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EVALUATION OF TILMICOSIN AS A TREATMENT FOR PNEUMONIA CAUSED BY *PASTEURELLA MULTOCIDA* IN EWES

By

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ABSTRACT

Tilmicosin is a semi-synthetic antibiotic used for treatment of respiratory infections in cattle, however, a little is known about the therapeutic use of the drug in sheep. In the present study, fifteen non-pregnant ewes, 2-3 years old with average body weight 40-50 kg. from a private farm at kafr-abohesen, Sharkia province were suffering from broncho-pneumonia caused by *pasteurella multocida* infection. The diseased ewes were divided into three equal groups, each of 5 animals, and treated with a single subcutaneous dose of tilmicosin at a dose level of 10, 20 and 30 mg/kg. B.wt-respectively. A fourth group of ten apparently healthy, free from *Pasteurella multocida* infection, non-pregnant ewes of about the same age and weight was served as a control. One week post-treatment with different doses of Tilmicosin, no *Pasteurella multocida* could be detected in the blood of treated ewes, in addition, the drug was effective in control of broncho-pneumonia and the ewes regained its signs of normal health conditions, however, the third group which treated with 30 mg/kg. B.wt. was depressed, move slowly with reduced appetite and tachycardia. The study revealed that the drug at 10 and 20 mg/kg. B.Wt had no adverse effects on haemoglobin concentration, erythrocytic count, total leucocytic count, platlets and some liver and kidney functions. The drug at a dose level of 30 mg/ kg. B. wt. resulted in significant increase of serum alkaline phosphatase and lactic dehydrogenase coupled with decreased haemoglobin percentage and platlets. It could be concluded that Tilmicosin at a subcutaneouse dose less than 20 mg/kg. B. wt. Was effective for control of pneumonia caused by *Pasteruella multocida* in ewes with no adverse effects on blood picture and liver and kidney functions.

INTRODUCTION

Tilmicosin is a novel semi-synthetic macrolide antibiotic, developed specifically for veterinary use (**Bakirel *et al.*, 2000**). It has a good efficacy

against *Pasteurella* species, *Mycoplasma* species and Gram-positive microorganisms (**Walters *et al.*, 1994**). Several studies have established the outstanding clinical efficacy of Tilmicosin in treatment of bovine pneumonia caused by *Pasteurella* species (**Gorham *et al.*, 1990; Schumann *et al.*, 1990; Laven and Andrews, 1991 and Chin *et al.*, 1998**). In addition, **Vogel et al. (1998)** reported the effectiveness of Tilmicosin in decreasing the affection by acute undifferentiated bovine respiratory disease (BRD) and improvement of calves growth.

Pharmacokinetics of Tilmicosin in serum and milk of sheep and goats have been investigated by **Parker *et al.*, (1994 a & b)** and **Ramadan, (1997)**. However, a little is known about the therapeutic use of the drug in sheep. **Watson, (1999)** concluded that single injection of Tilmicosin at a dose level of 10 mg/ kg. B. wt. Was an effective treatment for lambs suffering from severe lameness due to partial or complete loss of the overlying horn from one or both claws of the limb. Respiratory affection are a common problem of sheep and responsible for severe economic losses and mortalities (**Lotfy *et al.*, 1977** and **Gilmour and Angus, 1983**). *Pasteurella* species are the main causative agents of respiratory infections and the close contact of sheep facilitates the spread of infection among the flock (**Callan *et al.*, 1991** and **Sadiek *et al.*, 1993**). The present study was directed to evaluate the efficacy of Tilmicosin in treating respiratory affections of ewes and its effect on blood picture and some serum biochemical values.

MATERIAL AND METHODS

The drug:

Micotil ® injection (Tilmicosin) from Elanco Animal Health, England, was used. Each ml contains 300 mg Tilmicosin.

Methods:

This study was conducted on a private sheep farm [illegible]afr-Abohesen, Sharkia province that contains about 200 animals. It was [illegible]ced that some animals were suffered from acute peumonia in the form of rapid breathings, moist rales, congested mucous membranes, mucopurulent nasal discharges, severe dyspnea and pyrexia. All animals wee fed on barseem and dry ration and water was supplied ad libitum. Fifteen diseased non pregnant ewes, 2-3 years old with average body weight 40-50 kg were isolated from the farm, all were suffering from bronchopneumonia with pyrexia (rectal temp. > 40 °C). The diagnosis was carried out by clinical signs and/or auscultation confirming sound. The diseased ewes were divided into three equal groups and treated with a single subcutaneous dose of Tilmicosin at a dose level of 10, 20 or 30 mg/kg. B. Wt. Respectively (**Modric *et al.*, 1998**). A fourth group of ten clinically healthy non-pregnant ewes, free from *Pasteurella*

multocida infection, of about the same age and weight was served as a control.

A) Bacteriological examination:

Blood and nasal swabs from the four groups of ewes were collected under aseptic conditions before treatment directly and one week post treatment for bacteriological examinations.

As quickly as possible, the collected nasal swabs were suspended in phosphate buffer saline (pH 7.2) and were then platted on 5% sheep blood agar plates while the blood samples were cultured directly on the same media. The inoculated plates were incubated aerobically at 37 °C for 48 hrs. Bacterial isolates were identified biochemically according to the methods described by **Carter, (1986)**. Isolated *Pasteurella* strains were subjected to mice inoculation tests on the lines of **Carter, (1986)**.

B) Blood and serum samples:

Two blood samples from the jugular vein before treatment directly and one week post-treatment were collected from each animal in all groups, the first sample was taken on EDTA for haematological studies and the second sample was collected without anticoagulant for separation of serum. Collected blood. Serum was used for determination of serum total proteins (**Doumas, 1975**), serum albumin (**Doumas *et al.*, 1971**) and globulins were calculated. Serum was also used for estimation of alanine aminotransferase (ALT) according to **Reitman and Frankel, (1957)**, creatinine (**Husdan and Rapoport, 1968**) urea (**Tabacco, 1979**) and LDH (**Kachmar and Moss, 1976**). Erythrocytes (RBCs) and platlets were counted according to **Wintrobe, (1967)**. Haenmoglobin (Hb) and total and differential leucocytic cells counts were made according to **Coles, (1986)**.

C- Statistical analysis:

The obtained data were analysed using analysis of variance (ANOVA) according to **Snedecor and Cochran, (1980)**.

RESULTS

Before treatment with Tilmicosin, *Pasteurella multocida* was isolated from blood and nasal swabs of all the diseased three groups on blood agar media and biochemically identified. All inoculated mice were died within twelve hours. Healthy ewes (4^{th} group) were free from *Pasteurella multocida*. One week post-treatment with different doses of Tilmicosin, no *Pasteurella multocida* could be detected in the blood of treated ewes. Moreover, the 1^{st} and 2^{nd} groups showed an improvement in the general health conditions, normal body temperature, absence of nasal discharges and respiration

regained its normal rate. In the third group, signs of bronchopneumonia were subsided one week post-treatment, however, the general health condition was not the same as the ewes were depressed, move slowly with reduced appetite and tachycardia.

In ewes infected with *Pasteurella multocida* a significant ($p<0.05$) increase of total leucocytic count, neutrophils, eosinophils, serum globulins, alkaline phosphatase and urea associated with decreased Hb%, RBCS count, lymphocytes, platlets, serum albumin and ALT were recorded (Table 1 and 2).

Administration of Tilmicosin at a dose level of 10 or 20 mg/ kg. B.Wt. resulted in an improvement of most altered haematological parameters and some liver and kidney functions in diseased ewes toward the normal levels when compared with the same parameters before treatment (Table, 1 and 2). On contrary, the drug at a dose level of 30 mg/kg B.wt. failed to correct the disturbed parameters in diseased ewes (group 3) beside a significant ($p<0.05$) decrease of platlets and eosinophils coupled with a significant ($p<0.05$) increase of serum LDH and alkaline phosphatase were recorded in treated ewes (Table 1 and 2)

DISCUSSION

Tilmicosin, a novel macrolide antibiotic, is approved for the control of respiratory diseases caused by *Pasteurella haemolytica* in cattle (**Ose and Tonkinson, 1988** and **Vogel *et al.*, 1998).**

In the present study, *Pasteurella multocida* was isolated from ewes showing clinical signs of pneumonia.

In several previous investigations *Pasteurella multocida* was incriminated as the causative agent responsible for respiratory disorders in sheep (**Lotfy *et al.*, 1977; Morad *et al.*, 1980; Callan *et al.*, 1991; Sadiek *et al.*, 1993 and Eman and Suzan, 2001**). The present work revealed that diseased ewes showed a significant ($P< 0.05$) increase in total leucocytic count, neutrophils and eosinophils associated with a significant ($P< 0.05$) decrease in erythrocytic count, lymphocytes and platlets. Similar findings were reported by **Sadiek *et al.*, (1993)** who detected a significant increase in the total leucocytic count and neutrophils with decreased lymphocytes in pneumonic sheep. The recorded changes in the haematological parameters could be attributed to the bacterial infection and the inflammatory reactions (**Bryson *et al.*, 1979; Coles, 1986 and Sadiek *et al.*, 1993**). In the diseased ewes, serum albumin level was decreased, while serum globulins, alkaline phosphatase and urea levels were increased. These results were partly in agreement with that obtained by **Sadiek *et al.*, (1993)**. The authors reported a significant decrease in serum albumin coupled with a significant increase of serum globulins in sheep suffered from pneumonia. The decreased albumin level might be attributed to the destructive effect of bacteria and bacterial

toxins on the liver cells producing impaired synthesis of serum albumin (**Mottelib, 1972**). On the other hand, the increased levels of globulins could be explained on the base of immune response antibodies (**Affonso *et al.*, 1960**). Subcutaneous injection of Tilmicosin at 10, 20 or 30 mg/kg. B.Wt. was effective in the treatment of pneumonia caused by *Pasteurella multocida* in ewes as manifested by absence of clinical signs of pneumonia in treated sheep and the improvement of general health condition except in the 3rd group which treated by 30 mg/kg.B.Wt.; the ewes were depressed, move slowly with reduced appetite and tachycardia denoting a hazard effect was evoked by the drug. The success of treatment with Tilmicosin had been attributed to its pharmaco-therapeutic effect in appropriate tissues at low concentrations (**Schumann *et al.*, 1990; Hartman and Geryl, 1993** and **Morck *et al.*, 1997**). Moreover, previous studies suggested that some macrolides may have anti-inflammatory properteries by modulating functions of inflammatory cells such as polymorph onuclear leukocytes (PMN), macrophages and lymphocytes beside affecting functions of airway secretory cells and epithelial cells (**Umeki, 1993** and **Tamaoki *et al.*, 1994**). This concept was supported by **Chin *et al.*, (1998)** who referred to the anti-inflammatory benefit of Tilmicosin in calves infected with *Pasteurella haemolytica*.

Regarding the effect of different doses of Tilmicosin on the blood picture, it was noticed that the alterd haematological parameters in diseased treated ewes were improved toward the normal levels when compared with the infected non treated ewes. This effect was attributed to the improvement of general health condition of treated ewes with 10 or 20 mg/kg. B.Wt. of the drug and control of the infection. However, the drug at a dose level of 30 mg/kg.B.Wt. resulted in a significant ($P< 0.5$) decrease of haemoglobin percentage, platlets count associated with a significant ($P< 0.05$) increase of serum Alkaline phosphatase and lactic dehydrogenase levels. Based on this finding and the observed clinical signs, Tilmicosin at a high dose level might had a cardiotoxic effect. This suggestion was supported with the increased level of LDH in this study. In addition, **Ramadan, (1997)** observed that the subcutaneous injection of the drug at a dose level of 10 mg/kg. B.Wt. had no cardiac adverse effect in goats. Moreover, **Modric *et al.*, (1998)** mentioned that Tilmicosin can be used safely and effectively in sheep at a subcutaneous dose of 10 mg/kg.B.Wt. with no adverse cardiopulmonary effects. The obtained data in the present work supported the fore mentioned authors where the drug at 10 and 20 mg/kg/B.Wt. had no adverse effects on some liver and kidney functions and blood picture of treated infected ewes, however, the drug at a dose level of 30 mg/kg.B.Wt. had a success in control of *Pasteurella multocida* infection but it failed to correct the disturbed parameters in diseased ewes beside the adverse effects on the levels of serum alkaline phosphatase and lactic dehydrogenase.

In conclusion, Tilmicosin was an effective antibiotic for the treatment of pneumonia caused by *Pasteurlla multocida* in non pregnant ewes. However, the subcutaneous dose of the drug must not exceed 20 mg/kg.B.Wt.

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Table (1): Effect of single subcutaneous different doses (10, 20 and 30 mg/kg. B. Wt.) of Tilmicosin on haematological parameters of pneumonic ewes.

Parameter \ group	Healthy, non treated (n=10)	Diseased and treated with:					
		10 mg/kg. B. Wt. (n=5)		20 mg/kg. B. Wt. (n=5)		30 mg/kg. B. Wt. (n=5)	
		B	A	B	A	B	A
Haemoglobin (gm/dl)	a 9.9 ± 0.22	bc 9.1 ± 0.24	a 10.08 ± 0.17	b 9.28 ± 0.14	ab 9.6 ± 0.18	bc 9.11 ± 0.17	c 8.6 ± 0.19
RBCs (10^6/ul)	a 9.856 ± 0.13	cd 3.29 ± 0.12	b 3.82 ± 0.16	bc 3.518 ± 0.11	b 3.62 ± 0.083	cd 3.34 ± 0.024	d 3.26 ± 0.014
Total leucocytes (10^3/ul)	b 8.527 ± 0.23	a 11.14 ± 0.15	b 8.11 ± 0.11	a 11.328 ± 0.12	b 8.448 ± 0.10	a 11.148 ± 0.061	b 8.25 ± 0.151
Neutrophils (10^3/ul)	e 3.146 ± 0.11	a 7.375 ± 0.14	b 4.222 ± 0.13	a 7.669 ± 0.16	d 4.824 ± 0.12	d 7.261 ± 0.15	c 5.264 ± 0.13
Lyphocytes (10^3/ul)	a 4.843 ± 0.09	cd 2.816 ± 0.12	b 3.255 ± 0.10	cd 2.730 ± 0.11	bc 3.117 ± 0.13	bc 2.988 ± 0.10	d 2.514 ± 0.09
Monocytes (10^3/ul)	bc 0.307 ± 0.018	ab 0.343 ± 0.022	ab 0.340 ± 0.015	ab 0.346 ± 0.014	c 0.287 ± 0.019	a 0.392 ± 0.021	c 0.285 ± 0.017
Eosinophils (10^3/ul)	bc 0.230 ± 0.008	a 0.534 ± 0.012	b 0.279 ± 0.021	a 0.527 ± 0.035	cd 0.219 ± 0.013	a 0.508 ± 0.029	d 0.188 ± 0.016
Platlets (10^3/ul)	a 388.2 ± 16.3	b 334.2 ± 15.09	a 369.2 ± 8.67	b 327 4 ± 11.48	a 383.4 ± 8.41	b 318.14 ± 9.66	c 270 ± 11.4

Different letters in the same column indicate significant changes ($p < 0.05$)

A: After treatment B: Before treatment

Table (2): Effect of single subcutaneous different doses (10, 20 and 30 mg/kg. B. Wt.) of Tilmicosin on some serum biochemical values of pneumonic ewes.

Group / Parameter	Healthy, non treated (n=10)	Diseased and treated with:					
		10 mg/kg. B. Wt. (n=5)		20 mg/kg. B. Wt. (n=5)		30 mg/kg. B. Wt. (n=5)	
		B	A	B	A	B	A
Total proteins (gm/dl)	a 7.09 ± 0.06	a 6.96 ± 0.12	a 7.1 ± 0.10	a 7.02 ± 0.10	a 6.92 ± 0.11	a 6.88 ± 0.08	a 6.83 ± 0.093
Albumin (gm/dl)	a 3.8 ± 0.12	b 3 ± 0.21	a 3.63 ± 0.18	b 2.95 ± 0.16	a 3.52 ± 0.11	b 3.02 ± 0.23	b 3.2 ± 0.14
Globulins (gm/dl)	b 4.26 ± 0.13	a 4.09 ± 0.10	a 4.06 ± 0.13	a 4.08 ± 0.09	a 4.56 ± 0.21	a 4.82 ± 0.11	a 4.63 ± 0.18
A/G ratio	a 0.89 ± 0.03	c 0.61 ± 0.02	b 0.79 ± 0.03	c 0.61 ± 0.03	b 0.77 ± 0.03	c 0.63 ± 0.02	bc 0.69 ± 0.03
Alkaline phosphatase (kau/dl)	c 16.4 ± 0.53	b 22.5 ± 1.2	c 19.2 ± 0.92	bc 21.8 ± 1.7	cd 18.3 ± 0.77	bc 20.4 ± 1.4	a 27.1 ± 2.02
AL.T (u/L)	a 39.2 ± 2.62	bc 24 ± 1.99	bc 22 ± 1.65	b 27 ± 2.01	bc 23.1 ± 1.18	bc 23.9 ± 1.45	c 19.2 ± 1.73
LDH Iu/L	b 395 ± 23.5	b 408 ± 31.4	b 421 ± 27.3	b 416 ± 29.8	b 432 ± 16.7	b 399 ± 22.3	a 486 ± 18.4
Creatinine (mg/dl)	a 2.47 ± 0.08	ab 2.35 ± 0.06	ab 2.44 ± 0.02	a 2.52 ± 0.06	ab 2.37 ± 0.11	a 2.49 ± 0.16	b 2.28 ± 0.09
Urea (mg/dl)	bc 26 . ± 2.1	a 44 ± 3.2	b 31 ± 2.82	a 49 ± 2.91	bc 24 ± 1.96	a 43 ± 3.11	c 22 ± 1.72

Different letters in the same column indicate significant changes ($p < 0.05$)

B: Before treatment A: After treatment

الملخص العربي

تقييم التلميكوزين كعلاج للالتهاب الرئوي الذي تسببه الباستريلا مالتوسيدا في النعاج

رفعت خضرى محمد أسامة السعيد عبد الله

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التلميكوزين مضاد حيوي نصف مخلق يستعمل لعلاج الإصابات التنفسية في الماشية إلا أنه يتوفر القليل عن استعمالاته العلاجية في الأغنام.

في هذه الدراسة تم استخدام ٢٥ نعجة غير حامل بمزرعة خاصة في كفر أبو حسين محافظة الشرقية ويبلغ متوسط أوزانها ٤٠ إلى ٥٠ كجم للرأس وكانت خمسة عشر نعجة تعانى من التهابات رئوية سببتها العدوى بميكروب الباستريلا مالتوسيدا.

تم تقسيم النعاج المريضة إلى ثلاث مجموعات متساوية كلا منها يضم ٥ حيوانات وعولجت بجرعة واحدة تحت الجلد من التلميكوزين تحتوى على ١٠ و ٢٠ و ٣٠ مجم/ كجم وزن حي من الدواء على الترتيب كما تم تخصيص مجموعة رابعة تحتوى على عشر من النعاج السليمة وفى نفس العمر والوزن وخالية من ميكروب الباستريلا مالتوسيدا كمجموعة ضابطة.

بعد أسبوع من العلاج لم يتم عزل الميكروب من الدم واختفت علامات الالتهاب الرئوي واستعادت النعاج علامات الصحة إلا أن المجموعة الثالثة المعالجة بجرعة ٣٠ مج /كجم وزن حي كانت غير طبيعية وتتحرك ببطء مع فقدان الشهية وزيادة ضربات القلب. أثبتت الدراسة أن العقار عند استعماله بجرعات أقل من ٢٠مجم / كجم وزن حي ليس له آثار غير مرغوبة على تركيز الهيموجلوبين وعدد كرات الدم الحمراء والعد الكلى لكرات الدم البيضاء والصفائح الدموية وبعض وظائف الكبد والكلى بينما نتج عن استعمال الدواء بجرعة ٣٠مجم/كجم وزن حي زيادة معنوية فى مستوى أنزيم الفوسفاتيز القلوي واللاكتيك دى هيدروجنيز في مصل النعاج المعالجة مع نقص نسبة الهيموجلوبين والصفائح الدموية.

تشير هذه الدراسة إلى إمكانية استعمال التلميوكوزين بجرعة أقل من ٢٠ مجم / كجم وزن حي لعلاج الالتهابات الرئوية التي تسببها الباستريلا مالتوسيدا في النعاج بالإضافة إلى أن استعمال الدواء عند هذه الجرعة ليس له آثار غير مرغوبة على صورة الدم وبعض وظائف الكبد والكلى في هذه النعاج. إلا أنه يجب عدم تجاوز الجرعة الموصى بها نظراً للتأثير الضار الذي تحدثه الجرعة العالية .