Haemophilus somnus and reproductive disease in the cow: A review

Jacek M. Kwiecien, Peter B. Little

Abstract

Haemophilus somnus causes inflammatory disease in the genital tract of cows as reported in several field surveys and experimental trials. This organism can also innocuously colonize the healthy genital mucosa of the cow, which indicates its dual relationship with the host, that of pathogen and commensal. Experimental data indicate embryocidal capability of this pathogen suggesting a possible role in early embryonic death. *Haemophilus somnus* also causes sporadic abortions after a bacteremia in the dam. Retrograde infection of the pregnant uterus from the lower genital tract appears unlikely; however, this process can account for post-parturient endometritis. Detection of high homologous IgG_2 serum antibody titers using an ELISA test may be useful for the diagnosis of current or recent genital inflammation. Experimental laboratory data indicate that a proportion of genital strains of *H. somnus* are pathogenic and capable of causing thrombotic meningo-encephalitis and perhaps pneumonia. *In vivo* testing of the pathogenicity of genital strains remains to be conducted.

Résumé

Haemophilus somnus et les maladies du système reproducteur chez la vache : une revue

Haemophilus somnus a été identifié et cité comme agent causal de maladies inflammatoires du tractus génital chez la vache dans plusieurs études expérimentales et lors d'évaluation dans le champs. Cet agent peut aussi coloniser avec innocuité la muqueuse saine du tractus génital, soulignant la dualité de son association avec son hôte, soit celle d'un agent pathogène et d'un agent commensal. Les données des études expérimentales indiquent que cet agent pathogène aurait une capacité embryocide suggérant ainsi la possibilité d'un rôle dans le processus de mortalité embryonnaire précoce. *Haemophilus somnus* a aussi été responsable d'avortements sporadiques suite à une bactériémie chez la femelle. L'infection par voie rétrograde de l'utérus gravide à partir du tractus génital inférieur est peu probable; toutefois, ce processus pourrait expliquer l'endométrite postpartum. L'utilisation d'un test Elisa pourrait être utile pour détecter un titre élevé d'anti-corps sériques homologues IgG_2 pour diagnostiquer les maladies inflammatoires du système génital en cours ou récentes. Les données expérimentales de laboratoire montrent qu'une proportion d'*H. Somnus* de souches d'origine génitale sont pathogènes et peuvent causer une méningo-encéphalite thrombo-embolique et possiblement une pneumonie. Les tests de pathogénicité *in vivo* des souches d'origine génitale demeurent à effectuer.

(*Traduit par Dr Thérèse Lanthier*)

Introduction

Haemophilus somnus is associated with a complex of diseases including thrombotic meningoencephalitis (TME) (1-4), bronchopneumonia (2,5-9), myocardial abcessation (10), arthritis (2,4) and mastitis (11-13). The *H. somnus* complex has been reviewed by Humphrey and Stephens (14) and more recently by Harris and Janzen (15). Although this organism can be isolated frequently from semen and the prepuce of bulls, it generally causes no inflammatory genital lesions in the male reproductive tract (16-21).

Does *H. somnus* cause genital disease in cows? We address this controversial issue through this review of reports on various aspects of the interaction between *H. somnus* and the genital tract of the cow.

Reproductive disease associated with *H. somnus* in cattle has already been reviewed in 1983 by Miller *et al* (22). However, more recent field and experimental data have enriched our knowledge of *H. somnus*-associated reproductive disease in cows. The papers reviewed herein pertain to research published since 1983 and were selected through manual searching of published references with the aid of the Index Veterinarius and Medline® on SilverPlater® information system (1990 edition, US National Library of Medicine, SilverPlater International N. V., Boston, London, Amsterdam).

Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Reprint requests to Dr. P.B. Little.

Vaginitis, cervicitis, and endometritis

Surveys on the incidence of *H. somnus* in the genital tracts of slaughtered cows in Switzerland (23) and Canada (24) identified this organism in association with lesions of vaginitis, cervicitis, and endometritis (23), and in either diseased or normal reproductive tracts (24). The role of *H. somnus* as a reproductive pathogen is controversial because the organism has been isolated from live cows with both suppurative genital lesions and normal genital mucosae (9,25). However, *H. somnus* is commonly isolated from the diseased female bovine reproductive tract either in pure or mixed culture, which indicates its association with concurrent inflammatory lesions (9,26,27).

In Australia, *H. somnus* was frequently isolated from the lesions of purulent vaginitis, cervicitis, and endometritis in dairy cows and identified as a major pathogen responsible for an apparently contagious reproductive disease and lowered fertility (25, 28, 29). The purulent vaginal discharge was due mainly to vaginitis and cervicitis rather than to endometritis (25); it was significantly associated with the natural mating of bulls carrying *H. somnus* in the preputial cavity, and the use of the same artificial insemination (AI) catheter in more than one cow (29). Use of separate AI catheters in each cow apparently decreased venereal transmission of the disease (25,29).

The rates of isolation of *H. somnus* from the genital tract of slaughtered cows have been reported from Germany as 6.8% from the vagina (30), and from Canada as 8% from the vagina, vestibular glands, cervix, and uterus combined (31). In bovine vaginal field surveys in the USA, *H. somnus* was isolated from 15% of cows (9), 15.6% from vagina and cervix in Polish cows (32), and 28.6% in Australian cows (33). The data from Australia were collected during an outbreak of *H. somnus*-associated inflammatory genital disease in dairy cattle, and were strongly related to natural insemination by *H. somnus*-infected bulls (29). The Polish data are composed of a rate of incidence in one dairy herd with a history of four abortions and a number of cases of vaginitis (32). In general the rate of isolation of *H. somnus* appears to be lower in the genital tract of cows than that of bulls and steers (9), but it may vary depending on the geographical area surveyed and the type of cows sampled. More epidemiological surveys on genital prevalence of *H. somnus* in beef cattle are needed to establish its occurrence and better understand its role in bovine reproductive disease under conditions of natural mating (16).

After conducting a survey on the prevalence of *H. somnus* in the genital tract of slaughtered cows, Miller *et al* (31) suggested that the highest probability of isolation of *H. somnus* from healthy cows was from the vestibular glands, with the frequency of isolation decreasing towards the uterine tube. Bacteriological sampling of the glands, however, is difficult in live cows due to fecal contamination and culture overgrowth by *Escherichia coli* and *Proteus* spp. (32,34). The presence of high numbers of *Actinomyces pyogenes*, another important genital pathogen, may also lower isolation of *H. somnus* (35).

Isolation of *H. somnus* from the bovine genital tract is a demanding task which prompted attempts to develop selective media (33,36–38). Guarded swabbing was proposed by Stephens *et al* (29) as superior to that of sampling by aspiration of genital mucus for isolation of *H. somnus*. The use of a guarded swabbing technique and an adequate selective medium would be important in future field and abattoir surveys to obtain reliable prevalence data on *H. somnus* in the genital tract of cows.

Several studies of experimental genital infection with *H. somnus* have been reported. In one study of *in utero* persistence of *H. somnus* in the genital tract of virgin heifers inoculated at estrus, the organism was isolated for up to 10 days but not after the subsequent estrus (26). In a separate study, the intrauterine inoculation of virgin heifers with a pathogenic genital strain of *H. somnus* resulted in a transient infection which in some cases was associated with a mild to moderate endometritis (38). Using separate posterior intracervical inoculation of two TME and one abortion strains of *H. somnus* into pregnant cows, Miller *et al* (35) reported that clearance of the infection from the genital tract occurred within 8 to 87 days. Some of the inoculated cows had fever and a transient purulent vaginitis and vulvitis, but none aborted. Although *H. somnus* could not be reisolated from the apparently healed vagina during gestation, some cows developed a severe metritis associated with *H. somnus* at the time of parturition. The authors suggested that *H. somnus* might have persisted in the vestibular glands and invaded the uterus through the dilated cervix at the time of calving (35). The vestibular glands, however, were not sampled before parturition in this study.

In another study using heifers at estrus, one group was inoculated intracervically with an *H. somnus* isolated from a cow with endometritis and the other group with another strain isolated from the vagina of a clinically normal cow (39). All challenged animals developed purulent cervicitis and vaginitis within one day, but returned to normal within a week after the subsequent estrus. The histopathological examination of two heifers conducted five weeks after intracervical inoculation revealed a chronic suppurative vaginitis, vestibular adenitis, and squamous metaplasia of the vestibular glands which were associated with isolation of *H. somnus*. It was concluded that *H. somnus* can cause vaginitis and cervicitis in cattle, and further suggested that the infection can persist for a long time, particularly in the vestibular glands, an observation similar to that made by Miller *et al* (35).

It is clear that pathogenicity of various *H. somnus* isolates should be addressed more carefully in future research, since the results and observations cited vary considerably with regard to the reproducibility of the genital disease. Experimental inoculation of *H. somnus* into the genital tract of the cow readily produces inflammatory lesions which may vary in severity in individual animals. Although lesions are transient and likely due to a peculiar but unexplained influence of the estrus cycle, the organism may persist in the major vestibular glands and become a potential source of infection for bulls and cows (26,28). The isolation of *H. somnus* from normal genital tracts can be interpreted as a normal finding considering the apparent commensal behavior of this organism (21), but the

potential of genital isolates to cause TME and pneumonia, although not confirmed, should be considered seriously.

Infertility

Haemophilus somnus has been proposed as a causative factor of infertility in cows (26,40). However, the organism has been isolated from the genital tract of both infertile (26) and fertile cows (28). The association of *H. somnus* infection with infertility in cattle has been doubted on the basis of its presence in vaginal cultures from both diseased and normal genital tracts (9,25), and because of lack of previous experimental evidence indicating a direct influence of the organism on conception rate.

In a study by Kaneene *et al* (41), artificially inseminated heifers were inoculated *in utero* with an abortion isolate of *H. somnus* and yielded a significantly higher proportion of degenerated embryos than noninfected controls eight days later. Only four of 12 infected heifers had bacterial uterine lesions when tested by uterine biopsies. Embryos from infected heifers had a significantly shorter survival time than embryos from control heifers when cultured *in vitro*. The authors concluded that, although *H. somnus* does not always produce endometritis after an intrauterine inoculation, it may specifically affect developing embryos. They suggested that this might be due to an alteration of local conditions in the uterus, presumably due to bacterial growth, and the presence of a specific toxin (41). In another study by Kaneene *et al* (38), low numbers of normal-appearing embryos were recovered from some superovulated heifers 21 days after insemination and intrauterine inoculation of *H. somnus*. The authors suggested that a proportion of embryos died early, soon after infection with *H. somnus*, and the recovered live embryos persisted as a result of some protective but unknown uterine factors (38).

Thomson *et al* (42) demonstrated *in vitro* that *H. somnus* can adhere to preimplantation bovine embryos with an intact zona pellucida, whereas no such effect was demonstrated in zona pellucida-free embryos. Adherence to the zona pellucida-intact embryos allowed the persistence of *H. somnus* on some embryos after several repeated washes with antibiotic-free culture medium, but antibiotics used in the embryo transfer media eliminated *H. somnus*. All zona pellucida-intact and zona pellucida-free embryos degenerated after exposure *in vitro* to *H. somnus*. The authors suggested that this detrimental effect might have been associated with a change in pH, oxygen tension, or nutrient depletion in the medium as a result of the bacterial growth, but these suggestions were not explored. A specific embryocidal toxin produced by *H. somnus* has been suggested as a cause of embryonal degeneration (42). The source of isolation of the strain used in this study was not indicated.

Experimental evidence indicates that *H. somnus* is embryocidal (38,41,42), therefore identification of bacterial embryocidal factors should be addressed in a number of strains isolated from diseased and normal genital tracts of cows and bulls. Field studies are also needed to investigate the role of *H. somnus* in early embryonic death in cattle since this appears controversial in light of the report by Patterson *et al* (28). These authors reported an 82% pregnancy rate in a herd of heifers that had a high incidence of mucopurulent cervicitis and vaginitis following mating with bulls carrying preputial *H. somnus*. *Haemophilus somnus* was isolated from the vagina of most of the clinically affected animals, indicating that infection at the time of coitus did not have a major influence on fertility in this herd (28).

Kaneene *et al* (38) reported development of cystic ovarian disease in a substantial number of cows following intrauterine inoculation of *H. somnus*. This observation, however, requires a larger experiment for confirmation.

Abortion

Sporadic spontaneous abortions associated with *H. somnus* are reported from several countries (9, 26, 28, 43-45) and are associated with retention of the placenta (9) and a severe placentitis from which large numbers of *H. somnus* can be cultured (43). The organism can be isolated from tissues of the aborted fetus (9, 26, 28, 43, 44), which may have vasculitis and thrombosis accompanied by a mononuclear leukocytic infiltrate in several organs (43).

The pathogenesis of *H. somnus*-associated abortion has been investigated by Miller *et al*. They inoculated abortion or TME strains of *H. somnus* into the uterine artery (24) or the amnion (46) of pregnant cows and induced abortion accompanied by a severe necrotizing placentitis and retention of the placenta but with few or no lesions observed in fetal tissues. Widders *et al* (47) produced abortion in five of 11 pregnant cows following jugular intravenous inoculation of an abortion isolate of *H. somnus*. In another experiment by Stuart *et al* (48) only one of five cows inoculated intravenously with a Swiss abortion strain of *H. somnus* aborted and retained its placenta; necrotizing placentitis and suppurative metritis were also present. Although the presence of the organism was demonstrated with an immunocytochemical stain in endothelial cells of some placental vessels, lesions of vasculitis or thrombosis were not observed. The authors also reported identification of lesions antecedent to the challenge in the aborted fetus and placenta (48). The strain of *H. somnus* inoculated intravenously in this study therefore should be considered a rather questionable abortifacient.

The experiments of Miller *et al* (24,46) and Widders *et al* (47) provide strong evidence that *H. somnus*-induced abortion results from bacteremia in the dam. This is supported in a report by Miller *et al* (35) who demonstrated that intracervical infusion of *H. somnus* did not permit bacterial penetration of the cervix of the pregnant cow. The report by Stuart *et al* (48), on the contrary, may indicate that not all abortion isolates of *H. somnus* can readily cause abortion in intravenously inoculated cows. This may suggest a lower pathogenicity of the isolate used by Stuart *et al* (48) than that of isolates used by Miller *et al* (24) and Widders *et al* (47). Variable susceptibility of individual animals to intravenous infection may have also played a role in the outcome of all three experiments.

Work by Miller et al (35) suggests that isolation of H. somnus from the vagina or cervix of a pregnant cow may be an incidental finding and irrelevant to the fetus. The frequency of vaginal isolation of H. somnus from cows appears to be higher than that of abortion associated with this organism. Investigation of the role of H. somnus as an infectious agent of abortion in cattle should be continued under field conditions and correlated to the occurrence of vaginal infection and the nature of the strains involved.

Genital disease and immunity
Antibodies against H. somnus are found commonly in the blood of cattle and are assumed to develop from exposure to various strains of the organism that cause clinical or subclinical respiratory or genital disease (49,50) as well as exposure to organisms with cross-reacting antigens.

Using the microagglutination test (MAT), Ruegg et al (51) examined anti-H. somnus antibody titers in a herd of Holstein cows with a history of early embryonic death, cystic ovarian disease, and clinically normal genital tracts. High titers of anti-H. somnus agglutinins in some cows were interpreted as being compatible with an ongoing infection in the reproductive tract, but no attempt was made to isolate H. somnus from the genital tract of these cows.

Hoblet et al (52) induced a suppurative genital disease in virgin heifers after the intrauterine infusion of a genital strain of H. somnus. However, they were not able to demonstrate any increase in serum anti-H. somnus antibodies when measured by MAT even though they isolated H. somnus from the infected genital tracts on many occasions. On the other hand, Kaneene et al (38,41,53) showed a significant increase of serum anti-H. somnus antibody titers using a microtiter plate agglutination assay following intrauterine infusion of H. somnus which produced transient genital inflammatory lesions in some challenged heifers. The latter authors concluded that the strain used for the challenge had significant antigenicity but recorded no protective action in the same animals against a subsequent similar infection conducted five months later with the same strain (38).

An enzyme-linked immunosorbent assay (ELISA) using soluble H. somnus antigen has proven to be more specific than agglutination and complement fixation tests for examining H. somnus antibody titers (54). Using MAT, Corbeil et al (9) frequently found antibody titers against H. somnus in tested sera. They concluded, however, in their report with Widders et al (47) that the ELISA test was more specific than the MAT for screening anti-H. somnus antibody titers. Using ELISA and MAT, these researchers demonstrated the greatest rise of the IgG_2 isotype in pregnant cows after an intravenous inoculation with an abortion strain of H. somnus as compared to IgG_1 and IgM titers. High titers of IgG_2 persisted in postchallenge sera for several weeks and the ELISA test was identified as being more specific than the MAT. Anti-H. somnus IgG_2 titers had been higher in prechallenge sera of cows which did not abort than in those which did, and the authors concluded that IgG_2 isotype may play a role in protection against systemic infection with H. somnus. They suggested that measurement of IgG_2 titers using the ELISA test may be helpful diagnostically in the detection of current or recent systemic infection with H. somnus and could be useful in diagnosing abortion associated with this organism (47).

Using convalescent sera from cows that aborted, the same group of authors (55) identified two antigens on the outer membrane of various strains of H. somnus isolated from TME, pneumonia, abortion, and the normal genital tract. They suggested that both antigens might be suitable for use in protection against H. somnus-induced systemic infection; however, no subsequent work has elaborated on this suggestion.

Using an anionic antigen vaccine prepared from an H. somnus isolate from Australia (56), Patterson et al (39) vaccinated heifers to attempt prevention of clinical genital disease induced by intracervical instillation of H. somnus. The homologous antibody titer, as measured using an ELISA test, was increased in the serum of two of the three vaccinated heifers but no such increase was observed in their vaginal mucus. All heifers developed suppurative vaginitis and cervicitis after vaginal infection, regardless of the prechallenge antibody titers. Although the authors demonstrated a large increase in homologous antibody titers in the vaginal mucus of some heifers three weeks after inoculation, the rise was present in both vaccinated and nonvaccinated heifers (39). No difference in susceptibility to the cervical infection was demonstrated between either group of animals.

High serum anti-H. somnus antibody titers are a frequent finding in cows, a fact that complicates serological confirmation of infection. However, reported data have been based on surveys using agglutination tests with no determination of the subclass of antibody. This appears to be important in light of the studies by Corbeil et al (9) and Widders et al (47) who proposed the measurement of serum IgG_2 to detect a current or recently active infection with H. somnus. This test may be suitable in field studies on prevalence of genital infection with H. somnus in conjunction with culturing of genital specimens. The experimental data on the role of genital infection and its effect on systemic and local antibody response is limited and confusing. More experimental work in this area is indicated.

Pathogenicity and virulence factors of genital isolates
The group of organisms called "H. somnus" is antigenically heterogenous. Using a complement fixation and ELISA test in cross absorption studies of soluble outer membrane antigens, Stephens et al (57) examined 62 isolates of H. somnus from the healthy genital tracts of cows and bulls, cows with endometritis, and an abortion case. They found at least 11 serotypes of the organism but could not relate antigenic characteristics to the conditions of isolation. After comparing their study to similar work by Canto and Biberstein (58) and Corboz (59), who used not only genital but also pneumonic and TME isolates, Stephens et al (57) suggested that genital strains of H. somnus form a more heterogenous group than do TME and pneumonia isolates. However, no distinction could be

made between isolates from the healthy genital tract and those from inflammatory genital, pneumonic, and encephalitic lesions using their antigenic characterization.

Humphrey (60) suggested that genital strains of *H. somnus* are nonpathogenic, unlike encephalitic isolates, and demonstrated that one preputial and two seminal isolates caused only nonfatal meningitis after an intracisternal (I/C) inoculation into susceptible calves. In contrast, when one pneumonic and two TME isolates were inoculated, they caused severe peracute meningitis and death of the animals (60). Groom *et al* (61) have also obtained similar results and suggested that, in general, pneumonic and encephalitic strains of *H. somnus* are pathogenic, whereas preputial and seminal strains are not. The I/C calf assay appears to be an important and reliable method of examination of pathogenicity of *H. somnus* isolates and should be used to test genital isolates from cows.

Using inoculation of bacterial suspensions into artificially created subcutaneous chambers in calves, Groom (62) demonstrated a marked increase in colony forming unit numbers in two TME and two pneumonic isolates. In contrast to that, one preputial and two seminal strains of *H. somnus* failed to proliferate and decreased in numbers in the same *in vivo* test. This test allowed for differentiation between pathogenic and nonpathogenic strains, similar to that obtained in the I/C calf assay, and its application in testing large numbers of genital strains should be explored.

Corboz and Wild (44) and Little (63) have proposed that most genital isolates in carrier cattle are probably benign commensals but some may have the ability to cause clinical disease such as pneumonia or septicemia. However, the former (44) indicated that, due to the lack of reliable criteria to differentiate pathogenic from nonpathogenic isolates from any organ, all of the isolates should be regarded as potential pathogens. Webber *et al* (40), who may have been dealing with an *H. somnus* isolate from a case of bovine endometritis, were the first to report pathogenicity of a genital isolate after I/C inoculation into two calves. This work however, suffers from inadequate identification of the organism (40), and natural cases of TME have not been reported from Australia (29,64).

Corbeil *et al* (9) implied that female bovine genital isolates act as both pathogens and commensals. In a previous study Corbeil *et al* (65) identified 25% of the preputial isolates of *H. somnus* as susceptible to killing by fresh bovine serum containing complement. Most vaginal isolates of *H. somnus* from healthy cows were also susceptible to bovine serum after prolonged exposure. The authors suggested that the serum-susceptible genital strains are unlikely to invade the bloodstream and cause systemic disease, whereas those preputial and vaginal isolates showing serum resistance similar to that of TME, abortion, and pneumonic isolates should be considered potential pathogens (65).

In a study of three genital isolates from the bull by Humphrey (60), two were serum resistant but all were nonpathogenic in the I/C calf assay. This finding suggests that serum susceptibility does not correlate with pathogenicity in the intracisternal assay. The capacity of isolates of *H. somnus* to cause genital disease in

experimental cows discussed previously was not correlated to a test of serum susceptibility.

Widders *et al* (66,67) and Yarnall *et al* (68,69) determined that most pathogenic isolates of *H. somnus* express Fc receptors on their surface for the nonspecific binding of bovine immunoglobins, and concluded that their expression is consistently associated with the resistance of *H. somnus* to serum killing tests (67). Widders *et al* (67) suggested that the presence of the surface Fc binding receptors in pathogenic isolates and genital carrier isolates of *H. somnus* play a protective role allowing the organisms to escape immunological defense mechanisms of the host. These include the avoidance of opsonization by specific antibody, and of complement-fixation, and thus may influence the development of systemic disease or the persistence of the organism on the mucosal surface. They concluded that genital carrier isolates of *H. somnus* expressing Fc receptors should be considered potential pathogens. In this valuable work the authors also discovered that some preputial strains did not express Fc binding receptors, but only some of these strains were serum susceptible (67). Therefore, lack of Fc binding receptors does not correlate with susceptibility to bovine serum. It is obvious that some of the strains that are serum resistant and lack Fc binding capacity can be potentially pathogenic, as suggested previously (65).

Haemophilus somnus appears to contain a factor that impedes the normal microbicidal functions of neutrophils. This assumption is based on the fact that bovine neutrophils readily phagocytized, *in vitro*, an opsonized TME isolate of *H. somnus* but failed to kill it (70). Opsonized *H. somnus* triggered a reduced oxidative microbicidal response in phagocytizing neutrophils when compared to neutrophils stimulated with opsonized *E. coli* (71). Also, an apparently pathogenic strain of *H. somnus* was readily phagocytized by bovine monocytes *in vitro*, but was not killed and in fact multiplied inside the infected leukocytes leading to cell death (72).

It has been determined for a TME strain *in vitro* that it can secrete soluble fractions of low molecular weight including purine and pyrimidine bases, ribonucleotides, and a ribonucleoside which apparently cause degranulation of neutrophils after bacterial phagocytosis (73). Hubbard *et al* (74) demonstrated with the same TME strain that low molecular weight soluble fractions were able to inhibit bactericidal functions of neutrophils, and also a large >300 kilodalton cell surface fragment was able to inhibit phagocytosis by neutrophils. These studies provide evidence that *H. somnus* is capable of producing molecular virulence factors. Further similar studies need to be conducted with a larger number of *H. somnus* strains.

Humphrey (60) used bovine arterial endothelial cells cultured *in vitro* to examine cytotoxic capacity of two TME isolates, one pneumonic and three genital carrier isolates, and found that only one preputial isolate was unable to produce toxic changes and exfoliation of the endothelial monolayer. This study (60) also showed a lack of correlation between serum resistance and the capacity of one genital isolate for causing cytopathic effects. Endothelial tissue experimentation

appears to be particularly suitable for examination of virulence of *H. somnus* isolates, considering the thrombogenic potential of isolates examined *in vitro* by Thompson and Little (75). The mechanism of the contraction of endothelial cells exposed to *H. somnus* shown in their work needs clarification.

Although the serum bactericidal assay has been putatively linked with the ability of *H. somnus* to invade the blood stream and cause systemic disease, some serum resistant strains were nonpathogenic on the I/C calf assay, thus contradicting the prediction (60). The absence of Fc receptors did not consistently correlate with serum susceptibility in some genital strains. These findings indicate that serum-susceptibility of isolates of *H. somnus* is not the sole mechanism of virulence.

Summary
Incontrovertible evidence has been accumulated through field and experimental research that *H. somnus* is a cause of genital inflammatory disease and abortion in cows. Experimental data suggest that this organism is an important agent in early embryonic death but this possibility needs to be explored in field studies. Some isolates from the female bovine genital tract have characteristics that parallel those of strains isolated from cattle with TME and pneumonia, as indicated by various *in vitro* tests. The presence of pathogenic strains in the genital tract, however, should be explored through *in vitro* testing.

Acknowledgments

References

1. Kennedy PC, Biberstein EL, Howarth JA, Frazier LM, Dungworth DL. Infectious meningo-encephalitis in cattle caused by a *Haemophilus*-like organism. Am J Vet Res 1960; 21: 403-409.
2. Panciera RJ, Dahlgren RR, Rinker HB. Observations on septicemia of cattle caused by a *Haemophilus*-like organism. Pathol Vet 1968; 5: 212-226.
3. Smith PB, Biberstein EL. Septicemia and meningoencephalitis in pastured cattle caused by a *Haemophilus*-like organism ('*Haemophilus somnus*'). Cornell Vet 1977; 67: 300-305.
4. Stephens LR, Little PB, Wilkie BN, Barnum DA. Infectious thromboembolic meningoencephalitis in cattle: A review. J Am Vet Med Assoc 1981; 178: 378-384.
5. Van Dreumel AA, Thomson GW, Kierstead M. *Haemophilus somnus* infections in cattle in Ontario: A review with emphasis on the respiratory form. Proc 1st Int Symp Vet Lab Diagnost 1977: 482-492.
6. Andrews JJ, Slife LN, Stevenson GW. Naturally occurring *Haemophilus somnus* pneumonia in cattle. Proc 3rd Int Symp Vet Lab Diagnost 1983: 377-384.
7. Lancaster MJ, McGillivery DJ, Patterson RM, Irwin S. Pneumonia associated with *Haemophilus somnus* in a calf. Aust Vet J 1984; 61: 269.
8. Andrews JJ, Anderson TD, Slife LN, Stevenson GW. Microscopic lesions associated with isolation of *Haemophilus somnus* from pneumonic bovine lungs. Vet Pathol 1985; 22: 131-136.
9. Corbeil LB, Widders PR, Gogolewski R, Arthur J, Inzana TJ, Ward ACS. *Haemophilus somnus*: Bovine reproductive and respiratory disease. Can Vet J 1986; 27: 90-93.
10. Guichon PT, Pritchard J, Jim GK. *Haemophilus somnus* myocarditis in a feedlot steer. Can Vet J 1988; 29: 1012-1013.
11. Armstrong KR, Osborne AD, Janzen ED. *Haemophilus somnus* mastitis in a dairy cow. Can Vet J 1986; 27: 211-212.
12. Higgins R, Martin JR, Larouche Y, Goyette G. Mastitis caused by *Haemophilus somnus* in a dairy cow. Can Vet J 1987; 28: 117-119.
13. Greer D, McConnell W, Ball H. Isolation of *Haemophilus somnus* from bovine milk. Vet Rec 1989; 125: 381-382.
14. Humphrey JD, Stephens LN. '*Haemophilus somnus*': A review. Vet Bull 1983; 53: 987-1004.
15. Harris FW, Janzen ED. The *Haemophilus somnus* disease complex (Hemophilosis): A review. Can Vet J 1989; 30: 816-822.
16. Janzen ED, Cates WF, Barth A. Nechala L. Pawlyshyn V, Saunders JR, Osborne AD. Prevalence of *Haemophilus somnus* in the semen of bulls in Saskatchewan. Can Vet J 1981; 22: 361-362.
17. Humphrey JD, Little PB, Barnum DA, Doig PA, Stephens LR, Thorsen J. Occurrence of *Haemophilus somnus* in bovine semen and in the prepuce of bulls and steers. Can J Comp Med 1982; 46: 215-217.
18. Stefaniak T, Molenda J, Krolinski J, Chelmonska A. Wstepne badania nad zakazeniem narzadu rozrodczego buhajów, wywolanych paleczka *Haemophilus somnus*. Med Weter 1987; 43: 621-623.
19. Krogh HV, Pedersen KB, Blom E. *Haemophilus somnus* in semen from Danish bulls. Vet Rec 1983; 112: 460.
20. Humphrey JD, Little PB, Stephens LR, Barnum DA, Doig PA, Thorsen J. Prevalence and distribution of *Haemophilus somnus* in the male bovine reproductive tract. Am J Vet Res 1982; 43: 791-795.
21. Corbeil LB, Woodward W, Ward ACS, Mickelsen WD, Paisley L. Bacterial interactions in bovine respiratory and reproductive infections. J Clin Microbiol 1985; 21: 803-807.
22. Miller RB, Lein DH, McEntee KE, Hall CE, Shin S. *Haemophilus somnus* infection of the reproductive tract of cattle: A review. J Am Vet Med Assoc 1983; 182: 1390-1392.
23. Fisher W. Comparative studies of cows with and without endometritis. (Dissertation). Zürich, Switzerland, 1977. (Abstr) Vet Bull 1977; 49: 6188.
24. Miller RB. An investigation into the prevalence, pathogenesis and pathology of *Haemophilus somnus* infections of the reproductive tract in cows (Dissertation). Ithaca, New York: Cornell University, 1980.
25. Stephens LR, Slee KJ, Poulton P, Larcombe M, Kosior E. Investigation of purulent vaginal discharge in cows with particular reference to *Haemophilus somnus*. Aust Vet J 1986; 63: 182-185.
26. Klavano GG. Observations of *Haemophilus somnus* infection as an agent producing reproductive disease: Infertility and abortion. Proc Soc Theriogenology 1980: 139-149.
27. Luginbuhl A, Kupfer U, Nicolet J. Bacteriologische Befunde im Geschlechtsapparat von Kuchen im Puerperium. Schweiz Arch Tierheikd 1981; 123: 629-637.
28. Patterson RM, Hill JF, Shiel MJ, Humphrey JD. Isolation of *Haemophilus somnus* from vaginitis and cervicitis in dairy cattle. Aust Vet J 1984; 61: 301-302.
29. Stephens LR, Webber J, Humphrey JD. Diagnosis and control of infertility in dairy cattle due to *Haemophilus somnus* infection. Australian Dairy Research Council. Final Report on a terminated project (DAV 81), Australia, July 1984-June 1987: Victorian Department of Agriculture and Rural Affairs.
30. Weisser W, Albert K. *Haemophilus somnus* und *Corynebacterium pyogenes* Vorkommen im Vaginalsecret fruchtbarkeitsgestörter Rinder in Nordwürttemberg. Tierärztl Umsch 1987; 42: 596-600.
31. Miller RB, Barnum DA, McEntee KE. *Haemophilus somnus* in the reproductive tracts of slaughtered cows: location and frequency of isolations and lesions. Vet Pathol 1983; 20: 515-521.
32. Nowacki W, Molenda J, Stefaniak T, Chelmonska A, Nikolajczuk M. Izolacja *Haemophilus somnus* z dróg rodnych krów. Med Weter 1988; 44: 36-39.
33. Slee KJ, Stephens LR. Selective medium for isolation of *Haemophilus somnus* from cattle and sheep. Vet Rec 1985; 116: 215-217.
34. Brewer RA, Smith RA, Corbel MJ. Development of an improved medium for the isolation of '*Haemophilus somnus*'. Lett Appl Microbiol 1986; 2: 57-59.

35. Miller RB, Barnum DA. Effects of *Haemophilus somnus* on the pregnant bovine reproductive tract and conceptus following cervical infusion. Vet Pathol 1983; 20: 584–589.
36. Ward ACS, Corbeil LB, Mickelsen WD, Sweet VF. A selective medium for Gram-negative pathogens from bovine respiratory and reproductive tracts. Proc 28th Am Assoc Vet Lab Diagnost 1983: 103–112.
37. Brewer RA, Corbel MJ, Stuart FA. Development of improved methods for the transport and isolation of *Haemophilus somnus*. Res Vet Sci 1985; 39: 299–306.
38. Kaneene JB, Coe PH, Gibson CD, Yamini B, Morrow DA, Marinez RO. The role of *Haemophilus somnus* in bovine early embryonic death. III. The effect of the organism on embryos by day 21 postbreeding. Theriogenology 1987; 27: 737–749.
39. Patterson RM, Mitchell GM, Humphrey JD, Stephens LR. Experimental induction of vaginitis in heifers by infection with *Haemophilus somnus*. Aust Vet J 1986; 20: 163–165.
40. Weber JJ, Pritchard DH, Gordon LM, Dean HF. Isolation of bacteria resembling *Histophilus ovis* from cattle. Aust Vet J 1983; 60: 276.
41. Kaneene JB, Coe PH, Gibson CD, Yamini B, Marinez RO, Morrow DA. The role of *Haemophilus somnus* in bovine early embryonic death. I. The effect of the organism on embryos by day 8 postbreeding. Theriogenology 1986; 26: 189–198.
42. Thomson MS. Stringfellow DA, Lauerman LH. Adherence of *Haemophilus somnus* to bovine embryos after *in vitro* exposure. Am J Vet Res 1988; 49: 63–66.
43. Van Dreumel AA, Kierstead M. Abortion associated with *Haemophilus somnus* infection in a bovine fetus. Can Vet J 1975; 16: 367–370.
44. Corboz L, Wild P. Epidemiologie der *Haemophilus somnus*-Infektionem beim Rind: Vergleich von Stämmen in der Polyacrylamidegel-Electrophorese (PAGE). Schweiz Arch Tierheilkd 1981; 123: 79–88.
45. Chladek DW. Bovine abortion associated with *Haemophilus somnus*. Am J Vet Res 1975; 36: 1041.
46. Miller RB, Van Camp SD, Barnum DA. The effects of intra-amniotic inoculation of *Haemophilus somnus* on the bovine fetus and dam. Vet Pathol 1983; 20: 574–583.
47. Widders PR, Paisley LG, Gogolewski RP, Evermann JF, Smith JW, Corbeil LB. Experimental abortion and the systemic immune response to '*Haemophilus somnus*' in cattle. Infect Immun 1986; 54: 555–560.
48. Stuart FA, Corbel MJ, Richardson C, Brewer RA, Bradley R, Bridges AW. Experimental *Haemophilus somnus* infection in pregnant cattle. Br Vet J 1990; 146: 57–67.
49. Hoerlein AB, Goto K, Young S. *Haemophilus somnus* agglutinins in cattle. J Am Vet Med Assoc 1973; 163: 1375–1377.
50. Sanfaçon D, Higgins R. Epidemiology of *Haemophilus somnus* infection in dairy cattle in Quebec. Can J Comp Med 1983; 47: 456–459.
51. Ruegg PL, Marteniuk JV, Kaneene JB. Reproductive difficulties in cattle with antibody titers to *Haemophilus somnus*. J Am Vet Med Assoc 1988; 193: 941–942.
52. Hoblet KH, Haibel GK, Kowalski JJ, Rojko JL. Culture positive persistence and serum agglutinating antibody response after intrauterine inoculation of *Haemophilus somnus* in virgin heifers. Am J Vet Res 1989; 50: 1008–1014.
53. Kaneene JB, Gibson CD, Coe PH, Morrow DA. The role of *Haemophilus somnus* in bovine early embryonic death. II. Persistence of the organism in the uterus following intrauterine exposure. Theriogenology 1986; 26: 795–801.
54. Canto J, Biberstein EL, Schulte TA, Behymer D. Cross-reactivity of *Haemophilus somnus* antibody in agglutination and complement fixation tests and in the enzyme-linked immunosorbent assay. J Clin Microbiol 1983; 17: 500–506.
55. Corbeil LB, Arthur JE, Widders PR, Smith JW, Barbett AF. Antigenic specificity of convalescent serum from cattle with *Haemophilus somnus*-induced experimental abortion. Infect Immun 1987; 55: 1381–1386.
56. Stephens LR, Little PB, Wilkie BN, Barnum DA. Isolation of *Haemophilus somnus* antigens and their use as vaccines for prevention of bovine thromboembolic meningoencephalitis. Am J Vet Res 1984; 45: 234–239.
57. Stephens LR, Aukema R, Murray LJL. Antigenic heterogeneity of *Haemophilus somnus*. Aust Vet J 1987; 64: 113.
58. Canto GJ, Biberstein EL. Serological diversity in *Haemophilus somnus*. J Clin Microbiol 1982; 15: 1009–1015.
59. Corboz L. Serologische Untersuchung von *Haemophilus somnus*-Isolaten aus Verschiedenen Organen von Rindern. Proc 3rd Int Symp Vet Lab Diagnost 1983: 385–391.
60. Humphrey JD. '*Haemophilus somnus*': Colonization of the bovine reproductive tract (Dissertation). Guelph, Ontario: University of Guelph, 1982.
61. Groom SC, Little PB, Rosendal S. Virulence differences among three strains of *Haemophilus somnus* following intratracheal inoculation of calves. Can J Vet Res 1988; 52: 349–354.
62. Groom SC. *Haemophilus somnus*: Studies of virulence and pathogenesis (Dissertation). Guelph, Ontario: University of Guelph, 1990.
63. Little PB. *Haemophilus somnus* complex: Pathogenesis of the septicemic thrombotic meningoencephalitis. Can Vet J 1986; 27: 94–96.
64. Stephens LR, Humphrey JD, Little PB, Barnum DA. Morphological, biochemical, antigenic and cytochemical relationship among *Haemophilus somnus*, *Haemophilus agni*, *Haemophilus haemoglobinophilus*, *Histophilus ovis* and *Actinobacillus seminis*. J Clin Microbiol 1983; 17: 728–737.
65. Corbeil LB, Blau K, Prieur DJ, Ward ACS. Serum susceptibility of *Haemophilus somnus* from bovine clinical cases and carriers. J Clin Microbiol 1985; 22: 192–198.
66. Widders PR, Smith JW, Yarnall M, McGuire TC, Corbeil LB. Non-immune immunoglobulin binding by *Haemophilus somnus*. J Med Microbiol 1988; 26: 307–311.
67. Widders PR, Dorrance LA, Yarnall M, Corbeil LB. Immunoglobulin-binding activity among pathogenic and carrier isolates of *Haemophilus somnus*. Infect Immun 1989; 57: 639–642.
68. Yarnall M, Gogolewski RP, Corbeil LB. Characterization of two *Haemophilus somnus* Fc receptors. J Gen Microbiol 1988; 134: 1993–1999.
69. Yarnall M, Widders PR, Corbeil LB. Isolation and characterization of Fc receptors from *Haemophilus somnus*. Scand J Immunol 1988; 28: 129–137.
70. Czuprynski CJ, Hamilton HL. Bovine neutrophils ingest but do not kill *Haemophilus somnus in vitro*. Infect Immun 1985; 50: 431–436.
71. Czuprynski CJ, Hamilton HL. Failure of bovine neutrophils to kill ingested *Haemophilus somnus*. (Abstract) J Leukoc Biol 1985; 38: 166.
72. Lederer JA, Brown JF, Czuprynski CJ. '*Haemophilus somnus*', a facultative intracellular pathogen of bovine mononuclear phagocytes. Infect Immun 1987; 55: 381–387.
73. Chiang YW, Kaeberle ML, Roth JA. Identification of suppressive components in *Haemophilus somnus* fractions which inhibit bovine polymorphonuclear leukocyte function. Infect Immun 1986; 52: 792–797.
74. Hubbard RD, Kaeberle ML, Roth JA, Chiang YW. *Haemophilus somnus*-induced interference with bovine neutrophil functions. Vet Microbiol 1986; 12: 77–85.
75. Thompson KG, Little PB. Effect of *Haemophilus somnus* on bovine endothelial cells in organ culture. Am J Vet Res 1981; 42: 748–754.