

ISOLATION OF RHODOCOCCUS EQUI FROM A HORSE WITH CORNEAL ULCER

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Geliş Tarihi : 25.04.2002

Summary: R.equi isolated and identified in a horse with keratitis ulcerosa. Direct microscopic examination and cultural methods were used to isolate the bacteria. The Kirby-Bauer disc diffusion method was used for antibiotic sensitivity test. Rhodococcus equi was isolated from the horse with keratitis ulcerosa. The isolate was sensitive to erythromycin, rifampycin, vancomycin, azithromycin, ciprofloxacin, clindamycin, gentamicin, but intermediate sensitive to penicillin. Although, various nonspecific and specific bacteria are commonly reported in equine keratitis, however this is the report describing the involvement of R. equi isolation has been reported rarely in such a case.

Key Words: Rhodococcus equi, horse, keratitis ulcerosa, isolation, antibiotic sensitivity.

Korneal Ülserli Bir Attan Rhodococcus Equi İzolasyonu

Özet: Sağ gözü keratitis ülserosalı olan bir attan Rhodococcus equi izole ve tanımlanmıştır. Numunenin direkt mikroskopik incelemesi yapıldı. Bakterinin izolasyonu amacıyla kültürel yöntemlerden yararlanıldı. Kültürel yoklama sonucu keratitis ülserosalı bölgeden R. equi izole edildi. Antibiyotik duyarlılık testinde Kirby-Bauer disk difüzyon metodu kullanıldı. Bu yöntemle göre izole edilen bakteri, eritromycin, rifampicin, vankomycin, azithromycin, ciprofloxacin, clindamicin, gentamicine duyarlı ve penicilline orta derecede duyarlı bulundu. Atların keratitis olgularından nonspesifik ve spesifik bakteriler izole edilmesine rağmen, böyle olgulardan R. equi izolasyonu çok seyrek olarak bildirilmiştir. Böyle bir olgudan hastanın 9 yaşında oluşu kadar infeksiyon etkeni olarak R. equi izole edilmiş olması da ilginç bir bulgu olarak değerlendirildi.

Anahtar Sözcükler: Rhodococcus equi, at, keratitis ülserosa, izolasyon, antibiyotik duyarlılığı.

INTRODUCTION

Infections caused by Rhodococcus equi occur sporadically in foals less than three months of age and are characterized primarily by bilateral bronchopneumonia, diarrhoea and lymphadenitis¹⁻³. R. equi is an encapsulated Gram-positive, pleomorphic rod and extremely resistant to soil pH, chemical treatment and drying for extended periods^{4,5}. The organisms exist in the soil and are frequently isolated from the faeces of normal horses and other herbivores. The natural route of R.equi transmission is unknown, but it is generally thought to be transmitted via inhalation and alimentary tract. The agent also causes other extrapulmonary pathological conditions, including septic arthritis, ulcerative lymphangitis, mesenteric lymph node abscesses, cellulitis, pleuritis and uterine infections^{4,6}. R. equi occurs worldwide and is generally considered to be a soil contaminant. Several reports have also described contamination by R.equi⁷.

Rhodococcus equi infections of eye are rarely reported. In this report, we aimed to establish the presence of R.equi in the right eye of a 9 year old horse with keratitis purulenta.

CASE REPORT

Horse was examined and found to have had an increase of opacity and ulceration, vasculitis, lacrimation and pain on cornea. The lesion was subsequently determined to be keratitis ulcerosa. The condition had been present to several months according to the history taken from the owner of horse. Eye swabs were used as the study material obtained from the right eye a horse with keratitis ulcerosa.

Isolation and identification of the bacterium: Smear samples were stained using Gram staining technique and cultured aerobically on sheep blood agar, EMB agar, trypticase soy broth and nutrient broth with sera at 37°C for 24-48 hours. The isolate was tested for urea and aesculine hydrolysis, catalase production, maltose, sucrose, mannitol and glucose fermentation, nitrate reduction, haemolysis, CAMP test and growth in Cystein Tellurite Agar^{4,8}. Bacteriologic culture; Gram positive pleomorphic rods were seen in direct microscopic examinations of Gram-stained swab samples. The colonies on blood agar were large, moist, mucoid, non-haemolytic and yellowish after 48 hours of incubation. In differential biochemical tests, isolate was catalase, nitrate reduction and CAMP test

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positive, maltose, sucrose, mannitole and glucose fermentation, urea and esculine hydrolysis negative. The isolate was identified as *R. equi* based on colony morphology, various biochemical reactions, microscopic appearance, growth on Cystein Tellurite Agar and the CAMP test result. *R. equi* was the only pathogen isolated from the swab sample of the horse.

Antibiotic sensitivity test: Antimicrobial sensitivity testing was performed on the isolated strain by the Kirby-Bauer disc diffusion method⁹, on Muller Hinton Agar. Eritromycin (15 μ g), rifampycin (2 μ g), vankomycin (30 μ g), azithromycin (15 μ g), ciprofloxacin (1 μ g), clindamicin (2 μ g), gentamicin (10 μ g) and penicillin(10IU) discs were used for antibiotic susceptibility test. Testing on *R. equi* isolate by the Kirby-Bauer disc diffusion method showed that the isolate was sensitive to eritromycin, rifampycin, vankomycin, azithromycin, ciprofloxacin, clindamicin, gentamicin and intermediately sensitive to penicillin.

DISCUSSION

R. equi is the primary cause of pneumonia in foals, it also causes naturally occurring infections in a wide variety of species¹⁰⁻¹². It is well known that *R. equi* can affect several organs including the eye, digestive tract and associated with lymph nodes, bones, joints and various organs^{2,3,11,12}. The presence of *R. equi* infection has been reported particularly in various organs of newborn and immuno-deficient horses^{4,5}. Also, immunologic status associated with age, antibody levels, environmental stress may contributed to the susceptibility to infection and lead to variations in immunologic reactivity. Gram-positive bacteria predominate in the normal conjunctival microflora of horses, also Gram-negative bacteria and fungi are more often isolated from equine ulcers such as *Streptococcus* spp.¹³, *Staphylococcus* spp.¹⁴, *Pseudomonas aeruginosa*¹⁵ and *Listeria*¹⁶. In this case, the naturally occurring *R. equi* keratitis, in which there was no accompanying pneumonia indicated that the organism was capable of causing infection and disease in the eyes as well. This rare situation may be related to the age of the horse, its immunologic status and lack of proper environmental conditions.

The susceptibility of *R. equi* to various antibiotics has been determined using the Kirby-Bauer disc diffusion method. According to previous reports, gentamicin, rifampicin, erytromycin, neomycin, kanamycin and streptomycin are effective agents against *R. equi* in vitro^{4,5}, but penicillin is relatively

ineffective¹. We found that the isolate was sensitive to eritromycin, rifampycin, vankomycin, azithromycin, ciprofloxacin, clindamicin, gentamicin however, intermediately sensitive to penicillin. Our antimicrobial susceptibility test yielded results parallel to those of Hillidge¹.

R. equi is ubiquitous in the environment but is not reported as a cause of keratitis in animals. In this case, a horse was presented with keratitis ulcerosa and evidence of increased lacrimation in the right eye. *R. equi* was isolated from a corneal lesion. This is reported case of ulcerative keratitis associated with *R. equi* in a horse. The control of environmental dust should be priority in preventing *R. equi* exposures. Preventing of trauma to corneal epithelium and dust control can be achieved by irrigation of paddocks and pastures particularly in hot and dry months when incidence of *R. equi* infection is high. In addition to dust control, trauma, wounds, overcrowding, housing, nutrition, parasite control and individual immunity level are among the factors which must be considered in the control of *R. equi* infection.

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