Evaluation of levamisole efficacy as immune-stimulant on respiratory diseases treatment in calves

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A B S T R A C T

This study was performed on fifteen newly born calves, 3-5 month old with clinical signs of bovine respiratory disease (BRD) in addition to five clinically healthy which considered as control group. After bacterial isolation and identification of the causative microorganisms, fifteen calves from which were found infected with *E.coli*, *P. multocida*, *Klebsiella* and *S. aureus.* The diseased calves were divided randomly into three equal groups: Gp.1: were treated dose of draxxin (2.5 mg/kg body weight) by s/c injection in the neck; Gp.2: were received Draxxin and Meloxicam (single I/M injection of 0.5 mg/kgb.wt.), while Gp.3: were received draxxin (single s/c injection of 20 mg/kg bwt.) +Levamisole (single I/M injection at a dose of 1mg/50kg bwt). Blood and serum samples were collected from all calves just before treatment, 3 and 10 days post treatment. TLC, neutrophils and monocyte count revealed significant decrease in all treated groups in comparison with diseased group before treatment. While, lymphocyte count recorded significant increase in all treated groups compared with diseased group and lastly, esinophil recorded significant decrease in treated groups with draxxin plus meloxicam and draxxin plus levamisole in comparison with diseased group. Total protein demonstrated significant increase in all treated in comparison with diseased group. Serum albumin, total globulin, α-globulin, β-globulin and γ-globulin showed significant decrease when compared with diseased calves before treatment. Haptoglobin and fibrinogen showed significant decrease in all treated groups in comparison with diseased group. There were significant increase in immunoglobulins and cellular immunity (phagocytic index and phagocytic percentage) in all treated groups especially in group treated with draxxin plus levamisole when compared with diseased group before treatment.

Keywords: Bovine respiratory disease, Levamisole, Haptoglopin, Fibrinogen, Non-steroidal anti-inflammatory drug.

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1. INTRODUCTION

Diseases of the respiratory system are a major cause of illness and death in cattle from 6 weeks to two years of age. Sadly, this is as true today as it was 30 years ago despite development of new and improved vaccines, new broad spectrum antibiotics, and increased fundamental knowledge as to the cause of disease (Kasimanickam, 2010).

A wide variety of antibacterial agents, most of which are antibiotics are employed for the prevention and treatment of infection in livestock. These antibiotics can cause various problems, such as residues, the emergence of bacterial resistance as well as suppression to the host resistance. A depressed immune function causes marked increase in the incidence of opportunistic

infection (Nakagawa et al., 1993). Initial field trials with a tulathromycin (Draxxin) suggested a high level of efficacy in treatment of respiratory diseases in newly born calves (Ragbetli et al., 2009).

NSAIDs also have an anti-inflammatory effect, but are not immunosuppressive. Some of them have been used with therapeutic success in naturally occurring BRD (Lockwood et al., 1996 and Balmer et al., 1997).

Many drugs have been also used recently to increase the resistance of animals by improving the humoral and cell mediate immune response such as levamisole hydrochloride (Hany et al., 1999).

The aim of this study was to evaluate bovine respiratory disease (BRD) in calves through using immune-stimulant drug as alternative drug and other drugs on calves suffering from naturally occurring bronchopneumonia and the reflection of these drugs on: leukogram, some inflammatory markers and lastly, humeral and cellular immunity

2. MATERIALS AND METHODS

2.1.Experimental Animals:

Twenty newly born calves, up to 3 month old belonging to a private farm in Sharkia province, from December till february were used in this study. Five of these animals were apparently healthy and free from any external parasites and kept as a control group. The remaining calves were suffering from bronchopneumonia (fever over 40 ◦C, cough, nasal discharge from mucous, mucopurulent to purulent, dyspnea and anorexia).The first group were treated with long acting Draxxin; the second group were received Draxxin and Meloxicam (NSAID) while, the third group were received Draxxin and Levamisole.

2.2. Drug:

2.2.1.Draxxin®

Tulathromycin (Pfizer-Company) contain 100 mg/ml tulathromycin in propylene glycol/water injectable solution ready for use.The recommended dose is a single dose of 2.5 mg/kg bwt by s/c injection in beef and non-lactating dairy cattle (Skogerboe et al., 2005).

2.2.2. Meloxicam (Metacam)®

Solution for injection contains 20 mg/ml meloxicam produced by Boehringer Ingelheim Vetmedica Compan, Germany. The recommended therapeutic dose is a single intramuscular injection at a dose of 0.5 mg/kg of body weight (Friton et al., 2005).

2.2.3. Levamisole®

It's commercial product used as anti-helminthic and immune-stimulant for large animals in farms. Levamisole injection(B.P.Vet 85) , Levamisole HCl 75 mg/ml. The recommended therapeutic dose is A single intramuscular injection at a dose of 1mg/50kg b.wt.(Egyptian Co. for Chemical and Pharmaceutical-ADWIA, 10th of Ramadan City (Pekmezci and Cakiroglu , 2009).

2.2.4. Isolation and identification of bacteria:

Nasal swap samples were collected following the antisepsis procedure to prevent possible bacterial contamination risk and placed in transport mediums (Eurotubo collection swab, Delta lab, Spain). Swab sample were cultured in 5% sheep blood agar and MacConkey Agar. Plates were inoculated and incubated aerobically and anaerobically at 37°C for 24-72 hours. Bacteria were identified (Ozkanlar et al., 2012). *Streptococcus spp*. and *Staphylococcus spp*. growing in the media were examined in terms of colony structure, Gram staining, microscopic appearance.

2.2.5.Blood samples :

Blood samples were collected from the jugular vein of each animal just before treatment, 3 and 10 day post treatment. Sample (1) was 2ml of blood collected in tube containing heparin (50i.u/ml) used for plasma fibrinogen estimation and cellular immunity tests. Sample (2) was 1 ml of blood collected on di-potassium salt of EDTA for hematological examination. Sample (3) was 3

ml of blood taken without anticoagulant collected in a clean and dry tube and left to clot at room temperature for about 2 hours and centrifuged at 3000 rpm for 15 min. Serum samples were drawn in dry clean capped tubes and kept in deep freeze at – 20 ^{o}C for protein electrophoresis, IgG, IgM and estimation of serum haptoglobin.

I- Hematological studies:

Total and differential leukocytic counts determined by automatic vet CBC counter (Sysmex xt 2000 iv Corporation, KOBE, Japan) in Animal Health Research Institute (Zagazig provincial Lab.) according to the manufacturer instructions.

II-Acute phase proteins:

-Plasma fibrinogen concentrations was measured according to a method of (Becker et al., 1984).

- Serum haptoglobin was determined photometrically as described by Makimura and Suzuki (1982).

III-Immunological studies :

• Humoral immune response

a- Immunoglobulin:

Serum samples were used for determination of immunoglobulin M (IgM) according to Naito (1986), while immunoglobulinG (IgG) were measured according to Dati et al. (1989).

b- Proteinogram:

Total protein was measured in Animal Health Research Institute (Dokki, Lab.) according to Henry (1964). Electrophoretic analysis was carried out for determination of serum albumin, alpha, beta and gamma globulins according to the technique described by Davis (1964).

• Cellular immune response:

Phagocytic activity and phagocytic index:

a) Separation of peripheral blood mononuclear cells:

Peripheral blood mononuclear cells (PBMC) were isolated according to the method described by Goddeeris et al. (1986). In brief, 2 ml heparinized blood was harvested from each calf and then diluting the blood 1:2 in heparinized (10 I.U. heparin/ml) PBS. The diluted blood was overlaid on the surface of lymphocyte separation medium Ficoll-Hypaque (1:1) in sterile centrifuge tubes, and centrifuged for 15 min at 80 xg. The PBMC was aspirated by 5 ml pipette from the interphase layer, diluted with an equal volume of heparinized PBS and pelleted by centrifugation at 800 xg for 15 min. The PBMC was washed three times (3x) in heparinized PBS by repeated pipetting with 10 ml heparinized PBS, resuspension and centrifugation.

b) Phagocytic Assay:

To assess the cell phagocytic activity, was added 0.25 ml of adjusted viable leukocytes suspension to 0.25 ml heat inactivated *C. albicans* in a sterile plastic tubes. The tubes were incubated at 37ºC for 30 minutes in a humidified CO_2 incubator. Subsequently, the tubes were centrifuged at 2500 rpm for 5 minutes and the supernatant was removed with Pasteur pipette leaving a drop .Smears were prepared from the deposit, dried in air and stained with Leishman`s stain.

c) Evaluation of phagocytic activity:

Under a light microscope using oil immersion lens, a total number of 100 phagocytic cells were counted randomly in about ten microscopic fields. The number of ingested yeast cells in each individual phagocytes were determined to calculate the phagocytic cell activity in each of the tested group. The phagocytic-activity is considered as the percentage of phagocytic cells by microscope field. The phagocytic-index is the mean number of *C.albicans*, ingested by one phagocytic cell.

Statistical analysis:

Statistical analysis was performed using the statistical software package SPSS for windows (Version 18.0; SPSS Inc., Chicago, IL). The significance of differences between the experimental groups were evaluated by one-way analysis of variance (ANOVA) (Kinnear and Gray, 2006).

3. RESULTS

3.1. Results of bacteriological examination:

The bacteriological examination of the cultured swabs from the diseased animals revealed that the isolated bacterial pathogens were *E.coli, Pasteurella* spp., *Klebsiella and Staph Aureus* .Table (1).

3.2.Leukogram results:

Concerning to leukogram, diseased calves with BRD showed leukocytosis associated with neutrophilia, monocytosis, eosinophilia and lymphocytopenia before treatment. Treated calves with draxxin in regard to TLC, eosinophil and monocyte and neutrophils count revealed significant decrease, lymphocyte count and basophil recorded non-significant changes when compared with diseased group. While, treated groups with draxxin plus meloxicam and draxxin plus levamisole showed significant decrease in TLC, eosinophil , monocyte and neutrophils count decreased, lymphocyte count showed significant increase when compared with infected control group .Table (2&3).

3.3.Protein electrophoresis results:

The protein electrophoresis of diseased calves showed significant decrease in total protein and albumin. Total protein demonstrated significant increase in all treated groups especially in group treated with draxxin plus levamisole when compared with diseased group. Serum albumin showed significant increase while, total globulin, α-globulin, β-globulin and γ-globulin showed significant decrease in group treated with draxxin plus meloxicam when compared with diseased calves. Table (4&5).

3.4.Inflammatory markers results:

Diseased calves suffering from pneumonia revealed significant increase in haptoglobin and fibrinogen. Haptoglobin and fibrinogen exhibited significant decrease in all treated groups when compared with infected control group. Table (6&7).

3.5.Immunity results:

Concerning to immunoglobulin (IgG and IgM) results in diseased calves revealed significant increase when compared with control group. While, in all treated groups showed significant decrease when compared with diseased group before treatment . Cellular immunity (phagocytic index and phagocytic%) showed significant decrease in diseased groups in comparison with control group . While, in all treated groups revealed significant increase which more pronounced in group treated with draxxin plus levamisole in comparison with diseased group before treatment. Table (8&9).

Table (1): Prevalence of the isolated microorganisms from the nasopharyngeal swabs of the pneumonic cattle-calves: (Total number of the examined animals = 20)

Isolated bacteria	*E.coli*	*P. multocida*	*Klebsiella*	*S. aureus*	*Total No.*
No. of positive animals	5	8	4	3	20
%	25	40	20	15	100

Table (2):Leukogram of healthy and diseased calves before and 3 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
T.L.C.	9.14	13.43	11.07	10.47	11.44
$x10^3$ /µl	± 0.50 [c]	± 0.14 [a]	± 0.35 [b]	± 0.20 [b]	± 0.46 [b]
Neutrophils	4.50	7.72	5.22	4.55	5.02
$x10^3$/µl	± 0.53 [b]	± 0.24 [a]	± 0.27 [b]	± 0.21 [b]	± 0.20 [b]
Lymphocytes	3.89	3.77	4.03	4.47	5.03
$x10^3$/µl	± 0.18 [b c]	± 0.22 [c]	± 0.28 [b c]	± 0.14 [b]	± 0.12 [a]
Monocytes	0.35	0.99	0.92	0.74	0.75
$x10^3$/µl	± 0.04 [c]	± 0.02 [a]	± 0.03 [a]	± 0.03 [b]	± 0.04 [b]
Eosinophils	0.26	0.84	0.82	0.61	0.52
$x10^3$/µl	± 0.03 [c]	± 0.03 [a]	± 0.05 [a]	± 0.03 [b]	± 0.05 [b]
Basophils	0.14	0.11	0.09	0.10	0.12
$x10^3$/µl	± 0.02 [a]	± 0.03 [a]	± 0.02 [a]	± 0.03 [a]	± 0.02 [a]

Results are expressed as mean ± S.E.M.
Different superscripts (a,b,c) within the same rows indicate significant differences at $P \leq 0.05$.

Table (3):Leukogram of healthy and diseased calves before and 10 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
T.L.C.	9.04	13.43	10.13	9.78	9.85
$x10^3$ /µl	± 0.18 [c]	± 0.14 [a]	± 0.26 [b]	± 0.27 [b]	± 0.22 [b]
Neutrophils	3.83	7.72	4.06	3.95	4.01
$x10^3$/µl	± 0.11 [b]	± 0.24 [a]	± 0.09 [b]	± 0.24 [b]	± 0.19 [b]
Lymphocytes	4.37	3.77	4.29	4.63	4.49
$x10^3$/µl	± 0.29 [a]	± 0.22 [b]	± 0.11 [a b]	± 0.06 [a]	± 0.28 [a]
Monocytes	0.47	0.99	0.90	0.53	0.77
$x10^3$/µl	± 0.04 [d]	± 0.02 [a]	± 0.04 [b]	± 0.05 [d]	± 0.04 [c]
Eosinophils	0.27	0.84	0.79	0.58	0.50
$x10^3$/µl	± 0.14 [c]	± 0.03 [a]	± 0.04 [a]	± 0.03 [b]	± 0.04 [b]
Basophils	0.10	0.11	0.09	0.09	0.08
$x10^3$/µl	± 0.01 [a]	± 0.03 [a]	± 0.02 [a]	± 0.02 [a]	± 0.02 [a]

Results are expressed as mean ± S.E.M.
Different superscripts (a,b,c) within the same rows indicate significant differences at $P \leq 0.05$.

Table (4):Proteinelectrophoresis of healthy and diseased calves before and 3 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
Total protein g/dl	7.06 ± 0.06 [b]	7.35 ± 0.06 [a]	7.36 ± 0.05 [a]	7.32 ± 0.05 [a]	7.44 ± 0.03 [a]
Albumin g/dl	3.70 ± 0.03 [a]	3.26 ± 0.01 [d]	3.47 ± 0.03 [c]	3.56 ± 0.02 [b]	3.52 ± 0.02 [b c]
α-Globulin g/dl	0.86 ± 0.02 [c]	1.02 ± 0.02 [a]	0.94 ± 0.01 [b]	0.90 ± 0.02 [b c]	0.93 ± 0.02 [b]
β-Globulin g/dl	0.88 ± 0.01 [d]	1.02 ± 0.02 [a]	0.98 ± 0.01 [b]	0.92 ± 0.01 [c]	0.99 ± 0.01 [a b]
γ -Globulin g/dl	1.62 ± 0.04 [b]	2.04 ± 0.04 [a]	1.97 ± 0.04 [a]	1.93 ± 0.04 [a]	2.00 ± 0.03 [a]
T-globulin g/dl	3.36 ± 0.06 [d]	4.09 ± 0.05 [a]	3.89 0.04 [b c]	3.75 ± 0.05 [c]	3.92 ± 0.04 [b]

Results are expressed as mean ± S.E.M.
Different superscripts (a,b,c,d) within the same rows indicate significant differences at *P≤ 0.05.*

Table (5):Proteinelectrophoresis of healthy and diseased calves before and 10 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
Total protein g/dl	7.08 ± 0.04 [c]	7.35 ± 0.06 [ab]	7.25 ± 0.04 [b]	7.30 ± 0.04 [b]	7.43 ± 0.02 [a]
Albumin g/dl	3.74 ± 0.03 [a]	3.26 ± 0.01 [d]	3.51 ± 0.03 [c]	3.65 ± 0.02 [b]	3.59 ± 0.01 [b]
α-Globulin g/dl	0.85 ± 0.01 [d]	1.02 ± 0.02 [a]	0.92 ± 0.01 [b]	0.88 ± 0.01 [c d]	0.90 ± 0.01 [bc]
β-Globulin g/dl	0.88 ± 0.01 [c]	1.02 ± 0.02 [a]	0.94 ± 0.01 [b]	0.90 ± 0.01 [c]	0.96 ± 0.004 [b]
γ -Globulin g/dl	1.61 ± 0.04 [c]	2.04 ±0.04 [a]	1.87 ±0.02 [b]	1.86 ± 0.03 [b]	1.97 ± 0.02 [a]
T-globulin g/dl	3.34 ± 0.05 [d]	4.09 ± 0.05 [a]	3.73 ± 0.02 [b c]	3.64 ± 0.03 [c]	3.83 ± 0.02 [b]

Results are expressed as mean ± S.E.M.
Different superscripts (a,b,c,d) within the same rows indicate significant differences at *P≤ 0.05*.

Table (6): Inflammatory markers of healthy and diseased calves before and 3 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
Haptoglobin *mg/dl*	1.30 ± 0.04 [e]	6.10 ± 0.30 [a]	4.40 ± 0.07 [b]	2.11 ± 0.09 [d]	3.61 ± 0.07 [c]
Fibrinogen *mg/dl*	512.80 ± 1.59 [e]	844.60 ±3.14 [a]	746.80 ±5.29 [b]	551.80 ±4.97 [d]	621.00 ±5.14 [c]

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

Table (7): Inflammatory markers of healthy and diseased calves before and 10 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
Haptoglobin *mg/dl*	1.30 ±0.04 [e]	6.1 ±0.3 [a]	3.5 ±0.09 [b]	1.6 ±0.03 [d]	3.10 ±0.03 [c]
Fibrinogen *mg/dl*	511.80 ±2.89 [b]	844.60 ±3.14 [a]	532.80 ±8.90 [b]	518.80 ±3.62 [b]	524.80 ±2.46 [b]

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at $P \leq 0.05$.

Table (8):Hummoral and cellular immunity of healthy and diseased calves before and 3 days post treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin + Levamisole
IgM *g/L*	6.14 ± 0.62 [e]	18.71 ±0.26 [a]	15.35 ±0.47 [b]	11.59 ±0.53 [d]	13.68 ±0.22 [c]
IgG *g/L*	2.22 ± 0.24 [c]	7.88 ±0.37 [a]	7.21 ±0.24 [a]	4.26 ±0.09 [b]	4.98 ±0.28 [b]
Phagocytic index	6.70 ± 0.12 [a]	3.19 ± 0.11 [d]	3.74 ± 0.05 [c]	4.74 ± 0.09 [b]	4.78 ± 0.08 [b]
Phagocytic %	76.60 ± 0.93 [a]	59.00 ± 0.71 [d]	64.00 ± 0.55 [c]	71.00 ± 0.95 [b]	74.60 ± 0.51 [a]

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at *P≤ 0.05.*

Table (9):Hummoral and cellular immunity of healthy and diseased calves before and 10 dayspost treatment (n=5)

Groups / Parameters	Healthy control calves	Diseased calves before treatment	Diseased calves after treatment with		
			Draxxin	Draxxin+ Meloxicam	Draxxin +Levamisole
IgM *g/L*	6.38 ±0.69 [e]	18.71 ±0.26 [a]	11.19 ±0.42 [b]	8.33 ±0.27 [d]	9.26 ± 0.36 [c]
IgG *g/L*	2.10 ±0.18 [d]	7.88 ±0.37 [a]	5.54 ±0.16 [b]	3.45 ± 0.19 [c]	3.56 ±0.20 [c]
Phagocytic index	5.76 ± 0.098 [a]	3.19 ± 0.11 [d]	4.16 ± 0.05 [c]	5.00 ± 0.07 [b]	5.16 ± 0.07 [b]
Phagocytic %	74.00 ± 0.55 [a]	59.00 ± 0.71 [c]	68.80 ± 0.66 [b]	72.43 ± 0.75 [a]	73.68 ± 0.43 [a]

Results are expressed as mean ± S.E.M.

Different superscripts (a,b,c,d,e) within the same rows indicate significant differences at *P≤ 0.05.*

4. DISCUSSION

The bacteriological examination of the cultured swabs from the diseased animals revealed that the isolated bacterial pathogens were *E.coli, Pasteurella* spp., *Klebsiella and Staph* .This results the same as reported by Aytekin et al., (2010).

Concerning to the leukogram, diseased calves with BRD showed leukocytosis associated with neutrophilia, monocytosis, eosinophilia and lymphocytopenia before treatment. Such findings indicated the presence of inflammation and suppuration caused by the bacterial infection and the response of leukogram to the inflammatory lung disease (Coles, 1986). Our findings are similar to those previously cited by Faris et al. (2010) and Eleiwa et al.(2014).

Treated calves with draxxin in regard to TLC, eosinophil and monocyte and neutrophils count revealed significant decrease and lastly, lymphocyte count and basophil recorded non-significant changes when compared with diseased group. Similar results obtained by Crew et al. (2013) and Eleiwa et al. (2014). Neutrophilia may occur due to neutrophils provide the first line of defence against pathogens when calves suffer from BRD (Janeway et al., 2005).

Regarding to leukogram in treated groups with draxxin plus meloxicam showed significant decrease in TLC, eosinophil , monocyte and neutrophils count comparativly with diseased group while, lymphocyte count and basophil recorded non-significant changes when compared with diseased group. This results agree with Faris et al.(2010) who attributed this to meloxicam which has a non-steroidal structure, and acts on the archidonic acid cascade in the inflammatory process by inhibiting the production of prostaglandin H2 through the enzyme cyclo-oxygenase (COX).While, treated group with draxxin plus levamisole revealed that TLC, neutrophil, lymphocyte, monocyte count and eosinophil recorded significant decrease while, basophil showed non-significant change compared with infected control group. This attributed to that levamisole have cholinergic effect on leukocytes and also increases protein and nucleic acid synthesis in resting leukocytes,

thus causing leukocytosis Bourne et al. (1978).

The protein electrophoresis of diseased calves showed significant decrease in total protein and albumin. Similar results obtained by Csilla et al. (2013). The significant decrease of serum albumin in diseased calves could be attributed to the destructive effect of bacteria and its toxins on the liver cells which are the main sources of albumin and protein synthesis in the body McPherson (1984). A significant increase in α-globulin indicating tissue damage results from infection or inflammation (Faris et al., 2010) .The β-globulin and γ-globulin showed higher values indicating the activation of the immune defense of calves due to the infection (Coles, 1986).

Treated groups with draxxin revealed that serum total protein and albumin demonstrated significant increase compared to diseased calves before treatment. Meanwhile, total globulin (α-globulin, β-globulin and γ-globulin) revealed significant decrease compared to diseased calves before treatment. Similar results reported by Bednarek et al.(1999).Although many antimicrobial agents have been reported to cause immune-suppression effect in animals, the macrolide antibiotics enhance the immune function by activation of the peripheral blood leukocytes function, cytokine production, increase random migration of neutrophils and their phagocytic activity after one week post-treatment of the bronchopneumonia in calves (Bednarek et al., 1998).

Treated groups with draxxin plus meloxicam the serum albumin showed significant increase and reached to the highest value. Meanwhile, total globulin (α-globulin, β-globulin and γ-globulin) revealed significant decrease compared to diseased calves and reached to the lowest value.This results attributed to that meloxicam produce analgesia and reduce inflammation by inhibiting the enzyme of cyclooxygenase (COX) and subsequent prostaglandin production in the peripheral tissues and central nervous system (Friton et al., 2005 and Francoz et al., 2012).

Treated group with draxxin plus levamisole revealed that total protein demonstrated significant increase and recorded the highest value. Serum albumin showed significant increase. Meanwhile, total globulin, α-globulin, β-globulin and γ-globulin showed significant decrease compared to diseased group. Our finding agree with Garszon (1992) and Holcombe et al.(1998) This results attributed to that levamisole enhance the immune response and act as immune-stimulants, immune-potentiators (Blecha, 1988).

Diseased calves suffering from pneumonia revealed significant increase in haptoglobin and fibrinogen. Our results were paralleled to the finding of Angen et al. (2009) who reported that the haptoglobin might be the best choice for detecting respiratory diseases under field conditions and to distinguish between calves requiring anti-inflammatory drug.

Treated groups with draxxin alone, draxxin plus meloxicam ,draxxin plus levamisole exhibited significant decrease in haptoglobin and fibrinogen in all treated groups which were more pronounced decrease in group treated with draxxin plus meloxicam. Similar results agree with Lynch et al., (2010) and Richeson et al. (2013).

Treated groups with draxxin, draxxin plus meloxicam and lastly draxxin plus levamisole IgM and IgG results revealed significant decrease compared to diseased group but reached the lowest value in group treated with draxxin plus meloxicam. These results agree with Perino (1997) and Galyean et al. (1999) who reported that feedlot respiratory morbidity was associated with lower serum plasma protein, but not serum IgG, at 24 h after birth. Calves with inadequate plasma proteins at 24 h after birth had a greater risk of morbidity and respiratory tract morbidity in the feedlot compared with calves that had adequate plasma protein.

Concerning to cellular immunity of the diseased calves phagocytic index and phagocytic percent showed significant decrease .This results agree with Chirase et al. (2004) and Carroll et al. (2007). Treated

group with draxxin plus levamisole results of cellular immunity (phagocytic index and phagocytic%) revealed significant increase. Similar results were obtained by Bellavite et al. (2006) who reported that levamisole has a broad range of effects on the immune system by stimulating cell mediated immunity through potentiating the rate of T-lymphocyte differentiation, the responsiveness to antigen and mitogens and the activity of effector lymphocytes. Furthermore, levamisole stimulate phagocytosis and chemotaxis for neutophil and monocytes.

5. CONCLUSION

It could be concluded that, the use of draxxin plus levamisole as alternative drug in calves which suffered from BRD provide enhancement of cellular immunity. Also, combination of meloxicam with draxxin was superior to the antibiotic alone.

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