Comparative evaluation of the effects of florfenicol and tulathromycin on clinical recovery and acute phase proteins in undifferentiated natural bovine respiratory disease

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SUMMARY

In this study, the effects of florfenicol and tulathromycin on clinical recovery and acute-phase proteins in bovine respiratory diseases (BRD) were evaluated under the field conditions. A total of 30 local race feedlot cattle were used in the study. Twenty naturally infected beef and 10 healthy beef were used for the experimental and control groups, respectively. Sick animals were randomly divided into two groups of 10 and subcutaneously 40 mg per kg florfenicol was administrated in group I and 2.5 mg per kg tulathromycin was administrated in group II as single dose. The animals were clinically examined before (0 h) and 9 days after the treatment. Also, blood samples were taken and serum haptoglobin and serum amyloid A concentrations were measured before and 9 days after the treatment. It was concluded that although the clinical findings and recovery were not always correlated with serum acute phase proteins levels, florfenicol and tulathromycin can be effective for BRD therapy under field conditions.

Keywords: Acute-phase proteins, bovine respiratory disease, clinical findings, florfenicol, tulathromycin.

RÉSUMÉ

Comparaison des effets du florfénicol et de la tulathromycine sur la rémission clinique et les protéines de la phase aiguë chez les bovins atteints de maladie respiratoire bovine

Dans cette étude, les effets de florfénicol et tulathromycine sur la rémission clinique et les protéines de la phase aiguë de maladies respiratoires bovines (BRD) ont été évalués dans les conditions de terrain. Un total de 30 bovins de race locale ont été sélectionnés pour l'étude. Vingt bovins naturellement infectés et 10 bovins sains ont constitué les groupes expérimentaux et témoins, respectivement. Les bovins malades ont été répartis aléatoirement en deux lots de 10: le lot 1 a reçu une administration unique de florfénicol par voie sous cutanée à la dose de 40 mg par kg tandis que le lot 2 recevait une administration unique de tulathromycine par voie sous cutanée à la dose de 2.5 mg par kg. Les animaux ont subi un examen clinique avant et 9 jours après le traitement. A ces périodes, des échantillons de sang ont été prélevés et les concentrations sériques en haptoglobine et sérum amyloïde A ont été déterminées. Il a été conclu que, bien que les résultats cliniques et la rémission ne soient pas toujours en relation avec les taux sériques des protéines de la phase aiguë, le florfénicol et la tulathromycine peut être utilisés pour traiter les BRD dans les conditions de terrain.

Mots clés : Protéines de phase aiguë, maladies respiratoires bovines, les constatations cliniques, florfénicol, tulathromycine.

Introduction

Bovine respiratory disease (BRD) is a multifactorial disease usually resulting from the interaction of bacterial and viral agents, combined with stress [26]. This disease is a major health problem of cattle worldwide and causes large economic loss every year in cattle industry [1, 2, 14, 26]. BRD incidence is related to genetic, environmental, and economic factors in feedlot calves [5, 25].

BRD is characterized by an acute-onset pneumonia caused by Pasteurella haemolytica, Pasteurella multocida, Histophilus somni, or Mycoplasma spp. [18, 19, 26]. Also, especially respiratory syncytial viruses (RSV), para influenza 3 viruses (PI-3), bovine herpes virus 1 and bovine viral diarrhoea/mucosal disease virus (BVDV) play an important role in the infection [1].

Antimicrobials are the primary tool for prevention, control, and treatment of this disease. As common bacterial strains are resistant to many antibiotics and chemotherapeutics worldwide, there is a need for new antibiotics [23, 26].

Florfenicol is a synthetic, fluorinated chloramphenicol derivative. It inhibits protein synthesis by binding to ribosomal subunits of susceptible bacteria, leading to the inhibition of peptidyl transferase. Florfenicol is a broad-spectrum antimicrobial agent and it is active against Gram-positive and -negative bacteria. Florfenicol is proposed for the treatment of BRD [10, 23, 27].

Tulathromycin is a semi-synthetic macrolide antibiotic, prepared via fermentation followed by organic synthesis. It is
a member of the triamilide subclass of macrolides [9]. It has been approved for use in the treatment and prevention of BRD and in the treatment of swine respiratory disease in European Union and United States [8, 11]. In the present study, these antibiotics were chosen because of various reasons, such as florfenicol and tulathromycin are relatively newly antibiotics in Turkey, antibacterial resistance developed for a lot of other antibiotics and these two antibiotics are administered only once in the treatment (their applications may be economical and practical).

The early stages of the host response to infectious agents include a number of physiologic changes, collectively known as the acute-phase response. The term “acute phase” was introduced in 1941 to describe serum in which an acute-phase protein (APP), the C-reactive protein, was present. Today, the acute-phase response is considered to be a dynamic process involving systemic and metabolic changes providing an early non-specific defence mechanism against insult before specific immunity is achieved [13, 21]. Several experiments showed that both viral and bacterial respiratory infections can induce acute-phase responses [12].

The measurement of APPs has potential as a diagnostic and prognostic tool in cattle [2]. The most sensitive APPs are haptoglobin (Hp) and serum amyloid A (SAA) in cattle, and concentrations of these proteins increase particularly in response to acute inflammatory conditions as well as to subclinical inflammation [4, 28].

The aim of this study was comparative evaluation of the effects of florfenicol and tulathromycin on clinical recovery and serum APPs, which are Hp and SAA, in natural BRD infection under field conditions. This study also investigates the importance of serum Hp and SAA concentrations on determination of illness and recovery level in BRD.

Materials and Methods

ANIMALS AND EXPERIMENTAL DESIGN

In this study, a total of 30 local race feedlot cattle, which were in different herds in Samsun province, Turkey, were used. Ages of the animals were 1–3 years and their weights were 80–280 kg. Twenty natural infected beef and 10 healthy beef were used for the experimental and control groups, respectively. Sick animals were housed under the similar conditions. All animals were collected at the beginning of September and treated. No other disease symptom was observed in the animals.

The clinical criteria used in the initial diagnosis of acute pneumonia were body temperature ≥39°C, respiratory rate >50 breaths/min, coughing and/or nasal discharge, and anorexia.

TREATMENT

Sick animals were randomly divided into two groups and subcutaneously 40 mg/kg florfenicol (Nuflor, Intervet Shering-Plough) was administered in group I (n=10) and 2.5 mg/kg tulathromycin (Draxxin, Pfizer) was administered in group II (n=10) as single dose.

CLINICAL EXAMINATIONS AND BLOOD SAMPLES

The animals were clinically examined by the same investigator before the treatment (0 h/pretreatment) and 9 days after the treatment. Observed clinical symptoms were body temperature (°C), respiratory rate, general condition, hyperpnoea, nasal discharge, abnormal lung sounds, and cough. Each parameter was scored using a scale of 0 (normal) to 4 (severely) modified from ELITOK and ELITOK [7]. Clinical scoring criteria are shown in Table I.

Blood samples were collected from all sick animals before and 9 days after antibiotic treatment and also from control animals. After blood clotting at 37°C for 10 minutes in an incubator, serum was collected by centrifugation (10 minutes, 1550 g at 4°C) for the detection of serum APPs.

ACUTE PHASE PROTEIN ANALYSIS

Serum Hp and SAA concentrations were detected at the beginning and 9 days after the treatment. Because, it was considered that an animal can completely clinically recovers and APP levels significantly change about 9 days after the antibiotic treatment [12]. Serum concentrations of Hp and SAA were measured with commercially available kits (Tridelta Development Limited, Ireland), according to the manufacturer’s instructions and manual by ELISA microplate reader (Digital and Analog Systems, Rome, Italy). The principal of the haptoglobin assay based on free haemoglobin exhibits peroxidase activity, which is inhibited at low pH. Haptoglobin present in the specimen combines with haemoglobin and at low pH preserves the peroxidase activity of the bound haemoglobin. Preservation of the peroxidase activity of haemoglobin is directly proportional to the amount of haptoglobin present in the specimen. Also, the principal of the SAA assay based on the solid phase sandwich ELISA. A monoclonal antibody specific for SAA has been coated onto the wells of the microtitre strips provided. Samples, including standards of known SAA content, were added into microwells along with biotinylated anti-SAA monoclonal antibody. Any SAA present in the well is both captured on the plate by the immobilized antibody and labelled with the conjugate antibody in a one step procedure. After washing to remove all of the unbound material, Streptavidin-Horse Radish Peroxidase conjugate was added to the well and incubated. Following the second incubation, TMB substrate solution was added. The intensity of the colour produced is proportional to the concentration of SAA present in the original specimen.

DATA EVALUATION

Mann–Whitney U-test for two independent samples or Wilcoxon test for two related samples was used for comparison.
Results

The mean body weights of cattle in group I and group II were 141±14.17 and 187±29.13 kg, respectively, and no statistically significant difference was observed (P>0.05). All animals were clinically examined at the beginning and 9 days after the treatment. Mean body temperatures were determined 39.23±0.13°C in group I and 39.52±0.30°C in group II before the treatment. Nine days after the treatment, these values were measured 38.41±0.14 and 38.63±0.13°C in group I and group II, respectively, and an statistical difference was found between body temperatures of experimental groups before and after the treatment (P<0.05).

The mean values of clinical (coughing, nasal discharge, respiratory auscultation, and appetite) and recovery scores of the groups before and 9 days after the treatment are shown in Figure 1.

Mean recovery scores were determined 2.90±0.34 and 2.60±0.22 in Groups I and II, respectively. Ten animals (50%) were evaluated as completely clinically recovered (6 animals in group I and 4 animals in group II) and 9 animals (45%, 3 animals in group I and 6 animals in group II) were partially recovered (generally body condition better than before the treatment but lung sound was not to get well completely) and only one animal (5%) in group I did not recover 9 days after the treatment. However, that animal completely recovered 13 days after the antibiotic injection.

No statistical difference was observed in the clinical scores between the experimental groups before the treatment (P>0.05). Clinical scores were decreased in all observed findings (coughing, nasal discharge, respiratory auscultation, and appetite) and statistically significant differences were determined in both experimental groups before and 9 days after the treatment (P<0.05).

Determined mean serum APP concentrations are shown in Table II. Serum Hp and SAA concentrations of the control animals were ranging from 1.29 to 1.98 mg/ml (1.55±0.08 mg/ml) and from 20 to 42 µg/ml (27.90±2.42 µg/ml), respectively. Compared to the healthy animals, statistically significant differences in the serum Hp and SAA levels were determined in all the sick animals before the treatment (P<0.05). The concentrations of APPs decreased in both antibiotic groups after the treatment, but these decreases were not determined statistically important from the pre-treatment Hp and SAA levels (P>0.05). In consequence, decreasing of the serum APPs could not reach the control group level.

No statistical significant difference was observed in the serum acute phase protein concentrations between two groups after the treatment (P>0.05).

Discussion

Antimicrobial therapy is the most effective method for the prevention and treatment of BRD. Ampicillin, erythromycin, tetracycline, spectinomycin, and sulfamethazine are anti-
microbial agents commonly used in the treatment of BRD. Previous studies have indicated that resistance to these compounds is frequently encountered. Therefore, currently several new antibiotic agents have been introduced or under development for the treatment of BRD [26].

SHIN et al. [23] detected that florfenicol is therapeutically valuable in the treatment of primary or complicated bacterial pathogens causing the bovine and swine respiratory tracts. ASLAN et al. [1] also reported that florfenicol has a high bacteriological and clinical efficacy in the treatment of calf respiratory tract disease. In addition, SKOGERBOE et al. [24] found that tulathromycin was more efficacious in the treatment of undifferentiated BRD than florfenicol and tilmicosin. KILGORE et al. [17] reported that tulathromycin demonstrated superior efficacy compared with tilmicosin and florfenicol when treating groups of high-risk cattle before the onset of signs of BRD.

In the present study, florfenicol and tulathromycin had similar efficacy on the clinical recovery in BRD. These findings showed that florfenicol and tulathromycin were effective in BRD, in the working area, Samsun province, Turkey. No statistically significant difference was observed between clinical scores of the florfenicol and tulathromycin groups after the treatment and it was concluded that both antibiotics can be used for BRD therapy under field conditions.

APPs can be used as an indicator in various infections and inflammations. Base levels of serum Hp, SAA, and fibrinogen concentrations were established and may be used for evaluating calf health in herds [12].

Hp shows a significant increase during an acute-phase response in both experimental and natural occurring infections and inflammatory conditions [4]. CARTER et al. [3] reported that serum Hp concentrations have a value for use in assessing feedlot cattle that may become ill as a result of respiratory tract disease and for use in monitoring treatment efficacy. WITTUM et al. [29] said that Hp concentration may be an indicator of response to antibiotic therapy, although it appears to be untreated to case severity or need for treatment.

In addition, SAA has been suggested as being a valuable acute-phase protein for assessment of bovine medicine [6]. SAA can be beneficial for the veterinarians in helping to eval-

<table>
<thead>
<tr>
<th>APPs</th>
<th>Control (n=10)</th>
<th>Before the treatment</th>
<th>After the treatment</th>
</tr>
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<tbody>
<tr>
<td>Hp (mg/ml)</td>
<td>1.55±0.08a</td>
<td>3.52±0.23b</td>
<td>2.57±0.28b</td>
</tr>
<tr>
<td>SAA (µg/ml)</td>
<td>27.90±2.42a</td>
<td>157.20±28.08b</td>
<td>98.60±29.17b</td>
</tr>
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Table II: Serum APPs concentrations (mean ± SEM).

In the present study, similar to previous reports, an important difference was seen in the serum Hp and SAA levels in all the sick animals in comparison with control group. APP concentrations were statistically significantly different from the control group before the treatment and no significant difference was observed between two experimental groups.

Serum Hp and SAA concentrations decreased in both antibiotic groups after the treatment. But, this decrease was not satisfactory when compared with the control group. WITTUM et al. [29] reported that feedlot cattle with clinical respiratory tract disease have a large and variable Hp response. In addition, GANHEIM et al. [12] and ORRO et al. [20] reported that the acute-phase response after single infection of bacteria or virus did not parallel the clinical symptoms of the animals. ORRO et al. [20] also indicated that APPs could show long half life in blood after BRD infection. The authors evaluated bovine APPs, in 10 dairy calves with BRD caused by bovine respiratory syncytial virus (BRSV) and emphasized that SAA and Hp concentrations increased at week 1 probably due to BRS virus and at weeks 3 and 4 due to secondary bacterial infections.

HUMBLET et al. [15] reported that Hp response was quite variable in cattle with bronchopneumonia. Hp is very specific for disease but it is not sensitive for some findings such as recovery duration. Similar with previous studies [12, 15, 20], although some sick animals were observed clinically recovered, their serum APP levels were determined higher than the control animals in the present study. These findings showed that serum Hp and SAA may be an indicator for monitoring healthy and sick animals in BRD, but they may not be used for monitoring all the clinical recovery stage.

Consequently, florfenicol and tulathromycin showed sufficient therapeutic effect in naturally occurred BRD in cattle and no statistically significant difference was seen between efficiency of two antibiotics in the working area, Samsun province, Turkey. Also, serum Hp and SAA concentrations were determined very high in the sick animals when compared with the control group before the treatment. Although, the acute phase protein concentrations of the experimental groups decreased, these levels were still high from the control group after the treatment. It was concluded that florfenicol and tulathromycin effective and can be used for BRD.
therapy under field conditions. Although the clinical findings were not always correlated with serum APP levels, serum Hp and SAA concentrations may give an opinion about illness in BRD. However, these parameters may not be sufficient for evaluation of the clinical stage of antibiotic therapy.

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References