



***Corynebacterium pseudotuberculosis* Case in a Boer Goat X Turkish Hair Goat Crossbred**

Gülşah AKGUL¹, Serpil KAHYA DEMIRBILEK², Özgür Yaşar ÇELİK¹, Kıvanç IRAK³,
Mustafa Barış AKGUL⁴, Seyrani MERSİN⁵

¹University of Siirt, Faculty of Veterinary, Department of Internal Medicine, Siirt-TURKEY

²University of Uludag, Faculty of Veterinar, Department of Microbiology, Bursa-TURKEY

³University of Siirt, Faculty of Veterinary, Department of Biochemistry, Siirt-TURKEY

⁴University of Siirt, Faculty of Veterinary, Department of Surgery, Siirt-TURKEY

⁵University of Siirt, Faculty of Veterinary, Department of Artificial Insemination, Siirt-TURKEY

Summary: In this case report, it is intended to report the results of the clinical examination and microbiological analysis of *Corynebacterium pseudotuberculosis* infection which was detected (determined) in a goat. At Goat Application and Research Center at the University of Siirt, after the clinical examination carried out on a two-year-old female Boer goat x Turkish Hair goat crossbred, orange sized lymph nodes in a fluctuating viscosity were found in the right submandibular and left prescapular lymph nodules. Cream and slightly greenish malodorous content was taken from the lump from puncture. Swab sample taken from the content was sent to the University of Uludag, Faculty of Veterinary, Microbiology Laboratory in Stuart transport medium by cold chain. Swabs were inoculated onto % 7 sheep blood agar, and incubated at 37°C for 24-48 hours in both aerobic and microaerophilic conditions. After the microscopic morphology of the colonies was examined, biochemical tests were performed according to the suspected factors and the isolate of suspected bacteria was defined as *Corynebacterium pseudotuberculosis* which was first isolated from Boer goat x Turkish Hair goat crossbred.

Key words: Boer goat, *C. pseudotuberculosis*, Turkish Hair goat

Bir Boer-Kıl Keçisi Melezi Keçide *Corynebacterium pseudotuberculosis* Olgusu

Özet: Bu olgu sunumunda bir keçide tespit edilen *Corynebacterium pseudotuberculosis* enfeksiyonunun klinik muayene ve mikrobiyolojik analiz sonuçlarının rapor edilmesi amaçlanmıştır. Siirt Üniversitesi Keçi Uygulama ve Araştırma Merkezi'nde ki iki yaşlı dişi Boer x Kıl Keçisi melezi bir keçide yapılan klinik muayeneler sonucunda sağ submandibular ve preskular lenf yumrusunda fluktuasyon kıvamlı portakal büyüklüğünde bir şişkinlik saptandı. Şişkinliğin punksiyonu yapıldığında krema kıvamında hafif yeşilimsi pis kokulu bir içerik alındı. İçerikten alınan svap örneği Stuart transport besiyeri içinde soğuk zincirde Uludağ Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı Laboratuvarı'na gönderildi. Alınan svap örnekleri %7 koyun kanı katılmış kanlı agarına ekildi ve hem aerobik hem de mikroaerofilik koşullar altında 24-48 saat boyunca 37°C'de inkübe edildi. Kolonilerin mikroskopik morfolojileri incelendikten sonra kuşku edilen faktörlere göre biyokimyasal testler yapıldı. Şüpheli bakteri izolatu *Corynebacterium pseudotuberculosis* olarak tanımlandı ve ilk kez Boer x Kıl keçisi melezinde ortaya çıktığı görüldü.

Anahtar kelimeler: Boer keçisi, *C. pseudotuberculosis*, Kıl keçisi

Introduction

Caseous lymphadenitis (CLA) is a chronic disease of sheep and goats caused by *C. pseudotuberculosis* characterized by the formation of apshenian superficial or visceral lymph nodes (6,8). The disease causes economical loss with a decrease in the quantity and quality of wool obtained from sheep and goats, resulting in a decrease in carcass quality resulting from apseler of infected carcasses and a decrease in milk yield (8). Sources of infection are pus and

infected material released from the lymph nodes in infected animals. The disease spreads from infected animals and / or from other contaminated material to other herds (11).

Corynebacterium species are pleomorphic Gram positive, catalase positive, phospholipase -D (PLD) exotoxin-secreting, non-spore forming, facultative anaerobic and inert bacteria classified within the family of Corynebactericea, which includes *Corynebacterium spp.*, *Mycobacterium spp.* and *Nocardiaspecies* that called CMN group (*Corynebacterium spp.*, *Mycobacterium spp.* and *Nocardiaspp.*). *C. pseudotuberculosis* colonies having the properties of being cream-

colored and easily degradable, and forming a small, narrow hemolysis space; and the strains isolated from sheep and goats are nitrate negative (11,12).

In recent years, studies have been reported on the use of PCR methods in the etiologic diagnosis of CLA (3,10). In addition, it has also been reported that the use of ELISA-based diagnostic kit is effective in the control and eradication of caseous lymphadenitis, and interferon γ -ELISA is more sensitive than ELISA test and is not affected by antibodies induced by immunization (7,9). The antibiotic treatments for causes of disease are ineffective and once infected, the animal remains infected throughout life (15).

CLA has been reported to be widespread throughout the world for more than a century, and is also reported to be widespread in Turkey, and a study published in 2012 reported a 63% incidence in small ruminants (3,6,14).

In line with the literature studied, thinking that each study on this subject done in our country will help to obtain new information on diagnosis and treatment of the disease, we intended to contribute to the diagnosis of the disease and to the prevalence in our country by reporting clinical examination and microbiological analysis of lymphadenitis case detected for the first time in a two years old female Boer goat x Turkish Hair goat crossbred.

Case History

Case Description

After the clinical examination carried out on a two-year-old, female Boer goat x Turkish Hair goat crossbred in Goat Application and Research Center at the University of Siirt, a fluctuating viscous orange-sized lump was detected in the right submandibular and prescapular lymph nodules. Cream and slightly greenish malodorous content was taken off by puncture of the lump (Figure 1). The blood sample was taken from vena jugularis of the patient to the sterile disposable biochemistry tubes and the patient's hematologic findings were obtained with automatic blood cell counter (VETSCAN HM5®, Abaxis Inc., USA). Swab samples of the content were sent to University of Uludag, Faculty of Veterinary, Department of Microbiology Laboratory, for examination in the Stuart transport medium with cold chain.

Clinical Findings

The body temperature of 39.3°C, the respiratory rate of 78 breaths/min, heart rate of 108 beats/



Figure 1: Creamy and slightly greenish malodorous content was taken from the lump by puncture.

min were determined by the clinical examination of the patient and it was observed that the right submandibular and prescapular lymph nodules were in the size of an orange among the palpable lymph nodes (Figure 2). In the respiratory system examination, it has been determined that cough assessment is positive and sounds of sclerosis were heard in the lung auscultation.

Hematological Findings

Leukocytosis and neutrophilia were detected by hematological examination and it was determined that the erythrocyte and thrombocyte index were normal.



Figure 2: Image of the lump detected in the right submandibular and prescapular lymph nodeles by clinical examination.

Microbiological Findings

The suspected lymph nodes were sampled. Creamy slightly greenish malodorous content was taken off by puncture of the lump. Swab sample taken from the content in Stuart transport medium with cold chain was sent to the University of Uludag, Faculty of Veterinary, Microbiology Laboratory. The swab was cultured on 7% sheep blood agar at 37°C for 48h. After incubation; B-hemolytic, small, white, easily degradable colonies were observed in the agar. After obtaining a pure culture from the suspected colonies, Gram positive coryneform looking bacteria were detected by staining, bacterial colonies were characterized and suspected colonies were tested with API systems. Suspected colonies were positive for catalase, urease, maltose and glucose, but by inhibiting beta hemolysin of *Staphylococcus aureus* which were obtained from the culture collection of University of Uludag, Faculty of Veterinary, Department of Microbiology, and were negative for trehalose and xylose considered as *C. pseudotuberculosis* (12).

Discussion

Caseous lymphadenitis (CLA) has been widely observed all over the world more than a century and there are studies showing that the disease is also common in Turkey (6,14). In Siirt region, the goat and sheep population is higher and also the case presented in this study has been seen in this region, thus we think that the rate of incidence of CLA is higher in the regions where the small cattle population is higher.

In various studies, it has been reported that the CLA has two forms, mainly superficial and visceral (1,3,8). In those studies, it has been reported that mediastinal and visceral abscess rates for all cases are approximately between 3% and 25% (1,2). In this presented case superficial form of CLA has been reported. It has been detected by detailed clinical examinations a fluctuating, orange-sized content in the right submandibular and prescapular lymph nodules which has slightly greenish, malodorous, creamy content in it, and it was consistent with the superficial form of CLA. Other studies demonstrated that *C. pseudotuberculosis* was not the only bacterium isolated, and there were some other bacteria (1). For example, the distinguishing from actinobacillosis, which is limited to the head region, was done by the color and consistency, after the macroscopic examination

of the content, and the separation from simple abscess was done by defining suspicious bacterial isolates as *C. pseudotuberculosis*, with the microbiological examination.

There are many problems encountered in the control of CLA, because the epidemiology, pathogenesis and immunogenesis of the disease are still not fully known (4,5). Because of the fact that the bacteria are in a thick capsule surrounding the abscess, this generally leads to failure of the treatment (1).

Consequently, it has been reported that the most important virulence factor is phospholipase D (PLD) in *C. pseudotuberculosis* strains, which cause classic caseous lymphadenitis in sheeps. Also it has been reported that PLD negative *C. pseudotuberculosis* strains were defined as "toxminus", as a result of chromosomal deletions or mutations of PLD genes. And, those cannot create abscesses specifically for classic CLA in lymph nodes that were identified (4). So the basic and golden diagnosis of *C. pseudotuberculosis* is culture, but it is strengthened with molecular and serological techniques. For example; exotoxin ELISA, sonicated ELISA and dot-blot ELISA techniques were found to be useful screening tests for the routine diagnosis of CLA (5). Also, *C. pseudotuberculosis* has various sensitivity to different antimicrobials (13). For effective control of CLA, it is initially required to separate the infected animals identified in the herd and the vaccination of the healthy animals must be done (2). Once the sick animals are separated, precautions should be taken to comply with hygiene rules and animals found to be diseased, should be sent to slaughter house in the most appropriate time. In this presented case, when the final diagnosis established after microbiological examination and definitive diagnosis, the animal with CLA was separated from the herd as soon as possible and it was sent to slaughter house.

For the case presented in this report, we think that the isolated strain may be a "toxminus" strain and the frequently encountered CLA cases are still a problem in our country and that it is needed to give more weight to the vaccination and eradication studies on this disease. It is also important that this case was seen for the first time on a Boer goat x Turkish Hair goat cross breed. After this case, we plan to expand our work with ongoing cases and prepare a new study involving molecular and serological tests.

Since the effect of the disease is very common across the world and also among the sheeps in our country, it is necessary to concentrate on the studies and to detect toxins, determine the antimicrobial susceptibility and eradication in accordance with all these results.

References

1. Adebbe A, Sisay Tessema T. Determination of *Corynebacterium pseudotuberculosis* prevalence and antimicrobial susceptibility pattern of isolates from lymph nodes of sheep and goats at an organic export abattoir, Modjo, Ethiopia. *Lett Appl Microbiol* 2015; 61(5): 469-79.
2. Al-Gaabary MH, Osman SA, Oreiby AF. Caseous lymphadenitis in sheep and goats: Clinical, epidemiological and preventive studies. *Small Ruminant Res* 2009; 87(1): 116-21.
3. Cetinkaya B, Karahana M, Atila E, Kalina R, Baereb TD, Vanechoutteb M. Identification of *Corynebacterium pseudotuberculosis* isolates from sheep and goats by PCR. *Vet Microbiol* 2002; 88(1): 75-83.
4. Dorella FA, Pacheco LG, Seyffert N, Portela RW, Meyer R, Miyoshi A, Azevedo V. Antigens of *Corynebacterium pseudotuberculosis* and prospects for vaccine development. *Expert Rev Vaccines* 2009; 8(2): 205-13.
5. İlhan Z. Koyunlarda *Coynebacterium pseudotuberculosis*'in ELISA ve Dot-Blot ELISA ile teşhisi. *Turk J Vet Anim Sci* 2003; 27(6): 1327-33.
6. İlhan FS. Koyunların kazeöz lenfadenitis enfeksiyonunda patolojik bulgular. *Van Vet J* 2008; 19(1): 23-8.
7. Kaba J, Kutschke L, Gerlach GF. Development of an ELISA for the diagnosis of *Corynebacterium pseudotuberculosis* infections in goats. *Vet Microbiol* 2008; 78(2): 155-63.
8. Kumar J, Singh F, Tripathi BN, Kumar R, Dixit SK, Sonawane GG. Epidemiological, bacteriological and molecular studies on caseous lymphadenitis in Sirohi goats of Rajasthan. *Trop Anim Health Pro* 2012; 44(7): 1319-22.
9. Menzies PI, Hwang Y, Prescott JF. Comparison of an interferon-gamma to a phospholipase D enzyme-linked immunosorbent assay for diagnosis of *Corynebacterium pseudotuberculosis* infection in experimentally infected goat. *Vet Microbiol* 2004; 100(1): 129-37.
10. Pacheco LGC, Roberta RP, Thiago LPC, Fernanda AD, Robson CB, Renato C, Marcilio NLF, Sergio CO, Roberto M, Francisco SFA, Anderson M, Vasco A. Multiplex PCR assay for identification of *Coynebacterium pseudotuberculosis* from purecultures and for rapid detection of this pathogen in clinical samples. *J Med Microbiol* 2007; 56(4): 480-6.
11. Quinn PJ, Markey BK, Leonard FC. *Corynebacterium* species. Fitzpatrick ES, Hartigan PJ. eds. In: *Veterinary Microbiology and Microbial Disease*. UK: John Wiley & Sons LTD, 2004; pp. 207-12.
12. Quinn PJ, Carter ME, Markey BK, Carter GR. *Clinical Veterinary Microbiology*. First Edition. London: Mosby International Limited, 1994; pp. 820-5.
13. Sakmanoğlu A, Hadimli HH, Erganiş O, Pınarkara Y, Sayın Z, Kav K. Koyunlardan izole edilen *Corynebacterium pseudotuberculosis* suşlarının identifikasyonu ve antibiyotiklere duyarlılıkları. *Eurasian J Vet Sci* 2015; 31(1): 116-21.
14. Ural K, Haydardedeoglu AD, Cedden F, Guzel M, Ozyildiz Z, Cantekin Z. *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 Ruminant Res* 2008; 77(1): 84-8.
15. Voigt K, Baird GJ, Munro F, Murraya F, Brülisauer F. Eradication of caseous lymphadenitis under extensive management conditions on a Scottish hill farm. *Small Ruminant Res* 2012; 106(1): 21-4.

Correspondence

Gülşah AKGÜL
University of Siirt, Faculty of Veterinary,
Department of Internal Medicine, Siirt- TURKEY
Phone: 0484 223 3255
E-mail: gulsahvet@hotmail.com