of *S. zooepidemicus* and 24 isolates of *S. equi* revealed that *S. equi* is most closely related to a cluster of *S. zooepidemicus* strains that were significantly associated with isolation from cases of uterine infection or abortion rather than the upper

respiratory tract (Webb *et al.*, 2008). This suggests that the ancestor of *S. equi* may have been better suited to bind a set of host ligands different to those available on the surface of the upper respiratory tract. Analysis of the *S. equi* genome provides a possible explanation for its decreased ability to colonize the nasopharynx. *S. zooepidemicus* strain H70, isolated from the equine upper respiratory tract, encodes 39 sortase-processed proteins putatively attached to the cell wall by virtue of a C-terminal LPxTG motif. In contrast, *S. equi* strain 4047 encodes 29 such proteins and lacks functional homologues of several *S. zooepidemicus* sortase-processed cell surface proteins, including FimII, FimIII and FimIV pilus loci (Beres *et al.*, 2008; Holden *et al.*, 2009). Pili play an important role in the adherence of streptococci to host tissues (Abbot *et al.*, 2007; Pinton *et al.*, 2010), and the reduced complement of pili and other sortase-processed proteins on the surface of *S. equi* may limit its ability to bind to an array of host tissues and to occupy diverse pathogenic niches.

Although the number of putative sortase-processed cell surface proteins is reduced in *S. equi*, it retains the FimI locus, whose accessory pilin, CNE, shares homology to CNA of *Staphylococcus aureus* and has been similarly shown to bind collagen (Lannergård *et al.*, 2003). The gene encoding the putative TetR-like repressor of the FimI locus of *S. equi* was found to contain a nonsense mutation at codon 43, which hypothetically would lead to deregulation and constitutive or elongated pilus production (Holden *et al.*, 2009; Swierczynski & Ton-That, 2006). This pilus locus is present in the genomes of every strain of *S. equi* and *S. zooepidemicus* examined to date, as determined by PCR (Lannergård *et al.*, 2003), quantitative PCR or genome sequencing (K. Steward, unpublished data), and is likely to play an important role in general adhesion to host tissues. Such a function may enable *S. equi* to attach to tonsillar epithelium. In support of this, a new multi-component subunit vaccine containing recombinant CNE conferred protection against *S. equi* infection in Welsh mountain ponies (Guss *et al.*, 2009).

In contrast to the majority of *S. zooepidemicus* strains, *S. equi* lacks the ability to ferment ribose, sorbitol and lactose as a consequence of independent lesions in its genome (Holden *et al.*, 2009). This difference in metabolism is commonly used to differentiate cultures of *S. equi* from *S. zooepidemicus* (Bannister *et al.*, 1985). Utilization of different carbohydrates plays an important role in the ability of streptococci to colonize mucosal surfaces (Shelburne *et al.*, 2008), and reduction of its metabolic capabilities could further reduce the potential of *S. equi* to persist on the surface and in the crypts of the equine tonsil.

S. equi does not possess a clustered regularly interspaced short palindromic repeat (CRISPR) locus, which confers resistance to phage attack and invading DNA (Barrangou *et al.*, 2007; Holden *et al.*, 2009; Sorek *et al.*, 2008). Interestingly, the SeP9 bacteriophage, which is lysogenic in *S. equi*, does not replicate in *S. zooepidemicus* (Tiwari &

Timoney, 2009). *S. equi* also lacks several putative competence loci, whose presence may be incompatible with lysogeny (Beres *et al.*, 2008; Holden *et al.*, 2009). Consequently, *S. equi* is polylysogenic and temperate phage is easily demonstrated (Spanier & Timoney, 1977). The strain 4047 genome contains four different prophages, which may each contribute to the adaptation of *S. equi* to its unique pathogenic niche (Holden *et al.*, 2009). Unlike the other three prophage, ϕ Seq1 contains no 'cargo' genes and was induced with mitomycin C. Interestingly, the sixth CRISPR spacer of *S. zooepidemicus* strain H70 exactly matches part of SEQ0163 of ϕ Seq1, suggesting that this strain of *S. zooepidemicus* has previously been attacked by a ϕ Seq1-like prophage (Holden *et al.*, 2009).

The cargo of ϕ Seq2 encodes a putative phospholipase A₂ with 98% predicted amino acid sequence identity with SlaA of *S. pyogenes* M3 strain MGAS315. The acquisition of *slaA* by *S. pyogenes* M3 was associated with increased morbidity and mortality in humans (Brüssow *et al.*, 2004). Deletion of *slaA* reduced the ability of *S. pyogenes* to colonize the upper respiratory tract of a macaque model of pharyngitis and reduced its virulence in a mouse intraperitoneal infection model (Sitkiewicz *et al.*, 2006). *slaA* was present in 31% of a diverse population of *S. zooepidemicus* strains, suggesting that this toxin may also be important to this opportunistic pathogen (Holden *et al.*, 2009). A second gene encoding a putative phospholipase A₂ toxin, SlaB, sharing 70% amino acid sequence identity with SlaA and associated with a phage remnant, suggesting horizontal acquisition, was identified in all strains of *S. equi* and *S. zooepidemicus* examined by sequencing or quantitative PCR (Holden *et al.*, 2009). The contribution of these toxins to both *S. equi* and *S. zooepidemicus* virulence is unknown (Fig. 1).

Invasion and abscessation of lymph nodes

Rather than colonizing the epithelial surface, *S. equi* quickly invades tonsillar tissue where it multiplies rapidly producing many extracellular microcolonies. It has been detected in the lymph nodes of the head and neck within as little as 3 h post-infection (Timoney & Kumar, 2008). An array of factors are produced that putatively enhance its ability to replicate *in vivo* and misdirect the equine immune response.

Acquisition of iron is an essential process for all pathogenic bacteria (Brown & Holden, 2002). In mammalian hosts tissue iron is sequestered by transferrin, lactoferrin, or inside red blood cells (Wooldridge & Williams, 1993). Bacterial pathogens access this limited iron supply through the production of cell surface receptors for transferrin and lactoferrin, utilizing haem-containing compounds, iron transporters, and the synthesis and secretion of iron-sequestering siderophores (Wandersman & Delepelaire, 2004).

A unique *S. equi* iron-capture mechanism that is absent from all *S. zooepidemicus* strains examined to date is encoded

Fig. 1. The prophage ϕ Seq2 contains the 'cargo' gene *slaA*, *slaB* is associated with the remnants of a hypothetical prophage gene. The genes *slaA* and *slaB* encode putative phospholipases A_2 that may play a role in *S. equi* attachment and invasion.

by a novel integrative conjugative element ICESe2 (Holden *et al.*, 2009). The cargo region of this ICE encodes proteins sharing similarity with the non-ribosomal peptide synthesis (NRPS) system of *Clostridium kluyveri* and *Yersinia* sp. that produce an unnamed siderophore (Seedorf *et al.*, 2008) and the ferric iron-binding siderophore yersiniabactin (Bobrov *et al.*, 2002), respectively. The *S. equi* NRPS system produces an as yet undefined secreted molecule, provisionally named equibactin, which enhances the ability of *S. equi* to acquire iron *in vitro* (Heather *et al.*, 2008) (Fig. 2). Siderophore biosynthesis has not previously been identified in any *Streptococcus* sp. (Eichenbaum *et al.*, 1996), but plays an important role in the virulence of many important pathogens (Brown & Holden, 2002), including *Yersinia pestis*, the causative agent of bubonic plague (Bearden *et al.*, 1997). Acquisition of ICESe2 may increase the ability of *S. equi* to acquire iron *in vivo*, and enhance its ability to proliferate in tonsil and lymph node tissue.

The *S. equi* and *S. zooepidemicus* genomes also encode an HtsABC haem-binding system (Nygaard *et al.*, 2006), a putative MtsABC Mn^{2+} and Fe^{3+} metal transport system with 80 to 91 % amino acid sequence identity to that of *S. pyogenes* (Janulczyk *et al.*, 1999) and a putative FtsABCD Fe^{3+} ferrichrome transport system with 59 to 77 % amino acid sequence identity to that of *S. pyogenes* (Hanks *et al.*, 2005). Although a recent report suggested that the HtsABC system is functional in *S. equi* (Nygaard *et al.*, 2006), the

Fig. 2. The ICESe2 locus may improve iron acquisition in *S. equi* through the production of a potential siderophore, equibactin. The genes *eqbH*, *eqbI* and *eqbJ* encode a putative ABC transporter.

gene in the *S. equi* strain 4047 genome encoding the first haem-binding protein of this system, Shr (Zhu *et al.*, 2008), contains a frameshift mutation at codon 442 that truncates this protein (Holden *et al.*, 2009) resulting in its secretion. Secreted Shr lacks haem- or fibronectin-, but retains haemoglobin- and haemoglobin-haptoglobin-binding capabilities (Meehan *et al.*, 2010). Truncation of Shr either has an as yet unknown functional benefit for *S. equi*, or reflects redundancy in iron-uptake mechanisms following gain of the equibactin NRPS system.

S. equi and *S. zooepidemicus* produce streptolysin S (SLS), which is responsible for the characteristic zone of β -haemolysis surrounding colonies on blood agar plates (Flanagan *et al.*, 1998). The toxin is almost identical to that produced by most strains of *S. pyogenes*, which has a direct cytopathic effect on many host cell types (Wannamaker, 1983). *S. pyogenes* also produces a streptolysin O (SLO), which is not made by *S. equi* or *S. zooepidemicus* (Flanagan *et al.*, 1998). Deletion of SLO or SLS alone or in combination has shown that each contributes to the early stages of *S. pyogenes* infection in a subcutaneous mouse infection model (Fontaine *et al.*, 2003). In agreement with these results, deletion of *sagA*, encoding SLS in *S. equi* strain 4047 abolished β -haemolytic activity *in vitro* and delayed the onset of clinical signs in a murine intranasal infection model by 24 h (C. Robinson, unpublished data). These data suggest that SLS plays a role in *S. equi* virulence, but indicate that other factors are also important to the ability of this organism to cause disease.

Two other prophage of *S. equi* strain 4047 encode four bacterial sAgs – SeeL and SeeM encoded on ϕ Seq3, and SeeH and SeeI encoded on ϕ Seq4, which share homology with the mitogenic toxins of *S. pyogenes* (Artiushin *et al.*, 2002; Paillot *et al.*, 2010b; Proft & Fraser, 2003; Proft *et al.*, 2003). sAgs are potent immuno-stimulatory molecules that disrupt innate and adaptive immune responses through non-specific T lymphocyte proliferation and the generation of an overzealous pro-inflammatory response (Llewelyn & Cohen, 2002; Sriskandan *et al.*, 2007). sAg activities are based on their ability to bypass the mechanism of MHC-restricted antigen presentation (Dellabona *et al.*, 1990). Conventional exogenous antigens are processed and presented by antigen presenting cells within the antigen groove of specific MHC class II molecules and recognized by antigen-specific TCRs (T-cell receptors), which results in highly specific T-cell activation (1 out of 1×10^6 T lymphocytes activated). Secreted sAgs bind as intact proteins directly to the MHC class II molecule outside the peptide-binding site and to one or more specific TCR V β chains leading to the activation of 5–20% of the T-cell population (Li *et al.*, 1999; Llewelyn & Cohen, 2002) (Fig. 3).

SeeI, SeeL and SeeM of *S. equi* have been shown to stimulate proliferation of equine peripheral blood mononucleated cells (PBMC) *in vitro* (Anzai *et al.*, 1999a; Artiushin *et al.*, 2002; Paillot *et al.*, 2010b) (Fig. 4). A febrile response in ponies was elicited by SeeI but not by SeeH. There is also some discrepancy in the level of mitogenic activity induced by SeeH *in vitro*, which was active on equine PBMCs in one study (Artiushin *et al.*, 2002), but active only on asinine PBMCs in another

(Paillot *et al.*, 2010b). Such differences might be explained by variation in the affinity of sAgs for different MHC class II and TCR molecules. HLA class II polymorphisms are known to influence the nature of T-cell responses to *S. pyogenes* sAgs (Llewelyn *et al.*, 2004) and the risk of severe streptococcal infection in humans (Kotb *et al.*, 2002). The recently sequenced equine genome will provide the opportunity to identify equine MHC class II molecules recognized by *S. equi* sAgs and quantify the risks associated with the production of particular alleles thereof (Wade *et al.*, 2009). Recent sequencing of *S. zooepidemicus* strain BHS5 identified three new sAg-encoding genes: *szeF*, *szeN* and *szeP*, the presence of which was significantly higher in isolates from non-strangles lymph node abscesses (Paillot *et al.*, 2010a), suggesting that sAgs play an important role in the ability of these isolates to cause disease in lymph nodes.

The truncated fibronectin-binding protein, FNE, of *S. equi* lacks its C-terminal cell wall sorting LPxTG motif resulting in its secretion (Lindmark *et al.*, 2001). However, despite this truncation secreted FNE maintained its fibronectin- and collagen-binding activity enabling the induction of cell-mediated collagen gel contraction by linking fibronectin to collagen type-I fibres and normalization of interstitial fluid pressure (Lidén *et al.*, 2008). Acute strangles is often accompanied by oedema on the afferent side of infected mandibular and supratharyngeal nodes, which may be an effect of contraction of collagen in the capsule of these nodes.

One consequence of the acute phase inappropriate immune response generated by *S. equi* is a rise in body temperature

Fig. 3. The prophages ϕ Seq3 and ϕ Seq4 contain the 'cargo' genes *seeL/seeM* and *seeH/seeI*, respectively. *S. equi* sAgs interact with the equine MHC class II molecules and TCR, bypassing the mechanism of MHC-restricted antigen presentation.

Fig. 4. *S. equi* sAgs have been shown to stimulate the proliferation of equine PBMCs and an overzealous cytokine production. The potential consequences are the impairment of T lymphocyte responses through anergy or deletion and misdirection of adaptive immune response to *S. equi*. Production of sAg-specific antibodies has been detected after *S. equi* infection. However, their neutralizing activity *in vitro* is variable.

(Artiushin *et al.*, 2002), which can be monitored and detected in the absence of bacterial shedding. Therefore, once a strangles outbreak is confirmed, presumptively infected pyrexemic horses may be identified and isolated before the organism is passed to in contact animals.

Resistance to phagocytosis

S. equi deploys an array of anti-phagocytic factors and mechanisms that frustrate and confound the innate immune response. In common with many other pathogenic bacteria, *S. equi* produces a hyaluronic acid capsule that mimics the molecule in vertebrate tissue and shields the bacterium from immune recognition (Woolcock, 1974). Although it appears that strains producing different levels of capsule *in vitro* retain the same ability to infect horses (Anzai *et al.*, 1999b) either because the multitude of anti-phagocytic mechanisms exploited by *S. equi* confer a level of redundancy or because other factors expressed by strains may compensate. The high level of capsule production in *S. equi* relative to many *S. zooepidemicus* strains (Fig. 5) may reflect differences in operon structure (Blank *et al.*, 2008; Holden *et al.*, 2009) that affect biosynthesis, or differences in hyaluronic acid turnover (Holden *et al.*, 2009). *S. equi* encodes and produces a non-secreted hyaluronate lyase on ϕ Seq4 with low activity (Lindsay *et al.*, 2009), but lacks a functional copy of the genome-encoded hyaluronate lyase (Holden *et al.*, 2009), whose orthologues have a broader substrate range and greater activity (Pritchard *et al.*, 1994). Reduced hyaluronic

acid and chondroitin turnover may explain why *S. equi* infection rarely spreads beyond the lymphatic system and why the majority of horses recover from strangles despite possessing extremely high bacterial loads during the acute phase of disease.

The anti-phagocytic surface protein SeM binds fibrinogen, and IgG4 and IgG7 subclasses (Lewis *et al.*, 2008; Meehan *et al.*, 2001). Fibrinogen binding masks C3b-binding sites on the bacterial surface reducing the rate of phagocytosis (Boschwitz & Timoney, 1994; Lewis *et al.*, 2008; Meehan *et al.*, 2001). The C-terminal two-thirds of SeM is predicted to have an alpha-helical coiled-coil structure and shares close sequence similarity with SzM of *S. zooepidemicus* (Kelly *et al.*, 2006) whereas, the N-terminal third is predicted to possess non-coiled-coil single strands and is unique to *S. equi* (Timoney *et al.*, 1997). SeM binds fibrinogen through residues located at the extreme N terminus of SeM, but requires the stabilizing coiled-coil structure (Meehan *et al.*, 2000). In contrast, the IgG-binding site of SeM is located in the central region (amino acid residues 273–357) between the A and B repeats (Meehan *et al.*, 2001). Studies utilizing recombinant SeM proteins containing defined internal deletions and protein chimeras consisting of the non-IgG-binding *S. pyogenes* M5 protein fused to SeM sequences identified a region of 14 amino acids (335–348) that was critical for IgG, but not fibrinogen binding (Meehan *et al.*, 2009). It was also noted that binding of IgG to SeM did not reduce its ability to bind fibrinogen.

Fig. 5. Hyaluronate lyase activity is predicted to be reduced in *S. equi* when compared with *S. zooepidemicus*. This provides an explanation for increased levels of hyaluronate capsule that may enhance resistance to phagocytosis and reduce adhesion to the mucosal surface. SeM: *S. equi* M protein; SzM: *S. zooepidemicus* M protein and homologue of SeM; CNE: collagen-binding accessory pilin subunit.

Se18.9 binds factor H and inhibits phagocytosis by reducing C3 deposition on the bacterial surface (Tiwari *et al.*, 2007). Se18.9 was produced by all 26 strains of *S. equi* examined, but by only 1 of 140 strains of *S. zooepidemicus* (Holden *et al.*, 2009), suggesting that this gene could have been important to the evolution of *S. equi*.

S. equi encodes two different IgG endopeptidases – IdeE and IdeE2 (Hulting *et al.*, 2009; Lannergård & Guss, 2006) – which share amino acid sequence identity with IdeZ and IdeZ2 of *S. zooepidemicus* and IdeS/Mac IgG endopeptidase of *S. pyogenes* (Lei *et al.*, 2001; Von Pawel-Rammingen *et al.*, 2002). These enzymes cleave IgG produced by a variety of animal species, thereby reducing IgG recognition and targeting of bacteria by the immune response. IdeE2 and IdeZ2 cleave equine IgG more efficiently than IdeE and IdeZ (Hulting *et al.*, 2009), but there remains some uncertainty as to whether the main antiphagocytic mechanism exploited by IdeE is reduction of opsonophagocytosis via IgG cleavage (Liu & Lei, 2010; Timoney *et al.*, 2008). Rather, its dose dependent inhibitory effect on the bactericidal activity of neutrophils for both *S. equi* and *S. zooepidemicus*, which is neutralized by a specific antibody response, suggests a different mechanism of reaction. Interestingly, the inclusion of recombinant IdeE and IdeE2 significantly enhanced the efficacy of a multi-component subunit vaccine against strangles, suggesting that antibodies that target these secreted proteins are important for the development of protective immunity against *S. equi* in the horse (Guss *et al.*, 2009).

Interference with neutrophil recruitment theoretically should enhance the ability of *S. equi* to evade immune

clearance. *S. equi* encodes a sortase-processed cell envelope proteinase (SeCEP) with 59% amino acid identity to SpyCEP of *S. pyogenes* (Turner *et al.*, 2009), which cleaves and inactivates interleukin 8 and other CXC chemokines (Edwards *et al.*, 2005; Turner *et al.*, 2009). Heterologous production of SpyCEP in *Lactococcus lactis* transformed this normally avirulent organism into one that caused rapid onset of systemic disease in a mouse infection model (Kurupati *et al.*, 2010). However, although dissemination of *S. pyogenes* to the lungs of infected mice was SpyCEP-dependent, its deletion did not affect persistence in the upper airways (Kurupati *et al.*, 2010). Immunization of mice with SpyCEP significantly reduced dissemination of both *S. pyogenes* and *S. equi* in an intramuscular mouse infection model (Turner *et al.*, 2009), suggesting that immunization with CEP could provide cross-protection against a range of streptococci that produce such proteins.

Resolution of lymph node abscessation and persistence

The inability of the innate immune response to kill *S. equi* results in the release of complement-derived chemotactic factors and a massive influx of neutrophils into tonsillar tissues and draining lymph nodes to which *S. equi* has metastasized. As *S. equi* numbers increase and abscesses form, so the lymph nodes swell and eventually (approximately 4 to 21 days post-infection) rupture either externally through the horse's skin or, in the case of the retropharyngeal lymph nodes, internally into the guttural pouch. This air-filled sack is an enlargement of the eustachian tube and drains into the nasal cavity. Drainage of abscess material

into the nasal cavity from the guttural pouch contributes to the mucopurulent nasal discharges commonly observed during strangles. In most horses clearance of infection accompanies drainage. However, in up to 10% of cases complete drainage does not occur. Residual pus becomes inspissated to form chondroids, which may contain live *S. equi* that remain in the guttural pouch for several years (Newton *et al.*, 1997; Verheyen *et al.*, 2000). These persistent infections in 'carrier' horses are likely to have been critical to the global success of *S. equi* because intermittent shedding from the guttural pouches of otherwise normal horses contributes to the interepizootic maintenance of the disease. However, with the exception of SeM (Chanter *et al.*, 2000), little is known about the selective pressures exerted on the surface proteins of *S. equi* as it persists for long periods in the face of local acquired immune responses.

The SeM protein is of special interest because its N terminus may be truncated or mutated over time in isolates recovered from the guttural pouch (Chanter *et al.*, 2000; Kelly *et al.*, 2006) with 87 alleles currently listed on the online seM database [http://pubmlst.org/perl/mlstdbnet/agdbnet.pl?file=sz_seM.xml (accessed 28/03/11)]. Truncation leads to loss of fibrinogen binding, suggesting this function is not important in the guttural pouch. Changes in the N terminus of SeM may alter a conformational epitope of significance in mucosal IgA and T-cell responses, but does not affect the overall susceptibility of the organism to opsonic antibody (Timoney *et al.*, 2010). It is not known if SeM allelic variants of *S. equi* vary in virulence. It was thought that strains lacking the N terminus of SeM may be avirulent, and therefore be suitable candidates for the development of live attenuated vaccines. However, challenge of Welsh mountain ponies with a dose of 2×10^{10} c.f.u. of one such mutant led to the rapid onset of clinical signs (N. Chanter, unpublished data).

Vaccination

Although *S. equi* can be resolved into 87 different types based on the sequence of the 5' region of the SeM-encoding gene, isolates of *S. equi* are actually very closely related and can only be differentiated into two sequence types by multilocus sequence typing (Webb *et al.*, 2008). Vaccination of mice with recombinant SeM was protective (Meehan *et al.*, 1998), but these promising results were not repeated on vaccination and challenge of horses (Sheoran *et al.*, 2002). A live attenuated strain of *S. equi*, Equilis StrepE, containing an *aroA* gene deletion (Kelly *et al.*, 2006), protected 50% of horses from developing lymph node abscesses (Jacobs *et al.*, 2000) suggesting that vaccines that generate responses against multiple bacterial targets could be effective at preventing *S. equi* infection. Exploiting the emerging *S. equi* genome sequence data and a mouse infection model (Flock *et al.*, 2004, 2006) led to the identification of a combination of seven *S. equi* antigens that proved to be highly protective in Welsh mountain ponies (Guss *et al.*, 2009). These components share similar function or amino acid identity with their *S. pyogenes*

counterparts, and a similar combination of *S. pyogenes* antigens may prove efficacious in humans.

Conclusions

Long-term occupation of the guttural pouch environment may be a driving force behind several of the observed gene loss events from the *S. equi* genome. Analysis of strains recovered from this environment may lead to further improvements in the efficacy of emerging multicomponent subunit vaccines and shed light as to how and why *S. equi* has evolved to become a pathogen restricted to one host.

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