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ISOLATION AND CHARACTERIZATION OF HAEMOPHILUS SOMNUS FROM COWS WITH METRITIS

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Metritisli ineklerden Haemophilus somnus izolasyonu ve identifikasyonu

Özet: *Metritisli iki inek uterusundan Haemophilus somnus izole ve identifiye edildi. Mezbahada kesilen metritisli iki inek uterusundan steril eküvyonlarla kanlı agar, triptoz agar ve kanlı-maya özetli-beyin kalp infüzyon (BHEBYE) agara ekimler yapıldı. Sadece kanlı-maya özetli-beyin kalp infüzyon agarda üreme belirlendi. Koloniler, 24 saat inkübasyondan sonra 0.5 mm çapında, yarı saydam, grimsi, yuvarlak ve konveks şekilli olarak görüldüler. Saf halde izole edilen bakteriler, morfolojik ve üreme özelliklerine ve biyokimyasal test sonuçlarına göre Haemophilus somnus olarak identifiye edildiler. Gram negatif, pleomorfik, küçük kokobasil şeklindeki mikroorganizmnın üremek için serum ve karbondiokside gereksinim gösterdiği saptandı. İzole edilen suşlar glukozu fermente ettiler oksidaz pozitif ve katalaz negatif bulundular. İzolatların H. somnus olduğu, standart bir H. somnus suşuna karşı hazırlanmış hiperimmun serum kullanılarak yapılan aglütinasyon testi ile doğrulandı. Saf olarak H. somnus izolasyonu, metritise bu etkenin neden olabileceğini gösterdi.*

Summary: *Haemophilus somnus was isolated from two cows with metritis slaughtered in a local abattoir. Uterine samples collected with steril swabs were seeded onto several culture media. The organisms grew well only on brain-heart infusion agar supplemented with blood and yeast extarct (BHIBYE) in pure culture. The colonies were translucent, greyish, round, and reached the diameter of 0.5 mm after 24 hours of incubation. The isolated bacteria were identified as Haemophilus somnus on the basis of morphological, cultural and biochemical characteristics. The organism requiered serum and carbon dioxide*

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for growth. The isolate was Gram negative, small, pleomorphic coccobacilli and fermented glucose, produce oxidase but did not catalase. For the confirmation of isolates as H. somnus a slide agglutination test was also made. The isolation of agent in pure culture suggested that this organism was the cause of pathologic condition.

Introduction

Haemophilus somnus is the aetiological agent of several disease conditions of cattle. This organism was initially isolated from a case of thromboembolic meningoencephalitis (TEME) in feedlot cattle (10). Subsequently it has became clear that this pathogen is involved in the pathogenesis of a variety of syndromes including septicemia (11), arthritis (17), laryngitis-tracheitis (9), bronchopneumonia-pneumonia (1,7), mastitis (12) and genital diseases (15).

Reproductive disease syndromes in cattle caused by *H. somnus* are metritis (6,8,14), vulvovaginitis (16), abortion (4), "weak calf syndrome" (19) and infertility (7). These syndromes beginning with a primary *H. somnus* infection and completed with invasion by secondary bacterial pathogens are referred to as "*H. somnus complex*" (18).

H. somnus is a pleomorphic, microaerophilic, Gram negative bacterium that has the same taxonomic features in common with members of the genus Haemophilus (6,21). But, it differs from this genus by the ability of growth in the absence of "X" and "V" factors. In 1969, the name of *H. somnus* was proposed by Bailie (3). It is a widely used but unsatisfactory classification for this organism. Unclassified microorganisms isolated from sheep including *Histophilus ovis* and *Haemophilus agni* together with H. somnus may constitute a single species (13,20).

The isolation and identification of *Haemophilus somnus* from cows with metritis is reported in this paper. No report on the recovery of this organism in Turkey has appeared in literature.

Materials and Methods

Two bovine uteruses with metritis were collected from an abattoir. Uterine infections were diagnosed by post-mortem examination.

Samples were collected aseptically from uterus mucosas by swabbing. Swabs were seeded onto following media: sheep blood agar, tryptose agar and brain-heart infusion agar supplemented with 0.5

% yeast extract and 7 % defibrinated sheep blood (BHIBYE). The plates were incubated at 37 °C in an atmospher containing 10 % carbon dioxide for 48 hours.

The isolated organisms were examined by means of growth and biochemical characteristics described by Humphrey and Stephens (13). The isolates were tested for their ability to grow on/in the following solid and liquid media: sheep blood agar, tryptose agar, triple sugar iron agar, McConkey agar, thiolglycolate agar, BHIBYE agar, nutrient broth, thiol medium, brain-heart infusion broth with yeast extract (BHIBYE) and these 3 broth media with the addition of serum. In addition, the following biochemical tests were used for identification: carbohydrate fermentation, nitrate reduction, oxidase, catalase, urease, indol and hydrogen sulfite production, MR/VP, citrate, ornithin and lysin decarboxylase tests. All biochemical tests were performed in BHIYE broth supplemented with serum.

H. somnus starin 43826 was used as control strain in all tests and for the production of immunizing antigen in rabbits. This strain was isolated from the brain of a steer with naturaally occuring TEME and obtained from Dr. S.C. Groom, Canada. The hyperimmun serum to this reference strain obtained from rabbit was tested against the colonies of the both isolates on a slide.

Results

Pure cultures of small, less than 1 mm, round, glistening, nonhemolytic, translucent and grey colonies grew only on BHIBYE agar incubated in carboxyphilic condition. Gram negative, pleomorphic, small coccobacilli were seen by Gram staining. The isolates grew well only BHIBYE agar and BHISYE broth but not on/in other media tested. The organism required additional carbon dioxyde for growth. Motility was not observed under phase contrast and dark field illumination.

The isolated organisims produced oxidase and indol but did not produce catalase, urease and hydrogen sulfite. They fermented glucose, sorbitol, mannitol and maltose but did not ferment lactose and arabinose. Nitrate was reduced to nitrites. MR/VP and ornithin decarboxylase tests were negative and lysin decarboxylase test was positive. The isolates did not utilize citrate.

Both isolates had the same morphological, growth and biochemical features.

In the slide agglutination test, hyperimmun serum agglutinated the both isolates.

Discussion and Conclusion

In this study, two *H. somnus* strains were isolated from cows with metritis. Morphological and cultural characteristics of isolates were found identical with the reference strain and other strains reported by several investigators (3,7,16).

Asmussen and Daugh (2) have described that serum and yeast extract were the growth factors for *H. somnus*. In this study, isolated strains also did not grow on/in the media without these supplements. Cultural and biochemical examination of two isolates haves also indicated that both strains belonged to the same species.

The identification of isolates as *H. somnus* has been confirmed by slide agglutination test. Since, Humphrey and Stephens (13) reported that *H. somnus* had a single serotype and did not cross react with other bacteria except close related species, the confirmation by agglutination can be regarded as a suitable way. No report on the slide agglutination test of this organism has appeared in the literature.

Humphrey and Stephens (13) have reviewed the biochemical features of *H. somnus* and listed the constant ones. Two strains isolated in this case showed close similarity to these criteria. The differentiation of isolates from related bacteria such as *H. agni* and *Histophilus ovis* was also achieved. Webb (22) has reported some biochemical tests which can be used for the differentiation of *H. somnus* from related bacteria. Agents isolated in this study were indol, sorbitol, lysin decarboxylase positive, but urease and ornithin decarboxylase were negative. They were separated from *H. agni* and *Histophilus ovis* according to these results.

Reproductive type diseases associated with *H. somnus* infection are less well characterized than the nervous system type. Corboz and Wild (8) and Miller et al (14) have isolated *H. somnus* from cows with metritis and endometritis. These workers found that in the case from which *H. somnus* was isolated, endometritis was almost always present. Though experimental induction of metritis has not been reported yet, observations in natural infections indicated that *H. somnus* was an important cause of metritis.

Miller et al (15) reported that bulls and cows might carry the organism in their reproductive tracts without evidence of macroscopic lesions. In addition, the frequecny of *H. somnus* in uterus of healthy cows has been found lesser than the other parts of reproductive tract. But, Stephens et al (18) found that *H. somnus* in reproductive tract of clinically healty cattle was low in number.

Ward et al 21) and Corboz (6) have compared the strains of *H. somnus* isolated from cattle with clinical cases and from clinically normal cattle. No differences between strains were reported using morphological, cultural and biochemical criteria. In the present study, such a comparison could not be executed. On the other hand, Corbeil et al (5) showed that all isolates of *H. somnus* from cattle with clinical disease were not inhibited by serum. In this case, since the isolated strains had the ability of growth in serum, at least these strains may not be considered as nonpathogenic.

In this study, the isolation and identification of *H. somnus* strains in pure culture from cows with metritis strongly suggested that *H. somnus* was cause of these conditions.

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