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Detection of specific antibodies and some biochemical changes in bovine ephemeral fever virus in El-Wady El-Gedid governorate

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Abstract

Bovine ephemeral fever (BEF) existed in the Egyptian oases (El-Wady El-Gedid) during the period 2017-2018. Aimed to the present study the document existence of (BEF) in this area and to define the blood biochemical alterations associated with the natural BEF in cattle based on clinical symptoms and confirmation of the disease by seroconversion using antibodies ELISA test. Forty animals cross-bred (Friesian x Balady) were selected randomly in their natural habitat (El-Dakhla oasis). The blood samples collected from unvaccinated naturally infected and apparently healthy animals without anticoagulant from the different village of El- Dakhla oasis to collect serum for detection of BEF specific antibodies by ELISA kit and biochemical analysis. Sera were collected during viremic stages and after two weeks of clinical symptoms appear and examined by ELISA test for determination of BEFV antibodies. The result of tested sera revealed that out of 40 samples 8 (20%) were positive and 32 (80%) were negative in viremic stages and seroconverted after two weeks to 24 (60%) out of 40 samples were positive and 16 (40%) were negative for BEFV antibodies and according to the disease history, clinical examinations and ELISA results the samples were classified into two groups for biochemical analysis, BEFV infected group and compared with the apparently healthy control one. the result of this analysis showed a significant reduction in concentrations of (Ca, P, Fe and Zn), and a significant increase ($P<0.05$) in blood serum Cu and the biomarker concentrations of the liver and kidney functions (γ-GT, AST, urea and renal function). The conclusion, this study act as the first study for documented the existence of BEFV in the Egyptian oasis. Further studies are needed to explore the real reasons for the persistence of the disease all over the year in such a dry desert environment. These findings could help in understanding the complex pathogenesis for naturally BEFV infection.

Keywords: *Detection antibodies, Biochemical in BEFV.*

Introduction

Bovine ephemeral fever (BEF) is a viral disease of cattle and buffaloes (**Seddon, 1966, Davies *et al.,* 1975)**, caused by bovine ephemeral fever virus (BEFV, order Mononegavirales, family Rhabdoviridae, genus Ephemerovirus). BEFV sub clinically infects a greater range of ruminant species (**Walker & Cybinski, 1989**). There is also evidence of infection by BEF virus in camels (Camelus dromedaries) in Egypt (**Elbayoumy *et al.,* 2013**). It causes an acute febrile illness of cattle and water buffalo known as bovine ephemeral fever (BEF) or various other local names such as 3-day sickness, bovine enzootic fever, bovine influenza or stiffseitk **(Walker, 2005).**

The BEFV has been described in many tropical and sub-tropical regions around the world. It is enzootic and seasonally epizootic in Australia, Asia, Africa and the Middle East, usually not extending beyond a zone limited by the latitudes if 38°N to 36°S **(Walker 2005; Hsieh *et al.,* 2005).** Epizootics commonly move northwards or southwards in a wave-like fashion, commencing in tropical enzootic foci in the

spring or early summer and subsiding in autumn.
Infection may be clinically unapparent or result in mild to severe clinical signs including a biphasic fever, salivation, ocular and nasal discharge, Sternal and lateral recumbency, muscle stiffness, lameness and anorexia **(St George *et al.* 1984 and Uren, 1993).**

The disease is characterized by rapid onset and rapid recovery, lasting only 1–3 days, but there are reports of prolonged paralysis and ataxia in some animals following the acute phase of infection.

It has been reported that the clinical signs of ephemeral fever were related to biochemical, cellular and serological changes in the blood. There was a rise in peripheral blood neutrophils and fall in lymphocytes counts, a fall in serum calcium levels that directly proportionate with the severity of the clinical picture. Also, bovine ephemeral fever (BEF) in cattle has been reported to be associated with a range of biochemical changes which may affect the animal health **(Young *et al.,* (1980) and Amin, (2016).**

The most severe cases can result in mortality which may be due to exposure, starvation or pneumonia, but little is currently known about the direct cause of death. Morbidity rates can be very high (approaching 100%) and mortality rates are typically low (<1%). However, in recent years there have been reports from several countries of alarmingly high case-fatality rates, sometimes exceeding 20% (**Hsieh *et al.,* 2005; Tonbak *et al.,* 2013)**

Epizootic evidence indicates that BEF virus is spread by an insect bite, and it is clear from the distribution that more than one vector is capable of transmitting the disease **(Walker, 2005; Thabet *et al.,* 2011)**. A large body of evidence suggests that BEFV is transmitted by hematophagous insects including biting midges and mosquitoes (**Muller and Standfast. 1986).**

The economic impacts of BEF can be considerable and are due primarily to cessation of lactation in dairy cattle, loss of condition in beef cattle and the immobilization of water buffalo used for draught power A recent study has estimated an average net loss of 175.9 kg milk per cow affected by BEF **(Davis *et al.,* 1984; St George, 1986; Walker, 2005**). BEF also impacts on trade in live cattle from infected zones and there is some evidence that the risks of inter-continental spread of BEFV through animal transport or vector translocation may be increasing (**Aziz *et al.,* 2012).**

In Egypt, BEF was known as “dengue fever” of cattle in 1896 but the first detailed report was of an epizootic in 1909 that commenced at Aswan, travelled down the Nile Valley to Cairo and spread across the Delta to the coast **(Ragbagliati, 1924).** In 1991, the disease affected 250,000 imported cattle and a smaller number of indigenous cattle and water buffalo all along the Nile Valley, in the Delta and at several oases west of the Nile (**Davies *et al.,* 1992**). Subsequent outbreaks affecting hundreds of cattle, and recent outbreaks were reported during the summers of 2000-2015. The disease in cattle is characterized by the acute febrile reaction, severe respiratory distress, oedema and swelling of the lymph nodes under the skin, stiffness, lameness and spontaneous recovery in three days (**St George, 1990**). These clinical signs can be exacerbated by severe environmental stress or forced exercise **(Radostits *et al.,* 2006).** The morbidity may be high but the mortality is low (**Walker and Klement, 2015**). Mortality was reported previously in several governorates in lower and upper Egypt that characterized by 2.5% (**Walker and Klement, 2015).** Higher mortality (5.6%) was reported by **(Amin, 2016).**

Ephemeral fever is usually diagnosed from history and clinical signs **((Zaghawa *et al.,* 2000).** However, a confirmatory diagnosis, which can be obtained by the competitive ELISA test using monoclonal antibodies, is the most specific test developed so far and should be made more widely available **(Zaghawa *et al.,* 2000).**

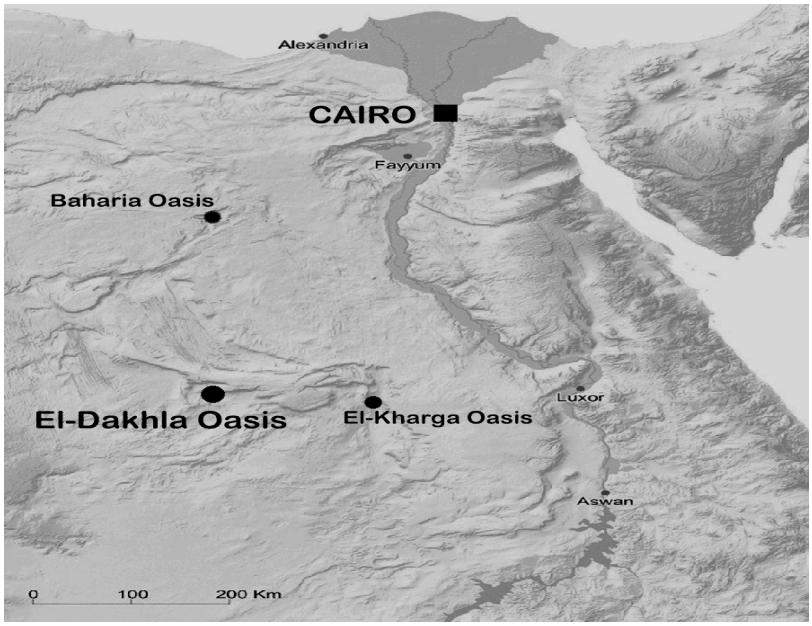
The greater simplicity and sensitivity of the blocking ELlSA, when compared with the virus neutralization test, makes it the preferred test for the diagnosis and monitoring of clinical bovine ephemeral fever **(Uren, 1993).**

A number of investigators have examined changes in blood chemistry during experimental BEF infection in an effort to characterize the pathogenesis of the disease **(Burgess and Spradbrow, 1977; Uren *et al.* 1992, 2002)**. The data presented here extend earlier observations on biochemical changes occurring in natural BEF affected cattle in their habitat.

Despite the subsequent outbreaks affecting cattle in the Egyptian oases as a part of the countrywide intermittent outbreaks in Egypt since 1991, there are no reports that documented the existence of BEF in this area. Therefore, the aim of the present study was to document the existence of the disease in 2017-2018 the Egyptian oases. In addition, to define the blood biochemical alterations associated with the natural BEF infection in calves, with particular emphasis on the change in blood mineral levels, renal and liver function tests based on confirmation by ELISA test kit

Materials and Methods

Study area and agronomic data:



Map (1): Egypt map showing El-Dakhla oases.

This study was carried out in El-Dakhla Oasis (El-Wahat El-Dakhla). This oasis belongs to El -Wadi El-Gadid depression (Map 1). GPS coordinates showed that El- Dakhla Oasis is a depression of a minus 77.8 m altitude, lies between 25° 30′ and 29° 30′ N latitudes and between 25° 42′ and 30° 47′ E longitudes. The climate is arid, essentially that of the desert. The temperature ranges from 49°C during summer days to 2°C in the chilly winter nights. Rainfall is almost negligible. Watering and irrigation depend absolutely on the ground wells. Crossbred Holstein-Friesians with Native Balady cattle constitutes the majority of cattle breeding in this area. There are no extensive feeding systems or investment stations in this area but smallholders (1–5 animals) represent the bovine industry.

Animals and blood sampling:

During an outbreak of BEF infection that extended from October 2017 to October 2018, forty cross-bred animals (Friesian x Balady) were selected randomly in their natural habitat

(the corral).

-Blood samples were collected from naturally infected (diseased) unvaccinated and apparently healthy animals (as a control) without anticoagulant from deferent villages of El-Dakhla during viremic stages and after about two weeks of clinical symptoms appear. the samples were centrifuged to obtain sera and stored at-20˚C until serological and biochemical analysis.

BEFV (ELISA) test

-ELISA Kit for bovine ephemeral fever virus antibodies detection BEFV (Wuhan Unibio test Co., Ltd. Wuhan city, China-Lanzhou Veterinary Research Institute of CAAS) pre-coated plates with antigen.

-Forty sera sample were tested by (BEFV) ELISA Kit as a rabid test to determine antibody against BEFV and to validate the new commercial kit (**Wuhan *et al.* , 2016).**

Principle:

Bovine ephemeral fever virus is an arthropod-borne virus, the BEFV spread quickly and has extensive epidemicity as well as certain periodicity. This kit can be used as immune evaluation and epidemiological investigations for BEFV.

The test was conducted according to manufacturer instruction.

Results analysis:

Calculate the mean value of absorbance for positive and negative control and test serum

Negative control OD_{450} <0.3, positive control OD_{450}>0.8.

(1) If $OD_{450}\leq$0.22 the serum is negative (- ve)

(2) If OD_{450}>0.30 the serum is strong. Positive +ve).

(3) If 0.30>OD_{450}>0.22 the serum is incredible, need to test again.

The samples were classified according to history, clinical examinations **(Radostits *et al.*, 2000)** and the results of BEFV-ELISA test into two groups, animals clinically diagnosed as having EFV infection(diseased) (N=24), and animals clinically diagnosed as healthy used as a control group (N=16).

-Biochemical analysis

The macro-minerals, calcium (Ca) and inorganic phosphorus (IP) in serum were determined calorimetrically using test kits supplied by Eltech Co. Egypt according to manufacturer instructions and the methods described by **Henry *et al.* (1974)** and **(Burgess and Spradbrow, 1977; Uren *et al.* 1992, 2002)**. respectively.

Blood serum trace elements iron (Fe), copper (Cu), and zinc (Zn) were determined by an atomic absorption spectrophotometer (GBC 932 AA; GBC Scientific Equipment, Australia) according to **(Piper and Higgins, 1967**) after washing in perchloric, nitric and sulfuric acids **(AOAC, 1990).**

Hepatic and renal function biomarkers including γ-GT, AST, urea and creatinine were measured by using test kits (Boehringer Mannheim, Germany) after the methods described by (**Henry *et al.* 1974)** and manufacture instructions.

Statistical analysis

The packaged SPSS program for windows version 10.0.1 (SPSS, Chicago, IL, USA) was used for statistical analysis according to (**Borenstein *et al.* 1997).** Data were expressed as mean ± standard deviation (SE). Differences between groups were determined by means of a Student t-test. Significance level was set at P ≤0.05

Results

Clinical signs:

All animals with BEF exhibited typical clinical signs of ephemeral fever, including pyrexia, stiff gait, lameness, swelling of the joints, anorexia, shivering, tremors, hurried respiration, rapid pulse rate, recumbency, ruminal stasis, salivation, nasal and ocular discharges.

Tabl*e* (1). Result of ELISA test:

Time of collected serum samples	Total No. of serum samples	No. of positive (+) samples	%	No. of negative (-) samples	%
During viremic stages	40	8	20	32	80
After about two weeks of infection	40	24	60	16	40

Serum samples were collected from naturally infected (diseased) and apparently healthy animals were tested for BEFV antibodies detection ELISA the results revealed that out of 40 serum samples 8 (20%) were positive and 32 (80%) were negative at viremic stage while after about two weeks out of 40 serum samples 24 (60%) were positive, 16 (40%)were negative for BEFV antibodies**. (Table1)**

Biochemical results:

Table (2). Macro-minerals, Ca and P in blood serum of BEF infected animals and healthy control animals

Serum samples of	Ca (mmol/l)	IP (mmol/l)
BEF infected animals	1.89 ± 0.021	1.43 ± 0.017
healthy control animals	2.17 ± 0.019	1.71 ± 0.019
P	0.027	0.031

Table (3). Micro-minerals, Fe, Zn and Cu in blood serum of BEF infected animals compared with healthy control animals

Serum samples of	Fe (µmol/l)	Zn (µmol/l)	Cu (µmol/l)
BEF infected animals	27.51 ± 0.251	8.82 ± 0.246	13.83 ± 0.320
Healthy control animals	31.81 ± 0.226	10.43 ± 0.301	11.32 ± 0.215
P	0.027	0.009	0.016

Table (4). Blood serum concentrations of the hepatic and renal function biomarkers in blood serum of BEF infected animals and healthy control one.

Serum samples of	AST (IU 1^{-1})	γ-GT (IU 1^{-1})	Urea (mmol 1^{-1})	Creatinin (µmol 1^{-1})
BEF infected animals	91.61 ± 1.196	25.05 ± 0.947	7.01 ± 0.056	79.46 ± 0.152
healthy control animals	78.85 ± 1.244	22.61 ± 0.831	5.94 ± 0.061	66.82 ± 0.177
P	0.034	0.041	0.029	0.038

Results of macro-minerals, Ca and P in the serum of diseased animals are Shown in a table (2). Compared with the healthy control group, animals with BEF showed a significant reduction in blood serum Ca and P ($P<0.05$).

Micro-mineral concentrations in blood serum are shown in table (3). It is clear that blood serum Fe and Zn are significantly reduced ($P<0.05$) and Cu increased in animals infected with BEF when compared with a healthy control group.

Table (4) shows blood serum concentrations of the hepatic and renal function biomarkers including γ-GT, AST, urea and creatinin. These biomarkers are significantly increased ($P<0.05$) in animals infected with BEF when

compared with healthy control group.

Discussion

Bovine ephemeral fever virus is (BEFV) an arthropod born Rhabdo virus which is classified as the type species of the genus Ephemeovirus. It causes an acute febrile illness of cattle and water buffalo known as bovine ephemeral fever (BEF) or various other local names such as3-day sickness, bovine enzootic fever, bovine influenza or stiffseitk **(Walker 2005).**

Bovine Ephemeral Fever (BEF) is an inflammatory disease affecting mainly cattle and buffaloes (**Henry *et al.* 1974)** The maximum prevalence of BEF occurs during hot humid season, which is an active period for insect vector that resulting in high rates of virus transmission.

There was a countrywide epidemic of ephemeral fever in Egypt in 1991 (**Davies *et al*., 1992).** In the last decade, there is a dramatic increase in the number of BEF outbreaks in the Nile-Valley **(Kasem *et al,* 2014; El-Bagoury, *et al.,* 2014).** An El-Dakhla oasis is an isolated area in the western Egyptian desert (map 1) and faraway removed about 200 km from the nearest oasis (El-Kharga oasis) and about 450 km from the Nile-Valley. A severe attack of BEFV occurred in 1991 followed by intermittent outbreaks of BEF (unpublished data). The high intermittent incidence of BEF outbreaks in El-Dakhla oases raises an important question regarding their source and route of introduction. It is suggested there are three alternative routes for the introduction of the disease. The first is that BEFV may be enzootic in the area, where a severe attack occurred in 1991. The second is that BEFV may be introduced into this area throughout animal transmission from endemic areas such as Nile-Valley provinces. The third suggests the aerial movement of the virus. **Aziz-Boaron *et al*. (2012)** suggested that both winds and animal transport might have an important role in the transboundary transmission of BEFV in the Middle East.

The repeated number of BEF outbreaks in Egypt occurred seasonally usually in summer, where the transmitting vector is active **(El-Bagoury *et al.,* 2014).** In El-Dakhla oases, the pattern of the current storm of BEF is not seasonal, but its period extended from October 2017 to October 2018 (time of reporting this manuscript). It is suggested that there are other factors rather than insects that may favour the existence of the disease. The long period of the hot summer season with a concomitant dispersion of the insect vector in El-Dakhla oases may be a reason for the continuity of the BEF storm. Further, the use of the same needle for medical or vaccine injection among more than one animal during the storm of BEF may be also incriminated. (vaccination other than BEF).

Natural BEFV infection has been reported to result in durable immunity **(Mackerras *et al.,* 1940)**. There have been observations of multiple episodes of clinical ephemeral fever in the same cattle (**St George** TD (1986); (1993) but it is not known if other ephemeroviruses may have been responsible for the disease**.**

The study conducted to evaluate the humoral immune response of cattle using ELISA test, the competitive ELISA test using monoclonal antibodies is the most specific test developed so far and should be made more widely available **(Zakrzewski *et al.,* 1992)**.

In the present study, all animals with BEF exhibited typical clinical signs of ephemeral fever as previously reported **(Nandi and Negi, 1999).** and the results of ELISA test as shown in **(table 1)** revealed that the rate of antibodies detected against BEF in naturally infected animals was higher after about two weeks of infection than during viremic stages where about 60% and 20% of tested cases respectively These results are in agreement with those reported by (**Zakrzewski *et al.,* 1992)**. The level of antibodies increased gradually and reach the maximum on the tenth day of infection

The clinical characteristics of the disease are the expression of inflammatory mediators common to various numbers of acute febrile disease **(Georg, 1994)** and massive interferon production that results in cellular damage (**Burgess and Spradbrow, 1977**). Recent studies showed empirically defined cytokine networks that underlie the acute phase of the disease **(Barigye *et al.,* 2015).** During the acute BEF, there is a kinetic activation of pro-inflammatory cytokines such as IL-1β, IL-2, IL -6, TNF-α, and IFN-γ **(Barigye *et al.,* 2016)**. Accordingly, the host's response to the infection is considered as a reflection of the effects of these mediators on the host tissues **(Gu *et***

***al.,* 2018).**
Ephemeral fever is a biochemical model for inflammatory disease in cattle (**Murphy *et al.,* 1990).** A number of investigators have examined changes in blood chemistry during experimental BEF infection in an effort to characterize the pathogenesis of the disease. The data presented here extend earlier observations on biochemical changes occurring in BEF affected cattle **(Murphy *et al.,* 1990).**
Accordingly, this study is the first, which record the biochemical changes in the febrile host naturally affected by BEF as a method to follow the systematic reaction of the body to the infection.
Hypocalcemia in cattle infected with BEF is similar to that seen in milk fever **(St George *et al.,* 1995; Charbonneau *et al.,* 2008),** in which the great stresses on Ca homeostasis is associated with hypocalcemic parturient paresis among high producing dairy cows **(Goff and Horst, 2003).** In BEF, despite detailed investigation of the clinical signs and the pathology of the disease, the reason for the lowered serum calcium levels is not well known. Laminitis, the sensitivity of synovial membranes to minor disturbances of capillary permeability or a toxic change leading to damage to these cells, are suggested **(Burgess and Spradbrow 1977; Uren, 1989).** However, in our study, the sever drop in blood calcium may be a contributing cause. **St George *et al.* (1984)** found that the serum calcium level declined on the day of clinical disease and reached its lowest point on the second day of the disease. The mean level of 2.13 mmol/l is only slightly below the lower level of the normal range of 2.25-2.75 mmol/l. **Nandi and Negi (1999)** found a significant drop in plasma calcium in cattle with BEF. The reason for the lowered serum calcium levels is not known, but metabolic in acid-base balance may have affected the ionized calcium level **(St George *et al.,* 1984**).
Alkalosis will cause a decrease in ionized calcium concentration and may lead to the symptoms of hypocalcemia **(Radostits *et al.,* 2006).** Ruminal stasis and hypomotility of the digestive tract may have contributed to the lower serum calcium levels, by reducing calcium intake, but are not likely to be initiating causes **(Radostits *et al.,* 2006).** On the other hand, **Amin (2016)** noticed coagulative necrosis of the superficial layer of the intestinal villi and necrosis of the intestinal glands in cattle infected with BEF, which might be a co-factor of Ca mal-absorption and reduction of serum Ca **(Ettinger *et al.,* 2017).**
Our results showed a significant decrease in the mean levels of serum phosphorus in diseased when compared with healthy animals. These results are in agreement with those reported by **(St. George *et al.,* 1984, Uren *et al.,* 1992; 2002 and Thabet *et al.,* 2011).** The decrease in serum phosphorus concentrations seemed to be secondary to phosphorus redistribution as a result of respiratory alkalosis **(Burtis and Bruns, 2014; Kurtz and Travlos, 2017).** During respiratory alkalosis, intracellular CO2 decreases, causing an increase in the intracellular pH. This mechanism stimulates the glycolytic pathway, specifically phosphofructokinase, a key rate-limiting enzyme of glycolysis. Production of sugar phosphates is enhanced, which in turn induces intracellular phosphorus entry, thus decreasing serum phosphorus concentration (**Marshall *et al.,* 2014).** Respiratory alkalosis also has been reported to enhance phosphorus uptake by muscle which largely accounts for the hypophosphatemia (**McPherson and Pincus, 2017; de Morais and DiBartola, 2017; Orban and Ichai, 2018).**
Plasma Zn and Fe values fell while plasma Cu levels rose markedly in cattle with BEF when compared with healthy animals. These results are similar to those reported by (**Uren *et al.* 1992**) the reduction of iron limits microbial growth and, therefore, the ability of the host to reduce plasma Fe levels have a beneficial effect on the host response **(Egli, 2015; Rose, 2016).** Iron is delivered to the actively dividing and metabolizing cells of the immune system by transferrin **(Kurtz and Travlos, 2017)** or sequestered by lactoferrin released by degranulating neutrophils occurs during inflammation (**Muras-Szwedziak and Nowicki, 2018).**
Similarly, zinc is required for rapid microbial metabolism and is known to inhibit phagocytosis **(Teixeira *et al.,* 2014).** Its depletion from the plasma, therefore, is considered to be of benefit to the host. Copper plays a prominent role in hepatic synthesis of the copper-containing protein, ceruloplasmin, which has

antioxidant activity and has a beneficial effect on the host response **(Harris, 2018).**
In the present work, we found a significant increase in hepatic and renal function biomarkers including γ-GT, AST, urea and creatinine. These results contradict the findings of **Uren and Murphy (1985), Young and Spradbrow (1990**) who reported that hepatic enzyme levels, urea and creatinine concentrations remained within normal limits. Probably these differences resulted from the nature of the infection, where these authors studied the pathogenesis of bovine ephemeral fever in experimental cattle, whereas our study based on field cases with natural BEF infection. However, **Burgess and Spradbrow (1977)** isolated BVEF from the blood, lung, kidney and liver. Further, post mortem observations showed congested, fragile kidneys and liver with micro-abscesses, accompanied histologically by marked diffuse degeneration of the hepatocytes, necrosis and degeneration of the of renal tubules **(Guifang *et al.,* 1992; Amin, 2016)**. So that the elevated hepatic and renal function biomarkers are a primary consequence of tissue damage in these organs **(Burtis and Bruns, 2014; McPherson and Pincus, 2017).**

As a conclusion, this study is the first that documented the existence of BEFV in the Egyptian oasis. Further studies are needed to explore the real reasons for the persistence of the disease all over the year in such a dry desert environment. These findings could help in understanding the complex pathogenesis of the naturally occurring BEFV, and clarify that BEF represents a biochemical model for the study of inflammation in cattle. It is becoming increasingly apparent that BEF occurs as a direct result of disruption to the normal functioning of the physiological systems of the host.

References

Amin, A. (2016). Histopathological and Immunohistochemical Studies on Bovine Ephemeral Fever in Cattle. Journal of Advanced Veterinary Research, 6, 93-100.

AOAC (1990). Official Methods of Analysis, vol. 1, 15th ed. Association of Official Analytical Chemists, Washington DC.

Aziz-Boaron, O.; Klausner, Z.; Hasoksuz, M.; Shenkar, J.; Gafni, O.; Gelman, B. and Klement, E. (2012). Circulation of bovine ephemeral fever in the Middle East—strong evidence for transmission by winds and animal transport. Veterinary microbiology, 158(3-4), 300-307.

Barigye, R.; Melville, L.F.; Davis, S.; Walsh, S.; Hunt, N. and Hunt, R. (2016). Kinetics of selected plasma cytokines during innate-adaptive immune response transition in adult cattle infected with the bovine ephemeral fever virus. Veterinary microbiology, 186, 111-116.

Barigye, R.; Melville, L.F.; Davis, S.; Walsh, S.; Hunt, N.; Hunt, R. and Elliot, N. (2015). Kinetics of pro-inflammatory cytokines, interleukin-10, and virus neutralising antibodies during acute ephemeral fever virus infections in Brahman cattle. Veterinary immunology and immunopathology, 168(3-4), 159-163.

Borenstein, M.; Rothstein, H. and Cohen, J. (1997). Sample Power Statistics 1.0. SPSS Inc., Chicago.

Burgess, G.W. and Spradbrow, P.B. (1977). Studies on the pathogenesis of bovine ephemeral fever. Australian veterinary journal, 53: 363-368.

Burtis, C.A. and Bruns, D.E. (2014). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics-E-Book. Elsevier Health Sciences.

Charbonneau, E.; Pellerin, D. and Oetzel, G.R. (2008). Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. Journal of Dairy Science, 89: 537–548.

Davies, F.G.; Shaw, T. and Ochieng, P. (1975). Observations on the epidemiology of ephemeral fever in Kenya. Epidemiology & Infection, 75(2), 231-235.

Davies, F.G.; Moussa, A. and Barsoum, G. (1992). The 1990–1991 epidemic of ephemeral fever in Egypt and the potential for spread to the Mediterranean region. In: St George TD, Uren MF, Young PL, Hoffmann D (eds) Proceedings of the 1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses, Beijing, August 1992. Australian Centre for International Agricultural Research Proceedings 44, pp 54 –56.

Davis, S.S.; Gibson, D.S. and Clark, R. (1984). The effect of bovine ephemeral fever on milk production. Aust Vet. J. 61: 128 –129.

De Morais, H.A. and DiBartola, S.P. (2017). Advances in Fluid, Electrolyte, and Acid-base Disorders, An Issue of Veterinary Clinics of North America: Small Animal Practice, E-Book (Vol. 47, No. 2). Elsevier Health Sciences.

Egli, T. (2015). Microbial growth and physiology: a call for better craftsmanship. Frontiers in microbiology, 6, 287.

El-Bagoury, G.F.; El-Nahas, E.M. and El-Habbaa, A.S. (2014). Serological and molecular identification of Bovine Ephemeral Fever virus isolates from cattle and buffaloes in Qaluobia province, Egypt 2014. Egyptian Journal of Virology, 11, 176 -182.

Elbayoumy, M.K.; Allam, A.M. and Albehwar, A.M. Elsayed EL (2013). Investigation of the immune status of camels (Camelus dromedarius) against some viral diseases. Alex. J. Vet. Sci., 39: 12–17.

Ettinger, S.J.; Feldman, E.C. and Cote, E. (2017). Textbook of Veterinary Internal Medicine-eBook. Elsevier health sciences.

Goff, J.P. and Horst, R.L. (2003). Role of Acid-Base Physiology on the Pathogenesis of Parturient Hypocalcaemia (Milk Fever) - the DCAD Theory in Principal and Practice. Acta Veterinary Scandnavian, 97: 51-56. (Supplementary).

Gu, W.; Wang, Y.; Qiu, Z.; Dong, J.; Wang, Y. and Chen, J. (2018). Mitogen-activated protein kinase signaling is involved in nonylphenol-induced proinflammatory cytokines secretion by BV2 microglia. Journal of Applied Toxicology, 38(7), 958-967.

Guifang, C.; Zigang, Z.; Xunnan, Y. and Wenbin, B. (1992). Pathological Changes in Cattle Experimentally Infected with Bovine Ephemeral Fever Virus. In: St George TD, Uren MF, Young PL, Hoffmann D (eds) Proceedings of the 1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses, Beijing, August 1992. Australian Centre for International Agricultural Research Proceedings 44, pp 60–62.

Harris, Z.L. (2018). Ceruloplasmin. In Clinical and Translational Perspectives on Wilson Disease (pp. 77-84). Academic Press.

Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974). Clinical Chemistry, Priciples and Techniques.4th Ed., Harper and Row; Hagerstown M D.

Hsieh, Y.C.; Chen, S.H.; Chou, C.C.; Ting, L.J.; Itakura, C. and Wang, F.I. (2005). Bovine ephemeral fever in Taiwan (2001–2002). J. Vet. Med. Sci., 67: 411–416.

Kasem, S.; Dawod, A.; Khodier, M.; Kotb, A.; Abouelyazeed, M.; Kareem, S.A. and Ibrahim, B. (2014). Isolation and characterization of bovine ephemeral fever virus in Delta Provinces, Egypt 2012. Glob Vet, 13, 972-976.

Kurtz, D.M. and Travlos, G.S. (2017). The Clinical Chemistry of Laboratory Animals. CRC Press.

Mackerras, I.M.; Mackerras, M.J. and Burnet, F.M. (1940). Experimental studies of ephemeral fever in Australian cattle. CSIRO Bull 136: 1–116.

Marshall, W.J.; Lapsley, M.; Day, A. and Ayling, R. (2014). Clinical Biochemistry E-

Book: Metabolic and Clinical Aspects. Elsevier Health Sciences.

McPherson, R.A. and Pincus, M.R. (2017). Henry's Clinical Diagnosis and Management by Laboratory Methods E-Book. Elsevier Health Sciences.

Muller, M.J. and Standfast, H.A. (1986). Vectors of ephemeral fever group viruses. In: St George TD, Kay BH, Blok J (eds) Proceedings of the Fourth Symposium on Arbovirus Research in Australia, Brisbane, May 1986. CSIRO-QIMR, pp 295–300.

Muras-Szwedziak, K. and Nowicki, M. (2018). Associations between intravenous iron, inflammation and fgf23 in non-dialysis patients with chronic kidney disease stages 3-5. Kidney and Blood Pressure Research, 43(1), 143-151.

Murphy, G.M.; St. George, T.O. and Uren, M.F. (1990). Ephemeral fever -a biochemical model for inflammatory disease in cattle and sheep. Proceedings Fifth Symposium Arbovirus Research in Australia, 28 August -1 September 1989. CSIRO/QIMR Brisbane, Australia. (Uren, M.F., Blok, J., and Manderson, L.H., Eds) pp.268- 274 (1990).

Nandi, S. and Negi, B.S. (1999). Bovine ephemeral fever: a review. Comparative immunology, microbiology and infectious diseases, 22(2), 81-91.

Orban, J.C. and Ichai, C. (2018). Alkalosis: Diagnosis and Treatment. In Metabolic Disorders and Critically Ill Patients (pp. 195-214). Springer, Cham.

Piper, H.G. and Higgins, G. (1967). Estimation of trace metals in biological material by atomic absorption spectrophotometry, Proc. Assoc. Clin. Biochem. 7: 190–195.

Radostits, O.M.; Mayhew, I.G. and Houston, D.M. (2000). Veterinary clinical examination and diagnosis. WB Saunders.

Radostits, O.M.; Blood, D.C. and Gay, C.C. (2006). Veterinary Medicine, 8th Ed. Bailliier Tindall, London.

Ragbagliati, D.S. (1924). Three day's fever or stiff sickness in cattle. Vet. Rec. 4: 503–505.

Rose, J. (2016). Trace elements in health: a review of current issues. Butterworth-Heinemann.

Seddon, H.R. (1966). Ephemeral fever. In: Diseases of domestic animals in Australia. Part 4: protozoan and virus diseases. Commonwealth Department of Health, Canberra, pp 118–126.

St George, T.D. (1990). Bovine ephemeral fever virus. In: Dinter Z, Morein B (eds) Virus infections of vertebrates, Vol 3. Virus infections of ruminants. Elsevier, Amsterdam, pp 405–415.

St George, T.D.; Cybinski, D.H.; Murphy, G.M. and Dimmock, C.K. (1984). Serological and biochemical factors in bovine ephemeral fever. Australian journal of biological sciences, 37(6), 341-350.

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.

St George, T.D. (1986). The epidemiology of bovine ephemeral fever in Australia and its economic effect. In: St George TD, Kay BH, Blok J (eds) Proceedings of the 4th Symposium on Arbovirus Research in Australia, Brisbane, May 1986. CSIRO-QIMR, pp 281–28.

St George, T.D. (1993). The natural history of ephemeral fever of cattle. In: St George TD, Uren MF, Young PL, Hoffmann D (eds) Proceedings of the1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses, Beijing, August 1992. Australian Centre for International Agricultural Research Proceedings 44, pp 13–19.

Teixeira, A.G.V.; Lima, F.S.; Bicalho, M.L.S.; Kussler, A.; Lima, S.F.; Felippe, M.J. and Bicalho, R.C. (2014). Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on immunity, health, and growth of dairy calves. Journal of dairy science, 97(7), 4216 -4226.

Thabet, N.S.; Ghazy, E.W.; Nayel, M.A. and Abo-Elkhair, M. (2011). Molecular and biochemical studies on bovine ephemeral fever. Research Opinions in Animal & Veterinary Sciences, 1(5), 269-275.

Tonbak, S.; Berber, E.; Yoruk, M.D.; Azkur, A.K.; Pestil, Z. and Bulut, H.A. (2013). Large-scale outbreak of bovine ephemeral fever in Turkey, 2012. J Vet Med Sci. 2013; 75(11): 1511-4.

Uren, M.F. and Murphy, G.M. (1985). Studies on the pathogenesis of bovine ephemeral fever in sentinel cattle. II. Haematological and biochemical data. Vet. Microbiol., 10: 505-515.

Uren, M.F.; St George, T.D. and Murphy, G.M. (2002). Studies on the pathogenesis of bovine ephemeral fever III: Virological and biochemical data. Vet. Microbiol. 30: 297-307.

Uren, M.F. (1989). Bovine ephemeral fever. Aust Vet. J. 66: 233–236.

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. Vet Microbiol 30: 297–307.

Uren, M.F. (1993). Bovine ephemeral fever: pathology, virology, serology. In: Corner LA, Bagust TJ, editors. Australian standard diagnostic techniques for animal diseases. East Melbourne, Vic., CSIRO.

Walker, P.J. and Cybinski, D.H. (1989). Bovine ephemeral fever and rhabdo viruses endemic to Australia. Australian veterinary journal, 66, 398-400.

Walker, P.J. and Klement, E. (2015). Epidemiology and control of bovine ephemeral fever. Veterinary research, 28, 46: 124. doi: 10.1186/s13567-015-0262-4.

Walker, P.J. (2005). Bovine ephemeral fever in Australia and the world. Curr Top Microbiol Immunol 292: 57–80

Young, P.L. and Spradbrow, P.B. (1980). The role of neutrophils in bovine ephemeral fever virus infection of cattle. J. Infect. Dis. 142: 50–55.

Young, P.L. and Spradbrow, P.B. (1990). Clinical response of cattle to experimental infection with bovine ephemeral fever virus. The Veterinary record, 126(4), 86-88.

Zaghawa, A.; Akla, M.A.; Khader, A.M. and Hassan, H.Y. (2000). An outbreak of bovine ephemeral fever in Egypt during 2000. Clinical and Epidemiological Investigation 9th Sci. Cong. Fac. Vet. Med. Assiut Univ., Egypt, pp. 346-353.

Zaher, K.S. and Ahmed, W.M. (2011). Investigations on bovine ephemeral fever virus in Egyprian cows and buffaloes with emphasis on isolation and identification of a field strain. Glob Vet 6: 447–452.

Zakrzewski, H.; Cybinski, D.H. and Walker, P.J (1992). A blocking ELISA for the detection of specific antibodies to bovine ephemeral fever virus. J Immunol Methods (1992);151: 289–297.