Efficacy of doxycycline in a goat model of *Pasteurella* pneumonia

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ABSTRACT

The clinical efficacy of doxycycline (Doxycen®, Cenavisa, Spain), a long-acting preparation, was evaluated for treatment of *Pasteurella haemolytica* infection in 6 goats. One goat was not infected and served as a control. The disease was induced by intratracheal inoculation of 10^7 to 10^9 cfu of *P. haemolytica.* Confirmation of respiratory disease was based on evidence of appropriate clinical signs. Before and after initiation of doxycycline treatment on day 10, each goat was examined daily. Three clinical responses to doxycycline treatment were noted. Mean rectal temperatures decreased from 40.1 °C to normal, while mean respiratory rate decreased from the pre-treatment value of 32 to 27/min after 4 days. Other clinical signs associated with pneumonia resolved within 3–5 days post treatment. In addition the minimum inhibitory concentration of DOTC for the *P. haemolytica* isolate was found to be <0.5 µg/mℓ. The present study indicates that DOTC may be a useful antimicrobial agent in the treatment of caprine pasteurellosis.

Key words: doxycycline, goat, *Pasteurella haemolytica,* pneumonia.

Ole-Mapenay I M, Mitema E S **Efficacy of doxycycline in a goat model of *Pasteurella* pneumonia**. *Journal of the South African Veterinary Association* (1997) 68(2): 55–58 (En.). Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, PO Box 29053, Nairobi, Kenya.

INTRODUCTION

Doxycycline (DOTC), most often available as hydrochloride, is a tetracycline of recent vintage. On account of lipophilicity it is characterised by comparatively better tissue penetration, which is reflected by a large volume of distribution[1,9] and enhanced *in vivo* and *in vitro* antimicrobial activity[7,15].

Widespread use of doxycycline in human medicine has confirmed the therapeutic efficacy and safety of the drug[5]. However, few studies have been conducted in food animals[14,18,19], and there is no published information on its clinical efficacy in goats.

The purpose of this study was to determine the efficacy of a long-acting formulation of doxycycline in goats infected with *Pasteurella haemolytica.* Since efficacy of the drug is determined by its ability to resolve the prevailing clinical signs, response to doxycycline or lack thereof may have profound implications for the use of this relatively new veterinary formulation as a first-line chemotherapeutic agent in caprine pneumonia.

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Received: July 1995. Accepted: February 1997.

MATERIALS AND METHODS

Animals

Seven small East African goats, 5 males and 2 females, varying in age from 1–2 years, were used in this study. The animals were purchased from a farm on the outskirts of Nairobi. The average body weight of the goats was 21.0 ± 2.5 kg (18–26 kg). After arrival, the goats were acclimatised in their new environment for 2 weeks. During this time the goats were healthy and remained clinically normal without any signs of respiratory disease. The goats were dewormed with fenbendazole (Panacur®, Hoechst), a broad-spectrum anthelmintic, and were housed individually indoors in pens on concrete floors. They were offered pelleted concentrates once daily and had free access to hay and water throughout the trial.

Infectious agent and experimental infection

The *P. haemolytica* strain was previously isolated from the respiratory tract of a goat that had died of severe fibrinous pneumonia at the Department of Pathology and Microbiology of the Faculty of Veterinary Medicine, University of Nairobi. *P. haemolytica* was initially passaged by growing it on blood agar plates that were checked for purity after overnight incubation at 37 °C. A pure colony was transferred from the blood agar plate and inoculated in 10 mℓ brain heart infusion (BHI) broth (Difco Labs, Detroit). After overnight incubation the 10 mℓ was added to 40 mℓ BHI broth. After another 6-hour incubation at 36 °C the culture was used for experimental infection. Serial dilutions were made of the 12-hour culture, inoculated on blood agar plates and after 24 hours the infective dose was enumerated. The optical density of the infective dose was measured spectrophotometrically (Spectronic-20, Bausch & Lomb) at 537 nm wavelength.

A 12-hour culture of *P. haemolytica,* containing approximately 10^7 to 10^9 cfu/mℓ suspended in 5 mℓ BHI broth was inoculated intratracheally by tracheopuncture in 6 goats. The control animal was inoculated with the same volume of sterile broth and served as an uninfected and untreated control.

Drug administration

The goats were injected intramuscularly with the 20 % doxycycline (Doxycen®, Cenavisa, Spain) solution at the manufacturer's recommended dose rate of 20 mg/kg body weight. The drug was administered on day 10 post infection during the febrile reaction.

Clinical observation

Goats were examined daily for clinical signs following inoculation and after treatment with DOTC. The following clinical parameters were observed daily: rectal temperature, respiratory rate (breaths/min), appetite, general behaviour, nasal discharge, cough and lung auscultation.

Haematological analysis

Blood samples of 5 mℓ each were collected by jugular venipucture in heparinised vacutainer tubes for haematology. Samples were collected on days 0, 4, 8, 12, 16 and 20. The white blood cell (WBC) counts were performed with a Coulter counter (Coulter Electronics). Plasma was harvested by centrifugation from heparinised blood. Total protein concentration (TPC) was measured by means of a refractometer (Atago, Japan).

Antibiotic susceptibility test

The *in vitro* minimum inhibitory concentration (MIC) of DOTC for the *P. haemolytica* isolate used in our study was determined by the broth dilution technique (BHIB, Difco Labs, Detroit). For the sensitivity testing an inoculum concentration of 10^6 cfu/mℓ from an overnight culture was used. Serial 2-fold dilutions of DOTC concentrations ranging from 0.03 to 16 µg/mℓ were incorporated into the BIH broth. All tubes were prepared simultaneously. The antibiotic-containing tubes were inoculated using a micropipette that delivered an inoculum of about 10 µℓ, giving an inoculum size of 10^4 cfu per tube. Each concentration was tested in triplicate. The tubes were then incubated aerobically at 37 °C. The MIC was read after 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent at which there was no visible growth.

Statistical analysis

Haematological parameters and clinical signs (respiratory rates and temperatures) obtained before and after DOTC treatment were subjected to analysis of variance (ANOVA) using SAS (SAS Institute, Cary, USA) statistical package. Tukey's highest significant difference (HSD) test[16] was used to determine whether there were significant differences among the group means. Significance was tested at the level of $p \leq 0.05$.

RESULTS

Clinical signs following infection

Intratracheal inoculation of infectious doses ranging from 10^7 to 10^9 cfu/mℓ consistently produced a marked febrile response (Fig. 1), increased respiratory rates and accentuated bronchovesicular sounds in all infected goats. Based on the observed clinical findings, the response to challenge with *P. haemolytica* was similar in all the goats despite the fact that different doses of the infecting organism were used. The control uninfected and untreated animal remained clinically normal throughout the experimental period.

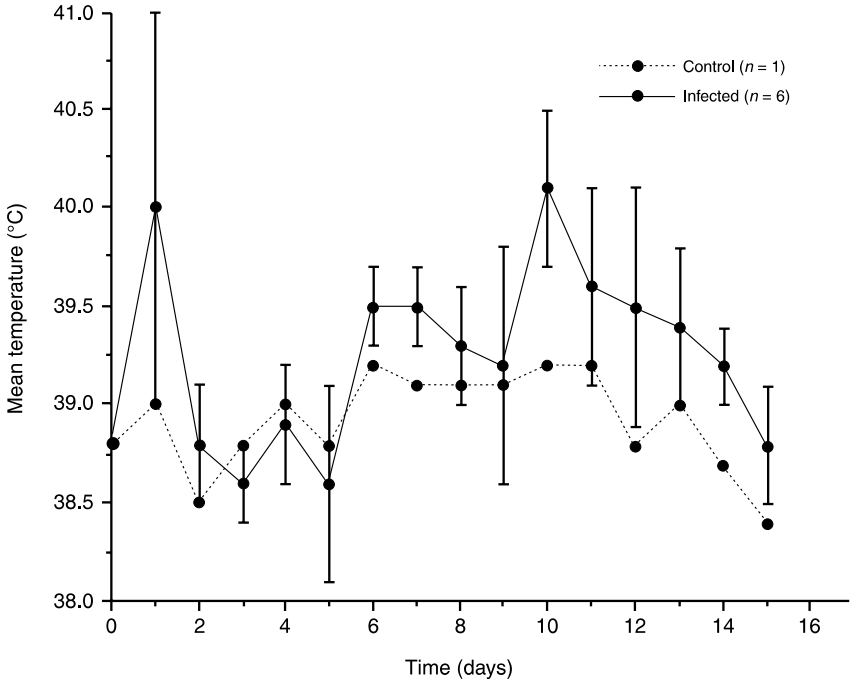

Fig. 1: **Mean diurnal rectal temperature following intratracheal inoculation of *Pasteurella haemolytica* in goats** ($n = 6$). **One goat served as control.**

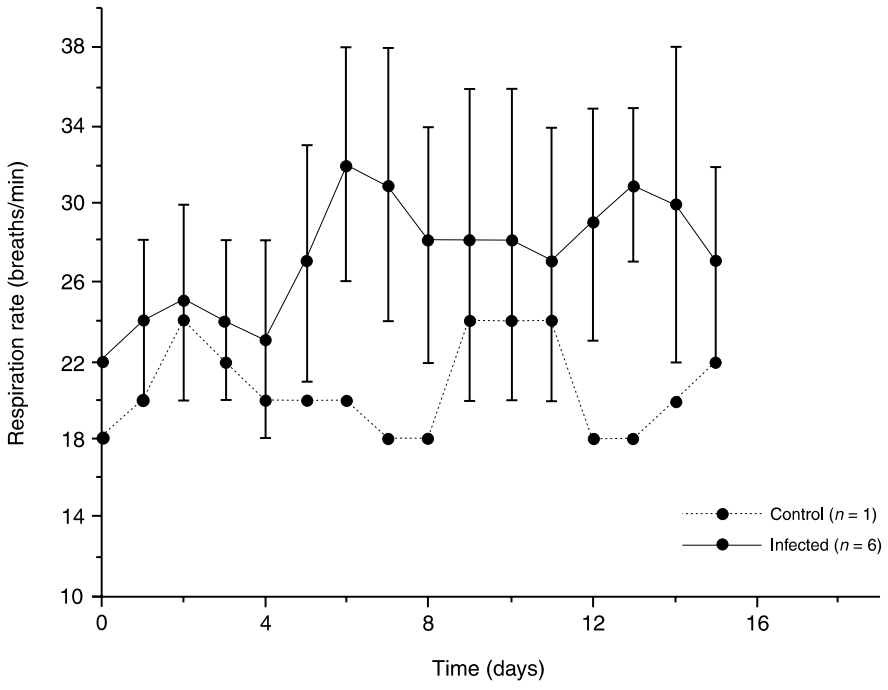

Fig. 2: **Mean daily respiration rates following intratracheal inoculation of *Pasteurella haemolytica* in goats** ($n = 6$). **One goat served as control.**

Temperature

Before the goats were infected their mean daily rectal temperature was 38.7 ± 0.1 °C. Following infection there was a rise in temperature on day 6 (39.5 °C) that persisted over a period of 6 days. All the infected animals ($n = 6$) developed pyrexia (>39 °C) by day 10 post-inoculation (Fig. 1). The highest individual rectal temperature recorded during the course of infection was 41 °C on days 10 and 11. In the control animal, temperatures fluctuated within the normal range throughout the experimental period.

Respiratory signs

In addition to the febrile response, respiratory changes were manifested clinically in challenged goats. Prior to infection the mean daily respiratory rate in the infected group was 22 breaths/min. Following infection there was a progressive rise in respiratory rate from day 2 to day 6, when a maximum mean rate of 32/min was recorded (Fig. 2). During the course of infection, 5 goats developed moist rales either uni- or bilaterally by day 12 post infection. Thoracic auscultation

Table 1: **Clinical findings in 6 goats infected with *Pasteurella haemolytica.***

Clinical signs[1]	Goat No. 36	37	38	41	42	46
Pyrexia	+	+	+	+	+	+
Anorexia	–	–	–	+	–	–
Dyspnoea	++	++	+	++	++	++
Moist rales	++	++	++	+	++	++
Coughing	–	+	+	–	+	+
Nasal discharge	–	+	+	–	+	+
Diarrhoea	–	–	+	–	+	–

[1] ++ = severe; + = mild; – = absent.

Table 2: **Haematological parameters** (mean ± SD) **of 6 goats before and after *Pasteurella haemolytica* infection and after DOTC treatment.**

Parameter	Unit	Pre-infection	Post infection	
			Before DOTC treatment	After DOTC treatment
WBC	$10^3/\mu\ell$	12.0 ± 2.67	15.9 ± 3.22*	11.8 ± 2.74
TPC	g/dℓ	68.8 ± 3.02	62.0 ± 3.65*	66.8 ± 3.63
RBC	$10^6/\mu\ell$	14.1 ± 1.67	15.29 ± 1.68	15.26 ± 2.17
PCV	%	30.6 ± 5.32	27.3 ± 3.25	28.86 ± 3.13
Hb	mg/dℓ	11.54 ± 1.54	10.33 ± 1.22	10.24 ± 1.06

*Significant differences ($p < 0.05$) between pre- and post-treatment groups.

initially revealed increased vesicular sounds and later coarse crackles in the lower portion of the chest. Moreover, coughing coupled with a nasal discharge was observed in 4 goats from day 10. Wheezing and expiratory grunting was observed in 1 goat on day 11 post infection (Table 1). In contrast to the infected goats, the control animal remained normal and showed only minor changes in respiratory rate.

Response to DOTC treatment

Five of the 6 goats responded very well to doxycycline treatment. Mean rectal temperatures decreased from 40.1 ± 0.24 °C to 38.8 ± 0.22 °C within 4 days following treatment. Mean ± SD respiratory rates decreased from 32 ± 5 to 26 ± 3 after a 5-day post-treatment period. Clinical signs associated with pneumonia (coughing, sneezing, nasal discharge and harsh lung sounds) resolved by day 5 post treatment in all 6 goats. There were no adverse reactions to the drug following intramuscular administration.

Haematology

Haematological parameters were evaluated prior to entry into the study, during infection and after DOTC treatment. WBC counts that were significantly increased ($p < 0.05$) after challenge with *P. haemolytica* decreased dramatically while TPC increased ($p < 0.05$) after the goats were treated with DOTC. However, baseline PCV and Hb concentrations were within the species range before and after DOTC administration (Table 2).

In vitro testing

The MIC value of doxycycline for the *P. haemolytica* isolate used in the study was 0.4 µg/mℓ (0.25–0.50 µg/mℓ; $n = 3$).

DISCUSSION

The long-acting preparation of doxycycline used in the study was effective in reversing the clinico-pathological signs observed in all the goats with induced pneumonic pasteurellosis. There were no physical or clinico-pathological findings at initial examination that predicted the subsequent response of the infected goats to DOTC treatment. Thus the speedy recovery exhibited by these animals following treatment confirms the efficacy of doxycycline as a chemotherapeutic agent. Moreover, the *in vitro* (MIC) assay of DOTC for the *P. haemolytica* demonstrated the susceptibility of a common respiratory tract pathogen to doxycycline. In a recent study, Abric *et al.*[1] reported an MIC value of 2 µg/mℓ for OTC for 92 field strains of *Pasteurella.* In a similar study the MIC values for doxycycline and metacycline were lower than for OTC[13].

Reports on the efficacy of doxycycline in the treatment of infectious diseases in food animals are few. Favourable response to a single intramuscular injection was reported for sheep with induced heartwater (cowdriosis)[8], for splenectomised calves with induced anaplasmosis[10] and for *P. haemolytica* infection in calves[18].

The efficacy of DOTC against respiratory tract pathogens has been reported[11,13,18]. In addition, earlier studies had demonstrated the superiority of DOTC to conventional tetracyclines at lower doses[4,6]. These findings confirm the usefulness of doxycycline in the treatment of respiratory tract infections. The efficacy of doxycycline observed in the present study may be attributed to its faster and complete absorption[3], comparatively better tissue penetration[2,9] and its ability to penetrate infected tissues[17]. Preliminary studies on the pharmacokinetics of DOTC in healthy and diseased goats have given support to this hypothesis[12].

The results of the present study indicate that doxycycline is very effective in the treatment of caprine pneumonic pasteurellosis as demonstrated by a good *in vivo* antimicrobial activity. It may thus be used as an alternative to oxytetracycline in caprine pneumonias at the recommended dose rate of 20 mg/kg intramuscularly.

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Book review – Boekresensie

Proceedings of a symposium on the African buffalo as a game ranch animal

Edited by B L Penzhorn

1996. South African Veterinary Association Wildlife Group, Onderstepoort, South Africa, 198 pp. Price: R70.00 (excl. postage). ISBN 1 875088 08 3.

The proceedings were bound and made available to delegates of the symposium at registration, on 26 October 1996, for which the editor should be complimented. This symposium held at Onderstepoort, was one of a series that deals with management and veterinary issues of one of the 'Big Five' game species as a game ranch animal. The 18 authors who contributed to the symposium were carefully selected experts in their field and they provide a comprehensive and balanced overview of current management practices and diseases that are pertinent to the African buffalo. Papers include aspects on socioecology, applied anatomy, hunting and meat production, management of large buffalo populations, capture, boma management and translocation, reproduction, diseases, parasites, and of particular interest, the breeding of 'corridor disease-free' buffalo.

Most South Africans reading this review will be aware that the African buffalo are carriers of foot-and-mouth disease (FMD), corridor disease (CD), brucellosis (CA) and more recently tuberculosis (TB), which due to veterinary regulations restrict the movement and therefore sale of buffalo in South Africa. With the growing tourist industry and the desire for game farmers to provide the 'Big Five' for the market, the so-called 'disease-free' buffalo are in great demand and fetch prices between R60 000 and R75 000 per animal at auctions. This has stimulated research to establish and breed herds of buffalo that do not carry the four diseases mentioned above. The paper on breeding corridor disease-free buffalo reports on this aspect. The paper on reproduction mentioned the failure of embryo transfer from buffalo cows to domestic cattle. Even though two pregnancies out of 15 transfers were obtained, they supposedly failed owing to immune responses in the placenta. The possibility of overcoming this would be to use inner-cell-mass transfer, where the inner mass of the buffalo embryo is transplanted into the recipient species embryo so that the buffalo embryo will develop from the recipient's placenta. This is exciting research and has potential for many other scarce or endangered species. The paper on FMD gives details on current trends in FMD control and research relating to the buffalo, with the following summary: 'Although African buffalo are central to the FMD problem in southern Africa, there are developments in the international approach to the control of FMD as well as on the technological front that provide optimism for the old concept of "buffalo = FMD" being consigned to history.'

Included in the proceedings is an extensive bibliography of the African buffalo, consisting of 677 references, which was compiled by the Price Forbes Chair in Wildlife, Faculty of Veterinary Science, University of Pretoria. The index at the back of the proceedings is comprehensive and well structured using 'key-words', but the reader is left to guess that the numbers refer to the references in the bibliography and not to the page numbers in the text.

In conclusion, the papers are well written with diagrams and tables where required, with most having their own list of references for further reading. The proceedings succeed in bringing together and consolidating for the reader the most up-to-date information on the management and problems associated with the African buffalo as a game ranch animal, and I would therefore recommend this publication as a useful source of information for wildlife experts and the game rancher on this highly sought-after species.

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