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. IgG2 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 patógenas del microorganismo, principalmente en la mucosa genital. Las causas de las diferencias en la virulencia entre cepas no están defini-
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 (Griener 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 (Griener 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 and 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.

BIOLGICAL PROPERTIES OF H. SOMNI AND TAXONOMIC CONSIDERATIONS

H. somni is a small, Gram-negative, non-spor 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; Chladek 1975; Smith and Biberstein 1977). 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% CO₂ atmosphere (Kennedy et al. 1960; Claus and 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-pilius 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 pneumonia 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, lppB, 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* lipooligosaccharides (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; Siddarammpa 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 (Ig-BPs). 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 IgG2b, but not IgG2a (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* 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 (Bretscher 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 (Czuprynski and Hamilton 1985; Hubbard et al. 1986). Bovine neutrophils phagocytize H. somni (Andrews et al. 1985). However, they are unable to kill the bacterium (Czuprynski 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 difficulty 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 (Czuprynski 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 pneumatic 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 °C. 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 IgG₂ response during natural infection and after intra-venous and intra-bronchial administration of H. somni. Therefore, it was proposed that IgG₂ 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 IgG₁ and IgG₂ 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
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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. 2002; Kennedy et al. 1960; Momotani et al. 1985). Microabscessation and myelitis have also been reported (Griner et al. 1956; 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. 1983b). 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 (Bretscheider 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; Pfeifer 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 serological 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 difficulty 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 IgG1 and IgG2 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 IgG₂, 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 sulfachloropyridazine. 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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