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Prevalence of *Haemophilus somnus* in breeding bulls: Bacteriological and immunological methods

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**ABSTRACT**

In addition to being an import pathogen, *Haemophilus somnus* is routinely isolated from mucus surfaces in apparently normal animal. Isolation of *H. somnus* is possible only under specific conditions, selective media and 5 or 10% of CO2 in the atmosphere.

As material for the isolation, a native bull sperm was used. For the cultivation of *H. somnus*, chocolate agar, Columbia agar, PPLO agar and brain-heart infusion agar were used. The method of agglutination (tube and micro agglutination) and m-CF test were used for proving the antibodies in blood sera from 93 breeder bulls.

Out of 93 examined bull blood sera, by the method of tube-agglutination (TA), the positive results were found in 2.15%, and by the method of m-CF in 4.3% of the samples. The highest positive percentage was gained by the method of micro agglutination 9.68%. Gained results suggest that there is a presence of the infection with *H. somnus* in breeding bulls.

**Key words:** *Haemophilus somnus*, breeding, bulls, reproduction

**INTRODUCTION**

*Haemophilus somnus* is known to be an etiological agent in several disease syndromes of cattle including thromboembolic meningoencephalitis, respiratory disease, abortion and possibly infertility (Ward 1995, Corbeil 2007). A great number of countries in the world have confirmed the existence of this disease, in addition to being an important pathogen, this organism can be routinely isolated from mucosal surfaces of apparently normal animal. Most bulls carry the organism asymptomatically in the prepuce, many cows are vaginal carriers. In the pathology of reproductive system of the cattle produced by this bacillus, the male is, first of all, the reservoir of the microorganism. Sparadically *H. somnus* was isolated in pure culture from purulent ejaculates and from the calf orchitis. Setting *Haemophilus* diagnosis is very complicated, and depends on combining the information from the clinical and laboratory investigations (Humphrey 1982.). Isolation of *H. somnus* is possible only under specific conditions, selective media and in the atmosphere with 10% CO2. Besides this, bacteria isolation itself is not a sure sign of pathogen influence, having in mind different virulence of the isolated strains. Certainly, in detecting this disease serological methods would be of great help, first of all because of their variety in epizootiological investigations (Stefaniak et al 1993, Akhtar, 1997.).

**EXPERIMENTAL METHODS**

As material for isolation we used native bull sperm. The sperm was received by applying artificial vagina. For cultivating *H. somnus* we used several culture media: chocolate agar, Columbia agar, PPLO agar and Brain-heart infusion agar. To the mentioned nutritive media neomycin sulfate (5 mikro g/ml), nistatin 100 IJ/ml, yeast extract (0,5%) and horse sera (5%) were added. Incubation was done at the temperature of 37oC with the presence of 10% CO2. Colonies which are morphologically corresponded to *H. somnus* were further identified.
For proving antibodies in the blood sera of 93 breeding bulls, the method of agglutination (tube- and mikroagglutination) and m-CF test was applied. The antigens were prepared out of the reference strain Hjomnus 6280 CAPM-Bmo. Findings of the agglutinin in diluted sera 1:64 or 1:80 and greater were considered positive. Findings of CF-antibodies in sera solution 1:8 and higher were considered positive.

RESULTS A>T> DISCUSSION

H. somnus was isolated in two samples of bull sperm. After 24 hours on chocolate agar at the atmosphere with 10% of CO2 small colonies were noticed 0.4-0.6 mm large, rounded ,with smooth straight edges. After 48 and 72h the colonies were 1.0-1.5 mm yellow pigmented, without shine’nildly granulated surface (Figure 2). After 48 and 72h on Columbia agar the colonies were rounded, smooth, but in prolonged incubation colony got yellow color, with mildly granulated surface (Figure 3). On nutritive agar, with adition of horse serum, without blood, after 48 hours colonies were rounded, shiny, smooth and lightly opalescent. The organism grew in the absence of both X i V factors. The organism was a pleomorphic Gram-negative coccobacillus, was catalase negative, oxidase positive, it reduced nitrates and split aesculine. Sugar fermentation reactions 48h after the inoculation were as follows: acid, but no gas , was produced in dextrose, and there was no fermentation of maltose, lactose, raffinose, or rhamnose.

Out of 93 examined bull blood sera, by the method of tube-agglutination (TA) positive results were registered in 2.15%, and by the method of micro complement fixation (mCF) in 4.3% of the samples (Table 1). The highest positive percentage was gained by the method of microagglutination, 9.68% (Figure 1), and the highest antibody liter, was up to 1:128. The results suggest the presence of infection with H. somnus in breeding bulls.

![Figure 1. The result of investigated sera from bulls – agglutination and complement fixation](image1)

![Figure 2. Haemophilus somnus- a 72h old culture on chocolate agar](image2)

![Figure 3. Haemophilus somnus- a 72h old culture on Columbia agar 2](image3)
Table 1. Findings of agglutination and CF-antibodies in bull blood sera

<table>
<thead>
<tr>
<th>Method</th>
<th>tube agglutination</th>
<th>total</th>
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<th>pos.</th>
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<tr>
<td></td>
<td>&lt;10</td>
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<tr>
<td>No.anim.</td>
<td>74</td>
<td>20,14</td>
<td>40</td>
<td>80</td>
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<td>%</td>
<td>79,57</td>
<td>15,05</td>
<td>3,23</td>
<td>2,15</td>
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<tr>
<td>method</td>
<td>microagglutination</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;4</td>
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<td></td>
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<tr>
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<td>8,17</td>
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<td>32,19</td>
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<td>26,88</td>
<td>18,28</td>
<td>24,73</td>
<td>20,43</td>
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<tr>
<td>method</td>
<td>micro CF test</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>&lt;4</td>
<td></td>
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<tr>
<td>No.anim.</td>
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<td>8,4</td>
<td>160</td>
<td>32</td>
</tr>
<tr>
<td>%</td>
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REFERENCES