

SERUM BIOCHEMICAL AND HAEMATOLOGICAL STUDIES IN CATTLE INFECTED WITH EPHEMERAL FEVER (THREE DAYS SICKNESS) VIRUS

A.D. HELAL * and M.H.H. AHMED **

*Animal Health Research Institute (Banha Branch, Chemistry Department)

** Animal Health Research Institute (Virology Department, Dokki,Giza)

Received: 12. 10. 2002.

Accepted: 2. 3. 2003.

SUMMARY

Eighty male cattle (6 - 8 months age) were divided into four equal groups, the 1st., group is the normal (negative control) group, the 2nd., 3rd., and 4th., groups showed clinical signs of Bovine Ephemeral Fever (BEF) disease. The 2nd., group was left untreated and considered as the positive control, the 3rd., and 4th., groups were therapeutically treated with flumequine and oxytetracycline hydrochloride antibacterials, respectively (once a day for 3-successive days). Results revealed isolation and identification of the BEF-virus from the 2nd., 3rd., and 4th., groups. The oxytetracycline treated group showed significant increase of the specific antibody titer against BEF-viral infection, lymphocyte percentage and the immunoglobulins than that of BEF-infected non-treated positive control cattle. The alpha- (α) globulins increased significantly in positive control animals than that of normal negative control

ones. Total globulins decreased significantly and the neutrophil percentage increased significantly in all BEF diseased cattle than that of normal animals. Serum iron, cobalt, copper and zinc were significantly decreased in all BEF infected groups than that of normal group. Serum Lead significantly increased only by either flumequine or oxytetracycline treated animals than in normal animals. Except by flumequine treatment, serum sodium increased significantly and potassium decreased significantly in BEF diseased cattle than that of normal negative control animals. Total bilirubin significantly increased only by either flumequine or oxytetracycline treatment than that in normal cattle.

It could be concluded that oxytetracycline antibiotic should be the recommended therapy during the course of BEF disease in cattle because it improves hypogammaglobulinemia and lymphopenia that induced in BEF infected cattle. Also,

copper, iron, cobalt, zinc, selenium and potassium therapy should be administered to substitute their deficiencies in BEF infected cattle. Alkaline diet and calcium gluconate therapy should be advised to demobilizing the excess of lead from circulation into the bones and soft tissues in cases of oxytetracycline treatment of BEF-diseased cattle.

INTRODUCTION

Bovine Ephemeral Fever (Three days sickness or stiff sickness) is a non-contagious viral disease of cattle and buffalo caused by unclassified arbovirus of the family Rhabdoviridae (Mathews, 1982). The mosquitoes of various anopheline or culicine species are likely to be the more efficient vectors of the virus transmission (St-George et al., 1993). Also, a potential for wind-borne spread of this viral disease has been recorded (Davies et al., 1993).

The major clinical signs of Bovine Ephemeral Fever disease are sudden pyrexia, watery nasal discharges, lacrimation, diarrhoea, recumbency (especially in calves), lameness (especially in heifers) and sudden drop in milk yield in lactating cattle (Sharma, 1992). Differential diagnosis should be done with botulism, emphysema, acute pulmonary oedema, diploëdiosis and milk fever (Coetzer et al., 1993 and St-George et al., 1995).

The principal post mortem findings of the Bovine Ephemeral Fever (BEF) disease in Chinese cattle

were serofibrinous arthritis (59%), interstitial emphysema (31%), nephritic infarcts (69%), bronchopneumonia (9%) (Guifang et al., 1992). Neutrophilic leucocytosis and lymphopenia were the major haematological findings in BEF-infected cattle (Odiawo, 1989 and Prasad et al., 1997).

Chemical changes of the blood of BEF infected cattle included selenium deficiency which was detected only in animals with respiratory distress and subcutaneous emphysema (Odiawo, 1989). Serum calcium, zinc, iron and the pH were reduced, while serum copper was elevated (Uren et al., 1992). Plasma ammonia and the non-esterified fatty acids were elevated (St. George et al., 1995). Plasma fibrin was elevated and inorganic phosphate was reduced (St. George et al., 1995). Activities of serum alanine- and aspartate-aminotransferases and creatine phosphokinase enzyme were increased together with the concentrations of serum bilirubin and urea (Sayed et al., 2001).

Trials for prophylactic and/or supportive treatment of BEF aiming at reducing fever, inflammation and secondary bacterial infections were recorded by several authors. Such trials included administration of novalgine, oxytetracycline and liver extract-B complex (Sharma, 1992), ketoprofen for treatment of locomotor symptoms (Fetrick and Daniel, 1996), and bovirum as digestive system stimulant (Prasad et al., 1997).

The BEF-viral disease was isolated and identified

n Egypt by (Nawal et al., 2001 and Sayed et al., 2001) following the outbreak in summer 2000. The present study was established to examine the efficiency of the treatment of secondary bacterial agents which may be associated with BEF disease with flumequine antibacterial (which was previously proven as an immunostimulant drug against Newcastle viral disease in birds by Rzedzicki et al., 1991) . Also to compare this treatment with that of oxytetracycline which has been used as a field antibiotic by many veterinarians in Egypt to reduce the mortality and morbidity rates of BEF disease in cattle.

MATERIAL AND METHODS

Eighty small male cattle (6 - 8 months age) from a private farm in Kaleubia province were used in the present study. The animals were divided into four groups (20 each) as follow:- the 1st., group was apparently healthy and was considered as normal (negative) group, the 2nd., group showed the clinical signs of BEF disease and not given any treatment and considered as a positive control group, the 3rd., group showed the clinical signs of BEF disease and intramuscularly (I/M) injected with 10mg/kg. B. wt. of the antibacterial drug Flumequine (Uniquine, from Amoun Pharmaceutical Co.) once a day for three successive days, the 4th., group showed also the clinical signs of BEF disease and I/M injected with 3mg/kg. b. wt. of oxytetracycline hydrochloride antibiotic (Pan Terramycine, from Pfizer Co.) once a

day for three successive days. At the 4th., day from starting of treatment, blood samples were taken from each animal of the four groups and sera were separated and kept frozen at - 20°C till carrying out the immunochemical investigations. Blood samples (with EDTA anticoagulant) were taken also from each animal of the 4 groups for carrying out the differential leucocytic count (Schalm, 1961) and for isolation and identification of BEF-virus. The reference BEF-virus and BEF-antiserum were kindly obtained from Virology Department of the Animal Health Research Institute, Dokki, Cairo (origin: Plum Island, USA). Buffy coat of the whole blood from each animal was intracerebrally injected into a baby mouse (1-3 day age), the inoculated mice were observed daily for any nervous manifestations or deaths. Virus isolation was attempted from the buffy coats preparations of EDTA treated blood by inoculation of three blind passages into confluent sheet of VERO-cells which were observed daily for cytopathic effect (CPE) according to (ST. George et al., 1978). Corresponding serum from cattle groups were used for serological identification of BEF-virus using Serum Neutralization Test (SNT). The reciprocal of the highest serum dilution that completely inhibited the appearance of the viral cytopathic effect (CPE) was taken as the SN-titer. The SNT was carried out according to (Cybinski, et al., 1978). Serum samples were also used for determination of some micro-(trace) and macro elements (copper, iron, cobalt, zinc, selenium, lead, sodium and

potassium) by Atomic absorption spectroscopy according to Schrenk (1975), determination of the different protein fractions using polyacrylamide gel electrophoresis (Gordon, 1980), total protein (Doumas et al., 1971), total lipids (Schmit, 1964), total bilirubin (Jendrassiki, 1938) and serum alanine amino transferase (ALT) enzyme activity (Reitman and Frankel, 1957).

Statistical evaluation of data was performed by analysis of variance (ANOVA) according to (Snedecor and Cochran, 1969).

RESULTS

1- Clinical signs:

Diseased cattle with BEF showed clinical signs of: fever, loss of appetite, cessation of rumination, stiff gait, respiratory manifestations, salivation and heavy movement.

2- BEF-Virus Isolation:

Isolated BEF-virus from cattle in relation to the total number of the tested groups were: 0.00%, 100%, 95% and 85% in the normal (negative) control, positive (non-treated) control, flumequine and oxytetracycline treated group respectively, as shown in table (1).

3- The specific antibody titers against BEF infection measured by Serum Neutralization Test (SNT):

Titers (log₁₀-values of the reciprocal titers) of the specific antibodies against BEF viral infection of the four groups of cattle determined by

SNT revealed that the oxytetracycline treated group showed significant increase of the antibody titers than that of either positive control and the flumequine treated group, as shown in table (2) and fig (1).

4- Immuno electrophoresis of the serum protein fractions:

Alpha (α) Globulin fraction showed a significant increase in the BEF-infected non treated (positive control) group than that of any other groups. The Beta - (β) - Globulin fraction showed non-significant changes between various groups of cattle. Gamma (γ)-Globulins (Immunoglobulins) decreased significantly in all BEF viral infected groups of cattle than that of the normal (negative) control ones. However, the γ -globulins of the oxytetracycline treated animals was significantly increased than that of either positive control or flumequine treated group of cattle. Total Globulins were decreased significantly in all BEF infected cattle than that of the normal negative control cattle. Both albumin and total protein showed no significant changes between groups, as shown in table (3) and figures (2 and 3).

5- Differential leucocytic count:

Neutrophil percentages significantly increased in BEF-infected cattle than that of the normal (negative control) cattle. Lymphocytes percentage decreased non significantly in positive control and oxytetracycline treated groups and significantly in flumequine treated group than that of the negative control one. In contrast-

the lymphocyte percentage of oxytetracycline treated group was increased significantly than that of either positive control or flumequine treated groups of cattle. Monocyte percentage decreased by all BEF infected groups than that of negative (normal) control, but the decrease only significant by oxytetracycline treated group. Both eosinophils and basophils non-significantly changed, as shown in table (4) and Fig. (4).

Serum Micro (Trace) and Macro-Elements:

The trace elements cobalt, copper, iron and zinc showed significant decrease in all BEF-diseased and treated groups of cattle than that of normal negative control (except the non-significant decrease of copper in positive control group). Also, the flumequine or oxytetracycline treated cattle showed significant decrease in cobalt, zinc and copper than that of positive control cattle. Lead significantly increased in groups treated with either flumequine or oxytetracycline than that of either

normal control or infected, non-treated control group of cattle. The macroelement potassium significantly decreased only by flumequine treated cattle than of negative control and other groups, but sodium increased significantly by all BEF infected cattle (except in flumequine treated group where the increase was non-significant). Selenium was deficient in all studied animals as tabulated in table (5) and illustrated in Figure. (5).

7- Some Biochemical Constituents of Serum:

Concentration of total bilirubin was increased significantly in either flumequine or oxytetracycline treated groups than that of either negative or positive control animals. Concentrations of total lipids, total proteins and creatinine and the activity of alanine amino transferase (ALT) enzyme were non-significantly changed than normal (negative) control group as tabulated in table (6) and illustrated in Figure (6).

Table (1): Isolation of Bovine Ephemeral Fever (BEF) -virus from the four groups of examined cattle.

Cattle Groups	Total Examined Animals	Number of BEF infected Cattle	Percent of infected animals to the total No.
Normal (negative) control	20	0.00	0.00%
Positive control (infected-non-treated)	20	20	100%
Flumequine treated (infected)	20	19	95%
Oxytetracycline treated (infected)	20	17	85%

Table (2) : The Specific antibody titers (log₁₀ - values of the reciprocal titers) against BEF-viral infected cattle as measured by Serum Neutralization Test (SNT).

Cattle Groups	No. of examined animals	Antibody Titers (Means)	Log ₁₀ -values of the Reciprocal Titers (mean + SE)
Negative (normal control)	10	0.00	0.00 + 0.00 A
Positive control (infected-non-treated)	10	1/32	1.505 + 0.120 B
Flumequine treated (infected)	10	1/32	1.445 + 0.179 B
Oxytetracycline treated (infected)	10	1/128	2.107 + 0.085 C
F-Test			*
LSD (At P ≤ 0.05)			0.361

N.B - Different litters in the last column denote significant change at P < 0.05.

- * = Significant change at P ≤ 0.05 LSD = Least Significant Difference

Table (3): Serum protein fractions determined by polyacrylamid gel immunoelectrophoresis of serum of normal and BEF-infected cattle.

Cattle Groups	Alpha (α-) Globulins g./dl.	Beta (β) Globulins g./dl.	Gamma (γ) Globulins g./dl.	Total Globulins g./dl.	Albumin g./dl.	Total Proteins g./dl.
Negative control (non infected)	0.629 A ±0.014	0.562 A ±0.063	1.423 A ±0.181	2.614 A ±0.099	3.463 A ±0.113	6.077 A ±0.792
Positive control (infected)	0.889 B ±0.013	0.489 A ±0.102	0.783 B ±0.165	2.161 B ±0.140	5.224 A ±0.309	7.385 A ±0.759
Flumequine treated (infected)	0.636 A ±0.063	0.457 A ±0.114	0.868 B ±0.030	1.961 B ±0.245	4.411 A ±0.129	6.372 A ±0.382
Oxytetracycline treated (infected)	0.446 C ±0.070	0.508 A ±0.022	1.131 C ±0.101	2.083 B ±0.222	3.247 A ±0.245	5.330 A ±0.356
F-Test	*	N.S.	*	*	N.S.	N.S.
LSD (At P ≤ 0.05)	0.116	-	0.252	0.284	-	-

N.B - Different litters in columns denote significant change at P ≤ 0.05.

- * = Significant change at P ≤ 0.05 N.S. = Non-Significant change LSD = Least Significant Difference

Table (4): Differential leucocytic counts of normal and BEF- infected cattle.

Cattle Groups	Neutrophil %	Lymphocyte %	Monocyte %	Eosinophil %	Basophil %
Negative control (non infected)	22.966 A ±1.925	54.226 AC ±4.920	11.258 A ±2.340	9.098 A ±2.648	3.236 A ±0.706
Positive control (infected)	34.026 B ±0.550	48.42 AB ±2.224	6.296 AB ±0.746	9.800 A ±1.796	61.460 A ±0.232
Flumequine treated (infected)	38.18 C ±2.433	41.82 B ±2.433	10.91 A ±2.433	7.270 A ±1.649	1.820 A ±0.165
Oxytetracycline treated (infected)	29.63 D ±2.433	61.73 C ±3.225	3.700 B ±0.632	3.090 A ±0.316	1.850 A ±0.385
F-Test	*	*	*	N.S.	N.S.
LSD (At P ≤ 0.05)	3.956	10.348	6.032	-	-

N.B - Different litters in columns denote significant change at P ≤ 0.05.

- * = Significant change at P ≤ 0.05 N.S.= Non-Significant change LSD = Least Significant Difference

Table (5): Values of some serum macro - and micro(trace) - elements in normal and BEF-diseased cattle.

Cattle Groups	Iron ppm	Cobalt ppm	Zinc ppm	Copper ppm	Potassium ppm	Sodium ppm	Lead ppm	Selenium ppm
Negative control (non infected)	78.67 A ±3.162	1.450 A ±0.063	22.62A ±0.632	9.830A ±0.345	183.600A ±4.948	1230.1A ±6.325	3.500A ±0.228	0.00A ±0.00
Positive control (infected)	49.020 B ±3.373	0.930 B ±0.046	15.910B ±1.020	9.760A ±0.632	187.900A ±6.778	2545.1B ±5.831	2.130A ±0.118	0.00A ±0.00
Flumequine treated (infected)	53.360 B ±7.793	0.840 C ±0.033	5.080C ±0.316	8.230B ±0.707	164.000B ±4.492	1232.4A ±8.944	6.530B ±0.316	0.00A ±0.00
Oxytetracycline treated (infected)	49.060 B ±2.500	0.670 D ±0.032	5.650C ±0.316	8.240B ±0.570	189.500A ±7.094	1907C ±9.055	7.728B ±1.101	0.00A ±0.00
F-Test	*	*	*	*	*	*	*	N.S.
LSD (At P ≤ 0.05)	10.703	0.063	1.823	0.953	10.197	9.733	1.572	-

N.B - Different litters in columns denote significant change at P ≤ 0.05.

- * = Significant change at P ≤ 0.05 N.S.= Non-Significant change LSD = Least Significant Difference

Table (6) : Some Serum Biochemical constituents of Normal and BEF- infected Cattle.

Cattle Groups	Total lipids g./dl .	Total protein g./dl .	Total Bilirubin m mol./l .	ALT- Enzyme U/L	Creatinine mg./dl.
Negative control (non infected)	3.044 A ±0.021	6.077 A ±0.792	1.234 A ±0.182	11.948 A ±0.442	1.391 A ±0.087
Positive control (infected)	3.271 A ±0.222	7.385 A ±0.759	1.373 A ±0.030	14.673 A ±1.066	1.311 A ±0.096
Flumequine treated (infected)	3.070 A ±0.315	6.372 A ±0.382	1.562 B ±0.031	12.182 A ±0.473	1.376 A ±0.081
Oxytetracycline treated (infected)	3.302 A ±0.106	5.330 A ±0.356	1.816 B ±0.029	14.005 A ±0.555	1.301 A ±0.081
F-Test	N.S.	N.S.	*	N.S.	N.S.
LSD (At P ≤ 0.05)	-	-	0.267	-	-

N.B - Different litters in columns denote significant change at $P \leq 0.05$.

- * = Significant change at $P \leq 0.05$ N.S.= Non-Significant change LSD = Least Significant Difference

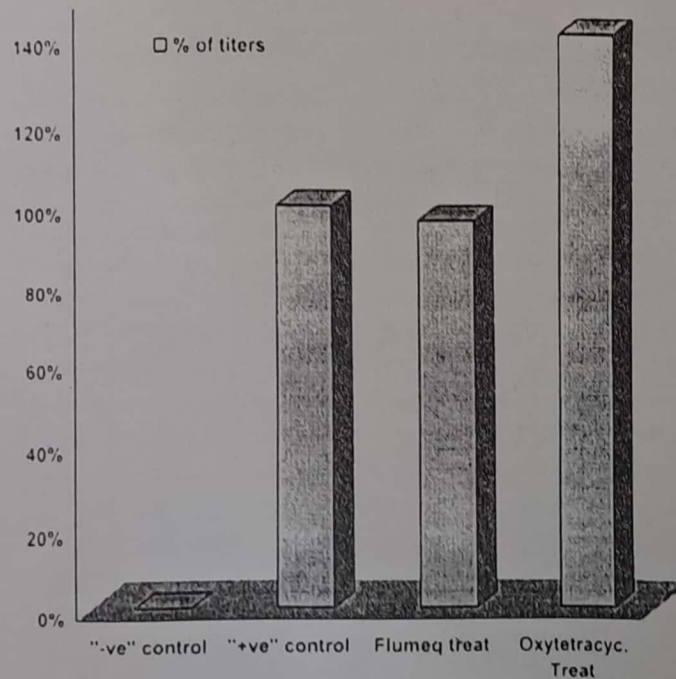


Fig. (1): The percentages of the specific antibody titers against BEF viral infected groups of cattle.

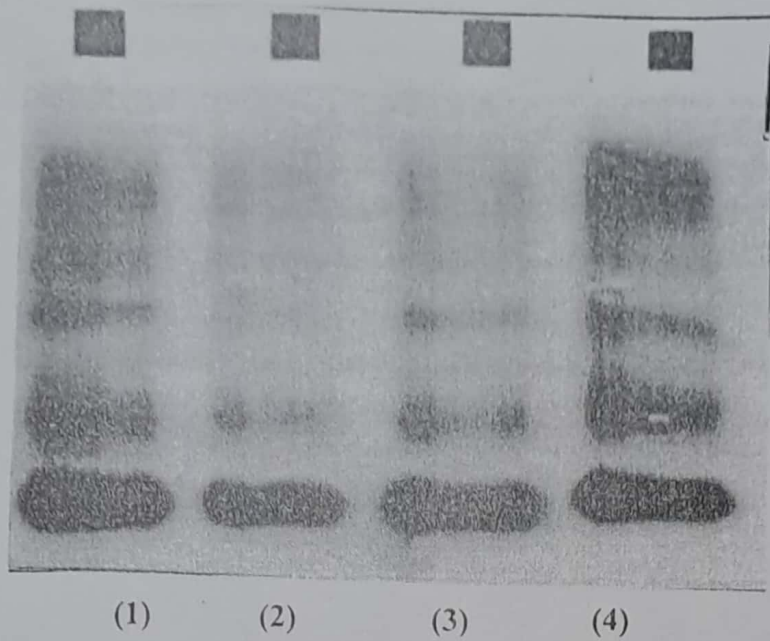


Fig. (2): The different protein fractions of the normal and the BEF infected cattle by polyacrylamid gel electrophoresis (1= oxytetracycline treated), (2= flumequine treated), (3= positive control, (4= negative control)

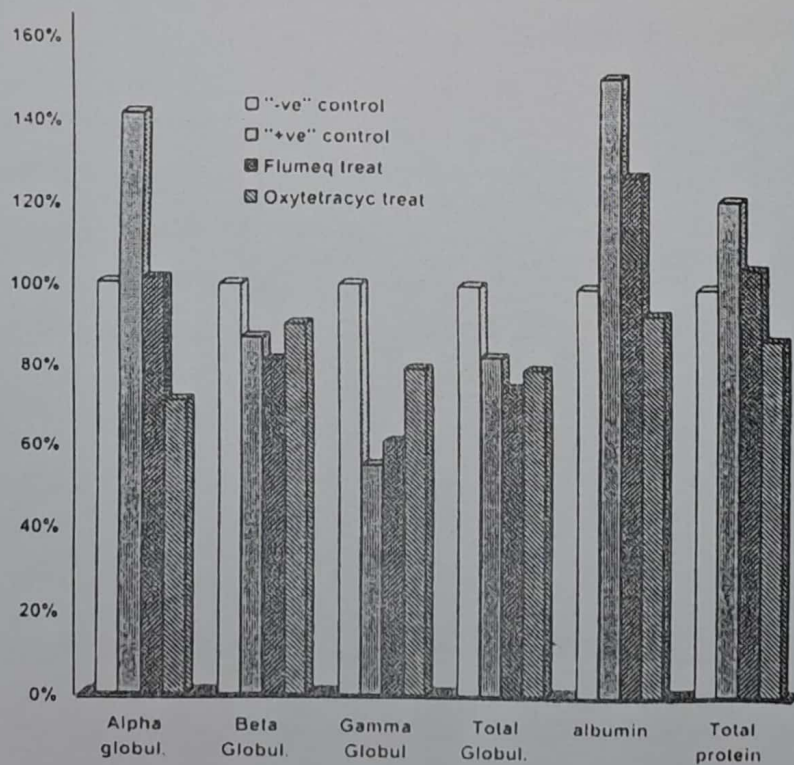


Fig. (3):The percentages of the different protein fractions in the normal and the BEF infected groups of cattle

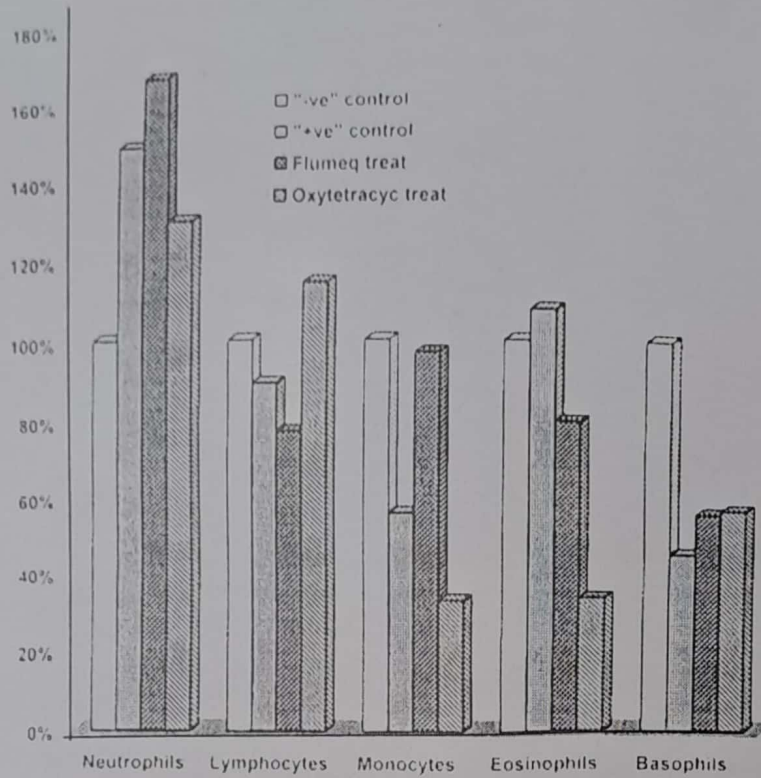


Fig. (4):The percentages of the differential leucocytic counts of the normal and the BEF infected groups of cattle.

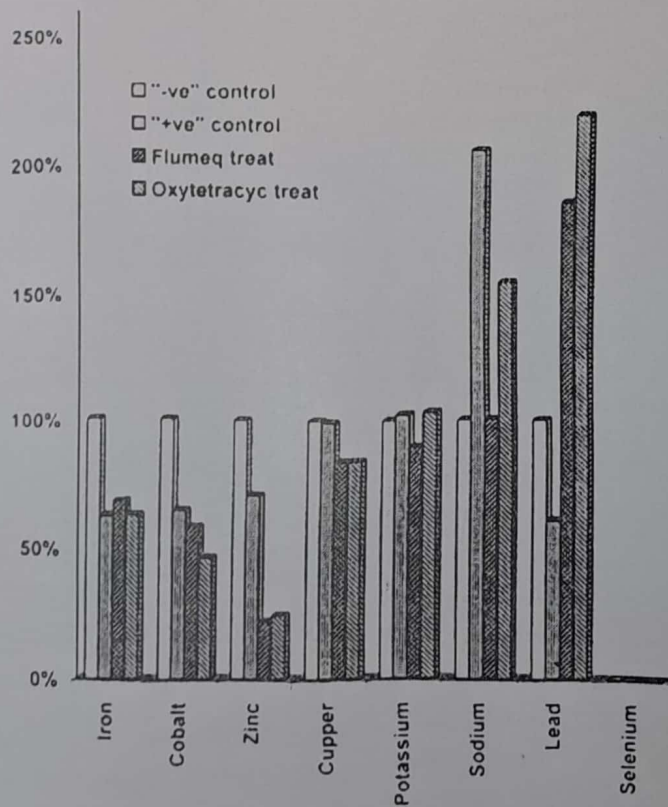


Fig. (5):The percentage of some serum micro- and macro elements in the normal and the BEF infected groups of cattle.

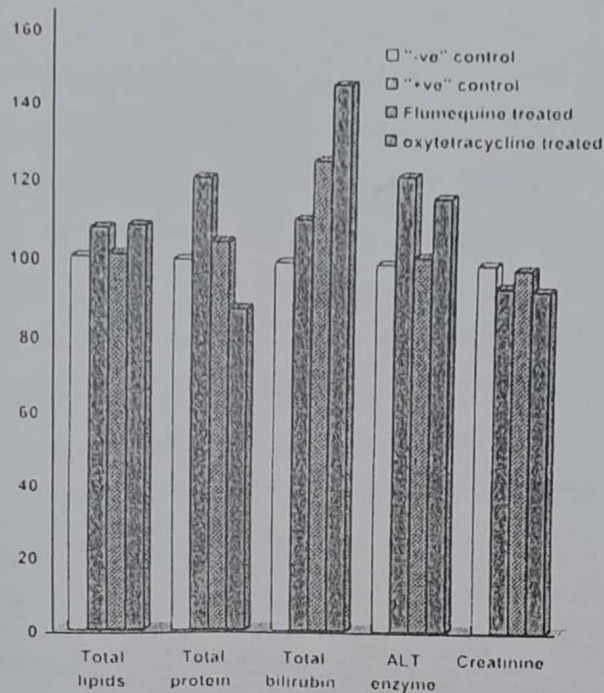


Fig. (6): The percentage of some serum biochemical constituents in the normal and the BEF infected groups of cattle

DISCUSSION

In Egypt, the Bovine Ephemeral Fever (BEF) disease was firstly described by (Piot, 1896) then by Rabagliati (1924) and recently by Hassan et al. (1991). In the summer 2000 and over, the disease was identified by Nawal et al. (2001) and Sayed et al. (2001) in most of the Egyptian Governo-rates. The disease resulted in great economic losses in Egyptian cattle. The BEF-virus was isolated and identified in cattle under the present study which gave positive Serum Neutralization Test compared to normal negative control cattle.

There were several methods used for treatment of BEF disease in cattle, aiming for controlling the secondary bacterial complications, pyrexia, ab-

normal metabolism and abnormal plasma constituents (especially in micro-and macro - elements) and for supporting the diseased animals (Sharma, 1992, ST - George et al., 1995 and Prasad et al., 1997).

In order to improve the morbidity and mortalities among BEF-infected Egyptian cattle, the efficiency of either flumequine or oxytetracycline antibacterials was evaluated in the present study. Oxytetracycline was used by many veterinarians in Egypt and the flumequine was previously proved as an immunostimulant against one of the avian viral diseases. Only oxytetracycline treatment could induce significant increase of immuno (Gamma)-globulins, lymphocyte percentage and specific antibody titer against BEF than that

of BEF infected non-treated (positive) control, suggesting that the oxytetracycline antibiotic treatment could improve the immunosuppressive effects caused by BEF viral infection in cattle.

There was a direct relationship between the increased levels of gamma (γ) globulins (Immunoglobulins) and the lymphocyte percentages as recorded by the present study. The γ -globulins are synthesized in the plasma cells which matured from B-lymphocytes in the spleen, bone marrow and lymph nodes (Mepheron, 1984). 20% of the circulating lymphocyte population are B-lymphocytes (Jain, 1986).

Flumequine treatment failed to significantly improve the hypogammaglobulinemia and lymphopenia (i.e. the immunosuppressive effects) caused by BEF viral infection in spite of its previously inducing an immunostimulant effect against Newcastle viral disease vaccine in birds as recorded by Rzedzicki et al. (1991). The reasons of the difference in results perhaps due to species difference or to the difference in the nature of the two antigens or to the difference between the actual infection response and the vaccination response. The response of the immune system of the host depends largely on the nature of the antigen and on the site where the host exposed to it (Loveren and Vos, 1989).

The present study revealed increase of alpha-globulin fraction in BEF infected cattle (positive

control) than that of the normal (negative) control cattle. Also, the flumequine and oxytetracycline treated groups showed significant decrease of alpha-globulins than that of positive (infected, non-treated) control. Kaneko (1989) reviewed that there are 13-types of alpha-globulins of diagnostic value, the elevated levels of some alpha-globulins have reported with some toxic chemicals (Dolezalova et al., 1983), protein catabolism or adrenal stimulation (Schalm, 1975).

The current work revealed presence of a significant increase of neutrophil percent in all BEF virus infected groups of cattle than that of normal control cattle. Neutrophils are the first line of cellular defence that respond to infectious agents, tissue injury, parasites and inflammatory or foreign materials. Neutrophils eliminate foreign materials via phagocytosis and may also have a role in cytotoxicity or lymphocyte stimulation (Jain, 1986).

The current study revealed that total bilirubin was increased in BEF-viral infected cattle than of normal control. This increase became significant by treatment with either flumequine or oxytetracycline. Sayed et al. (2001) recorded an elevation of serum bilirubin and ALT-enzyme activity in cattle infected with BEF- virus. Bilirubin is the bile pigment in which haemoglobin from damaged or aged erythrocytes is metabolized through a series of biochemical reactions (Hayes, 1989), so that the significant increase of bilirubin in the

groups treated with flumequine or oxytetracycline may be attributed to increased RBCs hemolysis by drug treatment, infection and/or hepatic dysfunction (Zimmerman, 1984).

Serum cobalt was significantly decreased in all BEF-viral infected cattle than that of normal control cattle as recorded by the current study, so that the cobalt should be supplied in diet of BEF infected cattle in order to avoid any sign of cobalt deficiency which are manifested by loss of appetite, reduced growth, emaciation, anaemia, fatty degeneration of liver and hemosiderosis of the spleen, as cobalt is a constituent of vitamin B12 (Church and Pond, 1988).

The current study revealed presence of significant decrease of serum iron in all BEF-infected cattle than that of normal control cattle. Uren et al. (1992) recorded also a fall of plasma iron levels in BEF infected cattle. 60 - 70% of the body iron is present in haemoglobin in RBCs and myoglobin of muscle, 20% of iron is stored in labile form in liver, spleen and other tissues to be available for haemoglobin formation, the remaining 10 - 20% is fixed firmly in unavailable forms as a component of muscle myosin and actomyosin and as a constituent of metalloenzymes (Moore and Dubach, 1962). Therefore Fe-dextrin should be administered to BEF infected cattle to avoid the microcytic hypochromic anemia (iron-deficiency anemia) as recorded by Underwood (1977).

Serum zinc was significantly decreased in all BEF-infected cattle than that of the negative control animals as revealed by our study. Zinc deficiency may lead to retardation of growth and bone formation, early embryo mortality or difficult parturition, hypogonadism in males, delayed wound healing, and decreased hepatic enzyme activities (Underwood, 1977), so that zinc therapy must be supplied during the course of BEF infection of Cattle.

Serum copper was significantly decreased in both flumequine or oxytetracycline treated cattle than either negative control or positive control groups of cattle. Copper deficiency may be attributed to antibacterial therapy. Copper deficiency induce anemia in cattle and sheep, shortening of RBCs life span, reduce absorption and utilization of iron and reproductive failure in Cattle (Cooke, 1983). Copper therapy should be accompanied oxytetracycline or flumequine treatment of BEF infected cattle. BEF diseased or drug treated cattle showed elevation of plasma copper levels as recorded by Uren et al. (1992).

Serum Lead was increased significantly by treatment with either flumequine or oxytetracycline. The increased serum lead levels by such antibacterial therapy may be attributed to the possible stimulation of RBCs hemolysis by these drugs, where more than 90% of lead in the blood is found in the haemoglobin and cell membrane of RBCs (Barltrop and Smith, 1971). This perhaps

explain the significant increase of total bilirubin in BEF infected cattle which were treated with either flumequine or oxytetracycline. The increased plasma lead should be demobilized from circulation into the bones and soft tissues with the help of alkaline diets and calcium gluconate (Kaye, 1980).

No selenium containing diet was given to all cattle under study, and no selenium could be detected by the method described in all studied animals. Odiawo (1989) reported that BEF infected cattle which suffered from selenium deficiency showed respiratory distress or subcutaneous emphysema. Selenium present essentially in the liver, kidney and muscle and its values in the tissue are affected by dietary intake. The concentration generally is less than 1 ppm., and its function is to maintain the integrity of cellular membrane (Church and pond, 1988). Addition of inorganic selenium to the diet of BEF infected cattle is essential to reduce the severity of the disease.

Flumequine treatment induced significant hypokalaemia. A critical osmotically active substance of the blood plasma is sodium and that of intracellular contents is potassium ions. Potassium provides for a stable osmotic pressure of the intracellular fluid, acetylcholine synthesis, generation of rest, and action potentials (Georgieva, 1989). In contrast to potassium, sodium of BEF-infected cattle was significantly increased than that of normal (negative) control (except by flumequine treated animals where the increase was non-significant). Sodium maintains a stable

osmotic pressure of the extracellular fluid, regulate acid-base status and generates membrane action potentials (Georgieva, 1989). Excess Sodium chloride may disturb water balance as a result of reducing the ability of kidney and intestine tract to remove excess water from the blood coupled with an excess of sodium ions (Medway and Kare, 1959).

According to our study, it could be concluded that oxytetracycline hydrochloride therapy should be recommended during the course of BEF- viral infection in cattle because it induced significant increase of gamma (immuno) globulins (gammaglobulinemia), lymphocyte percentage (lymphocytosis) and the specific antibody titer against BEF viral infection. Also, copper, iron, cobalt, zinc and selenium therapy should be recommended (parallel with oxytetracycline therapy) to substitute their significant decrease in the serum of infected animals. Alkaline diet and calcium gluconate may be advised to demobilize excess lead to the bones and soft tissues during the treatment of BEF - infected cattle.

REFERENCES

- Barltrop and smith (1971): Cited by Klassen et al. (1986).
- Church, D.C. and Pond, W.G. (1988): Basic Animal Nutrition and Feeding, 3rd., Ed., John Wiley & Sons PP. 181.
- Clarke, E.G.C. and Clarke, M.L. (1975): Veterinary Toxicology, 1st., Ed. The English language Book Society and Baillier Tindall PP: 50.
- Cooke, B.C. (1983): In Recent Advances in Animal Nutrition. W. Haresign (ed). Butter worths, London, Eng

Vet.Med.J.,Giza.Vol.51.No.2(2003)

- land.
- Doetzer, J.A.W.; ST-George, T.D.; Uren, M.F., Young, P.L. and Hoffmann, D. (1993): The Diagnosis and Differential Diagnosis of bovine ephemeral fever in southern Africa. Proceedings of the 1st., International symposium, Beijing, PRC, 25 - 27 August 1992 - 1993, 70 - 73; ASIAR Proceedings, No. 44; 34 ref.
- Cybinski, D.H.; ST-George, T.D. and Paull, N.J. (1978): A microtiter Serum Neutralization Test for bovine ephemeral fever virus. Aust. Vet. J. 54 : 1.
- Davies, F.G; Moussa, A.; Barsoum, G.; ST-George, T.D.; Uren, M.F.; Young, P.L and Hoffman, D. (1993): The 1990 - 1991 epidemic of ephemeral fever in Egypt and the potential for spread to the Mediterranean region. Proceedings of the 1st., International symposium held in Beijing, PRC, 25 - 27 August 1992 - 1993, 54 - 56; 11 ref; ACIAR proceedings No. 44.
- Dayan, A.D. and paine, A.J. (1989): Advances in Applied Toxicology, Chapter (7) Tylor and Francis Ltd.
- Doumas, B.T.; Watson, W.A. and Bigs, H.G. (1971): Kits for Determination of Serum Total Protein. Clin. Chem., Acta, 31 (1): 87.
- Dolezalova, V.; Stratil, P.; Simickova, M. et al., (1983): α -fetoproteins and macroglobulin as a markers of distinct response of hepatocytes to carcinogens in the rat: Carcinogenesis. Ann. N.Y. Acad. Sci. (417): 294-306.
- Fenwick, D.C. and Daniel, R.C.W. (1996): Evaluation of the effect of ketoprofen on experimentally induced ephemeral fever in dairy heifers. Australian veterinary Journal, 74 (1): 37 - 41.
- Georgieva, S.A. (1989): Essentials of physiology, English Translation, Mir publishers, Moscow. PP: 195.
- Gordon, A.H. (1980): Electrophoresis of proteins in polyacrylamide and starch gels. In laboratory techniques in biochemistry and molecular biology, T.S. work and E. work (Eds). Elsevier North Holland Biomedical press, Amesterdam, 213 pages.
- Guifang, C.; Zigang, Z.; Xunnan, Y.; Wenbin, B.; Chu, G.F.; Zhang, Z.G.; Yin, X.N.; Bia, W.B.; ST-George, T.D.; Uren, M.F; Young, P.L. and Hoffmann, D. (1993): Pathological changes in cattle experimentally infected with bovine ephemeral fever virus. Proceedings of the 1st., International symposium, Beijing, PRC, 25 - 27 August 1992 - 1993, 61 - 62 ACIAR proceedings, No. 44.
- Hassan, H.B.; El-Danaf, N.A.; Hafez, A.M.; Ragab, A.M. and Fatchia, M.M. (1991): Clinico-diagnostic studies on bovine ephemeral fever with detection of its virus for the first time in Egypt. J. Egypt. Vet. Méd. Ass., 51 (4): 873 - 887.
- Hayes, A.W. (1989): Principles and Methods of Toxicology, 2nd., Ed. Raven press, New York, PP: 485.
- Jain, N.C. (1986): Schalm's Veterinary Haematology, 4th., Ed. Lea & Febiger, Philadelphia.
- Jendrassiki, G.P. (1938): Vereinfachte photometrische methoden Zur bestimmung de blutbilirubins. Biochemical, 2. (297): 81.
- Kaneko, J.J. (1989): Clinical Biochemistry of Domestic Animals, chapter (6) PP: 142, 4th. Ed., Academic press, Inc.
- Kaye, S. (1980): Handbook of Emergency Toxicology (A guide for the Identification, Diagnosis and Treatment of Poisoning), 4th., Ed., Charles C. Thomas publisher, Springfield, Illinois, USA PP: 357.
- Klaassen, C.D.; Andur, M.O. and Doull, J. (1986): Casarett and Doull's Toxicology (The Basic science of poisons), 3rd., Ed. Macmillan Publishing Company, New York, PP: 598.
- Loveren, H.V. and Vos, J.G. (1989): Cited by Dayan and paine (1989).
- Mathews, R.E.F. (1982): Virus classification. Inter virology, 17, 109.
- McPherson, R.A. (1984): Specific protein. In clinical diag-

- nosis and management by laboratory methods. Edited by Henry, J.B. PP: 204 - 216, Saunders, W.B., Philadelphia.
- Medway, W. and Kare, M.R. (1959): Cited by: Clarke and Clarke (1975).
- Moore, C.V. and Dubach, R. (1962): In Mineral Metabolism, Vol. II, Part B. Academic Press, N.Y.
- Nawal, M. Ali; Lamia, A. Ahmed and Shahein, M.A. (2001): Isolation and identification of three-day sickness virus in Egypt. *Vet. Med. J., Giza*, 49(3): 425 - 434.
- Odiawo, G.O. (1989): The relationship between selenium deficiency and the development of pulmonary and subcutaneous emphysema in bovine ephemeral fever virus-infected cattle. *Onderstepoort Journal of Veterinary Research*, 56(2): 123 - 125.
- Piet, J.B. (1896): Epizootic of Dengue fever of cattle in Egypt. *Prix Manbinne, Paris, France: National Academy of Medicine*.
- Prasad, B.; Manuja, S.; Kishwaria, R.S. Rao, V.N., Singh, R.J. and Manuja, S. (1997): Clinical report on ephemeral fever in cattle. *Indian veterinary journal*, 74(8): 685 - 686.
- Rabagliati, D.S. (1924): Three days fever or stiff sickness in cattle. *Vet. Res*, 4(23): 503 - 505.
- Reitman, S. and Frankel, S. (1957): Kits for Determination of SGOT and SGPT., *J. Clin. Path.* (28): 56.
- Rzedzicki, J.; Cybulska, R.; Trawinska, B. and Jaworska Adamu, J. (1991): Effect of some antibacterial agents on the immune response and intestinal mucosa of chickens. *Zeszyty Naukowe Akademii Rolniczej we weterynaria* (49): 179 - 89.
- Sayed, A.S.; Sadiq, A.H. and Ali, A.A. (2001): Bovine Ephemeral Fever in Assiut Governorate: Clinical, Laboratory and Therapeutic studies. *Assiut Veterinary Medical Journal*, 44(88): 157 - 175.
- Schalm, O.W. (1961): *Veterinary Haematology*, Lea and Febiger, Philadelphia, U.S.A.
- Schalm, O.W. (1975): *Veterinary Haematology*, London: Bailliere Tindall and Cassel LTD., 2nd. Ed., London.
- Schmit, J.M. (1964): *Kits for Determination of Serum Total Lipid Conc.*, Thesis, Lyon.
- Schrenk, W.G. (1975): *Analytical Atomic spectroscopy*, plenum press, New York and London.
- Sharma, L.K. (1992): A report on Bovine Ephemeral Fever in cattle. *Indian veterinary journal* 69(6): 544 - 546.
- Snedecor, G.W. and Cochran, W.G. (1969): *Statistical Methods*, 6th., Ed. IOWA State University Press, Ames, IOWA.
- ST-George, T.D.; Standfast, H.A. and Cybinski, D.H. (1978): Bovine Ephemeral Fever and related rhabdoviruses, *Aust. Vet. J.*, 54 : 558.
- ST-George, T.D.; Uren, M.F.; Young, P.L. and Hoffmann, D. (1993): The natural history of ephemeral fever of cattle. *Proceedings of the 1st., International symposium, Beijing, PRC, 25 - 27 August 1992 - 1993*, 13 - 19. ACIAR proceedings, No. 44; 48 ref.
- ST-George, T.D.; Murphy, G.M.; Burren, B. and Uren, M.F. (1995): Studies on the pathogenesis of bovine ephemeral fever: IV: A comparison with the inflammatory events in milk fever of cattle. *Veterinary microbiology*, 46 (1-3): 131 - 142.
- Underwood, E.J. (1977): *Trace Elements in Human and Animal Nutrition*, 4th., Ed. Academic press, N.Y.
- Uren, M.F.; ST-George, T.D. and Murphy, G.M. (1992): Studies on the Pathogenesis of Bovine Ephemeral Fever in experimental cattle. III. Virological and Biochemical Data, *Veterinary Microbiology*, 30(4): 297-307.
- Zimmerman, H.J. (1984): Function and Integrity of the liver. In *Clinical Diagnosis and Management by Laboratory Methods*, Edited by Henry J.H., Saunders, W.B. Philadelphia.