EFFECT OF FLORFENICOL ON SOME BLOOD CONSTITUENTS OF BUFFALO CALVES

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ABSTRACT

Forty-eight buffalo calves 3-6 months age at Helia Sharkia Governorate were divided into four healthy groups each of 8 calves and a diseased group suffered from respiratory symptoms. Group I was left as control. Groups II and III were treated with a therapeutic regimen of florfenicol (two intramuscular doses of the drug each 20 mg/kg. B.wt. 48 hours apart) and double therapeutic regimen respectively. Group IV was daily injected with 20mg/kg. B.wt. of florfenical for 5 successive days. The fifth diseased group was equally subdivided into untreated subgroup and a treated one with the therapeutic regimen of florfenicol, each subgroup was 8 calves. Effects of the drug on haematological picture and some liver and kidney functions of all treated groups were studied. The obtained results indicated that florfenicol at the recommended therapeutic regimen had no adverse effects on liver and kidney functions of buffalo calves. however bone marrow suppression had been detected in treated calves manifested by normocytic normochromic anaemia and decreased total leucocytic count mainly granulocytopenia.

INTRODUCTION

Florfenicol is a novel broad-spectrum antibiotic for the treatment of bovine respiratory disease (BRD) caused by multiple bacteria and mycoplasma (**Lockwood et al, 1996**), bacterial meningitis and enteritis of calves (**Craene et al, 1997 and Sheldon, 1997**). Florfenicol is belonging to the family that including thiamphenicol and chloramphenicol (**Richard, 1994**). These drugs are similar in structure with substitution of fluorine atom in the site of hydroxyl group of chloramphenicol in case of florfenicol. they act by inhibition of bacterial protein synthesis at the ribosome (**Cannon et al, 1990, Paape et al, 1990**). Bacterial resistance to both chloramphenicol and thiamphenicol is due to the presence of chloramphenicol acetyltransferase (CAT) in these bacteria. Several bacterial strains that are highly resistant to both chloramphenicol and thiamphenicol due to CAT production are highly sensitive to florfenicol. Also florfenicol has a superior

in vitro bacterial activity compared with chloramphenicol and thiamphenicol (**Syropoulou et al., 1981**). The administration of chloramphenicol resulted in aplastic anaemia due to bone marrow suppression in humans by a mechanism involving inhibition of mitochondiral protein synthesis, this suppression was related to the presence of the nitro group (**Varma et al., 1986**). Hence, since August, 1994, its use in food producing animals was banned in the European union (**Martel, 1994**). Florfenicol and Thiamphenicol don't contain this nitro group.

The objective of the present study was to detect any side effects of florfenicol on some blood parameters of buffalo-calves as most of the available literature was directed towards its pharmcokinetic aspect and efficacy.

MATERIALS AND METHODS

I- Drugs:

Florfenicol (Nuflor)$^{(R)}$ is injectable solution obtained from Schering Plough Animal Health (U.S.A.). Each 20 ml vial contains 300 mg florfenicol per ml.

II- Animals:

Forty eight buffalo calves 3-6 months old in Hehia, Sharkia Governorate, were divided into 6 equal grops (4 healthy (gps. 1-4) and two ill (gps. 5 & 6)). The diseased groups were suffering from respiratory signs in the form of nasal discharge, cough, fever, congested mucous membranes, lacrimation, bronchial rales and abnormal lung sound. Faecal and blood samples were examined to exclude infestation with gastrointestinal and blood parasites. Gp. (1) was left as control. Gps. (2 and 3) were treated with the therapeutic regimen of florfenicol (two intramuscular doses of the drug each of 20 mg/kg B.Wt. 48 hours apart) and double therapeutic regimen respectively. Gp. (4) was daily injected with 20 mg/kg B.wt. of the drug for 5 successive days. Gp. (5) was kept as affected control. Gp. 6. was treated with the therapeutic regimen of florfenicol.

Sampling:

Heparinized blood (for haematological studies) and blood to separate serum (for biochemical studies) were collected from each calf on the 7th and 14th days post drug administration.

Haematological studies:

The erythrocytic count (RBC-s), haemoglobin (Hb) and haematocrit value (PCV) were estimated beside the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Moreover total (TLC) and differential leucocytic count were determined according to Schalm et al. (1975).

Biochemical Studies:

Serum samples were analysed for estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) according to Reitman and Frankel (1957), alkaline phosphatase (ALP) after Kind and King (1954), total proteins (Doumas, 1975) and albumin (Doumas et al., 1971). -Serum globulins were calculated by substraction of albumin from serum total proteins. Serum urea (Tabacco, 1979) and creatinine (Husdan and Rapoport, 1968) were also determined.

Statistical analysis was carried out using Student "t" test according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

A significant decrease was encountered in the RBSc ($P < 0.001$), Hb ($P < 0.05$), PCV ($P < 0.05$), TLC ($P < 0.001$), neutrophils ($P < 0.001$), and eosinophils ($P < 0.001$), of healthy calves (gps. 2,3 and 4) on the 7th day post administration of the drug (Table 1). Similar results were found on the 14th day post treatment, however a slight improvement in the mean values of all measured parameters towards the normal levels were observed (Table 2).

A significant increase was found in serum AST, ALT ($P < 0.01$), ALP ($P < 0.01$) and BUN ($P < 0.05$) in calves of gp. (4). On the 7th day post drug administration (Table 4).

The respiratory affection was associated with a significant decrease ($P < 0.001$) in RBCs, Hb and PCV with a significant increase ($P < 0.001$), in TLC, neutrophils and monocytes beside a significant decrease ($P < 0.05$) in lymphocytic count (Table 3). A significant increase in serum AST, ALT, ALP ($P < 0.05$), globulin ($P < 0.001$), and BUN ($P < 0.01$) with a significant decrease ($P < 0.001$) in albumin in calves showing respiratory signs were recorded (Table 5).

The administration of florfenicol to buffalo-calves produced a normocytic normochromic anaemia manifested by a significant decrease of RBCs count, Hb and PCV. This effect may be attributed to depression of mitochondrial synthesis of protein in bone marrow. Inhibition of mitochondrial protein synthesis ultimately disrupts mitochondrial function, cellular function and cellular proliferation (Yunis, 1988). The obtained results show that the decrease in the TLC was associated with granulocytopenia, which could be due to a reversible bone marrow suppression elicited by florfenicol with a dose-dependent inhibition, as the measured parameters post-treatment were improved toward the normal level in comparison with those parameters at 7th day after injection of the last therapeutic dose. The effect of florfenicol, in this study, on bone marrow was similar to its analogue thiamphenicol. Yunis, (1969) reported that thiamphenicol produced, reversible bone marrow suppression by

concentration dependent inhibition of mitochondrial protein synthesis. Moreover, the present results are in agreement with Richard, (1994) who mentioned that florfenicol may cause some reversible bone marrow suppression in the myeloid series.

At the mean time, this study showed that florfenicol at therapeutic or double therapeutic regimens had no adverse effects on liver and kidney functions. These findings coincide with several studies that referred to safety of the drug in addition to its effectiveness in the treatment of respiratory diseases in cattle (**Almajano et al., 1998; Hoar et al., 1998 and Varma et al, 1998**). Treatment of buffalo calves suffering from undifferentiated respiratoy symptoms with two doses of florfenicol each of 20 mg/kg. B.wt. 48 hours apart resulted in an improvement of liver and kidney functions when compared with the untreated diseased animals. This effect could be due to the efficiency of the drug in the treatment of the bovine respiratory disease, as it was associated with alterations of the liver and kidney functions. In the present work, diseased animals showed elevation of serum AST, ALT, ALP, globulin and urea coupled with a decrease of serum albumin level. However the total serum proteins were non significantly altered due to the increase of serum globulin in response to respiratory infection. The significant decrease of serum albumin could be attributed to the destructive effect of bacteria and bacterial toxins on the liver cells; thus impaires the synthesis of serum albumin (**Mottelib, 1972**). In addition, in diseased animals a significant increase in the total leucocytic count (TLC) coupled with a relative increase in the mature neutrophils and a relative decrease in lymphocytes were detcted indicating the presence of inflammation and suppuration caused by the bacterial infection (**Bryson et al., 1979 and Coles, 1980**). Treatment of diseased calves with the therapeutic doses of florfenicol aggrevinted the alteration in the haematologic picture due to the stress effect elicited by the disease and also the suppressive effect of the drug on bone marrow.

Based on these results, it could be concluded that florfenicol at the recommended therapeutic regimen had no adverse effects on the liver and kidney functions of buffalo calves, however reversible bone marrow suppression, manifested by normocytic normochromic anaemia and leucocytopenia due to granulocytopenia was evident.

Table 1 : Effect of intramuscular injection of florfenicol at therapeutic regimen (two doses of 20mg/kg.B.wt, 48 hrs. apart), double therapeutic regimen and 20mg/kg.B.wt. for five successive days on haematological picture of healthy buffalo calves on the 7th day post treatment (n = 8) (Mean ± SE).

Parameter / Group	RBCs 10^6/ul	Hb gm/dl	PCV ml/dl	MCV fl	MCH pg/cell	MCHC gm/dl	TLC 10^3/ul	Absolute differential leucocytic count 10^3/ul				
								N	L	E	B	M
1 (Control untreated)	10.44 ± 0.35	12.58 ± 0.18	43.89 ± 2.43	41.89 ± 2.17	12.05 ± 0.97	28.86 ± 0.63	8.66 ± 0.28	3.12 ± 0.2	4.94 ± 0.45	0.35 ± 0.03	0	0.26 ± 0.02
2 (Treated with therapeutic dose)	7.97 ± 0.10***	11.10 ± 0.62*	36.60 ± 1.91*	45.92 ± 1.93	13.94 ± 0.83	30.40 ± 0.91	7.02 ± 0.12***	2.0 ± 0.18***	4.92 ± 0.38	0.06 ± 0.008***	0	0.14 ± 0.019***
3 (Treated with double therapeutic dose)	6.80 ± 0.17***	9.92 ± 0.16***	31.04 ± 1.83***	45.65 ± 1.25	14.56 ± 0.93	30.40 ± 1.52	5.15 ± 0.18***	1.00 ± 0.10***	3.90 ± 0.30	0.04 ± 0.003***	0	0.16 ± 0.016***
4 (Treated with 20mg/kg. B. Wt. for 5 successive days	6.25 ± 0.18***	9.44 ± 0.19***	29.02 ± 1.69***	46.40 ± 1.64	15.08 ± 1.06	32.40 ± 1.63	5.10 ± 0.30***	0.92 ± 0.08***	4.00 ± 0.37	0	0	0.18 ± 0.010***

* Significant at P<0.05 *** Significant at P<0.001

N : Netrophils L : Lymphocypes E. eosinophils B : Basoophils. M : Monocyles.

Table 2 : Effect of intramuscular injection of florfenicol at therapeutic regimen (two doses of 20mg/kg.B.wt, 48 hrs. apart), double therapeutic regimen and 20mg/kg.B.wt for five successive days on haematological picture of healthy buffalo calves on the 14th day post treatment (n = 8) (Mean ± SE).

Parameter / Group	RBCs 10^6/ul	Hb gm/dl	PCV ml/dl	MCV fl	MCH pg/cell	MCHC gm/dl	TLC 10^3/ul	Absolute differential leucocytic count 10^3/ul				
								N	L	E	B	M
1 (Control untreated)	10.80 ± 0.29	13.09 ± 0.20	45.12 ± 2.18	41.81 ± 1.59	12.18 ± 0.79	29.10 ± 0.89	8.91 ± 0.16	3.21 ± 0.13	4.81 ± 0.25	0.15 ± 0.01	0	0.43 ± 0.03
2 (Treated with therapeutic dose)	8.28 ± 0.15***	11.06 ± 0.12***	37.10 ± 2.00*	44.81 ± 1.26	13.36 ± 0.49	31.27 ± 1.46	7.41 ± 0.18***	2.22 ± 0.16***	4.56 ± 0.29	0.10 ± 0.006***	0	0.52 ± 0.08
3 (Treated with double therapeutic dose)	7.11 ± 0.14***	10.09 ± 0.09***	31.61 ± 1.85***	44.46 ± 2.02	14.19 ± 0.64	31.86 ± 1.38	5.86 ± 0.21***	1.47 ± 0.09***	4.01 ± 0.28	0.11 ± 0.01*	0	0.17 ± 0.02***
4 (Treated with 20mg/kg. B. Wt. for 5 successive days	6.91 ± 0.26***	10.01 ± 0.10***	30.02 ± 2.72***	43.44 ± 2.98	14.39 ± 0.9	33.27 ± 2.43	5.62 ± 0.24***	1.20 ± 0.10***	4.16 ± 0.21	0.11 ± 0.009*	0.02 ± 0.001	0.16 ± 0.01***

* Significant at P<0.05 *** Significant at P<0.001

N : Netrophils L : Lymphocypes E. eosinophils B : Basoophils. M : Monocyles.

Table 3 : Effect of intramuscular injection of florfenicol at therapeutic regimen (two doses of 20mg/kg.B.wt, 48 hrs. apart), on haematological picture of healthy buffalo calves suffering from respiratory symptoms on the 7th day post treatment (n = 8) (Mean±SE).

Parameter / Group	RBCs 10^6/ul	Hb gm/dl	PCV ml/dl	MCV fl	MCH pg/cell	MCHC gm/dl	TLC 10^3/ul	Absolute differential leucocytic count 10^3/ul				
								N	L	E	B	M
1 (Control untreated healthy calves)	10.80 ± 0.29	13.09 ± 0.20	45.12 ± 2.18	41.81 ± 1.59	12.18 ± 0.79	29.10 ± 0.89	8.91 ± 0.16	3.21 ± 0.13	4.81 ± 0.25	0.15 ± 0.01	0	0.43 ± 0.03
5 (Affected untreated calves)	8.02 ± 0.51***	10.61 ± 0.1***	35.06 ± 1.39***	43.72 ± 1.04	13.21 ± 0.18	30.25 ± 0.80	12.18 ± 0.25***	7.64 ± 0.29***	3.80 ± 0.19*	0.20 ± 0.02*	0	0.73 ± 0.069***
6 (Afected treated calves)	5.90 ± 0.46***	8.00 ± 0.18***	25.24 ± 1.91***	42.78 ± 2.00	13.56 ± 0.26	31.70 ± 1.08	7.64 ± 0.16***	4.20 ± 0.18***	3.13 ± 0.49**	0.08 ± 0.01***	0	0.35 ± 0.02*

* Significant at P<0.05 *** Significant at P<0.001
N : Netrophils L : Lymphocypes E. eosinophils B : Basoophils. M : Monocytes.

Table 4 : Effect of intramuscular injection of florfenicol at therapeutic regimen (two doses of 20mg/kg.B.wt, 48 hrs. apart), double therapeutic regimen and 20mg/kg.B.wt for five successive days on some liver and kidney functions of healthy buffalo calves on the 7th day post treatment (n = 8) (Mean ± SE).

Parameter / Group	AST	ALT	ALP	Total protein	Albumin	Globulin	Urea	Creatinine
	U/L		U/L	gm/dl			mg/dl	
1 (Control untreated)	16.99 ± 1.60	56.83 ± 2.54	28.66 ± 1.73	7.15 ± 0.23	4.12 ± 0.19	3.02 ± 0.14	27.43 ± 2.4	1.37 ± 0.12
2 (Treated with therapeutic dose)	17.01 ± 0.98	61.83 ± 2.48	32.13 ± 1.07	7.50 ± 0.28	4.23 ± 0.17	3.32 ± 0.24	31.24 ± 3.60	1.35 ± 0.14
3 (Treated with double therapeutic dose)	18.65 ± 1.09	62.83 ± 3.17	28.25 ± 1.78	7.40 ± 0.36	4.08 ± 0.19	3.32 ± 0.30	35.26 ± 2.92	1.39 ± 0.12
4 (Treated with 20mg/kg. B Wt. for 5 successive days	27.81 ± 1.20***	79.17 ± 3.66***	36.50 ± 1.98**	6.29 ± 0.20	3.19 ± 0.18	3.10 ± 0.20	40.01 ± 3.69*	1.68 ± 0.18

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.001

Table 5 : Effect of intramuscular injection of florfenicol at therapeutic regimen (two doses of 20mg/kg.B.wt, 48 hrs. apart), on some liver and kidney functions of buffalo calves suffering from respiratory symptoms on the 7th day post treatment (n = 8) (Mean ± SE).

Parameter / Group	AST	ALT	ALP	Total protein	Albumin	Globulin	Urea	Creatinine
	U/L		U/L	gm/dl			mg/dl	
1 (Control untreated healthy calves)	16.99 ± 1.66	56.83 ± 2.54	28.66 ± 0.73	7.15 ± 0.23	4.12 ± 0.19	3.02 ± 0.14	27.43 ± 2.4	1.37 ± 0.12
5 (Affected untreated calves)	21.04 ± 1.3*	68.85 ± 3.78*	35.85 ± 1.83*	7.70 ± 0.30	3.41 ± 0.16***	4.29 ± 0.16***	38.18 ± 2.31**	1.43 ± 0.12
6 (Afected treated calves)	20.56 ± 1.14	62.50 ± 3.31	33.24 ± 1.95	7.46 ± 0.20	4.08 ± 0.09	3.38 ± 0.19	32.94 ± 2.62	1.55 ± 0.16

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.001

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الملخص العربى

تأثير الفلورفينكول على بعض مكونات الدم فى عجول الجاموس

المشتركون فى البحث

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معهد بحوث صحة الحيوان - معمل الزقازيق

تم تقسيم ٤٨ عجل جاموس عمر ٣-٦ شهور فى ههيا بمحافظة الشرقية إلى ٦ مجموعات متساوية أربع مجموعات سليمة (رقم١ إلى ٤) ومجموعتين تعانى أعراض تنفسية (رقمى ٥ ، ٦). عولجت المجموعات الثانية والثالثة بالجرعات العلاجية للفلورفينيكول (جرعتين فى العضل ٢٠ مجم / كجم وزن حى بينهما ٤٨ ساعة) وضعف الجرعات العلاجية على الترتيب بينما تم ترك المجموعة الأولى كمجموعة ضابطة سليمة، تم حقن المجموعة الرابعة بجرعة ٢٠كجم / مجم وزن حى فى العضل يومياً لمدة خمسة أيام متتالية بينما إستخدمت المجموعة الخامسة كمجموعة ضابطة مريضة وعولجت المجموعة السادسة بالنظام العلاجى.

تم دراسة صورة الدم وبعض وظائف الكبد والكلى فى الحيوانات المعالجة، أظهرت الدراسة عدم وجود تأثيرات ضارة للفلورفينكول على وظائف الكبد والكلى فى العجول الجاموس بينما حدث تثبيط لنخاع العظام تم الاستدلال عليه بالأنيميا ونقص العدد الكلى لخلايا الدم البيضاء خاصة النوع المحبب.