

***Haemophilus somnus* - Unlikely to be a Causative Microbiological Agent in Bovine Clinical Mastitis in Denmark**

Haemophilus somnus is a Gram-negative bacterium, which is the cause of a clinical syndrome in cattle that may include pneumonia, myocardial abscessation, arthritis, thrombotic meningoencephalitis, genital infections and mastitis (Humphrey & Stephens 1983, Armstrong *et al.* 1986, Higgins *et al.* 1987, Harris & Janzen 1989, Kwiecien & Little 1992). In Denmark the infection is mainly found in the respiratory tract of calves (Krogh *et al.* 1986, Wedderkopp 1991).

H. somnus is wide-spread in the cattle population, but growth of the bacteria on agar plates presupposes an atmosphere containing a minimum of 5% CO₂ (Merino & Biberstein 1982), a technique which is not used routinely in laboratory examinations of mastitis cases. In a traditional microbiological study of cases of clinical bovine mastitis, no growth of any microbiological agents was seen in 11.8% of the samples (Aalbak *et al.* 1994). The present study was undertaken in order to investigate whether *H. somnus* can be involved in cases of bovine mastitis.

Bovine mammary secretions from 6809 quarters, representing 3783 cows suffering from clinical mastitis were carefully examined. The samples were collected from May 1994 to April 1995, and the milk samples had been submitted to the laboratory by veterinary practitioners for bacteriological examination. The samples re-

present herds from all parts of Denmark. In this study no control group was included, like cases of subclinical mastitis. Also, the disease history was often scarce leaving the laboratory uninformed about the severity of the cases.

For routine examination drops of milk were spread on esculin blood agar without and with 0.1 IU penicillin. The plates were incubated overnight at 37°C in a "candle jar", which approximately generates an atmosphere containing 2.5% CO₂. Parallel to the routine examinations another plate without penicillin was incubated for 20 h at 37°C in an atmosphere containing 5% CO₂.

Esculin agar, used for routine examinations of mastitis cases in Denmark, consists of peptone (Difco) 11.66 g, agar 12.0 g, meat extract (Fleischextrakt rocken, Merck) 4.0 g, Na₂HPO₄·2H₂O 1.5 g, NaCl 5.0 g, yeast extract (Difco) 3.0 g, esculin (Merck) 1.0 g, tryptone (Difco) 8.3 g, glucose (Merck) 0.44 g, distilled water 1000 ml (pH 7.4), and 5% sterile bovine stabilized blood.

At the routine examination bacteriological growth was seen in 4266 of the cases, in 430 of the cases it was possible to demonstrate 2 different pathogens in secretions from one milk quarter. The bacteriological diagnoses are shown in Table 1. In the group "other pathogens" only a few bacteria from different species were found, such as various types of haemolytic *Streptococcus*, *Klebsiella*, *Listeria mono-*

Table 1. Bacteriological diagnosis after routine examination of mammary secretions from 6809 milk quarters in 3783 individual cows suffering from clinical mastitis. In the group "Other pathogens" only a few bacteria representing different species were found.

Diagnosis	Number	Per cent
<i>Streptococcus dysgalactia</i>	827	11.4
<i>Streptococcus uberis</i>	685	9.5
<i>Staphylococcus aureus</i>	1067	14.7
Coagulase negative staphylococci and micrococci	456	6.3
Penicillin resistant <i>Staphylococcus aureus</i>	217	3.0
<i>Actinomyces pyogenes</i>	195	2.7
Yeast cells	96	1.3
Coliform group	727	10.0
Faecal streptococci	137	1.9
Contaminated samples	179	2.6
No pathogen found	2543	35.1
Other pathogens	110	1.5
Total	7239	100.0

cytogenes, *Pasteurella multocida*, *Proteus* and *Pseudomonas*.

Due to no growth of any microbiological agent, drops from 2543 of the samples were spread on esculin blood agar for a second time and incubated at 37°C in an atmosphere containing 5% CO₂. All the plates were read twice, after 20 h and after 44 h.

H. somnus was isolated in only one case and in pure culture from a cow showing a rather mild clinical mastitis.

In this study the growth conditions for *H. somnus* on agar plates were optimal. Prior to this study isolation of *H. somnus* has only been reported twice in connection with acute bovine mastitis in Denmark (Wedderkopp & Nylin 1994). Experimentally *H. somnus* is capable of producing either a chronic form or a severe gangrenous form of bovine mastitis (Hazlett *et al.* 1983). Two cases of severe clinical mastitis in dairy cows, where *H. somnus* was isolated in pure culture, have been reported from Canada (Armstrong *et al.* 1986, Harris & Janzen 1989). The fact that only one isolate of *H. somnus* was demonstrated in this material indicates that *H.*

somnus cannot be a major cause of bovine clinical mastitis in Denmark.

At the Cattle Health Laboratory, the CO₂-incubator is now being used instead of the "candle jar" for routine examination of milk samples from cows suffering from clinical mastitis.

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