

Short Communication

Frequency of *haemophilus somnus* in the semen of bulls in Iran as determined by polymerase chain reaction

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Haemophilus somnus is a small, pleomorphic, gram-negative and coccobacillus bacterium that has been identified as a common inhabitant of the sheath in bulls and the vagina and major vestibular gland in cows. The purpose of this study was to determine the frequency of *H. somnus* infection in the semen samples of bulls in Iran. *H. somnus* was isolated from 40 of 185 (21.62%) bull's semen. Results showed high frequency of *H. somnus* infection in artificially prepared semen that is used for artificial insemination. The findings of this study indicate that semen samples of bulls that are used for artificial insemination are important sources of *H. somnus* infection in Iran. In addition, it can be concluded that this bacterium may normal flora of the bovine prepuce and that dissemination from the male bovine reproductive tract and it can be related to *H. somnus*-associated diseases. To control and prevention of the distribution of *H. somnus*, examination of bull's semen for artificial insemination seems to be necessary.

Key words: Bulls, *Haemophilus somnus*, semen, artificial insemination, polymerase chain reaction, Iran.

INTRODUCTION

Haemophilus somnus (*Histophilus somni*) is a member of the family *Pasteurellaceae* as the cause of encephalitis in cattle and isolated for the first time in 1956 (Diaz-Aparicio et al., 2009). *H. somnus* is a gram-negative, fastidious, non acid-fast, rod-shaped, encapsulated, non-motile and facultative pathogenic bacterium. It had many former names (*H. ovis*, *H. agni*, and *H. somnus*) that were used parallel resulting to certain confusion (Appuhamy et al., 1997). The taxonomical position 1 of the microorganism was not clarified for a long time, the name *H. somni* was suggested a few years ago (Angen et al., 2003). *H. somnus* is a common disease-causing bacterium that spreads throughout cattle herds in a very elusive manner.

H. somnus causes a wide variety of diseases including pneumonia, abortion, thrombotic meningoencephalitis, arthritis, myocarditis and septicemia in cattle, sheep, and some of other domestic animal (Hazirolu et al., 2000). This organism is commensal in the genital tract of bulls. Isolation has been made from normal and inflamed female genital tract and aborted fetuses. *H. somnus* can be either opportunistic pathogens or commensal of bovine mucosal surfaces (Akhtar et al., 1997).

Infections related to *H. somnus* are similarly related to stress factors and the situation of farm and feed pats at fall and winter. These bacteria commonly live in the upper respiratory tract, prepuce and vagina of cattle and can also be found circulating in the bloodstream (Orr, 1992). Sources of infection are therefore endogenous to herds or individuals (Atyabi et al., 2005). Diseases due to *H. somnus* account for millions of dollars in losses to the cattle industry, and are particularly prevalent in feedlots. This pathogenic potential of *H. somnus* and the economic importance of the above diseases indicate that this

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Abbreviations: *H. somnus*, *Haemophilus somnus*.

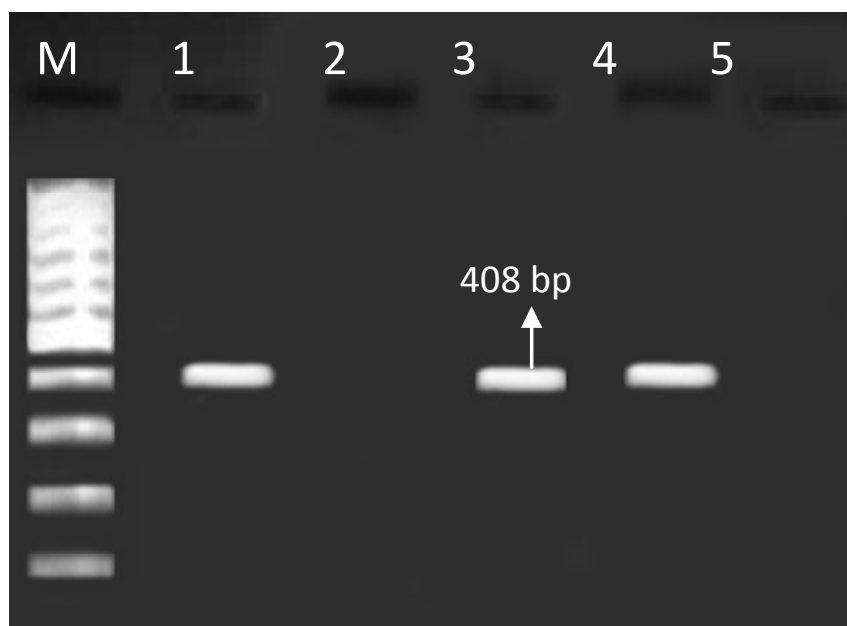


Figure 1. Gel electrophoresis for identification of *H. somnus* infection in artificially prepared semen samples (Line M is 100 bp DNA ladder, line 1 is positive control, line 2 is negative control, lines 3 to 4, are positive samples, and line 5 is negative sample).

organism is a significant bovine bacterial pathogen (Corbeil, 2007). Beside cattle, sheep and specially bulls neither the isolation nor the etiological roles of *H. somnus* have ever been reported in any other domesticated animal species.

The objective of this study was to determine the frequency of *H. somnus* infection in the semen samples of bulls used for artificial insemination in Iran.

MATERIALS AND METHODS

Sampling

Semen samples were obtained from 185 bulls between May and August 2010, being careful of avoiding contamination with bacteria present in the prepuce. Prior to taking the samples, the prepuce was washed with a 1% benzalkonium chloride solution drying with sterile cotton. Semen samples were stored at -70°C until use.

DNA extraction

Genomic DNA was extracted from semen samples with DNA extraction kit (Qiagen, Germany), according to the manufacturer's instructions. The total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001).

Gene amplification

Specific primers for "16S rRNA" of *H. somnus* (Angen et al., 2003) were used for gene amplification. Primer 1 begins at base 453 with

the following sequence H-somn-F: 5'-GAAGGCGATTAGTTTAAGAG-3' and primer 2 begins at base 860 with the sequence H-somn-R: 5'-TTCGGGCACCAAGTATTCA-3', therefore it is intended to amplify a fragment of 408 base pairs. PCR amplification was performed in a 25 μL reaction volume containing 1 μg of genomic DNA, 1 μM of each primers, 2 mM MgCl_2 , 200 μM dNTP, 2.5 μL of 10X PCR buffer and 1 unit of Taq DNA polymerase (Fermentas, Germany). The samples were placed in a thermal cycler (Mastercycler gradient, Eppendorf, Germany) with an initial denaturation step at 95°C for 5 min, then amplified for 30 cycles of denaturation at 94°C for 1 min, alignment at 55°C for 1 min, extension at 72°C for 1 min and, final extension step at 72°C for 5 min. Reaction mixture without DNA template was used as a negative control. The PCR amplification products (10 μL) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with Ethidium bromide, and images were obtained in a UVIdoc gel documentation systems (UK).

RESULTS

In this study 185 semen samples were examined for the presence of *H. somnus* infection. Analysis of PCR products for presence of "16S rRNA" gene of *H. somnus* on agarose gel revealed a 408 bp fragment (Figure 1). The frequency of *H. somnus* infection in semen samples of bulls was 40 of 185 (21.62%). The results showed a high frequency of *H. somnus* infection in bull's semen that is used for artificial insemination.

DISCUSSION

H. somnus is associated in some cases with respiratory

diseases, abortions, infertility, weak calf syndrome, arthritis and sudden death (Miller and Barnum, 1983). *H. somnus* diseases have also occurred in the ears and eyes of cattle and it is dangerous for calf (Odugbo et al., 2009). Results of this study indicate that high frequency of *H. somnus* infection in semen samples that is used for artificial insemination in Iran. Preliminary studies at Iran Veterinary College to assess the role of genital infection with *H. somnus* in the epizootiology of *H. somnus* – associated diseases have indicated that urogenital dissemination may be a significant means of transmission (Atyabi et al., 2005). The previous studies showed the presence and associated *H. somnus* with respiratory diseases and abortions in the United States, Australia, parts of Western and Eastern Europe, Scandinavia, Argentina, Japan and New Zealand (Atyabi et al., 2005), African countries namely, Egypt and South Africa (Last et al., 2001).

H. ovis infection was observed in Great Britain and the United States, with epididymitis cases, at the present time are grouped together as *H. somnus* (Odugbo et al., 2009). Atyabi et al. (2005) showed the presence of *H. somnus* in the artificially prepared semen as a causative agent of infection in Iran (Atyabi et al., 2005). Odugbo et al. (2009) showed *H. somnus* pneumonia for first time in Nigerian dairy cattle (Odugbo et al., 2009).

Conclusions

The results of the present study indicated that *H. somnus* may normally form part of the flora of the bull prepuce and can be transmitted from the male bovine reproductive during natural mating or artificial insemination and it can cause *H. somnus* -associated diseases in cattle. Furthermore, semen samples of bulls that are used for artificial insemination are important sources of *H. somnus* infection in Iran. According to these findings vaccinations (two doses) and use of antibiotics or antibacterial drugs for prevention of bulls and cattle from *H. somnus* infection it seems to be necessary.

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