

## **SOME CLINICAL, EPIDEMIOLOGICAL AND LABORATORY STUDIES ON BOVINE EPHEMERAL FEVER (THREE DAYS SICKNESS)**

**F.K. HAMODA; S.S. KHALAF-ALLAH\* and M.H. KHODEIR \*\***

Dept. of Anim. Med., Fac. Vet. Med., Zagazig University, Benha Branch, Egypt

\* Dept. of Hygiene, Animal Behaviour and Management, Fac. Vet. Med.,

Zagazig University, Benha Branch, Egypt.

\*\* Veterinary serum and vaccine Research Institute, Abbasia, Cairo, Egypt

Received: 15-8-2001.

Accepted: 1-10-2001.

### **SUMMARY**

This study was carried out on a total number of 672 animals (551 cows and 121 calves), belonging to two private farms at Sharkia governorate, 352 of them showed an apparent clinical manifestations referred to bovine ephemeral fever. The disease was swept throughout Egypt during the period from April to July 2000.

Virological examination revealed that, buffy coat samples obtained from viremic cattle on inoculation into BHK cell cultures elicited cytopathic effect (CPE) while in suckling mice resulted in appearance of neurological signs and subsequent death. The detected virus was confirmed by performing virus neutralization test. Meanwhile examination of buffy coats samples collected from incontact animals (buffaloes, sheep, goats, dogs

and donkeys) revealed negative findings.

Serological investigation declared that, infected and convalescent cattle had a significant titer of serum neutralizing antibodies while it did not detected in the sera of incontact animals except buffaloes had serum neutralizing antibodies without clinical evidence of the disease.

Hematological panel of the infected cows showed anemia associated with leukocytosis and neutrophilia with lymphopenia. Serum biochemical profile revealed significant alteration in the values of the total protein, albumin, globulin, calcium, phosphorus, ALT, AST, creatinine, urea, copper, iron and zinc.

The complicated cases declared pulmonary and subcutaneous emphysema broncho-pneumonia, abortion and prolonged recumbency. The percentages of subclinical mastitis and reduction of milk

reduction among infected cows were studied during infection and at different intervals post recovery. Symptomatic therapy was adopted and the suggested line of control of this problem was traced and discussed.

## INTRODUCTION

Bovine ephemeral fever (BEF) is an acute insect-borne viral disease of cattle and water buffaloes, caused by an arbovirus of the family rhabdoviridae, characterized by fever, depression, inappetence, difficult in swallowing, serous ocular and nasal discharges, dyspnea, salivation, joint pain, stiffness, lameness and sometimes temporary or permanent paralysis of limbs (Burgess and Sparadbrow., 1977; St.George., 1988; Hungerford., 1990; Abu-Elzein et al., 1997; Murray., 1997; Farag et al., 1998; Nandi and Negi., 1999 and Radostits. et al., 2000).

The virus does not survive long outside its vertebrate or invertebrate host and is rapidly inactivated by the high concentration of lactic acid, which develop in the muscles of cattle after death. Fomites, body discharges or tissues therefore play no part in the transmission of the disease but the seasonal occurrence of the disease suggested insect transmission (Coetzer et al., 1994).

The disease causes great economic losses such as mortalities, decreased body weight, fall in milk production and most cows could not return to

preillness production levels on convalescence, except in those at very early stage of lactation, abortion as well as the expenses of care or treatment and vaccination (Parsonson and Snowden 1974a ; Davis et al., 1984; Sharma., 1992 and Coetzer et al., 1994).

The disease may be followed by various complications as pneumonia, mastitis, hindquarter paralysis, abnormal gait, abortion in late pregnancy, temporary bull infertility for up to six months and rarely pulmonary and subcutaneous emphysema (Hill and Schultz., 1977 and Radostits et al., 2000). However Hungerford., (1990) reported that subcutaneous emphysema is considered as a characteristic sign in severe ephemeral fever outbreaks.

The more spectacular epidemics of the disease have been occurred in summer and autumn in tropical and temperate regions of Africa (St. George., 1988). The disease was firstly described in Egypt in 1865 under the name of Dengue fever (Paton., 1869) and was described by Rabagliati., (1924). Since that time no publications about the occurrence of the disease in Egypt could be traced until the summer of 1991, where typical form of the disease has been recorded in different governorates (Hassan, et.al., 1991; Nagi, et.al., 1992; Fayed., 1993 and Banoub., 1994).

The objectives of this paper are to investigate a field problem swept among cattle during summer

mer 2000 in Egypt through description of clinical signs and the postmortem lesions, recording some epidemiological data (including assessment of morbidity and mortality rates, animals susceptibility, determination of incubation period and course of the disease and declaring the role of incontact buffaloes, sheep, goats and donkeys), virological and serological investigations, determination of some hematological and serum biochemical parameters, following up the possible subsequent complications and a trial of symptomatic therapy and planning the line of disease control.

## MATERIAL and METHODS

**1-Animals:** A total number of 672 Holstein-Friesian and native breed animals (551 cows and 121 calves) belonging to two farms at Sharkia governorate were investigated, out of which 352 animals (294 cows and 58 calves) showed the suspected clinical signs of bovine ephemeral fever. These animals were kept under close clinical observation during the period of study. Clinical assessments of the diseased animals, as well as the postmortem examination of dead and emergency slaughtered animals were done.

**2-History and some epidemiological data:** History of the investigated animals declared that, the disease appeared suddenly during the period from April to July 2000. The disease was rapid in spread and recovery or transit in na-

ture, the period between appearance of new cases was about 1-9 days, there was a high morbidity rate with low mortality and the majority of clinical cases were recovered within one week. The disease affected cows only although there was direct and indirect contact between cows and other domestic animals as buffaloes, sheep, goats, dogs and donkeys. As well, historical details regarding to changes in management and diet were recorded.

### 3-Sampling and analytical procedures:

**a-Heparinized blood samples:** It were collected from the diseased cows (at the febrile stage) as well as from apparently healthy cow. They were divided into two portions, the first one was used for some hematological studies (red cells, total and differential leukocytic count and hemoglobin estimation) according to Sastry., (1985). The second portion was used for virus isolation and identification by inoculation of buffy coat samples into baby hamster kidney cell culture (BHK) and suckling mice of 2-4 days old intracerebrally (Glyn Davies and Walker., 1974) and by performing virus neutralization test using standard hyperimmune sera (Sigma chemical.co.USA) according to the method of Rossiter and Jessette., (1982). Also buffy coat samples were collected from incontact animals to investigate the role of these animals in epidemiology of the disease.

**b-Serum samples:** It were separated from collect-



ed blood samples of diseased and convalescent cows and apparently healthy one as well as from incontact buffaloes, sheep and goats and donkeys and were subjected to the serum neutralization test (SNT) using standard hyperimmune sera (Rossiter and Jessette., 1982). In addition, sera of diseased and apparently healthy cows were used for estimation of total protein (Henry., 1964), albumin (Dumas et al., 1971), globulin was calculated as the difference between total protein and albumin, inorganic phosphorus (EL-Merzabani et al., 1977), calcium ( Glinder and king., 1972), creatinine, (Giorio., 1974), blood urea nitrogen (Richterich., 1968), AST and ALT (Reitman and Frankel., 1957) and zinc, copper and iron (AOAC., 1980).

**c-Milk samples:** It were collected from lactating diseased and recovered cows for detection of subclinical mastitis and decrease in milk production (Schalm et al., 1971).

**4-Therapeutic trials:** Diseased cows were isolated in insect proof stables whenever it was possible with complete rest. Ancillary and symptomatic treatment of infected cows by antipyretic (Analgin, 8ml/100kg, b/w, I/V, Memphis. Co) together with cold fomentation, anti-inflammatory (Phenylbutazone with sodium salicylate, 5ml/calf, 20ml/cow, I/V, Virbac.Co), calcium therapy for recumbent non feverish animals (Cal-D-Mag, 200ml/calf,

500ml/cow, slowly I/V, Pfizer.co), fluid therapy (Dextrose and normal saline, 1/2 liters/calf, 1-3liters/cow, I/V, Adwia.co), vitamins (Vitamin AD3E, 5ml/calf, 30ml/cow, I/M, Norbrook. co) and antibiotic (Oxytetracycline hydrochloride 5%, 1ml/10kg, b/w, I/M, Arab Veterinary Industrial co) for combating of secondary bacterial infection. Special care and nursing of recumbent cows was advocated (Uren et al., 1989; St. George et al., 1997; and Hassan., 2000). It should be remembered that nothing should be given by mouth unless swallowing reflex is observed to be functional.

**5-Statistical analysis:** It was carried according to the method of Sendecor and Cochran., (1982).

## RESULTS

**1- Clinical signs:** The infected cows were showed two febrile phases, each lasted for 1-2 days, the first phase was often mild and accompanied by a sharp decrease in milk production and depression. More severe clinical signs were evident in the second febrile phase typically included; severe dejection, decrease of appetite or anorexia, general weakness, salivation, serous ocular and nasal discharges, rapid respiration, accelerated pulse rate, joint swelling, edematous swelling of superficial lymph nodes, muscular shivering or tremors, stiffness in gait or shifting lameness and moving their weight from limb to limb when standing and

later on became in sternal or lateral recumbency and assuming the posture of cows that had milk fever. Some of diseased animals showed loss of the swallowing reflex, bloat, ruminal stasis, constipation, excessive salivation, coma and death.

### **2-Virological and serological investigation:**

Buffy coats collected from viremic animals on inoculation in BHK cell cultures elicited cytopathic effect (CPE) and causes neurological signs and subsequent death of suckling mice. Virus neutralization test was performed using standard hyperimmune sera to confirm that the isolate is BEF virus. Samples from incontact animals were negative. Serological examination of the serum samples collected from acutely infected and convalescent cows showed significant rise in neutralizing antibody titer. The serum samples collected from incontact animals showed negative results except buffaloes had serum neutralizing antibodies.

**3-Epidemiological data:** Table (1) illustrated that, the disease was sudden in onset, spread rapidly, occurred in summer with an incubation period ranged from 1 to 9 days and the course of the disease was about one week in uncomplicated cases and more than one week in complicated cases.

In table (2), the incidence was 53.36 and 47.93 % among cows and calves respectively while

the total incidence was 52.38 %. Table (3) revealed that, the incidence among males and females was 43.64 and 61.66 %, respectively and those in foreign breed and native breed was 73.8 and 33.71%, respectively. In table (4), the mortality rate was 2.18 and 3.31 % among cows and calves while the percentage of emergency slaughtered animals due to prolonged recumbency and extreme loss of weight was 4.54 and 2.48 % among cows and calves respectively.

**4-Hematological studies:** The hematological parameters (table 5) of the diseased animals showed significant reduction in total erythrocytic count, hemoglobin concentration and lymphocyte percent and non significant elevation in total leukocytic count and significant increase in neutrophils percent.

**5-Serum biochemical analysis:** Table (6) declared significant decrease in total protein, albumin, globulin, calcium, phosphorous, iron and zinc and significant elevation in copper, creatinine, blood urea-nitrogen and highly significant increase in AST and ALT.

**6-Postmortem findings:** The most prominent lesions were focal necrosis of the larger muscles of the shoulder and back, accumulation of large quantities of serous fluid rich in fibrin in peritoneal, pleural, pericardial and joint cavities, most of lymph nodes were edematous and

enlarged, lung have patches of edema, consolidation or pneumonic foci and areas of emphysema in some animals with evidence of subcutaneous emphysema.

**7-The possible complication:** Table (7) showed the type and percentage of different complications among infected cattle. There were pulmonary emphysema (2.56 %), subcutaneous emphysema (1.95 %), broncho-pneumonia (16.59 %), abortion (2.82 %) and prolonged recumbency (3.11).

**8-Estimation of subclinical mastitis and reduction in milk:** The results in table (8) declared the percentage of subclinical mastitis and reduction in milk production during infection and at 1W, 3W and 2M post-recovery were 54.91, 28.56, 11.22 and 6.49 % and 51.3, 40.1, 23.6 and 4.5 %, respectively.

**9-Therapeutic trials:** The treated animals were showed clinical improvement within one week in uncomplicated cases while those manifesting complication, the improvement was achieved in more than one week or may be emergency slaughtered or died.

Table (1): Some epidemiological dynamics of bovine ephemeral fever

Animals susceptible	Onset	Spread	Seasonal incidence	Incubation period	Course of the disease	
Cattle	Sudden	Rapid	Summer	1-9 days	Within one week in uncomplicated cases	More than one week in complicated cases

Incubation period was determined as the period between appearance of old and new cases.

Table (2): Percentage of bovine ephemeral fever among examined animals

Animals	No. of animals at risk	No. of clinically normal animals	No. of clinically diseased animals	Percent ill
Adult cattle	551	257	294	53.36
Calves	121	63	58	47.93
Total	672	320	352	52.38



Table (3): Relation between sex and breed of cattle and the incidence of bovine ephemeral fever

Animals	Sex				Breed			
	Males		Females		Foreign		Native	
	No	%	No	%	No	%	No	%
Infected animals	151	43.64	201	61.66	231	73.8	121	33.71
Clinically normal animals	195	56.36	125	38.34	82	26.2	238	66.29
Total	346		326		313		359	

Table (4): Mortality rate and emergency slaughtered animals percentages among infected animals.

Animals	No. of animals at risk	No. of slaughtered animals	Percentage	No. of dead animals	Percentage
Adult cattle	551	25	4.54	12	2.18
Calves	121	3	2.48	4	3.31
Total	672	28	4.17	16	2.38

Table (5): Some hematological parameters in apparently clinically healthy control and diseased cows (M  $\pm$  S.E).

Parameters	Control cows	Diseased cows
RBCs $10^6/\text{cumm}$	$7.23 \pm 0.24$	$6.12 \pm 0.04^*$
Hb g %	$11.95 \pm 0.45$	$10.24 \pm 0.15^*$
Total WBCs $10^3/\text{cumm}$	$9.82 \pm 0.08$	$10.1 \pm 0.4$
Neutrophils %	$47.7 \pm 0.87$	$52.4 \pm 0.74^*$
Lymphocytes %	$42.6 \pm 0.53$	$38.9 \pm 0.69^*$
Monocytes %	$4.1 \pm 0.33$	$4.8 \pm 0.43$
Eosinophils %	$3.6 \pm 0.41$	$3.4 \pm 0.29$
Basophils %	$1.67 \pm 0.11$	$1.61 \pm 0.13$

\* Significant at  $p < 0.05$

Table (6): Some Serum biochemical parameters in apparently clinically healthy control and diseased cows (M  $\pm$  S.E).

Parameters	Control cows	Diseased cows
Total protein gm/dl	8.24 $\pm$ 0.75	6.21 $\pm$ 0.93*
Albumin gm/dl	3.22 $\pm$ 0.41	2.30 $\pm$ 0.27*
Globulin gm/dl	5.02 $\pm$ 0.22	3.9 $\pm$ 0.04*
Calcium mg/dl	8.93 $\pm$ 0.13	7.13 $\pm$ 0.96*
Phosphorus mg/dl	4.5 $\pm$ 0.72	3.11 $\pm$ 0.04*
ALT IU/L	22.89 $\pm$ 3.19	35.2 $\pm$ 2.57**
AST IU/L	64.43 $\pm$ 2.78	91.8 $\pm$ 1.1**
Urea mg/dl	24.1 $\pm$ 1.64	27.59 $\pm$ 1.89*
Creatinine mg / dl	1.10 $\pm$ 0.05	2.1 $\pm$ 0.08*
Iron ug/dl	141.8 $\pm$ 4.7	134.4 $\pm$ 3.9*
Copper ug/dl	118.4 $\pm$ 3.5	126.8 $\pm$ 4.3*
Zinc ug/dl	98.2 $\pm$ 2.4	91.5 $\pm$ 3.9*

\* Significant at p < 0.05

\*\* Highly significant at p < 0.01

Table (7): Type and percentage of different complications among diseased animals

Type of complication	Percent
1-Pulmonary emphysema	2.56
2-S/C emphysema	1.95
3-Broncho-pneumonia	16.59
4-Abortion	2.82
5-Prolonged recumbency (more than one week)	3.11

Table (8): Percentages of subclinical mastitis and reduction in milk production among infected cows as well as at different intervals post recovery.

Duration	Subclinical mastitis %	Reduction in milk production %
1-During infection	54.91	51.3
2-1 WPR	28.56	40.1
3-3 WPR	11.22	23.6
4-2 MPR	6.49	4.5





Photo (1) A diseased cow showing nasal discharge



Photo (2) A diseased cow showing ocular discharges



Photo (3) A diseased recumbent cow

## DISCUSSION

Bovine ephemeral fever is an overt clinical ailment of cattle and buffaloes. It has a bad effect on the economy due to high morbidity rate and subsequent complications although it has a low mortality rate. (St. George., 1993 and Radostitis et al., 2000). It has occurred during summer 2000 in Egypt in a number of occasions (Attia and selim., 2000, Hassan, 2000; Zaghawa et al., 2000 and Sayed et al., 2001).

The results of virological and serological investigation are in agreement with that reported by Snowdon., (1970); Burgess and Spradbrow., (1977) and Nagi et al., (1992).

The developed clinical signs were previously

described in the prior work of Nagano et al., (1990); Hassan et al., (1991); Nagi et al., 1992; Banoub., (1994); Radostits et al., (2000), Zaghawa et al., (2000) and Attia and Selim., (2000).

The release of pyrogens from leukocytes and Kupfler cells acting on the hypothalamus resulting in pyrexia and ruminal atony with subsequent anorexia and cessation of rumination (Stoner., 1972), moreover interference in swallowing resulting in drooling of saliva (Burgess and Spradbrow., 1977). The lameness and recumbency contributed to the increase in the amount of synovial fluids and degenerative changes in synovial membranes due to vascular damage results in joint pain, shifting lameness or paresis (St.George et al., 1995), as well, the inflammatory nature of the disease includes tendon sheaths, muscles, fasciae



and skin (Basson et al., 1970). The edematous enlargement of the lymph nodes could be attributed to lymphadenitis and alteration in the permeability of blood vessels (Hungerford., 1990 and Radostits, et.al., 2000).

The data of interest recorded in this investigation revealed that some of infected animals had a double bouts of clinical disease after apparent clinical recovery from initial infection and the second one was more severe, these results are supported by conclusion of Coetzer, et.al ., (1994). This phenomena was also observed by MacFarlane and Haig (1955) who found that in a single epidemic, double or triple bouts of clinical disease have been occurred and which may be attributed to different strains of bovine ephemeral fever virus or to insufficient antigenic stimulus in first mild infection episode to produce adequate antibody level.

The result tabulated in table (1) simulated that reported by Spradbrow and Francis., (1969), Hungerford., (1990) and Banoub., (1994). Epidemiological information and indirect evidence suggested that bovine ephemeral fever is an insect-born infection where the disease spread during summer in Egypt, this conclusion is highly augmented by the previous work of Uren., (1989); Coetzer, et.al., (1994) and Murray., (1997).

The obtained data in table (2&3) are higher than that recorded in 1991 outbreak in Egypt where

Hassan et al., (1991) reported morbidity rate ranged from 32 to 43 % and Banoub., (1994) recorded morbidity rate of 49.4%. The incidence rate was lower in calves than that in adults. This finding is supported by the conclusion of Gibbs., (1981) ; Coetzer et al., (1994) and Nandi and Negi., (1999) who reported that fat cows and bulls were worstly affected while calves were least affected.

It is obvious that, the increase in incidence rate in this outbreak may be contributed to the increased virulence of the causative virus due to serial passage in cattle or other reservoirs, so it is advisable to study the antigenic and immunogenic characters of the virus isolated during the outbreak of 1991 compared to that isolated during this outbreak. There is no evidence of antigenic or immunogenic diversity within the virus population (Snowdon., 1970), but preliminary epitope mapping suggested some variation (Cybinski., 1988). The results in table (4) were in a harmony to that reported by the work of other workers (Hassan et al., 1991 and Banoub., 1994).

Interestingly, the only infected animal species in this outbreak and in previous outbreak during 1991 was cattle. The role of buffaloes as a reservoir of infection has not been declared in Egypt, however in this study, the incontact buffaloes to infected cattle had antibodies in their sera without evidence of clinical findings, Tomar and Tripathi., (1986) recorded bovine ephemeral fever virus



infection in 2.2 % of lactating buffaloes and suggested that buffaloes might have a genetic resistance to this ephemeral fever virus infection. The antibodies are not detected in sheep, goats, dogs or donkeys that are in contact with infected cows, a data which agreed with those recorded by Gibbs., (1981) and Uren., (1989).

The results presented in table (5) are in parallel to those reported by many authors (Nandi and Negi., 1990; Hassan et al., 1991; St.George et al., 1995; Abu-Elzein et al., 1997; Attia and Selim., 2000 ; Hassan., 2000; and Mahmoud., et al. 2000). The decreased values of hematological parameters may be attributed to anorexia or to hemosiderosis of lymph nodes and spleen (Burgess., 1971) or due to endothelial hypoplasia that caused by the virus (Combs., 1987).

On the other hand, neutrophilia may be contributed to tissue damage and secondary bacterial infection, while lymphopenia to stress situation (Inaba., 1968) or to release of large quantities of endogenous corticosteroids during the disease (Schalm., 1965). The above mentioned results are nearly similar to those obtained by St.George et al., (1995) who found leukocytosis with relative increase in neutrophils during the acute stage of the disease with lymphopenia and hypocalcemia. In this respect, Coetzer et al., (1994) reported that, the most useful direct confirmatory test for ephemeral fever was a differential leukocyte count.

The results in table (6) were nearly similar to those obtained by the prior work of St.George et al., (1984); Uren et al., (1992); St.George et al., (1995); Mahmoud et al., (2000) and Sayed et al., (2001). The reduction in the above parameters might be attributed to the state of anorexia during initial stage of the disease (Galycan et al., 1981) or to hepatic insufficiency (Hassan., 2000). The increase in blood urea nitrogen could be attributed to the increase of protein breakdown during pyrexia (Varley., 1969) or due to tissue destruction caused by the virus and subsequent renal insufficiency (Heuschele and Barber., 1966). While elevation of creatinine might be due to the effects of the virus on the kidney (Hassan., 2000). However, the increase in ALT and AST could be attributed to inflammatory and destructive changes in liver, muscles and myocardium due to the virus infection (Hungerford., 1990 and Radostitis et al., 2000).

The reduction in serum zinc might be exaggerated through the inflammation process (Mills., 1987) while the increase of serum copper may be attributed to inflammation stress (Chandra and Daylon., 1982). Moreover, the decrease of iron might be contributed to endothelial hypoplasia that caused by the virus (Combs ., 1987).

Results of the postmortem findings confirm the major systemic inflammatory response (Coetzer et al., 1994). Similar observations were described by Nagi et al., (1992) ; Zaghawa et al., (2000) and

Sayed et al., (2001).

Results presented in table (7) are highly augmented and simulate those described by Brown (1955); Burges., (1971) and Venbin et al (1991). In this field, Radostits et al., (2000) mentioned that, cows recovered from ephemeral fever disease showed protracted motor disturbance in the hind limbs and sometimes prolonged recumbency. Moreover, Snowdon., (1970) reported that, cattle recovering from acute bovine ephemeral fever were continuing to show protracted motor disturbances usually of hind limbs or prolonged recumbency, also Hill and Schultz., (1977) recorded ataxia and paralysis associated with bovine ephemeral fever infection.

Regarding to complications (Table,7). The subcutaneous emphysema occurred due to interstitial pulmonary emphysema with partial blockage of the air passages with exudate and wall rupture of the necrotic bronchioles and alveoli resulting in gasses escaping beneath pleura into mediastinum and via fascial plane to under skin of the neck (Burgess and Sparadbrow., 1977; Theodorides and Coetzer., 1979 and Gibbs., 1981).

The results of abortion percentage was higher to that recorded by Banoub., (1994) who found that three cows out of 256 infected cows had aborted and lower to that recorded by Coetzer et al., (1994) who reported that abortion occurred in about 5 % of pregnant cows.

The reduction in milk yield (table 8) is attributed to anorexia and to the subclinical mastitis developed (Losos., 1986), these findings are nearly similar to those recorded by Theodorides et al., (1973); Davis et al., (1984) and Stomdfast and Miller (1985) who recorded 35 % and 58.7 % reduction in milk yield during infection respectively. Also, Zaghlawa et al., (2000) recorded that milk yield was reduced to about 25 % during the peak of the outbreak.

The symptomatic and ancillary treatment of infected cases resulted in clinical improvement of the infected cases within one week but some of recumbent cases did not respond and emergency slaughtered or died. In this respect, Uren et al., (1989); St.George et al., (1986) and St.George et al., (1995) mentioned that, the intravenous administration of calcium preparations can be considered a justifiable addition to the treatment regimen together with prolonged phenylbutazone therapy, to relief temporary paralysis.

Following this epizootic, the data records in the investigated farms recorded a temporary infertility in bulls and decrease conception rate. Such reports would appear to be sustained by the demonstration of changes in the midpiece of spermatozoa or mid-piece abnormalities, detached normal or abnormal heads and tails and spermatozoa with bent tails shown to occurs following bovine ephemeral fever virus infection in bulls (Chenoweth and Burges., 1972 and Parsonson

and Snowdon., 1974b).

We should list diseases that may cause threat to Egypt and put necessary regulations to prevent its introduction to the country through improvement of the diagnostic facilities, laws dealing with animal movement, quarantine regulations and training of field veterinarian on recent advances in the field of veterinary medicine (Fayed., 1993).

Yet many unsolved questions associated with this and previous outbreak of bovine ephemeral fever in Egypt as following:

1-Epidemiological analysis of the disease on large scale should be applied to declare the reasons of occurrence of this outbreak. Is the virus present during interepizootic period (1991-2000) circulating subclinically in cattle or other reservoirs? and are genetic variation of the virus occurred ? or is the disease entering Egypt from importation of animals or introduction of infected arthropods by any way? and is bovine ephemeral fever exotic or endemic in Egypt?

molecular studies of bovine ephemeral virus from the last two outbreaks and different geographic locations should resolve many of these important questions.

3-The possible use of vaccine prepared from the local virus strain and use as emergency or routine vaccine to protect valuable breeding stock and

dairy cows in future outbreaks should be investigated.

Finally, this study declared that bovine ephemeral fever disease affect cattle only, the onset of the disease is sudden and rapidly spread during summer season suggesting that it may be transmitted by insects, transit in nature and most of infected cases were clinically improved within one week. Buffaloes in contact with infected cattle had serum neutralizing antibody titers in their sera without showing clinical evidence of the disease and this indicated that buffaloes have a role in epidemiology of the disease.

The disease causes disturbance of the general health condition of the affected cows where there are decrease in body weight and milk production besides deaths, abortion and culling of the recumbent cows from the herd and deleterious alteration in hematological picture and serum biochemical panel where there were anemia, hypocalcemia and hypoproteinemia. Therefore improving feeding programs and supplementation of the diet with the deficient elements to compensate the deficiency and administration of hematinics to compensate anemia and a fluid therapy containing calcium is indicated especially for recumbent cases. It could be concluded that preparation and evaluation of vaccine prepared from local strain to be used under field condition together with an insect control program are now recommended to combat bovine ephemeral fever.



## REFERENCES

- Abu-Elzein, E.M.E.; Gameel, A.A.; AL Afaleq, A.I. AL-Gundi, O. and Bukhari, A. (1997) : Bovine ephemeral fever in Saudi Arabia., 14: 630-631.
- AOAC. (1980): Official methods of analysis. 13 th Ed. Office and Cham., Washington DC.
- Attia, H. and Selim, H. (2000): Clinical-Hematological and biochemical studies on bovine ephemeral fever. 9th.Sci. Cong. Fac.Vet. Med., Assiut Univ: 619-627
- Banoub, S.M. (1994): Ephemeral fever (Three day sickness) in closed milking cow farm at Sharquia. 6th Sci.Cong.Fac.Vet.Med., Assiut : 558-563.
- Basson. P.A.; Pienaar, J.C. and Van der Westhuizen, B. (1970): The pathology of ephemeral fever: A study of the experimental disease in cattle. J. South Afr. Vet. Med. Assoc., 40: 385-397.
- Brown, J.H.M. (1955): Bacterial endocarditis in cattle a possible sequel to three days stiffness sickness. J. South Afr. Vet. Med. Assoc., 26: 29-37.
- Burges, G.W. (1971): Bovine ephemeral fever: A review. Vet.Bull., 41: 887-895.
- Burgess, G.W. and Sparadrow, P.B. (1977): Studies on the pathogenesis of bovine ephemeral fever. Aust.Vet.J. 53: 636-639.
- Chandra, R.K. and Daylon, D.H. (1982): Trace elements regulation of immunity and infections. Nutr. Res., 2: 733-734.
- Chenoweth, P. and Burges, G.W. (1972): Aust.Vet.J., 48 : 37. Cited by Parsonson, I.M. and Snowden, W.A. (1974b) : Ephemeral fever virus : Excretion in the semen of infected bulls and attempts to infect female cattle by the intrauterine inoculation of virus. Aust.Vet.J., 50 : 329-334
- Coetzer, J.A.W.; Thomson, G.R. and Tustin, R.C. (1994): Infectious diseases of livestock with special reference to South Africa. Cape Town. Oxford New York. Oxford University Press
- Combs, G.P. (1987): Bovine ephemeral fever. Proc.US Anim.Health. Assoc., 82: 29-35.
- Cybinski, D.H. (1988): Commonwealth scientific and industrial researches organization, Canberra, Australia. Unpublished data.
- 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
- Doumas, B.; Watson, W. and Biggs, H. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin .Chem .Acta. 81: 87.
- EL-Merzabani, M.M.; EL-Aaser, A.A. and Zakhary, N.I. (1977): J.Clin.Chem.Clin. Biochem., 15: 715-718.
- Farag, M.A.; AL-Sukayran, A.; Mazloum, K.S. and AL-Bukomy, A.M. (1998): Epizootic of bovine ephemeral fever on dairy farms in Saudi Arabia. Rev Sci.Tech., 17: 713-722.
- Fayed, A.A. (1993): Exotic animal diseases reported in Egypt. 2nd Sci.Cong.Egyptian Society for cattle diseases., Assiut.,: 249-256.
- Galyean, M.L.; Lee, R.W. and Hubbert, M.E. (1981): Influence of fasting and transit on ruminal and blood metabolites in beef steer. J.Anim.Sci., 53: 7-17.
- Gibbs, E.P.J. (1981) : Virus diseases of food animals., Volume II. Academic press, London, New York
- Giorio, J.D. (1974): Clinical chemistry. Principles and Techniques. Henry et., Ed., Harper and Row. Hagerstown. pp: 543.

- Glinder, E.M. and King, J.D. (1972): Rapid calorimetric determination of calcium in biological fluids with methylene blue. *Amm.J.Clin.Path.*, 58 : 376-382.
- Glyn Davies, F. and Walker, A.R. (1974): The isolation of ephemeral fever virus from cattle and culicoides midges in Kenya. *Vet.Rec.*, 20: 63-64.
- Hassan, H.B.; EL-Danaf, N.A.; Hafez, M.A.M.; Ragab, A.M. and Fathia, M.M. (1991): Clinico-diagnostic studies on bovine ephemeral fever with detection of virus for first time in Egypt. *J.Egypt.Vet.Med.Ass.*, 51 (4) : 873-887.
- Hassan, H.Y. (2000): An outbreak of bovine ephemeral fever in Egypt during 2000: II-Clinico-chemical and hematological alterations before and after symptomatic treatment trials. 9th.Sci. Cong.Fac.Vet.Med., Assiut Univ: 325-336
- Henry, R.J. (1964): Clinical chemistry. Harper & Row Publishers., New York, p: 181.
- Heuschele, W.P. and Barber, T.L. (1966) : Changes in certain blood components of rinderpest infected cattle. *Am.J.Vet.Res.*, 180(6) : 570-572
- Hill, M.W. and Schultz, K. (1977): Ataxia and paralysis associated with bovine ephemeral fever infection. *Aust. Vet.J.*, 53 : 217-221
- Hungerford, T.G. (1990): Diseases of live stock. 9th. Ed. McGraw-Hill Book Co., Sydney.
- Inaba, Y. (1968): *Jap.Agric. Q.*, 3:36. Cited by Burgess, G.W. and Sparadrow, P.B. (1977): Studies on the pathogenesis of bovine ephemeral fever. *Aust.Vet.J.*, 53 : 636-639.
- Loson, G.J. (1986): Infectious tropical diseases of domestic animals. Longman Scientific and Techn., England. pp: 452-477.
- MacFarlane, I.S. and Haig, D.A. (1955): Some observations on three-day stiff-sickness in the Transvaal. 1954. *J.Sou.Afr.Vet.Med.Assoc.* 36 :1-7.
- Mahmoud, A.A; Abd-El-Raof, Y.M. and Aboul EL-Enen, G.F. (2000): Utility of blood picture and serum metabolic profile in cases of ephemeral fever in Holstein-Friesian dairy cows. *Benha.Vet.J.*, 11(2) :60-69
- Mills, C.F. (1987): Biochemical and physiological indicators of mineral status in animals: Copper, cobalt and zinc. *J.Anim.Sci.* 65 : 1702-1711.
- Murray, M.D. (1997): Possible vectors of bovine ephemeral fever in the 1967/68 epizootic in northern Victoria. *Aust.Vet.J.* 75 : 220.
- Nagano, H.; Hayashi, K.; Kubo, M. and Miura, Y. (1990): An outbreak of bovine ephemeral fever in Nagasaki prefecture in 1988. *Japanese J.Vet.Sci.* 52(2) : 307-314.
- Nagi, A.A.; EL-Mashed, A.I.; EL-Bagoury, G.F. and Moustafa, A.M. (1992): An investigation on ephemeral fever (three-days sickness) in cattle at Kalyubia governorate (Egypt). *Annals of Agric. Sc., Moshtohor.* 30 (2): 1159-1169.
- Nandi, S and Negi, B.S. (1999): Bovine ephemeral fever: A review. *Comp.Immun.Microbiol.Infect. Dis.*, 22: 81-91.
- Parsonson, I.M. and Snowdon, W.A. (1974a): Experimental infection of pregnant cattle with ephemeral fever virus. *Aust.Vet.J.*, 50 : 335-337
- Parsonson, I.M. and Snowdon, W.A. (1974b) : Ephemeral fever virus : Excretion in the semen of infected bulls and attempts to infect female cattle by the intrauterine inoculation of virus. *Aust.Vet.J.*, 50 : 329-334
- Piot, J.B. (1869): Epizootic of dengue fever of cattle in Egypt. *Prix Monbinne. Paris, France: National Academy of medicine.*

- Labaghiati, D.S. (1924): Three days fever or stiffness sickness in cattle. *Vet.Rec.* 4 (23): 503-505.
- Radostits, O.M.; Gay, C.C, blood, D.C. and Hinchcliff, K.W. (2000): *Veterinary medicine A textbook of the diseases of cattle, sheep, pigs, goats and horses*. 9 th Ed. W.B.Saunders Company Ltd.
- Reitman, S. and Frankel, S. (1957) : Calorimetric determination of GOT and GPT activity.*Am.J.Clin.Path.*, 18 : 26
- Richterich, E. (1968): Estimation of urea by Bertholet reaction.*Klinische Chemie Akademische verlagsgesellschaft, Frankfurt/main*. 2nd., p : 254
- Rossiter, P.B. and Jesette, D.M., (1982): Microtitre techniques for the assay of rinderpest virus and neutralizing antibody. *Res. Vet. Sci.*, 32:253-256.
- Sastry, G.A. (1985): *Veterinary clinical pathology*.3rd Ed.CBS publishers distributors. Delhi, India
- Sayed, A.S.; Sadiq, A.H. and Ali, A.A. (2001): Bovine ephemeral fever in Assiut governorate: Clinical, laboratory and therapeutic studies. *Assiut.Vet.Med.J.*, 44 (88):157-175.
- Schalm, O.W; Carroll, E.J. and Jain, N.C. (1971): *Bovine mastitis*. Lea and Febiger, Philadelphia.
- Sendecor, G.W. and Cochran, W.G. (1982): *Statistical methods*.8th Ed.The Iowa state, University Press, USA.
- Sharma, L.K. (1992): A report on bovine ephemeral fever in cattle. *Indian Vet.J.*, 69: 544-546.
- Snowdon, W.A. (1970): Bovine ephemeral fever: The reaction of cattle to different strains of ephemeral fever virus and the antigenic comparison of two strains of virus. *Aust.Vet.J.* 46: 258-266.
- Spradbrow, P.B. and Francis, J. (1969): Observations on bovine ephemeral fever and isolation of virus.*Aust.Vet.J.* 45 : 325-327.
- St. George, T.D. (1988): Bovine ephemeral fever: A review. *Trop. Anim.Health.Prod.* 20 : 194-202.
- St. George, T.D.; Cybinski, D.H.;Murphy, G.M. and Dimmock, C.K. (1984): Serological and biochemical factors in bovine ephemeral fever.*Aust.J of.Biological.Sci.* 37 : 341-349.
- St. George, T.D.; Uren, M.F. and Zakrzewski, H. (1986): The pathogenesis and treatment of bovine ephemeral fever. In St.George, T.D.; Kay, B.H. and Blok, J., *Proceedings of the fourth symposium on arbovirus research in Australia, Brisbane, Australia 6-9 may 1968*.Canberra: Commonwealth Scientific and Industrial Research Organization.
- St. George, T.D. (1993): The natural history of ephemeral fever of cattle. In bovine ephemeral fever and related Rhabdovirus.Eds. St.Georg, T.D.; Uren, M.F.; Young, A P.L and Hoffman, D. Canberra, Australian center for international Agricultural research.pp :13
- St. George, T.D. (1997): Effective treatment of bovine ephemeral fever. *Aust.Vet.J.* 75: 221-223.
- St. George, T.D.; Murphy, G.M.;Burren, B. and Uren, M.F. (1995): Studies on pathogenesis of bovine ephemeral fever. IV: A comparison with the inflammatory events in milk fever of cattle.*Vet.Microbiol.* 46 : 131-142.
- Stoindfast, H.A. and Miller, K. (1985): *Veterinary viral diseases*. Ed. of Dell-Porta, New York.Academic press. p:394
- Stoner, H.B. (1972): *Microbial pathogenesis in man and animals*. Cambridge University Press. London.
- Theodorides, A. and Coetzer, J.A.W. (1979): Subcutaneous and pulmonary emphysema as complications of bovine ephemeral fever. *Onderstepoort of Vet.Res.* 46 : 125-127.



- Theodorides, A., Giesecke, W.H. and Du Toit, I.J. (1973): Effect of ephemeral fever on milk production and reproduction of dairy cattle. Onderstepoort J. of Vet.Res. 40 : 83-91.
- Tomar, S.S. and Tripathi, V.N. (1986): Genetic resistance for ephemeral fever in Murrah buffaloes. 3rd World Congress on genetics applied to livestock production, Lincoln, Nebraska, USA. Genetics of reproduction, lactation, growth, adaptation, disease and parasite resistance: 671-675.
- Uren, M.F. (1989): Bovine ephemeral fever. Aust.Vet.J. 66 (8): 223-236.
- Uren, M.F.; St. George, T.D. and Zakrzewski, H. (1989) : The effect of anti-inflammatory drugs on bovine ephemeral fever. Vet.Microbiol., 19 : 99-111
- Uren, M.F.; St. George, T.D. Murphy, G.M. (1992). Studies on the pathogenesis of bovine ephemeral fever in experimental cattle. Vet.Microbiol. 30 : 297-307.
- Varley, H. (1969): Practical Clinical Biochemistry. 4th ed. Williams Heinemann Medical Book Ltd. London and Intr science Book Inc, New York.
- Venbin, B.; Chunling, J. and Danis, S.S. (1991): Preliminary observations on the epidemiology of bovine ephemeral fever in China. Trop.Asian. Health. Prod (1) : 22-26.
- Zaghawa, A.; Akela, M.A.; Khader, A.M. and Hassan, H.Y. (2000): An outbreak of bovine ephemeral fever in Egypt during 2000: II-Clinical and epidemiological investigations. 9th. Sci. Cong.Fac.Vet.Med.Assiut Univ.: 353.