

The Effect of Tulathromycin Treatment on Antioxidant Vitamins in Montofon Calves with Pneumonia

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Abstract: The purpose of this study is to examine changes in some vitamin levels and clinical symptoms of treatment with tulathromycin in Montofon calves with pneumonia. For this purpose, 30 calves exhibiting clinical signs of pneumonia such as fever $>39^{\circ}\text{C}$, elevated respiratory rate >40 (min^{-1}), pulsation >100 , cough, lack of appetite, nasal flaring, mandibular lymph node enlargement, respiratory difficulties and nasal discharge were studied. A control group of 20 healthy calves were also studied. Blood samples were collected from the diseased calves before and after treatment with tulathromycin (Draxxin-Pfizer), as well as from the healthy calves in order to determine retinol ($\mu\text{g mL}^{-1}$), α -tocopherol ($\mu\text{g dL}^{-1}$) and vitamin D₃ ($\mu\text{g dL}^{-1}$) levels by High Performance Liquid Chromatography (HPLC). Compared to the control group, there were increase of both retinol and α -tocopherol levels in the diseased animals and the increase in retinol levels were significant ($p<0.001$). Vitamin D₃ levels weren't affected in the diseased animals ($p>0.05$). Abnormal rectal temperatures, respiratory rates and pulsation levels decreased significantly ($p>0.001$).

Key words: Pneumonia, vitamin levels, calf, tulathromycin, montofon calves

INTRODUCTION

Calf pneumonia is a multifactorial respiratory infection that frequently occurs in young calves (Peters, 1986). Pneumonia is caused by a range of pathogens and many pathological conditions such as warm, wet weather, poor ventilation, stress, mixed age groups and high stocking densities (Roy, 1990; Tjornehoj *et al.*, 2003; Caswell and Archambault, 2007). Low vitamin A levels may enable bacterial adherence and colonization, thereby increasing the risk of bacterial infections, including pneumonia (Coles *et al.*, 2005). In young calves, vitamin A deficiency symptoms usually begin with watery eyes, along with mild symptoms of respiratory problems such as nasal discharge often accompanied by a cough. If this condition is allowed to persist, pneumonia usually follows (Haris, 2009). Another experiment at the Michigan station found that young calves invariably died of pneumonia before reaching 3 months-age when placed on a vitamin A-deficient ration (Jacobson *et al.*, 1949). Vitamin A, D and E supplementation has beneficial impacts on the incidence of clinically-defined common cold and pneumonia (Massaro and Massaro, 1996; Hemila and Kaprio, 2008; Haris, 2009).

Calf pneumonia represents a complex and economically damaging disease and is the leading cause of calf morbidity and mortality in Turkey and around the world. Therefore, the early treatment of pneumonia is very important in calf barns (Ozen *et al.*, 2009). Changes such as vaccination, housing conditions and alteration in calf management strategies may be instituted in order to reduce the impact of calf pneumonia on the farm. Veterinary involvement, such as antibiotics, should be applied towards the effective management of an outbreak, coupled with investigations to enable long term strategies to prevent future outbreaks (Potter, 2007).

Currently, many new antimicrobial agents have been introduced for pneumonia therapy. Tulathromycin is a member of the triamilide subclass of macrolide antibiotics and is a semi-synthetic macrolide. The common dose is a single dose of 2.5 mg kg^{-1} by subcutaneous injection to calves (CVMP, 2002). An antibiotic treatment, which could be administered as a single dose injection, would offer numerous advantages for veterinary management. Tulathromycin was more efficacious in the treatment of pneumonia in calves compared with other antibiotics in recent years (Godinho *et al.*, 2005a, b; Nutsch *et al.*, 2005; Skogerboe *et al.*, 2005; Robb *et al.*, 2007).

Calf pneumonia has long been recognized as a widespread problem leading to production loss through a reduced growth rate and a predisposition to pleurisy and leads to substantial economic losses in Turkey as well as globally (Hasoksuz *et al.*, 2002; Ozen *et al.*, 2009). Thus, this study was designed to determine the effects of single dose 2.5 mg kg⁻¹ subcutaneously tulathromycin on plasma levels of retinol, α -tocopherol, vitamin D₃ and some clinical symptoms both before and after treatment of Draxxin in Montofon calves with pneumonia.

MATERIALS AND METHODS

This was a prospective study that included 30 Montofon calves with pneumonia between 2 and 3 months of age and 20 clinically healthy calves of similar age. All animals were from Van district, Turkey and were subjected to similar management conditions.

A complete physical examination was performed on each animal. The diagnosis of pneumonia was established based on clinical symptoms. Diseased animals showed some or all clinical signs of pneumonia, including fever, cough, dullness, an increase in pulsation and respiration rates, lack of appetite, nasal flaring, signs of auscultation, mandibular lymph node enlargement and respiratory difficulties.

Venous blood samples from the jugular vein were collected from both the control and infected groups by jugular venipuncture before treatment (0 days) and one week after treatment (7 days) with subcutaneous (sc) injections of tulathromycin (Draxxin-Pfizer) at a single dose of 2.5 mg kg⁻¹. Sera were separated and stored at -20°C, until required for testing blood samples collected from all of the calves. Some calves were given a second dose if they continued to show moderate or severe clinical signs of abnormal temperature, increased respiratory rate, increased pulsation and depression after 72 h. After and before Draxxin injection, retinol ($\mu\text{g mL}^{-1}$), α -tocopherol ($\mu\text{g dL}^{-1}$) and vitamin D₃ ($\mu\text{g dL}^{-1}$) were measured using the High Performance Liquid Chromatography (HPLC, Agilent-1100, Germany) method (Tanritanir *et al.*, 2009). Statistical analysis was performed using the SPSS statistical program. Values were expressed as mean

standard error. The Duncan-ANOVA test was used to compare the parameters between the groups. The significant level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Generally, the symptoms observed in the clinically examined calves with pneumonia were fever, cough, dullness, increased pulse and respiration, lack of appetite, mandibular lymph node enlargement, nasal flaring, conjunctival hyperemia, signs of auscultation and difficulty in respiration. After treatment with tulathromycin, none of these clinical signs were observed in the calves with pneumonia. The clinical efficacy of tulathromycin was evaluated both before and after the injections were administered in diseased calves.

Diseased calves that were treated with tulathromycin (Draxxin-Pfizer) were observed 8 h after treatment. As noted in the results, no adverse clinical reactions were observed in any of the animals treated with tulathromycin.

Rectal temperature, respiratory rates and pulsation had reduced significantly ($p < 0.001$). Only three calves needed to receive a second injection of the treatment for persistent symptoms.

One week after treatment with Draxxin, retinol levels were significantly altered. Furthermore, retinol levels of the diseased animals increased to be statistically the same as the retinol levels in the healthy animals. The study's results, including standard deviation and differences in values of parameters obtained in this research, are summarized in Table 1.

Pneumonia is a significant respiratory disease, marked by the filling up of the lungs' alveoli with edema and exudative materials. The precise factors that cause pneumonia are not yet fully known. What is known is that it appears as a result of a complicated interaction between primary factors of the respiratory system and dispositional factors affecting the immunity of the host, consequently causing bacteria to grow in the lower respiratory tract (Giles *et al.*, 1991). Globally, the susceptibility of calves to pneumonia is higher during the first 1-3 months of age than at any other time. Instances of pneumonia vary based on the conditions where, the

Table 1: Antioxidant vitamin levels of 2-3 months old montofon calves with pneumonia that were treated with Draxxin (Tulathromycin)

Parameters	Control	Before draxxin treatment	After draxxin treatment	p-value
Retinol ($\mu\text{g mL}^{-1}$)	0.412±0.105	0.168±0.010	0.375±0.017	<0.001
α -Tocopherol ($\mu\text{g dL}^{-1}$)	2.083±0.026	1.968±0.036	2.043±0.083	>0.05
Vitamin D ₃ ($\mu\text{g dL}^{-1}$)	0.0294±0.0077	0.0293±0.0037	0.0242±0.0019	>0.05
Body temperature (°C)	38.51±0.16	40.16±0.51	38.54±0.32	<0.001
Respiratory rate	23.68±5.22	45.56±8.67	22.53±3.21	<0.001
Pulsation	78.89±4.95	116.78±12.26	80.74±5.43	<0.001

Values are expressed as means±SD. The significant differences between healthy and diseased animals are set at $p < 0.05$

calves are held. Housing systems, feeding, climate and management are some examples of the factors that influence calves' susceptibility (Meglia *et al.*, 2001).

Early detection and treatment of low respiratory track disease is challenging (Poulsen and McGuirk, 2009). Antimicrobial treatment is the most effective method for the prevention and management of pneumonia. Tulathromycin (Draxxin-Pfizer) was approved for the treatment of pneumonia and for the reduction of morbidity in calves in Turkey and around the world (Skogerboe *et al.*, 2005; Robb *et al.*, 2007; Schunicht *et al.*, 2007; Icen *et al.*, 2009).

The nutritional status of the animals has been associated with etiology, aggravation of infections and the ability to resist infections (Coles *et al.*, 2005). Calves suffering from pneumonia displayed a reduction in serum concentrations of vitamins A, D and E. This situation requires deeper scrutiny, owing to the fact that pneumonia is responsible for considerable economic losses for calf farmers (Meglia *et al.*, 2001; Maas, 2008; Haris, 2009).

Micronutrients are very important for modulate in altering the cause and outcome of pneumonia. Poor nutritional status or selective nutrient deficiencies like retinol, α -tocopherol and vitamin D₃ have been shown to suppress several facets of immune response to pneumonia (Jacobson *et al.*, 1949; Cannell *et al.*, 2006; Hemila and Kaprio, 2008). Several studies on the diagnosis, treatment and prognosis of respiratory system diseases in calves have documented low serum levels of these vitamins during acute infections (Blood and Studdert, 1989; Hammell *et al.*, 2000). Vitamin A deficiency especially interferes with immunity and with the respiratory epithelium. Vitamin A deficiency may lead to squamous metaplasia with subsequent loss of defense mechanisms against microbial invasion and may cause the development of obstructive pneumonia caused by bronchial hyper responsiveness (Biesalski and Nohr, 2003; Cannell *et al.*, 2006). Vitamin D₃ and α -tocopherol deficiency are also, associated with pneumonia in calves and thus, vitamin supplements would give them more resistance to the ill effects caused by the stress factors on the immune system (Cannell *et al.*, 2006; Haris, 2009).

In this study, both retinol and α -tocopherol levels in infected calves increased, but only retinol levels increased significantly after the treatment of a single dose of Draxxin. There is statistically significant association between the deficiency of serum retinol levels and recovery, as demonstrated by many studies similar to ours (Nonnecke *et al.*, 2000; Thurnham *et al.*, 2003). The level

of vitamin D₃ in infected calves wasn't affected by the treatment of tulathromycin. Plasma levels of vitamin D₃ in the treated animals were similar to levels in the control group.

After treatment with a new tulathromycin drug (Draxxin-Pfizer), none of the clinical signs of pneumonia were observed in the infected calves (Icen *et al.*, 2009). Only three calves needed a second antimicrobial injection. Rectal temperature, respiratory rates and pulsation levels in the infected calves decreased to be statistically the same as the healthy animals ($p < 0.001$). These findings are in accordance with previous studies (Godinho *et al.*, 2005a, b; Icen *et al.*, 2009). This study is similar to Schunicht *et al.* (2007) study of calves that were treated with tulathromycin where there were also no observed side effects in any of the calves.

The results of this study indicate that it is effective to use a single dose of 2.5 mg kg⁻¹ s.c. tulathromycin in calves and that this dose altered their antioxidant vitamin status. All of the diseased calves were cured with a single dose of tulathromycin and only 3 calves needed a second injection. Additional studies are necessary to more accurately characterize the relative efficacy of tulathromycin compared to other commonly used antimicrobials for the treatment of pneumonia in calves.

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