ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of a combined Oxytetracycline HCl / Flunixinmeglumine therapy On undifferentiated respiratory disease in calves

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Manuscript Info *Abstract*

Manuscript History:

Received: 12 January 2014
Final Accepted: 22 Febuary 2014
Published Online: March 2014

Key words:
Floxon®, Calves, BRD, efficacy, anti-inflammatory, immunity,antioxidant

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A study was carried out to evaluate the clinical efficacy of Floxon® ; a combination of oxytetracyclineHCl and flunixinmeglumine; compared with treatment with two different doses of oxytetracyclineHCl alone in calves suffering from undifferentiated respiratory disease (BRD). Twenty newly born calves were used in this study. Five of these calves were apparently healthy and kept as a control group. The remaining fifteen calves were suffering from respiratory signs in the form of nasal discharge, cough, fever, congested mucous membrane, lacrimation, bronchial rales and abnormal lung sound. Those diseased calves were divided into three equal groups; each of 5. The first group (A) was treated I/M with oxytetracycline ; 5 mg/kg b.w.daily for 3 consecutive days .The second group (B) was treated with long-acting oxytetracycline ; 20 mg/kg b.w.twice with an interval 48 hours, while the third group(C) was treated with Floxon® ;0.5ml/10kg b.w. daily for 3 consecutive days. Clinical illness index score (CIIS) was recorded on daily basis and Blood & serum samples were collected from calves of each group just before treatment, three and ten days post-treatment for immunological and biochemical studies.The results revealed that, diseased calves before treatment showed significant increase in the TLC, Neutrophils, monocytes, eosinophils, basophils, serum MDA, haptoglobin and C-reactive protein with significant decrease in the lymphocytes , Phagocytic activity (%) ,phagocytic index, serum CAT and SOD levels compared with the healthy calves.Treatment of diseased calves with Floxon®as well as Long acting oxytetracycline were associated with significantly faster improvements in CIIS (cough, nasal discharge, dyspnea, depression and anorexia), especially on the 3rd day of treatment that associated with significant betterment in all the measured immunological, antioxidant and anti-inflammatory parameters compared with the results obtained from calves treated with oxytetracycline 5 mg/kg alone so , the study strongly recommend the use of Floxon® or Long acting oxytetracycline20 mg/kg b.w in dealing with BRD in calves

INTRODUCTION

Bovine respiratory disease (BRD) or “shipping fever” is a complex disease of feedlot young cattle that causes major economic losses to the livestock industry ***(Van der Fels-Klerx et al., 1998).*** The disease is characterized by depression, lack of appetite, fever, cough, nasal discharge and dyspnea. The primary lesions observed at necropsy include severe bronchopneumonia or fibrinous pneumonia***(Esslemont and Kossaibati, 1997).***

The etiology is multifactorial and generally believed to be an interaction between viruses (bovine respiratory syncytial virus, Para influenza virus 3, bovine herpes virus 1…etc) that may facilitate the invasion of

bacteria such as Pasteurellamultocida, MannheimiahaemolyticaorMycoplasma spp, in addition to physiological and environmental stress factors ***(Caldow, 1996 and Griffin, 1996).***

Effective prophylaxis of the syndrome is limited because of the multifactorial etiology and immunosuppressive effect of viruses and Mycoplasmas.Conventional treatment for BRD usually consists of antimicrobial therapy with the application of mucolytics and bronchodilators ***(Smith, 1996).*** Several injectable antibacterials can be used to treat animals with respiratory disease (RD) such as tilmicosin (TIL), florfenicol, oxytetracycline (OXY) and others ***(Goubin etal., 1991).***

Treatment of RD using oxytetracycline has been largely accepted and published ***(Musser et al., 1996).***Oxytetracycline is a wide spectrum bacteriostatic antibiotic, acts against large number of Gram-negative, Gram-positive bacteria, *Rickettsia* and *Mycoplasma*, and its bacteriostatic effect is based on the inhibition of bacterial protein synthesis by binding with 30S ribosomal subunit.It is used for the treatment of local and systemic infections with newly discovered additional properties including anti-inflammatory and immunosuppressive activities ***(Olszewska, 2006).***

Since the inflammatory response that occurs in BRD forms a significant part of the disease process in the early and especially chronic stage, anti-inflammatory drugs have been recommended ***(Lekeux, 1996).*** Some, such as flunixinmeglumine or ketoprofen combined with an antibiotic have been used with therapeutic success in naturally occurring respiratory diseases ***(Doherty et al., 2001; Lockwood et al., 2003; Fritonet al., 2004 and Brentnall et al., 2013)***

Flunixinmeglumine (FLU) is considered to be one of the most potent non-steroidal anti-inflammatory drugs that has analgesic, anti-inflammatory and antipyretic activities and commonly used as adjunctive therapy for the treatment of respiratory diseases worldwide ***(Anderson, 1988 and Guzelet al., 2010).***

The objective of this study was to study the clinical efficacy of Floxon® ; a combination of oxytetracyclineHCl and flunixinmeglumine ; compared with treatment with two different doses of oxytetracyclineHCl alone; in calves suffering from undifferentiated respiratory disease (BRD).

MATERIALS AND METHODS

Drugs:

1. **Oxytetracycline HCL 5%injectable solution ;**a product ofArab Company for Medical Products.
   It was administered intramuscularly at a dose of 5mg/kg bodyweight daily for 3 consecutive days ***(Elsheikh et. al., 1997)***
2. **Long- acting oxytetracycline (oxytetracycline 20%L.A.)®** (Arab Company for Medical Products). It was given as a 20% solution; each ml contains 200 mg oxytetracycline as hydrochloride. It was administered intramuscularly by a dose of 20 mg/kg bodyweighttwice with an interval 48 hours ***(Deleforge et al., 1994).***
3. **Floxon®** (Pharma Swede Animal Health Products), Each 1 ml contains 108 mg OxytetracyclineHCl and 33 mgFlunixinMeglumine.The recommended therapeutic dose in cattle is 0.5 ml / 10 kg B.wt. (5.4mg oxytetracycline and 1.6 mg flunixin / kg)intramuscularly daily for 3 consecutivedays.

Animals:

Twenty newly born calves (3-5 month old) belonging to a private farm in Sharkia province, were used in this study. Five of these calves were apparently healthy and kept as a control group. The remaining fifteen calves were suffering from respiratory signs in the form of nasal discharge, cough, fever, congested mucous membrane, lacrimation, bronchial rales and abnormal lung sound. Those diseased calves were divided into three equal groups; each of 5.The first group (A) was treated intramuscularly with oxytetracycline formulation at a dose of 5 mg/kg bodyweight daily for 3 consecutive days .The second group (B) was treated with long-acting oxytetracycline at a dose of 20 mg/kg bodyweight twice with an interval 48 hours, while the third group(C) was treated with Floxon® at a dose of 0.5ml/10kg bodyweight daily for 3 consecutive days.

The following clinical observations were recorded and scored as shown in table (1) on daily basis in a form of clinical illness index score (CIIS): body temperature (◦C), breathing (breaths/min) ,heart rate (beat/min), nasal discharge (mucous, mucopurulent, purulent), soft coughing, dyspnea, appetite (anorexia), signs of depression and mortality***(Bednarek et al., 2003)***.

Table (1): Clinical Illness Index Scores (CIIS) for Calves

Score	Description	Appearance
1	Normal	No abnormalities noted
2	Slightly ill	Mild depression, gaunt, +/- cough
3	Moderately ill	Severe depression, labored breathing, ocular/nasal discharge, +/- cough
4	Severely ill	Moribund, near death, little response to human approach

After the end of the treatment course, the calves were re-examined again by clinical examination.

Blood samples

Two blood samples were collected from each calf through jugular vein puncture just before treatment and on the3rd and10th day post treatment.The first one was taken on heparin as anticoagulant for leukogram examination and to determine the phagocytic activity and phagocytic index.The second blood sample was left to clot at room temperature for about 2 hours, stored overnight in a refrigerator at 4ºC and centrifuged at 3000 rpm for 15 min. Serum samples were then collected in dry clean capped tubes and kept at -20°C until used for biochemical analysis.

Total and differential leukocytic counts:

Total and differential leukocytic counts were determined according to the standard techniques described by ***Coles (1986).***

Phagocytic activity and phagocytic index:

Phagocytic activity was determined according to ***Kawahara et al. (1991).****Candida albicans*culture (50 µg) was added to 1ml of heparinized blood collected from animals and shaken in water bath at 23-25ºC for 3-5 hours. Blood smears were then made and stained with Giemsa stain. Phagocytosis was estimated by determining the proportion of macrophages, which contain intracellular yeast cells in a random count of 300 macrophages and expressed as percentage of phagocytic activity (PA). The number of phagocytozed organisms were counted in the phagocytic cells and called phagocytic index (PI).

Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytozed}}{\text{Number of phagocytic cells}}$$

Oxidant/antioxidant markers

L- Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase activity (CAT) were calorimetrically assayed in the serum according to ***(Sinha, 1972; Satoh, 1987 and Packer&Glazer, 1990)*** respectively**.**

Anti-inflammatory markers

Serum haptoglobin level was determined by means of sodium dodecyl sulphat-polyacrylamide gel electrophoresis (SDS-PAGE) according to ***Yoshino et al., (1992).***Serum C-reactive protein was determined according to the methods reported by kits of Biosystems S.A. (Spain) & Bio-Med Diagnostics (Egypt).

Statistical analysis

The obtained data were analyzed statistically using an ANOVA test according to ***(SPSS, 2001).***

RESULTS AND DISCUSSION

Bovine respiratory disease is the most common illness affecting housed cattle and is a major limiting factor in animal production .In addition to the economic loss, high morbidity respiratory disease also represents a significant animal welfare issue ***(Healyet al., 1993).***

The acute bovine respiratory disease described in the study was undifferentiated: establishing the etiology was not a component of the scientific protocol as bovine respiratory disease is a multi-factorial problem and there is usually no simple microbiological pattern of infection. ***(Caldow, 1996)***

Treatment of bovine respiratory disease involves specific antimicrobial therapy as well as treatment aimed at alleviating symptoms and enhancing respiratory exchange mechanisms. Thus, non-steroidal anti-inflammatory drugs (NSAIDs) are frequently administered concurrently with antibiotics for symptomatic improvement in the treatment of acute respiratory disease ***(Clarke et al., 1991).***

The objective of this work was to study the clinical efficacy of Floxon®; a combination of oxytetracyclineHCl and flunixinmeglumine; compared with treatment with two different doses of oxytetracyclineHCl alone; in calves suffering from undifferentiated respiratory disease (BRD)**.**

The results revealed that, treatment of diseased calves with Floxon® at a dose of 0.5ml/10kg bodyweight daily for 3 consecutive days(Group C) was associated with a significantly (P <0.05) faster improvement in CIIS (cough, nasal discharge, dyspnea, depression and anorexia), especially on the 3rd day of treatment (Fig. 1). Also on the 4th and 5th day of treatment, the improvement in CIIS was more pronounced in Group B & C. At the end of the observation, body temperature in all groups returned to normal, but breathing and heart rates as well as CIIS were still the highest in Group A, treated with oxytetracycline 5mg/kg alone.

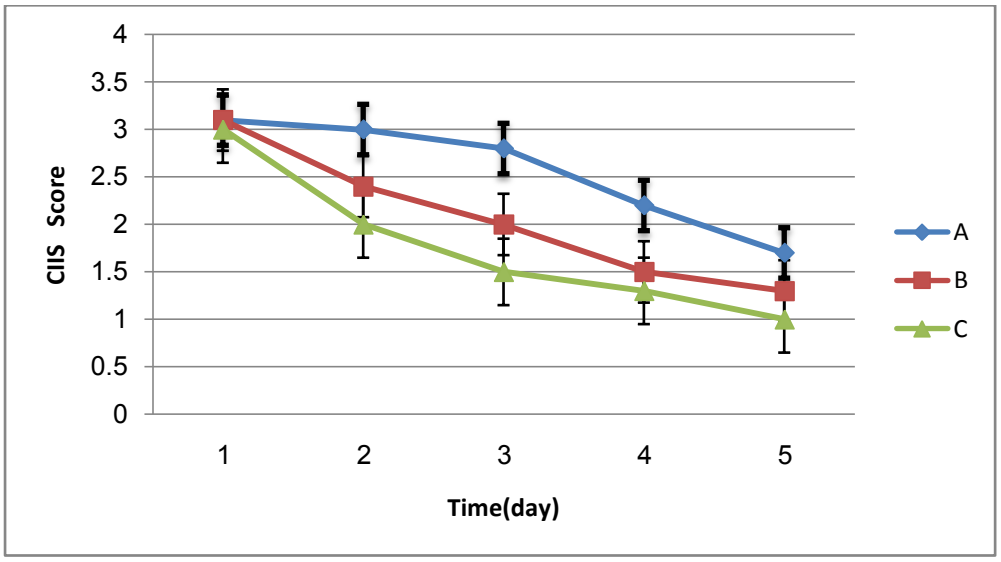

Fig. (1): Clinical illness index scores in calves suffering from respiratory disease and treated intramuscularly with oxytetracycline at a dose of 5 mg/kg bodyweight daily for 3 consecutive days (A), long-acting oxytetracycline at a dose of 20 mg/kg bodyweight twice with an interval 48 hours (B) and Floxon® at a dose of 0.5ml/10kg bodyweight daily for 3 consecutive days (C) showing faster improvement in CIIS (cough, nasal discharge, dyspnea, depression and anorexia) in calves belonged to group (C) , especially on the 3rd day of treatment. On the 4th and 5th day of treatment, the improvement in CIIS was more pronounced in Group (B) & (C) while CIIS was the highest in group (A).

In facts, earlier studies supported our results, ***De Haas et al. (1998) and Zundel et al. (1996)*** compared three daily injections of a single product combining oxytetracycline and flunixin with daily injections of oxytetracycline only. The combination therapy produced a more significant clinical response in the diseased animals characterized by a reduction in body temperature, fewer relapses, increased appetite and live weight gain.

On similar ground, ***Bednarek et al., (2003)*** reported that, treatment of calves with the combination of oxytetracycline and meloxicam caused a significantly faster improvement in the clinical illness index score (CIIS: cough, nasal discharge, dyspnea, depression and anorexia) and a faster normalization of body temperature compared with the group treated with oxytetracyclinealone.

Selman et al., (1986) and Anderson and Young, (1986) compared separate injections of oxytetracycline and flunixin for three days with injections of oxytetracycline only and they found improved clinical responses characterized by a significant reduction of respiratory rates as well as fewer fevers in cattle experimentally-infected with *Pasteurellahaemolytica*.

The positive effects of flunixin as an adjunctive therapy are supported by a large number of published studies ***(Doherty et al., 2001; Friton et al., 2004 and Keita et al., 2007)***. This positivity was reflexed by a faster improvement of clinical parameters.

Our results demonstrated that, diseased calves showed significant increase ($p>0.05$) in the TLC, neutrophils, monocytes, eosinophils and basophils with significant decrease in the lymphocytes compared with the control healthy calves (table, 2).

Actually, leukocytosis including neutrophilia, monocytosis, eosinophilia and lymphocytopenia observed in diseased calves indicating the presence of inflammation caused by bacterial infection ***(Coles 1986).*** The previous findings were similar to those obtained by ***Galhoom et al., (2002).***

From table (2) also we can observe that, treatment of diseased calves with oxytetracycline at a dose of 5 mg/kg bodyweight daily for 3 consecutive days(group A) or with long-acting oxytetracycline at a dose of 20 mg/kg body weight twice with an interval 48 hours (group B) or with Floxon® at a dose of 0.5ml/10kg bodyweight daily for 3 consecutive days (group C) resulted in an improvement in leukogram picture that represented by significant decrease ($p>0.05$) in the TLC, neutrophils and monocytes levels with significant increase in the lymphocytes counts compared with the diseased calves before treatment and the best results were belonged to those of group C; especially on the 10^{th} day post-treatment where the levels of all leukocytes returned to reach the levels of the healthy control calves .As for groups A & B , there were non significant differences between them in their results concerning leukogram on the 3^{rd} day post treatment while on the 10^{th} day post treatment, group B showed more favorable results compared with group A.

Fortunately, our results came in consistency with those obtained by ***Bednarek et al., (2003)***who reported that, treatment of diseased calves with the combination of oxytetracycline and meloxicam caused a slow decrease in white blood cell (WBC) count, the number of neutrophils, monocytes, eosinophils and basophils.This improvement in the leukogram picture of diseased calves post treatment may be attributed to the general betterment in the CIIS observed in such calves as a result of a decline in the curve of the infectioncourse and the inflammatory process proved in our findings.

Data presented in table (3) revealed that, diseased calves showed significant increase ($p>0.05$) in the Phagocytic activity (%) and phagocytic index compared with the healthy calves.Calvesbelonged to groups (B&C) displayed significant decrease ($p>0.05$) in the Phagocytic activity (%) and phagocytic index on the 3^{rd} and 10^{th} days post treatment while calves of group (A) showednon significant changes compared with the diseased calves before treatment

In fact, our results was supported by those obtained by***Bednarek et al., (2003)*** who stated that, treatment of calves with the combination of oxytetracycline and flumethasone exhibited a decrease in gamma-globulin concentration and phagocytic activity.

There isa growing evidence from human studies that some non steroidal anti-inflammatory drugs as flunixin might haveadditionalimmunomodulatory properties concerning leukogram, cytokines and phagocyticactivities. For example,***Iñiguez et al., (1999)*** reported that COX-2 inhibitors regulate Tcell activation.

In the present work, diseased calves before treatment showed significant increase ($p>0.05$) in the mean values of serum MDA level accompanied with significant decrease ($p>0.05$) in the levels of serum CAT and SOD compared with the healthy ones.Calves belonged to groups A,B and C showed significant decrease ($p>0.05$) in the MDA level and significant increase ($p>0.05$) in the CAT and SOD levels compared with diseased calves before treatment and the most favorable results were belonged to those of groups C & B on the 10^{th} day post treatment while group B displayed better aftermaths on the 3^{rd} and 10^{th} days post treatment compared with those of group A.(table, 4).

Similar results were previously observed in cattle ***(Bliznetsova et al., 2008 and Megahed et al., 2006)***as it is well known that inflammatory diseases are associated with enhanced oxidative reactions and reduced antioxidant defense capabilities ***(Behima etal., 2001).***

In general, oxidative stress is an imbalance between radical-generating and radical-scavenging activity, resulting in oxidation products and tissue damage ***(Bernabucci et al., 2002)***. Normally, the body is protected by a wide range of antioxidant systems working in concert.

The excessive lipid peroxidation in plasma and cells ascribed to many factors or diseases lead to excessive formation of NADPH, which in turn promote lipid peroxidation in the presence of cytochrome p-450 system ***(Jain, 1989 and Ahmed et al., 2010).***

Malondialdehyde (MDA), a major and stable end product formed of peroxidation, is regarded as a marker of lipid peroxidation ***(Del et al., 2005).***

Cells have evolved different antioxidants to neutralize reactive oxygen species (ROS) which can suppress lipid peroxidation; hence these antioxidants are absolutely critical for inhibiting oxidative stress-induced cytotoxicity. Antioxidant enzymes, such as CAT, GPx and SOD,are a class of enzymes capable of inhibiting the oxidation and are major intracellular antioxidant defenses in cells. It has been shown that the over expression of antioxidant enzymes can provide protective effects against the ROS-induced cardiomyocytes damage ***(Liu et al., 2009).***

In keeping with this line, ***Topsakal et al. (2003)*** stated that, oxytetracycline is effective in preventing lipid peroxidation in spinal cord injury.

On the other hand,whenflunixinmeglumin was administered simultaneously with lipopolysaccharide (LPS), it inhibited ($p<0.05$) the increase of MDA levels in all tissues. As results, LPS caused MDA levels and oxidative stress to increase. However, flunixinmeglumindepressed the LPS induced increase of MDA levels in addition to increasing the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) ***(Sibel et al., 2007).***

Concerning the anti-inflammatory markers, the present investigation demonstrated that, diseased calves before treatment disclosed a significant increase ($p>0.05$) in serum haptoglobin and C-reactive protein compared with healthy calves and their levels were significantly decreased in all treated calves specially those belonged to group B &C on the 10^{th} day post treatment where the levels of the two markers nearly reached to their levels in the healthy calves (Table, 5).

In this context, it is fitting to mention that C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex ***(Thompson et al., 1999).***CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes)***(Lau et al., 2005).***

On similar ground, Haptoglobin is the protein that is encoded by the HP gene. In blood plasma, haptoglobin binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity. The haptoglobin-hemoglobin complex will then be removed by the reticuloendothelial system (mostly the spleen). As haptoglobin is indeed an acute-phase protein, any inflammatory process (infection, extreme stress, burns, major crush injury, allergy, etc.) can increase the levels of plasma haptoglobin***(Wassell, 2000)*** so, measuring of C - reactive protein and haptoglobulin is indicative in the inflammatory evaluation studies.

Results of our study are in harmony with the previous findings of ***Wittum et al. (1996) and Orro et al., (2011).***The authorsreported that serum concentrations of haptoglobin in diseased calves increased significantly. The concentration change of this acute phase protein wasoften accompanied with respiratory disease conditions. The concentration rapidly decreased in calves that recovered quickly. Haptoglobin values were 10 to 100 times higher than in the normal calves.

Treatment either with LA oxytetracycline or in combination with flunixin displayed the best anti-inflammatory outcome that could be explained by ***Olszewska, (2006)*** who stated that, oxytetracycline is a bacteriostatic antibiotic with newly discovered, additional mechanisms of action include antioxidant, antiinflammatory and immunosuppressive activity.

On the other hand, The NSAID flunixinmeglumine is an aminonicotinicacidderivative that has anti-inflammatory, analgesic and anti-pyreticproperties in cattle ***(Verhoeff et al., 1986).*** The acute inflammatory component of pneumonia results in impaired gas exchangeand the aim of modulating pulmonary inflammationby the use of NSAIDs are to block the production and/or theeffects of inflammatory mediators and modulators which have adeleterious effect on alveolar exchange of gases ***(LekeuxandVanDeWeerdt, 1996).***

Studies in calves by ***Landoni et al., (1995a)*** showed that, in addition to thenonselectiveinhibition of cyclooxygenase activity, flunixin decreased the oedematousresponse to intradermally-injected bradykinin and inhibited the generationof superoxide radicals by neutrophils *in vitro*.

Landoni et al., (1995b) found that flunixin had a long elimination half-life ofapproximately seven hours in calves and that it attained concentrations in inflammatory exudates that were four timesgreater than the concentration found in transudate or plasma.

In conclusion,the biochemical and immunological changes observed in calves suffering from undifferentiated respiratory disease and treated with (Floxon)® or long acting oxytetracycline alone ,with faster normalization of their body temperature and faster disappearance of BRD clinical signs, indicated that such treatments are superior to the application of oxytetracyclineHCl 5mg/kg alone.

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Table (2) :Leukogram of healthy and diseased calves before and after treatment with oxytetracycline ; 5 mg/kg b.wt. daily for 3 consecutive days (A); long-acting oxytetracycline;20 mg/kgb.wt.twice with an interval 48 hours (B) and Floxon®; 0.5ml/10kg b.wt. daily for 3 consecutive day (C) (Mean± S.E) n=5

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day posttreatment			Diseased calves on 10th day post treatment		
			A	B	C	A	B	C
TLC 10^3/UL	8.74 ± 0.13^{f}	12.35 ± 0.23^{a}	11.44 ± 0.13^{b}	11.06 ± 0.08^{bc}	10.65 ± 0.15^{cd}	10.21 ± 0.16^{d}	9.25 ± 0.13^{e}	9.07 ± 0.18^{ef}
Neutrophil 10^3/UL	3.37 ± 0.11^{e}	7.48 ± 0.09^{a}	5.93 ± 0.08^{b}	5.71 ± 0.15^{b}	5.19 ± 0.10^{c}	4.92 ± 0.15^{c}	3.94 ± 0.19^{d}	3.77 ± 0.20^{de}
Lymphocyte 10^3/UL	4.76 ± 0.06^{a}	3.72 ± 0.17^{b}	4.48 ± 0.13^{a}	4.48 ± 0.06^{a}	4.61 ± 0.09^{a}	4.40 ± 0.10^{a}	4.51 ± 0.14^{a}	4.65 ± 0.06^{a}
Eosinophil 10^3/UL	0.15 ± 0.01^{d}	0.30 ± 0.03^{a}	0.27 ± 0.02^{ab}	0.23 ± 0.02^{abcd}	0.24 ± 0.03^{abc}	0.25 ± 0.02^{abc}	0.20 ± 0.01^{bcd}	0.19 ± 0.02^{cd}
Monocytes 10^3/UL	0.38 ± 0.03^{c}	0.73 ± 0.02^{a}	0.66 ± 0.04^{a}	0.54 ± 0.02^{b}	0.51 ± 0.04^{b}	0.53 ± 0.04^{b}	0.50 ± 0.03^{b}	0.37 ± 0.01^{c}
Basophil 10^3/UL	0.08 ± 0.007^{c}	0.12 ± 0.003^{a}	0.10 ± 0.004^{ab}	0.10 ± 0.005^{ab}	0.10 ± 0.005^{ab}	0.11 ± 0.004^{ab}	0.10 ± 0.002^{ab}	0.09 ± 0.006^{bc}

Different letters in the same row means significant difference at ($p\leq0.05$)

Table (3):Mean values of Phagocytic activity (%) and Phagocytic index in healthy and diseased calves before and after treatment with oxytetracycline ; 5 mg/kg b.wt. daily for 3 consecutive days (A); long-acting oxytetracycline ; 20 mg/kg b.wt twice with an interval 48 hours (B) and Floxon® ;0.5ml/10kg b.wt daily for 3 consecutive days (C). (Mean± S.E) n=5

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day Post-treatment			Diseased calves on 10th day Post-treatment		
			A	B	C	A	B	C
Phagocytic activity (%)	69.20 ± 1.24^{d}	83.00 ± 0.89^{a}	81.80 ± 0.91^{a}	77.80 ± 0.66^{bc}	75.40 ± 1.07^{c}	82.20 ± 0.80^{a}	78.60 ± 0.87^{b}	77.20 ± 0.96^{bc}
Phagocytic index	1.16 ± 0.04^{d}	2.44 ± 0.12^{a}	2.24 ± 0.07^{a}	1.66 ± 0.04^{c}	1.48 ± 0.09^{c}	2.34 ± 0.13^{a}	1.94 ± 0.09^{b}	1.68 ± 0.06^{bc}

Different letters in the same row means significant difference at ($p\leq0.05$)

Table (4):Mean values of serum L- Malondialdehyde (MDA), catalase activity (CAT) and superoxide dismutase (SOD) in healthy and diseased calves before and after treatment with oxytetracycline ; 5 mg/kg b.wt. daily for 3 consecutive days (A); long-acting oxytetracycline ; 20 mg/kg b.wt twice with an interval 48 hours (B) and Floxon® ;0.5ml/10kg b.wt daily for 3 consecutive days (C). (Mean± S.E) n=5

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day Post-treatment			Diseased calves on 10th day Post-treatment		
			A	B	C	A	B	C
MDA mmol/L	29.26±0.40^{d}	41.18±0.62^{a}	35.42±0.49^{b}	33.92±0.99^{bc}	32.04±0.77^{c}	35.30±0.42^{b}	33.46±0.88^{bc}	29.56±0.40^{d}
CAT µ/gmHb	1.27±0.06^{a}	0.33±0.07^{e}	0.59±0.06^{d}	0.86±0.05^{c}	0.95±0.05^{bc}	0.88±0.06^{c}	1.12±0.07^{ab}	1.30±0.06^{a}
SOD µ/L	31.40±0.80^{a}	5.52±0.79^{e}	15.12±0.91^{d}	18.22±0.96^{c}	18.90±0.88^{c}	26.28±1.54^{b}	29.20±0.69^{a}	30.24±0.48^{a}

Different letters in the same row means significant difference at ($p \leq 0.05$)

Table (5):Mean values of serum haptoglobin and C-reactive protein in healthy and diseased calves before and after treatment with oxytetracycline ; 5 mg/kg b.wt. daily for 3 consecutive days (A) ; long-acting oxytetracycline ; 20 mg/kg b.wt. twice with an interval 48 hours (B) and Floxon® ; 0.5ml/10kg b.wt. daily for 3 consecutive days (C). (Mean± S.E) n=5

	Healthy control calves	Diseased calves before treatment	Diseased calves on 3rd day Post-treatment			Diseased calves on 10th day Post-treatment		
			A	B	C	A	B	C
Haptoglobin (gm/dl)	0.17±0.008^{e}	1.06±0.035^{a}	0.50±0.051^{b}	0.34±0.017^{c}	0.29±0.013^{c}	0.26±0.028^{cd}	0.20±0.007^{de}	0.17±0.010^{e}
C-reactive protein (gm/dl)	0.66±0.039^{e}	3.82±0.053^{a}	2.90±0.117^{b}	1.68±0.196^{cd}	1.52±0.223^{d}	1.95±0.117^{c}	0.88±0.148^{e}	0.65±0.038^{e}

Different letters in the same row means significant difference at ($p \leq 0.05$)