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László Fodor

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## Short communication

### First isolation of *Histophilus somni* from goats

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## 1 Abstract

2

3 *Histophilus somni* (former name: *Haemophilus somnus*) is a Gram-negative,  
4 facultative pathogen bacterium that colonises the mucous membranes of cattle and sheep,  
5 however it was also described in American bison and bighorn sheep. It can cause local or  
6 generalised diseases and asymptomatic carriers can also occur. The presence and the  
7 etiological role of this microorganism have not been confirmed in any other domesticated  
8 species yet. The purpose of this study was to prove the presence of *H. somni* in goats by  
9 bacterial isolation. Nasal, vaginal or praeputial swab samples were collected from 205 goats  
10 in 10 flocks. *H. somni* strains were isolated from 2 out of 10 flocks; in one flock 10 *H. somni*  
11 strains were isolated from the genital mucosa of 17 goats, while a single *H. somni* strain was  
12 cultured from a vagina of 26 animals in the other flock. Partial amplification and sequencing  
13 of the 16S rRNA gene of three *H. somni* strains verified the identification. The comparative  
14 examination of carbon source metabolism using the BIOLOG MICROSTATION™ ID SYSTEM  
15 (Biolog, Ca) showed a close relationship of the caprine strains, while they were less related to  
16 *H. somni* type strain CCUG-36157 of bovine origin. *H. somni* strains were isolated only in the  
17 oestrus season from goat flocks with sheep contact. This is the first paper on isolation of *H.*  
18 *somni* from goats.

19

20 *Keywords: Histophilus somni; goat; genitals.*

21

## 22 1. Introduction

23

24 *Histophilus somni* is a Gram-negative, fastidious, facultative pathogenic bacterium. It  
25 had many former names (*Histophilus ovis*, *Haemophilus agni*, *Haemophilus somnus*) that

1 were used parallel resulting certain confusion. The taxonomical position of the microorganism  
2 was not clarified for a long time, the name *Histophilus somni* was suggested a few years ago  
3 (Angen et al., 2003).

4 *H. somni* is a coloniser of the respiratory and genital tract of cattle and sheep. It can  
5 cause thromboembolic-meningoencephalitis – TEME – (Kennedy et al., 1960), pneumonia  
6 (Andrews et al., 1985; Blackall et al., 2007), metritis (Miller et al., 1983) and abortion (Van  
7 Dreumel and Kierstead, 1975) in cattle. In some cases arthritis (Panciera et al., 1968),  
8 myositis and myocarditis (Janzen, 1987) were described as a consequence of the septicaemic  
9 form (Panciera et al., 1968). Mastitis (Higgins et al., 1987) and suppurative otitis media and  
10 interna (McEwen and Hulland, 1985) were also reported. In sheep *H. somni* can cause  
11 septicaemia (Kennedy et al., 1958), meningitis (Lees et al., 1994), mastitis (Roberts, 1956),  
12 polysynovitis and neonatal losses (Rahaley and White, 1977). Epididymitis of young rams  
13 (Low and Graham, 1985; Saunders et al., 2007), abortus (Hajtós, 1987) or vulvitis (Ball et al.,  
14 1991) of ewes were also described. Asymptomatic carriers can occur in both animal species  
15 (Walker and LeaMaster, 1986; Humphrey et al. 1982). *H. somni* was also reported in  
16 respiratory problems of American bison (*Bison bison*) (Dyer, 2001) and in bighorn sheep  
17 (*Ovis canadensis*) (Ward et al. 2006).

18 Beside cattle and sheep neither the isolation nor the etiological role of *H. somni* have  
19 ever been reported in any other domesticated animal species. The aim of this study was to  
20 examine the presence of *H. somni* on the mucous membranes of the respiratory or genital tract  
21 of goat.

22

## 23 **2. Materials and methods**

24

### 25 *2.1. Specimen sampling.*

1

2 Ten goat flocks were sampled in the northern part of Hungary between March and September  
3 2007. Nasal and vaginal or praeputial swab samples were taken from 205 animals, including  
4 191 female-, 13 male goats and 1 hermaphrodite animal (Table 1). They were transported in  
5 physiologic saline under refrigeration to the laboratory for bacteriological examination within  
6 3 hours. Six flocks (No. 1-6) were sampled before the oestrus season and 4 ones (No. 7-10)  
7 during it. In two flocks goats were in contact with sheep; there was one ram in flock No. 8  
8 while about the same number of goats and sheep were kept together in flock No. 9.

9

## 10 2.2. Bacteriological examination.

11

12 Swab samples were inoculated on chocolate agar containing 100 µg/ml cyclohexamide (Slee  
13 and Stephens, 1985). All samples were incubated for 48 h at 37°C, in an atmosphere  
14 containing 10% CO<sub>2</sub>. After proper incubation *H. somni* had typical, round shaped, plane  
15 colonies about 1-1,5 mm in diameter with characteristic yellow pigmentation. Examination of  
16 morphology, motility, production of catalase, oxidase and indole were carried out using  
17 standard methods (Barrow and Feltham, 1993).

18

## 19 2.3. DNA extraction, 16S rRNA PCR and sequencing.

20

21 The 16S rRNA gene of 3 *H. somni* strains (8/17, 9/13, 9/15) was partially sequenced. DNA  
22 was extracted from pure cultures, using commercial kit (Quiagen, Protocol C) following the  
23 manufacturer's instructions, then a 382 bp part of the 16S rRNA gene was amplified as  
24 described by Angen et al. (1998) and sequenced ([Biomi Ltd.](#), Hungary; NCBI-GenBank:

1 [EU708473](#)). The 16S rRNA gene partial sequence was analysed using programs, based on the  
2 BLAST algorithm (Altschul et al., 1997) at NCBI ([NCBI-BLAST](#)).

3

#### 4 2.4. Metabolic fingerprint.

5

6 The examination of the metabolic fingerprint of 11 *H. somni* isolates from goat and a *H.*  
7 *somni* type strain of bovine origin (Culture Collection, University of Göteborg Sweden;  
8 CCUG-36157) was carried out using the BIOLOG MICROSTATION™ ID SYSTEM (Biolog, Ca).  
9 The bacteria were processed according to the BIOLOG MICROSTATION™ ID SYSTEM's protocol  
10 for Gram-negative, fastidious bacteria and a dendrogram was made on the basis of the results  
11 of the metabolic fingerprinting.

12

### 13 3. Results

14

15 Eleven bacterial strains were isolated and identified as *H. somni* on the basis of  
16 cultural, morphological and biochemical characteristics. *H. somni* strains were isolated from  
17 genital swabs collected in two out of ten goat flocks. In flock No. 9 eight *H. somni* strains  
18 were isolated from vaginal samples out of 14 and one strain was cultured from the praeputium  
19 out of two bucks. A swab sample from the sexual orifice of a single hermaphrodite animal  
20 also contained *H. somni*. In this flock 58.8% of the sampled animals carried *H. somni* on the  
21 genital mucous membranes. One vaginal sample (3.8%) was positive for the presence of *H.*  
22 *somni* out of 26 in the other goat flock (No. 8). Both positive flocks had contact with sheep  
23 and the positive flocks were sampled in the main mating season. No *H. somni* strains were  
24 cultured from the nasal samples. 162 sampled animals of eight goat flocks proved to be free  
25 from *H. somni* when examined with cultural methods.

1 The isolated strains were uniform, they were Gram-negative, small (1-3  $\mu\text{m} \times 0.5\text{-}0.6$   
2  $\mu\text{m}$ ), non motile coccobacilli, however sometimes filaments could be seen in smears made  
3 from 48-hour-old cultures. The most important cultural, morphological and biochemical  
4 characteristics are presented in Table 2 and 3.

5 The partial 16S rRNA sequence of 3 selected *H. somni* strains (8/17, 9/13, 9/15) were  
6 identical in 100% to that of an ovine septicaemic isolate (CCUG-46772) when compared with  
7 sequences in the NCBI databases.

8 There were 11 carbon sources that could be utilized by all examined *H. somni* strains  
9 of goat origin, and there were 76 carbon sources that could not be metabolized by any of  
10 them. A dendrogram made on the basis of the results of the metabolic fingerprinting is given  
11 in Figure 1.

#### 13 4. Discussion

14  
15 The present paper describes the first isolation of *H. somni* from goats. *H. somni* was  
16 isolated only from those flocks, where goats were kept together with sheep and the sampling  
17 took place in the main mating season. According to the results, the presence of *H. somni* on  
18 goat genital mucous membranes is in correlation with the oestrus season and the intensity of  
19 sheep contact. When the same number of goats and sheep were kept together 58.8% of the  
20 goats carried *H. somni* on the genital mucous membranes, while in the case of limited sheep  
21 contact the carrier rate was lower, as well. The interspecies sexual activity of rams in the  
22 mating season can explain the infection of goats. The dendrogram based on the carbon source  
23 utilisation of the strains shows the close relationship of the caprine *H. somni* strains and the  
24 distinct position of *H. somni* type strain of bovine origin. The partial sequencing of the 16S

1 rRNA verified the identification, the homology of this sequence and the high level of  
2 conformity of the metabolic fingerprints shows common source of infection.

3

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5

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7 technical work of Mrs Teréz Halasi is gratefully acknowledged.

8

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1 Table 1. Specimen sampling and summarized results

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2	Flock	Sex of the goats			Origin of the sample			
3	No.	Female	Male	Hermaphrodite	Vagina	Praeputium	Sexual orifice	Nasal cavity
4	1	20	0	0	20/0 <sup>a</sup>	0/0	0/0	20/0
5	2	19	1	0	19/0	1/0	0/0	20/0
6	3	20	0	0	20/0	0/0	0/0	20/0
7	4	24	1	0	24/0	1/0	0/0	25/0
8	5	15	1	0	15/0	1/0	0/0	16/0
9	6	13	1	0	13/0	1/0	0/0	14/0
10	7 <sup>b</sup>	18	2	0	18/0	2/0	0/0	20/0
11	8 <sup>b,c</sup>	26	4	0	26/1	4/0	0/0	30/0
12	9 <sup>b,c</sup>	14	2	1	14/8	2/1	1/1	17/0
13	10 <sup>b</sup>	22	1	0	22/0	1/0	0/0	23/0
14	Σ	191	13	1	191/9	13/1	1/1	205/0

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16 <sup>a</sup>Total No. of samples/No. of positive samples for the presence of *H. somni* by culturing  
 17 methods

18 <sup>b</sup>Flocks sampled in oestrus season

19 <sup>c</sup>Goat flocks kept together with sheep

1 Table 2. Cultural, morphological and primary biochemical characteristics of *H. somni* strains  
2 isolated from goat genital mucous membranes

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3	Enhancement of growth in 10%	+ (11) <sup>a</sup>
4	Production of yellow pigment	+ (11)
5	Motility	- (0)
6	Gram-staining (48h)	1-3 $\mu\text{m}$ $\times$ 0,5-0,6 $\mu\text{m}$ coccobacilli
7	Catalase	- (0)
8	Oxidase	+ (11)
9	Indole production	+ (11)

10

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11 <sup>a</sup>Number of positive strains in parenthesis

1 Table 3. Carbohydrate utilization of *H. somni* strains isolated from goat genital mucous  
2 membranes

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3	D-Cellobiose	+ (11)
4	Dextrin	+ (11)
5	D-Fructose	+ (11)
6	$\alpha$ -D-Glucose	+ (11)
7	D-Glucose-6-Phosphate	+ (11)
8	N-Acetyl-D-Glucosamin	+ (11)
9	$\alpha$ -Ketobutyric-Acid	+ (11)
10	D-Mannitol	+ (11)
11	D-Mannose	+ (11)
12	D-Sorbitol	+ (11)
13	Turanose	+ (11)
14	D,L-Lactic-Acid	+ (10)

---

16 Number of positive strains in parenthesis

1 Figure caption

2 Figure 1. Dendrogram based on the carbohydrate metabolism of the 11 *H. somni* strains of  
3 caprine origin and 1 *H. somni* type strain of bovine origin.

4 \_\_\_\_\_

5 Flock No./Sample No.

6 \**H. somni* type strain: CCUG-36157 = ATCC-43625 = 8025; USA, cattle, brain.

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