HISTOPHILUS SOMNI: PATHOGENICITY IN CATTLE. AN UPDATE

Histophilus somni: actualización de la patogenicidad en vacuno

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ABSTRACT

Histophilus somni (*H. somni*) is a Gram-negative bacterium currently classified as a member of the *Haemophilus-Actinobacillus-Pasteurella* group. Clinical syndromes associated with *H. somni* infection involve thromboembolic meningoencephalitis, pneumonia and disease of the reproductive tract in cattle. Animals can be carriers of non-pathogenic variants of the organism, mainly in the genital mucosa. The causes of these differences in virulence between strains are not defined. Several determinants of virulence of the pathogen are proposed. However, many of these factors cannot be clearly related to clinical disease. *H. somni* avoids killing by phagocytic cells. Thus, it is able to evade the immune response by intracellular survival in the infected host. The bovine adaptive immune response against *H. somni* is not completely characterized. IgG_2 antibodies are thought to be protective. However, the major antigen determinants of the bacterium are still unknown. Studies with *H. somni* bacterins have inconsistent results, especially because the factors involved in pathogenesis and immune response remain unclear.

Key words: *Histophilus somni*, cattle, clinical syndromes, virulence factors, carriers, diagnosis, vaccines.

RESUMEN

Histophilus somni (*H. somni*) es una bacteria Gram negativa actualmente clasificada como un miembro del grupo *Haemophilus-Actinobacillus-Pasteurella* (HAP). Los síndromes clínicos en el ganado bovino asociados con la infección por *H. somni* incluyen meningoencefalitis tromboembólica, neumonía y enfermedad del tracto reproductivo. Los animales pueden ser portadores de variantes no patogénicas del microorganismo, principalmente en la mucosa genital. Las causas de las diferencias en la virulencia entre cepas no están defini-

das. Se propusieron varios determinantes de la virulencia del patógeno. Sin embargo, muchos de estos factores no pueden relacionarse claramente con la enfermedad clínica. *H. somni* elude la destrucción por parte de las células fagocíticas. De este modo, es capaz de evadir la respuesta inmunitaria a través de la supervivencia en forma intracelular en el huésped infectado. La respuesta inmune adaptativa del bovino contra *H. somni* no está caracterizada completamente. Se estima que los anticuerpos IgG_2 son protectores. Sin embargo, los principales determinantes antigénicos de la bacteria se desconocen. Los estudios con las bacterinas de *H. somni* han tenido resultados inconsistentes, especialmente debido a que los factores involucrados en la patogénesis y en la respuesta inmunitaria permanecen sin esclarecer.

Palabras claves: *Histophilus somni,* vacuno, síndromes clínicos, factores de virulencia, portadores, diagnóstico, vacunas.

INTRODUCTION

Histophilus somni (*H. somni*; ex-*Haemophilus somnus*) is a Gram-negative bacterium responsible for a variety of clinical syndromes in cattle. These syndromes are particularly common in feedlot and dairy calves, although they can also be observed in grazing cattle (Griner *et al.* 1956; Crandell *et al.* 1977; Smith and Biberstein, 1977; Bastida-Corcuera *et al.* 1999). Thromboembolic meningoencephalitis (TEME), pneumonia and pathological disorders of the reproductive tract are the most frequently observed clinical manifestations (Griner *et al.* 1956; Firehammer 1959; Kennedy *et al.* 1960; Chladek 1975; Smith and Biberstein 1977; Humphrey *et al.* 1982; Miller *et al.* 1983a, 1983b; Andrews *et al.* 1985; Corbeil *et al.* 1985; Ames 1987; Jackson *et al.* 1987; Gogolewski *et al.* 1987a, 1987b, 1988; Potgieter *et al.* 1988; Haritani *et al.* 1990; Kwiecien and Little 1990; Butt *et al.* 1993; Tegtmeier *et al*. 2000; Descarga *et al.* 2002). The occurrence of asymptomatic carriers, mainly in the genital tract, is relevant for the epidemiology, diagnosis and control of the disease complex (Corbeil *et al.* 1985; Rosendal y Boyd 1986; Groom *et al.* 1988; Greer *et al.* 1989; Kwiecien and Little 1990; Nielsen 1990; Corbeil 1990; Sylte *et al.* 2001). Although the existence of pathogenic and non-pathogenic strains is clearly defined, the factors that determine these different phenotypes are still unknown. In this work, the clinical syndromes associated with *H. somni*, as well as the current knowledge on the carrier state, virulence determinants and host immune response are described. Considerations on the epidemiology of the disease, diagnosis and vaccine development are also presented.

BIOLOGICAL PROPERTIES OF *H. SOMNI* AND TAXONOMIC CONSIDERATIONS

H. somni is a small, Gram-negative, non-spore forming, pleomorphic bacilli (Kennedy *et al.* 1960; Rosendal and Boyd 1986). It does not have a polysaccharide capsule and it does not express pili or flagella (Firehammer 1959; Kennedy *et al.* 1960). Identification of *H. somni* by the routinely used biochemical tests is difficult due to its poor growth characteristic. The bacterium grows in a 5-10% CO_2 atmosphere (Kennedy *et al.* 1960; Claus y Rikihisa 1986; Inzana and Corbeil 1987) and growth is enhanced by the addition of pyridoxine, flavin mononucleotide, riboflavin and thiamine monophosphate to the medium (Inzana and Corbeil 1987). As quality control standards for *H. somni,* it is recommended cation-adjusted Mueller-Hinton broth and chocolate Mueller-Hinton agar for microdilution and disk diffusion testing, respectively (McDertmont *et al.* 2001). *H. somni* ferments glucose, reduces nitrate, and it is oxidase-positive and catalase-negative (Waldhalm *et al.* 1974). Bacteria of the *Haemophilus* group (*H. somni* was previously classified within this group) require either hemin (X factor) or nicotinamide adenine dinucleotide (NAD, V factor)

for optimal growth (Rosendal and Boyd 1960; Angen *et al.* 2003). Satellitism is a phenomenon that describes the ability of factor V-dependent *Haemophilus* colonies to utilize NAD secreted by other bacteria, such as *Staphylococcus aureus* (Fink and St. Geme III 2006) and it is used for the diagnosis of *H. somni*. However, the fact that *H. somni* isolates do not require any of these factors for growth made uncertain its taxonomic position (Humphrey *et al.* 1982; Claus and Rikihisa 1986; Rosendal and Boyd 1986). *H. somni* shares 60% and 44% of genetic homology with *Haemophilus influenzae* and *Actinobacillus lignieresii*, respectively (Rosendal and Boyd 1986). Nevertheless, its close phenotypic relationship with *Haemophilus agni* (*H. agni)* and *Histophilus ovis* (*H. ovis*) (Canto *et al.* 1983; Rosendal and Boyd 1986; Ounnariwo *et al.* 1990; Ward *et al.* 1995) suggests that these organisms should be considered in a separate taxon of the *Haemophilus-Actinobacillus-Pasteurella* (HAP) group. Protein profiles from *H. agni* and *H. ovis* are very similar to bovine *H. somni* (Ounnariwo *et al.* 1990). In addition, members of the family *Pasteurellaceae* have genomic patterns completely different from those of *H. somni* and *H. ovis*, whereas isolates of *H. ovis* are indistinguishable from those of *H. somni* (Appuhamy *et al.* 1995). Recently, it was determined that *H. somni*, *H. agni* and *H. ovis* represent the same species and it was proposed to allocate them to a novel genus within the family *Pasteurellaceae*, with the name *Histophilus somni* (Angen *et al.* 2003).

VIRULENCE FACTORS

H. somni can cause localized or septicemic disease (Corbeil 1990). However, the determinants of virulence and the mechanisms involved in the development of the different pathological conditions have not been clearly defined (Inzana and Todd 1992). The following are some of the factors that would contribute to *H. somni* infection:

Adherence. *H. somni* colonizes the surface of the mucous membranes and attaches to non-epithelial cells (Corbeil *et al.* 1985). Both live and formalin-killed *H. somni* adhere to bovine aortic endothelial cells (Thompson and Little 1981; Kwiecien *et al.* 1994). Likely, non-pilus adhesins are involved in the adherence of the organism to the cell surface (Sethi and Murphy 2001). A bacterial surface protein, p76, would play a role in adherence (Sanders *et al.* 2003).

Iron uptake. Competition of *H. somni* and the host for nutrients seems to be key for *in vivo* multiplication of the pathogen (Corbeil 1990). In the host, most of the extracellular iron is bound to transferrin or lactoferrin and the bacterium utilizes these iron pools in order to sustain its growth (Ounnariwo *et al.* 1990; Sethi and Murphy 2001). Studies on experimentally-induced *H. somni* pneumonia demonstrated that calves with the lowest pre-exposure serum transferrin levels developed the most severe pneumonic lesions. Thus, serum transferrin concentration at the time of exposure may influence the outcome of the infection (McNair 1998). The acquisition of iron from transferrin depends on transferrin-binding proteins, which are present in the bacterial outer membrane (Sethi and Murphy 2001). The iron-regulated outer membrane proteins (OMPs) of *H. somni* seem to differ among strains. Particularly, genital isolates showed a high variability in their iron-regulated protein profiles (Wedderkopp *et al.* 1993). *H. somni* utilizes a receptor-mediated mechanism for iron acquisition and it is able to obtain iron for growth from transferrin of bovine origin, but not from other mammalian species (Ounnariwo *et al.* 1990). For example, *H. somni* binds bovine transferrin, but not ovine transferrin. This was proposed as one of the causes of the host specificity of *H. somni*. However, *H. somni* is also associated with respiratory disease in American bison and domestic sheep, which harbor the microorganism in their reproductive tract (Ward *et al.* 1995).

Antigenic variation of surface proteins. OMPs are important immunological structures because they are accessible to the host defense mechanisms (Nielsen 1990; Tagawa *et al.* 1993). The major OMP of *H. somni* strains are diverse in molecular mass and antigenic reactivity (Tagawa *et al.* 2003) and this may play an important role in the ability of the organisms to cause infection (Sethi and Murphy 2001). Two *H. somni* OMPs with molecular mass of 46 and 14 kDa, common to all isolates tested, were identified (Thomson *et al.* 1988). Moreover, a 37 kDa heat-modifiable OMP (Tagawa *et al.* 1993) and a 78 kDa OMP (Kania *et al.* 1990), which are surface exposed and highly conserved between isolates from carrier animals and animals with clinical disease were also described (Kania *et al.* 1990). A lipoprotein, *lpp*B, is also predominantly expressed in the outer membrane fraction of *H. somni* (Theisen *et al.* 1993). However, the involvement of these proteins in *H. somni* pathogenesis is unknown. Immunization of calves with a 40 kDa fraction of OMP generates a humoral response (Corbeil *et al.* 1987). Although this molecule can be an important virulence factor, the mechanisms involved in protection are not determined (Corbeil 1990).

Heterogeneity and phase variation of lipooligosaccharides. *H. somni* lipopolysaccharides (LPS) lack the long, repeated polysaccharide side chains, typically described for enteric Gram-negative bacteria. Thus, lipooligosaccharides (LOS) is a more accurate designation for these structures. *H. somni* is able to incorporate neuraminic acid into its LOS. This process, known as sialylation, may block the binding of antibodies to certain epitopes and enhances the resistance to the bactericidal activity of serum (Corbeil 1990; Inzana *et al.* 2002). Most clinical isolates are capable of LOS sialylation, whereas preputial isolates are not (Inzana *et al.* 2002; Siddaramppa and Inzana 2004). *H. somni* LOS can also be altered by phase variation (Inzana *et al.* 1992), a mechanism that appears to enable pathogenic strains of *H. somni* to persist systemically by escaping recognition by the host immune response (Corbeil 1990; Inzana *et al.* 1992).

Immunoglobulin-binding proteins (IgBPs). IgBPs are thought to enable *H. somni* to evade antibody defenses (Corbeil and Bon-Durant 2001). High molecular weight (HMW) IgBPs and p76 are present in virulent strains of *H. somni*, (serum-resistant or SR strains), but not in the isolates from asymptomatic carriers (serum-sensitive or SS strains) (Corbeil *et al.* 1997a). Likely, HMW IgBPs are the main component of the fibrillar network that morphologically characterizes IgBP-positive strains and which is absent in IgBP-negative strains. IgBP-positive strains are able to bind IgG_2b, but not IgG_2a (Corbeil *et al.* 1997a, 1997b). This difference in Fc binding between immunoglobulin allotypes to *H. somni* IgBPs was suggested to be relevant to disease since pathogenic *H. somni* strains with IgBP fibrils are resistant to complement-mediated killing (Bastida-Corcuera *et al.* 1999).

Non-immune mechanism of immunoglobulin binding. This mechanism enables *H. somni* to bind both IgM and IgG and it may represent a microbial specific adaptation for evasion of the host effector functions (Gogolewski *et al.* 1988), by masking bacterial surface structures that are target of antibodies or complement and by reducing opsonization and susceptibility to phagocytosis and killing (Widders *et al.* 1989).

Immunoglobulin Fc receptors. Immunoglobulin Fc receptors are identified both on the surface of *H. somni* and in the fluid phase (Corbeil 1990). It is known that both pathogenic and vaginal carrier isolates display binding of immunoglobulin Fc. However, this activity was not detected in preputial isolates (Widders *et al.* 1989).

Histamine and hemolysin production. *H. somni* is able to synthesize and secrete histamine. Thus, an IgE response might be involved in the pathogenesis of some infections, especially

in the pneumonic form (Ruby *et al.* 2002). A 31 kDa protein that possesses hemolytic activity was identified (Won and Griffith 1993). However, studies associating *H. somni* hemolysin and virulence have not been conducted.

SEROGROUPING OF *H. SOMNI* STRAINS

Information on serogroups of *H. somni* would be a valuable tool to assist in transmission studies and in the development of vaccines (Canto and Biberstein 1982; Won and Griffith 1993). However, the attempts for grouping the serological variants of *H. somni* have been inconclusive. Six different variants of *H. somni* have been described. Variants 1, 2 and 3 have been isolated from cattle (Bretschneider and Cipolla 1999). The major antigens detected by serology are heat-stable and are probably located outside the LOS fraction since purified LOS are un-reactive (Won and Griffith 1993). Moreover, antigens containing *H. somni* LOS are not useful for serotyping due to their antigenic phase variation (Howard *et al.* 2000). Tagawa *et al.* (2000) grouped *H. somni* variants according to their molecular mass and monoclonal antibody reactivity of the major OMPs. They were able to group the strains isolated from different pathological conditions. However, they found that isolates from asymptomatic carriers were distributed among all groups. These results suggest that it will be necessary to identify the major sero-reactive antigens of *H. somni* and to develop proper serological tests for an accurate classification of the different variants.

INTERACTION OF *H. SOMNI* WITH PHAGOCYTIC CELLS

Lesions observed in natural infections by *H. somni* are characterized by the presence of a large number of polymorphonuclear (PMN) cells (Czuprynzki and Hamilton 1985; Hubbard *et al.* 1986). Bovine neutrophils phagocytize *H. somni* (Andrews *et al.* 1985). However, they are unable to kill the bacterium (Czuprynzki and Hamilton 1985; Hubbard *et al.* 1986; Lederer *et al.* 1987; Czuprynski and Sample 1990; Pfeifer *et al.* 1992; Yang *et al.* 1998). The survival of *H. somni* within PMNs would protect the pathogen from host defenses, allowing the invasion of different tissues (Pfeifer *et al.* 1992). It would also explain the difficultness to isolate the organism from infected tissues or during bacteremia (Pfeifer *et al.* 1992) and would contribute to the establishment of chronic, multisystemic infections (Lederer *et al.* 1987). *H. somni* interferes with the oxidative burst of bovine neutrophils (Czuprynzki and Hamilton 1985). Hubbard *et al.* (1986) also suggested that *H. somni* would inhibit either the process of degranulation of primary granules or the myeloperoxidase activity of neutrophils. *H. somni* stimulates the production of nitric oxide (NO) by the infected PMNs. However, NO production is not an effective bactericidal mechanism against the pathogen (Gomis *et al.* 1997). Furthermore, Yang *et al.* (1998) demonstrated that only direct contact of intact, non-viable bacterium with bovine neutrophils is sufficient to induce cellular apoptosis.

PATHOGENESIS OF THE SEPTICEMIC DISEASE

Clinical disease associated with *H. somni* septicemia is classically divided into peracute, acute, subacute and chronic. Only the peracute and acute forms of the disease involve the nervous system (Ames 1987). Invasion of the bloodstream is the first step in the acute infections induced by *H. somni*. It is not known whether the bacteria harbored in the respiratory or genital tract are invasive. Although respiratory disease preceding development of TEME has been described (Rosendal and Boyd 1986), it was shown that after intra-tracheal inoculation, *H. somni* is localized in the alveoli and bronchiolar lumen without detection of bacteremia (Groom *et al.* 1988). Thus, invasion and spread

from the lungs seem uncommon. Thrombi formation in the brain and kidney is observed after bacteremia (Stephens *et al.* 1981; Rosendal and Boyd 1986). Apoptosis of endothelial cells caused by the bacterium might be responsible for the induction of thrombosis (Sylte *et al.* 2001). *H. somni* LOS induce cell death by promoting the generation of intracellular reactive oxygen species and NO (Sylte *et al.* 2001, 2004). Apoptosis would also contribute to *H. somni* pathogenesis through the impairment of immune surveillance and promotion of bacterial survival (Sylte *et al.* 2006). LOS-induced apoptosis and adherence of the organism to the vascular endothelium are enhanced by TNF-α (Sylte *et al.* 2006). Likely, this cytokine, produced during *in vivo H. somni* bacteremia, causes pro-coagulant activation and vascular damage that facilitates the development of lesions (Kwiecien *et al.* 1994). Thompson and Little (1981) suggested a probable sequence of events involved in the vascular pathology: a) adhesion of *H. somni* to vascular endothelium; b) contraction and desquamation of cells with exposure of sub-endothelial collagen and; c) thrombosis and vasculitis with the consequent ischemic necrosis of the adjacent parenchyma. The high antibody titers observed after intravenous inoculation of *H. somni* might also lead to the formation of antigen/antibody complexes in the bloodstream, generating a type III hypersensitivity reaction that causes vasculitis (Stephens *et al.* 1981). However, vasculitis was also observed in *H. somni* antibody-negative calves, suggesting that bacteria/antibody complexes are unlikely to contribute to the pathology.

EXPERIMENTAL MODELS

Appropriate laboratory animal models for the study of infections by *H. somni* are not available. Mice and hamsters are susceptible to *H. somni*-induced abortion after intra-peritoneal inoculation (Inzana and Todd 1992). Intravenous and intra-peritoneal inoculation of an encephalitic strain of *H. somni* in a wide range of laboratory animal species only caused some non-characteristic lesions in hamsters (Dewey and Little 1984b). The intra-cisternal calf assay is regarded as the only reliable method to differentiate pathogenic from non-pathogenic strains of *H. somni*. However, this is a costly assay and it is usually subject to human concerns (Kwiecien and Little 1992). Endo-bronchial and endo-tracheal inoculation of *H. somni* in calves consistently resulted in pneumonic lesions that resemble the naturally occurring disease (Jackson *et al.* 1987; Potgieter *et al.* 1988). However, the appearance of an unusual number of abscesses after endo-tracheal inoculation, suggests that a high number of bacteria is introduced into focal sites when this methodology is used (Jackson *et al.* 1987). These data suggest that the elaboration of *in vitro* tests would be useful for studies of pathogenicity of *H. somni* strains and for the screening of carrier animals, especially for exported live cattle and semen samples (Kwiecien and Little 1992).

EPIDEMIOLOGY

H. somni is a commensal of the mucosal surface of the respiratory and genital tracts and the agent usually spreads by direct contact (Rosendal and Boyd 1986; Kwiecien and Little 1992). Broncho-alveolar lavage fluids might contain a high number of bacteria, even when nasal cultures are negative. This finding implies that once established, *H. somni* survives more readily in the broncho-alveolar area than in the nasal mucosa and, as a consequence, coughing is a likely route for the transmission of the infection in animals that are negative to nasal cultures (Gogolewski *et al.* 1989). Whole blood and nasal mucus are also a potential source of infection since the bacterium is viable in these exudates up to 70 days at 23.5 ºC. In a lesser extent, vaginal and nasal mucus can also be potentially infective, with bacterial survival detected for 5 days (Dewey and Little 1984a). Carrier animals

must also be considered as another potential source of transmission of *H. somni* (Rosendal and Boyd 1986).

Carrier state. *H. somni* is an organism for which both pathogenic and non-pathogenic variants exist since cattle carrying the bacterium in absence of clinical disease is commonly detected. *H. somni* can be isolated from semen and preputial or vaginal mucosa from asymptomatic cattle, whereas pneumonic and encephalitic isolates always cause disease (Corbeil 1990; Groom *et al.* 1988). These findings demonstrate that *H. somni* is a commensal adapted to the genital mucosa, which rarely produces a local inflammatory response (Kwiecien *et al.* 1994). Corbeil *et al.* (1985) suggested that the presence of specific bacteria in the normal flora from one animal to another may account for some of the differences in strain virulence. Changes in the normal flora might lead to the development of disease in carrier animals. For example, *Bacillus spp*., which is usually present in the regular mucosal flora, can affect the growth of *H. somni* and *Pasteurella spp*. *H. somni* is rarely isolated from the normal upper respiratory tract, although it is able to adhere to the nasal epithelium (Rosendal and Boyd 1986). Therefore, enhancer species present among the genital, but not in the nasal flora, are a likely cause for the higher number of genital carriers (Corbeil *et al.* 1985). Asymptomatic carrier isolates do not cause septicemia and vasculitis. Thus, it seems that *in vivo*, these isolates are unable to reach the vascular endothelium (Starost 2001).

IMMUNE RESPONSE TO *H. SOMNI*

The common occurrence of *H. somni* in the reproductive tract states the possibility that many clinically normal cattle possess antibodies against the organism (Nielsen 1990). According to these findings, Stephens *et al.* (1981) found that 91.2% of asymptomatic cattle had titers to *H. somni*. Widders *et al.* (1986) demonstrated a high and persistent IgG_2 response during natural infection and after intra-venous and intra-bronchial administration of *H. somni*. Therefore, it was proposed that IgG_2 antibodies might have a role in limiting the hematogenous dissemination of the organism. Antibodies in convalescent phase sera are directed to one or more antigenically variable determinants of the major OMP (Gogolewski *et al.* 1987b; Tagawa *et al.* 2000) and this protective antibody activity may be strain specific (Tagawa *et al.* 1993). *In vivo* studies demonstrated that convalescent serum from pneumonic cattle protects against *H. somni*-induced acute pneumonia and protection was correlated with both high IgG_1 and IgG_2 antibody titers (Gogolewski *et al.* 1987b). High levels of agglutinins have been detected in cattle that died after experimentally induced TEME. Agglutinins antibodies are described as bactericidal antibodies that act by a complement-independent mechanism (Simonson and Maheswaran 1982).

CLINICAL SYNDROMES

Thromboembolic meningoencephalitis (TEME)

TEME is the most important condition caused by *H. somni* (Rosendal and Boyd 1986; Descarga *et al.* 2002). It was first described in 1956, in Colorado (Griner *et al.* 1956). The causative agent was identified in 1960, as a *Haemophilus*-like organism (Kennedy *et al.* 1960). TEME occurs typically in feedlot calves, especially a few weeks after their arrival (Griner *et al.* 1956; Rosendal and Boyd 1986; Descarga *et al.* 2002). However, the syndrome has also been described in grazing cattle (Griner *et al.* 1956; Smith and Biberstein 1977). The morbidity is usually low but high mortality is observed (Rosendal and Boyd 1986). Death can occur within 12 hours of the onset of clinical signs (Kennedy *et al.* 1960; Smith and Biberstein 1977; Descarga *et al.* 2002) or the clinical manifestations can last as long as

three weeks (Griner *et al.* 1956). Clinical signs involve blindness, recumbency, depression, fever, anorexia and periods of excitement and irritability (Griner *et al.* 1956; Kennedy *et al.* 1960; Smith and Biberstein 1977; Stephens *et al.* 1981; Descarga *et al.* 2002). Although less frequently, strabismus, nistagmus and opisthotonus can occur (Kennedy *et al.* 1960). Gross lesions are mainly present in the brain and are usually bilateral and asymmetrical (Griner *et al.* 1956; Stephens *et al.* 1981). Commonly, brain lesions consist of multifocal, dark-red areas of necrosis and fibrinopurulent meningitis (Kennedy *et al.* 1960; Smith and Biberstein 1977; Stephens *et al.* 1981; Momotani *et al.* 1985). Pericarditis, myocarditis, bronchopneumonia, hemorrhagic lymphadenitis, renal infarcts and polyarthritis are also present in cases of TEME (Griner *et al.* 1956; Kennedy *et al.* 1960; Smith and Biberstein 1977). Microscopic lesions are characterized by vasculitis with neutrophilic infiltration, degeneration of macrophages, and numerous lymphocytic perivascular cuffs and areas of focal necrosis (Griner *et al.* 1956; Descarga *et al.* 2002; Kennedy *et al.* 1960; Momotani *et al.* 1985; Smith and Biberstein 1977; Stephens *et al.* 1981). Randomly distributed thrombi, composed of fibrin, leukocytes and bacterial colonies are present in all cases (Griner *et al.* 1956, Kennedy *et al.* 1960; Stephens *et al.* 1981; Momotani *et al.* 1985). Microabscessation and myelitis have also been reported (Griner *et al.* 1956; Smith and Biberstein 1977). Polioencephalomalacia, lead poisoning and listeriosis must be considered in the differential diagnosis of TEME (Ames 1987; Smith and Biberstein 1977).

Pneumonia

Diseases of the respiratory tract are of important economical concern both in beef and dairy cattle. Stress, concomitant infections or immunosuppression are required for the establishment of bacterial pneumonia (Tegtmeier *et al.* 2000). Septicemic invasion rarely occurs after a pneumonic *H. somni* infection (Rosendal and Boyd 1986; Potgieter *et al.* 1988; Groom *et al.* 1988). Pneumonic disease can be easily reproduced with pneumonic *H. somni* isolates. In contrast, preputial and encephalitic strains fail to induce the disease after intra-tracheal inoculation. In addition, these strains seem to be more efficiently cleared from the lung parenchyma (Groom *et al.* 1988). *H. somni* is commonly associated with the undifferentiated bovine respiratory disease (UBRD) complex. Nevertheless, it is not considered as the main agent associated with this condition (O´Connor *et al.* 2001). In some cases, *H. somni* is the sole agent recovered from pneumonic lesions (Tegtmeier *et al.* 2000). However, concurrent infections with *P. multocida*, *M. haemolytica* and *C. pyogenes* can also be detected (Haritani *et al.* 1990). Lesions observed in natural cases of *H. somni* respiratory disease consist of fibrinous suppurative pneumonia and bronchopneumonia (Andrews *et al.* 1985; Jackson *et al.* 1987). Commonly, necrotizing bronchiolitis, vasculitis and interstitial inflammation are observed (Andrews *et al.* 1985; Gogolewski *et al.* 1987a). By immunohistological staining, *H. somni* can be detected in large aggregates within alveoli, perivascular connective tissue, airway exudates, and within the cytoplasm of alveolar macrophages (Gogolewski *et al.* 1987a; Tegtmeier *et al.* 1999). Degeneration of alveolar macrophages is described as characteristic of *H. somni* pneumonia (Gogolewski *et al.* 1989). Contrary to the rapid spread of *M. haemolytica* in the respiratory tract, *H. somni* has little tendency to disseminate within the lungs, a fact that would contribute to the chronic progression of the disease (Potgieter *et al.* 1988).

Reproductive tract disease

Both pathogenic and non-pathogenic strains of *H. somni* colonize the bovine reproductive tract (Bastida-Corcuera *et al.* 1999; Corbeil

1990; Kwiecien and Little 1992). Although the differences in virulence between isolates from neurological disease and from the reproductive tract are evident, there are no biochemical features that allow distinction between these strains (Miller *et al.* 1983a).

Female reproductive tract. Reproductive failure caused by *H. somni* can occur either via systemic hematogenous dissemination (abortion) or by ascending route from vagina (Corbeil and BonDurant 2001). *H. somni* causes vulvovaginitis, endometritis, cervicitis and abortion (Firehammer 1956; Chladek 1975; Widders *et al.* 1986; Hoblet *et al.* 1989). However, under natural conditions, the pathological significance of the isolation of *H. somni* from vaginal discharges is difficult to interpret. *H. somni* is isolated at higher rates from inflamed uterus and cervixes than from normal organs (22% vs. 8% and 39% vs. 10%, respectively). The fact that the pathogen is also isolated from normal reproductive tracts implies that these strains cannot always be associated with disease. *H. somni* persists in the cervico-vaginal area from periods ranging from 8 to 87 days post-inoculation, even in the presence of humoral response (Hoblet *et al.* 1989). The main site of bacterial persistence is unknown; however, the major vestibular gland is considered a significant reservoir of *H. somni* (Miller *et al.* 1983). The detrimental effect of *H. somni* on embryo development was proposed as a possible cause of infertility in subclinically infected cows (Van Donkersgoed *et al.* 1990).

Abortion. Firehammer (1959) reported for the first time the isolation of a member of the genus *Haemophilus* from a bovine fetus aborted at 7 months of pregnancy and from the vaginal secretions of the aborted cow a month after abortion. Chladek (1975) reported the isolation of *H. somni* from the placenta and lung of a fetus aborted at 8.5 month of gestation. Gross lesions were not observed and microscopic lesions consisted of focal necrosis of placental cotyledons and leukocyte infiltrates in fetal alveoli and bronchioles. Experimental abortion after intravenous inoculation of *H. somni* demonstrated that the bacterium is able to reach the pregnant uterus and placenta by hematogenous dissemination (Widders *et al.* 1986). *H. somni*-induced abortion was also reproduced by intra-amniotic inoculation and the lesions observed in placenta resembled the vascular changes associated with bacterial septicemia (Miller *et al.* 1983b).

Male reproductive tract. *H. somni* is commonly recovered from the male bovine reproductive tract in the absence of pathological lesions. The preputial prevalence of the pathogen is very high (71%), particularly in young bulls. The organism is also harbored in accessory glands (19%) and in the ampulla of the ductus deferens (10%) (Humphrey *et al.* 1982). Some strains can cause purulent posthitis and distal urethritis and spermatic abnormalities, with slow and non-progressive motility (Bretschneider and Cipolla 1999).

Mastitis

The mature beef cow udder is not an important site for the persistence of *H. somni*. However, experimental inoculation of the mammary gland with an encephalitic strain of *H. somni* produced acute gangrenous mastitis with systemic dissemination of the organism. Chronic, subclinical mastitis can also be observed (Hazzlet *et al.* 1989). Isolation of the organism from milk samples from cows with clinical mastitis has also been demonstrated (Greer *et al.* 1989).

OTHER SYNDROMES ASSOCIATED WITH *H. SOMNI* INFECTION

H. somni has been isolated in pure culture from an urachal abscess in a 2 months old calf. Lesions in other organs were not observed. An infected birth canal or contacts of the calf umbilicus with a contaminated environment were possible causes of infection (Starost 2001).

Although the etiology of the weak calf syndrome remains unknown, in some instances *H. somni* has been associated with this manifestation. *H. somni* was isolated from 24% of cows that delivered weak calves and only from 6.5% of cows with normal calves in the same herd (Stauber 1986). The condition has also been experimentally reproduced by intra-uterine inoculation of *H. somni* at breeding (Waldhalm *et al.* 1974).

H. somni has been isolated from clinical cases of severe conjunctivitis. However, there is no evidence whether the organism was present as a commensal in the conjunctival sac or whether it plays a role in the disease (Lamont and Hunt 1982).

Schuh and Harland (1991) described for the first time myocarditis due to *H. somni* in Canada. Haines *et al.* (2004) demonstrated that *H. somni* is the main agent associated with myocarditis in feedlot calves. In contrast, Gagea *et al.* (2006) indicated that from 8 cases of myocarditis in calves, *H. somni* was isolated from the lung of one case with concurrent bronchopneumonia and TEME, but never from the heart.

CONSIDERATIONS ON THE DIAGNOSIS

H. somni is difficult to grow in culture, especially from samples of infected tissues or from environmental samples (Angen *et al.* 1998; Pfiefer *et al.* 1992). The identification of *H. somni* in genital secretions is improved when a guarded swabbing technique, which reduces the contamination level, in combination with KD-columbia agar as a selective medium, is used (Kwiecien and Little 1992). A PCR test that uses primers specific for the 16S rRNA of *H. somni* was shown to be useful for the identification of the bacterium in mixed populations (Angen *et al.* 1998). Tegtmeier *et al.* (2000) also demonstrated that the PCR technique was the most sensitive method for the identification of *H. somni*-induced calf pneumonia. Tests for the measurement of antibodies against *H. somni* are not sufficiently sensitive or specific. Therefore, it is suggested to use multiple serologic tests in research situations (Stephens *et al.* 1981; Groom and Little 1988).

VACCINES

The development of an effective vaccine against all clinical forms of the *H. somni* complex requires a proper characterization of the differences between strains (Primal *et al.* 1990). The fact that OMPs and LOS are antigenically variable imposes an obstacle in the elaboration of immunogens (Howard *et al.* 2000; Corbeil and BonDurant 2001). Moreover, the lack of sensitive and specific serological tests contributes to the difficultness in the evaluation of vaccine efficacy (Groom and Little 1988). Results from studies with commercial bacterins are variable. Van Donkersgod *et al.* (1994) did not observe a significant change in antibody titers after vaccination with a combined *M. haemolytica-H. somni* bacterin, whereas Groom and Little (1988) demonstrated that two vaccinations with a whole cell *H. somni* bacterin were able to reduce the clinical and pathological effects of *H. somni*-induced pneumonia after intra-tracheal challenge. An experimental vaccine composed of an anionic antigen present in the outer membrane complex of *H. somni*, induced a high level of serum antibodies. However, control animals that developed TEME after challenge also had a detectable increase in antibody response. Therefore, it was concluded that protection against TEME might be independent of the humoral response (Stephens *et al.* 1984). Butt *et al.* (1991) demonstrated that intra-muscular immunization with OMP-270 from *H. somni* in cows, at oestrus, contributed to the presence of IgG_1 and IgG_2 in uterine secretions. Although intra-uterine infusion of OMP-270 at oestrus did not elicit a highly significant PMN effusion, these findings are important for

the development of protection in the bovine reproductive tract since bovine PMNs express Fc receptor for IgG_2, which would assist in the clearance of infection (Butt *et al.* 1993).

TREATMENT

Prophylactic mass medication is an alternative management practice to reduce infection disease in feedlot calves. Van Donkersgoed *et al.* (1994) showed that long-acting oxytetracycline did not reduce the risk of haemophilosis mortality. According to Welsh *et al.* (2004) has a variable susceptibility to spectinomycin and sulfachloropyridizine. However, it is highly susceptible to other antibiotics of regular use, such as ampicillin and tetracycline (Harris and Janzen 1989; Welsh *et al.* 2004).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

More than 50 years ago, *Histophilus somni* was described as an organism associated with the occurrence of TEME in cattle. Since this first report, the pathogen has been associated with a variety of clinical syndromes. Its relevance in veterinary medicine is mainly related to the economical losses it causes in the feedlot industry. However, despite its recognized significance as a pathogen of cattle, little is known about the biological properties of the bacterium. The development of an effective vaccine against the disease complex will require a thorough understanding of the determinants of virulence responsible for the different clinical conditions. Identification of the relevant antigens of *H. somni* that are recognized by the host immune system is also essential. New methodologies to test differences in virulence between strains and to evaluate the contribution of the non-pathogenic strains to the immunological response and epidemiology will also provide new tools for the control of the disease.

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