[Frontiers in Bioscience 9, 1157-1188, May 1, 2004]

EXTRACELLULAR VIRULENCE FACTORS OF STREPTOCOCCI ASSOCIATED WITH ANIMAL DISEASES

Mariela Segura and Marcelo Gottschalk

Groupe de Recherche sur les Maladies Infectieuses du Porc (GREMIP) and Canadian Research Network on Bacterial Pathogens of Swine, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada

TABLE OF CONTENTS

1. ABSTRACT

A virulence factor denotes a bacterial product or strategy that contributes to virulence or pathogenicity. Streptococci produce a variety of protein toxins and enzymes that are capable of killing host cells and breaking down cell constituents, presumably to provide nutrients for the bacteria or to promote their spread. Some of these secreted products are hemolysins, streptokinases, hyaluronidases, exotoxins and proteases. In some cases, they play an important role in resistance to the host immune system, acting alone or in combination with cell-associated virulence factors (such as the capsule and surface proteins). Thus, the virulence of streptococci is considered as a multifactorial process. In contrast to well known human pathogens, and in spite of their veterinary importance, knowledge of virulence factors of most animal disease-associated streptococci is limited or almost inexistent. In the present article, the available information regarding the extracellular virulence factors of the most important animal disease-related streptococci is reviewed.

2. INTRODUCTION

The genus *Streptococcus* could be taxonomically divided into clusters or groups which included more than 30 species, as reviewed by Facklam (1). These are:

I. the pyogenic group, which includes several beta-hemolytic species, such as *S. agalactiae, S. canis*, *S. dysgalactiae, S. equi*, *S. iniae, S. porcinus*, and *S. pyogenes*.

II. the bovis group, which includes *S. bovis/S. equinus, S. gallolyticus* and the new described species: *S. infantarius, S. pasteurianus* and *S. lutetiensis*.

III. the salivarius group, which includes, *S. salivarius*, *S. thermophilus, S. vestibularius*, *S. infantarius, S. alactolyticus*, and *S. hyointestinalis*.

IV. the mutans group, which includes *S. mutans, S. cricetus, S. downei, S. sobrinus, S. ferus, S. macaccae, S. ratti,* and *S. hyovaginalis*.

V. the anginosus group (also referred to as the milleri group), which includes *S. anginosus, S. constellatus*, and *S. intermedius*.

VI. the mitis group, which includes *S. mitis*, *S. oralis*, *S. pneumoniae, S. cristatus, S. infantis*, *S. perois*, and *S. orisratti*.

VII. the sanguinus group, which includes *S. sanguinis* (formerly known as *S. sanguis*), *S. parasanguinis*, and *S. gordonii*.

The salivarius, mutans, anginosus, mitis, and sanguinus groups are all grouped in the major group of viridans *Streptococcus* species. Finally, the group of unusual *Streptococcus* species includes, among other species, *S. uberis* and *S. parauberis* (1). *S. suis*, an important swine pathogen, is not classified in any of the above mentioned groups or clusters. No single system of classification suffices for the differentiation of this heterogeneous group of organisms. Instead, classification depends on a combination of features including patterns of hemolysis observed on blood agar plates, antigenic composition, growth characteristics, biochemical reactions, and more recently, genetic analysis (1-3).

Several of these species are of exclusive importance in human medicine, whereas others are important agents of different animal diseases as well as zoonotic agents. The most important streptococci related to animal disease are *S. suis*, *S. porcinus*, *S. dysgalactiae, S. agalactiae, S. uberis*, *S. equi*, *S. pneumoniae*, *S. canis*, *S. iniae*, and *S. gallolyticus*. Most of these pathogenic streptococcal species possess a panel of virulence factors, including extracellular toxins or enzymes, surface associated proteins and adhesins and, in most cases, a capsule covering the bacterial surface. As for several other pathogens, virulence in streptococci could be considered as multifactorial. In this review, data about extracellular virulence factors of the most important streptococci associated with animal diseases will be summarized. The capsule, which is the most external layer of the bacterial surface, will also be considered under the definition of extracellular factors; as well as M- or M-like-proteins, that are fibrillar proteins largely exposed at the cell surface. In fact, M proteins are major virulence determinants and protective antigens of the human pathogen S. pyogenes. Besides several well known functions of M proteins (reviewed in reference (4)), wall-anchored M protein has recently been implicated in the maturation of a cysteine proteinase, which in turn releases biologically active fragments of M protein (5, 6). In this regard, it could be considered an extracellular factor. Interestingly, several streptococcal species of Lancefield groups A, C and G streptococci express M-like proteins on their cell surface. Finally, even rare among Gram-positive species, surface fimbriae when present, will also be reviewed. The most common extracellular virulence factors present in streptococci associated with animal diseases are summarized in Table 1.

3. STREPTOCOCCI OF PORCINE ORIGIN

In this section, the most important streptococcal species associated with diseases in pigs and of importance for the swine industry are described.

3.1. *Streptococcus suis*

S. suis is a worldwide cause of a variety of porcine infections. It is one of the most important agent of swine meningitis. In addition, it is a cause of meningo-encephalitis, septicemia, arthritis, endocarditis, pericarditis, polyserositis, rhinitis, and abortion (7). Although *S. suis* is commonly isolated from the respiratory tract of pigs with respiratory disease, its relationship to pneumonia is unclear because *S. suis* is usually isolated in combination with other recognized respiratory pathogens (8). Thus, in these cases, *S. suis* may act as an opportunistic pathogen or a secondary pulmonary invader. In addition, *S. suis* is considered as a zoonotic agent related to human cases of meningitis, endocarditis, septicemia and toxic-shock syndrome. *S. suis*

Table 1. Most common extracellular virulence factors of streptococci associated with animal diseases [1]

Virulence factor	*Streptococcus sp.*	Main function or suggested function	Comments
Capsule	*S. suis*	phagocytosis R	polysaccharide (with sialic acid)
	S. porcinus	unknown	unknown composition
	S. dysgalactiae[2]	unknown	unknown composition
	S. uberis	phagocytosis R (?); intracellular killing R (?)	hyaluronic acid
	S. agalactiae	opsonin-dependent intracellular killing R	polysaccharide (with sialic acid)
	S. equi[3]	phagocytosis R (?); intracellular killing R (?)	hyaluronic acid
	S. zooepidemicus[4]	phagocytosis R (?); intracellular killing R (?)	hyaluronic acid
	S. pneumoniae	unknown role in equine IAD	polysaccharide (type III)
	S. iniae	phagocytosis R (?); intracellular killing R (?)	polysaccharide
	S. gallolyticus	unknown	unknown composition
M-like proteins	*S. porcinus*	phagocytosis R (?)	named as APF, fibrillar
	S. equisimilis[5]	unknown	only in human isolates
	S. dysgalactiae	Fg/IgG-binding: phagocytic killing R (?)	named as Dem A, and Dem B
	S. uberis	adhesion to and invasion of epithelial cells	M protein type 24-like
	S. equi	Fg/IgG-binding: phagocytic killing R	named as FgBP/SeM, and SzPse
	S. zooepidemicus	Fg-binding: phagocytic killing R (?)	SzP (antigenic variation)
	S. canis	plasma protein-binding (?); phagocytic killing R (?)	M-like gene, fibrillar protein (?)
Hemolysin[6]	*S. suis*	cytotoxicity; inflammation; ↑ BBB permeability (?)	cholesterol-binding toxin family
	S. equisimilis	unknown (characterized only from human isolates)	cholesterol-binding toxin family
	S. agalactiae	unknown role in animal diseases	beta-hemolysin, cell-associated
	S. equi	unknown	Streptolysin S-like activity
	S. zooepidemicus	unknown	nature (?) - contradictory reports
	S. pneumoniae	pneumolysin is absent in equine IAD isolates	cholesterol-binding toxin family
	S. iniae	local tissue necrosis; ↑ BBB permeability (?)	Streptolysin S-like activity
	S. canis	unknown	SLO-like gene, protein unknown
CAMP-factor	*S. porcinus*	unknown	non-characterized
(or co-hemolysin)	*S. uberis*	unknown role in mastitis	lethal for mice/rabbits
	S. agalactiae	IgG/IgM-binding: impaired immune responses (?)	lethal for rabbits; pore-forming
	S. canis	unknown	protein of 18 kDa (*cfg* gene)
Streptokinases	*S. porcinus*	unknown	species-specific Pg activation
	S. equisimilis	matrix protein-degradation; ↑ invasive properties (?)	species-specific Pg activation
	S. dysgalactiae	matrix protein-degradation; ↑ invasive properties (?)	broad Pg activation
	S. uberis	↑ invasive properties (?); nutritional advantage	broad Pg activation
	S. equi	matrix protein-degradation; ↑ invasive properties (?)	species-specific Pg activation
	S. zooepidemicus	matrix protein-degradation; ↑ invasive properties (?)	species-specific Pg activation
Hyaluronidase	*S. equisimilis*	unknown	non-characterized
	S. dysgalactiae	unknown	protein of 55 kDa
	S. uberis	tissue invasion (?); affects epithelial cell functions	protein of 54 kDa
	S. agalactiae	tissue invasion (?)	no-random cleavage mechanism
	S. equi	tissue invasion (?); nutritional advantage (?)	protein of 55 kDa
	S. zooepidemicus	unknown	protein of 55 kDa
Pyrogenic	*S. equisimilis*	unknown (present only in human isolates)	SpeG-like gene, protein unknown
exotoxins	*S. dysgalactiae*	unknown role in mastitis	SDM (*sdm* gene), 25 kDa
(superantigens)	*S. agalactiae*	unknown (present only in human TSLS isolates)	two exotoxins described to date
	S. equi	strangles-associated inflammatory activities	SePE-I and SePE-H

[1] Extracellular virulence factors most commonly expressed among streptococcal species related to animal diseases. The capsule as well as the M-like proteins are included in the definition of "extracellular".
[2] *S. dysgalactiae* is used here as the abbreviated name for *S. dysgalactiae subsp. dysgalactiae.*
[3] *S. equi* is used here as the abbreviated name for *S. equi subsp. equi*.
[4] *S. zooepidemicus* is used here as the abbreviated name for *S. equi subsp. zooepidemicus*.
[5] *S. equisimilis* is used here as the abbreviated name for *S. dysgalactiae subsp. equisimilis*.
[6] In this part we included only streptococcal species for which a hemolysin has been purified or characterized.
Abbreviations used in this table. R = resistance. (?) = non clearly established in the literature (suggested role). IAD = inflammatory airway diseases. ↑ = increased. BBB = blood-brain-barrier. SLO = Streptolysin O (which is the prototype of the cholesterol-binding toxin family). Fg = fibrinogen. Pg = plasminogen. SpeG = streptococcal pyrogenic exotoxin G of *S. pyogenes* TSLS = toxic shock-like syndrome.

infection in humans is considered as an occupational disease with a probable underestimated importance (7). *S. suis* isolates have also been recovered from ruminants, horses, wild boar, cats, dogs, and birds. This suggests that *S. suis* may be pathogenic for more than one animal species (9-11).

S. suis possesses cell wall antigenic determinants related to Lancefield group D, although it is genetically unrelated to other members of this group. All strains are alpha-hemolytic on sheep blood agar, and many strains produce a beta-hemolysis on horse blood agar. *S. suis* can be classified into capsular types according to capsular polysaccharide antigens. The original classification of *S. suis* into Lancefield groups R, S, RS and T, which actually correspond to capsular types 2, 1, 1/2 and 15, respectively (12, 13), is obsolete and should be avoided, since it was realized that the polysaccharides involved in serotyping originated from the capsular material rather than from the cell wall (13). To date 35 capsular types have been described, and among them, capsular type 2 has always been considered as the most virulent and prevalent type isolated from diseased pigs in most countries where the swine industry is important (7).

Knowledge on virulence factors and the pathogenesis of *S. suis* infection is still limited. *S. suis* is often transmitted via the respiratory route and remains localized in the palatine tonsils. Some animals will become healthy carriers and will never develop disease, whereas others will, sooner or later, develop bacteremia, sometimes septicemia, and finally meningitis. Hence, in these cases, bacteria gain access to the bloodstream, where they persist until they reach the central nervous system (14). An early theory suggested uptake of bacteria by monocytes, intracellular survival and invasion of the central nervous system by the "Trojan horse theory" (15). However, most studies carried out during the last decade suggest that bacteria may also use (an) other mechanism(s) to disseminate. A review on the recent proposed theories of *S. suis* pathogenesis was published in 2000 (14). It has to be noted that most studies on *S. suis* virulence factors have been carried out with capsular type 2 strains. Although there is confusion in the definition of "virulence" for *S. suis* strains, researchers agree at least on one point: the existence of virulent and avirulent strains of *S. suis* type 2. To date, several proposed virulence factors have been described for *S. suis* type 2 strains. However, the mere presence of these virulence factors does not necessarily define a strain as virulent (14).

3.1.1. Polysaccharide capsule

Encapsulated bacteria are responsible for causing some of the most serious invasive infections, including septicemia, pneumonia, and meningitis. The role of the capsule in bacterial virulence is to protect bacteria from the host's inflammatory response, i.e. complement activation and phagocyte-mediated killing (16). *S. suis* possesses an integral, cell associated capsule (17). The capsular polysaccharide (CPS) of *S. suis* type 2 is composed of five different sugars: rhamnose, galactose, glucose, *N*-acetyl glucosamine, and *N*-acetyl neuraminic acid (sialic acid) (18, 19). The conformational structure of the capsule is largely unknown, although the sialic acid moiety has been suggested as being at a possible terminal sequence and probably responsible of antigenicity (19-21). Even though antibodies against the type 2 CPS have been shown to have opsonizing activity, the CPS itself is a poor immunogenic material (22). In addition, antibodies against the capsular material only partially protect against infection (21) and convalescent (protected) animals produce low levels of these antibodies (23).

Despite the fact that the Trojan horse theory was largely addressed in the literature, findings suggesting that monocytes can phagocyte encapsulated *S. suis* cells in the absence of specific antiserum appears to be controversial (15). Indeed, early studies suggested that pathogenic strains were able to resist phagocytosis in porcine or human blood in the absence of anti-*S. suis* specific antibodies. It was thought, therefore, that the capsule of the pathogenic strains was responsible for the resistance to phagocytosis, allowing pathogenic strains to become septicemic in pigs early in the infection, before an antibody response had been launched (24). These observations were recently confirmed with the production of isogenic acapsular mutants from virulent *S. suis* type 2 strains. The absence of CPS correlated with increased hydrophobicity and phagocytosis rates by both murine and porcine phagocytes. In addition, unencapsulated mutants were shown to be avirulent in murine and pig models of infection (25, 26).

Isolates of *S. suis* type 2 recovered from diseased animals were shown to possess a thicker capsule than those isolated from clinically healthy animals (27). An increase of capsular material thickness following *in vivo* growth was noted for virulent strains but not for avirulent ones (28). On the other hand, other reports do not demonstrate any correlation between the thickness of capsular material and virulence. Capsular type 2 is considered to be one of the most virulent serotype, but cells of the type reference strain were not covered by a thick layer of CPS, compared to other capsular types. It was suggested that the invasive ability of strains of this capsular type may be due to the composition of the capsular material which contains sialic acid (17). This latter component has already been related to virulence for other bacterial agents of meningitis (29). However, Charland *et al.* (30) further demonstrated that virulent and avirulent strains possess a capsule of similar size with similar concentrations of sialic acid. In fact, resistance to clearance from the bloodstream does not rely only on the presence of the CPS, since a well encapsulated avirulent strain is eliminated from the blood within 48 h, whereas a virulent strain can remain in relatively high numbers for more than five days (unpublished observations). In conclusion, despite the fact that the CPS seems to be a major virulence factor, most avirulent strains are encapsulated, indicating that other important virulence factors are essential.

Different strains within a single capsular type may vary in virulence and tropism, both within countries and among countries. There are also differences in pathogenicity among capsular types. Several capsular types are found mainly in healthy pigs, notably type 21 and to a lesser extent, types 17, 18, and 19 (31). Correlation between capsular antigens and virulence is the basis for the

suggestion that these antigens play a role in the pathogenesis of the disease (32). However, this suggested correlation is not widely accepted. Indeed, some strains belonging to less common capsular types have been associated with severe cases of infection (unpublished observations).

3.1.2. Muramidase-released protein and extracellular protein factor

In addition to the CPS, cell-wall and extracellular proteins have been associated with virulence of *S. suis*. Two proteins, a muramidase-released protein (MRP) and an extracellular protein factor (EF), originally associated with virulent strains, have been reported in type 2 strains (33). MRP is a 136-kDa cell wall-associated protein, which is also released during bacterial growth, whereas EF is a 110-kDa protein only detected in culture supernatants (33). Type 2 strains with the phenotype MRP+EF+ induced severe clinical signs of disease, whereas strains with the phenotype MRP-EF- did not (34, 35). Molecular weight variants of these two proteins were later described. Enlarged or reduced forms of MRP, respectively called MRP* (> 136-kDa) and MRP^s (< 136-kDa), and higher molecular weight EF proteins, called EF* (> 150-kDa) can be found in phenotypes as MRP*EF-, MRP^{s}EF-, MRP^{s}EF+, MRP-EF*, and MRP+EF* of uncertain or variable virulence (36-38). Strains of the last phenotype were isolated at high frequency from human patients, but caused almost no clinical signs of disease in experimentally infected pigs (36).

Although the genes encoding the EF and MRP proteins have been cloned and characterized, the amino acid sequences did not provide information with respect to the possible functions of these proteins (36, 39). One particular region within the MRP amino acid sequence, however, showed some similarity with the fibronectin (FN)-binding protein of *Staphylococcus aureus*, although binding of MRP to human FN could not be confirmed (39). To date, the function of these two proteins in the pathogenesis of the *S. suis* infection remains unclear.

Isogenic mutants lacking both these proteins appeared to be as virulent as the wild type strain after experimental infection of newborn germfree piglets (40). The authors suggested that the virulence of *S. suis* is a multifactorial process in which particular functions can be fulfilled by alternative factors. They also suggested that the synthesis of these proteins may only be coincidentally associated with virulence rather than being virulence factors *per se*. However, this association of MRP and EF with virulence is observed with strains from certain countries but not with others. For example, most North American strains isolated from acute cases of septicemia and/or meningitis (of either pig or human origin) were MRP and/or EF negative (38, 41). Interestingly, as demonstrated by randomly amplified polymorphic DNA analysis, the few MRP+EF+ North American strains were clustered in the same group as European strains which shared the same phenotype (41). Thus, the absence of one or more of these proteins cannot necessarily be associated with a lack of virulence. Again, since the term "virulence" is poorly defined for *S. suis*, it is also possible that, under standardised conditions, MRP+ EF+ strains are potentially more virulent than MRP- EF- strains.

Both MRP and EF proteins are major antigens recognized by convalescent sera of infected pigs (33). A subunit vaccine containing MRP and EF protected pigs against challenge with a homologous or a heterologous MRP+ EF+ *S. suis* type 2 strain (42). The possible cross-protection of this type of vaccine would be of interest, since in addition to capsular type 2, strains of different types (from 1 to 22) from several European countries have been reported to produce MRP and/or EF (37, 43). On the other hand, this kind of vaccine would not be useful in North America since, as previously mentioned, most virulent strains lack MRP and EF (38, 44).

3.1.3. Hemolysin

Hemolysin activity is associated with virulence and pathogenicity of several bacterial species. A 54-kDa hemolysin, also known as suilysin, was identified in *S. suis* type 2 (45); subsequently a 65-kDa hemolysin from the same type was described (46). These two proteins were shown to be the same toxin and the molecular mass variation was in fact related to purification methods (46). Suilysin belongs to the family of toxins known as thiol-activated toxins, or more recently named as antigenically related cholesterol-binding cytolytic toxins. The most widely known members of this family are streptolysin O, listeriolysin, perfringolysin, and pneumolysin (47). Suilysin possesses several characteristics in common with these toxins, such as loss of activity upon oxidation, reactivation upon reduction, inhibition by small amounts of cholesterol, formation of transmembrane pores and a "multi-hit" mechanism of action (46). Membrane cholesterol is thought to be the toxin-binding site on the surface of eukaryotic cells (47, 48). In addition, the N-terminal amino acid sequence of suilysin shows many similarities with the respective deduced sequences of the above mentioned toxins (45). Furthermore, the gene encoding suilysin (*sly*) has been sequenced, revealing a relative high similarity with the pneumolysin (49).

While several of these cytolysins have been shown to be determinants of bacterial pathogenicity, their biological roles may vary, as do the lifestyles of the bacteria secreting them (47). Despite the fact that a culture supernatant from a hemolysin-positive *S. suis* injected intraperitoneally failed to cause death in mice (50), a role of suilysin in virulence has been suggested since it has been shown to be cytotoxic to endothelial, epithelial and phagocytic cells (51-54). In addition, purified suilysin was shown to induce the release of several pro-inflammatory cytokines by human brain microvascular endothelial cells (55), by pig blood cells (unpublished observations), and by pig alveolar macrophages (56). It was also shown to induce the up-regulation of adhesion molecules on human monocytes (57). Thus, it may play an important role in bacterial dissemination and host inflammation, which is a hallmark of *S. suis* infections. Recently, a defined allelic-

replacement mutant of the *sly* gene was shown to be non-toxic for murine macrophages cells, and thus further proves that the suilysin is probably the only cytolysin produced by *S. suis* (58). Despite its suilysin gene deletion, this mutant was still virulent in an intravenous pig model of infection. Since, total lesion scores were higher in pigs infected with the wild type strains than those infected with the mutant, it was suggested by the authors that suilysin may have a role in increasing the severity of clinical signs, and allowing bacterial colonization of infected organs to reach higher levels (58). Recently, an independent group also reported the production of suilysin knockout mutants (56). *In vitro* bactericidal test showed that both wild type and suilysin mutants were resistant to bactericidal factors in whole pig blood. Furthermore, in a model of experimental pig infection, either high or low doses of the mutant strains applied by aerosol to the pharynx induced disease similarly to the wild type strains. All diseased pigs showed fever, clinical signs and developed septicemia. *S. suis* was isolated from tissue samples such as brain, submandibular lymph node, lung, spleen, liver, heart or joint (56). Thus, the results of this study confirm that suilysin is not a critical virulence factor for *S. suis* serotype 2 infections.

Antibodies against the suilysin could not be detected in pigs experimentally infected with a suilysin-positive strain of *S. suis* type 2 (46); however, a vaccine containing purified suilysin was highly immunogenic and induced an increase in hemolytic-neutralising antibodies. This vaccine protected mice against a lethal *S. suis* type 2 challenge and induced protection against clinical signs in pigs (45, 59). Since several strains of different capsular types (from 1 to 22) and field isolates from diseased pigs were shown to have hemolytic activity (45, 50, 60), suilysin was suggested as a promising cross-protection factor for use in vaccines (60). However, as observed for MRP and EF, most European strains are suilysin-positive, whereas variable production of this protein has been observed with North American strains (38, 61). Indeed, the presence of the *sly* gene was demonstrated only in 7% of North America strains (49). In a recent study in France, authors reported that most of the type 2 isolates recovered form diseased pigs carried MRP+EF-suilysin- phenotype. Thus, despite the fact that suilysin, EF and MRP are highly associated with pathogenicity, they should not be universally used to determine virulence of *S. suis*; and, as demonstrated by the use of knockout mutants, they cannot be considered as essential virulence factors (43, 56, 62, 63).

3.1.4. Hyaluronidase

Hyaluronan is the main polysaccharidic component of the host connective tissues, thus the enzyme hyaluronidase, also called the spreading factor, is considered an important pathogenic factor for several hyaluronidase-producing streptococci. Hyaluronidase activity was reported once in the literature for some *S. suis* isolates (64), but the presence of this hydrolytic activity could not be confirmed by using either the *S. suis* type 2 reference strain or a representative strain of the North American phenotype (personal communication, Dr. D. Grenier, GREB, Université de Laval, Québec, Canada). Thus, it is difficult to predict the possible prevalence of this enzyme among *S. suis* isolates.

3.1.5. Proteases

Proteolytic enzymes have been identified as important virulence factors in a number of microbial pathogens. Protease activity on casein has been detected in the culture supernatant of a *S. suis* strain isolated from a septicemia patient, however further studies on the identification of this activity were not reported (65). Recently, four major *S. suis* proteolytic activities (Arg-aminopeptidase, DPP IV, chymotrypsin-like and caseinase) have been reported and partially characterized (66). These activities were produced not only by some *S. suis* type 2 strains, but also by the reference strains of serotypes 1, 1/2 and 3. The Arg-aminopeptidase of *S. suis* was found to be both extracellular and cell-associated and showed a molecular mass of 55 kDa. It was suggested that the presence of this proteolytic activity would be useful in the production of ATP and other essential metabolic precursors. The DPP IV activity was found in the culture supernatant and on the cell surface of *S. suis*, having a molecular mass of 70 kDa. This activity is also present in mammals on activated T cells (CD26) and in a soluble form in plasma where it regulates bioactive peptides like cytokines (67). However, further studies are needed to evaluate the potential contribution of DPP IV in virulence of *S. suis*. The cell-associated caseinase activity, belonging to the class of metalloproteases, was detected in all tested strains of *S. suis* type 2. The protease responsible for this caseinase activity showed a molecular mass of 36 kDa and may have a nutritional function. In addition, it was suggested that it may participate in the maturation of bacterial protein precursors. However, further studies are needed to evaluate this hypothesis (66). The cell-associated chymotrypsin-like activity, belonging to the class of serine proteases, was detected in European ,but not in North American, strains of serotype 2 tested in this study. The authors suggested that these proteases may help *S. suis* to meet nutritional requirements, to neutralize the host defense system and to contribute to tissue invasion and destruction. Further analysis should be done to determine their physiological and pathological functions (66).

On the other hand, preliminary results indicate that *S. suis* type 2 does not produce IgG or IgA proteases, nor other hydrolytic activities, such as fibrinogen and fibronectin digestion, or phospholipase D activity. In contrast, the presence of phospholipase C activity was found (personal communication, Dr. D. Grenier, GREB, Université de Laval, Québec, Canada). Further studies should be done to determine the prevalence of this later activity, as well as its role in *S. suis* infection.

3.1.6. Superoxide dismutase

Superoxide dismutases convert superoxide anions to molecular oxygen and hydrogen peroxide, which, in turn, is metabolized by catalases and/or peroxidases. These enzymes constitute one of the major defense mechanisms of cells against oxidative stress and hence play a role in the pathogenesis of certain bacteria. The presence of a gene

encoding superoxide dismutase (SOD), as well as SOD activity was reported in *S. suis* type 2 strains (2, 68). Superoxide is a major antibacterial substance in macrophage phagolysosomes, and thus the capacity to secrete this type of bacterial enzyme may be important in virulence. However, no correlation was observed between specific SOD activity and virulence. It is unlikely, therefore, that the production of this enzyme by *S. suis* would be related to phagocytic killing resistance of virulent isolates (68). Another report indicated that *S. suis* requires manganese, but not iron, for *in vitro* growth, and that manganese availability during growth affects the activity of the superoxide dismutase enzyme (69). Further studies are needed concerning the specific role of this enzyme in *S. suis* virulence.

3.1.7. Fimbriae

Fimbriae are important in bacterial adhesion to host surfaces; however, very few Gram-positive bacterial species have been shown to carry fimbriae, compared with the large number of Gram-negative species known to possess them (70). Interestingly, ultrastructural studies of surface components of *S. suis* revealed the presence of peritrichous, thin, and flexible fimbriae (17). Morphologically similar fimbriae were observed on hemagglutinating as well as on non-hemagglutinating strains of *S. suis* (71). Thus the possible role of fimbriae in hemagglutination and/or cell adhesion remains unclear.

3.1.8. Bacteriocins

Bacteriocins are a heterogeneous group of antibacterial peptides characterized by their ability to inhibit other related or unrelated bacterial strains. Thus, they play a major role in the natural defense systems of several bacterial species (72). Since a number of bacterial species are found in the upper respiratory tract of pigs, the secretion of bacteriocins may be of competitive advantage for the producing strain. In fact, it was recently reported the production of bacteriocin-like substances by some isolates of *S. suis* type 2 (73). These substances, with molecular masses in the range of 14 to 30 kDa, were characterized as heat-stable and proteinase K, pronase, and elastase sensitive, but trypsin and chymotrypsin resistant. Maximum production was reported during the mid-log phase of bacterial growth and the bacteriocins were active against other *S. suis* isolates and other swine pathogens. Only 4 of 38 *S. suis* strains tested produced a bacteriocin, and three of them were originally isolated from healthy carrier pigs (73). However, the role of bacteriocins in *S. suis* infection is still unclear.

3.2. *Streptococcus porcinus*

S. porcinus strains belong to serological groups E, P, U, or V, and are widely distributed in animals. *S. porcinus* group E is mainly associated with disease in pigs, and it is the etiological agent of the streptococcal lymphadenitis of swine, an important disease also known as jowl abscess, cervical lymphadenitis, feeder boils, or swine strangles. SLS is a problem for the swine industry, because of the losses from trimming and condemnation of infected carcasses. *S. porcinus* strains do not appear to be the primary cause of any other important disease or condition (74, 75). Among strains of group E, several serotypes have been described (II, IV, V, VI, VII, VIII), with type IV most commonly isolated from swine lymphadenitis (74, 75). *S. porcinus* is rarely reported from human infections. In these rare cases, the majority of the isolates were recovered from the genitourinary tract of reproductive-age female patients, some being associated with complications of pregnancy and delivery problems (76). Whether these isolates are significant pathogens is unknown because very little clinical information is available to date.

S. porcinus enters the swine host through the mucosa of the pharyngeal or tonsillar surfaces, and are carried to the lymph nodes, primarily of the head and neck region, where abscesses are formed. There is little effect upon the overall state of health of the animal, but the finding of such abscesses at slaughter is a cause for condemnation of the head or, occasionally, the entire carcass. Cell wall antigens induce the formation of serum agglutinins; a microtitration agglutination test, based upon reactions involving the type IV antigen, is considered a reliable test for detection of infection in animals. Antigenic components include group and type antigens, extracellular enzymes, and an antiphagocytic factor (APF) which may be associated with virulence of the organism and with protective immunity (74).

The APF was described as a surface protein with morphological characteristics of fimbriae and considered to be analogous to the M-protein (also named for this reason as the group E streptococcal M protein). It develops on cells cultured *in vivo* or in media fortified with serum, and it was suggested to render the bacterial cells resistant to phagocytosis by porcine leukocytes (for a review see reference (74)). On the other hand, there are conflicting data regarding the formation of capsules. Freshly isolated organisms from cervical abscesses were reported to be encapsulated but capsule disappeared upon *in vitro* passages, although some nutritional conditions could induce its formation (74). The contribution of the capsule to phagocytosis resistant is so far unknown.

In general, group E streptococci are not considered to be strongly proteolytic (74) and this observation may be extended to *S. porcinus*. Although a streptokinase has been described with an apparent specificity for swine plasminogen, its role in *S. porcinus* virulence is so far unknown. Streptokinases of other streptococcal species have been shown to be important plasminogen activators, and thus contribute to tissue damage and bacterial spread across tissue barriers, as described below (77, 78). Only a low number of isolates show neuraminidase activity, but no hyaluronidase activity has been detected so far (64, 74). The presence of a gene encoding for a manganese-dependent SOD has been reported (2); however, no further information is available with respect to the possible role of this enzyme in *S. porcinus* virulence.

Concerning the presence of toxins, a CAMP-like reaction was reported among several isolates of *S. porcinus*. As discussed below; this reaction is mediated by an

extracellular protein, also named as co-hemolysin, in several streptococcal species. However, the putative toxin or co-hemolysin of *S. porcinus* involved in this reaction has not been characterized (64, 75). Although this bacterium shows beta-hemolysis on blood agar, to date, no hemolysin has been described.

3.3. *Streptococcus dysgalactiae* subsp. *equisimilis*

The species *S. dysgalactiae* was originally proposed to accommodate a heterogeneous group of streptococci associated with infections in animals and human beings. This taxon includes now animal isolates of alpha-hemolytic group C streptococci, previously called *S. dysgalactiae*; animal and human isolates of beta-hemolytic group C streptococci, previously called '*S. equisimilis*'; beta-hemolytic group L strains associated with infections in animals and, rarely, in humans; and beta-hemolytic group G strains isolated from humans. The exact composition of the taxon *S. dysgalactiae* has been in a state of flux for the past few years till the separation of this group of strains into two subspecies, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*. Strains of the first supspecies group belong to Lancefield group C and are not beta-hemolytic, while the second subspecies group includes strains of Lancefield group C, L and G, as well as recently reported group A strains, which are beta-hemolytic and commonly isolated from human infections. It is important to state that both subspecies possess virulence factors similar to those found in *S. pyogenes*, including M proteins (1).

S. dysgalactiae subsp. *equisimilis* (which will be referred throughout the text as *S. equisimilis*) is an important pathogen for horses and pigs. Beta-hemolytic *S. equisimilis* of group C and L are associated with endocarditis, arthritis and lymphadenitis in pigs. These isolates are reported as CAMP-negative, but hyluronidase positive (64, 79). Some animal isolates of *S. equisimilis* were shown to produce bacteriocin-like substances (80). This activity could be of selective advantage since this bacterium is present as part of the normal flora of pigs (64). The latter activity, however, has not been further characterized. Indeed, little information is available regarding the virulence factors of animal isolates of *S. equisimilis*. This is in contrast with the greater information about human isolates. In this regard, high hemolytic variants have been reported among human clinical isolates of *S. equisimilis*. These variants were associated to clinical symptoms of pharyngitis and to the development of serious streptococcal sequelae (81). The hemolysin, which can be neutralized by adding cholesterol, was purified and characterized as a streptolysin O secreted toxin (82). Despite the fact that *S. equisimilis* strains of animal origin are reported as beta-hemolytic, no hemolysin has been characterized to date. In addition, the presence of M-like proteins and a superantigen-like gene homologous to the gene *spe*G of *S. pyogenes* have been reported for human isolates of *S. equisimilis* (83, 84), although no further characterization of this exotoxin is available in the literature. The presence of exotoxins or M-like proteins in isolates of animal origin has not been reported so far.

Group C *S. equisimilis* isolates from non-human hosts have been regarded as non-streptokinase producers simply on the basis of the inability to activate human plasminogen. However, it was demonstrated that group C streptococci isolated from non human sources secrete streptokinases which preferentially activated plasminogen obtained from the host from which the isolate had been obtained (85). However, they all bound plasminogen regardless of the host source. These observations suggested that there are two major events in the activation of plasminogen by streptokinases; a primary event (binding) which is not species specific and a secondary event (activation) which is species-specific (86). It has been suggested that species-specific plasminogen activation may account for the species preference of certain streptococci (85). In this regard, *S. equisimilis* isolates from equine origin were shown to specifically activate equine plasminogen, while isolates from swine origin activate porcine plasminogen, with trace activity for human plasminogen. *S. equisimilis* not only produces a plasminogen activator but also binds the generated plasmin, thus providing the bacteria with unregulated proteolytic activity and enhanced invasive properties (85). The streptokinase genes from *S. equisimilis* strains of different origins have been cloned and characterized. The streptokinase secreted by equine isolates, which was purified as a protein of 49 kDa, has little sequence or amino-acid similarities to any known streptokinases secreted by either human or porcine isolates (86-88). The streptokinase secreted by porcine isolates has limited structural and functional similarities to streptokinases secreted by human isolates (86). The epidemiological associations between streptokinase activity and the species of mammal subject to infection have suggested a possible cause-and-effect relationship.

4. STREPTOCOCCI OF BOVINE ORIGIN AND ASSOCIATED WITH MASTITIS

Mastitis is an inflammatory disease of the mammary gland and is one of the most significant limiting factors to profitable dairying throughout the world. Several bacterial genera and species capable of causing mastitis are widespread in the environmental of dairy cows. Mastitis organisms have been categorized as contagious or environmental pathogens based on their distinct characteristicts of distribution and interaction with the teat and teat duct. Contagious pathogens, such as *S. agalactiae*, live and multiply on and in the cow's mammary gland and are spread from animal to animal primarily during milking. Environmental pathogens, such as *S. dysgalactiae* and *S. uberis*, are those whose primary reservoir is the environment where cows live (89, 90).

4.1 *Streptococcus dysgalactiae* subsp. *dysgalactiae*

S. dysgalactiae subsp. *dysgalactiae* (which will be referred throughout the text as *S. dysgalactiae*) is one of the most common pathogens of bovine mastitis and causes large economic losses in the dairy industry. It is capable of survival in the mouth, vagina, and skin of healthy animals as well as bedding and pastures. Because of its environmental location, normal hygiene methods and antibiotic therapy are less effective in preventing

S. dysgalactiae infections than infections with other contagious pathogens (89, 90). Isolation of this bacterium from human infections has not been documented (1). In spite of its high prevalence, little is known about factors that contribute to the virulence of *S. dysgalactiae*. The bacterium expresses several cell-associated and extracellular factors; yet, the relative importance of these factors in the transmission and pathogenesis of mastitis caused by *S. dysgalactiae* has not been defined. *S. dysgalactiae* can interact with several plasma and extracellular host-derived proteins such as immunoglobulin G, albumin, fibronectin, fibrinogen, collagen, vitronectin, plasminogen, and alpha 2-macroglobulin. These interactions are mediated by bacterial surface proteins. In addition, *S. dysgalactiae* adheres to and is internalized by bovine mammary epithelial cells *in vitro*. These aspects will not be discussed here (for a review see reference (90)). On the other hand, this organism also produces extracellular enzymes which may be involved in promoting dissemination of the organism into host tissue, as discussed below.

4.1.1. Capsule

Presence of capsule was detected in only some fresh isolates from intramammary infections; however, capsule expression was lost upon storage. Attempts to induce *in vitro* capsule expression in *S. dysgalactiae* strains using supplemented media were unsuccessful. As discussed below, expression of capsule in *S. uberis*, another important mastitis pathogen, has been associated with resistance to phagocytosis by bovine mammary gland macrophages. In this regard, a hydrophilic strain of *S. dysgalactiae* was significantly more resistant to phagocytosis by bovine mammary gland macrophages than a hydrophobic strain. The contribution of the capsule to these two phenotypes was not specified (91). Thus, further studies are needed to determine conditions for capsule expression by *S. dysgalactiae*, as well as its role in virulence (90).

4.1.2. M-like proteins

The M proteins have for a long time been considered one of the major virulence factors of *S. pyogenes* by mediating resistance to phagocytosis. Although not a prerequisite for inhibition of phagocytosis, fibrinogen (Fg) and IgG binding is a common feature among these proteins (4, 92). Fg binding is common among mastitis isolates of *S. dysgalactiae* (93, 94). Indeed, a gene called *demA*, encoding for a protein (DemA) with a molecular mass of ~58 kDa, was characterized. DemA displays both plasma protein binding properties and sequence similarities with the M and M-like proteins of other streptococcal species. In competitive binding assays, purified recombinant DemA protein was found to completely inhibit Fg-binding to *S. dysgalactiae* (95). A gene similar to *demA* was isolated from another strain of *S. dysgalactiae* and named *demB*. Directly upstream of the respective *dem* genes, two other genes, called *dmgA* and *dmgB*, were described. The proteins encoded by these genes show similarities to Mga proteins in *S. pyogenes* which are known to be involved in the regulation of the expression of various virulence factors (92). Thus, it was suggested that the *dmg* and *dem* genes are members of a regulon similar to the described *mga* regulon in *S. pyogenes*. The presence of *demA* and *dmg*-like genes in several mastitis isolates of *S. dysgalactiae* suggests that the encoded proteins are of importance for the virulence of this species (95). Fg binding by *S. dysgalactiae* has been related to inhibitory effects of phagocytic killing by neutrophils in a similar manner as M protein carrying *S. pyogenes* isolates from human infections (96).

4.1.3. Fibrinolysin and streptokinase-like activity

S. dysgalactiae produces a fibrinolysin specific for bovine but not for human fibrin. Fibrinolysin activity was due to a proteolytic enzyme rather than to streptokinase-like activity (90, 97). On the other hand**,** the ability of this bacterium to activate mammalian plasminogen and to bind either plasmin or its zymogen was reported (98). Activation of bovine plasminogen was dependent on both the strain and the growth medium used for cultivation. Several strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from horse, rabbit and pig origins, but none activated human plasminogen. *S. dysgalactiae* is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin. It was suggested that the activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection (98).

4.1.4. Other putative virulence factors

Extracellular hyaluronidase has been isolated and purified from *S. dysgalactiae* as a protein of approximately 55 kDa. However, the importance of this protein has not been evaluated (99). Since hyaluronic acid is an important intercellular component, a possible role of bacterial hyaluronidases in tissue invasion has been proposed for other streptococci (see below). In addition, *S. dysgalactiae* has been shown to posses a gene that encodes a manganese-dependent SOD (2), as well as to produce a bacteriocin-like activity (80). No further characterization of these factors was reported and their putative role in virulence is so far unknown. As mentioned above, the production of a bacteriocin may give the producing strains a competitive advantage by killing bacteria in the same environment. Finally, a mitogenic substance (superantigen), designated *S. dysgalactiae*-derived mitogen (SDM), has recently been purified from the ATCC43078 strain. The *sdm* gene consists of two segments encoding a signal peptide and a mature 25 kDa protein. Three out of 34 animal isolates of *S. dysgalactiae* tested showed mitogenic activity and were positive for *sdm*. Further investigations are required to determine the pathogenic role of SDM in animal infections caused by this bacterial species (100).

4.2. *Streptococcus uberis*

S. uberis is the causative agent of a significant proportion of clinical episodes of bovine mastitis worldwide. In the United Kingdom, it may be responsible for as many as 33% of all clinical cases of bovine mastitis (101), and in Denmark, 23% of the mastitis cases in dairy herds could be related to an infection by *S. uberis* (102). Pathologic findings of experimentally induced *S. uberis*

infection in the mammary gland of cows revealed acute inflammatory response involving accumulation of large numbers of neutrophils in the secretory acini followed by infiltration of septa by lymphocytes, septal edema, extensive vacuolation of secretory cells, focal necrosis of alveoli, small outgrowths of the secretory and ductular epithelium, and widespread hypertrophy of the ductular epithelium. Streptococci are found phagocytosed inside macrophages in several types of affected tissues, highlighting the importance of the macrophage as the primary phagocytic cell. In fact, the value of the exuberant neutrophil response by the host in defense of the gland has been questioned (103).

It has been suggested that for intramammary infections to occur, bacteria need mechanisms associated with avoidance of phagocytic defenses, rapid growth, adherence to epithelial cells, and/or colonization of mammary tissue (104). During the last decade, several potential virulence factors of *S. uberis* have been identified and previously reviewed (101, 104). Some of these factors are cell-associated while others are extracellular. Proposed antiphagocytic factors of *S. uberis* include capsule, neutrophil toxin, M-like and R-like proteins. Potential virulence factors produced by *S. uberis* and released extracellularly include hyaluronic acid capsule, hyaluronidase, plasminogen activator or streptokinase, and Uberis (CAMP) factor.

4.2.1. Hyaluronic acid capsule

Phagocytosis and killing of bacteria by neutrophils constitute major defense mechanisms of the lactating mammary gland. This system is responsible for controlling other infections within the bovine udder, such as that produced by *Staphylococcus aureus*. The role of phagocytes is less clear with regard to *S. uberis* infections. However, strains of *S. uberis* that resist phagocytic killing *in vitro* can establish infection more effectively than susceptible strains (105, 106). The ability of certain strains of *S. uberis* to resist the bactericidal action of neutrophils has been reproduced *in vitro* when the organism is grown in the presence of casein-derived peptides (107). Indeed, the production of a hyaluronic acid containing capsule was correlated with the ability to resist *in vitro* phagocytic killing by neutrophils (107-109). Researchers recently identified the genes responsible for capsule formation in *S. uberis* which are placed in a novel genomic arrangement compared to that observed in *S. pyogenes* (110). The hyaluronic acid capsular operon in *S. pyogenes* includes three genes: hyaluronate synthase (*has*A); UDP-glucose dehydrogenase (*has*B); and UDP-glucose pyrophosphorylase (*has*C), with only *has*A and *has*B being essential for capsule production (111). However, the *S. pyogenes hasABC* capsular operon structure is not conserved in *S. uberis*, and two discrete loci comprising homologues of either *hasAB* or *hasC* were instead identified. Disruption of *S. uberis hasA* or *hasC* resulted in complete cessation of hyaluronic acid capsule production. Correspondingly, these mutants were found to have lost their resistance to *in vitro* killing by bovine neutrophils (110).

Most studies have been done with neutrophils; however, the importance of macrophages as the primary phagocytic cell has been highlighted (103). In this regard, studies of interaction of *S. uberis* with bovine mammary macrophages revealed that encapsulated strains are "to some extent" ingested and killed by macrophages; however, phagocytic and killing rates of non-encapsulated strains are considerably higher than those observed with encapsulated strains (112). Opsonization of encapsulated strains with homologous or heterologous antiserum increases the phagocytic and killing rates (112, 113). On the other hand, differences in interactions between *S. uberis* and either neutrophils or macrophages have been reported and showed that macrophages isolated from mammary secretions collected during the mid-dry period are capable of engulfing and killing strains known to be either resistant or susceptible to phagocytic killing by neutrophils, in the presence of serum and skimmed milk (114). Complement does not seem to influence the opsonization of *S. uberis* while both, IgG1 and IgG2 isotypes, were shown to opsonize both types of strains. However, bacteria were not killed in the absence of opsonin (114). Differences in expression and distribution of Fc receptors would be responsible for such distinct pattern of phagocytosis observed between the two cell types (114).

The precise mechanism by which the capsule layer confers *in vitro* resistance to phagocytosis and/or resistance to intracellular killing remains unclear. In fact, the use of the "bactericidal test or phagocytic killing assays" does not allow to differentiate between intracellular or extracellular bacteria (specially in the above mentioned studies with neutrophils), thus it is difficult to predict the role of the *S. uberis* capsule in resistance to phagocytic uptake or in resistance to intracellular killing (if the bacterium is phagocytosed). Indeed, the importance of the capsule as a virulence factor during *in vivo* infection has been recently questioned (115). In fact, both clinical and environmental isolates posses *hasABC* genes (115); and differences in the ability to cause clinical mastitis and in phagocytic killing resistance do exist among encapsulated strains, suggesting that other virulence factors are also essential (106). Furthermore, a capsular mutant strain induced inflammatory responses and milk bacterial shedding similar to those observed with the wild type strain in an experimental model of bovine mastitis. It was suggested that the acapsular mutant strain is able to withstand the bactericidal action of incoming neutrophils, since it persisted in the mammary gland despite the presence of a overwhelming neutrophil response (115). This is in marked contrast to the sensibility of this mutant strain to bovine neutrophil killing reported previously in bactericidal studies conducted *in vitro* (110). Since reversion of the mutant sensibility to neutrophil killing *in vitro* could be obtained by pre-growing of the mutant in raw skim milk, which more closely mimic the situation *in vivo*, it was suggested that the capsule may not be the sole effector of resistance to neutrophil killing. This also raises the possibility of the concomitant expression of additional cell surface-associated proteins that could influence the interaction of the bacterium with phagocytes (110). In this regard, it was suggested that the resistance of encapsulated

S. uberis to neutrophil killing was due to a protease-resistant molecule of 500-1000 Da that could inhibit or lyse neutrophils. Such a molecule, named the "neutrophil toxin", appeared to be produced by encapsulated strains and it would be retained in the capsular matrix (104). On the other hand, an "additional hypothesized antiphagocytic component" was recently suggested, since supernatants of bacterial cultures in milk were shown to prevent the bactericidal action of neutrophils (115). The nature and precise mode of action of these inhibitory activities (if they are different) are yet to be established.

4.2.2. M-like protein

Reports indicating the presence of a M-like protein in strains of *S. uberis*, screened by using antibodies against the type 24 M protein of *S. pyogenes*, have been previously reviewed (104). Since the M-like protein of *S. uberis* has been suggested to have a role in bacterial adherence to and invasion into bovine mammary epithelial cells, it could be an important factor in the pathogenesis of *S. uberis* infection.

4.2.3. Streptokinases

Discrepances in streptokinase gene and protein nomenclature in the literature are extremely confusing. A plasminogen activator (streptokinase) from *S. uberis*, with activity towards bovine, equine and ovine plasminogen, but not human or porcine ones was described and purified (116, 117). This activity was related to a protein of 29 kDa, which could also be found as a dimer of 57 kDa. This protein was later reported by the same group as having a molecular weight of 32 kDa and as being different to the above described streptokinase of *S. equisimilis* (118). The gene encoding the plasminogen activator was cloned and characterized by two independent groups (119, 120), and named as *pau*A or *skc*, respectively. The *pau*A nomenclature will be retained in this review. The *pau*A gene encodes for a protein of 33.4 kDa, consisting of 286 amino acids including a signal peptide of 25 amino acids, and it seems to be highly conserved within the species. The protein was named as PauA (but also found in the literature as SUPA or SKu), and shows only weak amino acid sequence homology to the streptokinases isolated from *S. equisimilis* and *S. pyogenes*; and no significant genomic homology to any member of the streptokinase family of plasminogen activators. PauA has been considered a new class of bacterial plasminogen activator that may act through a unique mechanism (119-122). In the course of cloning *pauA*, a single isolate (SK880) from a panel of 11 strains tested was reported to display plasminogen-dependent fibrinolysis due to a plasminogen activator of 45 kDa (119). This molecule failed to cross-react with antibody raised to PauA, suggesting that significant differences between the two *S. uberis* plasminogen activators exist. This second plasminogen activator was named PauB and shown to display an unexpectedly broad specificity profile for bovine, ovine, equine, caprine, porcine, rabbit, and human plasminogen. Clinical and nonclinical field isolates from United Kingdom or Danish herds were screened for the *pauB* gene and none were identified as carrying it. Therefore, PauB represents a novel but rare bacterial plasminogen activator which displays very broad specificity (123).

By activation of plasminogen to plasmin through the action of its plasminogen activator, *S. uberis* was also shown to be able to acquire surface-localized plasmin activity (124). For a mastitis-inducing pathogen, the production of a plasminogen activator could be of importance in two ways. In addition to the generation of plasmin activity needed for degradation of extracellular matrix proteins and subsequent colonization, the activation of endogenous plasminogen present in milk would lead to hydrolysis of milk proteins and, thereby, liberation of peptides from which a nutritionally fastidious microorganisms such as *S. uberis* could obtain essential amino acids and colonize environments such as the bovine mammary gland (125).

4.2.4. Hyaluronidase

An early study reported the production of free hyaluronidase by *S. uberis*. The purified hyaluronidase has a molecular weight of approximately 54 kDa (126). It was suggested that the presence of this enzyme could facilitate bacterial dissemination into the mucosal barrier and other tissues leading to spreading infection (104). As shown with hyaluronic acid capsule, *S. uberis* hyaluronidase also influence mammary epithelial cell proliferation *in vitro*, which could have important consequences *in vivo* during the periparturient period, when mammary tissue undergoes rapid differentiation and growth (127). In addition, hyaluronidase would be responsible of the release of soluble capsular hyaluronidate, which may affect phagocytic capabilities and block surface receptors of phagocytic cells (108).

4.2.5. CAMP-factor

The CAMP reaction is a synergistic lysis of erythrocytes by the interaction of an extracellular protein, named as co-hemolysin or CAMP factor, produced by some streptococcal species with the *Staphylococcus aureus* sphingomyelinase C (beta-toxin). It has been reported that some cultures of *S. uberis* present CAMP-like synergistic hemolytic activities on sheep blood agar (128). The co-hemolytic (CAMP) factor was partially characterized and named as Uberis-factor. It was shown to share similar properties with the *S. agalactiae* CAMP-factor. The Uberis-factor was initially reported as a protein of 42 kDa (129). However, the gene coding for the Uberis-factor (*cfu*) from *S. uberis* has now been cloned and the gene product has been shown to be a protein of 28 kDa. The deduced amino acid sequence is highly homologous to the corresponding *S. agalactiae* CAMP factor (130, 131). The Uberis-factor has been shown to exert lethal effects when administered parenterally to rabbits and white mice (132). Thus it may represent a significant component within the complex of pathogenic factors of this bacterium. However, the role of this activity in the pathogenesis of bovine mastitis has not been established; neither the production of Uberis-factor not the presence of the *cfu* gene has been correlated to virulence of individual strains (101).

4.2.6. Other putative virulence factors

Several reports showed adherence and/or invasion of *S. uberis* to bovine mammary epithelial cells in

culture. Proteins induced or having increased expression in both supernatant and surface-associated samples from *S. uberis* co-cultured with mammary epithelial cells have been suggested to be regulated during *in vivo* infection (133, 134). Some of these proteins were recognized by antibodies in serum obtained from a convalescent cow. In supernatant samples, two distinct protein bands at 35 and 36.8 kDa were identified. These two bands were absent when bacterial protein synthesis was inhibited by chloramphenicol. The identity and function of these secreted proteins are unknown (133). In addition, the possible mechanisms of adhesion, invasion and interaction of this bacterial species with host cells and extracellular matrix proteins have been previously reviewed (101, 104) and are out of the scope of the present review.

4.3. *Streptococcus agalactiae*

S. agalactiae or Group B *Streptococcus* (GBS) is a common cause of sepsis and meningitis in newborns, and causes disease in pregnant women and non-pregnant adults. The incidence of disease among non-pregnant adults, particularly those with underlying conditions, is increasing. In addition, many individuals are asymptomatically colonized with GBS. Epidemiological aspects of human infections, as well as phenotypic and genotypic characteristics were recently reviewed (135). In addition, an extensive review of the pathogenesis and virulence factors involved in humans infections was reported (136).

From a veterinary point of view, this bacterium is considered one of the major causes of bovine intramammary infections, particularly in North America, and a source of economic loss for the industry. Infection with *S. agalactiae* is associated with elevated somatic cell and total bacteria counts and a decrease in the quantity and quality of milk products produced (137). In addition, *S. agalactiae* could be responsible for meningitis in fish, and was also isolated from pathological processes in various other animal species, indicating that this bacterium has a broad host range (138-141). However, less information is available regarding the pathogenesis of animal infections caused by GBS, specially the virulence factors which may be involved. Interestingly, in a comparative study in experimental mouse models, it was shown that lethality and levels of colonization in spleens or livers were significantly greater for human isolates than for bovine isolates. However, no statistically significant differences in the ability to colonize placentas and in the induction of abortions were noted between isolates of either origin (142). On the other hand, human and bovine GBS isolates were characterized as two distinct populations (143), although isolates from other animal species behaved similarly to those from humans, regarding cultural, biochemical, serological and bacteriocin sensitivity properties (141).

4.3.1. Polysaccharide capsule

GBS strains associated with disease are almost invariably encapsulated (144). Antigenic variation in the capsular polysaccharide (CPS) is responsible for the serotype classification. Most GBS strains that cause human infection in the United States are encapsulated by one of five antigenically distinct polysaccharides (serotype Ia, Ib, II, III, or V). Whereas human isolates belong to a variety of serotypes, most bovine isolates belong to serotype III or are non-typable (143, 145).

The CPS is well established as a critical virulence factor which protects bacteria against the immune response of the host. However, studies on GBS phagocytosis are somehow controversial. Despite that the CPS was described as an antiphagocytic factor (29), it has been lately shown that well encapsulated GBS serotype III strains are easily ingested by murine macrophages under non-opsonic conditions (146). The CPS would rather protect bacteria against intracellular killing by modulation of opsonin deposition on the bacterial surface. With minor exceptions, the various GBS CPS antigens are composed of the same four component monosaccharides: glucose, galactose, *N*-acetylglucosamine and sialic acid. The biochemistry and immunology of GBS capsular polysaccharide has been studied most thoroughly in serotype III organisms, and this, in the context of human infections. The native type III CPS is a high-molecular-weight polymer composed of more than 100 repeating pentasaccharide units. Sialic acid is known to be a critical element in the epitope of type III GBS capsule which confers protective immunity (29, 136). In addition, direct evidence for the role of type III GBS capsule in virulence is provided by the construction of isogenic capsule-deficient mutants. These mutants were shown to be significantly less virulent in animal models of GBS infection (29, 147).

As it is the case with most other pathogenic bacteria, effective elimination of GBS by neutrophils and macrophages requires opsonization. Without the participation of specific antibodies and serum complement, phagocytic killing of GBS is dramatically reduced (136). The presence of naturally acquired antibodies against *S. agalactiae* was reported in normal bovine serum (NBS), while levels of antibodies in milk wheys were lower than in sera, and pre-colostral calf serum (PCS) was shown to lack antibodies to type II and III *S. agalactiae*. Antibodies present in NBS were required for the efficient ingestion of both human and bovine isolates of type II and III by bovine neutrophils. Nevertheless, greater than or equal to 35% of bacteria remained viable at the end of the phagocytosis incubation in 10% NBS. In contrast to human isolates, type II and III bovine isolates does not seem to require complement opsonization. These findings suggest that human isolates have higher opsonic requirements (148).

Phase variation in CPS expression has been clearly demonstrated for human isolates and was also reported for bovine isolates of type IV. Phase variation was suggested as playing an important role during colonization and adhesion to epithelial cells (nonencapsulated variants), and in phagocytosis resistance (encapsulated variants) (149).

4.3.2. Hemolysin

The beta-hemolysin of *S. agalactiae* is expressed by the vast majority of human strains and it appears to be a surface-associated molecule (150). Due to a rapid loss of

hemolytic function in bacterial extracts, the biochemical nature of the molecule has not been elucidated yet. Attempts to identify and characterize the hemolysin by generating antibodies have not been successful. A strong correlation exists between the amount of hemolytic activity and the production of an orange-red pigment, suggesting a very close genetic linkage between these two properties (151, 152). Indeed, it was recently reported that the *cyl* operon of GBS are involved in both the production of hemolysin and pigment (153). In contrast to human strains, bovine isolates are usually non-pigment producing and non-hemolytic, while isolates from other animals were shown to behave similarly to those from humans (143). Nevertheless, beta-hemolysin production in bovine GBS has been reported for some isolates (141, 154). In human infections, production of beta-hemolysin by GBS has been correlated with lung epithelial cell injury *in vitro*, suggesting a possible pathogenic role of this enzyme in the invasive step of early-onset GBS disease (136). The role of GBS hemolysin in animal infections, if produced, is so far unknown.

4.3.3. Pyrogenic exotoxins

Production of a novel pyrogenic toxin was reported in GBS strains from patients with streptococcal toxic shock-like syndrome (TSLS). The purified toxin presents a molecular weight of 12 kDa and was pyrogenic in rabbits, enhanced the susceptibility of the animals to lethal endotoxin shock, and caused the proliferation of rabbit splenocytes; these properties define pyrogenic toxins. When given to rabbits via a subcutaneous miniosmotic pump, the toxin caused TSS-like symptoms ending in death (155). The presence of a polysaccharide exotoxin, named CM101, was also reported from infants with sepsis, as well as from GBS culture media. CM101 induces a complement-activated cytokine-driven inflammatory response (156). No such a toxin was reported to date in animal isolates of GBS.

4.3.4. Hyaluronidase

It should be noted here that the extracellular GBS enzyme described in numerous reports as a neuraminidase is in fact a hyaluronidase, also named in the literature as hyaluronate lyase (157). The enzyme was purified and characterized as a 116 kDa secreted protein encoded by the gene *hyl*B (158, 159). Production of hyaluronidase is found at high frequency in bovine isolates (141, 160). *S. agalactiae* isolates from diseased pigs and an equine isolate of serotype Ia/cbeta were also shown to be hyaluronidase positive (64, 161). In contrast, isolates of GBS type III of canine and feline origin, and isolates of serotype III/Rib of equine origin were shown to be hyaluronidase negative. Canine, feline and equine origin GBS seem to be more related to human isolates, which were also reported as hyluronidase negative in the same study, indicating some epidemiological relationship (141, 161). This is in contrast to a previous study reporting production of hyaluronidase in 75% of human clinical isolates of GBS (160).

Little is known about the exact role of GBS hyaluronidase in the pathogenesis of the infections caused by GBS. Hyaluronidase was suggested as an important virulence factor that helps this pathogen to break through the biophysical barrier of the host tissues by the enzymatic degradation of hyaluronan and certain chondroitin sulfates at beta-1,4 glycosidic linkages. Characterization of the purified enzyme revealed that it degrades hyaluronan by a mechanism different from that of other previously studied hyaluronidases. Instead of randomly cleaving hyaluronan chains leading to a continuous decrease in average chain size, the GBS enzyme initially yields primarily unsaturated disaccharides. Such a function of the enzyme would destroy the normal connective tissue structure of the host and expose the tissue cells to various bacterial toxins (162-164). Strains producing high levels of hyaluronidase were frequently found to be more virulent. Although it was not possible to experimentally demonstrate a relationship between the increased virulence in mice and the enzyme production (165), it could be involved in bacterial dissemination as already discussed for the above mentioned mastitis-associated pathogens. In addition, it has been suggested that there is a strong possibility that the enzyme may also subvert some normal host defense mechanisms since hyaluronan and molecules that bind to it are involved in numerous immune system functions (159).

4.3.5. CAMP-factor

The CAMP factor (co-hemolysin) of GBS is a secreted 25.3-kDa protein encoded by the gene *cfb* (166), and is one of the most studied co-hemolysin. GBS from human and animal origins were reported to be CAMP-positive (143, 161). However, phenotypically CAMP-negative *S. agalactiae* isolates from cows with mastitis have also been reported; although some isolates did not carry the *cfb* gene, others were shown to harbour a normal sized CAMP-factor encoding *cfb* gene, indicating a reduced expression of CAMP-factor or a gene defect elsewhere along the pathway of expression (130, 166, 167). A close relationship was reported between the CAMP gene of *S. agalactiae* and those of *S. pyogenes*, *S. canis*, and *S. uberis* (130).

Besides its role in co-hemolysis, the CAMP protein binds in a nonimmune reaction with the Fc part of immunoglobulins G and M, leaving the Fab sites active (168). Although the role of CAMP factor in pathogenesis is unclear, several investigations suggest that the release of this protein during systemic infections could impair the host immune response. In addition, partially purified CAMP factor was found to be lethal for rabbits (132), and purified CAMP factor, although not lethal in mice, did increase the virulence of various GBS isolates (169). Recently, it was shown that *S. agalactiae* CAMP factor forms discrete transmembrane pores on susceptible membranes by an oligomeric mode of action; however the role of CAMP factor as a pore-forming toxin remains unclear (170).

4.3.6. Superoxide dismutase

S. agalactiae has been shown to possess a gene that encodes a manganese-dependent SOD (2, 171). *S. agalactiae* is a facultative anaerobe, which, like all streptococci, lacks catalase. The absence of this enzyme in

this bacterial genus suggests that SOD could play an important role against oxidative stress, affecting both the survival and, consequently, the virulence of the bacteria. The presence of SOD could be of pathogenic advantage for the bacterium, since it was shown that in the absence of opsonins, GBS is able to enter and survive inside phagocytes (172). The construction of a *sodA*-disrupted mutant by allelic exchange allowed for an examination of the role of this enzyme in the pathogenicity of GBS. In mouse bone marrow-derived macrophages, the *sodA* mutant showed an increased susceptibility to bacterial killing by macrophages. In a mouse infection model, the survival of the *sodA* mutant in the blood and the brain was markedly reduced in comparison to that of the parental strain. These results suggest that SodA plays a role in the pathogenesis of GBS infection by enabling bacteria to survive in the phagosome of macrophages or to the oxidative burst triggered by phagocytosis (173). In addition, the involvement of toxic oxygen intermediates in the bacteriostatic effect of milk has been reported, and thus the presence of SOD could be of adaptative advantage for GBS during infection of the mammary gland. However, this hypothesis remains to be confirmed.

4.3.7. Bacteriocins

Bacteriocin-like activity was reported in few GBS isolates of animal and human origins (80). No further information is available on the nature or the role of these inhibitory products in GBS colonization or survival as part of the normal flora in animals or humans.

5. STREPTOCOCCI OF EQUINE ORIGIN

5.1. *Streptococcus equi* subsp. *equi*

Streptococcus equi (Lancefield group C) comprises the two subspecies, *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus*. Subspecies *equi* is the causative agent of strangles, a worldwide-distributed and serious disease of the equine upper respiratory tract. Subspecies *zooepidemicus* is considered an opportunistic commensal, often present in the upper respiratory tract of healthy horses; however, after stress or a virus infection, it can cause a secondary infection, which results in strangles-like symptoms. Subspecies *equi* is virtually confined to horses, whereas subspecies *zooepidemicus* also infects a wide range of other animals, such as pigs, dogs, cats, sheep and cows. Human cases with infection due to subspecies *zooepidemicus* have also been reported (174, 175). Isolates of subspecies *equi* are serologically and genetically very homogeneous, whereas isolates of subspecies *zooepidemicus* display a high degree of heterogeneity. Subspecies *equi* is thought to be a clone derived from subspecies *zooepidemicus* (176, 177).

As mentioned above, *S. equi* subsp. *equi* (which will be referred through out the text as *S. equi*) is the aetiological agent of strangles, a highly contagious purulent disease of the upper respiratory tract of the family *Equidae* (178). The disease is initially characterized by nasal discharge and fever, followed by abscess formation in local lymph nodes. In fact, *S. equi* spreads rapidly to the lymph nodes of the head where bacterial multiplication proceeds unhindered by a massive infiltration by polymorphonuclear leukocytes. Early lymphadenitis progresses to abscessation and finally pus drain through the nearest site of egress, either through the skin or upper respiratory tract mucosa. This process, involving one or more lymph nodes, can last for 2-3 weeks and is usually associated with depression, loss of apetite, pyrexia, mucopurulent nasal discharge and inspiratory dyspnoea. Abscesses can form in other body organs and their rupture is fatal in up to 10% of cases (bastard strangles; (179)). Morbidity rates of up to 100% have been reported, and current antibiotic therapy is often ineffective and thus recent attention has focused on vaccine development (180). Harrington *et al.* (180) suggested that a systematic understanding of *S. equi* virulence, leading to the identification of targets to which protective immunity can be directed, is a prerequisite for the development of a successfully vaccine. In a recent publication (180), the authors extensively reviewed the knowledge on *S equi* virulence factors which will be summarized and updated below.

5.1.1. Hyaluronic acid capsule

S. equi is described as being highly encapsulated giving it a "honey dew" appearence. The *S. equi* hyaluronic acid capsule is constitutively expressed and is an important virulence factor which protects bacteria from phagocytic killing (180). Indeed, *in vivo* pathogenicity and resistance to intracellular killing of *S. equi* strains correlate with levels of capsule expression (181, 182). As mentioned aboved, capsule synthesis in the pyogenic streptococci is directed by genes of the hyaluronic acid synthesis (*has*) operon and has been extensively studied in *S. pyogenes* (111). Analysis of the *has* operon in *S. equi* indicated that *has*A, *has*B and *has*C have sizes and organisation similar to those of *S. pyogenes*. An interesting, and as yet unexplained finding was that of an additional copy of *has*C in another region of the *S. equi* genome. Since *has*C is not essential for capsule production in *S. pyogenes*, it is possible that the additional copies in *S. equi* are indicative of another role for the *has*C gene product (183).

The hyaluronic acid capsule is non-antigenic and so is not involved in protective immunity (184). However, a hyaluronate associated protein (HAP) is present in the capsule and confers partial protection in murine models of *S. equi* infection (185). Recently, a specific mutation of the hyaluronate synthase (*has*A) gene in Pinnacle (an avirulent strain widely used for vaccination in North America), permanently abolished the production of capsule and provided an easily recognisable genetic marker. This deletion mutant should serve as a useful candidate to replace Pinnacle since it cannot revert to a mucoid phenotype and can be genetically distinguished from wild type strains (183). The use of this mutant in phagocytosis and killing assays would further confirm the importance of the capsule in bacterial virulence, as suggested by previous studies using non-encapsulated variants or hyaluronidase-treated strains. Unfortunately, these assays have not yet been performed.

5.1.2. M-like proteins

It has been early shown that the adherence of *S. equi* to cheek and tongue cells isolated from adult ponies was trypsin-sensitive and could be inhibited by pre-incubation of cells with a purified M-like protein of *S. equi*

or by an antiserum raised to this M-like protein (186). It was later reported that *S. equi* possesses genes encoding two M-like proteins, one of which (FgBP or SeM) is unique to *S. equi* while the other (SzPSe) is a homologue of the M-like protein SzP produced by subspecies *zooepidemicus*. Both FgBP and SzPSe show strong binding to equine Fg (180, 187). Of these, FgBP is by far the most dominant wall-associated protein expressed by virulent *S. equi* (187, 188). FgBP is structurally and functionally similar to the well-studied M protein of *S. pyogenes*, with the ability to bind equine Fg, as well as the Fc fragment of equine IgG. Binding activity to human, rabbit, pig and cat IgG has also been reported (189). The efficiency with which FgBP binds Fg from different animal species decreases in the order with horse, mouse, pig, rat, sheep, dog, bovine, and human Fg (190). The protein reacts with convalescent horse serum and protects mice against lethal infection with virulent *S. equi* (188, 191, 192). The sequence of the corresponding gene (*fbp*) has been determined and shown to encode a protein of 534 amino acids (58.3 kDa), which possesses some structural and sequence similarities to other streptococcal cell wall proteins. However, the protein shows little significant sequence similarity to other M-like proteins, except for the Fg/IgG-binding DemA protein from *S. dysgalactiae*, where some similarities in the A-repeat and C-terminal regions have been noted (95). The acquisition of FgBP, in *S. equi* but not in subspecies *zooepidemicus*, has been postulated to be one of those key elements which suggests that *S. equi* is a clone derived from subspecies *zooepidemicus*. Furthermore, the absence of variation in the immunogenic M-like proteins FgBP and SzPSe of *S. equi* is striking and contrasts with the situation in *S. pyogenes* and *S. zooepidemicus* in which the M and M-like SzP proteins, respectively, are highly variable (177, 187).

Early studies showed that *S. equi* cells are resistant to non-immune phagocytosis, and there is some indirect evidence indicating that M-like proteins may be involved in this process (180, 182, 191, 192). Recently, an *fbp* knockout mutant which does not express FgBP on the cell surface has been constructed. The mutant does not bound equine Fg or IgG-Fc, it is rapidly killed in whole horse blood, and showed greatly decreased virulence in a mouse model. The use of this mutant convincingly demonstrated that FgBP plays a role in resistance to phagocytic killing and in virulence (189). Additional supporting evidence that FgBP is important in *S. equi* infection of the target species comes from a recent study of horses that were outwardly healthy but were shown to be persistent carriers of *S. equi*. Streptococcal isolates from about 25% of these carrier animals expressed truncated forms of FgBP (lacking the N-terminal Fg-binding region) and were more sensitive to phagocytosis (193). Full resistance to phagocytosis, however, is likely to be the sum of the activities of a number of *S. equi* products, including the capsule. Consequently, the reduced resistance to phagocytosis of the variant *S. equi* strains may only be partial and insufficient to compromise the ability to persist in the guttural pouches. The precise mechanism by which FgBP contributes to phagocytosis resistance remains to be elucidated. There is evidence that reduction in C3b deposition and Fg binding contribute to the ability of *S. equi* to resist killing by equine neutrophils (191, 192). These aspects of the antiphagocytic activity of M-like proteins have been previously reviewed (180). The ability of FgBP to bind IgG-Fc suggests another possible mechanism for resistance to phagocytosis (189). On the other hand, the contribution of the second M-like protein, SzPSe, to the virulence of *S. equi* is little known and seems to be less important to that of FgBP (187).

5.1.3. Secreted fibronectin-binding protein

S. equi has the potential to express several wall-associated proteins with host-protein binding properties, such as the FN-binding protein (FNE or SFS; (194, 195)), and the alpha$_2$-macroglobulin/albumin/IgG-binding protein (ZAG), which is also present in the subspecies *zooepidemicus* (176). Interestingly, the FNE protein was found to be secreted into the culture medium (195). Sequence comparison of the *fne* and *fnz* genes (coding for a FN-binding protein in subspecies *zooepidemicus*) revealed only minor differences (195). The reading frame of *fne* is interrupted due to a one-base deletion present in the middle of the gene and, as a consequence, only the 5'-terminal half of *fne* is translated in *S. equi*. Thus, FNE protein was found to have a signal peptide, but is lacking the COOH-terminally located sequence motifs involved in cell wall anchoring, which is logical for a secreted protein (195). Whether it is cell surface-attached protein that has been released during the cultivation or is actively secreted into the growth medium is not clear, but it could be a mechanism similar to that reported in *S. pyogenes* for the release of large biologically active fragments of cell surface proteins (6). It has been shown that FNE inhibits the binding between FN and collagen which could have as consequence the disturbance of physiologic processes like wound healing, where the interaction between collagen and FN constitutes an important step (194).

As already mentioned it has been proposed that *S. equi* is a clone derived from subspecies *zooepidemicus* and that certain evolutionary changes turned it from a commensal to a pathogen. The finding that *S. equi* isolates secrete an Fn-binding protein whereas the tested subspecies *zooepidemicus* isolates do not, also reflects a distinct difference between the two subspecies. Thus, there is a possibility that the deletion of one base in *fnz* has also contributed to change a commensal to a pathogen (195).

5.1.4. Hemolysin

On blood agar *S. equi* shows a zone of beta-hemolysis. This hemolytic activity has been characterized as being dependent on an equine serum supplement and the logarithmic phase of growth, after which activity declined sharply (196). Hemolysis was not affected by cholesterol, was only slightly increased in reducing conditions and was completely inactivated by trypan blue, identifying the hemolytic activity as streptolysin S-like (SLS-like). On the other hand, a lambda phage library of *S. equi* was shown to contain plaques whose hemolytic activity was enhanced by reducing conditions and inhibited by cholesterol, suggesting a streptolysin O-like (SLO-like) activity. However, it was later demonstrated by the authors that

isolates of *S. equi* in medium without SLS-like inducers show no SLO-like activity and no evidence for an SLO-like toxin could be found by immunoblotting with pneumolysin antiserum and monoclonal antibodies, or by polymerase chain reaction with primers derived from sequences conserved between the SLO genes of Lancefield group A, C and G streptococci. Thus, *S. equi* does not appear to possess a SLO but does make a SLS-like toxin whose production can be interrupted at just one genetic locus (196). The precise role of this toxin in the virulence of *S. equi* is so far unknown.

5.1.5. Pyrogenic exotoxins

The recent discovery of potent mitogenic and pyrogenic activity in culture supernatants of *S. equi* isolates (197) lead to the characterization of two pyrogenic mitogens, SePE-H and SePE-I, which have molecular masses of 27.5 and 29.5 kDa, respectively, and each is almost identical to its counterpart in *S. pyogenes* (198). The pyrogenic exotoxins of the later species, including SpeA, C, and F, are members of a family of proteins with high immunomodulating capacity and hence have been termed superantigens. Superantigens are crucial molecules in toxic shock syndrome (199). In this regard, many of the clinical features of acute strangles, including fever, neutrophilia and fibrinogenemia, are characteristic of an acute phase response associated with proinflammatory cytokine release induced by pyrogenic exotoxins. The genes coding for the two *S. equi* exotoxins were shown to be adjacent to a gene encoding a phage muramidase of 49.7 kDa and are located immediately downstream from a phage genomic sequence almost identical to a similar phage sequence in *S. pyogenes*. Strong mitogenic responses were elicited by both proteins from horse peripheral blood mononuclear cells. However, although both were pyrogenic for rabbits, only SePE-I was pyrogenic in ponies. Convalescent sera contained antibody to each mitogen and horses recovered from strangles or immunized with SePE-I were resistant to the pyrogenic effect of SePE-I. The immunogenicity of SePE-I suggests that it should be included in new generation strangles vaccines. *Sepe-I* and *sepe-H* were consistently present in isolates of *S. equi*, but they were absent from the closely related subspecies *zooepidemicus*, suggesting that phage mediated transfer was an important event in the formation of the clonal, more virulent, *S. equi* from its putative *S. zooepidemicus* ancestor (197, 198). In addition, the presence of mitogenic exotoxins in *S. equi* would explain the difference in clinical severity of strangles compared to the generally milder, less systemic disease caused by subspecies *zooepidemicus* infection of the upper respiratory tract (180). Recently, two novel streptococcal superantigen genes (*spe*L_{Se} and *spe*M_{Se}) were identified from the *S. equi* genome database. However, the novel superantigen genes could not be found in any of the analyzed *S. equi* isolates, suggesting that they are rare in this species (200).

5.1.6. Streptokinase

As mentioned above, the group C streptococci *S. equi*, isolated from non-human hosts, have been regarded during years as non-streptokinase producers simply on the basis of the inability to activate human plasminogen. However, it was demonstrated that *S. equi* isolates from equine origin secreted a streptokinase which specifically activates plasminogen obtained from equine plasma but not from human or porcine plasma. It was suggested that species-specific plasminogen activation may be an early step in events resulting in infection and may determine the selective virulence of this bacterium for certain host (85). As shown for *S. pyogenes*, as well as other group C streptococci, *S. equi* not only produces a plasminogen activator but also binds the generated plasmin, thus providing the bacteria with unregulated proteolytic activity (85). This process may be an important contributor to the tissue-invasive properties of this pathogen.

5.1.7. Hyaluronidase

Some isolates were shown to produce extracellular hyaluronidase activity which was associated with a protein of approximately 55 kDa (99). It was suggested that hyaluronidase activity may be required for penetration of mucous membranes and tissue spread but it is also likely to be important in the utilisation of host hyaluronic acid, an abundant carbon source and possibly in the recycling of released capsular hyaluronic acid (180). Conversely, hyaluronidase can remove hyaluronic acid capsule, and perhaps render encapsulated organisms more sensitive to phagocytosis (201). The hyaluronidase expression was related to a lysogenic bacteriophage in some isolates of *S. qui* (201).

5.1.8. Proteases

Clinical isolates of *S. equi* have been shown to produce extracellular enzymes capable of degrading number of protein substrates including Fg, gelatin, and casein. In fact, *S. equi* was shown to produce at least two easily detectable proteases, and the caseinolityc activity was associated with a 29 kDa putative cysteine protease that could be detected in both cell-associated and supernatant fractions. It was suggested that this activity could be involved in the cleavage of M-proteins (202).

5.1.9. Superoxide dismutase

S. equi has been shown to posses a gene that encodes a manganese-dependent SOD although its role in combating oxidative stress has not been investigated (2).

5.2. *Streptococcus equi* subsp. *zooepidemicus*

The very closely related organism *S. equi* subsp. *zooepidemicus* (referred here as *S. zooepidemicus*) is associated with several syndromes including endometritis in susceptible mares and it is one of the most frequent causes of infectious abortion and placentitis, pneumonia and shipping fever. It is also associated with lower airway inflammation in foals and Thoroughbred horses in training, and there is growing evidence of close involvement with repeated respiratory tract infections from life as a foal to horses of 3 to 4 years of age. It was also related to outbreaks of bovine mastitis (203). As it is the case of *S. equi*, despite the considerable animal suffering and economic burden associated with these diseases, there is little information regarding the molecular basis of virulence, and there are presently no effective vaccines against this organism (178).

5.2.1. Hyaluronic acid capsule

As described for *S. equi*, the capsule of *S. zooepidemicus* was suggested to be antiphagocytic as it inhibits the ingestion of bacteria by pulmonary phagocytes and leads to increased severity of infection. It was reported that isolates with high concentrations of cell-associated hyaluronic acid were more resistant to phagocytic killing (204). *In vivo* up-regulation of capsule expression has been suggested as an important factor in opportunistic invasion by *S. zooepidemicus*. In fact, colonies of *S. zooepidemicus* isolated from tonsillar specimens were uniformely nonmucoid, while the organism is encapsulated in smears of lungs and mucopurulent material from foals with pneumonia or bronchitis. In addition, *S. zooepidemicus* quickly becomes encapsulated and more virulent in the lungs of mice experimentally infected via aerosol exposure. It was concluded that capsule expression is important for survival and proliferation in tissues (204, 205).

It was reported that the hyaluronic acid capsular material not only helps bacteria to resist phagocytosis by macrophages, but also contributes to adherence properties of *S. zooepidemicus* (206). Indeed, whilst the direct role of the hyaluronic acid capsule in adherence of *S. equi* has received little attention, it is interesting to note that the hyaluronic acid capsule plays a significant role in the adherence of *S. zooepidemicus* to HeLa cells (180).

The hyluronic acid nature of the *S. zooepidemicus* capsule makes it non-immunogenic, and, as shown for *S. equi*, the hyaluronate-associated protein (HAP) is in fact responsible of confering partial protection in a murine model of *S. zooepidemicus* infection (185). To the best of our knowledge, isogenic non-encapsulated mutants of *S. zooepidemicus* have not been reported so far. The construction of this type of mutant will help to elucidate the role of the capsule in the pathogenesis of this bacterial infection.

5.2.2. M-like proteins

As mentioned above, *S. zooepidemicus* possesses a M-like protein, named SzP, responsible for great antigenic variation. Variations are found at its amino N-terminus, in a central hypervariable region, as well as in a proline-rich region close to the membrane anchor (207). This variation, together with sequence differences, is potentially useful in recognition of strains of *S. zooepidemicus* in clinical specimens and tissues of healthy horses. However, it was shown that SzP phenotype is not an important determinant of invasiveness or epizootic capabilities (204). On the other hand, opsonic and mouse-protective responses to SzP are, at least in part, not serovar (SzP phenotype) specific (207).

The M-like protein gene was cloned and it encodes for a protein with a molecular weight of 40 kDa which protects mice against *S. zooepidemicus* infection and stimulates the production of antibodies which opsonize *S. zooepidemicus* but not *S. equi*. The predicted amino acid structure shows significant homology with only the carboxy terminus of M proteins of streptococci of Group A and G. The M-like protein, although showing an extensive region of alpha helix, lacks the A, B, and C repeats found in M proteins of *S. pyogenes* and has a shorter signal sequence (208). The role in virulence of protein SzP has not yet been determined; however, this protein was shown to bind Fg, and thus may contribute to bacterial survival in tissues by blocking phagocytosis and/or phagocytic killing (187).

5.2.3. Other putative virulence factors

Streptokinase and hyaluronidase production have been reported in *S. zooepidemicus* isolates (85, 99, 209), as well as a gene that encodes a manganese-dependent SOD (2). Their role in virulence is poorly characterized. No hemolysin or cytotoxin has been fully characterized to date for this subspecies, except for a report of increased anti-streptolysin O titers in *S. zooepidemicus* infected horses (210), and a report of hemolytic activity produced by an animal isolate which was shown to be resistant to cholesterol treatment (81). Thus, these two reports are contradictory with regard to the possible nature of the *S. zooepidemicus* hemolysin.

5.2.4. Bacteriocins

S. zooepidemicus was shown to produce a lytic enzyme, zoocin A, which specifically target the cell walls of some closely related streptococcal species. The gene encoding zoocin A (*zooA*) was cloned and the sequence of *zooA* determined (211). The N-terminal region of zoocin A had a high degree of homology to the N-terminal region of lysostaphin, an endopeptidase produced by *Staphylococcus simulans* biovar *staphylolyticus* that specifically targets the cell walls of some staphylococcal species and to a number of other enzymes with peptidase activity (212). Flanking *zooA* in a back-to-back orientation was reported a gene named as zoocin A immunity factor (*zif*), responsible for protecting the producer strain from the otherwise lethal action of its own product (213). The functional role of enzymes such as lysostaphin and zoocin A has been widely debated. They are clearly related to cellular peptidoglycan hydrolases which are essential for various cellular functions, including cell wall expansion, cell wall turnover, and cell separation, but unlike these enzymes they do not remain cell associated. Their release into the extracellular medium, combined with their high target specificity, supports the concept that these enzymes are produced for the specific purpose of killing closely related bacterial species (214).

5.3. *Streptococcus pneumoniae*

Many of the capsular types of *S. pneumoniae* in man are important worldwide cause of pneumonia, meningitis and septicemia, as well as the non-invasive diseases otitis media, sinusitis and bronchitis in humans. Virulence factors and the pathogenesis of human disease caused by this microorganism have been previously reviewed (215, 216). *S. pneumoniae* was first isolated from horses during a study of the virological causes of respiratory diseases in Switzerland. Reports of sporadic isolation from various types and age of horses followed. Since then, the importance of this bacterium as a cause of

inflammatory airway diseases (IAD), a moderate to gross excess of mucopus in the trachea, complicated with cough and pyrexia, increased considerably. All equine isolates recovered to date have been reported as capsular type 3 (217, 218). Knowledge of virulence factors and pathogenesis of pneumococcal infection in horses is limited. Recently, molecular characterization of a group of *S. pneumoniae* isolates obtained from horses from geographically distinct locations showed that they represent a tight clonal group, virtually identical to each other but genetically distinguishable from more than 120 divergent isolates of human *S. pneumoniae*. A comprehensive analysis of known pneumococcal virulence determinants was undertaken in an attempt to understand the pathogenicity of equine pneumococci. Surprisingly, equine isolates appear to lack activities associated with both the hemolytic cytotoxin pneumolysin, often considered a major virulence factor of pneumococci, and the major autolysin gene *lytA*, also considered an important virulence factor. In support of phenotypic data, molecular studies demonstrated a deletion of parts of the coding sequences of both *lytA* and *ply* genes in equine pneumococci (219). Phylogenetic analysis clearly shows that equine pneumococci cluster within the normal human-infecting population and do not form a separate, deeply branching group. The apparent uniformity among isolates could suggest that the movement of pneumococci from humans to horses was a relatively recent event and that the isolates examined are part of a recent epidemic clone that has spread throughout Thoroughbreds (219). The implications of the alterations in genes believed to encode two of the major virulence factors of *S. pneumoniae* for that organism's pathogenicity in horses remain unclear. Although functional pneumolysin and autolysin are important in several murine models of infection and a functional pneumolysin is responsible for damage to the ciliated cells in the respiratory tract, there is evidence that the inactivation of pneumolysin in some human *S. pneumoniae* isolates has little impact upon their virulence in some models of infection. Although the extent of lung pathology seen in horses is generally less than that seen in lobar pneumonia in humans, the overall severity of pneumococcal disease seen in the two hosts may be similar.

6. STREPTOCOCCI FROM OTHER ANIMAL SPECIES

6.1. *Streptococcus iniae*

A large variety of Gram-positive cocci in chains has been isolated from aquatic animals, including frogs, crabs, prawn and shrimp, fish and mammals. In cultured fishes, streptococcal infections often develop into a lethal septicemia. Fish from both freshwater and marine environments may be affected, and farms in many parts of the world have consequently suffered serious economic losses. The taxonomic position of many aquatic isolates is still controversial but, as a whole, *Streptococcus* spp. has been recently listed among the emerging problems in aquaculture (220-222).

S. iniae, first isolated in 1976 from a subcutaneous abscess of a captive freshwater dolphin (223), causes meningoencephalitis and death in cultured fish species. *S. iniae* may colonize the surface of fish or cause invasive disease associated with 30 to 50% mortality in affected fish ponds. Infected tilapia become lethargic, swim erratically, have dorsal rigidity, and die within several days. Pathological studies show extensive infection in the central nervous system, with subarachnoid hemorrhages and parenchymal mononuclear infiltrates. These symptoms are not seen in the peracute form of the disease which is characterized by a sudden death and the diagnosis is possible only by recovering streptococci from infected organs, mainly the brain (138, 224). *S. iniae* has more recently been reported to cause fulminant soft tissue infection in humans, with associated bacteremia (225). However, results of a recent study do not support the contention that *S iniae* is a serious public health threat associated with commercially raised fish; rather, it represents a limited risk for older or immunocompromised people who incur puncture wounds while handling and preparing fish (226).

S. iniae has been well characterized biochemically (223). However, with regard to potential virulence factors, no clear phenotypic differences between disease-associated and commensal strains have been reported. Data on *S. iniae* infection in experimental animals are limited. Early studies reported that rabbits, guinea pigs, and mice were resistant to subcutaneous, intravenous, and intraperitoneal injection of 10^8 CFU of *S. iniae* type strain, ATCC 29178 (227). Conversely, meningo-encephalitis in cultured fish was reproduced in both trout and tilapia following fish-fish passage of a diseased-fish isolate (138). In this model, highly virulent strains did not differ from low virulent strains by any identifiable extrachromosomal elements (138). Experimental infection was later reproduced with isolates from diseased fish in different fish species (228-230).

Fuller *et al*. (231) recently reported a murine subcutaneous infection model for *S. iniae* local tissue necrosis and bacteremia. The ability to cause bacteremia in this model is associated with a genetic profile unique to strains responsible for disease in fish and humans (231). This suggests that specific, chromosomally encoded virulence determinants may account for the pathogenicity of the clonal (disease-associated) versus non-disease-associated (commensal) strains. Although a commensal strain does not induce invasive disease in mice, even at high inoculum, it does give rise to a localized necrotic lesion at the site of inoculation, as does a disease-associated strain (232). One of the few distinguishing phenotypes of *S. iniae* is the zone of beta-hemolysis surrounding colonies cultured on blood agar media. The *S. iniae* cytolysin that confers the hemolytic phenotype has recently been characterized (232). The cytolytic activity, examined by using a hemolysis assay, was found to be associated with whole-cell preparations but absent in culture supernatants. A chromosomal region with significant homology to the SLS biosynthetic operon *sag* in *S. pyogenes* was identified as being the genomic region responsible for cytolysin expression. The *sag*-like gene cluster in *S. iniae* was further characterized and comprises 9 genes named as *sag*A to

*sag*I. Confirmation that the *S. iniae* cytolysin is a functional homologue of SLS was achieved by PCR ligation mutagenesis, complementation of an SLS-negative *S. pyogenes* mutant, and use of the SLS inhibitor trypan blue. Transposon (Tn*917*) mutagenesis studies revealed that *S. iniae* SLS expression is required for local tissue necrosis but does not contribute to the establishment of bacteremia in the mouse model (232). Since the SLS is present in both, the commensal and the disease-associated strains tested so far (232), it could not explain by itself the differences in the capacity to induce invasive disease between these two groups of strains.

It was further reported that disease-associated strains are cytotoxic to human brain microvascular endothelial cell (BMEC) as measured by lactate dehydrogenase release from host cells. However, both disease-associated and commensal strains adhere to and invade cultured human epithelial HEp-2 cells and BMEC cells equally well. While cellular invasion may still contribute to the pathogenesis of invasive *S. iniae* disease, direct cytotoxicity appear to be a discriminating virulence attribute of the disease-associated clone (231). The authors suggested that damage to endothelial layers could aid bacterial access to the bloodstream and systemic spread, as observed in the murine model. Furthermore, the meningoencephalitis reported in fish disease may be attributed in part to the ability of *S. iniae* to promote injury and disruption of endothelial cells of the blood-brain barrier (231). It is not yet clear if SLS is related to cytotoxicity to BMEC cells or a second unknown toxin is in fact responsible of this discriminating phenotype.

One of the features that may allow *S. iniae* to stablish an invasive infection is related to the ability to induce high bacteremia and to overcome the immune response of phagocytic cells. In this regard, disease-associated strains have been found to be more resistant to phagocytic clearance in a human whole blood killing assay compared to commensal strains, which were almost entirely eradicated (231). However, this difference was not observed with trout whole blood (233). By using salmonid-specific cellular models, Zlotkin *et al.* (233) recently demonstrated that disease-associated strains are able to invade and persist within phagocytic cells and to induce their apoptosis. It was suggested that survival within phagocytes coupled to their apoptosis may play a crucial role in *S. iniae* infection. In addition, it may provide the pathogen an efficient mechanism of translocation into the central nervous system (233). The virulence factors, such as the capsule, involved in these processes remain to be characterized. Although *S. iniae* was originally described as an encapsulated organism (223), and mucoid colonies, characteristic of encapsulation, were reported in a fish infection model (138), the presence of a CPS has only recently been reported for the first time (234). In addition, a shift in "capsular composition" was suggested to be responsible of the emergence of a vaccine-resistant new serotype (235). However, the role of the CPS in the pathogenesis of *S. iniae* infection or in the above described interactions with phagocytes still remains unknown. In this regard, although M-like surface proteins, which render certain groups of streptococci resistant to phagocytosis, have not been identified in *S. iniae*, the existence of an uncharacterized surface component or even a secreted factor that might interfere with phagocytosis, as described for SpeB of *S. pyogenes* (236), cannot be excluded. The production of SLS by *S. iniae* does not seem to be involved in resistance to clearance in the human whole blood assay (232); and, although the presence of a gene encoding for a manganese-dependent SOD has been reported, the role of this enzyme in resistance to phagocytic killing is so far unknown (2).

Finally, it has been shown that some isolates of *S. iniae* increase necrosis of nonspecific cytotoxic cells (NCC), with consequent associated enhancement of inflammatory responses in tilapia fish. This would be a definite selection disadvantage for *S. iniae*. In contrast, other isolates negatively immunoregulated NCC activity, and in these cases infection and disease would likely occur (237, 238). To date the putative virulence factors implicated in immunoregulation of NCC cells are largely unknown.

6.2. *Streptococcus canis*

S. canis is a species originally proposed in 1986 (239) for streptococci isolated from dogs and cows possessing the Lancefield Group G antigen. It has since been isolated from a variety of other animals including cats, rats, mink, mice, rabbits, and foxes, and it causes opportunistic infections in these species. Human infections with *S. canis* are extremely rare (240, 241), although probably underestimated because the routine identification of beta-hemolytic streptococci is based only on Lancefield's serological grouping, which is often falsely considered to be species specific. The occurrence of such infections probably requires close contact between a domestic animal and a skin wound such as a burn or ulcers (242).

Although *S. canis* isolates may often represent commensal flora of the canine skin and mucosa, they have been involved in a variety of canine diseases associated with urinary tract infections, abortion, vaginitis, metritis, mastitis, polyarthritis, and skin infections. In addition, severe invasive *S. canis* infections in dogs, analogous to human streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (NF), have been reported, highlighting our lack of knowledge regarding the mechanisms of virulence of this microorganism (243, 244). Indeed, assessment of reports of virulence factors in *S. canis* is difficult since many authors refer to streptococcal isolates only as Lancefield serological group G, without clealry distinguishing by species, source or biotype between animal or human sources (243). An initial description of the first seven *S. canis* isolates from canine STSS and/or NF reported that they lacked genes homologous to *S. pyogenes* virulence determinants including the streptococcal pyrogenic exotoxins SpeA and SpeF, the streptococcal cysteine protease SpeB, the C5a peptidase, and two enzymes responsible for hyaluronic acid capsule synthesis, HasA and HasB (244). Other reports have also indicated

the absence of C5a peptidase activity and its encoding gene *scpA*, in group G strains isolated from dogs and cows (245), as well as the absence of streptococcal mitogenic factor (*mf*) gene in *S. canis* isolates (246). On the other hand, reports on the presence of M or M-like proteins in *S. canis* are contradictory. In fact, genes encoding M proteins, which are a major virulence factors in the human pathogen *S. pyogenes*, were reported in earlier studies to be present in human but not in animal group G streptococci (247, 248). However, in a more extensive study of a larger number of isolates of *S. canis* from cases of canine STSS and/or NF, the presence of genes encoding homologous to M protein as well as proteinaceous cell surface fibrillae consistent with M protein has been reported (243). The authors also confirmed lack of other recognized virulence genes with significant homology to those in *S. pyogenes* (including the superantigens SpeC and Ssa, and streptokinase A). However, these results do not rule out the possible presence of similar proteins with low nucleotide homology (243).

Thus, M-like proteins remain as putative virulence factors for *S. canis*. On the basis of the concept that the expression of M protein is the factor mainly responsible for conferring streptococci the ability to resist phagocytosis (92, 249), a correlation was established with the presence of M proteins and the ability of *S. canis* to resist phagocytic killing in a canine whole blood model, as deducted by increased bacterial multiplication (243). In addition, the expression of M proteins may also explain the ability of *S. canis* to bind several plasma proteins (250, 251).

It was also shown that *S. canis* hybridized with the SLO gene probe, suggesting that *S. canis* may encode for SLO (243). SLO is broad spectrum cytolysin responsible for the beta-hemolysis observed on blood agar in the absence of oxygen, is cytolytic to a range of nucleated cells and induces an inflammatory response (47). Thus, it is likely that this toxin is also a virulence factor in *S. canis* (243). Still, it is not known if it is functionally expressed in this bacterial species.

Another investigated phenotypic characteristic of *S. canis* is the CAMP-like reaction reported by independent studies (252, 253). While most canine isolates tested so far produce the CAMP factor, contradictory reports exist regarding this phenotypic characteristic in bovine isolates (239, 253, 254). It is of interest to note that false-positive CAMP-like reactions have been reported due to SLO in *S. pyogenes* (255). Nevertheless, the co-hemolysin or CAMP factor from the culture supernatant of a canine pathogenic *S. canis* strain has been purified and identified as a 18.6 kDa protein. The protein reacts with a homologous antiserum, appears to be trypsin-sensitive and relatively heat-stable (256). A close relationship was reported among the CAMP-factor gene (*cfg*) of *S. canis* and those of *S. pyogenes*, *S. uberis* and *S. agalactiae* (130). As described above, for *S. agalactiae*, the CAMP factor was proposed to play a role in virulence by binding IgG and IgM in a nonimmune fashion which could lead to an impaired host immune response (168). Although the *S. canis* CAMP-like factor did not show any non-specific IgG binding activities (256), its role in virulence remains to be investigated.

Almost all *S. canis* isolates tested to date produced proteases under different atmospheric conditions (252, 254). The identity of the proteases and their role in virulence, if any, has yet to be determined. Nevertheless, they seem to be distinct from the *S. pyogenes* cysteine protease and the C5a peptidase, as mentioned above. Finally, and diferent from what it was observed for human group G streptococci, the absence of hyaluronidase and fibrinolysin activities in *S. canis* has also been reported (254). The authors suggested that this may account for the increased virulence of human-origin isolates (254).

6.3. *Streptococcus bovis/equinus* and *Streptococcus gallolyticus*

The taxonomy of organisms designated *Streptococcus bovis* has a very complex history. *S. bovis* and *Streptococcus equinus* are listed as separate species in *Bergey's Manual of Systematic Bacteriology* (257) but were reported to be subjective synonyms (258). The conclusions of the latter study were primarily based on DNA-DNA hybridization values. The specific epithet *S. equinus* has priority over *S. bovis* but is rarely used in human clinical bacteriology. In veterinary medicine, both names are in use. Recently, the situation has become more complex by the description of two novel species for strains originally identified as *S. bovis: Streptococcus caprinus* (259) and *Streptococcus gallolyticus* (260). The epithet *gallolyticus* was derived from the ability of the strains to decarboxylate gallic acid. The presence of gallate decarboxylase, as well as tannase activities are characteristic of *S. gallolyticus* strains, confering them an adaptative advantage. The epithet *caprinus* referred to animals that forage on the same types of foods. However, later work revealed the synonymy of these two species, but the specific epithet *gallolyticus* has nomenclatural priority (261).

S. gallolyticus contains strains isolated from diverse habitats, including the feces of koalas, kangaroos, brushtail possums, ringtail possums, cows, horses, pigs, dogs, pigeons and guinea pigs, as well as animals with bovine mastitis, human clinical sources, and the sheep rumen, while most strains belonging to *S. bovis* are isolated from ruminants, such as cattle and horses (260-262). Indeed, the predominant *Streptococcus* isolated from the rumen of animals is *S. bovis*. *S. bovis* is of particular interest because of its role in the development of lactic acidosis in cattle and sheep fed an excess of starch (263). *S. bovis* strains are currently defined as a genomically heterogeneous group (3, 264).

The frequent association of *S. gallolyticus* with pathological conditions is noteworthy, not only among the animal strains but also among human lesion-derived strains. *S. gallolyticus* is also the most important etiological agent of septicemic streptococcosis in pigeons. The most important clinical signs of *S. gallolyticus* septicemia include sudden death, inability to fly, lameness, emaciation, polyuria and production of slimy, green droppings. Most

typical lesions consist of extensive, well-circumscribed areas of necrosis in the pectoral muscle and arthritis of the knee, hock and shoulder joints (265). However, the association of *S. gallolyticus* with pathogenicity is far from absolute, since these bacteria are also normal components of the intestinal flora of pigeons in many lofts, and certain serotypes are more virulent than others (262, 265). In fact, within the *S. gallolyticus* species, different types have been recognized in pigeons: five biotypes, two sub-biotypes, five serotypes and six supernatant-phenotypes. Supernatant-phenotypes are identified on the basis of the presence of either a T1 (70 kDa), T2 (68 kDa) or T3 (74 kDa) protein triplet and the presence (A+) or absence (A^-) of the named protein A of 185 kDa in culture supernatants (266, 267). There is a very strong correlation between the supernatant-phenotype and the virulence of a given strain. Indeed, experimental infection studies have demonstrated that strains belonging to the A+T1, A+T2, A+T3 and A^-T1 phenotype groups are highly virulent for pigeons. A^-T3 strains appeared moderately virulent, whereas strains belonging to the A^-T2 phenotype are low virulent for pigeons (266, 267). Later, in a comparative study of cell wall protein profiles of several isolates of *S. gallolyticus* of differing virulence for pigeons, a band with molecular mass of 114 kDa was observed in isolates that belong to the highly virulent A+T1, A+T2, A+T3 and A^-T1 culture supernatant phenotypes. A band with a slightly higher molecular mass (115 kDa) as well as a 207 kDa band were only detected in isolates that belong to the moderately A^-T3 or low A^-T2 virulent culture supernatant phenotypes. The 114- and 115-kDa bands were recognized by all homologous and heterologous pigeon sera used, whereas the 207 kDa band was only recognized by sera of pigeons infected with a A^-T2 strain. Authors suggested that the 114, 115- and 207-kDa bands are useful as additional virulence associated markers for pigeon *S. gallolyticus* strains (268). A similar association of protein virulence markers and pathogenicity of European *S. suis* strains was described in the literature (as discussed above). Like *S. suis*, the exact role of these extracellular and cell wall proteins in the pathogenesis of *S. gallolyticus* infections needs to be clarified. Nevertheless, in a study of prevalence, 94% of the strains lacking the A and T1 proteins were isolated from healthy pigeons, and only 6% were isolated from septicemia. Strains expressing A and/or T1, however, were isolated from septicemia in 57% of the cases. These observations may indicate that the A and/or T1 proteins are associated with virulence (266).

Vanrobaeys *et al.* (269) demonstrated that *S. gallolyticus* is an encapsulated organism and, that the capsule of the virulent strains had a regular, continuous appearance whilst irregularity of the capsule was a characteristic of the low virulence strains. Presence of fimbriae was also demonstrated in all strains belonging to the high virulence supernatant groups and in one strain of the moderately virulent group. The fimbriae are thin, flexible structures with a diameter of approximately 3-4 nm and a length of up to 700 nm. It was suggested that morphological differences in surface structure exist among virulent and low virulence pigeon *S. gallolyticus* strains, and that the capsule and/or fimbriae are possibly involved in virulence. In the *Enterobacteriaceae*, fimbriae serve as ligands during attachment to host cells. For the streptococci, the relationship between the presence of ultrastructural appendages and adhesion is less established. In pigeon *S. gallolyticus* strains, as well as all other streptococci, the role of fimbriae is not well known. In preliminary studies using pigeon, chicken, pig and sheep erythrocytes, pigeon *S. gallolyticus* strains were unable to cause hemagglutination (269).

Finally, *S. gallolyticus* and *S. bovis* were shown to inhibit the growth of other streptococci. The antibacterial compounds produced by these bacteria are protease sensitive, remained active in a pH range from 1 to 12, and did not lose activity after heating at 100°C for 15 min. The antibacterial peptide (named as bovicin 255) was shown to have characteristics that are very similar to those described for class II bacteriocins of Gram-positive bacteria (270). The diversity and density of the microbial population of the rumen suggest that this environment might favor the evolution of bacteriocins as competitive factors.

7. CONCLUDING REMARKS

The virulence of streptococci is considered as a multifactorial process. In contrast to well known human pathogens, and in spite of their veterinary importance, knowledge of virulence factors of several animal disease-associated streptococci is limited or almost inexistent. A common pattern for different streptococci seems to be the production of extracellular factors, such as capsule, hemolysins and other toxins, CAMP factor, M-like proteins and different proteases. Despite that these factors are present in many streptocci, their role in the pathogenesis of the respective infections is generally not known. In addition, as it is the case for *S. pneumoniae*, virulence factors usually present in strains of human origin are absent in those isolated from animals. For other streptococci, such as *S. suis*, it is still impossible to easily distinguish between virulent and non virulent strains. Further studies on virulence factors of different streptococci are granted. The genomic sequencing of members of streptococci associated with animal diseases, such as that of *S. suis* (http://www.sanger.ac.uk/Projects/S_suis/), as well as proteomic studies that will certainly follow, will probably clarify the pathogenesis of the infections caused by these important pathogens.

8. ACKNOWLEDGMENTS

We would like to thank Dr. Robert Higgins (Université de Montréal, Canada) for critical reviewing of this paper. Results on *S. suis* and *S. agalactiae* research carried out in our laboratory and briefly presented in this review have been funded by the Natural Science and Engineering Research Council of Canada and by the Canadian Network on Bacterial Pathogens of Swine.

9. REFERENCES

1. Facklam R.: What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev* 15, 613-630 (2002)

2. Poyart C., G. Quesne, S. Coulon, P. Berche & P. Trieu-Cuot: Identification of Streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase. *J Clin Microbiol* 36, 41-47 (1998)

3. Bentley R. W., J. A. Leigh & M. D. Collins: Intrageneric structure of *Streptococcus* based on comparative analysis of small-subunit rRNA sequences. *Int J Syst Bacteriol* 41, 487-494 (1991)

4. Navarre W. W. & O. Schneewind: Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol Mol Biol Rev* 63, 174-229 (1999)

5. Collin M. & A. Olsén: Generation of a mature streptococcal cysteine proteinase is dependent on cell wall-anchored M1 protein. *Mol Microbiol* 36, 1306-1318 (2000)

6. Berge A. & L. Björck: Streptococcal cysteine proteinase releases biologically active fragments of streptococcal surface proteins. *J Biol Chem* 270, 9862-9867 (1995)

7. Higgins R. & M. Gottschalk: Streptococcal diseases. In: Diseases of swine. Eds: Straw B E, D'Allaire S, Mengeling W L, Taylor D J, Iowa State University, Ames (1999)

8. Reams R. Y., D. D. Harrington, L. T. Glickman, H. L. Thacker & T. L. Bowersock: Multiple serotypes and strains of *Streptococcus suis* in naturally infected swine herds. *J Vet Diagn Invest* 8, 119-121 (1996)

9. Salasia S. I., C. Lammler & L. A. Devriese: Serotypes and putative virulence markers of *Streptococcus suis* isolates from cats and dogs. *Res Vet Sci* 57, 259-261 (1994)

10. Cruz Colque J. I., L. A. Devriese & F. Haesebrouck: Streptococci and enterococci associated with tonsils of cattle. *Lett Appl Microbiol* 16, 72-74 (1993)

11. Higgins R., A. Lagace, S. Messier & L. Julien: Isolation of *Streptococcus suis* from a young wild boar. *Can Vet J* 38, 114 (1997)

12. Perch B., K. B. Pedersen & J. Henrichsen: Serology of capsulated streptococci pathogenic for pigs: six new serotypes of *Streptococcus suis*. *J Clin Microbiol* 17, 993-996 (1983)

13. Higgins R. & M. Gottschalk: An update on *Streptococcus suis* identification. *J Vet Diagn Invest* 2, 249-252 (1990)

14. Gottschalk M. & M. Segura: The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. *Vet Microbiol* 75, 59-71 (2000)

15. Williams A. E. & W. F. Blakemore: Pathogenesis of meningitis caused by *Streptococcus suis* type 2. *J Infect Dis* 162, 474-481 (1990)

16. Moxon E. R. & J. S. Kroll: The role of bacterial polysaccharide capsules as virulence factors. *Curr Top Microbiol Immunol* 150, 65-85 (1990)

17. Jacques M., M. Gottschalk, B. Foiry & R. Higgins: Ultrastructural study of surface components of *Streptococcus suis*. *J Bacteriol* 172, 2833-2838 (1990)

18. Elliott S. D. & J. Y. Tai: The type-specific polysaccharides of *Streptococcus suis*. *J Exp Med* 148, 1699-1704 (1978)

19. Katsumi M., T. Saito, Y. Kataoka, T. Itoh, N. Kikuchi & T. Hiramune: Comparative preparation methods of sialylated capsule antigen from *Streptococcus suis* type 2 with type specific antigenicity. *J Vet Med Sci* 58, 947-952 (1996)

20. Charland N., J. T. Kellens, F. Caya & M. Gottschalk: Agglutination of *Streptococcus suis* by sialic acid-binding lectins. *J Clin Microbiol* 33, 2220-2221 (1995)

21. Charland N., M. Jacques, S. Lacouture & M. Gottschalk: Characterization and protective activity of a monoclonal antibody against a capsular epitope shared by *Streptococcus suis* serotypes 1, 2 and 1/2. *Microbiology* 143, 3607-3614 (1997)

22. Elliott S. D., F. Clifton-Hadley & J. Tai: Streptococcal infection in young pigs. V. An immunogenic polysaccharide from *Streptococcus suis* type 2 with particular reference to vaccination against streptococcal meningitis in pigs. *J Hyg Lond* 85, 275-285 (1980)

23. del Campo Sepulveda E. M., E. Altman, M. Kobisch, S. D'Allaire & M. Gottschalk: Detection of antibodies against *Streptococcus suis* capsular type 2 using a purified capsular polysaccharide antigen-based indirect ELISA. *Vet Microbiol* 52, 113-125 (1996)

24. Agarwal K. K., S. D. Elliott & P. J. Lachmann: Streptococcal infection in young pigs. III. The immunity of adult pigs investigated by the bactericidal test. *J Hyg* 67, 491-503 (1969)

25. Charland N., J. Harel, M. Kobish, S. Lacasse & M. Gottschalk: *Streptococcus suis* serotype 2 mutants deficient in capsular expression. *Microbiology* 144, 325-332 (1998)

26. Smith H. E., M. Damman, J. Van der Velde, F. Wagenaar, H. J. Wisselink, N. Stockhofe-Zurwieden & M. A. Smits: Identification and characterization of the *cps* locus of *Streptococcus suis* serotype 2: the capsule protects against phagocytosis and is an important virulence factor. *Infect Immun* 67, 1750-1756 (1999)

27. Gottschalk M., S. Petitbois, R. Higgins & M. Jacques: Adherence of *Streptococcus suis* capsular type 2 to porcine lung sections. *Can J Vet Res* 55, 302-304 (1991)

28. Quessy S., J. D. Dubreuil, M. Jacques, F. Malouin & R. Higgins: Increase of capsular material thickness following in vivo growth of virulent *Streptococcus suis* serotype 2 strains. *FEMS Microbiol Lett* 115, 19-26 (1994)

29. Wessels M. R., C. E. Rubens, V. J. Benedi & D. L. Kasper: Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule. *Proc Natl Acad Sci USA* 86, 8983-8987 (1989)

30. Charland N., M. Kobisch, B. Martineau-Doize, M. Jacques & M. Gottschalk: Role of capsular sialic acid in virulence and resistance to phagocytosis of *Streptococcus suis* capsular type 2. *FEMS Immunol Med Microbiol* 14, 195-203 (1996)

31. Gottschalk M., R. Higgins, M. Jacques, M. Beaudoin & J. Henrichsen: Isolation and characterization of *Streptococcus suis* capsular types 9-22. *J Vet Diagn Invest* 3, 60-65 (1991)

32. Robertson I. D., D. K. Blackmore, D. J. Hampson & Z. F. Fu: A longitudinal study of natural infection of piglets with *Streptococcus suis* types 1 and 2. *Epidemiol Infect* 107, 119-126 (1991)

33. Vecht U., H. J. Wisselink, M. L. Jellema & H. E. Smith: Identification of two proteins associated with virulence of *Streptococcus suis* type 2. *Infect Immun* 59, 3156-3162 (1991)

34. Vecht U., J. P. Arends, E. J. van der Molen & L. A. van Leengoed: Differences in virulence between two strains of *Streptococcus suis* type II after experimentally induced infection of newborn germ-free pigs. *Am J Vet Res* 50, 1037-1043 (1989)

35. Vecht U., H. J. Wisselink, J. E. van Dijk & H. E. Smith: Virulence of *Streptococcus suis* type 2 strains in newborn germfree pigs depends on phenotype. *Infect Immun* 60, 550-556 (1992)

36. Smith H. E., F. H. Reek, U. Vecht, A. L. Gielkens & M. A. Smits: Repeats in an extracellular protein of weakly pathogenic strains of *Streptococcus suis* type 2 are absent in pathogenic strains. *Infect Immun* 61, 3318-3326 (1993)

37. Wisselink H. J., H. E. Smith, N. Stockhofe-Zurwieden, K. Peperkamp & U. Vecht: Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. *Vet Microbiol* 74, 237-248 (2000)

38. Gottschalk M., A. Lebrun, H. Wisselink, J. D. Dubreuil, H. Smith & U. Vecht: Production of virulence-related proteins by canadian strains of *Streptococcus suis* capsular type 2. *Can J Vet Res* 62, 75-79 (1998)

39. Smith H. E., U. Vecht, A. L. Gielkens & M. A. Smits: Cloning and nucleotide sequence of the gene encoding the 136-kilodalton surface protein (muramidase-released protein) of *Streptococcus suis* type 2. *Infect Immun* 60, 2361-2367 (1992)

40. Smith H. E., U. Vecht, H. J. Wisselink, N. Stockhofe-Zurwieden, Y. Biermann & M. A. Smits: Mutants of *Streptococcus suis* types 1 and 2 impaired in expression of muramidase-released protein and extracellular protein induce disease in newborn germfree pigs. *Infect Immun* 64, 4409-4412 (1996)

41. Chatellier S., M. Gottschalk, R. Higgins, R. Brousseau & J. Harel: Relatedness of *Streptococcus suis* serotype 2 isolates from different geographic origins as evaluated by molecular fingerprinting and phenotyping. *J Clin Microbiol* 37, 362-366 (1999)

42. Wisselink H. J., U. Vecht, N. Stockhofe-Zurwieden & H. E. Smith: Protection of pigs against challenge with virulent *Streptococcus suis* serotype 2 strains by a muramidase-released protein and extracellular factor vaccine. *Vet Rec* 148, 473-477 (2001)

43. Berthelot-Hérault F., H. Morvan, A.-M. Kéribin, M. Gottschalk & M. Kobisch: Production of muraminidase-released protein (MRP), extracellular factor (EF) and suilysin by field isolates of *Streptococcus suis* capsular types 2, 1/2, 9, 7 and 3 isolated from swine in France. *Vet Res* 31, 473-479 (2000)

44. Galina L., U. Vecht, H. J. Wisselink & C. Pijoan: Prevalence of various phenotypes of *Streptococcus suis* isolated from swine in the U.S.A. based on the presence of muraminidase-released protein and extracellular factor. *Can J Vet Res* 60, 72-74 (1996)

45. Jacobs A. A., P. L. Loeffen, A. J. van den Berg & P. K. Storm: Identification, purification, and characterization of a thiol-activated hemolysin (suilysin) of *Streptococcus suis*. *Infect Immun* 62, 1742-1748 (1994)

46. Gottschalk M. G., S. Lacouture & J. D. Dubreuil: Characterization of *Streptococcus suis* capsular type 2 haemolysin. *Microbiology* 141, 189-195 (1995)

47. Palmer M.: The family of thiol-activated, cholesterol-binding cytolysins. *Toxicon* 39, 1681-1689 (2001)

48. Alouf J. E. & C. Geoffroy: The family of the antigenically-related cholesterol-binding ("sulphydryl-activated") cytolytic toxins. In: Sourcebook of Bacterial Protein Toxins. Eds: Alouf J E, New York Academic Press, New York (1991)

49. Segers R. P., T. Kenter, L. A. M. de Haan & A. A. C. Jacobs: Characterisation of the gene encoding suilysin from *Streptococcus suis* and expression in field strains. *FEMS Microbiol Lett* 167, 255-261 (1998)

50. Feder I., M. M. Chengappa, B. Fenwick, M. Rider & J. Staats: Partial characterization of *Streptococcus suis* type 2 hemolysin. *J Clin Microbiol* 32, 1256-1260 (1994)

51. Charland N., V. Nizet, C. Rubens, K. S. Kim, S. Lacouture & M. Gottschalk: *Streptococcus suis* serotype 2 interactions with human brain microvascular endothelial cells. *Infect Immun* 68, 637-643 (2000)

52. Lalonde M., M. Segura, S. Lacouture & M. Gottschalk: Interactions between *Streptococcus suis* serotype 2 and different epithelial cell lines. *Microbiology* 146, 1913-1921 (2000)

53. Norton P. M., C. Rolph, P. N. Ward, R. W. Bentley & J. A. Leigh: Epithelial invasion and cell lysis by virulent strains of *Streptococcus suis* is enhanced by the presence of suilysin. *FEMS Immunol Med Microbiol* 26, 25-35 (1999)

54. Segura M. & M. Gottschalk: *Streptococcus suis* interactions with the murine macrophage cell line J774: adhesion and cytotoxicity. *Infect Immun* 70, 4312-4322 (2002)

55. Vadeboncoeur N., M. Segura, D. Al-Numani, G. Vanier & M. Gottschalk: Pro-inflammatory cytokine and chemokine release by human brain microvascular endothelial cells stimulated by *Streptococcus suis* serotype 2. *FEMS Immunol Med Microbiol* 35, 49-58 (2003)

56. Lun S., J. Perez-Casal, W. Connor & P. J. Willson: Role of suilysin in pathogenesis of *Streptococcus suis* capsular serotype 2. *Microb Pathog* 34, 27-37 (2003)

57. Al-Numani D., M. Segura, M. Doré & M. Gottschalk: Up-regulation of ICAM-1, CD11a/CD18 and CD11c/CD18 on human THP-1 monocytes stimulated by *Streptococcus suis* serotype 2. *Clin Exp Immunol* 133, 67-77 (2002)

58. Allen A. G., S. Bolitho, H. Lindsay, S. Khan, C. Bryant, P. M. Norton, P. N. Ward, J. A. Leigh, J. Morgan, H. Riches, S. Eastty & D. Maskell: Generation and characterization of a defined mutant of *Streptococcus suis* lacking suilysin. *Infect Immun* 69, 2732-2735 (2001)

59. Jacobs A. A., A. J. van den Berg & P. L. Loeffen: Protection of experimentally infected pigs by suilysin, the thiol-activated haemolysin of *Streptococcus suis*. *Vet Rec* 139, 225-228 (1996)

60. Jacobs A. A., A. J. van den Berg, J. C. Baars, B. Nielsen & L. W. Johannsen: Production of suilysin, the thiol-activated haemolysin of *Streptococcus suis*, by field isolates from diseased pigs. *Vet Rec* 137, 295-296 (1995)

61. Okwumabua O., O. Abdelmagid & M. M. Chengappa: Hybridization analysis of the gene encoding a hemolysin (suilysin) of *Streptococcus suis* type 2: evidence for the absence of the gene in some isolates. *FEMS Microbiol Lett* 181, 113-121 (1999)

62. Staats J., P. Brandon, G. Stewart & M. M. Chengappa: Presence of the *Streptococcus suis* suilysin gene and expression of MRP and EF correlates with high virulence in *Streptococcus suis* type 2 isolates. *Vet Microbiol* 70, 201-211 (1999)

63. Allgaier A., R. Goethe, H. J. Wisselink, H. E. Smith & P. Valentin-Weigand: Relatedness of *Streptococcus suis* isolates of various serotypes and clinical backgrounds as evaluated by macrorestriction analysis and expression of potential virulence traits. *J Clin Microbiol* 39, 445-453 (2001)

64. Hommez J., L. A. Devriese, F. Castryck & C. Miry: Beta-hemolytic streptococci from pigs: bacteriological diagnosis. *J Vet Med B* 38, 441-444 (1991)

65. Yamaguchi M., M. Miyake, H. Nishiya & M. Noda: A cytotoxic factor of *Streptococcus suis* isolated from a septicemia patient. *Jpn J Med Sci Biol* 47, 318-319 (1994)

66. Jobin M.-C. & D. Grenier: Identification and characterization of four proteases produced by *Streptococcus suis*. *FEMS Microbiol Lett* 220, 113-119 (2003)

67. Mentlein R.: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul Pept* 85, 9–24 (1999)

68. Langford P., A. E. Williams & J. S. Kroll: Superoxide dismutases of pathogenic and non-pathogenic *Streptococcus suis* type 2 isolates. *FEMS Microbiol Lett* 61, 347-350 (1991)

69. Niven D. F., A. Ekins & A. A. W. Al-Samaurai: Effects of iron and manganese availability on growth and production of superoxide dismutase by *Streptococcus suis*. *Can J Microbiol* 45, 1027-1032 (1999)

70. Beachey E. H.: Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J Infect Dis* 143, 325-345 (1981)

71. Gottschalk M., A. Lebrun, M. Jacques & R. Higgins: Hemagglutination properties of *Streptococcus suis*. *J Clin Microbiol* 28, 2156-2158 (1990)

72. Jack R. W., J. R. Tagg & B. Ray: Bacteriocins of gram-positive bacteria. *Microbiol Rev* 59, 171-200 (1995)

73. Mélançon D. & D. Grenier: Production and properties of bacteriocin-like inhibitory substances from the swine pathogen *Streptococcus suis* serotype 2. *Appl Environ Microbiol* 69, 4482-4488 (2003)

74. Wessman G. E.: Biology of the group E streptococci: a review. *Vet Microbiol* 12, 297-328 (1986)

75. Lammler C., N. Cirak & J. Smola: Studies on biochemical, serological and further characteristics of *Streptococcus porcinus*. *J Vet Med B* 45, 235-243 (1998)

76. Facklam R., J. Elliott, N. Pigott & A. R. Franklin: Identification of *Streptococcus porcinus* from human sources. *J Clin Microbiol* 33, 385-388 (1995)

77. Kim D. M., S. J. Lee, S. K. Yoon & S. M. Byun: Specificity role of the streptokinase C-terminal domain in plasminogen activation. *Biochem Biophys Res Commun* 290, 585-588 (2002)

78. Lahteenmaki K., P. Kuusela & T. K. Korhonen: Bacterial plasminogen activators and receptors. *FEMS Microbiol Rev* 25, 531-552 (2001)

79. Katsumi M., Y. Kataoka, T. Takahashi, N. Kikuchi & T. Hiramune: Biochemical and serological examination of beta-hemolytic streptococci isolated from slaughtered pigs. *J Vet Med Sci* 60, 129-131 (1998)

80. Schofield C. R. & J. R. Tagg: Bacteriocin-like activity of group B and group C streptococci of human and of animal origin. *J Hyg (Lond)* 90, 7-18 (1983)

81. Dierksen K. P. & J. R. Tagg: Haemolysin-deficient variants of *Streptococcus pyogenes* and *S. dysgalactiae* subsp. *equisimilis* may be overlooked as aetiological agents of pharyngitis. *J Med Microbiol* 49, 811-816 (2000)

82. Gerlach D., W. Kohler, E. Gunther & K. Mann: Purification and characterization of streptolysin O secreted by *Streptococcus equisimilis* (group C). *Infect Immun* 61, 2727-2731 (1993)

83. Bisno A. L., C. Collins & J. C. Turner: M proteins of *Streptococcus equisimilis* strains isolated from pharyngitis patients. *Adv Exp Med Biol* 418, 745-748 (1997)

84. Sachse S., P. Seidel, D. Gerlach, E. Gunther, J. Rodel, E. Straube & K. H. Schmidt: Superantigen-like gene(s) in human pathogenic *Streptococcus dysgalactiae*, subsp *equisimilis*: genomic localisation of the gene encoding streptococcal pyrogenic exotoxin G (speG(dys)). *FEMS Immunol Med Microbiol* 34, 159-167 (2002)

85. McCoy H. E., C. C. Broder & R. Lottenberg: Streptokinases produced by pathogenic group C streptococci demonstrate species specificity plasminogen activation. *J Infect Dis* 164, 515-521 (1991)

86. Caballero A. R., R. Lottenberg & K. H. Johnston: Cloning, expression, sequence analysis, and characterization of streptokinases secreted by porcine and equine isolates of *Streptococcus equisimilis*. *Infect Immun* 67, 6478-6486 (1999)

87. Schroeder B., M. D. P. Boyle, B. R. Sheerin, A. C. Asbury & R. Lottenberg: Species specificity of plasminogen activation and acquisition of surface-associated proteolytic activity by Group C Streptococci grown in plasma. *Infect Immun* 67, 6487-6495 (1999)

88. Nowicki S. T., D. Minning-Wenz, K. H. Johnston & R. Lottenberg: Characterization of a novel streptokinase produced by *Streptococcus equisimilis* of non-human origin. *Thromb Haemost* 72, 595-603 (1994)

89. Fox L. K. & J. M. Gay: Contagious mastitis. *Vet Clin Norht Am Food Anim Pract* 9, 475-487 (1993)

90. Calvinho L. F., R. A. Almeida & S. P. Oliver: Potential virulence factors of *Streptococcus dysgalactiae* associated with bovine mastitis. *Vet Microbiol* 61, 93-110 (1998)

91. Calvinho L. F., R. A. Almeida & S. P. Oliver: Influence of *Streptococcus dysgalactiae* surface hydrophobicity on adherence to mammary epithelial cells and phagocytosis by mammary macrophages. *Zentralbl Veterinarmed [B]* 43, 257-266 (1996)

92. Fishetti V. A.: Streptococcal M proteins. *Sci Am* 264, 32-39 (1991)

93. Mamo W., G. Fröman, A. Sundås & T. Wadström: Binding of fibronectin, fibrinogen and type II collagen to streptococci isolated from bovine mastitis. *Microb Pathog* 2, 417-424 (1987)

94. Rantamäki L. K. & H. P. Müller: Phenotypic characterization of *Streptococcus dysgalactiae* isolates from bovine mastitis by their binding to host derived proteins. *Vet Microbiol* 46, 415-426 (1995)

95. Vasi J., L. Frykberg, L. E. Carlsson, M. Lindberg & B. Guss: M-like proteins of *Streptococcus dysgalactiae. Infect Immun* 68, 294-302 (2000)

96. Traore M. Y., P. Valentin-Weigand, G. S. Chhatwal & H. Blobel: Inhibitory effects of fibrinogen on phagocytic killing of streptococcal isolates from humans, cattle and horses. *Vet Microbiol* 28, 295-302 (1991)

97. Vandamme P., B. Pot, E. Falsen, K. Kersters & L. A. Devriese: Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae subsp. equisimilis* subsp. nov. *Int J Syst Bacteriol* 46, 774-781 (1996)

98. Leigh J. A., S. M. Hodgkindson & R. A. Lincoln: The interaction of *Streptococcus dysgalactiae* with plasmin and plasminogen. *Vet Microbiol* 61, 121-135 (1998)

99. Sting R., P. Schaufuss & H. Blobel: Isolation and characterization of hyaluronidases from *Streptococcus dysgalactiae, S. zooepidemicus* and *S. equi*. *Zentralbl Bakteriol* 272, 276-282 (1990)

100. Miyoshi-Akiyama T., J. Zhao, H. Kato, K. Kikuchi, K. Totsuka, Y. Kataoka, M. Katsumi & T. Uchiyama: *Streptococcus dysgalactiae*-derived mitogen (SDM), a novel bacterial superantigen: characterization of its

biological activity and predicted tertiary structure. *Mol Microbiol* 47, 1589-1599 (2003)

101. Leigh J. A.: *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis? *Vet J* 157, 225-238 (1999)

102. Vaarst M. & C. Enevoldsen: Patterns of clinical mastitis manifestations in Danish organic dairy herds. *J Dairy Res* 64, 23-37 (1997)

103. Thomas L. H., W. Haider, A. W. Hill & R. S. Cook: Pathologic findings of experimentally induced *Streptococcus uberis* infection in the mammary gland of cows. *Am J Vet Res* 55, 1723-1728 (1994)

104. Oliver S. P., R. A. Almeida & L. F. Calvinho: Virulence factors of *Streptococcus uberis* isolated from cows with mastitis. *Zentralbl Veterinarmed [B]* 45, 461-471 (1998)

105. Hill A. W.: Pathogenicity of two strains of *Streptococcus uberis* infused into lactating and non-lactating bovine mammary glands. *Res Vet Sci* 45, 400-404 (1988)

106. Leigh J. A., T. R. Field & M. R. Williams: Two strains of *Streptococcus uberis*, of differing ability to cause clinical mastitis, differ in their ability to resist some host defence factors. *Res Vet Sci* 49, 85-87 (1990)

107. Leigh J. A. & T. R. Field: *Streptococcus uberis* resists the bactericidal action of bovine neutrophils despite the presence of bound immunoglobulin. *Infect Immun* 62, 1854-1859 (1994)

108. Almeida R. A. & S. P. Oliver: Growth curve, capsule expression and characterization of the capsular material of selected strains of *Streptococcus uberis*. *Zentralbl Veterinarmed [B]* 40, 697-706 (1993)

109. Leigh J. A. & T. R. Field: Killing of *Streptococcus uberis* by bovine neutrophils following growth in chemically defined media. *Vet Res Commun* 15, 1-6 (1991)

110. Ward P. N., T. R. Field, W. G. Ditcham, E. Maguin & J. A. Leigh: Identification and disruption of two discrete loci encoding hyaluronic acid capsule biosynthesis genes *hasA*, *hasB*, and *hasC* in *Streptococcus uberis*. *Infect Immun* 69, 392-399 (2001)

111. Ashbaugh C. D., S. Alberti & M. R. Wessels: Molecular analysis of the capsule gene region of group A *Streptococcus*: the *has*AB genes are sufficient for capsule expression. *J Bacteriol* 190, 4955–4959 (1998)

112. Almeida R. A. & S. P. Oliver: Antiphagocytic effect of the capsule of *Streptococcus uberis*. *Zentralbl Veterinarmed [B]* 40, 707-714 (1993)

113. Almeida R. A. & S. P. Oliver: Phagocytosis of *Streptococcus uberis* by bovine mammary macrophages:opsonizing effect of bovine antiserum. *Zentralbl Veterinarmed [B]* 42, 331-337 (1995)

114. Grant R. G. & J. M. Finch: Phagocytosis of *Streptococcus uberis* by bovine mammary gland macrophages. *Res Vet Sci* 62, 74-78 (1996)

115. Field T. R., P. N. Ward, L. H. Pedersen & J. A. Leigh: The hyaluronic acid capsule of *Streptococcus uberis* is not required for the development of infection and clinical mastitis. *Infect Immun* 71, 132-139 (2003)

116. Leigh J. A.: Activation of bovine plasminogen by *Streptococcus uberis*. *FEMS Microbiol Lett* 114, 67-71 (1993)

117. Leigh J. A.: Purification of a plasminogen activator from *Streptococcus uberis*. *FEMS Microbiol Lett* 118, 153-158 (1994)

118. Lincoln R. A. & J. A. Leigh: Characterization of a novel plasminogen activator from *Streptococcus uberis*. *Adv Exp Med Biol* 418, 643-645 (1997)

119. Johnsen L. B., K. Poulsen, M. Kilian & T. E. Petersen: Purification and cloning of a streptokinase from *Streptococcus uberis*. *Infect Immun* 67, 1072-1078 (1999)

120. Rosey E. L., R. A. Lincoln, P. N. Ward, R. J. J. Yancey & J. A. Leigh: PauA: a novel plasminogen activator from *Streptococcus uberis*. *FEMS Microbiol Lett* 178, 27-33 (1999)

121. Sazonova I. Y., A. K. Houng, S. A. Chowdhry, B. R. Robinson, L. Hedstrom & G. L. Reed: The mechanism of a bacterial plasminogen activator intermediate between streptokinase and staphylokinase. *J Biol Chem* 276, 12609-12613 (2001)

122. Johnsen L. B., L. K. Rasmussen, T. E. Petersen, M. Etzerodt & S. N. Fedosov: Kinetic and structural characterization of a two-domain streptokinase: dissection of domain functionality. *Biochemistry* 39, 6440-6448 (2000)

123. Ward P. N. & J. A. Leigh: Characterization of PauB, a novel broad-spectrum plasminogen activator from *Streptococcus uberis*. *J Bacteriol* 184, 119-125 (2002)

124. Leigh J. A. & R. A. Lincoln: *Streptococcus uberis* acquires plasmin activity following growth in the presence of bovine plasminogen through the action of its specific plasminogen activator. *FEMS Microbiol Lett* 154, 123-129 (1997)

125. Kitt A. J. & J. A. Leigh: The auxotrophic nature of *Streptococcus uberis*. The acquisition of essential acids

from plasmin derived casein peptides. *Adv Exp Med Biol* 418, 647-650 (1997)

126. Schaufuss P., R. Sting, W. Schaeg & H. Blobel: Isolation and characterization of hyaluronidase from *Streptococcus uberis*. *Zentralbl Bakteriol* 271, 46-53 (1989)

127. Matthews K. R., J. J. Rejman, J. D. Turner & S. P. Oliver: Proliferation of a bovine mammary epithelial cell line in the presence of bacterial virulence factors. *J Dairy Sci* 77, 2959-2964 (1994)

128. Lammler C.: Biochemical and serological properties of *Streptococcus uberis*. *Zentralbl Veterinarmed [B]* 38, 737-742 (1991)

129. Lopes M. F., V. L. Merquior, J. M. Peralta & L. M. Teixeira: Partial characterization of the cohemolytic factor produced by *Streptococcus uberis* and comparison with the CAMP-factor. *FEMS Immunol Med Microbiol* 12, 205-212 (1995)

130. Hassan A. A., A. Abdulmawjood, A. O. Yildirim, K. Fink, C. Lammler & R. Schlenstedt: Identification of streptococci isolated from various sources by determination of *cfb* gene and other CAMP-factor genes. *Can J Microbiol* 46, 946-951 (2000)

131. Jiang M., L. A. Babiuk & A. A. Potter: Cloning, sequencing and expression of the CAMP factor gene of *Streptococcus uberis*. *Microb Pathog* 20, 297-307 (1996)

132. Shalka B. & J. Smola: Lethal effect of CAMP-factor and Uberis-factor: a new finding about diffusible exosubstances of *Streptococcus agalactiae* and *Streptococcus uberis*. *Zentralbl Bakteriol* 249, 190-194 (1981)

133. Fang W., D. A. Luther & S. P. Olivier: Protein expression by *Streptococcus uberis* in co-culture with bovine mammary epithelial cells. *FEMS Microbiol Lett* 166, 237-242 (1998)

134. Gilbert F. B., D. A. Luther & S. P. Olivier: Induction of surface-associated proteins of *Streptococcus uberis* by cultivation with extracellular matrix components and bovine mammary epithelial cells. *FEMS Microbiol Lett* 156, 161-164 (1997)

135. Manning S. D.: Molecular epidemiology of *Streptococcus agalactiae* (group B *Streptococcus*). *Front Biosci* 8, S1-18 (2003)

136. Spellerberg B.: Pathogenesis of neonatal *Streptococcus agalactiae* infections. *Microbes Infect* 2, 1733-1742 (2000)

137. Keefe G. P.: *Streptococcus agalactiae* mastitis: a review. *Can Vet J* 38, 429-437 (1997)

138. Eldar A., Y. Bejerano, A. Livoff, A. Horovitcz & H. Bercovier: Experimental streptococcal meningo-encephalitis in cultured fish. *Vet Microbiol* 43, 33-40 (1995)

139. Vandamme P., L. A. Devriese, B. Pot, K. Kersters & P. Melin: *Streptococcus difficile* is a nonhemolytic group B, type Ib Streptococcus. *Int J Syst Bacteriol* 47, 81-85 (1997)

140. Geyer A., A. Roth, S. Vettermann, E. Gunther, A. Groh, E. Straube & K. Schmidt: M protein of a *Streptococcus dysgalactiae* human wound isolate shows multiple binding to different plasma proteins and shares epitopes with keratin and human cartilage. *FEMS Immunol Med Microbiol* 26, 11-24 (1999)

141. Yildirim A. O., C. Lammler, R. Weiss & P. Kopp: Pheno- and genotypic properties of streptococci of serological group B of canine and feline origin. *FEMS Microbiol Lett* 212, 187-192 (2002)

142. Poutrel B. & J. Dore: Virulence of human and bovine isolates of group B streptococci (types Ia and III) in experimental pregnant mouse models. *Infect Immun* 47, 94-97 (1985)

143. Finch L. A. & D. R. Martin: Human and bovine group B streptococci: two distinct populations. *J Appl Bacteriol* 57, 273-278 (1984)

144. Klegerman M. E., K. M. Boyer, C. K. Papierniak, L. Levine & S. P. Gotoff: Type-specific capsular antigen is associated with virulence in late-onset group B streptococcal type III disease. *Infect Immun* 44, 124-129 (1984)

145. Martinez G., J. Harel, R. Higgins, S. Lacouture, D. Daignault & M. Gottschalk: Characterization of *Streptococcus agalactiae* isolates of bovine and human origin by randomly amplified polymorphic DNA analysis. *J Clin Microbiol* 38, 71-78 (2000)

146. Segura M. A., P. Cléroux & M. Gottschalk: *Streptococcus suis* and group B *Streptococcus* differ in their interactions with murine macrophages. *FEMS Immunol Med Microbiol* 21, 189-195 (1998)

147. Rubens C. E., M. R. Wessels, L. M. Heggen & D. L. Kasper: Transposon mutagenesis of group B streptococcal type III capsular polysaccharide: correlation of capsule expression with virulence. *Proc Natl Acad Sci USA* 84, 7208-7212 (1987)

148. Rainard P., Y. Lautrou & B. Poutrel: Ingestion and killing of *Streptococcus agalactiae* by bovine granulocytes in the presence of natural opsonins. *Vet Microbiol* 18, 41-50 (1988)

149. Salasia S. I., I. W. Wibawan, C. Lammler & M. Sellin: Phase variation in streptococci of serological group B.

Characteristic properties of isolates from human and bovine infection. *APMIS* 102, 925-930 (1994)

150. Platt M. W.: In vivo hemolytic activity of group B *Streptococcus* is dependent on erythrocyte-bacteria contact and independent of a carrier molecule. *Curr Microbiol* 31, 5-9 (1995)

151. Dal M. C. & H. Monteil: Hemolysin produced by group B *Streptococcus agalactiae*. *FEMS Microbiol Lett* 16, 89-94 (1983)

152. Spellerberg B., B. Pohl, G. Haase, S. Martin, J. Weber-Heynemann & R. Lutticken: Identification of genetic determinants for the hemolytic activity of *Streptococcus agalactiae* by ISS1 transposition. *J Bacteriol* 181, 3212-3219 (1999)

153. Spellerberg B., S. Martin, C. Brandt & R. Lutticken: The *cyl* genes of *Streptococcus agalactiae* are involved in the production of pigment. *FEMS Microbiol Lett* 188, 125-128 (2000)

154. Griffiths B. B.: Kinetics of hemolysin production in bovine group B streptococci. *J Basic Microbiol* 30, 241-250 (1990)

155. Schlievert P. M., J. E. Gocke & J. R. Deringer: Group B streptococcal toxic shock-like syndrome: report of a case and purification of an associated pyrogenic toxin. *Clin Infect Dis* 17, 26-31 (1993)

156. Sundell H. W., H. Yan, C. E. Carter, B. D. Wamil, K. Wu, R. Gaddipati, D. Li & C. G. Hellerqvist: Isolation and identification of the group B streptococcal toxin CM101 from infants with sepsis. *J Pediatr* 137, 338-344 (2000)

157. Pritchard D. G. & B. Lin: Group B streptococcal neuraminidase is actually a hyaluronidase. *Infect Immun* 61, 3234-3239 (1993)

158. Ozegowski J. H., E. Gunther & W. Reichardt: Purification and characterization of hyaluronidase from *Streptococcus agalactiae*. *Zentralbl Bakteriol* 280, 497-506 (1994)

159. Lin B., S. K. Hollingshead, J. E. Coligan, M. L. Egan, J. R. Baker & D. G. Pritchard: Cloning and expression of the gene for Group B Streptococcal hyaluronate lyase. *J Biol Chem* 269, 30113-30116 (1994)

160. Haug R. H., O. Olsvik & O. Hushovd: Hyaluronidase production, lactose and salicin fermentation and phage-typability in bovine group B Streptococci. *NIPH Ann* 4, 69-73 (1981)

161. Yildirim A. O., C. Lammler & R. Weiss: Identification and characterization of *Streptococcus agalactiae* isolated from horses. *Vet Microbiol* 85, 31-35 (2002)

162. Pritchard D. G., B. Lin, T. R. Willingham & J. R. Baker: Characterization of the group B streptococcal hyaluronate lyase. *Arch Biochem Biophys* 315, 431-437 (1994)

163. Mello L. V., B. L. De Groot, S. Li & M. J. Jedrzejas: Structure and flexibility of *Streptococcus agalactiae* hyaluronate lyase complex with its substrate. Insights into the mechanism of processive degradation of hyaluronan. *J Biol Chem* 277, 36678-36688 (2002)

164. Li S. & M. J. Jedrzejas: Hyaluronan binding and degradation by *Streptococcus agalactiae* hyaluronate lyase. *J Biol Chem* 276, 41407-41416 (2001)

165. Orefici G., A. De Stasio, M. Guarino, A. Martini & N. Orsi: Group B streptococci: extracellular neuraminidase production and virulence in mouse. *Microbiologica* 7, 75-78 (1984)

166. Podbielski A., O. Blankenstein & R. Lutticken: Molecular characterization of the *cfb* gene encoding group B streptococcal CAMP-factor. *Med Microbiol Immunol (Berl)* 183, 239-256 (1994)

167. Hassan A. A., O. Akineden, C. Lammler & R. Huber-Schlenstedt: Molecular characterization of phenotypically CAMP-negative *Streptococcus agalactiae* isolated from bovine mastitis. *J Vet Med B* 49, 257-259 (2002)

168. Jurgens D., B. Sterzik & F. J. Fehrenbach: Unspecific binding of group B streptococcal cocytolysin (CAMP factor) to immunoglobulins and its possible role in pathogenicity. *J Exp Med* 165, 720-732 (1987)

169. Fehrenbach F. J., D. Jurgens, J. Ruhlmann, B. Sterzik & M. Ozel: Role of CAMP-factor (factor B) for virulence. *Zentbl Bakteriol Hyg Suppl* 17, 351-357 (1988)

170. Lang S. & M. Palmer: Characterization of *Streptococcus agalactiae* CAMP factor as a pore-forming toxin. *J Biol Chem* June 30, [Epub ahead of print] (2003)

171. Gaillot O., C. Poyart, P. Berche & P. Trieu-Cuot: Molecular characterization and expression analysis of the superoxide dismutase gene from *Streptococcus agalactiae. Gene* 204, 213-218 (1997)

172. Valentin-Weigand P., P. Benkel, M. Rohde & G. S. Chhatwal: Entry and intracellular survival of group B Streptococci in J774 macrophages. *Infect Immun* 64, 2467-2473 (1996)

173. Poyart C., E. Pellegrini, O. Gaillot, C. Boumaila, M. Baptista & P. Trieu-Cuot: Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of *Streptococcus agalactiae*. *Infect Immun* 69, 5098-5106 (2001)

174. Barnham M., A. Ljunggren & M. McIntyre: Human infection with *Streptococcus zooepidemicus* (Lancefield

group C): three case reports. *Epidemiol Infect* 98, 183-190 (1987)

175. Boucher C., R. Higgins, M. Nadeau & C. Vincent: [A case of zoonosis associated with *Streptococcus equi* ssp. *zooepidemicus*]. *Can Vet J* 43, 123-124 (2002)

176. Lindmark H., P. Jonsson, E. Engvall & B. Guss: Pulsed-field gel electrophoresis and distribution of the genes *zag* and *fnz* in isolates of *Streptococcus equi. Res Vet Sci* 66, 93-99 (1999)

177. Jorm L. R., D. N. Love, G. D. Bailey, G. M. Bailey & D. A. Briscoe: Genetic structure of populations of -haemolytic Lancefield group C streptococci from horses and their association with disease. *Res Vet Sci* 57, 292-299 (1994)

178. Chanter N.: Streptococci and enterococci as animal pathogens. *J Appl Microbiol Symp Suppl* 83, 100S-109S (1997)

179. Timoney J. F.: Strangles. *Vet Clin North Am Equine Pract* 9, 365-374 (1993)

180. Harrington D. J., I. C. Sutcliffe & N. Chanter: The molecular basis of *Streptococcus equi* infection and disease. *Microbes Infect* 4, 501-510 (2002)

181. Anzai T., J. Timoney, Y. Kuwamoto, Y. Fujita, R. Wada & T. Inoue: In vivo pathogenicity and resistance to phagocytosis of *Streptococcus equi* strains with different levels of capsule expression. *Vet Microbiol* 67, 277-286 (1999)

182. Srivastava S. K., D. A. Barnum & J. F. Prescott: Production and biological properties of M-protein of *Streptococcus equi*. *Res Vet Sci* 38, 184-188 (1985)

183. Walker J. & J. Timoney: Construction of a stable non-mucoid deletion mutant of the *Streptococcus equi.* Pinnacle vaccine strain. *Vet Microbiol* 89, 311 (2002)

184. Timoney J. & M. M. Mukhtar: The protective M proteins of the equine group C streptococci. *Vet Microbiol* 37, 389-395 (1993)

185. Chanter N., C. L. Ward, N. C. Collin, J. A. Flanagan, M. Binns, S. B. Houghton, K. C. Smith & J. A. Mumford: Recombinant hyaluronate associated protein as a protective immunogen against *Streptococcus equi* and *Streptococcus zooepidemicus* challenge in mice. *Microb Pathog* 27, 133-143 (1999)

186. Srivastava S. K. & D. A. Barnum: Adherence of *Streptococcus equi* on tongue, cheek and nasal epithelial cells of ponies. *Vet Microbiol* 8, 493-504 (1983)

187. Timoney J. F., S. C. Artiushin & J. S. Boschwitz: Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. *Infect Immun* 65, 3600-3605 (1997)

188. Meehan M., P. Nowlan & P. Owen: Affinity purification and characterization of a fibrinogen-binding protein complex which protects mice against lethal challenge with *Streptococcus equi* subsp. *equi*. *Microbiology* 144, 993–1003 (1998)

189. Meehan M., Y. Lynagh, C. Woods & P. Owen: The fibrinogen-binding protein of *Streptococcus equi* subsp. *equi* additionally binds IgG and contributes to virulence in a mouse model. *Microbiology* 147, 3311-3322 (2001)

190. Meehan M., D. A. Muldowney, N. J. Watkins & P. Owen: Localization and characterization of the ligand-binding domain of the fibrinogen-binding protein (FgBP) of *Streptococcus equi* subsp. *equi*. *Microbiology* 146, 1187-1194 (2000)

191. Boschwitz J. S. & J. F. Timoney: Characterization of the antiphagocytic activity of equine fibrinogen for *Streptococcus equi* subsp. *equi*. *Microb Pathog* 17, 121-129 (1994)

192. Boschwitz J. S. & J. F. Timoney: Inhibition of C3 deposition on *Streptococcus equi* subsp. *equi* by M protein: a mechanism for survival in equine blood. *Infect Immun* 62, 3515-3520 (1994)

193. Chanter N., N. C. Talbot, J. R. Newton, D. Hewson & K. Verheyen: *Streptococcus equi* with truncated M-proteins isolated from outwardly healthy horses. *Microbiology* 146, 1361-1369 (2000)

194. Lindmark H. & B. Guss: SFS, a novel fibronectin-binding protein from *Streptococcus equi*, inhibits the binding between fibronectin and collagen. *Infect Immun* 67, 2383-2388 (1999)

195. Lindmark H., M. Nilsson & B. Guss: Comparison of the fibronectin-binding protein FNE from *Streptococcus equi* subspecies equi with FNZ from *S. equi* subspecies *zooepidemicus* reveals a major and conserved difference. *Infect Immun* 69, 3159-3163 (2001)

196. Flanagan J., N. Collin, J. Timoney, T. Mitchell, J. A. Mumford & N. Chanter: Characterization of the haemolytic activity of *Streptococcus equi*. *Microb Pathog* 24, 211-221 (1998)

197. Anzai T., A. S. Sheoran, Y. Kuwamoto, T. Kondo, R. Wada, T. Inoue & J. Timoney: *Streptococcus equi* but not *Streptococcus zooepidemicus* produces potent mitogenic responses from equine peripheral blood mononuclear cells. *Vet Immunol Immunopathol* 67, 235-246 (1999)

198. Artiushin S. C., J. F. Timoney, A. S. Sheoran & S. K. Muthupalani: Characterization and immunogenicity of pyrogenic mitogens SePE-H and SePE-I of *Streptococcus equi*. *Microb Pathog* 32, 71-85 (2002)

199. Alouf J. E., H. Knoll & W. Kohler: The family of mitogenic shock-inducing and superantigenic toxins from staphylococci and streptococci. In: Sourcebook of bacterial protein toxins. Eds: Alouf J E, Freer J H, Academic Press, London (1991)

200. Proft T., P. D. Webb, V. Handley & F. J. D.: Two novel superantigens found in both Group A and Group C *Streptococcus*. *Infect Immun* 71, 1361-1369 (2003)

201. Timoney J. F., P. J. Timoney & K. L. Strickland: Lysogeny and the immunologically reactive proteins of *Streptococcus equi*. *Vet Rec* 115, 148-149 (1984)

202. Collin N. C., N. Chanter & J. A. Mumford: Detection and characterisation of *Streptococcus equi* and *Streptococcus zooepidemicus* proteases to identify potential protective immunogens. Presented at the Equine Infectious Diseases VIII: Proceedings of the Eighth International Conference, Dubai, United Arab Emirates, (1998)

203. Sharp M. W., M. J. Prince & J. Gibbens: *S. zooepidemicus* infection and bovine mastitis. *Vet Rec* 137, 128 (1995)

204. Anzai T., J. A. Walker, M. B. Blair, T. M. Chambers & J. F. Timoney: Comparison of the phenotypes of *Streptococcus zooepidemicus* isolated from tonsils of healthy horses and specimens obtained from foals and donkeys with pneumonia. *Am J Vet Res* 61, 162-166 (2000)

205. Gilmour M. I., P. Park & M. K. Selgrade: Ozone-enhanced pulmonary infection with *Streptococcus zooepidemicus* in mice. The role of alveolar macrophage function and capsular virulence factors. *Am Rev Respir Dis* 147, 753-760 (1993)

206. Wibawan I. W., F. H. Pasaribu, I. H. Utama, A. Abdulmawjood & C. Lammler: The role of hyaluronic acid capsular material of *Streptococcus equi* subsp. *zooepidemicus* in mediating adherence to HeLa cells and in resisting phagocytosis. *Res Vet Sci* 67, 131-135 (1999)

207. Walker J. & J. F. Timoney: Molecular basis of variation in protective SzP proteins of *Streptococcus zooepidemicus*. *Am J Vet Res* 59, 1129-1133 (1998)

208. Timoney J. F., J. Walker, M. Zhou & J. Ding: Cloning and sequence analysis of a protective M-like protein gene from *Streptococcus equi* subsp. *zooepidemicus*. *Infect Immun* 63, 1440-1445 (1995)

209. Balke E., R. Weiss & A. Seipp: [Hyalurodinase activity of beta-hemolytic streptococci of the Lancefield group C]. *Zentralbl Bakteriol Mikrobiol Hyg [A]* 259, 194-200 (1985)

210. Romanowska D., Z. Szynkiewicz & J. Rita: Antistreptolysin O titer in horses. *Acta Microbiol Pol* 35, 91-95 (1986)

211. Simmonds R. S., W. J. Simpson & J. R. Tagg: Cloning and sequence analysis of *zoo*A, a *Streptococcus zooepidemicus* gene encoding a bacteriocin-like inhibitory substance having a domain structure similar to that of lysostaphin. *Gene* 189, 255-261 (1997)

212. Simmonds R. S., L. Pearson, R. C. Kennedy & J. R. Tagg: Mode of action of a lysostaphin-like bacteriolytic agent produced by *Streptococcus zooepidemicus* 4881. *Appl Environ Microbiol* 62, 4536-4541 (1996)

213. Beatson S. A., G. L. Sloan & R. S. Simmonds: Zoocin A immunity factor: a *femA*-like gene found in a group C *Streptococcus*. *FEMS Microbiol Lett* 163, 73-77 (1998)

214. Lai A. C., S. Tran & R. S. Simmonds: Functional characterization of domains found within a lytic enzyme produced by *Streptococcus equi* subsp. *zooepidemicus*. *FEMS Microbiol Lett* 215, 133-138 (2002)

215. Mitchell T. J.: Virulence factors and the pathogenesis of disease caused by *Streptococcus pneumoniae*. *Res Microbiol* 151, 413-419 (2000)

216. McCullers J. A. & E. I. Tuomanen: Molecular pathogenesis of pneumococcal pneumonia. *Front Biosci* 6, D877-8889 (2001)

217. Chanter N.: *Streptococcus pneumoniae* and equine disease. *Equine Vet J* 26, 5-6 (1994)

218. Burrell M. H., M. E. Mackintosh & C. E. Taylor: Isolation of *Streptococcus pneumoniae* from the respiratory tract of horses. *Equine Vet J* 18, 183-186 (1986)

219. Whatmore A. M., S. J. King, N. C. Doherty, D. Sturgeon, N. Chanter & C. G. Dowson: Molecular characterization of equine Isolates of *Streptococcus pneumoniae*: natural disruption of genes encoding the virulence factors pneumolysin and autolysin. *Infect Immun* 67, 2776-2782 (1999)

220. Colorni A., A. Diamant, A. Eldar, H. Kvitt & A. Zlotkin: *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Dis Aquat Org* 49, 165-170 (2002)

221. Austin B.: Emerging bacterial fish pathogens. *Bull Eur Assoc Fish Pathol* 19, 231-234 (1999)

222. Kitao T.: Streptococcal Infections. In: Bacterial diseases of fish. Eds: Inglis V, Roberts R J, Bromage N R, Blackwell, Oxford (1993)

223. Peir G. B. & S. H. Madin: *Streptococcus iniae* sp. nov., a beta-hemolytic streptococcus isolated from an Amazon freshwater dolphin, *Inia geoffrensis*. *Int J Syst Bacteriol* 26, 545-553 (1976)

224. Eldar A., Y. Bejerano & H. Bercovier: *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal

species causing a meningoencephalitis in fish. *Curr Microbiol* 28, 139-143 (1994)

225. Weinstein M. R., M. Litt, D. A. Kertesz, P. Wyper, D. Rose, M. Coulter, A. McGeer, R. Facklam, C. Ostach, B. M. Willey, A. Borczyk & D. E. Low: Invasive infections due to a fish pathogen, *Streptococcus iniae*. *N Engl J Med* 337, 589-594 (1997)

226. Shoemaker C. A., P. H. Klesius & J. J. Evans: Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. *Am J Vet Res* 62, 174-177 (2001)

227. Peir G. B. & S. H. Madin: Isolation and characterization of a second isolate of *Streptococcus iniae*. *Int J Syst Bacteriol* 28, 311-314 (1978)

228. Bromage E. S., A. Thomas & L. Owens: *Streptococcus iniae*, a bacterial infection in barramundi *Lates calcarifer*. *Dis Aquat Organ* 36, 177-181 (1999)

229. Eldar A. & C. Ghittino: *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. *Dis Aquat Org* 36, 227-231 (1999)

230. Neely M. N., J. D. Pfeifer & M. Caparon: Streptococcus-zebrafish model of bacterial pathogenesis. *Infect Immun* 70, 3904-3914 (2002)

231. Fuller J. D., D. J. Bast, V. Nizet, D. E. Low & J. C. S. de Azavedo: *Streptococcus iniae* Virulence Is Associated with a Distinct Genetic Profile. *Infect Immun* 69, 1994-2000 (2001)

232. Fuller J. D., A. C. Camus, C. L. Duncan, V. Nizet, D. J. Bast, R. L. Thune, D. E. Low & J. C. De Azavedo: Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of *Streptococcus iniae* disease. *Infect Immun* 70, 5730-5739 (2002)

233. Zlotkin A., S. Chilmonczyk, M. Eyngor, A. Hurvitz, C. Ghittino & A. Eldar: Trojan horse effect: phagocyte-mediated *Streptococcus iniae* infection of fish. *Infect Immun* 71, 2318-2325 (2003)

234. Barnes A. C., F. M. Young, M. T. Horne & A. E. Ellis: *Streptococcus iniae*: serological differences, presence of capsule and resistance to immune serum killing. *Dis Aquat Org* 53, 241-247 (2003)

235. Bachrach G., A. Zlotkin, A. Hurvitz, D. L. Evans & A. Eldar: Recovery of *Streptococcus iniae* from diseased fish previuosly vaccinated with a *Streptococcus* vaccine. *Appl Environ Microbiol* 67, 3756-3758 (2001)

236. Lukomski S., J. Burns, E. H. , P. R. Wyde, A. Podbielski, J. Rurangirwa, D. K. Moore-Poveda & J. M. Musser: Genetic inactivation of an extracellular cysteine protease (SpeB) expressed by *Streptococcus pyogenes* decreases resistance to phagocytosis and dissemination to organs. *Infect Immun* 66, 771-776 (1998)

237. Taylor S. L., L. Jaso-Friedmann, A. B. Allison, A. Eldar & D. L. Evans: *Streptococcus iniae* inhibition of apoptosis of nonspecific cytotoxic cells: a mechanism of activation of innate immunity in teleosts. *Dis Aquat Org* 46, 15-21 (2001)

238. Evans D. L., S. L. Taylor, J. H. Leary III, G. R. Bishop, A. Eldar & L. Jaso-Friedmann: In vivo activation of tilapia nonspecific cytotoxic cells by *Streptococcus iniae* and amplification with apoptosis regulatory factor(s). *Fish Shellfish Immunol* 10, 419-434 (2000)

239. Devriese L. A., J. Hommez, R. Kilpper-Bälz & K. H. Schleifer: *Streptococcus canis* sp. nov.: a species of group G streptococci from animals. *Int J Syst Bacteriol* 36, 422-425 (1986)

240. Takeda N., K. Kikuchi, R. Asano, T. Harada, K. Totsuka, T. Sumiyoshi, T. Uchiyama & S. Hosoda: Recurrent septicemia caused by *Streptococcus canis* after a dog bite. *Scand J Infect Dis* 33, 927-928 (2001)

241. Whatmore A. M., K. H. Engler, G. Gudmundsdottir & A. Efstratiou: Identification of isolates of *Streptococcus canis* infecting humans. *J Clin Microbiol* 39, 4196-4199 (2001)

242. Bert F. & N. Lambert-Zechovsky: Septicemia caused by Streptococcus canis in a human. *J Clin Microbiol* 35, 777-779 (1997)

243. DeWinter L. M., D. E. Low & J. F. Prescott: Virulence of *Streptococcus canis* from canine streptococcal toxic shock syndrome and necrotising fasciitis. *Vet Microbiol* 70, 95-110 (1999)

244. Miller C. W., J. F. Prescot, K. A. Mathews, S. D. Betschel, J. A. Yager, V. Guru, L. DeWinter & D. E. Low: Streptococcal toxic shock syndrome in dogs. *J Am Vet Med Assoc* 209, 1421-1426 (1996)

245. Cleary P. P., J. Peterson, C. Chen & C. Nelson: Virulent human strains of group G streptococci express a C5a peptidase enzyme similar to that produced by group A streptococci. *Infect Immun* 59, 2305-2310 (1991)

246. Yutsudo T., K. Okumura, M. Iwasaki, A. Hara, S. Kamitani, W. Minamide, H. Igarashi & Y. Hinuma: The gene encoding a new mitogenic factor in a *Streptococcus pyogenes* strain is distributed only in group A streptococci. *Infect Immun* 62, 4000-4004 (1994)

247. Simpson W. J., J. C. Robbins & P. P. Cleary: Evidence for group A-related M protein genes in human but not animal-associated group G streptococcal pathogens. *Microb Pathog* 3, 339-350 (1987)

248. Schnitzler N., A. Podbielski, G. Baumgarten, M. Mignon & A. Kaufhold: M or M-like protein gene polymorphisms in human group G streptococci. *J Clin Microbiol* 33, 356-363 (1995)

249. Fischetti V. A.: Streptococcal M protein: molecular design and biological behavior. *Clin Microbiol Rev* 2, 285-314 (1989)

250. Ullberg M., I. Karlsson, B. Wiman & G. Kronvall: Two types of receptors for human plasminogen on group G streptococci. *APMIS* 100, 21-28 (1992)

251. Lammler C., C. Frede, K. Gurturk, A. Hildebrand & H. Blobel: Binding activity of *Streptococcus canis* for albumin and other plasma proteins. *J Gen Microbiol* 134(Pt 8), 2317-2323 (1988)

252. DeWinter L. M. & J. F. Prescott: Relatedness of *Streptococcus canis* from canine streptococcal toxic shock syndrome and necrotizing fasciitis. *Can J Vet Res* 63, 90-95 (1999)

253. Soedarmanto I. & C. Lammler: Comparative studies on streptococci of serological group G isolated from various origins. *Zentralbl Veterinarmed [B]* 43, 513-523 (1996)

254. Clark R. B., J. F. Berrafati, J. M. Janda & E. J. Bottone: Biotyping and exoenzyme profiling as an aid in the differentiation of human from bovine group G streptococci. *J Clin Microbiol* 20, 706-710 (1984)

255. Tapsall J. W. & E. A. Phillips: *Streptococcus pyogenes* streptolysin O as a cause of false-positive CAMP reactions. *J Clin Microbiol* 19, 534-537 (1984)

256. Gurturk K. & C. Lammler: Purification and partial characterization of a cohaemolysin (CAMP-factor) produced by *Streptococcus canis*. *FEMS Microbiol Immunol* 2, 97-102 (1990)

257. Hardie J. M.: Other streptococci. In: Bergey's manual of systematic bacteriology. Eds: Sneath P H A, Mair N S, Sharpe M E, Holt J G, Williams & Wilkins, Baltimore (1986)

258. Farrow J. A. E., J. Kruze, B. A. Phillips, A. J. Bramley & M. D. Collins: Taxonomic studies on *Streptococcus bovis* and *Streptococcus equinus*: description of *Streptococcus alactolyticus* sp. nov. and *Streptococcus saccharolyticus* sp. nov. *Syst Appl Microbiol* 5, 467-482 (1984)

259. Brooker J. D., L. A. O'Donovan, I. Skene, K. Clarke, L. Blackall & P. Muslera: *Streptococcus caprinus* sp. nov., a tannin-resistant ruminal bacterium from feral goats. *Lett Appl Microbiol* 18, 313-318 (1994)

260. Osawa R., T. Fujisawa & L. I. Sly: *Streptococcus gallolyticus* sp. nov.; gallate degrading organisms formerly assigned to *Streptococcus bovis*. *Syst Appl Microbiol* 18, 74-78 (1995)

261. Sly L. I., M. M. Cahill, R. Osawa & T. Fujisawa: The tannin-degrading species *Streptococcus gallolyticus* and *Streptococcus caprinus* are subjective synonyms. *Int J Syst Bacteriol* 47, 893-894 (1997)

262. Devriese L. A., P. Vandamme, B. Pot, M. Vanrobaeys, K. Kersters & F. Haesebrouck: Differentiation between *Streptococcus gallolyticus* Strains of Human Clinical and Veterinary Origins and *Streptococcus bovis* Strains from the Intestinal Tracts of Ruminants. *J Clin Microbiol* 36, 3520-3523 (1998)

263. Stewart C. S., H. J. Flint & M. P. Bryant: The rumen bacteria. In: The rumen microbial ecosystem. Eds: Hobson P N, Stewart C S, Blackie Academic and Professional, London (1997)

264. Nelms L. F., D. A. Odelson, T. R. Whitehead & R. B. Hespell: Differentiation of ruminal and human *Streptococcus bovis* strains by DNA homology and 16S rRNA probes. *Curr Microbiol* 31, 294-300 (1995)

265. De Herdt P., F. Haesebrouck, R. Ducatelle, B. De Groote & L. A. Devriese: *Streptococcus bovis* infections in pigeons: virulence of different serotypes. *Vet Microbiol* 41, 321-332 (1994)

266. Vanrobaeys M., P. De Herdt, F. Haesebrouck, R. Ducatelle & L. A. Devriese: Secreted antigens as virulence associated markers in *Streptococcus bovis* strains from pigeons. *Vet Microbiol* 53, 339-348 (1996)

267. Vanrobaeys M., P. De Herdt, R. Ducatelle, W. Creten & F. Haesebrouck: Extracellular proteins and virulence of *Streptococcus bovis* isolates from pigeons. *Vet Microbiol* 59, 59-66 (1997)

268. Vanrobaeys M., F. Haesebrouck, R. Ducatelle & P. De Herdt: Identification of virulence associated markers in the cell wall of pigeon *Streptococcus gallolyticus* strains. *Vet Microbiol* 73, 319-325 (2000)

269. Vanrobaeys M., P. De Herdt, G. Charlier, R. Ducatelle & F. Haesebrouck: Ultrastructure of surface components of *Streptococcus gallolyticus* (*S. bovis*) strains of differing virulence isolated from pigeons. *Microbiology* 145(Pt 2), 335-342 (1999)

270. Whitford M. F., M. A. McPherson, R. J. Forster & R. M. Teather: Identification of bacteriocin-like inhibitors from rumen *Streptococcus* spp. and isolation and characterization of Bovicin 255. *Appl Environ Microbiol* 67, 569-574 (2001)

Key Words: Bacteria, microorganism, Streptococci, Animal Disease, Virulence Factors, Toxins, Capsule, M proteins, Review

Send correspondence to: Marcelo Gottschalk. GREMIP, Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, C.P. 5000, St-Hyacinthe, Québec, J2S 7C6, Canada, Tel: 450-773-8521 ext. 8374, Fax: 450-778-8108, E-mail: gottschm@medvet.umontreal.ca