CASE REPORT

Pulmonary nocardiosis in a fat-tailed sheep

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Özet

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Nocardia spp. sığırlarda mastit, kutanöz ve subkutanöz apseler, pneumoni ve yaygın hastalıklara neden olmaktadır. Bir koyunda *Nocardia* spp.'ya bağlı pulmoner enfeksiyon belirlendi. Makroskopide hasta akciğerinde çoklu beyaz, sabit, küçük nödüller gözlendi. Mikroskopide makrofajlı poligranulomatöz yangı odakları, çok çekirdekli (langhans-type) dev hücreler ve rozet şeklinde eosinofilik (Splendor-Hoeppli reaction) bölgeler gözlendi. Akciğer doku örnekleri sığır kanlı agarda 37 °C'de 48-72 saat aerobik şartlarda inkübe edildi. Dokular Gram ve modifiye Zeihl-Neelsen ile boyandı. Bakteriyolojik incelemede ince çok sayıda, gram-pozitif, boncuklu, filamentli ve non-asit hızlı organizmalar olduğunu gözlendi. Sonuçta bakteriyolojik, histopatalojik ve makroskobik bulgular pulmonary nokardiozis olgusunu doğruladı ve bu vaka İran'da koyunda gözlenen ilk pulmonar nokardiozis olgusudur.

Anahtar kelimeler: Pulmonar nokardiozis, koyun.

Abstract

Kheirandish R, Sami M, Ghanbarpour R, Alidadi S. Pulmonary nocardiosis in a fat-tailed sheep. **Eurasian J Vet Sci, 2013, 29, 4, 220-224**

Nocardia spp. are known as causes of bovine mastitis, pneumonia, cutaneous or subcutaneous abscesses and disseminated disease. Pulmonary infection by *Nocardia* spp. was diagnosed in a sheep. Grossly, the animal had multiple white, firm, and small nodules in the lung. Microscopically, the nodules consisted of foci of pyogranulomatous inflammation with the presence of macrophages, multinucleated (langhans-type) giant cells and eosinophilic amorphous material with rosette-shape configurations (Splendore-Hoeppli reaction). Tissue samples of the lung were streaked onto bovine blood agar, incubated at 37 °C for 48-72 hours under aerobic condition, and stained with Gram and modified Zeihl-Neelsen staining. Bacteriological investigations confirmed numerous thin, gram-positive, beaded, branching, filamentous and non-acid fast organisms. Finally, on the basis of characteristic gross, histopathological and bacteriological findings pulmonary nocardiosis was diagnosed, and this is the first reported case of pulmonary nocardiosis in a sheep in Iran.

Keywords: Pulmonary nocardiosis, sheep

Pulmonary nocardiosis is a rare subacute or chronic disorder of the lungs, caused by aerobic actinomycetes of the genus Nocardia (Taniguchi et al 1998, Hathur et al 2011). The Nocardiae are catalase-positive, gram-positive, delicate, non-motile, branching, filamentous bacteria that found in soil, dust, organic matter, fresh water and marine environmental. These organisms are partially acid fast by kinyoun's (modified Ziehl Neelsen) technique (Songer and Post 2005, Brown-Elliott et al 2006). Nocardial infections occur in several species including humans, dogs, cats, cattle, goats, horses, pigs, birds, monkeys and marine mammals (Jonas and Wyand 1966, Hamal 1974, Bacciarini et al 1999, Ramos-Vara et al 2007, Ribeiro et al 2008, St Leger et al 2009). In ruminants, *N. astreoides* is an uncommon cause of chronic mastitis, as well as abortion in horses, cattle and sheep (Vemireddi et al 2007, Bawa et al 2010). Bovine mastitis, cutaneous-subcutaneous abscess and pneumonia are usual clinical presentations of nocardiosis in animals. *Nocardia* spp. comprise of at least 12 several species that the more important pathogenic species include *Nocardia asteroids*, *N. brasiliensis*, *N. transvalensis*, *N. nova*, and *N. otitidis-caviarum* (formerly *N. caviae*). Of these, N. *asteroides* is considered as the most important pathogenic species for human and animal nocardiosis, followed by *N. brasiliensis* and *N. otitidis-caviarum*. *N. brasiliensis* has been isolated from horses suffering from pneumonia and pleuritis (Menendez et al 1997, Eshraghi and Amin 2001). In general, Nocardial species cause a large variety of diseases in both normal and immunocompromised humans and animals. Nocardiosis is often related to chronic obstructive pulmonary disease (COPD), acquired immunodeficiency syndrome (AIDS) and patients with pulmonary diseases are much more susceptible to Nocardial infections, particularly if they are receiving corticosteroids (Taniguchi et al 1998, Malladi et al 2010). The current case report describes a pulmonary nocardial infection in a sheep, in terms of its bacteriological and histopathological characteristics. To the authors' best knowledge, this is the first reported case of pulmonary nocardiosis in fat-tailed sheep.

A lung belonging to a four-year-old female fat-tailed sheep, found during meat inspection in Kerman slaughterhouse, Iran, was the subject of this investigation. Grossly, multiple white, well-circumscribed and small nodules that had a firm texture were present in the right apical lobe of the lung (Figure 1). The lesions were restricted to the lung and no lesions were noted in the other organs of sheep. To perform further investigations, this case was brought to the pathology department of the Faculty of Veterinary Medicine, Shahid-Bahonar University of Kerman, Kerman, Iran. For histopathological observations, tissue samples of the affected lung were taken and fixed in 10% neutral buffered formalin and processed according to the routine of histopathologic technique. All samples were embedded in paraffin, sectioned at 5 µm thicknesses and stained with hematoxylin and eosin (HE) for ordinary light microscope examination. Histologically, the lung showed multiple pyogranulomatous reactions, which were composed of a center of necrotic tissue, surrounded by a rim of macrophages, multinucleated (langhans-type) giant cells, neutrophils, and an outer layer of the connective tissue infiltrated by lymphocytes and plasma cells (Figure 2).

The bacteria surrounded by eosinophilic amorphous material with rosette-shape configurations (Splendor-Hoeppli reaction) (Figure 3). In order to microbiological examination, samples of the affected lung were inoculated onto the bovine blood agar, incubated at 37°C for 48–72 hours, under aerobic condition and examined daily for microbial growth. On culture the colonies appeared as white, dry, wrinkled, chalk like appearance (Figure 4). The organisms were identified on the basis of growth, morphological and biochemical

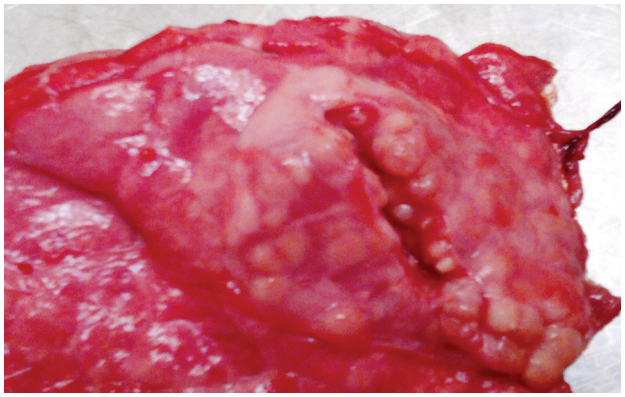

Figure 1. Lung. Multiple white, well-circumscribed nodules in the right apical lobe of the lung.

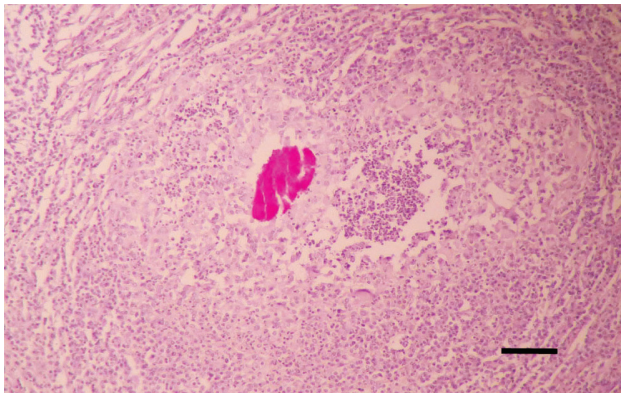

Figure 2. Photomicrograph shows pyogranulomatous reaction including necrotic tissue, surrounded by macrophages, multinucleated (langhans-type) giant cells, neutrophils, and an outer layer of the connective tissue infiltrated by lymphocytes and plasma cells (HE, 100 µm).

Figure 3. Rosette-shape configurations (Splendor-Hoeppli reaction) surrounded by neutrophils are seen (HE, 100 µm).

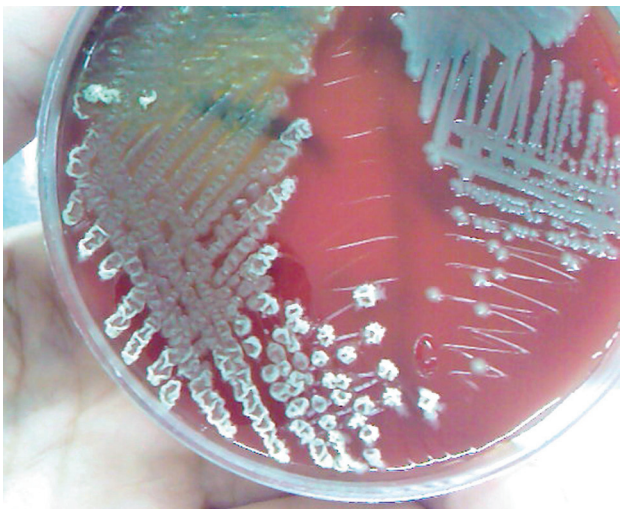

Figure 4. Bovine blood agar. White, dry, wrinkled, chalky colonies.

characteristics as well as microscopic examination of them. Thin, gram-positive, branching, beaded, filamentous and non-acid fast organisms were visible under the microscope with Gram's and modified Ziehl-Neelsen (ZN) staining, respectively. No other micro-organism was detected from the lung on the media. Hence, the diagnosis of pyogranulomatous nocardial pneumonia (i.e. pulmonary nocardiosis) was confirmed based on the characteristic gross, histological and bacteriological findings.

Nocardiosis occurs in a large variety of animals as well as humans. In mammals, 6 forms have been distinguished, such as pulmonary; central nervous system; extrapulmonary; cutaneous, subcutaneous or lymphocutaneous; actinomycetoma and systemic. The pulmonary form affects only the lungs and extrapulmonary form is characterized by any primary focus without involvement of the lung and CNS. Actinomycetoma is a distinct subcutaneous nodule and the systemic form involves several sites (St Leger et al 2009). Mortality rate is often high in both dogs (88.5%) and humans (50%), while in cattle is much lower (5–10%). The primary manifestation in cattle is mastitis and mortality results if it progress to systemic infection. Indeed, mortality is mostly associated with the presence of disseminated or pulmonary nocardiosis and the progression of conditions such as pulmonary diseases or immunosuppression (Vemireddi et al 2007, Ribeiro et al 2008). The lungs are the primary sites of nocardial infection in more than two-third of cases. Nocardia species are not naturally found in respiratory tract; therefore, isolation of Nocardia is nearly always representative of infection (Malladi et al 2010). Despite the occurrence of nocardiosis in many animals (e.g., cats, dogs, pigs, birds, fishes, buffaloes and cattle), there is no document of respiratory spread from affected animals to humans. There is also no evidence of person-to-person transmission among animals and human beings (Lerner 1996, Vemireddi et al 2007). Infection occurs in animals and persons of all ages, even neonates. Nevertheless, the present case was female, it has been stated that males are affected three times more commonly than females (McNeil and Brown 1994, Ribeiro et al 2008). Infection by *Nocardia* spp. may cause via inhalation, ingestion or through trauma. Since the widespread involvement of the lung, inhalation was considered to be the likely major route of entry for the organisms (Menendez et al 1997, Pal 1997, Tilak et al 2008). Nocardial infections in animals are either systemic or localized. In ruminants, reports of localized nocardiosis include mastitis, pneumonia and dermatitis (Pal 1997, Ramos-Vara et al 2007, Nahed et al 2011). Clinical signs of nocardial infections start with the appearance of firm nodules or pustules, which then rupture and suppurate (Songer and Post 2005). Dogs and cats usually develop localized infections, with cutaneous, subcutaneous lesions and mycetomas (Ribeiro et al 2008). *Nocardia* spp. have been isolated from acute and chronic mastitis in cows, with granulomatous lesions and from buffaloes with pneumonia (Pal 1997, Ribeiro et al 2008). Thoracic nocardiosis in dogs often involves purulent pleuritis and abscessation of heart; and diffusion to the central nercious system with brain abscesses is common (Songer and Post 2005, Ribeiro et al 2008). In horses, cutaneous infection is prevalent manifestation, with occasional respiratory or disseminated disease in immunocompromised animals. Nocardial infections in birds, fishes, reindeers and monkeys have been reported sporadically (Jonas and Wyand 1966, Bacciarini et al 1999, Vemireddi et al 2007, St Leger 2009). In addition, the organism has been isolated in raw milk samples collected from cattle, sheep, goats and buffaloes from Egypt

which is likely due to mastitis in them (Nahed et al 2011). In agreement with others, in the present case, the inflammation was primarily pyogranulomatous with the presence of macrophages and giant cells that surrounded the foci of necrosis (Bacciarini 1999, Hussein 2008). *Actinomyces* spp. cause similar gross and histologic lesions and resemble *Nocardia* spp. morphologically in tissue sections. Therefore, nocardiosis should be differentiated from other respiratory diseases, especially from tuberculosis and mycotic and other bacterial infections of the lung, by employing microbiological techniques (Lerner 1996, Eshraghi and Amin 2001). The clinical diagnosis of nocardiosis is relatively difficult; nonetheless, it may make the diagnosis but is not pathognomonic. Diagnosis is usually made base on culture, isolation and microscopic observations of the organisms. Colonies with aerial filamentous growth have a chalky white appearance on blood agar. However, confirmation of diagnosis and identification of species are done biochemically and also by molecular techniques such as PCR (Saubolle and Sussland 2003, Brown-Elliott et al 2006). Microbiologically, *Nocardia* spp. are gram positive, weak acid-fastness of the Nocardia species (by kinyoun's acid-fast staining) is very useful in differentiation from other actinomycetes such as the *Actinomyces* and Streptomyces species, which are also gram-positive branching bacteria (McNeil and Brown 1994).

In summary, diagnosis of pulmonary nocardiosis was made based on the presence of gross and microscopic lesions as well as isolation and identification of *Nocardia* spp. from the affected lung. Hitherto, has been not reported pulmonary nocardiosis in sheep in Iran; so, this is the first reported case of pulmonary nocardiosis.

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