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ISOLATION AND CHARACTERIZATION OF NEISSERIA OVIS FROM OVINE KERATOCONJUNCTIVITIS

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Keratokonjuktivitisli koyunlardan Neisseria ovis izolasyonu

Özet: *İnfeksiyöz keratokonjuktivitisli kuzulardan bir Neisseria ovis suşu izole edildi. Hasta hayvanların konjuktivalarından alınan eküvyoniar kanlı agar besiyeri üzerine ekildiler. Gram negatif, hemolitik koklar saf olarak izole edildiler. Koloniler, gri renkli, yarı saydam, "S" tipinde, düzgün kenarlı konveks ve belirgin olarak hemolitik idiler. Hareketsiz ve spor oluşturmayan bakteri çoğunlukla diploid şekilde görüldü. Penisilin varlığında, hücrelerde uzama görülmedi. Organizma, buyyonda parçalanabilir bir pelikül, biraz bulanıklık ve granüllü bir çüküntü oluşturdu. İzole edilen organizma oksidaz ve katalaz pozitif bulundu. Karbonhidratların hiçbirisinden asit üretimi gözlenmedi. Nitrat, nitrite indirgendi. İzolat indol ve hidrojen sülfit oluşturmadı. Ureaz, DNAaz, Fozfataz, MR ve VP testleri negatif bulundu. Eriyebilir bir hemolizin belirlenmedi. Organizma, denenen tüm antibiyotiklere duyarlı bulundu. Parenteral ve konjuktival yolla N. ovis verilen fareler hiçbir semptom göstermediler.*

Summary: *A Neisseria ovis strain was isolated from lambs with infectious keratoconjunctivitis. Swabs taken from the conjunctival sac of diseased animals were inoculated onto blood agar plates. The pure cultures of gram-negative hemolytic cocci were isolated. The colonies were greyish, translucent, smooth, convex with regular edges and hemolytic with a clear zone. The bacteriae were non-motile, non-spor forming which often occured in diploid arrangement. Elongation was not seen in the presence of penicilline. In broth culture, organism produce a fragile pellicle, some turbidity and a granular deposit. The isolated organism was oxidase and catalase positive. No acid production was observed from any of the carbohydrates tested. Nitrate was reduced to nitrite.*

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The isolate did not produce indole and hydrogen sulfide. Urease, DNAase, phosphatase, MR, and VP tests were negative. A filtrable hemolysin was not demonstrated. The organism was sensitive to all antibiotics tested. Mice exposed to parenteral and conjunctival administration of N. ovis did not show any symptom.

Introduction

Infectious keratoconjunctivitis (pink-eye) is an acute contagious disease of ruminants. The etiological agent of ovine keratoconjunctivitis has not been definetely demonstrated; some investigators (4, 7) have described *Rickettsia*, *Chylamidia* and various Gram-negative bacteria as being associated with the disease. In 1960, *Neisseria ovis*, as a new species of the genus *Neisseria* has been isolated from sheep with keratoconjunctivitis (6). Since then, a limited number of investigators have reported the isolation of *N. ovis* from both healthy and diseased eyes of sheep (1, 5, 7, 13, 14, 16). This organism has also been isolated from cattle with and without keratoconjunctivitis (8, 10, 12, 18) and from an aborted bovine fetus (11).

This paper describes the isolation and characterization of a *Neisseria ovis* strain from the eyes of lambs with keratoconjunctivitis in Turkey.

Materials and Methods

Two lambs with the symptoms of keratoconjunctivitis were brought to the clinic. There were lachrymation, swelling eye-lids, and corneal opacity in both eyes of effected lambs. Samples were collected from both eyes of two lambs, using a cotton wool swab. The swabs were inoculated onto 10 per cent sheep blood agar plates, which were incubated aerobically at 37°C for 24–48 hours.

After incubation period, isolated colonies were stained by Gram's method. Differentiation between cocci and rods was also carried out by observing cellular elongation in the presence of penicilline. A disc of penicilline G was placed on the surface of blood agar inoculated with suspected organism. After 18 h incubation at 37°C, a film was made from the edge of the zone of inhibition and examined by phase contrast microscopy for the evidence of elongation. Motility was

determined by phase-contrast illumination and in semi-solid broth. Pigment production was obseved on egg-yolk agar. The ability of growth at 20 °C and 22°C, on McConkey agar and in the presence of 2% and 5 % NaCl was also investigated.

The oxidase and catalase tests were carried out using Bactident Oxidase (Merck) and 5 per cent H_2O_2, respectively. Carbohyrate fermentation studies were performed in Trypticase Soy Broth (TSB) with 1 % of the sugar under test added. The sugars tested were adonitol, arabinose, dulcitol, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, rhamnose, sorbitol, sucrose and xylose. Nitrate reduction was tested in a medium containing 0.1 % Kalium nitrate in a TSB base. Tests for urease, DNAase, phosphatase, indol and hydrogen sulfide production and MR and VP tests were also performed.

The filtrate of broth culture was tested for hemolytic activity. Sensitivity to antibiotics was determined by disc diffusion method. Following antibiotics were used; ampicillin, carbenicillin, chloramphenicol, chlortetracyclin, colistin sulfate, erythromycin, gentamicin, neomycin, nitrofurontoin, oxytetracyclin, rifamycin, streptomycin, tetracyclin, trimethoprim and penicillin.

To test the pathogenicity of organism, an inoculum was prepared from 18 h broth culture. This inoculum was injected iv and ip the groups of 3 mice (0.1 ml each) and inoculated to the conjunctivae of mice by swabbing.

Results

The pure cultures of gram-negative, hemolytic cocci were obtained from each of blood agar plates. The isolated organism was named as *Neisseria ovis* according to the basis of following criteria.

The colonies were greyish, translucent, smoth, convex with regular edges and hemolytic with a clear zone. The colonies were difficult to emulsify and a suspension in saline was usually granular.

The isolated organism consisted exclusively of gram-negative cocci of irregular size with a tendency to retain variably the gram stain. The bacteriae were non-motile, non-spor forming which often occured in diploid arrangament. Elongation was not seen in the presence of penicilline.

Organism grew well on solid media with or without blood. No growth on McConkey agar and no pigmentation on egg yolk medium were observed. In broth culture, organism produce a fragile pellicle, some turbidity and a graular deposit. Very scant growth was detected at 20°C and 22°C. The organism had the ability to grow in the presence of 2 percent NaCI but not in 5 % NaCl.

The isolated organism was oxidase and catalase positive. No acid production was observed from any of the carbohydrates tested. Nitrate was reduced to nitrite. The isolate did not produce indol and hydrogen sulfite. Urease, DNA ase, phosphatase, MR and VP tests were negative. A filtrable hemolysin was not determined. The organism was sensitive to all antibiotics tested.

The mice exposed to parenteral and conjunctival administration of *N. ovis* did not show any symptom.

Discussion and Conclusion

In 1960, Lindqvist (6) described a *Neisseria* strain associated with infectious keratoconjunctivitis in Norwegian sheep and proposed the species name of *Neisseria ovis*. In this study, results obtained from the characterization of isolated strain from ovine keratoconjunctivitis justify the placing of this organism within the *N.ovis*. The morphological, cultural and biochemical characteristics of *N. ovis* isolated in this case were also similar to the *N. ovis* strains isolated by Fraser and Gilmour (3) and Wilcox (18). Differentiation of *N. ovis* from other *Neisseria sp.* and *Moraxella sp.* was readily performed by means of carbohdydrate fermentation, catalase ad nitrate tests and elongation in the presence of penicillin. Original *N. ovis* strain was sensitive to all antibiotics tested. Wilcox (18) has also found that *N.ovis* strains were sensitive to same antibiotics tested in the present study.

Madrid and Terzolo (7) and Buczek et al (1) have reported the outbreaks of infectious keratoconjunctivitis associated with *N. ovis* and showed some unique features in that the disease occured only in lambs and started in he first few hours of life. In the present study, *N. ovis* was similarly isoalted from lambs. However, other workers (6, 9, 15) have not pointed out the specific sensitivity of lambs to this infection.

The pathogenicity of *N. ovis* for experimental animals was investigated by several workers. Lindqvist (6), Moreno et al (9), Pedersen (12) and Spradbrow (15) have found that *N.ovis* in large doses was lethal to mice and could experimentally cause keratoconjunctivitis in sheep. However, in this study, attempts to produce an experimental infection in mice did not give positive result. Similarly, Fairlie (2) and Spradbrow and Smith (16) have reported that no symptoms were recorded in the experimental animals inoculated with *N. ovis*. Possibility of the strain of *N. ovis* became attenued after three or four passages on artificial medium, the minimum number necescary to prepare a pure culture and inoculum, may be cause the loss of pathogenicity.

It is more probable that *N. ovis* is a commensal organism well adapted to survival in the ovine conjunctival sac and a predisposing factor, either infective or traumatic, is required to initially lower the resistance of conjunctivae, before the establisment of *N. ovis* infection. The precise role of *N. ovis* in eye infections of sheep in this country requires further investigations.

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