STUDIES ON THE EFFICACY OF CEFTIOFUR SODIUM ON MANNHEIMIA "PASTEURELLA" HAEMOLYTICA INFECTION IN CALVES

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ABSTRACT

The present work was conducted to study the effect of ceftiofur sodium on the healthy and naturally Mannheimia haemolytica infected calves with the study the effect of the drug on blood picture and some parameters of liver and kidney functions. Fifteen calves from private farm in Facous City, Sharkia Governorate were examined for isolation of Mannheimia haemolytica. The result revealed that five calves (33.33%) were infected and showed respiratory signs with rise of body temperature as well as there were a significant increase in the level of AST, ALT and creatinine compared with the control group. Five of non-infected healthy calves as well as infected calves were treated with ceftiofur sodium (1 mg/kg b.wt.) intramuscularly injected for 5 successive days, whereas another five non infected calves were used as control. Treatment with ceftiofur sodium reduced the clinical signs, moreover the infected calves on the first week post-treatment with ceftiofur sodium showed a significant decrease in the level of AST, ALT and creatinine ($P \leq 0.05$) compared with those before treatment. It has been shown that ceftiofur sodium displayed a significant increase in RBCs counts, Hb concentration, PCV% with no response on WBCs counts, AST, ALT and serum creatinine in the non infected treated calves, compared with control calves. It could be concluded that ceftiofur sodium is considered as better treatment for respiratory diseases.

Key words: Ceftiofur sodium - Mannheimia "Pasteurella" haemolytica - RBCs counts -PCV%-WBCs- AST - ALT - Creatinine.

INTRODUCTION

Mannheimia haemolytica is the primary aetiological agent of pneumonic pasteurellosis, one of the most important respiratory diseases in cattle **(Ewers et al., 2004)**.

The infection of calves with Mannheimia haemolytica characterized by rise of body tempera-

ture. increase nasal discharge which changes in consistency from thin and clear to thick yellow and viscous. with breathing often rapid and labored. A cough may be noted early in the disease: however. as lung damage increases. coughing and breathing become very painful for the animal. If the disease process is not stopped. the lungs become irreversibly damaged. the body temperature drops to below normal and the animal usually dies **(Whiteley et al., 1992)**.

Ceftiofur sodium. a member of cephalosporins has been marketed for use in poultry and large animals. It is recommended for use in prevention of early chick mortality due to pasteurellosis. colibacillosis, salmonellosis and streptococcosis **(Schriemer et al., 1992)**. **Imaz et al., (1991)** compared the efficacy of ceftiofur sodium (1mg/kg b.wt.) for 3 days with oxytetracycline. amoxicillin and a combination of oxytetracycline and chloramphenicol in the treatment of 48 cases of respiratory diseases. They reported that calves treated with ceftiofur recovered earlier than calves treated with other antibiotics.

Anusz et al., (1992) found that ceftiofur injected daily at 1mg/kg. b.wt. was effective in treatment of respiratory disease in 38 naturally infected calves and 9 calves experimentally infected with Herpes virus and Mannheimia haemolytica. In most cases 3 injections were sufficient. although some animals required 4 to 5 injections to recover. Therapeutic effects of ceftiofur were compared with those of oxytetracycline used in 8 control calves. They concluded that both antibiotics are recommended in treatment of respiratory diseases of calves.

The antimicrobial activity of ceftiofur sodium as anti-infective agent belonged to the third generation cephalosporins against different bacterial pathogens were evaluated. The obtained results revealed that ceftiofur was more effective and superior in its action than the other compared antibacterial agent **(El-Naenaeey and Lotfy, 2000)**.

The aim of this study is to evaluate in-vitro and in-vivo the antibacterial efficacy of ceftiofur sodium, given intramuscularly at therapeutic regimen (1mg/kg b.wt) for 5 successive days against naturally Mannheimia haemolytica infected calves as well as in the healthy calves.

MATERIAL AND METHODS

MATERIALS :

(I) Animals

Fifteen calves from private farm in Facous City, Sharkia Governorate were used in this study. The animals in this farm were periodically vaccinated and prophylactically administered by anthelmintics drugs. They suffered from respiratory signs especially in calves. They were examined clinically according to **Radatits et al., (2007)**.

(II) Samples:

A total of 15 paired nasal and transtracheal swabs were collected and transferred directly to laboratory in ice box with minimum delay. Moreover, two blood samples were taken from all calves by jugular vein puncture as well as from the treated groups on the 7th day post-treatment for haematological studies, liver and kidney function tests. First sample was mixed with sodium citrate 3.8% (9:1) used for haematological studies and the second one was left to clot and centrifuged for 3000 r.p.m./20 minutes to obtain serum for liver and kidney function studies.

(III) Drugs "Ceftiofur sodium":

It is a cephalosporin from the third generation, obtained in the form of dry, lyophilized powder (Upjohn Co. Kalamazoo, U.S.A.). It is used intramuscularly in a dose of 1mg/kg b.wt. for 5 successive days (**Alms, 1988**).

METHODS:

(I) Bacteriological examination:

Nasal and transtracheal swabs were inoculated separately in tryptone soya broth (CM 13 "Oxoid") and incubated at 37°C over night. Loopfulls from incubated broth were cultured onto blood agar and incubated at 37°C for 24 hours. Isolated organisms were purified and identified microscopically using methylene blue stain and biochemically according to **Carter and Cole, (1991)**.

(II) Antibacterial activity in vitro:

1) Determination of minimum inhibitory concentration (MIC):

Determination of MIC of ceftiofur sodium against pathogenic strain of Mannheimia haemolytica was tested according to **Fingold and Martin (1982)**.

2) Sensitivity test:

Sensitivity test of Mannheimia haemolytica to ceftiofur sodium in comparison with other antimicrobial agents was studied in vitro using the disc diffusion method as described by **Bauer et al., (1966)**.

(III) Antibacterial activity in vivo:

The efficacy of ceftiofur sodium against Mannheimia haemolytica infection was studied. Fifteen calves were used in this study and classified as follows:

Group (1): Five calves were healthy, non-infected and non-treated (control group).

Group (2): Five calves were healthy, non infected and treated with ceftiofur sodium (1mg/kg. b.wt., for five days, intramuscularly).

Group (3): Five calves were naturally infected with Mannheimia haemolytica and treated with ceftiofur sodium (1mg/kg b.wt. five days, intramuscularly).

(IV) Haematological studies:

1) Blood cell count: Total erythrocytes and leucocytes were counted using the improved Neubauer chamber according to the method described by **Schalm, (1986)**.

2) Haemoglobin determination: was determined according to **Williams et al., (1983)**.

3) Packed cell volume (PCV): was determined using the microhaematocrit method according to **Coles, (1986)**.

(V) Biochemical studies:

1) Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT): were determined colorimetrically according to the method of **Reitman and Frankel, (1957)**.

2) Serum creatinine: was determined colorimetrically according to the method of **Folin (1934)**.

STATISTICAL ANALYSIS:

Statistical analyses of data were carried out by using "t" test according to Snedecor and **Cochran (1967)**.

RESULTS

(I) Isolation of Mannheimia haemolytica among examined calves:

From 15 calves, 5 (33.33%) were naturally infected with Mannheimia haemolytica.

(II) In vitro antibacterial activity:

(1) MIC of ceftiofur sodium against Mannheimia haemolytica:

The results showed that Mannheimia haemolytica was highly susceptible to ceftiofur sodium with MIC (0.10µg/ml).

(2) Sensitivity test:

The sensitivity of Mannheimia haemolytica to ceftiofur sodium, florphenicol, oxytetracycline and ciprofloxacin are illustrated in Table (1). Ceftiofur sodium exerted a more potent inhibitory effect compared with other tested antimicrobial agents.

(III) In vivo antibacterial activity:

Calves naturally infected with Mannheimia haemolytica displayed variable clinical symptoms as increase of body temperature (40-41°C), respiratory manifestations (painful cough and the breathing is rapid and difficult), diarrhea and loss of appetite. Treatment with ceftiofur sodium lead to improvement and disappearance of most clinical sings at 5 days post-treatment.

(IV) Haematological studies:

Non infected treated calves with ceftiofur sodium displayed a significant increase in RBCs counts, Hb concentration and PCV% with no response on WBCs counts ($P \leq 0.05$) compared with control group (Table 2). The infected calves before treatment showed a significant decrease in RBCs counts, Hb concentration and PCV% with significant increase in WBCs counts ($P \leq 0.05$) compared with the control group. Meanwhile, the infected calves pos-treatment with ceftiofur sodium displayed a significant increase in RBCs counts, Hb concentration, PCV% and WBCs counts ($P \leq 0.05$) compared with those before treatment.

(V) Biochemical studies:

Non-infected calves treated with ceftiofur sodium displayed a non significant response on the level of AST, ALT and serum creatinine ($P \leq 0.05$) on one week post treatment compared with control group. The group of calves which are naturally infected with Mannheimia haemolytica showed a significant increase in the levels of AST, ALT and creatinine ($P \leq 0.05$) compared with control group. Meanwhile, the group of infected calves on the first week post-treatment

with ceftiofur sodium showed a significant decrease in the level of AST, ALT and creatinine ($P \leq 0.05$) compared with those before treatment (Table 3).

DISCUSSION

It is clear from the present study that 5 (33.33%) calves were infected with Mannheimia haemolytica. Nearly similar results were recorded by **Ismail and El-Kattan, (1999) and DeRosa et al., (1999).**

In the present study, it has been shown that the naturally infected calves with Mannheimia haemolytica before treatment showed a significant decrease in RBCs counts, Hb concentration, PCV% with significant decrease in leucocytic counts, meanwhile, treatment with ceftiofur sodium adjust all the haematological parameters of infected calves. The results concordant with those recorded by **Abd El-Latif and Gamal El-Din, (1998)** who found that the treatment with ceftiofur sodium improved the adverse effects of Mannheimia haemolytica infection on haematological parameters as evidenced by improvement of macrocytic anaemia.

It has been shown that Mannheimia haemolytica infection in calves produced a significant increase the level of AST, ALT and creatinine. The increased serum AST, ALT and creatinine activity might be attributed to the muscular damage, liver damage and myocardial infarction **(Haines et al., 2004 and Radostits et al., 2007)**. A further support of previous concept is the fact that the main characteristic manifestation of hepatotoxicity is the increase in the serum AST and ALT levels consequent to degenerative changes in the cell membrane **(Pashor et al., 1987)**.

The results revealed that the infected calves on the first week post-treatment with ceftiofur sodium showed a significant decrease in the level of AST, ALT and creatinine ($P \leq 0.05$) compared with those before treatment. Our results was in agreement with that recorded by **Abdel-Latif and Gamal El-Din (1998)** who reported that ceftiofur sodium improved adverse effects of Mannheimia haemolytica infection on liver function evidenced by decrease in serum levels of AST and ALT.

Our results indicated that ceftiofur sodium injection improved all the effects of Mannheimia haemolytica clinically. These results agree with that obtained by **Imax et al., (1991)** who reported that calves treated with ceftiofur sodium recovered earlier than calves treated with other antibiotics.

It could be concluded that ceftiofur sodium is considered as better treatment of calves infected with Mannheimia haemolytica.

Table (1): Sensitivity of *Mannhelmia haemolytica* to ceftiofur sodium in comparison with some antibacterial agents (n = 5, Mean ± SE)

Antimicrobial agents discs	Potency of discs (µg)	Inhibition zone diameter (mm)
Ceftiofur sodium	25	31.5 ± 2.3
Florphenicol	30	25.6 ± 1.7
Oxytetracycline	30	15 ± 2.1
Ciprofloxacin	5	14.8 ± 1.5

Table (2): The effect of intramuscularly injected ceftiofur sodium (1.0mg/kg b.wt.) on RBCs counts (10^6/mm^3), Hb concentration (mg%), PCV (%) and WBCs counts (10^3/mm^3) of healthy and naturally infected calves with *Mannhelmia haemolytica*, one week post-treatment (n = 5, mean ± SE)

Parameters	Control group (non-infected non-treated)	Non infected treated	Infected calves	
			Before treatment	After treatment
RBCs counts	9.20 ± 0.64	12.95 ± 0.71*	8.31 ± 0.51*	11.92 ± 0.81**
Hb concentration	18.61 ± 0.54	21.61 ± 0.56*	19.74 ± 0.94*	20.61 ± 0.74**
PCV%	45.20 ± 1.82	50.92 ± 2.63*	40.60 ± 2.81*	43.6 ± 3.41**
WBCs counts	68.31 ± 3.22	67.31 ± 4.23	75.82 ± 3.51*	72.92 ± 4.12**

Significant when compared one parameter in the same row with control group (*P <0.05; **P <0.01)

Table (3): The effect of intramuscularly injected ceftiofur sodium (1.0mg/kg b.wt.) for 5 successive days on AST (U/ml), ALT (U/ml) and creatinine (mg/ml) of healthy and naturally infected calves with *Mannhelmia haemolytica* (n = 5, mean ± SE) .

Parameters	Control group	Non infected treated calves	Infected calves	
			Before treatment	After treatment
AST (U/ml)	64.81 ± 2.91	63.71 ± 3.92	90.92 ± 4.31*	66.71 ± 3.21**
ALT (U/ml)	45.92 ± 8.41	47.11 ± 2.63	82.81 ± 5.11*	48.93 ± 4.11**
Creatinine (mg/ml)	3.79 ± 0.24	4.62 ± 0.31	7.43 ± 0.66*	4.88 ± 0.11**

Significant when compared one parameter in the same row with control group (*P <0.05; **P <0.01)

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الملخص العربى

دراسات على كفاءة السفتيوفيور صوديوم على المانهيميا "الباستيريللا" هيموليتيكافى العجول

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أجرى الفحص البكتريولوجى على خمسة عشر عجلاً فى مزرعة عجول مصابة بمدينة فاقوس بمحافظة الشرقية لعزل ميكروب المانهيميا هيموليتيكا، تم عزل ميكروب المانهيميا هيموليتيكا من خمسة عجول بنسبة (٣٣,٣٣٪)، كما تم حقن العجول المصابة والعجول السليمة عضلياً بعقار السفتيوفيور صوديوم بجرعة (١مجم/كجم من الوزن) لمدة خمسة أيام متتالية وتركت خمسة عجول سليمة كمجموعة ضابطة "غير مصابة وغير معالجة".

ولقد وجد أن لدواء السفتيوفيور صوديوم تأثيراً فعالاً على ميكروب المانهيميا هيموليتيكا أقوى من عديد من المضادات الحيوية الأخرى وذلك من خلال قياس الحد الأدنى للكثافة المثبطة واختبار الحساسية.

وقد تمت الدراسة على أساس تأثير دواء السفتيوفيور صوديوم على الأعراض الظاهرية للمرض والتأثير على صورة الدم ووظائف الكبد والكلى وذلك بعد إيقاف الدواء بإسبوع واحد.

وقد وجد أن إصابة العجول بميكروب المانهيميا هيموليتيكا ينتج عن أعراض مميزة مثل فقدان الشهية وبعض الأعراض التنفسية، وعند إستخدام الدواء إنخفضت هذه الأعراض بشكل ملحوظ، ثم أخذ عينات دم من مجموعة العجول السليمة والمجموعة السليمة والمعالجة والمجموعة المريضة قبل وبعد العلاج بالسفتيوفيور صوديوم وذلك بعد إسبوع من إيقاف العلاج لدراسة تأثير الدواء على صورة الدم ووظائف الكبد والكلى ووجد أن إعطاء السفتيوفيور صوديوم للعجول السليمة يؤدى إلى زيادة معنوية فى عدد كرات الدم الحمراء وتركيز الهيموجلوبين وحجم كرات الدم المرصوصة وليس له أى تأثير معنوى على عدد كرات الدم البيضاء ونشاط خميرة الAST والALT والكيرياتينين وذلك بالمقارنة مع المجموعة السليمة والغير معالجة.

أما بالنسبة للمجموعة المصابة بالميكروب والمعالجة بالسفتيوفيور صوديوم لوحظ أن له تأثير علاجى جيد وزيادة معنوية فى عدد كرات الدم الحمراء وتركيز الهيموجلوبين وحجم كرات الدم المرصوصة ونقص معنوى فى عدد كرات الدم البيضاء ونشاط خميرة الAST والALT والكيرياتينين وذلك بالمقارنة بنفس المجموعة المصابة قبل العلاج.

ومن هذه النتائج يستخلص أن دواء السفتيوفيور صوديوم له تأثير قوى وفعال على ميكروب المانهيميا هيموليتيكا فى العجول المصابة بالأمراض التنفسية.