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Review article

The pathogenic equine streptococci

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Abstract – Streptococci pathogenic for the horse include *S. equi* (*S. equi* subsp. *equi*), *S. zooepidemicus* (*S. equi* subsp. *zooepidemicus*), *S. dysgalactiae* subsp. *equisimilis* and *S. pneumoniae* capsule Type III. *S. equi* is a clonal descendent or biovar of an ancestral *S. zooepidemicus* strain with which it shares greater than 98% DNA homology and therefore expresses many of the same proteins and virulence factors. Rapid progress has been made in identification of virulence factors and proteins uniquely expressed by *S. equi*. Most of these are expressed either on the bacterial surface or are secreted. Notable examples include the antiphagocytic SeM and the secreted pyrogenic superantigens SePE-I and H. The genomic DNA sequence of *S. equi* will greatly accelerate identification and characterization of additional virulence factors and vaccine targets. Although it is the most frequently isolated opportunist pyogen of the horse, *S. zooepidemicus* has been the subject of few contemporary research studies. Variation in the protectively immunogenic SzP proteins has, however, been well characterized. Given its opportunist behavior, studies are urgently needed on regulation of virulence factors such as capsule and proteases. Likewise, information is also very limited on virulence factors and associated gene regulation of *S. dysgalactiae* subspecies *equisimilis*. It has recently been shown that equine isolates of *Streptococcus pneumoniae* are clonal, a feature shared with *S. equi*. All equine isolates express capsule Type III, are genetically similar, and have deletions in the genes for autolysin and pneumolysin. In summary, the evolving picture of the interaction of the equine pathogenic streptococci and their host is that of multiple virulence factors active at different stages of pathogenesis. The inherent complexity of this interaction suggests that discovery of effective combinations of immunogens from potential targets identified in genomic sequence will be laborious.

streptococcus / equine / pathogenic

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1. INTRODUCTION

Streptococci pathogenic for equids include *S. equi* (*S. equi* subsp. *equi*), the agent of strangles, *S. zooepidemicus* (*S. equi* subsp. *zooepidemicus*), an important cause of respiratory disease and metritis, and *S. dysgalactiae* subsp. *equisimilis*, an infrequent cause of lymphadenitis and placentitis. These streptococci are beta hemolytic and belong to Lancefield group C. Alpha hemolytic streptococci have also been isolated from epizootics of abortion in Kentucky in 1981 and 2001 and *S. pneumoniae* capsule Type III has been associated with respiratory insufficiency in young horses in training.

2. *STREPTOCOCCUS EQUI*

S. equi causes strangles, a highly contagious infection of the upper respiratory tract and associated lymph nodes of solipeds. Isolates show remarkable antigenic conservation [24], and appear to constitute a clone or biovar of the closely related *S. zooepidemicus*, the DNA of which is almost identical to that of *S. equi*. This conclusion is supported by multilocus enzyme electrophoresis and 16S sRNA interspacer sequence studies [15, 30], which confirmed the genetic homogeneity of different isolates. Nevertheless, polymorphism in DNA restriction fragments suggests there is some limited genomic sequence variability [41]. Since small differences in nucleotide sequence may accumulate to the point of loss of or change in the protein encoded, continued survival of the clonal *S. equi* may depend on a specific phenotype which, when altered, results in disappearance of that variant. Recent sequence analysis has shown a much higher frequency of homologues of the IS 861 insertion sequence in *S. equi* than in *S. zooepidemicus* which suggests that replication of this insertion element may have played a role in the emergence of the clonal *S. equi* from a putative ancestral *S. zooepidemicus* (Timoney and Walker, unpublished data). For instance, insertional inactivation of a gene or genes required for DNA repair or recombination might have created a situation whereby the affected clone became "genetically stuck".

Virulence factors of *S. equi* include a non-antigenic hyaluronic acid capsule, hyaluronidase, streptolysin S, streptokinase, IgG Fc-receptor proteins, pyrogenic exotoxins including SePE-I and H, peptidoglycan, and the anti-phagocytic M protein (SeM). There is also evidence for the existence of a leukocidal toxin [39].

2.1. Virulence factors

The hyaluronic acid capsule is a high-molecular weight polymer consisting of alternating residues of N-acetylglucosamine and glucuronic acid. Virulent isolates of *S. equi* from cases of strangles are almost always highly encapsulated and produce very mucoid colonies, whereas non-encapsulated mutants are much less virulent [2, 22]. The anti-phagocytic capsule greatly reduces the numbers of streptococci that become associated with the surface of neutrophils and are subsequently ingested and killed. The capsule also increases the negative charge and hydrophilicity of the bacterial surface and produces a localized reducing environment that protects the activity of oxygen-labile proteases and toxins such as streptolysin S. Capsule is also required for the functionality of SeM and possibly other surface exposed hydrophobic proteins. In the absence of the hydrophilic capsule, these proteins

aggregate, losing the 3-dimensional conformation essential for functionality. Thus, non-encapsulated *S. equi* expressing normal amounts of the anti-phagocytic SeM are efficiently phagocytosed. Capsule synthesis is controlled by the *has* operon, composed of *has A* (hyaluronate sythase), *has B* (UDP-glucose dehydrogenase) and UDP-glucose pyrophosphorylase (has C). Deletions in either the *has A* or *B* genes result in loss of capsule synthesis and virulence [50].

Streptokinase released by *S. equi* interacts with the C-terminal serine protease domain of equine plasminogen to form active plasmin, which hydrolyses fibrin [34]. A role for plasmin in pathogenesis has not been proven but its lytic action on fibrin may aid in spread and dispersion of the bacteria in tissue. Other possible roles include in situ activation of complement and production of low-molecular weight nitrogenous substrates for bacterial growth. A receptor for plasmin also occurs on the surface of *S. equi*.

The oxygen stable 36AA oligopeptide, streptolysin S, is responsible for the beta hemolysis produced by *S. equi* [21]. Production of this bacteriocin-like cytotoxin is encoded by a 9-gene locus, and biologic activity requires stabilization by association with a carrier molecule such as double stranded RNA or albumin. Binding of the streptolysin S complex to erythrocytes results in formation of a transmembrane pore and irreversible osmotic lysis of the cell, a process similar to complement-mediated hemolysis [13]. Damage to keratinocytes has also been noted. *S. equi* and *S. zooepidemicus* do not have a gene for streptolysin O.

A proteinaceous cytotoxic activity unrelated to streptolysin S has been detected in culture supernatant of *S. equi* [5]. Equine PMN incubated in the presence of culture supernatant showed signs of toxicity and became chemotactically unresponsive [37]. The action of the toxin appears to be on mitochondrial membranes because suspensions of equine PMN exhibited intense respiratory activity shortly after exposure to culture supernatant, suggesting sudden release of respiratory enzymes. Nonhemolytic mutants of *S. equi* exhibited the same toxic effect, suggesting the existence of a toxin distinct from that of streptolysin S.

Peptidoglycan of *S. equi* is a potent activator of the alternative complement pathway, and chemotactic factors (C3a, C5a) released following incubation of peptidoglycan with plasma are strongly chemotactic for equine PMN [39]. This is the basic pathologic process in strangles – the outpouring of PMN in infected lymph nodes and onto the upper respiratory mucosa. Peptidoglycan is also a potent pyrogen by inducing release of pyrogenic cytokines such as interleukin-6 and tumor necrosis factor from leucocytes.

At least four pyrogenic mitogens, SePE-H, SePE-I, SePE-K and SePE-L, are expressed by *S. equi*. Genes for these pyrogenic exotoxins were originally acquired by phage mediated transfer, an event that may have been important in formation of the clonal more virulent *S. equi* from its putative *S. zooepidemicus* ancestor [1, 4]. Unlike conventional antigens, the pyrogenic mitogens have high immunomodulating capacity by binding simultaneously to the invariant region of Class II MHC molecules on antigen presenting cells and to the variable region of the β-chain of the T cell receptor. The result is non-specific T cell stimulation, proliferation, proinflammatory cytokine release and production of an acute phase response with high fever, neutrophilia and fibrinogenemia. These effects are characteristic of strangles and may be neutralized by antibody generated during convalescence or by active immunization with each mitogen [4].

The surface of *S. equi* carries numerous proteins anchored by their carboxy-termini (LPXTG motif), by their N termini (LYXC motif) or by other physico-chemical interactions. The functions and roles of most of these proteins are unknown. Based on

sequence homology, some are adhesins, others have enzymic or transporter functions and some such as SeM are antiphagocytic. Genes for immunogenic surface proteins show a marked tendency to be clustered in loci suggesting coregulation or enbloc acquisition. For instance, the gene for SzPSe, an M-like surface protein, is clustered with *Se73.9*, *Se51.9*, *Se46.8*, *Se44.2*, and *Se30.0*, genes for surface anchored proteins and a sortase.

Binding of host plasma proteins to the surface of the whole organism could be an effective mode of concealment from host cellular recognition mechanisms. The bound proteins might also block access of C3 or specific antibody to target sites on the organism. Moreover, IgG-binding proteins have been implicated in molecular mimicry and environmental sensing activities.

M proteins are antiphagocytic, acid-resistant, fibrillar molecules that project from the cell wall surface in an arrangement wherein two identical molecules are coiled around each other. A typical M protein molecule is about 50–60 nm long, with a long, coiled central region flanked by a short, random, coiled sequence at the N-terminus and by a specialized, highly conserved arrangement of hydrophobic and charged amino acids at the C-terminal cell wall anchor. The M protein (SeM) of *S. equi* has a molecular mass of about 58 kDa [23, 46]. The main fragments in acid extracts have molecular masses of 46 kDa, 41 kDa, and 29–30 kDa [11].

The antiphagocytic action is due to binding of fibrinogen to the N-terminal half of SeM and IgG to the central region [10, 35]. This interaction masks C3b-binding sites on the bacterial surface and inhibits the alternative C3 and classical C5 convertases [9]. Antibodies against specific linear epitopes override these effects and opsonize the streptococcus so that it is effectively phagocytosed. Unlike the M and M-like proteins of *S. pyogenes* or *S. zooepidemicus*, the M protein of *S. equi* is highly conserved and, with the exception of isolates from persistent guttural pouch carriers, shows no variation in size or antigenicity [24]. Some isolates from long term guttural pouch carriers have in-frame deletions representing about 20% of the SeM gene between the signal sequence and the central repeat region [17]. Isolates with truncated SeM proteins were more susceptible to phagocytosis, but their virulence in the horse has not been reported. The loss of SeM expression by *S. equi* results in loss of virulence but not infectivity for ponies [47], consistent with its role as an essential virulence factor.

2.2. Pathogenesis

S. equi enters via the mouth or nose and attaches to cells in the crypt of the tonsil and adjacent superficial lymphoid nodules (Fig. 1). The mechanism and ligands responsible for binding are unknown. Several fibronectin-binding proteins have been characterized on *S. pyogenes* that mediate attachment and invasion of host cells in concert with $\alpha\beta$ integrins. FNZ, a fibronectin binding protein produced by *S. zooepidemicus*, is also produced by *S. equi* but without a C-terminal anchor, so may not be functional [32]. After a few hours, the organism is difficult to detect on the mucosal surface because it is translocated below the mucosa into the local lymphatics where it may be found in one or more of the lymph nodes that drain the pharyngeal/tonsillar region. Complement-derived chemotactic factors generated after interaction of C1 with bacterial peptidoglycan attract large numbers of PMN [37]. The inability of PMN to phagocytose and kill the streptococci appears to be due to a combination of the hyaluronic acid capsule, antiphagocytic M protein, and a leukocidal toxin released by the organism. This culminates in accumulation of many extracellular streptococci in the form of long chains and large numbers of degenerating PMN. Final disposal of these organisms is dependent on lysis of the abscess capsule and evacuation of its contents.

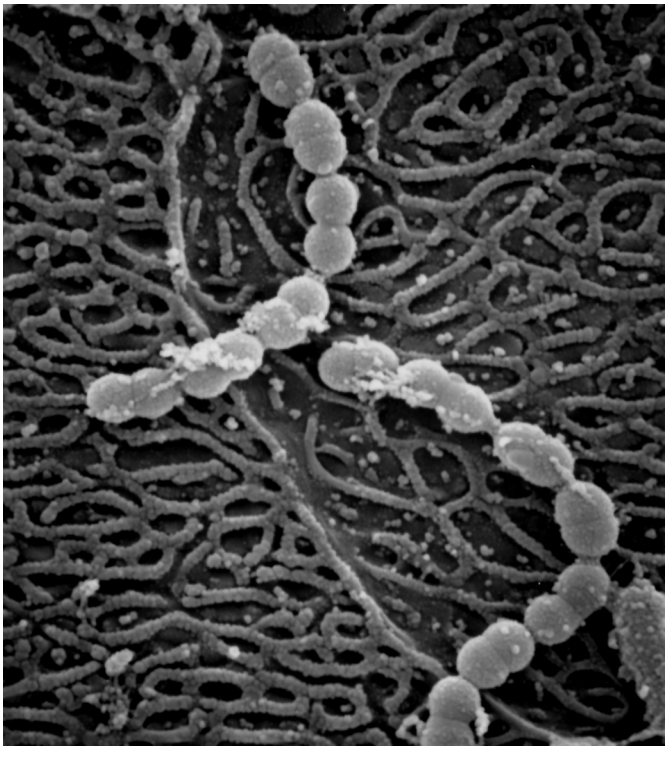

Figure 1. Scanning electron micrograph showing binding of *S. equi* to lingual tonsil epithelium (original magnification 10 000 ×). Courtesy Dr Pawan Kumar.

Streptolysin S and streptokinase may also contribute to abscess development and lysis by damaging cell membranes and activating the proteolytic properties of plasminogen. Although strangles predominantly involves the upper airways and associated lymph nodes, metastasis to other locations may occur. Spread may be hematogenous or via lymphatic channels, which results in abscesses in lymph nodes and other organs of the thorax and abdomen. This form of the disease is known as "bastard strangles". Metastasis to the brain has also been recorded. Evers [20] reported bacteremia in horses inoculated intranasally with *S. equi* and in noninoculated contact horses that became infected. Blood cultures were more likely to be positive on days 6 to 12 following inoculation. These interesting findings have not been confirmed but clearly show the potential for localization of *S. equi* in body sites other than the lymph nodes of the head and neck and for the formation of circulating immune complexes.

Nasal shedding of *S. equi* usually begins after a latent period of 4–14 days and ceases between 3–6 weeks after the acute phase. The guttural pouch is commonly infected during the early stages of strangles. A small percentage of animals may continue to harbor infection in the guttural pouch for months after clinical recovery and be a source of contagion for other susceptible horses [16, 38].

2.3. Immunity

Approximately 75% of horses develop a solid enduring immunity to strangles following recovery from the disease [26, 48]. Horses in the immediate post convalescent phase are resistant to experimental challenge with numbers of *S. equi* greatly exceeding those required to produce the original infection [22]. However, about 25% of these horses become susceptible to a second attack of the disease within months, which probably represents a failure to produce or maintain an adequate level of the appropriate mucosal and systemic antibodies. Strong serum IgGb responses to surface exposed proteins including SeM, Se44.2, Se46.8, Se45.5 and Se42.0, are produced during convalescence. Opsonophagocytic serum IgGb specific for the highly immunogenic SeM appears late in convalescence in some but not in all horses [43]. In addition, SeM specific IgGa is induced during and shortly after *S. equi* infection. There are strong SeM specific mucosal IgA and IgGb responses during the acute and convalescent phases but not following intramuscular vaccination [40].

Mucosal and systemic (serum) antibody responses are independent and local mucosal responses require local stimulation [22]. Anamnestic mucosal responses are elicited following reinfection and contribute to protection against a second or further occurrence of disease. The mode of action of locally produced SeM specific antibody is not known. Mucosal antibodies may prevent adhesion to receptors on tonsillar cells or prevent interiorization of the organism after it adheres.

Milk from mares that have recovered from strangles contains IgGb and IgA with specificities similar to those found in nasopharyngeal mucus of convalescent horses [25]. Suckling foals therefore benefit from the protective effects of this antibody until weaned. Colostral antibodies ingested during the first 24 h of life have also been shown to recirculate to the nasopharyngeal mucosa, thus providing an additional source of protection to the foal during its first weeks. Foals that suckle immune mares are usually resistant to *S. equi* infection until weaning.

The antiphagocytic SeM is widely believed to be a major protective antigen of *S. equi*. This heat- and acid-resistant protein antigen is protective for mice [45] and is an important component of commercial strangles vaccines. However, a SeM negative mutant of *S. equi* protected horses against experimental challenge following intranasal vaccination [47]. Thus, antigens other than SeM are involved in protective responses. Although the availability of genomic sequence has greatly facilitated identification of vaccine targets, determination of the optimum combination and presentation including route of delivery will be very laborious since it will involve systematic immunization and challenge of multiple groups of horses with pools of proteins.

2.4. Laboratory diagnosis

Culture of nasal swabs, nasal washes or pus from abscesses on Columbia CNA (colistin, nalidixic acid) agar with 5% sheep or horse blood added is essential for confirming the presence of *S. equi*. Where available, the polymerase chain reaction (PCR) based on primer sequences from *SeM* is about three times more sensitive than culture for detection of *S. equi* in clinical samples [42]. Nasal washes are more effective than swabs in detection of small numbers of organisms because a greater surface area within the internal nares is sampled. The technique involves instilling about 50 mL of warm normal saline via a 15 cm length of soft rubber tubing (5–6 mm diameter) about 12 cm into the internal nares and collecting the washings [25, 44]. These are centrifuged, and the pellet cultured. Culture may, however, be unsuccessful during the incubation and early clinical phases. *S. equi* is normally not present on the mucosa until 24 to 48 h after the onset of fever, and so horses monitored by rectal temperature during an outbreak may be recognized and isolated

before they engage in further transmission of *S. equi*.

Culture of nasal swabs or washes may fail to detect guttural pouch carriage in apparently normal horses following recovery from strangles. In Newmarket (UK), Newton et al. [38] have found that guttural pouch carriers may be undetectable for several months by culture of nasal swabs. Shedding of *S. equi* may resume later and continue sporadically. The only reliable mean of identifying these carriers is endoscopic examination to confirm empyema and/ or chondroids and to sample pouch content. However, this is not practical as a routine screening measure. The Newmarket group has shown that PCR combined with culture greatly increases the carrier detection rate, but that long-term guttural pouch carriers may remain PCR positive for months after viable organisms are cultured, suggesting that DNA persists for some time following death of *S. equi*. In contrast in convalescent horses, nasal swabs and washes become PCR negative shortly after viable organisms are no longer detectable [42]. This is explained by rapid mucociliary clearance from the nasopharynx. The value of PCR in rapid detection of *S. equi* in animals about to be exported or introduced to strangles-free premises is obvious.

Serology is not very useful in detection of infection with the possible exception of bastard strangles. A high level of *S. equi* antibody in combination with a history of loss of condition and intermittent fever and neutrophilia are helpful in diagnosis of this condition. Also, levels of SeM specific IgGa are usually high in convalescent sera but very low or absent in sera of vaccinated horses [40].

2.5. Control and prevention

The very contagious nature of strangles requires that measures to control spread be rigorous and uncompromising. Cases may appear following an incubation period as short as 4 to 5 days under conditions of climatic stress or overcrowding. However, the apparent incubation period in some horses may be as long as 12 to 14 days after exposure. These variations are accounted for by the numbers and virulence of *S. equi* to which the animals are exposed and their susceptibility. Thus the incubation period may become shorter as an outbreak develops and numbers of *S. equi* increase. Control measures to limit contagion and reduce disease severity and morbidity must include prompt identification and separation of infected animals. This principle, widely used in the military remount depots at the beginning of the 20th century, continues to be a most valuable aid in minimizing the effects of an epizootic [8, 48].

Carrier animals are very important in interepizootic maintenance of *S. equi* and in initiating new outbreaks [48]. Horses that develop persistent guttural pouch infections may shed intermittently for many months and introduce *S. equi* to premises previously free of the disease [38]. However, most new epizootics are initiated by horses that are in the incubation or late convalescent phases of the disease. Experience in the United States suggests that persisting carrier animals are not of major significance in the epizootiology of strangles based on the disappearance of the disease from large breeding farms and geographic areas for long periods. This situation is possibly explained by the fact that many carrier animals are intermittent shedders of small numbers of *S. equi*. In addition, deletions in the N-terminus of SeM may render *S. equi* less infectious for a new host [17].

Preventative measures should therefore include segregation and daily observation of new arrivals for two weeks before they are mixed with the resident population, prompt isolation of affected animals, daily measurement of rectal temperatures of in-contact horses so that they may be segregated immediately if pyrexia is noticed, and culture or PCR of nasal swabs or washes to establish cessation of nasal shedding by

recovering animals. Prophylactic use of antibiotics such as penicillin G will not prevent onset of strangles following cessation of prophylaxis.

Most horses develop a solid immunity during recovery from strangles which persists for up to five years in about 75% of animals. Acquired resistance appears to be primarily humoral and mediated by antibodies to SeM and other as yet unidentified antigens unique to *S. equi*. SeM specific antibodies produced systemically and locally do not by themselves protect against challenge. Moreover, intranasal immunization with a SeM negative mutant of *S. equi* stimulates a protective immune response effective against intranasal challenge.

Active immunization of horses against strangles was first attempted nearly a century ago but results were generally disappointing. Bacterin type vaccines were introduced in Australia in the early 1940's [6] and subsequently in the USA in the 1960's. These vaccines were produced by moderate heat inactivation of logarithmic phase cultures which should have resulted in preservation of all protective antigens available on cultured organisms. Frequent adverse reactions, including inflammation and abscess formation at the injection site and muscle soreness, were the impetus for subsequent replacement of bacterins with extract vaccines that lacked irritant cell wall constituents such as peptidoglycan. Protein rich vaccines produced either by hot acid treatment or mutanolysin, a muramidase that releases proteins from the cell wall were marketed in the United States in the 1970's and 1980's. Extract vaccines were better tolerated than bacterins. Studies on their efficacy suggested a reduction in the clinical attack rate of about 50% in vaccinates [27]. They have been shown to stimulate strong serum opsonic activity but not mucosal SeM specific IgGb or IgA responses [40, 43]. Since both SeM specific serum and mucosal immunoglobulin responses are correlated with resistance to reinfection [26], the lack of a mucosal response to parenterally injected strangles vaccines may explain their low level of efficacy. Based on this hypothesis a live, attenuated, non-encapsulated mutant of *S. equi* (709-27) was produced which stimulated mucosal and systemic antibody responses similar to those produced during convalescence [44]. Ponies vaccinated with the non-encapsulated mutant 709-27 were resistant to intranasal challenge with *S. equi* and made strong systemic and mucosal antibody responses to SeM. This mutant became the progenitor of an intranasal strangles vaccine marketed in the USA since 1998. The vaccine is administered intranasally in two doses at an interval of one to two weeks. Since it is a living mutant of *S. equi*, its entry into tissue results in abscessation. Hence, inadvertent contamination of injection sites remote from the nose has resulted in formation of deep abscesses. Transient nasal discharge, submandibular or pharyngeal lymphadenopathy, limb edema and bastard strangles abscesses have also been reported following vaccine administration.

Rapid progress is being made in the identification of protein immunogens of *S. equi* with potential as components of new generation vaccines that will mimic the immunity generated following infection with virulent *S. equi* but free of the hazards of live attenuated vaccines or bacterial extracts.

3. *STREPTOCOCCUS ZOOEPIDEMICUS*

Although *S. zooepidemicus* (*S. equi* subsp. *zooepidemicus*) shares very high DNA homology with its clonal derivative *S. equi*, they differ greatly in their biology and pathogenicity. Unlike *S. equi*, *S. zooepidemicus* is a mucosal commensal that opportunistically produces disease in situations of virus infection, heat stress or tissue injury. As implied by the specific name "zooepidemicus" it is not host adapted but is the most frequently isolated pyogen from equine joints, lymph nodes, nasal cavities, and

lungs [28]. In situations of concurrent influenza virus infection, high summer temperature or transport stress, it can be a devastating and rapidly fatal pathogen in the respiratory tract. Although rare, *S. zooepidemicus* is a very serious zoonotic pathogen of humans infected by contact with horses.

Virulence factors. Many of the same virulence factors described for *S. equi* are produced. As expected from the greater than 98% DNA homology shared by these species, their protein profiles are almost identical including expression of immunoglobulin and fibronectin binding proteins [29, 31]. Notable differences are the lack of the antiphagocytic SeM and the pyrogenic exotoxins SePE-I and H as well as homologues of a small number of other surface exposed or secreted proteins. Capsule synthesis is highly variable in *S. zooepidemicus* and is usually quickly lost following primary culture. Therefore, synthesis is tightly regulated, unlike *S. equi* in which expression is constitutive.

Isolates of *S. zooepidemicus* from the tonsil and other mucosal sites of healthy animals are almost always non-encapsulated. Hyaluronidase is produced by some strains, but it is questionable whether it should be regarded as a virulence factor since its production should render the organism more sensitive to phagocytosis by removing its capsule.

The M-like SzP proteins of *S. zooepidemicus* of equine origin are the basis of the Moore and Bryans typing system [49] and vary at their N-termini and central regions. These proteins are mouse protective, opsonogenic, and are found on isolates from different animal hosts. Fibrinogen is bound but in lesser amount than by the SeM protein of *S. equi*. Variations in the SzP proteins have been useful in establishing the clonal character of equine lung infections and their origin in the animal's own tonsil [3]. As expected, the SzP homologue (SzPSe) in *S. equi* is invariant, consistent with the latter's clonal character [46].

The severe necrotizing lesions seen in transit pneumonia in adult horses are suggestive of protease release by the invading clone [39], but this has not been established experimentally. Since serum antibodies to the SzP proteins of *S. zooepidemicus* are opsonic [14] and mouse protective, it is likely they contribute to protection in the horse and other animals. The SzP specific IgA and IgG in the nasopharyngeal secretions of horses may have a role in controlling numbers of *S. zooepidemicus* in the tonsil. Immunization of mares with bacterial extracts provides some resistance to endometritis caused by *S. zooepidemicus* [52].

Given the varied antigenicity of *S. zooepidemicus* and the opportunist nature of the infections it causes, vaccines and vaccination regimens will be difficult to develop. An added concern is the risk of glomerulonephritis associated with complexes of streptococcal proteins such as SzP and specific antibody [19].

4. *STREPTOCOCCUS DYSGALACTIAE* SUBSPECIES *EQUISIMILIS*

Equine isolates of *S. dysgalactiae* subspecies *equisimilis* are beta hemolytic and have the Lancefield group C carbohydrate antigen in their cell-walls. Although of Lancefield group C, they are genetically distinct from *S. equi* and *S. zooepidemicus* with which they share low DNA homology. Their normal habitat appears to be the skin and mucosal surfaces. Most isolations are made from aborted placentas; less frequently from abscessed lymph nodes and, very rarely, from cases of a strangles-like disease [8]. Protein and restriction fragment profiling has indicated that equine strains are phenotypically and genetically heterogeneous. Only limited information is available on virulence factors. Numerous surface exposed and secreted proteins that bind to plasma or host tissue components have been described on isolates from non-equine

hosts. These include protein G, fnb A, fnb B, ą2 macroglobulin, plasminogen and fibrinogen respectively. Surface coating with plasma proteins including immunoglobulins in combination with M-like proteins may serve to reduce phagocytosis. Release of hyaluronidase and fibrinolysin may be of value in tissue penetration and dissemination [12].

The pathogenesis of *S. dysgalactiae* subspecies *equisimilis* infections in the horse is poorly understood in part because these infections are very infrequent and opportunistic. It is unclear for instance whether strains found in the horse are unique to this host.

5. *STREPTOCOCCUS PNEUMONIAE*

Although a common and serious pathogen of humans, the highly host-adapted *S. pneumoniae* is seldom isolated from clinical disease in animals. However, a unique clone of capsular serotype 3 is found in the respiratory tract of normal horses and has been associated with lower airway disease in combination with other bacteria and respiratory viruses [18, 33]. A case of pneumonia in a neonatal foal has also been reported [36]. Equine isolates of *S. pneumoniae* are remarkable because they exhibit deletions in the *lytA* and *ply* genes for the virulence factors autolysin and pneumolysin and are genetically almost identical to each other. Moreover, they are genetically distinct from isolates of *S. pneumoniae* serotype 3 from humans [51].

Experimental intratracheal inoculation of ponies is followed by fever, cough, ocular and nasal discharge and lesions of lobar pneumonia [7].

5.1. Virulence factors

The well studied virulence factors of *S. pneumoniae* of human origin as observed in mouse models include the capsular polysaccharide, pneumolysin, autolysin, neuraminidase, hyaluronidase, cell wall peptidoglycan, teichoic acid and phosphorylcholine [7]. A large number of surface anchored proteins are also expressed including zinc metalloproteases involved in IgA proteolysis and in processing and export of other proteins, peptide permeases AmiA and PlpA, neuraminidases NanA and NanB, glycolytic enzymes, fibronectin binding enolase, an array of 12 choline binding proteins including PspA, LytA, a protective antigen, an autolysin, and CppA, an adhesin. A notable feature of the cell wall surface is the presence of free choline and choline covalently linked to teichoic and lipoteichoic acids. Proteins with choline binding repeats attach to these cholines on the bacterial surface.

5.2. Pathogenesis

Adhesion of *S. pneumoniae* to epithelium of the tonsil and soft palate of ponies has been noted following experimental infection [7]. Invasion may trigger a number of host responses including the coagulation cascade with thrombus formation, the complement cascade with accumulation of leucocytes, and the chemokine/cytokine cascade that ultimately leads to increased vascular permeability and leucocyte recruitment. Resistance to phagocytosis is mediated by a complex polysaccharide capsule that forms a hydrophilic gel on the surface of the organism. This gel shields the bacterium from antibodies and complement proteins. In addition, capsular sialic acid contributes to the antiphagocytic effect by inhibiting complement amplification and alternative pathway activation. Intrinsic complement inactivation mechanisms, which degrade C3b bound to the bacterial surface and prevent further C3 deposition, are also facilitated by capsular sialic acid. Capsular material has, however, been noted in the alveolar macrophages of ponies experimentally infected with *S. pneumoniae*, indicating that successful phagocytosis does take place. It is unclear how this relates to the clinically mild self-limiting nature of

the naturally occurring respiratory disease of young horses. Alveolar necrosis has also been observed in experimentally produced lesions in ponies [7]. Toxin involvement in pneumococcal pneumonia in humans is suggested by the acute fulminating and toxic clinical character of the disease. Neuraminidase may act both to decrease the viscosity of mucus and to alter oligosaccharides of mucosal cells by removing N-acetyl neuraminic acid residues and thus expose receptors for bacterial attachment.

Increased numbers of *S. pneumoniae* are associated with the stress of race training and with lower respiratory tract inflammatory disease suggesting that the host/parasite interaction is opportunistic. Increased respiration during intense exercise may result in aspiration of *S. pneumoniae* from the tonsil and soft palate. At the same time, impairment of the mucociliary escalator mechanism and fluid accumulation may contribute to failure to clear aspirated organisms. Bacteria that proliferate in the highly cellular exudates will generate highly inflammatory streptococcal cell wall products.

The significance in lesion development of the large numbers of *S. zooepidemicus* often found with *S. pneumoniae* in tracheal aspirates is unknown. It is possible that IgA protease produced by *S. pneumoniae* may destroy protective antibodies that control proliferation of *S. zooepidemicus*.

5.3. Immunity

Much of the information on protective immunity to *S. pneumoniae* must be interpreted with caution since it is based on mouse models. Type-specific capsular antibody produced during convalescence is opsonizing and protective. However, capsular polysaccharaide is often poorly immunogenic. Protein antigens including PspA, pneumolysin, PsaA, autolysin, the neuraminidases, NanA and B, and at least six other surface proteins reactive with human convalescent serum and mouse protective [53] may have potential as vaccine components.

6. CONCLUSION

Interaction of the pathogenic streptococci with their equine host is highly complex and requires the participation of multiple surface and secreted virulence factors that facilitate attachment, penetration and evasion of host defenses. Identification and regulation of expression of the factors has been or will be greatly facilitated by the availability of genomic DNA sequence. However, determination of factors essential to virulence, their functionality and their roles in protective immunity will require extensive experimentations in the natural host. Moreover, it is probable that effective immunization will require use of a combination of immunogens presented in a manner that will stimulate appropriate protective mucosal and systemic responses in the horse.

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