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Research article

Florfenicol Therapy During Naturally Occuring *Corynebacterium pseudotuberculosis* Infection in Sheep and Goats in Aydin, Turkey

Kerem Ural[1], Şükrü Kirkan[2], Deniz Alıç Ural[3], Göksel Erbas[2], Mehmet Gültekin[1], Uğur Parın[2], Canberk Balıkçı[1]

Adnan Menderes University, Faculty of Veterinary[1-3], Department of Internal Medicine[1], Department of Microbiology[2], Faculty Farm[3], Isikli, Aydin, Turkey

A B S T R A C T

Background/Aim: Caseous Lymphadenitis is a bacterial disease of chronic suppurative lymphadenitis of both goat and sheep resulting within major economic consequences. Given the distribution of the disease throughout the vast majority of the world, it has also been frequently decribed and found in Turkey. Condemnation of skins and carcasses due to abscess formation, severe losses in reproductive efficiency and in wool, meat and milk production all results in economic losses. The eradication is of difficult due to rapid spread of the disease once introduced into a flock. Treatment options with antibiotic application may not be efficicaous, indeed *C. pseudotuberculosis* is sensitive in vitro to the vast majority of antibiotics that have been tested. The intracellular location of the bacteria and the occurence of biofilm in naturally forming infections diminishes drug efficacy, making most of the antimicrobials inefficient. Anyhow it should not be unwise to suggest that there is clearly a need for an effective antimicrobial therapy. The present randomized field study was carried out to measure the effects of florfenicol treatment regime on *Corynebacterium pseudotuberculosis* infection in a mixed goat and sheep herd located in Aydin, Turkey.

Materials and Methods: All cases included were diagnosed cases of *C. pseudotuberculosis* abscesses located in several lymph nodes. The most prominent site of the abscesses were in the anterior half of the body both in sheep and goats. Two groups of animals were randomly assigned: Group F (n=8 goat, 6 sheep, treatment group) received florfenicol five daily subcutaneous doses of 40 mg/kg ; Group C (n=6 goat, 6 sheep, control group) served as positive (untreated) control.

Results and Conclusion: The efficacy of florfenicol was assessed clinically and based on a lesion score derived from the examination of the lesional lymph nodes. Clinical evaluation and lesion scoring were performed by a blinded researcher. Throughout the study florfenicol treatment significantly decrased (P<0.05) the clinical score while no significant changes were detected in the control group.

Keywords: Corynebacterium pseudotuberculosis, Identification,Sheep,Goat,Florfenicol.

Aydın, Türkiye'de Koyun ve Keçilerde *Doğal Corynebacterium pseudotuberculosis* Enfeksiyonunda Florfenikol Sağaltımı

Ö Z E T

Özbilgi/Amaç: Kazeöz lenfedanitis gerek keçi gerekse koyunlarda önemli ekonomik kayıplara yol açabilen, kronik suppuratif lenfadenitis ile seyreden bakteriyel bir hastalıktır. Hastalık dünyanın pek çok yerinde yaygın olarak seyretmekle birlikte Türkiye'de de sıklıkla tanımlanmış ve bildirilmiştir. Apse oluşumu deri ve karkas kullanımını sınırlandırmakta, üreme ve döl verimi ile yün, et ve süt üretimindeki azalmalar ciddi ekonomik kayıplara neden olmaktadır. Hastalığın sürüye girdikten sonra hızla yayılması eradikasyonunu zorlaştırmaktadır. *C. pseudotuberculosis* in vitro olarak test edilen antibiyotiklerin büyük çoğunluğuna hassas olmasına rağmen antibiyotik uygulaması ile sağaltım etkili olamayabilmektedir. Bakterinin intraselüler yerleşimi ve doğal enfeksiyonlarda biyofilm oluşumu, ilaç etkinliğini azaltarak pekçok antimikrobiyali etkisiz hale getirmektedir. Buna rağmen etkin bir antimikrobiyal sağaltıma ihtiyaç duyulduğunu açıkça ifade etmek yanlış olmayacaktır. Bu randomize saha çalışması Aydın, Türkiye'de bulunan ve *C. pseudotuberculosis* ile enfekte karışık bir koyun-keçi sürüsünde florfenikol sağaltım protokolünün etkilerini değerlendirmek amacıyla gerçekleştirilmiştir.

Materyal ve Metot: Çalışmaya dahil edilen tüm olguların çeşitli lenf yumrularında *C. pseudotuberculosis'*e bağlı apseler teşhis edildi. Koyun ve keçilerde apseler belirgin olarak vücudun ön yarısında tespit edildi. Hayvanlar rastgele iki gruba ayrılarak, Grup F (n=8 keçi, 6 koyun, sağaltım grubu) 40 mg/kg dozda, deri altı yolla, 5 gün süreyle florfenikol uygulanırken, Grup C (n= 6 keçi, 6 koyun, kontrol grubu) ise herhangi bir sağaltım uygulamadan kontrol grubu olarak ayırıldı.

Bulgular ve Sonuç: Florfenikol'ün etkinliği klinik ve lezyonlu lenf yumrumlarının muayenesinden elde edilen skorlama ile değerlendirildi. Klinik değerlendirme ve lezyon skorlaması kör bir araştırmacı tarafından gerçekleştirildi. Çalışma süresince florfenikol sağaltımı klinik skoru anlamlı olarak düşürürken (P<0.05), kontrol grubunda anlamlı bir değişiklik saptanmadı.

Anahtar kelimeler: Corynebacterium pseudotuberculosis, tanımlama, koyun, keçi, florfenikol

Correspondence to: Kerem Ural: Adnan Menderes Üniversitesi Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, Batı Kampüsü, 09016, Işıklı, Aydın, Türkiye. E-mail: uralkerem@gmail.com

Introduction

The genus *Corynebacterium* has been involved in a suprageneric group of actinomycetes, also including the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* (Hard, 1969; Songer et al., 1988; Songer, 1997; Paule et al., 2003). *Corynebacterium pseudotuberculosis (C. pseudotuberculosis*), *as* an important goat and sheep pathogen (Brown and Olander, 1987; Williamson, 2001; Carmen et al., 2006; Dorella et al., 2006), is the responsible agent of caseous lymphadenitis (CL) or cheesy gland (Williamson, 2001). The latter disease is recognized widely in major sheep and goat production areas, causing significant economic losses (Williamson, 2001; Paton et al., 2003). Both of the terms, *C. pseudotuberculosis* infection and CL, will be used as synonyms throughout the remainder of this article.

CL is characterized within abscess formation in the skin, therefore in superficial lymph nodes and visceral organs (Batey, 1986; Williamson, 2001). The disease may be endemic (Williamson, 2001), and has been considered to be one of the most economically important diseases of sheep and goats (Burrell, 1980; Paton et al., 1994; Stanford et al., 1998; Williamson, 2001; Paton et al., 2003) and is difficult to eradicate because of poor response to drug therapy, persistance to environment and limitations for detection of truly infected animals (Williamson, 2001; Dorella et al., 2006). In the present article we described *C. pseudotuberculosis* infection in sheep and goats and its effective florfenicol therapy.

Figure 1. Superfical abscess and caseous matter on infected sheep.
Şekil 1. Enfekte bir koyunda yüzeysel apse ve kazeöz içerik.

Materials and Methods

Study population

A total of 14 Angora goats (5 male, 9 female) and 12 Sakiz sheep (4 male, 8 female) at the age of 1 to 7 years were examined for the presence of abscesses in superficial lymph nodes at a private local farm located in Aydin (Cine province), Turkey, between March and May 2012.

Within the mixed herd there was a history of an outbreak of the development of superficial abscesses affecting almost all animals with no known appearent age or sex predisposition. The herd was certified as Brucellosis negative. According to the herdmen, who has been animal owner for almost 20 years, most of the animals have clinical signs of superficial abscesses often located on to the skin, besides previously slaughtered 4 goats and 2 sheep revealed no internal body organ involvement.

Clinical examination and sampling

Among all animals constituting the study all of them presented with large, spherical and superficially observed abscesses.

Lymph node samples with abscess formation were collected from all goats for smear and microbiological examination. Aspirated samples were Gram's stained and then examined microscopically. The samples taken for microbiological examination were immediately transported to the Department of Microbiology, Faculty of Veterinary University of Adnan Menderes and were processed for culture.

Diagnosis

For definitive diagnosis an aspirate of an intact abscess was brougt to Department of Microbiology diagnostic laboratory for microbiological identification. Differential diagnosis considered to account for probable microbiological content of the superficial abscesses included *Arcanobacter (Actinomyces) pyogenes*, *Staphylococcus aureus* and *Pasteurella multocida*. Identification of the *C. pseudotuberculosis* strains were based upon bacterial morphology, and in addition bacterial culture characteristic and biochemical reaction. Intant abscess aspirate sample was Abscessed inoculated on blood agar base supplemented with 5% defibrinated sheep blood. After the incubation of plate aerobically for 48 h at 37 °C, small, white, dry and crumbly colonies were searched for further identification. Pure cultures were prepared from the colonies that were Gram-positive and small curved rod-shaped in the microscopic examination. Then, routine biochemical tests, i.e. catalase, urease, trehalose, maltotriose and synergistic haemolysis with *Rhodococcus equi* and antagonistic haemolysis with *Staphylococcus aureus* were carried out to identify the isolates. Isolates positive for catalase and urease, enhancing the haemolysis with *R. equi*, but inhibiting beta haemolysin of *S. aureus* and negative for trehalose and maltotriose were considered as *C. pseudotuberculosis* (Koneman et. al., 1997).

Antimicrobial Susceptibility Test

For antibiotic susceptibility tests, Mueller-Hinton Agar (Oxoid) was used with Kirby-Bauer Disc Diffusion Method (Bauer et al., 1966; CLSI 2008).

The prepared Mueller-Hinton media were poured into 10 cm daimeter plates having 4 mm of thickness and then were left to freze. The 0.5 McFarland broth cultures of *Staphylococci* were inoculated to media and discs were placed after drying of media surface by heat sterilized forceps with 1.5 cm intervals.

Table 1. Site of lesions palpated and number of superfical abscesses on infected goat and sheep.
Tablo 1. Enfekte koyun ve keçilerdeki yüzeysel apselerin sayısı ve lezyonların palpe edildiği bölgeler.

	Lymph node							Total
Large active abscesses	Retropharyngeal (%)	Mandibular (%)	Parotid (%)	Prescapular (%)	Prefemoral (%)	Popliteal (%)	Superficial cervical (%)	
Goat (n=14)	5 (9.4)	11 (20.8)	10 (18.9)	8 (15.1)	6 (11.3)	13(24.5)	-	53
Sheep (n=12)	11 (26.8)	2 (4.9)	-	10 (24.4)	7 (17.1)	8 (19.5)	3 (7.3)	41
Total	16(17.0)	13(13.8)	10 (10.6)	18 (19.1)	13(13.8)	21 (22.3)	3 (3.2)	94

Table 2. On days 0, 14, 21 and 28 goats and sheep were given a clinical score and caseous abscesses were graded as: (1) normal sized-no enlargement-, (2) up to 2 cm in diameter, (3) 2-4 cm in diameter, (4) >4 cm.
Tablo 2. 0, 14, 21 ve 28. günlerde koyun ve keçilerin klinik skorlaması aşağıda verilmiş ve kazeöz apselerin derecelendirilmesi ise; (1) normal boyutlu-büyüme yok, (2) 2 cm çapına kadar, (3) 2-4 cm çapında, (4) >4 cm, olarak yapılmıştır.

	Day0	Day 14	Day 21	Day 28
Treatment (n=14)	3,50±0,75	1±0,0	1±0,0	1±0,0
Control (n=12)	2,66±0,77	2,33±071*	3,16±071*	3,50±0,85*

On days 0, 14, 21 and 28 goats and sheep were given mean clinical score of caseous abscesses. T test at each time point indicated that two groups were not different on day 0, while on subsequent days (14, 21 and 28) florfenicol group scores were significantly lower that control (P <0.05).
0, 14, 21 ve 28. günlerde keçi ve koyunlardaki kazeöz apselerin klinik skor ortalamaları verilmiştir. Her bir gün ortalamalarında uygulanan T testi ile 0. günde her iki grup arasında önemli bir değişiklik saptanmamış, devam eden günlerde (14, 21 ve 28) ise Florfenikol grubu Kontrol grubuna göre anlamlı olarak düşük bulunmuştur (P<0,05).

The used antibiotic discs and their ingredients are: Amoxicillin-Clavulanic Acid (AMC-10 µg), Oxacillin (OX-5 µg), Kanamycin (K-30 µg), Penicillin (P-10 IU), Ampicillin (AMP-10 µg), Florfenicol (FFC- 30 µg), Erythromycin (E-15 µg) ve Sulphametaxazol-Trimethoprim (SXT-25 µg).

The plates were incubated at room temperature for 24 hour. The inhibition zone diameters were calculated according to the Clinical and Laboratory Standards Institute (CLSI 2008).

Clinical lesion scoring

The scoring system used, was adapted to that of (Batey, 1986; Ellis et al., 1990), similarly to (Ural et al., 2008). Caseous abscesses were graded as:1) normal sized 2) up to 2 cm in diameter, 3) 2-4 cm in diameter, 4) >4 cm. The appearent size of the abscesses were categorized, by one of the authors blinded to treatment application or groups.

Treatment

Two groups of animals were randomly assigned Group F (n=8 goat, 6 sheep, treatment group) received florfenicol five daily subcutaneous doses of 40 mg/kg, and Group C (n=6 goat, 6 sheep, control group) served as positive (untreated) control. Supportive treatment included draning and lavaging of the abscesses with iodine solutions (Povidon iode, 10%) and subcutanoeus florfenicol (40 mg/kg once daily for 5 days duration) administration.

Statistical analysis

Differences in the clinical score between the florfenicol treatment group and the control group on the examination days were assessed by Student's *t*-test. Differences in the clinical score changes within-subject factor of time were analyzed using *Paired-Samples t*-test for multiple comparisons using computer software, SPSS Version 15.0 for Windows. The data were presented as mean ± standard error. The significance level was set as P<0.05.

Results

Localisation of abscesses

Within the herd superficial abscesses were most freqently located in anterior half of the body (Table 1), mostly involving retropharyngeal, mandibular, superficial cervical and prescapular lymph nodes (Fig. 1 and 2).

Clinical and bacteriological studies

Typically the gross lesions, comprising large swellings from sheep and goats, included easily palpated superficial abscesses with caseous, pale green, sometimes cream-white pus that has a soft, pasty consistency. Ten out 14 goats had three, and the rest had two large swellings.

Definitive diagnosis was based on bacteriological and cytological examination, aspiration of abcess content, and in addition on histopathological examination of abscessed lymph nodes. All smears revealed Gram-positive curved rods and cocci in a coryneform palisade arrangement within filamentous and/or intracellular forms, as was also reported previously (Soltys, 1963; Brown and Olander, 1987). *C. pseudotuberculosis* was isolated from all samples. All *C. pseudotuberculosis* isolates

were found to be susceptible to florfenicol accoding to the antimicrobial susceptibility tests within this research.

Treatment

The goat and sheep were randomly assigned to either florfenicol treatment group (n=14) or a control (n=12). The signs of resolution and the decrease in the size of lymph nodes were scored (Table 2) by a blinded investigator to the treatment that the animals received. Control group did not show a significant decrease over time (P >0.05). Between groups t test at each time point indicated that 2 groups were not different on day 0, while on subsequent days (14, 21 and 28) florfenicol group scores were significantly lower than control (P <0.05). Supportive treatment included draning and lavaging of the abscesses with iodine solutions and subcutanoeus florfenicol (40 mg/kg once daily for 5 days duration) administration. Florfenicol treatment had aparent effect on the natural course of *C. pseudotuberculosis* infection among enrolled animals.

Figure 1. Superfical abscess and caseous matter on infected sheep.
Şekil 1. Enfekte bir koyunda yüzeysel apse ve kazeöz içerik.

Discussion

The present authors interest to CL was aroused following receipt of large numbers of sheep and goat with caseous abscesses within the last years. During farm visits and occasionally on clinical referral, significant number of goat and sheep cases with *C. pseudotuberculosis* infection were observed within the last few years. Despite this, there have been very limited treatment surveys, furthermore to the present authors' knowledge no florfenicol therapy was established neither in sheep, nor in goats in Turkey.

It has been well recognized that controlling measures for CL with antibiotics is not very easy, since responsible agent invade into abscesses due to the thick capsule surrounding it (Piontkowski and Shivvers 1998; Stanford et al., 1998; Williamson, 2001). Thus the best strategy to control the disease may be of vaccination of healthy animals, accompanied by the identification/removal of infected ones (Mezies et al., 1989; Lindsay and Llyod, 1991; Paton et al., 1994; Williamson, 2001). Given the difficulties associated with the earlier recognition of infected animals could be a hindrance to above mentioned strategy (Dorella et al., 2006).

The vast majority of the commercially available vaccines for CL are combined with vaccines against other pathogens such as *Clostridium sp.* (Piontkowski et al., 1998; Stanford et al., 1998; Williamson, 2001; Paton et al., 2003). It was reported that although 43% of the farmers applied commercial CL vaccines, solely 12% used them correctly (Paton et al., 2003). It should be kept in mind that not all the vaccines licensed for use in sheep may be applied to goats. Besides the recommended vaccination program involving two initial doses in lambs and yearly boosters in sheep, and revaccination recommended at six-month intervals in goats (Paton et al., 2003; Williamson, 2001), may not be of beneficial for economic wellfare.

Regarding antimicrobial susceptibility pattern of *C. pseudotuberculosis*, controversial results were achieved (Connor et al., 2000; Foley et al., 2004). In a study of 26 strains isolated from lesions of caseous lymphadenitis in goats, it was detected that all strains were susceptible to the antibiotics penicillin G, ampicillin, gentamicin, lincomycin, chloramphenicol, tetracycline and sulfamethoxazole-trimethoprim (Muckle and Gyles, 1982). Garg et al. (1985) determined *C. pseudotuberculosis* strains that were strongly resistant to penicillin, indeed susceptible to neomycin. In another tiral of 22 isolates of *C. pseudotuberculosis* from sheep and goat abscesses, a strain highly resistant to streptomycin was observed in a study (Pepin et al., 1989). In United Kingdom (Connor et al., 2000) among different animal species, caprine isolates were found to be susceptible to kanamycin, as was also reported in a prievious study (Ural et al., 2008) from Ankara, Turkey among Saanen x Kilis crossbred goats performed by one of the authors (K.U.). Similar results indicating kanamycin susceptibility was previously reported in a dairy herd outbreak of *C. pseudotuberculosis* (Shpigel et al., 1993). However another trial suggested resistance against kanamycin (Connor et al., 2007). In Northern Hungary in a commercial sheep flock with CL, the isolated Corynebacterium strains were catalase- and urease-positive, and were sensitive to penicillin, erythromycin, doxycycline, oxytetracycline, cefotaxime, rifampicin and sulfamethoxazol-trimethoprim (Hajitos et al., 2005). In the present study based on antibiotic susceptibility test, florfenicol was the drug of choice. No observable side effect was evident.

The effectiveness of 3 different treatment regimens for sheep and goats with caseous lymphadenitis were evaluated in a randomized clinical trial. One group of involved animals were treated with opening, draining, and flushing the lesions and procaine penicillin G administration, whereas other group consisted of closed-system lavage and intralesional tulathromycin administration. The last group of animals were treated with closed-system lavage and tulathromycin administration. Proportions of lesions showing resolution of infection within 1 month following therapy did not differ significantly among the groups in that study (Washburn et al., 2009). In a sheep flock in Hungary surgical, antiseptic and local and parenteral oxytetracycline treatment of affected lymph nodes was apparently effective, as only one relapse was observed following therapy. In the latter study during the 18 months movement restriction period, solely two new

cases of CL were recognized (Hajitos et al., 2005). In a well designed prospective, randomized and double blinded study among Saanen x Kilis goats in Ankara, kanamycin treatment significantly decrased the investigator's clinical scores, resulting in recovery (Ural et al., 2008). In contrast the present authors adopted florfenicol therapy. In an attempt to make a major contribution to field veterinarians working on sheep and goat, clinical evaluation involving scoring of lymph node lesions was the main out come measure considered in this clinical trial. The scoring system used, was adapted to that of Batey (1986), Ellis et al. (1990), similarly to Ural et al. (2008). Comparison of control and florfenicol therapy groups revealed that the clinical scores did not present dignificance on day 0, at the beginning of the study, indeed florfenicol group possessed significantly lower clinical scores in contrast to control group on days 14, 21 and 28, suggesting effective treatment and maintained remission of caseous abscesses.

In view of those findings and based on clinical/bacetriological results, florfenicol treatment option may be effective, at least against ioslates detected in this study. Given the intracellular location of the bacteria and the formation of biofilm in natural infections (Brown and Olander, 1987; Olson et al., 2002), causes reduced drug efficacy and making antimicrobials inefficien, the efficacy of florfenicol resulting in releiving of lymphadenitis and clinical recovery, may hinder the cost of the latter antibiotic, making it of beneficial for herd-level disease management.

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