Florfenicol Therapy During Natural *Mannheimia haemolytica* Infection in Sakiz Sheep

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ABSTRACT

Background: Bronchopneumonia caused by *Mannheimia haemolytica* affects sheep of all ages worldwide and may be devastating especially in young animals undergone recent stress (i.e. transportation, weaning, mixing with animals from different farms. It is a common cause of morbidity and mortality in lambs and kids, especially in those that have not received adequate colostrum or in which passive colostral immunity is waning. Yearly herd losses costing millions of dollars have led to research focused on theuraputic trials and vaccine production because of various strains isolated demonstrating the continuing economic importance of *Mannheimia haemolytica* infection. A field trial was performed under commercial sheep farm located in Western Turkey, Aydin in an attempt to investigate the efficacy of an injectable formulation of florfenicol against naturally occuring *Mannheimia haemolytica*.

Materials, Methods & Results: A total of 27 Sakiz breed lambs at the age of 36–60 days old, of both sexes, with naturally occuring *M. haemolytica* infection were included in the present study. Prior to allocation into groups, a detailed clinical examination carried out in all the lambs, revealed a variety of abnormal findings: coughing, presence of nasal and ophthalmic discharge, increased respiratory rate and rectal temperature and abnormal sounds at lung auscultation. A total of 27 samples taken from transtracheal aspiration bronchoalveolar fluid in the Sakiz breed lambs were taken into sterile containers in an attemp to perform isolation and identification of *Mannheimia haemolytica*. The antibiotic susceptibility tests for *Mannheimia haemolytica* strains isolated from transtracheal aspiration bronchoalveolar fluid were carried out by disc diffusion as described previously. On days 0, 1, 7, 15 and 30 sheeps were given a clinical score. The clinical lesion score (scores from 0 to 3) was derived from the clinical signs (feed consumption oral and nasal discharges, coughing lung auscultation findings, complete clinical examination) and was carried out by an investigator blinded. The lambs were randomly allocated into 2 groups until the required number of lambs in each group was reached. Lambs in group F (n = 17) were subcutaneoulsy injected with a single dose of florfenicol (dosage 2 mg/kg bodyweight); lambs in group C (n = 10) did not receive an antibiotic treatment and were left as controls. The efficacy of florfenicol was assessed clinically and on the basis of a lesion score derived from the physical examination. Throughout the study florfenicol treatment significantly decreased (*P* < 0.01) the investigator's clinical scores while no significant changes were detected in the untreated control group.

Discussion: *M. haemolytica* is a commonly recognized responsible agent for respiratory diseases in sheep and several therapeutic antibiotics have been suggested for treatment applications. Florfenicol, that might have the potential of efficacy against *M. haemolytica* has not been used in sheep, therfore there is lack of data regarding its efficacy and safety. Despite the efficacy of the drug against respiratory infections of calves and pigs has been documented. Besides bioavailability and pharmacokinetics of florfenicol was evaluated solely in healthy sheep. Based on clinical scoring and complete remission, and supporting laboratory signs involving antibiogram studies, results of the present study may indicate that florfenicol may be a good and safe choice for treatment of respiratory infections due to *M. haemolytica*. The present results were supported by the gradual improvement and alleviation of clinical signs and, clinical remission within description of scores and the absence of pathogenic organisms in transtracheal aspiration after treatment. Furthermore, florfenicol-treated lambs had a significantly better clinical improvement and cure that that of untreated control group of animals.

Keywords: *Mannheimia haemolytica,* sheep, respiratory disease, florfenicol, Sakiz breed.

INTRODUCTION

Mannheimia haemolytica and *Pasteurella multocida* are the major etiological causes of respiratory infections in sheep [4,6,9]. Taking into account bacterial pneumonia in sheep, *Mannheimia haemolytica* is especially responsible either as primary or secondary infection [4]. The principal source of infection is carrrier adult sheep, and it has to be mentioned that this organism may survive in the environment [4]. There may be outbreaks at any duration along year and also at all age distribution, mostly affecting lambs aged up to 12 months [4].

Antimicrobial use in species such as sheep has long been recommended for therapy of these important respiratory tract disease microorganisms [5,8,11,15,19]. Therefore numerous antimicrobial choices have long been available commercially.

Florfenicol, a fluorinated thiamphenicol analogue antimicrobial agent [7], and has recently been approved for therapy of bovine respiratory disease [7,10,17] Although it may appear as a good choice in cases of respiratory infections in sheep, and a previous in vitro study reported that *M. haemolytica* isolates in sheep were susceptible to florfenicol [5], no documentation is available about its efficacy in sheep with respiratory tract disease. Therefore the objective of the field trial described in this paper, was to study the efficacy of florfenicol in lambs with respiratory infection due to *Mannheimia haemolytica*.

MATERIALS AND METHODS

### Animals and clinical signs

A total of 27 Sakiz breed lambs, 36-60 days old, of both sexes, were included in this clinical trial. Before allocation into groups, a detailed clinical examination carried out in all the lambs in the trial, revealed a variety of abnormal findings: coughing (70% of the lambs), presence of nasal (62%) and ophthalmic discharge (40%), respectively, increased (>40 breath min^{-1}) respiratory rate (85%), increased (> 39.9ºC) rectal temperature (60%) and abnormal sounds at lung auscultation (90%).

Percutaneous transtracheal aspiration

A practical field method involving percutaneous transtracheal aspiration was performed as described previously, that is adaptable to sheep. All cases were restrained in lying position with their neck slightly extended and an area over the trachea in about 4-5 cm distal to the larynx was clipped. Disinfection of the clipped area was performed within chlorhexidine gluconate 4 per cent, in an attempt to prevent scondary bacterial complication, and then followed by 70 per cent alcohol. This was followed by local anaesthesia within lidocaine hydrochloride.

A 12 gauge intravenous catheter was introduced, at 45º to the skin, into the trachea between two rings, as possible as lower on the neck as the trachea may be palpated and other tissues were eliminated. Within the entrance of the catheter into the tracheal lumen the needle was withdrawn and the stylet advanced. The sheep was restrained carefully with the help of the asisstants at this process as because of movements could cause the catheter to be broken. At this point some of the animals coughed, and 20 mL of sterile isotonic saline was infused steadily readily through the catheter. The fluid was witdrawn after 5 seconds within the gentle movements of the intratracheal tube forward and backward, for helping its recovery (Figure 1). When the aspiration fluid was clearly completed, the catheter was withdrawn in one occasion.
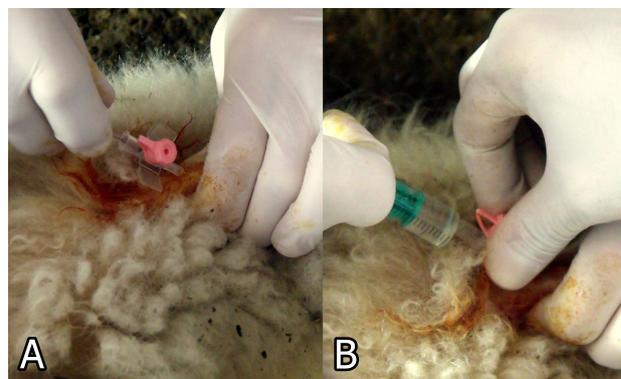
Figure 1. Transtracheal aspiration in a sheep during natural *Mannheimia haemolytica* infection in Sakiz sheep. **A)** percutaneous transtracheal aspiration application. **B)** isotonic infusion thorug catheter.

Isolation of Mannheimia haemolytica

A total of 27 samples taken from transtracheal aspiration bronchoalveolar fluid in the Sakiz breed lambs were taken into sterile containers. Isolation of *Mannheimia haemolytica* was obtained from transtracheal aspiration bronchoalveolar fluid. The samples were inoculated directly onto blood agar plates and incubating at 37ºC for 24 h. After incubation, the suspected colonies with Mannheimia-like morphology, colour, feature and haemolysis were stained by using Gram staining technique and Gram negative bipolar bacilli were examined at the microscopic examination [16,25].

Identification of Mannheimia haemolytica

Identification was performed on the basis of colony morphology, haemolysis, Gram staining and biochemical tests. Biochemical characteristics of the isolates were determined by using catalase, oxidase, nitrate reduction, ONPG, H_2S, ornithine decarboxylase, indol, urease, growth on MacConkey Agar, voges-proskauer and fermentation of glucose, lactose, mannitol, raffinose, salicine, trehalose, xylose and arabinose [16,25]. The isolates which were found positive for the catalase, oxidase, nitrate reduction, ONPG, H_2S, growth on MacConkey Agar, mannitol, xylose tests and found negative for the ornithine decarboxylase, indol, urease, voges-proskauer, arabinose, glucose, lactose, raffinose, salicine, trehalose tests were identified as *Mannheimia haemolytica*.

Antimicrobial Susceptibility of Mannheimia haemolytica strains

The antibiotic susceptibility tests for *Mannheimia haemolytica* strains isolated from transtracheal aspiration bronchoalveolar fluid were carried out by disc diffusion as described by Kirby-Bauer [3], using multidiscs (Oxoid) of amoxycillin + clavulanic acid (AMC-20 µg + 10 µg), oxytetracycline (OT-30 µg), ampicillin (AMP-10 µg), gentamicin (CN-10 µg), enrofloxacin (ENR-5 µg), erythromycine (E-15 µg) and florphenicol (Mast Diagnostics) (FFC-30 µg). Each isolate [10^5 CFU/ml in 0.1 mL as determined by Kirby-Bauer [3] was first poured on Mueller Hinton Agar. Then multidiscs were placed on the MH agar plates and then the plates were incubated at 37ºC for 24 h.

Treatment

The lambs involved in the present study were randomly allocated into 2 groups until the required number of lambs in each group was reached. Lambs in group F (n = 17) were subcutaneoulsy injected with a single dose of florfenicol (dosage 2 mg/kg bodyweight); lambs in group C (n = 10) did not receive an antibiotic treatment and were left as controls. Due to ethical concerns and commercial value of the Sakiz sheep in the Aydin region of Turkey, only a limited number of sheep (n = 10) served as controls (Figure 2).
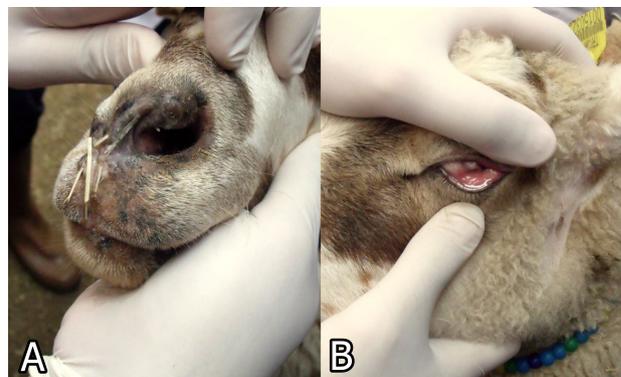
Figure 2. Clinical examination performed througout the study accompanied within the clinical scoring through **A)** the nostrils and the nose and **B)** conjunctivas.

Investigator's evaluation of clinical lesions

On days 0, 1, 7, 15 and 30 sheeps were given a clinical score. The clinical lesion score (scores from 0 to 3) was derived from the clinical signs (feed consumption oral and nasal discharges, coughing lung auscultation findings, complete clinical examination) (Figure 2). The findings were scored by using a system (Table 1) that we developed and have used before [24] and similar to

Table 1. Score details among lambs during clinical examination.

Clinical parameter	Score 0	Score 1	Score 2	Score 3
Feed consumption	No refusal	moderate consumption	few consumption	no consumption
Ocular discharge	No discharge	few discharge	moderate discharge	severe discharge
Nasal discharge	No discharge	few discharge	moderate discharge	severe discharge
Coughing	Absent	moderate	moderate	severe coughing
Lung auscultation findings	No AS	AS au	AS au m	AS au s
Complete clinical assessment	no clinical signs	few cs	moderate cs	severe cs

AS: abnormal sounds; au: audible; m: moderately; s:severely; cs:clinical signs.

that used by Christodoulopoulos *et al.* [8]. Detailed information was given on Table 1. The lesion scoring was carried out by an investigator blinded (Figure 3).

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. Clinical success rate of the florfenicol treatment were compared among groups on the examination days by using the *chi-square* test (Windows version of SPSS 13.0).

RESULTS

Bacteriological

Mannheimia haemolytica was identified from a total of 27 (100%) transtracheal aspiration bronchoalveolar fluid samples.

The antibiotic susceptibility test results showed that all *Mannheimia haemolytica* strains were susceptible to florfenicol and resistant to gentamicin and ampicillin. The results of the antibiotic susceptibility tests are given in Table 2.
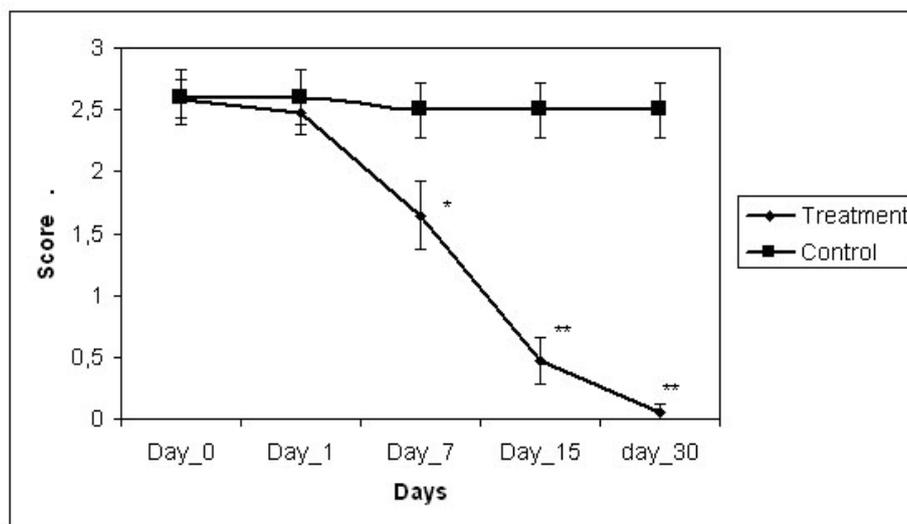
Figure 3. Investigator's clinical scores were assigned on days 0, 1, 7, 15 and 30 during the trial. The control group did not show significant changes in scores over time. Between the groups student's *t*-test at each time point indicated that the 2 groups were not different on days 0 and 1, while on day 7 ($P < 0.05$) and days 15 and 30 ($P < 0.01$) florfenicol treatment group scores were significantly lower than the control group. */**Indicate the time schedule at which two groups were significantly different. *$P <0.05$, ** $P <0.01$.

Table 2. Susceptibilities of *Mannheimia haemolytica* (n=27) isolated from respiratory infections in lambs, to various types of antibiotics.

Antibiotic	Resistance		Intermediate		Susceptible	
	N	%	N	%	N	%
Amoxycillin + Clavulanic acid (20 μg + 10 μg)	19	70	5	18	3	12
Oxytetracyclin (30 μg)	15	55	2	7	10	38
Ampicilline (10 μg)	27	100	-		-	
Gentamycine (10 μg)	27	100	-		-	
Enrofloxacin (5 μg)	13	48	8	30	3	22
Erythromycine (15 μg)	13	48	-		14	52
Florfenicol (30 μg)	-		-		27	100

Therapeutical trial

Seventeen lambs were allocated to the florfenicol group and 10 animals were allocated to the control group.

During and after treatment, no systemic nor adverse reactions were observed in any of the lambs. All treated lambs appeared healthy following florfenicol therapy; their heart and respiratory rates were within the physiological ranges. In addition, all lambs survived and concluded the trial. There were no local lesions observed at the injection site.

The efficacy of therapy and investigator's clinical scores (Figure 3)

Florfenicol treatment administered had apparent effect on the course of *M. haemolytica* infection in the present animals. It has been observed clinically that following the drug was administered during the course of infection, clinical signs were disappeared in the following clinical examination days, resulting in clinical recovery. Cure rates of the lambs after therapy were 100% by day 30. Table 1 showed the number of animals of each group with clinical scores at day 0, 1, 7, 15 and 30. Clinical remission and cure rates were deemed as disappearance of all the clinical signs. As a function of time, florfenicol group showed significant improvement in scores from day 7 ($P < 0.05$) to day 30 ($P < 0.01$) while the untreated control group showed a nonsignificant change in scores from day 7 to day 28. Comparison of the two groups revealed that the clinical scores did not differ between the groups on days 0 and 7, whereas the florfenicol group showed a significantly lower clinical score than control group on days 7, 15, and 30 ($P < 0.05$). No grossly visible side effects were noticed during treatment period.

DISCUSSION

Based on clinical scoring and complete remission, and supporting laboratory signs involving antibiogram studies, results of the present study may indicate that florfenicol may be a good and safe choice for treatment of respiratory infections due to *M. haemolytica*.

Mannheimia haemolytica is an important pathogen of ruminants in Turkey. Its control is an important measure in the prevention of pneumonic pasteurellosis of ruminants in Turkey and the rest of the world. Yearly herd losses costing millions of dollars have led to research focused on vaccine production because the number of strains isolated demonstrates the continuing economic importance of *Mannheimia haemolytica* infection [14]

The primary diseases associated with *Mannheimia haemolytica* were pneumonia in lambs and calves and septicaemia in lambs, as have been extensively recorded elsewhere [21]. The isolated serotypes in the present study are also in good conformity with the recognised patterns of disease in sheep [12].

M. haemolytica and *Mycoplasma* spp. are commonly recognized responsible agents for respiratory diseases in sheep [9]; several therapeutic choices involving antibiotics have been suggested for treatment applications [1,2,13,18]. Florfenicol, that have the potential of efficacy against *M. haemolytica*, has not been used in sheep, because of lack of data regarding its efficacy and safety. Despite this, the efficacy of the drug against respiratory infections of calves [23] and pigs [22] has been documented. Apart from those clinical studies, in a previous report regarding the antimicrobial susceptibility of common respiratory tract pathogens isolated from sheep and goats (with most common isolate was *Mannheimia hemolytica*), florfenicol was one of the drug that the isolates were susceptible [5]. A study was previously conducted regarding bioavailability and pharmacokinetics of florfenicol in 20 healthy sheep within intravenous and intramuscular doses of 20 and 30 mg/kg body weight [20].

Our results suggest that florfenicol could have beneficial effects against respiratory infections due to *M. haemolytica* in lambs. In this sense present results were supported by the gradual improvement and alleviation of clinical signs and, clinical remission within description of scores and the absence of pathogenic organisms in transtracheal aspiration after treatment. Furthermore, florfenicol-treated lambs had a significantly better clinical improvement and cure that of untreated control group of animals.

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