Szent István University

Postgraduate School of Veterinary Science

Comparative examination of *Histophilus somni* strains isolated from farm animals

PhD dissertation theses

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1. Introduction

Histophilus somni is a Gram-negative, fastidious, facultative pathogenic bacterium. It had many former names (*Histophilus ovis*, *Haemophilus agni*, *Haemophilus somnus*) that were used parallel resulting certain confusion. The taxonomical position of the microorganism was not clarified for a long time, the name *Histophilus somni* was suggested a few years ago.

H. somni is a coloniser of the respiratory and genital tract of cattle and sheep. It can cause local or generalised diseases, but asymptomatic carriers can also occur in both animal species. In cattle it can cause thromboembolic meningoencephalitis, pneumonia and reproductive problems as well as septicaemia, orchitis and epididymitis in sheep.

Diseases caused by *H. somni* are widespread in the world; they have been reported since the 1950's. Chronic respiratory disease of cattle causes the major losses of cattle industry in the USA as well in Hungary. About 5-20% of the losses of Hungarian cattle stocks can be explained by respiratory diseases. The first isolation of *H. somni* from pneumonic cattle lungs in Hungary was recorded by Forray et al. in 1984.

2. Objectives

Evaluation of the occurrence of *H. somni* strains in Hungarian cattle herds and goat flocks by isolation of the bacterium from respiratory and vaginal samples was the primary goal of the present work.

Identification of freshly isolated *H. somni* strains and strains from the culture collection of the Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University using BIOLOG MICROSTATION™ ID SYSTEM was planned.

We aimed to analyse the relationship of *H. somni* strains from different host species on the basis of metabolic fingerprinting using carbon source utilisation.

Analysing the macrorestriction patterns of *H. somni* strains of different origin was also intended.

A priority of the present project was the comparison of the results of carbon source utilisation and PFGE patterns of 100 *H. somni* strains.

To study the clinical and pathological effect of *H. somni*, development of an aerosol infection method and description of the pathological and histopathological findings was also one of our objectives.

Finally examination of antimicrobial susceptibility of *H. somni* strains using standard broth microdilution method was also planned.

3. Materials and methods

Specimen sampling and formerly isolated H. somni strains

During the 24 months of sample collecting we examined 9 cattle- and 10 goat flocks in 18 townships of Hungary. Altogether 652 respiratory- or genital swab samples were collected and examined by bacterial isolation for the presence of *H. somni*. A total of 27 *H. somni* strains isolated form sheep genitals and 33 bovine respiratory isolates collected in 10 cattle- and 6 sheep flocks of Hungary and stored in the culture collection of the Department of Microbiology and Infectious Diseases were. also included in the examinations.

Isolation and identification of H. somni

For the isolation of *H. somni* strains organ and swab samples were inoculated on chocolate agar plates containing 10% heat treated (80°C, 20 minutes) sheep blood. The agar plates were incubated in the presence of 5% CO_2, at 37°C for 48 hours. After proper incubation *H. somni* formed typical, round shaped, plane colonies about 1-1,5 mm in diameter with characteristic yellow pigmentation. Morphology, motility, production of catalase, oxidase and indole were examined using standard methods.

Using the BIOLOG MICROSTATION™ ID SYSTEM (Biolog Inc. Hayward, Canada) the isolates were identified on the basis of their carbon source utilisation.

In order to verify the identification, 15 *H. somni* strains were examined on the basis of analysing a highly similar part of the *H. somni* genome. A 400 bp part of the 16S rRNA gene was amplified and sequenced.

Examination of carbon source utilisation of H. somni

The carbon source utilisation of 98 clinical isolates and 2 *H. somni* type strains was examined using the BIOLOG MICROSTATION™ ID SYSTEM. The system analyses the ability of the utilisation of 95 single carbon sources simultaneously thus resulting a metabolic fingerprint of the examined bacterial strains. Dendrograms were made on the basis of metabolic fingerprints using the MicroLog3 (4.20.05) software. Several dendrograms were created from the *H. somni* strains of different origin to compare the relationship of the strains..

Examination of H. somni with pulsed field gelelectrophoresis (PFGE)

Ninety-seven clinical isolates and two *H. somni* type strains were digested using *SmaI* restriction enzyme. *Cfr9I* restriction enzyme, an isoschizomer of *SmaI*, was used to digest 7 undigested and 11 digested strains to compare the results of the two endonucleases and to digest the strains that were refractory to *SmaI* digestion. The complete genome of the examined bacteria was extracted in an agarose plug to avoid the damage of large DNA molecules. The PFGE was carried out using

contour-clamped homogeneous electric field (CHEF) electrophoresis. The results were analysed statistically by an independent expert.

Experimental infection of calves with H. somni

Twelve calves were infected were infected aerogenically with *H. somni* on three consecutive days using a vaporiser mask. Five animals served as controls. Observation period lasted for 14 days, and individual clinical data were recorded daily. Animals were exterminated on the 15th day of the trial and were necropsied followed by individual bacteriological and histopathological examinations.

Examination of antimicrobial susceptibility of H. somni

Examining the susceptibility of 39 clinical isolate and 1 *H. somni* type strain to enrofloxacin, florfenicol, gentamicine, penicillin-G, tetracycline and tilmicosin was carried following methods recommended by M31-A2 NCCLS document. Standard broth microdilution method was applied. Analysing the results minimal inhibitory concentrations (MIC) of antibacterial agents, as well as antibacterial susceptibility of the *H. somni* strains were determined.

4. Results

Isolation of H. somni strains

Out of the 652 samples 56 bacterial strains identified as *H. somni* on the basis of cultural, morphological and biochemical characteristics were isolated. Five cattle stocks out of six proved to be positive for the presence of *H. somni* by examining vaginal swab samples, while all flocks were positive according to the results of respiratory samples. We isolated 38 and 7 *H. somni* strains from vaginal and respiratory swab samples respectively. Out of 205 genital swab samples of goats 11 (5,4%) *H. somni* strains were isolated, while all examined nasal swab samples were negative.

Identification of H. somni

The BIOLOG MICROSTATION™ ID SYSTEM identified 83% of the examined bacterial strains as *H. somni*. Strains that could not be identified on the basis of carbon source metabolism proved to be *H. somni* by partial amplification of 16S rDNA.

Metabolic fingerprints of H. somni strains

Examining the carbon source metabolism of 100 *H. somni* strains there were two carbon sources – dextrin and α-D-glucose – that could be utilised by all of them. All bovine vaginal isolates could metabolise the D, L-lactic acid as well as of *H. somni* strains isolated from sheep utilised D-mannose and turanose. Studying the dendrogram based on the carbon source utilisation of 100 *H. somni* strains, several highly similar strains as well as different ones were found in certain cattle and sheep flocks. There were two highly similar *H. somni* strains of respiratory origin that could be isolated in the same cattle stock sampling it in different years.

PFGE patterns of H. somni strains

On the dendrogram consisting of the results of examined *H. somni* strains, there were many highly similar strains originated from cattle- and sheep genitals, while the distinct position of the strains could be detected in some cases. The 100% similarity of some strains of cattle lung origin could be observed in one cattle stock examined.

Clinical and pathological effect of H. somni

In infected groups the first clinical signs occurred on day 7, elevated rectal temperatures and mild respiratory distress could be observed. Catarrhal bronchopneumonia was seen in all groups post mortem. The character of lung lesions in challenged and control groups was different and difference was seen in their extension. Acute suppurative bronchopneumonia and acute lymphadenitis were detectable in the challenged animals rather than in the control ones, the difference was significant. At the end of the trial all infected animals proved to be positive for the presence of *H. somni* while there were no positive samples in the control group.

Antimicrobial-susceptibility of H. somni strains

Differences could be detected in the MIC-values of the examined antimicrobial agents. Bacteria isolated from sheep semen or testicles were slightly more susceptible to enrofloxacin and florfenicol than strains of cattle origin and the other hand cattle vaginal isolates were more susceptible to tilmicosin than strains of ovine origin. The susceptibility to penicillin-G and gentamicine was not uniform, while in the case of tetracycline similar MIC-values could be detected.

The examined *H. somni* strains proved to be susceptible to enrofloxacin, florfenicol, penicillin-G, tetracycline and tilmicosin in contrast the intermediate susceptibility to gentamicine.

5. Conclusions

The occurrence of H. somni in cattle- and goat flocks of Hungary

According to our results *H. somni* could be isolated more frequently from cattle flocks of Hungary than that of Finland, but the bacterium was not as widespread as in Denmark.

The occurrence of *H. somni* on cattle vaginal mucous membranes was 10-40%. During the four-time, monthly sampling of 20 heifers the isolation rate of *H. somni* increased from 40% to 90%, thus to the evaluation of vaginal occurrence of *H. somni* in cattle at least two sampling is recommended. Examination of respiratory samples of cattle for the presence of *H. somni* is also recommended, because of the 100% positivity of cattle flocks.

Identification of H. somni strains

Strains that could not be identified on the basis of carbon source metabolism proved to be *H. somni* on the basis 16S rRNA partial sequence. Compared to the results of 15 *H. somni* identified by partial amplification of 16S rDNA, some false negative but no false positive ones could be found. Developing the database of BIOLOG MICROSTATION™ ID SYSTEM will result more accurate identification of *H. somni* strains, however in the case of strains isolated from a new hosts or new diseases DNA-based identification is essential.

Carbon source utilisation of H. somni strains

The *H. somni* strains could utilise a wide range of mono- and disaccharides, sugar-alcohols, esters, acids, amino-sugars and phosphated sugar-derivates as well as amino acids and nucleosides. Analysing the dendrogram of 100 *H. somni* strains the presence of several highly similar strains as well as different ones in certain cattle and sheep flocks was proved. Two persistent respiratory isolates could be found in a cattle stock.

PFGE pattern of H. somni strains

Analysing the cumulative results of pulsed field gel electrophoresis (PFGE) the presence of similar, different and persistent *H. somni* strains in certain cattle and sheep flocks was also seen.

Comparison of metabolic fingerprinting and PFGE

Comparing the results of metabolic fingerprinting and PFGE, the methods complemented each other. The examination of carbon source utilisation was used successfully to evaluate epidemiological characteristics of *H. somni* strains.

Clinical and pathological effects of H. somni

Clinical signs in infected groups were similar to those described earlier in the case of *H. somni* pneumonia. Pathological examination of infected and control groups showed catarrhal

bronchopneumonia in all animals, thus histopathological examination is recommended to complete pathological results and evaluate the efficacy of the trial. In the control group no *H. somni* was isolated in contrast the 100% detection rate in infected groups. In conclusion according to the results the pathological and histological findings were mostly related to *H. somni*.

Antimicrobial susceptibility of H. somni strains

Examining the antimicrobial susceptibility of *H. somni* strains, they were found to be highly susceptible to enrofloxacin, florfenicol. The standardization of interpretive criteria of penicillin-G and tetracycline would be essential when determining the susceptibility of *H. somni* strains. There are no data on gentamicin-resistance of *H. somni* strains; we are planning to examine it in further studies.

The susceptibility to enrofloxacin, florfenicol and penicillin-G decreased in recent years, so it is very important to use the antimicrobial agents properly.

6. New scientific results

1. We reported the first isolation of *H. somni* from goat flocks. The dendrogram based on the carbon source utilisation of the strains indicated a close relationship among the caprine *H. somni* strains. The partial sequencing of the 16S rRNA verified the identification, the homology of this sequence and the high level of conformity of the metabolic fingerprints indicated a common source of infection. The partial 16S rRNA sequences of examined *H. somni* strains were 100% identical to that of an ovine septicaemic isolate, when compared with sequences of the GenBank, suggesting the sheep to be the common source of the infection.
2. Incidence of *H. somni* was 10-40% according to the results of vaginal swab samples collected in cattle herds of Hungary.
3. We verified that the BIOLOG MICROSTATION™ ID SYSTEM can be used for the identification and analysis of carbon source metabolism of *H. somni* strains.
4. We proved that the BIOLOG MICROSTATION™ ID SYSTEM is suitable for epidemiological investigation of *H. somni* strains.
5. The PFGE method was used for comparative analysis and characterisation of *H. somni* strains of different origin.
6. Comparing the carbon source utilisation and PFGE we found that metabolic fingerprinting can be used for epidemiological characterisation of *H. somni* strains isolated from similar origin.
7. We developed a new aerosol infection model for experimental infection of calves and utilized it successfully.
8. We were the first to report characterisation of pathological and histopathological findings of *H. somni* pneumonia in Hungary.
9. We examined the antimicrobial susceptibility of several *H. somni* strains using standard broth micro-dilution method, and found the majority of strains to have intermediate susceptibility to gentamicine.

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8. Articles published

K. Jánosi, I. Hajtós, L. Makrai, M. Gyuranecz, J. Varga, L. Fodor: First isolation of *Histophilus somni* from goats. Veterinary Microbiology (2009), 133: (4) 383-386.

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Posters and presentations:

K. Jánosi, L. Makrai, I. Hajtós, J. Varga, A. E. Tirián, L. Fodor (poster):

Comparative study of the metabolic fingerprint of *Histophilus somni* strains isolated from farm animals. 15th International Congress of the Hungarian Society for Microbiology, 18-20 July 2007. Budapest, Hungary. In: Proceedings of the 15th International Congress of the Hungarian Society for Microbiology. Acta Microbiol. Immunol. Hung. 2007. 54. Suppl.: 52-53.

K. Jánosi, I. Hajtós, L. Makrai, M Gyuranecz, J. Varga, L. Fodor (előadás):

First isolation of *Histophilus somni* from goat flocks in Hungary. Congress of the Hungarian Society for Microbiology 2008. 14-17. October 2008. Keszthely, Hungary. Abstracts 2008. 31-32.

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