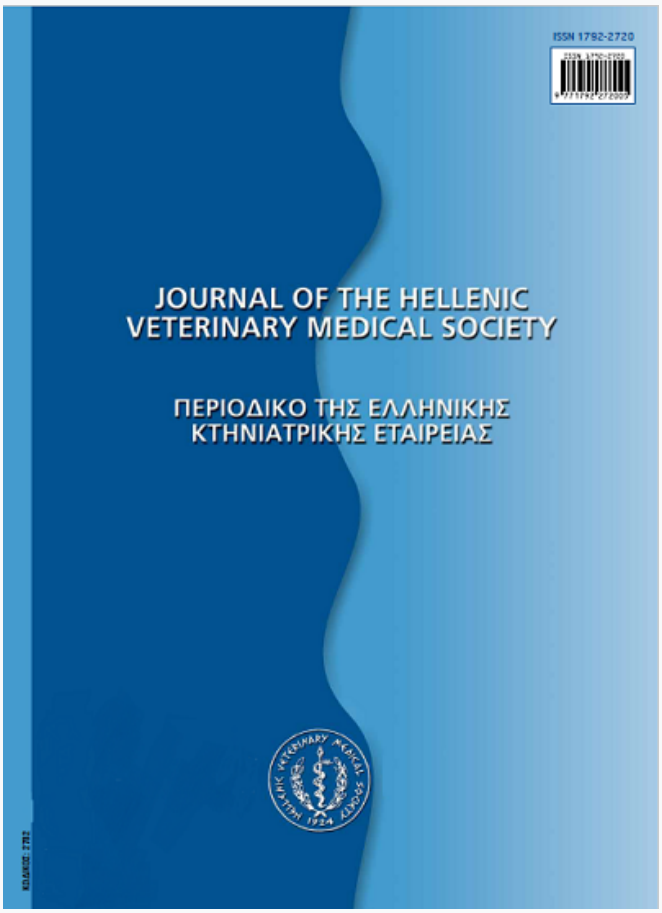
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 1 (2017)



Submandibular and parotid abscess due to Nocardia sp. and therapy with danofloxacin in sheep

S. KIRKAN, K. URAL, U. PARIN, O. ORTLEK, C. BALIKCI, M. GULTEKIN, U. KARADEMIR, I. AKIN

doi: 10.12681/jhvms.15552

To cite this article:

KIRKAN, S., URAL, K., PARIN, U., ORTLEK, O., BALIKCI, C., GULTEKIN, M., KARADEMIR, U., & AKIN, I. (2018). Submandibular and parotid abscess due to Nocardia sp. and therapy with danofloxacin in sheep. *Journal of the Hellenic Veterinary Medical Society*, *68*(1), 21–26. https://doi.org/10.12681/jhvms.15552

J HELLENIC VET MED SOC 2017, 68(1): 021-026
ΠΕΚΕ 2017, 68(1): 021-026

Research article
Ερευνητικό άρθρο

Submandibular and parotid abscess due to *Nocardia* sp. and therapy with danofloxacin in sheep

Kirkan S.[a], Ural K.b, Parin U.[a,*], Ortlek O.b, Balikci C.[b], Gultekin M.[b], Karademir U.[c], Akin I.[d]

[a]*Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology, AYDIN, TURKEY*

[b]*Adnan Menderes University Faculty of Veterinary Medicine Department of Internal Medicine, AYDIN, TURKEY*

[c]*Adnan Menderes University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology, AYDIN, TURKEY*

[d]*Adnan Menderes University Faculty of Veterinary Medicine Department of Surgery, AYDIN, TURKEY*

ABSTRACT. The aim of the present study was to report detection of *Nocardia* sp. from cutaneous lesions of sheep. Abscess material was collected from submandibular and parotid lesions in sheep for identification of *Nocardia* sp., which is well known as a causative agent for cutaneous or subcutaneous abscesses, disseminated disease, mastitis, and pneumonia. *Nocardia* sp. are Gram-positive, delicate, non-motile, branching filamentous bacteria that found in soil, dust, organic materials, water and aquatic environmental. Nocardia species generally yield a large variety of diseases in both normal and immunocompromised humans and animals. In the present study, bacterial staining methods and biochemical identification methods were applied for identification of *Nocardia* sp. in four sheep in Aydin. All cases had superficial abscesses in the submandibular and parotid region, mostly invading the head and neck regions. Neutrophilic leukocytosis was evident in three out of four sheep, and there was evidence of moderate anemia in two of the sheep. Samples were stained with Gram and modified Ziehl-Neelsen staining and streaked onto sheep blood agar, incubated at 37°C for 48-72 hours under aerobic condition, Sabouraud Dextrose Agar, incubated for one week under aerobic condition. *Nocardia* sp. was isolated from based on bacteriological investigation confirming numerous thin, Gram-positive, filamentous and nonacid fast organisms, and biochemical tests. *Nocardia* sp. strains were confirmed with 16s rRNA PCR method. Based on clinical appearance, hematological findings, and molecular analysis a definitive diagnosis cutaneous Nocardiosis was made in the sheep. Initial therapy involving danofloxacin, with regard to antibiogram, for 10 days and local iodine solutions, resulted in clinical recovery on 35-45 days post-treatment. This is the first reported case of cutaneous Nocardiosis and its effective danofloxacin therapy in sheep in Turkey.

Keywords: *Nocardia sp., sheep, goat, abscess, identification.*

Correspondence:
U. Parin
Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology, AYDIN, TURKEY

E-mail address: uparin@adu.edu.tr

Date of initial submission: 16-11-15
Date of revised submission: 4-2-2016
Date of acceptance: 3-3-2016

Ημερομηνία αρχικής υποβολής: 16-11-15
Ημερομηνία αναθεωρημένης υποβολής: 4-2-2016
Ημερομηνία αποδοχής: 3-3-2016

INTRODUCTION

Nocardia sp. are Gram-positive, delicate, non-motile, branching filamentous bacteria that found in soil, dust, organic materials, water and aquatic environment. These catalase-positive organisms are partially acid fast by Kinyoun's (modified Ziehl-Neelsen) technique (Brown-Elliott et al., 2006; Songer and Post, 2005). *Nocardia* infections occur in several species including humans, cattle, sheep, goats and other relevant species (Brown-Elliott et al., 2006; Ramos-Vara et al., 2007; Ribeiro et al., 2008; St Leger et al., 2009). Cutaneous-subcutaneous abscesses, pneumonia, and mastitis are common clinical presentations of Nocardiosis in animals. *Nocardia* sp. comprise of at least 12 different species involving pathogenic species i.e. *Nocardia asteroides, N. brasiliensis, N. transvalensis, N. nova, and N. otitidis-caviarum (formerly N. caviae). N. asteroides* is suggested as the most important pathogenic species for human and animal Nocardiosis, followed by *N. brasiliensis* and *N. otitidis-caviarum* (Eshraghi and Amin, 2001; Menendez et al., 1997). Nocardial species generally yield a large variety of diseases in both normal and immunocompromised humans and animals (Malladi et al., 2010). Infections due to *Nocardia* sp. are generally acquired through milk, inhalation, ingestion and trauma (Martínez, 2008).

This report presents the Nocardiosis in four sheep in terms of its bacteriological characteristics and clinical features. According to the authors' knowledge, this is the first reported case series of cutaneous Nocardiosis in sheep resided in Aydin province, Turkey.

MATERIALS AND METHODS

Sample collection

The sheep were referred to Adnan Menderes University Faculty of Veterinary Medicine Department of Internal Medicine Clinics for evaluation of swellings. In all cases, there were superficial abscesses within the submandibular and parotid region, mostly invading the head and neck regions. Tissue samples were collected by puncturing the specified lesions of submandibular and parotid abscesses. Abscessed materials were initially excised surgically and collected samples were brought to Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology Laboratory. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

Bacterial identification

Samples were stained with Gram and modified Ziehl-Neelsen staining. Thin, Gram-positive, branching, beaded, filamentous and non-acid fast organisms were visualized at first under light microscope. After that, the samples were streaked onto 5% sheep blood agar, MacConkey agar and EMB agar, incubated at 37°C for 48-72 hours under aerobic condition, Sabouraud Dextrose Agar, incubated at 25°C for one week under aerobic condition.

After growth period, the preliminary evaluation of the bacterial colonies was made on the grounds of colony appearance and preparation staining after Gram and modified Ziehl-Neelsen method. The cultures were observed as white, dry, wrinkled, and had chalk like appearance.

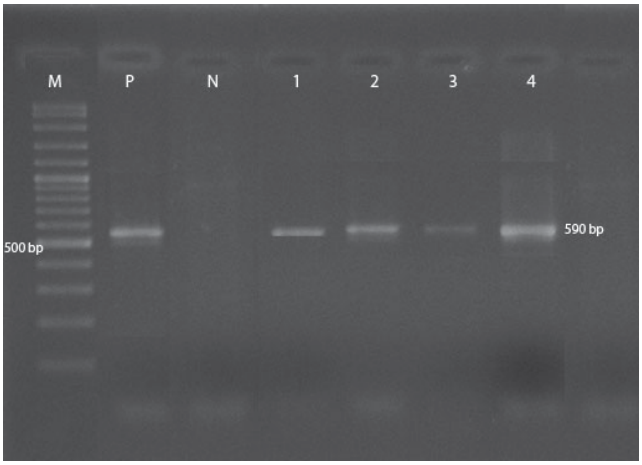
The organisms were identified on the basis of growth, morphological and biochemical properties as well as microscopic examination. Thin, Gram-positive, branching, beaded, filamentous and non-acid fast organisms were visible under the microscope with Gram's and modified Ziehl-Neelsen (ZN) staining, respectively. The bacterial cultures were identified with conventional biochemical and carbohydrate fermentation tests following the standard procedures described previously (Koneman et al., 1999).

All *Nocardia* sp. (n=4) strains were confirmed by PCR using a *Nocardia* genus-specific 590 bp fragment of 16S rRNA primers (Laurent et al., 1999). *Nocardia* sp. DNAs were extracted with Ultraclean Microbial DNA Isolation Kit® (MO BIO Laboratory, Inc.) as recommended by manufacturer. DNA extracts were used in PCR. 16S rRNA primers (NG1 5'-ACCGACCACAAGGGGG-3' and NG2 5'-GGTTGTAAACCTCTTTCGA-3') were designed according to Laurent et al. (1999). As a positive control *Nocardia asteroides* ATCC 19247 strain was used in the PCR. PCR amplification was carried out using final volumes of 50 µl PCR master mixes which contains 10 µl template DNAs as previously described (1999). Ten microliters of each amplification reaction mixture was analyzed by agarose gel electrophoresis performed with a 1% (wt/vol) agarose gel stained with ethidium bromide (0.7 µg/ml). After electrophoresis, PCR products were visualized and analyzed with UV trans-illuminator (Vilber Lourmat Infinity VX2®). PCR products of 590 bp for *Nocardia* sp. was considered evidence for identification.

Antimicrobial Sensitivity of the Nocardia Strains

All the Nocardia strains were tested for their sen-

Figure 1. 1% agarose gel electrophoresis of 16S rRNA PCR products



M: 100 bp DNA ladder, P: Nocardia asteroides ATCC 19247 strain as positive control, N: Negative control, 1-4: Nocardia sp. positive samples

Figure 2. The progression of abscess in relation to danofloxacin treatment and afterwards

sitivity against 8 antimicrobial agents. The antibiotics used in the study were danofloxacin, amoxicillin-clavulanic acid, streptomycine, cefoperazone, lincomycin, enrofloxacin, penicillin G, sulfamethoxazole and trimethoprim. The antibiotic susceptibility testing of all strains was determined on Mueller-Hinton medium. Each strain was inoculated to Brain-Heart infusion broth and incubated for 18 h. A volume of 100 µl from broth cultures were spread evenly on plates. The antimicrobial discs were placed on the plates at an appropriate distance from each other. The plates were then incubated at 37 °C for 24 h. The diameters of the inhibition zone were measured and matched with respective standard zone diameters to interpret the isolates as resistant, intermediate or sensitive according to the procedures of National Clinical Laboratory Standards Institute (NCCLS, 2008).

Haematological and serum biochemical examination

To those of sheep involved in the present study, hematological examination (erythrocyte, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, total leukocyte count) and serum biochemical analyses (AST, ALT, urea and creatinine concentrations) were performed by use of Abacus® Junior Blood Count Analyzer and Samsung® Automated Analyzer, respectively.

RESULTS

Clinical findings

At physical examination all cases had superficial abscesses in the submandibular and parotid region, mostly invading the head and neck regions.

Hematological and serum biochemical analysis

Regarding neutrophilic leukocytosis WBC:min-max 12.44-14.33 x 109/L (reference ranges 4-12x109/L); neutrophil: min-max 7.49-11.18 (reference ranges 0.7-7.3x109/L) was evident in 3 out of 4 sheep, and there was evidence of moderate anemia (PCV: 20.2-24 % (reference ranges 27-45 % in 2 of those sheep.

Microbiological findings

Clinical, hematological and molecular findings allowed us to detect cutaneous Nocardiosis from 4 sheep in Aydin province, Turkey. On first admission the abscesses were surgically excised, and then were analyzed microbiologically. The bacterial strains were identified as *Nocardia* sp. by biochemical examination and confirmed by PCR. Agarose gel electrophoresis of PCR products is shown below in Figure 1.

Therapy

The antibiogram tests revealed that all the isolates

were susceptible only to danofloxacin. Hence, therapy was initiated by application of danofloxacin (Advocin® flk., 50 ml, Zoetis) at a dose of 6 mg/kg s.c. daily (for 10 days) and local iodine solutions were applied by draining of the abscess for 15 days. Complete recovery was seen after 35-45 days (Figure 2). No side effect was noticed during drug administration.

DISCUSSION

Nocardiosis occurs in a large variety of animals as well as in humans. In mammals, six forms have been distinguished, such as pulmonary, central nervous system, extrapulmonary, cutaneous and subcutaneous or lymphocutaneous, actinomycetoma and systemic. Actinomycetoma is a distinct subcutaneous nodule and the systemic form involves several sites (St Leger et al., 2009).

The incidence of human Nocardiosis is not well established in Turkey and comprises mostly case reports and small case series (Yildiz et al., 2005; Mentes et al., 2006; Elmaci et al., 2007; Arslan et al., 2007; Karakan et al., 2007). Pulmonary Nocardiosis is the most common presentation, with dissemination to other parts of the body, especially the brain. Nine pulmonary and/or cerebral Nocardiosis cases in humans (Yildiz et al., 2005) were defined on the basis of a positive culture and consistent clinical presentation in a retrospective study which performed at Erciyes University Teaching Hospital. Long term treatment of cases was started with ceftriaxone plus amikacin. According to the susceptibility, antibiotic choice modified to amoxycillin–clavulanate, trimethoprim–sulphamethoxazole, iminepem or meropenem (Yildiz et al., 2005).

A rare cutaneous nocardiosis case as a superficial infection with cellulitis, on the right lower leg presented in a woman from with a history of travel to Turkey. This case was treated with trimethoprim–sulphamethoxazole for three months (Wong et al., 2003). On the other hand in animals, Nocardiosis was reported in some case series in cats (Erturk and Alibasoglu, 1974; Haziroglu et al., 2006).

Nocardiosis in ruminants have been well recognized in cattle (Pal, 1997; Ribeiro et al., 2008) and in sheep (Kheirandish et al., 2013) worldwide. Reports regarding localized Nocardiosis were composed of pneumonia, mastitis and dermatitis (Pal, 1997; Ramos-Vara et al., 2007; Ribeiro et al., 2008; Nahed et al., 2011). Hitherto, neither cutaneous Nocardiosis/submandibular and parotid abscess due to *Nocardia* species, nor danofloxacin treatment have never been reported in sheep in Turkey.

In sheep most subcutaneous abscesses exist as a result of trauma relevant skin penetration of invading microorganisms such as *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *A. pyogenes (*AL-Tuffyli and Shekhan, 2012), *C. pseudotuberculosis* (Ural et al., 2008; AL-Tuffyli and Shekhan, 2012), *Pasteurella* sp.*, E. coli* and *Klebsiella pneumonia* (Menzies and Muckle, 1989; Al-Harbi 2011; Tavassoli et al., 2012). The vast majority of the samples cultured revealed *S. aureus* as a major etiological reason, followed by *Arcanobacterium pyogenes* and *C. pseudotuberculosis* (Tadayon et al., 1980; Menzies and Muckle, 1989; Gezon et al., 1991; AL-Tuffyli and Shekhan, 2012). The positive culture of 4 samples in our study based on Gram and modified Ziehl-Nielsen Staining, incubation under aerobic conditions, identification with conventional biochemical and carbohydrate fermentation tests and finally via PCR, all confirmed that the responsible agent was *Nocardia* sp. in the present study.

In vitro activity of fluoroquinolones against clinical isolates of *Nocardia* species were tested previously (Mikami and Yazawa, 1989; Hansen et al., 2008; Mittal and Fernandes, 2012). A previous study in Turkey, investigated variations in antimicrobial susceptibility among several *Nocardia* species, indicating that all of the strains were susceptible to fluoroquinolones such as moxifloxacin and evofloxacin (Percin et al., 2011). Danofloxacin is another fluoroquinolone, which is preferred in some of the respiratory and gastrointestinal problems among ruminants, in the present authors' practice, was the drug of choice for treatment of abscesses, based on antibiogram. To the best of our knowledge' danofloxacin treatment in the present study is the first time that was tested such therapy. There was no apparent side effects due to danofloxacin administration, indicating that the latter compound may be safely used at the recommended dosage used in this study, although only a limited number of cases were involved.

Nocardiosis in animals are either systemic or localized. In ruminants, reports of localized Nocardiosis include mastitis, pneumonia and dermatitis (Nahed et al., 2011; Pal, 1997; Ramos-Vara et al., 2007). Clinical signs of Nocardiosis start with the appearance of firm nodules or pustules, which then rupture and suppurate (Songer and Post, 2005). In the present study enrolled sheep did not reveal the presence of pneumonia, nor mastitis were evi-

dent. Cutaneous lesions were solely present.

CONCLUSIONS

The clinical diagnosis of Nocardiosis is relatively difficult, since the cutaneous lesions are not pathognomonic. Diagnosis is usually based on culture, isolation and microscopic observations and PCR detection of the organisms. Confirmation of diagnosis and identification of species should be performed by biochemical features of the responsible agent. In conclusion, diagnosis of cutaneous Nocardiosis was accomplished based on the presence of gross lesions as well as clinical/hematological findings, besides isolation and identification of *Nocardia* sp. from the affected abscess materials. This is the first reported case series of cutaneous Nocardiosis for the Aydin province of Turkey. Further studies may be warranted in an attempt to evaluate the efficacy of danofloxacin in a larger sheep herd with Nocardiosis.

CONFLICT OF INTEREST STATEMENT

The authors hereby declare that there is no conflict of interests for this research.

REFERENCES

Al-Harbi KH (2011) Prevalence and etiology of abscess disease in sheep and goats at Qassim region, Saudi Arabia. Veterinary World 4 (11): 495-499.

AL-Tuffyli YI Kh, Shekhan MI (2012) Clinical and Bacteriological Study of Subcutaneous abscesses caused by gram positive bacteria in cow and sheep in Al-Qadissiyia province. AL-Qadisiya Journal of Vet Med Sci 11(2): 80-85.

Arslan U, Tuncer I, Uysal EB, Inci R (2007) Pleuropulmonary and soft tissue Nocardia cyriacigeorgici infection in a patient with Behcet's disease. Saudi Med J 28(9):1435-1437.

Bacciarini LN, Posthaus H, Pagan O, Miserez R (1999) Nocardia nova causing pulmonary nocardiosis of black crakes (Limnocorax flavirostra). Vet Pathol 36:345-347.

Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ (2006) Clinical and laboratory features of the Nocardia sp. based on current molecular taxonomy. Clin Microbiol Rev 19:259-282.

Elmaci I, Senday D, Silav G, Ekenel F, Balak N, Ayan E, Yazici S (2007) Nocardial cerebral abscess associated with mycetoma, pneumonia, and membranoproliferative glomerulonephritis. J Clin Microbiol 45(6): 2072-2074.

Erturk E, Alibasoglu M (1974) Fungus diseases of domestic animals in Ankara. Ankara Univ Vet Fak Derg 21 (3-4): 224-242.

Eshraghi S, Amin M (2001) Nocardia asteroides complex in patient with symptomatic pulmonary nocardiosis. Iranian J Publ Health 30:99-102.

Gezon HM, Bither HD, Hanson LA, Thompson JK (1991) Epizootic of external and internal abscesses in a large goat herd over a 16 – year period. J Am Vet Med Assoc 198(15): 257-263.

Hansen G, Swanzy S, Gupta R, Cookson B, Limaye AP (2008) In vitro activity of fluoroquinolones against clinical isolates of Nocardia identified by partial 16S rRNA sequencing. Eur J Clin Microbiol Infect Dis 27(2): 115-120.

Haziroglu R, Sahal M, Tunca R, Guvenc T, Duru SY, Ataseven L (2006) Pleuritis and pneumonia associated with nocardiosis and aspergillosis in a domestic short haired cat. Ankara Univ Vet Fak Derg 53: 149-151.

Karakan Y, Elbek O, Uyar M, Zer Y, Tulu M, Dikensoy O (2007) Nocardia transvalensis infection in an immunocompetent patient reported from Turkey. Tuberk Toraks 55(3): 295-298.

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

Koneman E, Winn W, Allen S, Janda W, Schreckenberger P, Procop G, Woods G (1999) The aerobic Gram positive Bacilli. In: Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins, New York: pp 691-695.

Laurent FJ, Provost F, Boiron P (1999) Rapid identification of clinically Relevant Nocardia species to genus level by 16S rRNA gene PCR. J Clin Microbiol 37:99-102.

Malladi SVS, Ankathi PK, Vemu L, Rao MN (2010) Disseminated nocardiosis in an advanced AIDS patient. JAPI 58:317-318.

Martínez R (2008) Pulmonary nocardiosis: risk factors, clinical features, diagnosis and prognosis. Curr Opin Pulm Med 14(3):219–227.

Menendez R, Cordero PJ, Santos M, Gobernado M, Marco V (1997) Pulmonary infection with Nocardia species: A report of 10 cases and review. Eur Respir J 10:1542-1548.

Mentes O, Zer Y, Balcı I, Ozaslan M (2006) First Report of Pulmonary Nocardia transvalensis Infection from Turkey. Pak J Biol Sci 9 (12): 2384-2386.

Menzies PI, Muckle CA (1989) The Use of a microagglutination assay for the detection of antibodies to Corynebacterium pseudotuberculosis in naturally infected sheep and goat flocks. J Vet Res 53: 313-318.

Mikami Y, Yazawa K (1989) Susceptibility patterns of pathogenic Nocardia to some selected antimicrobial agents and their usefulness in the identification work in a clinical laboratory. Bull JFCC 5: 89–95.

Mittal V, Fernandes M (2012) Cotrimoxazole-resistant Nocardia sclerokeratitis: effective therapy with fourth-generation fluoroquinolones. Can J Ophthalmol 47(6): 58-60.

Nahed MW, Neveen AGE, Nagah MS, Saad MN, Walaa MA (2011)

Incidence of Nocardia species in raw milk collected from different localities of Assiut City of Egypt. Vet World 4:201-204.

NCCLS (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard. 3rd ed. Document M31-A3, vol 28 No. 8. Villanova. Pa. National Committee for Clinical Laboratory Standards, Clinical and Laboratory Standards Institute.

Pal M (1997) Nocardia asteroides as a cause of pneumonia in a buffalo calf. Rev Sci Tech Off Int Epiz 16:881-884.

Perçin D, Sümerkan B, Inci R (2011) Comparative evaluation of e-test and disk diffusion methods for susceptibility testing of Nocardia species. Mikrobiyol Bul 45(2): 274-279.

Ramos-Vara JA, Wu CC, Lin TL, Miller MA (2007) Nocardia tenerifensis genome identification in a cutaneous granuloma of a cat. J Vet Diagn Invest 19:577-580.

Ribeiro MG, Salerno T, Mattos-Guaraldi AL, Camello TCF, Langoni H, Siqueira AK, Paes AC, Fernandes MC, Lara GHB (2008) Nocardiosis: An overview and additional report of 28 cases in cattle and dogs. Rev Inst Med Trop S Paulo 50:177-185.

Songer JG, Post KW (2005) Dermatophilus and Nocardia. In: Veterinary Microbiology. Bacterial and fungal agents of animal disease. 1st ed, Elsevier Saunders Inc., NorthCarolina: pp 84-85.

St Leger JA, Begeman L, Fleetwood M, JR Frasca S, Garner MM, Lair S, Trembley S, Linn MJ, Terio KA (2009) Comparative pathology of nocardiosis in marine mammals. Vet Pathol 46:299-308.

Tadayon RA, Cheema AH, Muhammed SI (1980) Microorganisms associated with abscesses of sheep and goats in the south of Iran. Am J Vet Res 41:798-802.

Tavassoli M, Imani A, Yousefnia Pasha M, Tukmechi A, Tajik H (2012) Bacteria associated with subcutaneous abscesses of cattle caused by Hypoderma spp larvae in north of Iran. J Vet Res Forum 1(2): 123-127.

Ural K, Alic D, Haydardedeoglu AE, Cedden F, Guzel M, Özyıldız Z, Cantekin Z (2008) Corynebacterium pseudotuberculosis infection in Saanen×Kilis crossbred (White) goats in Ankara, Turkey and effective kanamycin treatment: A prospective, randomized, double-blinded, placebo-controlled clinical trial. Small Rum Res 77(1): 84-88.

Yildiz O, Alp E, Tokgoz B, Tucer B, Aygen B, Sumerkan B, Couble A, Boiron P, Doganay M (2005) Nocardiosis in a teaching hospital in the Central Anatolia region of Turkey: treatment and outcome. Clin Microbiol Infect 11(6): 495-499.

Wong YW, Waghorn D, Ratnavel RC (2003) Antibiotic-resistant cellulitis acquired in Turkey. J R Soc Med 96 (1): 32-33.