Vet.Med.J.,Giza. Vol.55, No.2. (2007):435-450.

EPIDEMIOLOGICAL STUDIES OF TWO OUTBREAKS OF BOVINE EPHEMERAL FEVER IN MIDDLE EGYPT

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Received: 9. 8. 2006

Accepted: 21. 9. 2006

SUMMARY

In .two epidemics of bovine ephemeral fever (BEF) in Egypt, one in 2000 and the other in 2001, the virus was probably carried by vectors transported by air currents across the neighboring countries and through red sea or across Suez Canal trough. Then carried by vectors, transported to neighboring Governorates, until reached Middle Egypt (Beni-Suef and El-Fayoum Governorates). The disease broke out under different ecological conditions among cattle and buffaloes populations and spread rapidly; it developed in spring and summer and ended in November. The morbidity rate in native breed cattle was 39.5%, 67.5% while it was 44%, 73% in foreign breed cattle and in buffaloes was 4.68%, 80% in Beni-suef and EL Fayoum Governorates respectively, at 2000 while it was 20.38%, 19.5% in native breed cattle, 30.38%, 43%; foreign breed and 5.9%, 6.6% in Buffaloes in Beni-suef and El- fayoum Governorates at 2001. Case fatality rate 1.5%, 2.16% in native breed cattle while it was 3.14% , 4.1% in foreign breed cattle in BEF outbreak at 2000 while it was 0.0% , 0.5% in native breed cattle and 2,2 in foreign breed cattle in EL - fayoum and Beni-suef Governorates but buffaloes did not show any mortalities in either BEF outbreak at 2000 or 2001.The epidemic of BEF disease at 2001 was milder and shorter than that in 2000. All cattle and buffaloes affected in both outbreaks were more than 3 month old. The vectors unknown certainly but the outbreaks occurred in summer sesion where that the possible vectors mosquitoes and culicids species are evident.

INTRODUCTION

Bovine ephemeral fever (BEF) is an arthropod borne viral disease of Cattle and Water Buffalo characterized by short duration, fever, stiffness lameness, and sometimes paralysis. Both ons

and recovery are sudden. (St. George1988, St. George, 1994; Nandi & Negi, 1999; Radostits et al., 2000).

The disease has a variety of names including three days sickness, stiff sickness, dengue fever of cattle, bovine epizootic fever and lazy man's disease (St. George, 1994).

However, the name of bovine ephemeral fever is most commonly used and is very common (Combs, 1978; Doherty, 1978).

The causative virus was registered in the international catalogue of Arboviruses by Doherty (1978) as Bovine ephemeral fever (BEF) virus. The morbidity may be high but the mortality is low. The disease causes great economic losses such as moralities, abortion, decreased body weight, disruption of markets, drop in milk production ranges from 34-94 with an average of 46% and cows milk yield did not reach to pre-illness level on convalescence and from lowered fertility of bulls as well as the expenses of care or treatment and vaccination (Davis et al., 1984; St-George, 1986, St-George, 1996, Nandi and Negi, 1999, Sayed et al., 2001 and Yeruham et al., 2003) The disease was first described in Africa (Schwenfurth, 1867) it has been reported from many countries in Oceania and Asia (St. George, 1986).

Although a viral etiology was suspected, the Cause of the disease remained unknown unti Van-der Westhuizen (1967) succeeded in isola ing EF virus from defebrinated blood of cattl with syndrome of ephemeral fever by intracere bral inoculation of suckling mice. He also adapte the virus to growth in cell culture (BHK - 21 clone 13). The virus was isolated from cattle an mixed pool of culicids spp in 1972 - 1973 (Davie and walker, 1974).

The disease has been occurred in summer and autumn in subtropical and temperate regions of Africa, Asia and Australia. (St-George, 1984; Nandi & Negi, 1999). It spread by insect vectors, mosquitoes and culicids both biologically and mechanically (Burgess, 1971; Stand fast and Dyce, 1972).

The dynamics of transmission depend on the vector's abundance, distribution, host preference, and susceptibility to infection (Standfast and Dyce, 1972; Burgess, 1977; St. George 1993 and Radostitis et al., 2000).

The disease was firstly described in Egypt by Rabagliati (1924). Since that time no publications about the occurrence of BEF 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; and Banoub 1994) then summer 2000 severe outbreaks of BEF have been recorded in Egypt (Ali et al., 2001; Hamoda et al., 2002, Rah-

man et al., 2002 and Shehabe 2004).

The present work aimed to study the epidemiology of bovine ephemeral fever virus that causes three days sickness in bovine.

MATERIAL AND METHODS

1-Virus:

BEF virus (Webster's Strain) was obtained from Virology Department, Faculty of Veterinary Medicine, Cairo University.

2-Serum samples:

Blood samples, without anticoagulant were collected from diseased cattle and buffaloes, showed symptoms of BEF disease and located in small farms in different area in Beni-Suef and El-Fayoum Governorates, Separated and stored at -20°C, until used for serological studies.

3. Cell Cultures:

African green Monkey (Vero121) cell line, established by Yasumara and Kawatika (1963) serum neutralization test.

Methods :

Staphylococcus protein A (SPA) agglutination test :

Slow quantitative SPA. Agglutination test :

It was done according to(Barrow and felt ham 1995) two fold serial dilution of serum samples were prepared in (U-shaped bottom), microtiter plate then 25ul of 2% SPA suspension were added to each well at the plate. The plate was incubated at 37°C for 1 hour with periodical shaking, 25ul BEF virus suspension was add to SPA serum mixture, then the plate was incubated at 4°C for 2-6 hours. In positive cases lattice of aggregated SPA could be see at the bottom of microtiter plates, while negative wells shows only white button sediment SPA in suspension.

Virus neutralization test (VNT):

Virus neutralization was done according to Cybinski (1987) the reciprocal of the final dilution of serum inhibiting CPE was expressed as neutralizing antibody titers.

RESULTS:

1-Percent of diseased animal during BEF outbreak 2000

The morbidity rate in native breed cattle w 39.5% while it was 44% foreign breed cattle Beni-Seuf governorate while it was 67.2 and 73 native breed and foreign breed cattle respective in El-Fayoum Governorate. The morbidity rate buffaloes was 4.68 and 8% at Beni-Suef and Fayoum Governorates respectively. the morta percentage in native breed cattle ranged betw 0.9 and 3.77% while it was ranged between 0 6.66 in foreign breed cattle, buffaloes not sho

any mortality. The percentage of abortion in diseased native breed cattle ranged between 0.26 and 3.49 while it was 0-7.66% in foreign breed. The diseased buffaloes not showed any abortion. The percentage of emphysema in native breed cattle ranged from 0.83 - 4.57% while it was it was ranged from foreign breed 2. 08- 11.11%. Buffaloes not showed any emphysema.

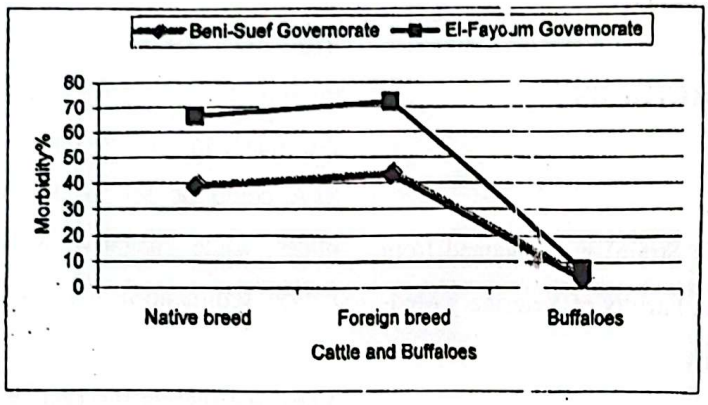

Fig. (1) Percent. of diseased animal during BEF outbreak 2000

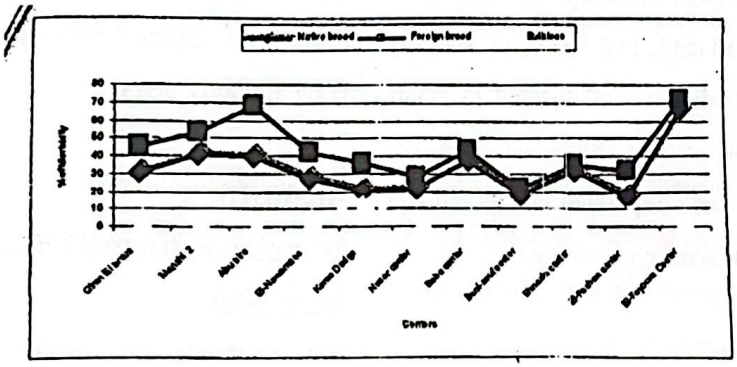

Fig (2): Percentage of morbidity rate (BEF) among animals in Beni-Suef and El-Fayoum Governorates during BEF outbreak at 2000.

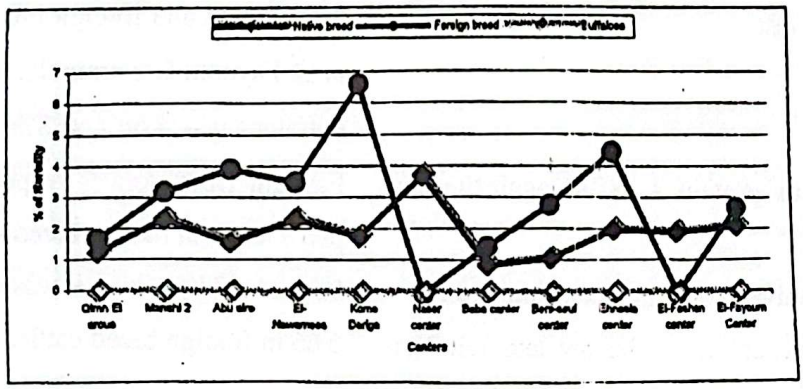

Fig. (3) : Percentage of case fatality in BEF outbreak during 2000.

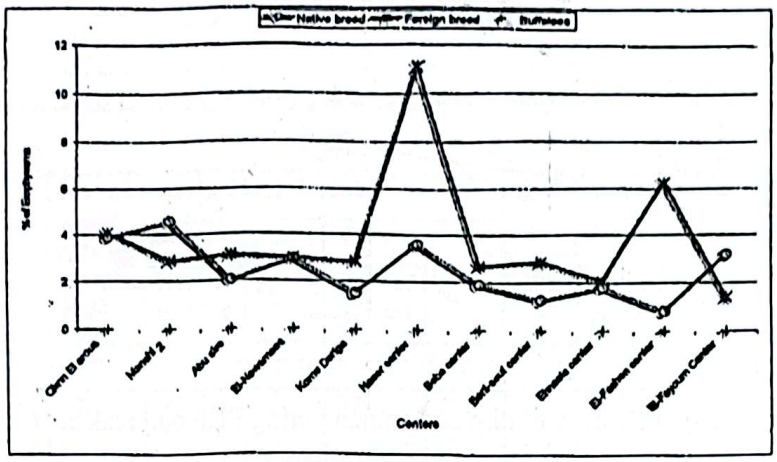

Fig (4) Percentage of Emphysema as in BEF outbreak during 2000.

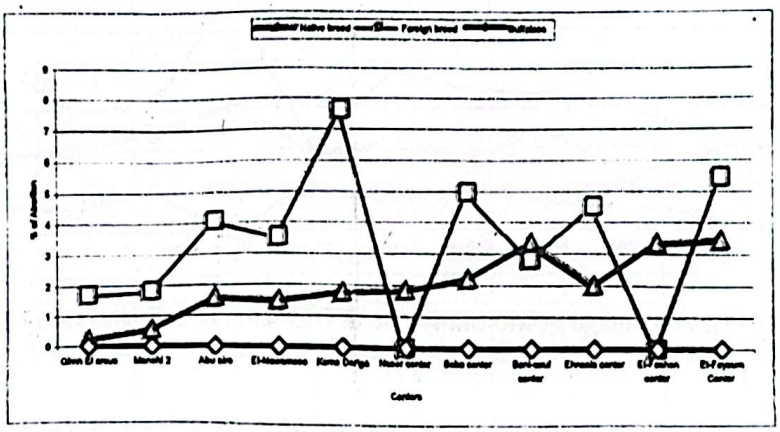

Fig. (5): Percentage of Abortion as in BEF outbreak during 2000

2- Percent of diseased animal during BEF outbreak 2001

the morbidly rate in native breed and foreign breed cattle was 20.38 and 30.38% at Beni-Suef Governarate while it was 19.5% to 43% at El-Fayoum Governorate. The morbidity rate in Buffaloes 0.06 to 0.14% at El-Fayoum and Beni-Suef and Governorate. the morbidity rate in native breed cattle was 13.7-81.1% while it was from 20-60 at foreign breed cattle at Beni-Suef Governorate But in El-Fayoum Governorates was 19.5-43% in native breed and foreign breed respectively the case fatality rate 0.5-1% in native breed and 1-2% in foreign breed at Beni-Suef while it was 0-2% in native breed and foreign breed at El-Fayoum Governorate. The morbidity in Buffaloe at Beni-Suef Governorate ranged between 4-10% while it was 6.6% at El-Fayoum Governorate The case fatality rate either in Beni-Suef and El Fayoum Governorates was zero.

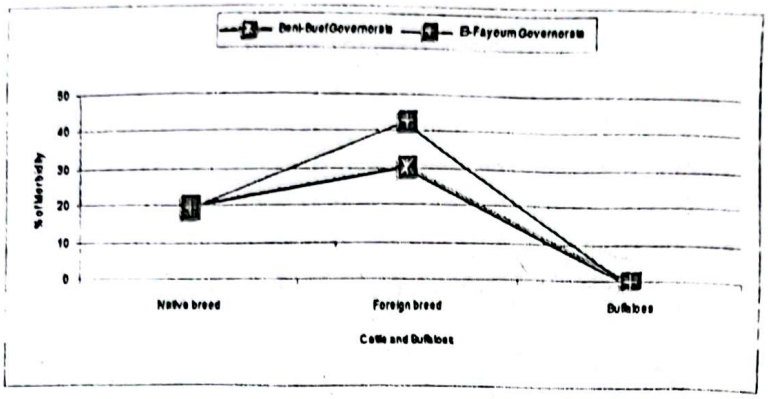

Fig. (6) : (Percent. of diseased animal during BEF outbreak at 2001

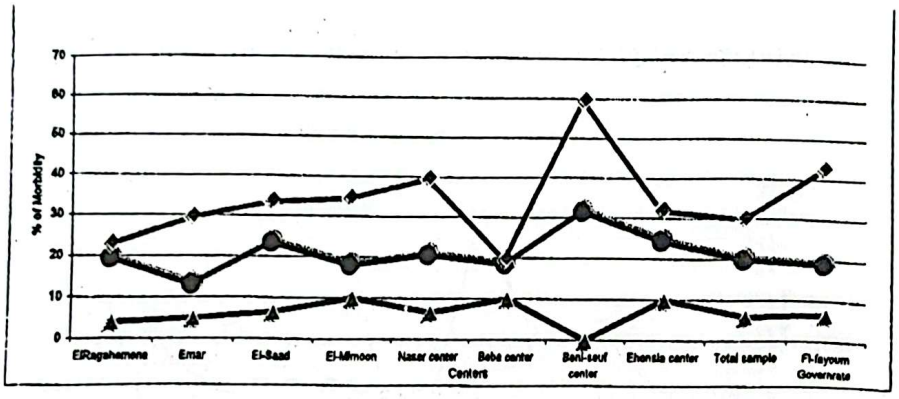

Fig. (7): Percentage of Morbidity rate of BEF among animals during BEF Outbreaks 2001 in Beni-Suef and El-Fayoum Governorate

Table (2) : Most important clinical signs observed during BEF outbreaks at (2000) and (2001)

Sings	*Cattle*		*Buffaloes*
	Native breed	*Foreign breed**	
Fever	40-42^{0}C	41-42°C	40-41 °C
Conjunctivitis	++	+++ sever	-
Salivation	Moderate ++	Profuse watery drooling +++	Little watery
Nasal discharge	Serous mucoid	Seromucoid to purulent	Serous
Respiration	Harried respiration & dy spneic	Harried respiration & dy spneic	Harried
Lacrimation	Little	Excessive	Excessive
Enlargement L.N	Mild	-	-
Dyspnra	2nd to 4th day (30-50%)		-
Stiff gait	++	++	+
Corneal opcity uni or bilateral	+ 0.5-1%	+ 1-2%	-
Subcutaneous emphysema	0.83 – 4.57%	2.08 – 11.11%	-
Submandibler edama	+	+	+
Recumbancy	Evident on the 5th day and on complicated untreated cases 0.5-3%		
Aboration	0.26-3.49%	1.73-6.66%	-

* Frisian and hybrid of Frisian and native breed.

Table (3) Percent of positive sera during BEF outbreaks in 2000 and 2001 by SPA and VNT

Governorates	No. of tested sera sample 2000	BEF outbreaks 2000				No. of sera 2001	BEF outbreak 2001			
		SPA		VNT			SPA		VNT	
		No. of positive	%	No. of positive	%		No. of positive	%	No. of positive	%
1. Beni-Suef Governorate										
- Foreign breed cattle	126	92	73	77	611	119	78	73.1	74	62.1
- Native breed cattle	258	174	67.4	146	56.58	210	166	79	152	72.3
• Buffaloes	45	12	26.6	8	17.7	53	22	41.5	16	30
Total	429	278	64.8	231	53.8	382	275	71.9	243	63.33
2. El-Fayoum Governorate										
- Foreign breed cattle	40	31	77.5	31	77.5	50	37	74	35	70
- Native breed cattle	65	49	75.3	45	64.5	78	64	82	60	76.9
• Buffaloes	14	5	35.7	3	21.4	20	8	40	5	25
Total	119	85	71.4	79	66.3	148	109	73.6	100	67.5
Total Bni-Suef & El-Fayoum Governrates	548	363	66.24	310	56.56	530	384	72.45	343	64.71

Data presented in table (3) showed clearly that the percentage of positive samples by either SPA or VNT in 2001 outbreak is more than those of 2000 outbreaks. the percentage of positive by SPA in Beni-Suef Governorate, were 64.8 and 71.9% in 2000 and 2001 outbreaks respectively. The same picture was found in El-Fayoum Governorate in that the percentage of positive sampled by SPA was 71.4 and 73.6% in 2000 and 2001 outbreaks. The picture by VNT is nearly to that by SPA the reacted positive samples by VNT were 53.8 and 63.33 at Beni-Suef Governorate during 2000 and 2001 outbreaks but it was 66.3 and 67.5% at El-Fayoum Governorate during the same outbreaks.

DISCUSSION

Bovine ephemeral fever virus is one of the arthr pod born virus that induces sever economic loss among cattle in areas showing epidemic of th disease. The disease has a high morbidity and lo mortality rate and characterized by short duratio

fever., stiffness, lameness and sometimes paralysis. Both onset and recovery are sudden and it has been occurred in summer and Autumn in subtropical and temperate region in Africa, Asia and Australia (St. George 1986, Uren et al., 1992) the virus mainly transmitted by arthropod vector and the severity of the disease depends upon the vector abundance and distribution beside host susceptibility and may be other stress factor (St. George 1993, Radostits et al., 2000).

In Egypt, the disease was first described by (Rebagliati 1924) and since this time and until summer 1991 no any publication about occurrence of the disease. After that typical forms of the disease has been occurred in different Governorates in Egypt (Hassan et al., 1991; Banoub 1994, Hassan 2000, Sayed et al., 2001, Shehab et al., 2004) However, the epidemiological studies of the disease provided us with the most important information about the distribution, incidence and prevalence of the disease and considered as a guide for controlling of the disease. Therefore, the present study conduct the epidemiology of the disease in two Governorates (Beni-Suef and El-Fayoum) during two outbreaks of the disease (2000 and 2001).

Concerning epidemiological, study the disease during outbreak 2000 (Fig 1 and 2) showed clearly that the morbidity rate in native breed cattle was 39.5% while it was 44.5% in foreign breed cattle in Beni-Suef Governorate while it was 67.2 and 73% in both breed respectively in El-Fayoum Governorate. The morbidity rate in buffaloes 4.88 and 8% at Beni-Suef and El-Fayoum Governorate respectively. The low morbidity rate in buffaloes either in Beni-Suef or El-Fayoum Governorates indicates clearly that, this species of animal, showing some resistance against BEF virus. On the other hand the highest morbidity rate in outbreak (2000) in foreign breed cattle indicates higher susceptibility of this breed than native breed to infection with BEFV. The incidence of the disease among foreign breed cattle ranged between 23.33%-69.33% while it was ranged from 18.21-67.2% in native breed cattle and it varied greatly in buffaloes as it ranged from 7-15% Fig (2) The highest percentage of incidence either in Abusir village at Beni-Seuf Governorate or El-Fayoum center at El-Fayoum Governorate may be referred to the cultivation of large scale of fadans with Rise crop that needs continuous irrigation with large amounts of water for several months during the period of cultivation which act as a good environmental conduction for multiplication, propagation and spread of Mosque toes and culicoids.

The mortality percentage in foreign breed cattle ranged between 0-6.60%, while it was ranged between 0.9-3.7% in native breed cattle but buffaloes did not show any mortality. The highest mortalities in foreign breed cattle may be due to it is

susceptibility for the disease while the highest mortalities in native breed cattle may be due to suppressive factors that induces over stress on these animals like heavy infestation with external and internal parasite, mal nutrition and poor management specially those reared in bens in separated cases while the majority of foreign breed cattle were reared in farms with closed stables, with good management and nutrition.

The percentage of abortion in native breed cattle 0.26% and 3.49% while it was ranged between 0.0-7.66% in foreign breed cattle.

The surprising event lies in buffaloes as they didn't show any abortion. The highest percentage in foreign breed cattle referred to the highest susceptibility while the absent of abortion in buffaloes, may be referred to its resistance.

The percentage of emphysema in native breed cattle 0.323-4.57% while it was ranged form 2.75- to 11% in foreign breed cattle but buffaloes did not show any emphysema

After the occurrence of BEF disease outbreak 2000 it followed by another mild outbreak during 2001.the two Governorates that observed and surveyed during outbreak 2000 where also observed and surveyed during outbreak 2001 (Beni-Suef and El-Fayoum Governorates). The picture in outbreak 2001 varied greatly from that occurred in outbreak 2000 in the severity of the disease, morbidity and mortality percentage. Concerning the severity of the disease, the morbidity rate in native breed cattle during 2001 outbreak was 20.38 versus. 39.5 In outbreak 2000 while 30.38 versus 44 in foreign breed cattle and its was 0.38 versus 0.14 in buffaloes at Beni-Suef Governarate respectively.

The occurrence of the disease in El-Fayoum Governorate was paralleled with that observed in Beni-Suef Governorate during 2001. The morbidity rate in native breed cattle 19.5% versus to 67.5%, foreign breed cattle 43% versus to 73%. While it was 0.06 versus to 8 in buffaloes in 2000-2001 respectively. The low morbidity and mortality rate during outbreak 2001 may be due to the protection afforded by previously infected animals during outbreak 2000 which act as immuned animals against the field strain. The observed clinical signs during outbreak 2001 were also differed than those observed during 2000,as the ani mals that mild to moderate signs but not sever a outbreak 2000 and this also denote to the presenc of some immune animals emerged from outbrea 2000 table (4and6)

Concerning the case fatality rate in native bre cattle, Foreign breed cattle and buffaloes duri outbreak 2001 it was 0.5%, 2% and 0% vers 1.5%, 3.14% and 0% in outbreak 2000 at Be Suef Governorate while they were 0%, 2%, 0

versus to 2.16%, 2.75% and 0% in El-Fayoum Governorate during outbreak 2000. Concerning the percentage of animals showed emphysema during outbreak 2001 it was 0.42% to 1& 1 to 2 in native breed and foreign breed cattle at Beni-Suef Governorate viruses to 3.30, 4.57 in the same species during outbreak 2000 respectively. while it was 1 and 2 at El-Fayoum Governorate outbreak 2001 versus to 1.14 and 3.17 in native breed and foreign breed at El-Fayoum Governorate outbreak 2000.

The low in percentage of animal showed emphysema during outbreak 2001 may be due to the presence of some neutralizing antibodies that prevent severing inflammatory reaction of the virus.

The reoccurrence of BEF disease outbreak after about nine years from the last outbreak (1991) is questionable why the disease absent in nine years then reoccurred with a sever from. To answer this question we look about the immune status and the duration of immunity followed BEF outbreaks, the outbreaks occurred in countries in vicinity of Egypt like Saudi Arabia, Israeli, Sudan, and Libya. The vaccination program, adopted in Egypt against the disease.

Concerning the duration of immunity following natural infection, the neutralizing antibodies and persists for several years and probably for life time of most cattle, and the immune dams transferred these antibodies to their off spring that suckle colostrums.The passive immunity can protect off spring from the clinical exposure but does not conferred life long immunity (Cybinski, 1987), St. George, 1988; Uren 1989; St. George 1993) .On the other hand, cattle are known to remain immune to BEF challenge for long period after natural or experimental infection Snowdon, 1970; Uren 1989; St. George 1993.

The humeral response to infection with BEF virus has been well documented (Cybinskik, 1987, Snowdon 1970) who mentioned that, IgG-specific neutralizing antibody can be detected 4 to 5 day after the onset of clinical signs and tends to peak 5, 30 days later BEF- specific antibody plays a questionable role in the recovery from the disease and protection from reinfection. Although maternal antibody will protect calves against experimental challenge with BEF virus (St. George et al., 1986, St. George 1993,), The extent to which cell mediated immunity influences the outcome of BEF infection is not known preliminary experiments indicate that BEF-specific T-cells can be reticulated in vitro and that high level of il-I and tissue necrosis factor (TNF) circulate during acute phase of infection (Uren and Murply 1985, Uern 1989). From this point of view duration above about the humraol and cell mediated immunity al can be concluded that the BEF- specific neutralizing antibody may play a role in protection of previously infection against reinfection but not pro-

tected calved against field infection subsequently their for the new born calves become susceptible for BEF disease after few months and this explain the reoccurrence of the disease within two year.

Concerning the virulence of BEF virus the reoccurrence of the BEF disease in cattle population followed pervious infection may explain on the other hand the change virulence of BEF virus or the introduction of others BEF virus strains that differs from the more endemic strain and this required certain studies to confirm any type of BEF strains are found. This explanation is parallel to (St. George 1988, sedden 1938, Macferalan and Haig 1955, St. George 1986) who found the same viation in occurrence of the double or triple bots of the BEF disease with in 6 weeks of the first infection other explanation for the recurrence of the disease in second year from the first infection may be due to the passage of BEF virus throw animal other than cattle like sheep, goat, camel or buffaloes a number of species of free living ruminant harbor neutralizing antibody BEF virus . In Australia, Marsupials, Which from the bulk of the indigenous wild life, are free of antibodies though introduced species of deer and water buffaloes Cybinski and Zakryewski, 1983 development antibodies without exhibiting diseases. Sheep appear to be unaffected even when pastured with cattle during an epidemic, and there no serological evidence that sub clinical infection occurs (St. George 1990, St. Gorge 1992).However, BEF virus has been passage through sheep and back to cattle under experiment natal conditions. There is a single report of neutralizing antibody in goats Biggs (1988). The infected cattle in El-Fayoum and Beni-Suef Governorates showed clinical symptoms which characterized by sudden onset of fever usually phasic and each peak 40, 42°C respectively that lasting 12-18 hours. In the first rise of body temperature, the milk production decreased to 50% and this finding agree with (Losses 1986., Coetzer et al., 1994 and St George 1995). While in the second rise of temperature caused, Nasal and ocular discharge were observed as in table (10) and photos (1, 2 & 3) beside hurried respiration, stiffness, lameness, recumbency table (10) photos (5, 9 & 10) as described by (Hill and Schaltz 1977., St. George 1986., St. George 1988 Prasad 1997., Liao et al., 1998., St. George 1998 and Ali et al., 2001). Edema in lower jaw as in photo (6), emphysema with accumulation of air under skin was noticed as in photos (7, 8 & 9) this finding agree with (Burgess and Spradbrow 1977., St. George 1995.,Sayed et al., 2001). Drooling of saliva as in photo (2) this drooling of saliva may be due to the direct effect of the virus in the lining epithelium of the oral cavity and salivary glands which render the affected animals unable to swallow and extends their head down. Myosititis and arthritis may be due to the inflammatory immune response of BEF virus that causing stiffness of gait, in coordination and subsequent lameness and recumbency as described by (St. George et al., 1995).

Interestingly, the most affected animal species in this outbreaks and previous outbreak during 1991 was cattle. The role of buffaloes as reservoir for BEFV has not been declared in previous outbreaks at Egypt. However in this study the contact infected buffaloes ranged from 0.7-15% this finding that agree with those recorded by (Sayed et al., 2001 and Tain et al 1989), who found that BEFV infection was 2.2% in lactating buffaloes and explained the lower percentage of infection in buffaloes may be due to these animals might have a genetic resistance to BEFV infection in Australia. The BEF symptoms not observed in sheep, goat, Dog, Donkeys, Mules, Horses, Poultry and Human. That are in contact with infected cattle and buffaloes this observation had been confirmed by testing the sera of these animals species and poultry by VNT and SPA without detection any amount of BEFV antibodies table (1) this observation can be explained by the insusceptibility of these animal species for BEFV and this may be due to lack of virus receptors for BEFV in these animal species, or the endothelial cell of the vascular system of these animal species are impermeable for BEF virus replication. The resistance of these animal species and / or poultry was previously observed by (Doherty 1978., St. George 1985., Uren 1989.,Nandi and Negi 1999).The causes of the observed subcutaneous emphysema were not actually understood, however, it may be results from the occurring of interstitial lung emphysema, which may be escape beneath the pleura, then pass into the mediastinum and flow through fascia into subcutaneous tissues as explained by (Burgess and Spradbrow 1977). Another explanation for the occurrence of subcutaneous emphysema may be due to partial block age of air passage with exudates as well as necrosis of the borncholer walls resulting in rupture of bronchioles and alveoli that allowing the air to reach the connective tissue septa and lymphatic of the lungs then extending sub pleural to reach the mediastinum, from here the air can spread through the thoracic inlet to the subcutaneous tissues as explained by (Coetzer et al., 1994 and St. George 1995).The paresis or paralyses which are characteristic of sever BEF virus affection is similar to parturient hypocalcaemia (milk fever). The hematological and biochemical changes that occur in bovine ephemeral fever and the per parturiem period of cows, suggesting that there is a common basis for hypocalcaemia which occurs in both conditions (Van Miert, 1985). It is not certain which change are part of a general stress response of inflammation. The associated hypocalcaemia that occurred incutenous myiasis of sheep (Depechin et al., 1985., Murphy et al., 1986,) and in toxic shock syndrome of women (Wagner et al., 1981) may referred to the inflammatory immune response as well as BEF virus. As a fact the decrease in serum calcium level may reflect on the amount of milk production therefore, the sudden drop in milk production which reached in some farms to 50% may be due to the drop cal-

cium level or to toxic effect at high amount circulating interferon which occur during the clinical phase of ephemeral fever (St. George et al., 1986). Most uncomplicated cases recovered spontaneously within 3 to 5 days of the first occurrence of the clinical signs. Signs persist for longer time and end with death are most common in fattened steers, milkier cattle and mature bulls. The cause of the increase mortality rate could be attributed to many factors, among of which the secondary bacterial infection, aspiration pneumonia after regurgitation of ruminal contents, or following recumbency (Andrews et al., 1992) in addition to other stress factors that may be present in our livestock, such as nutritional deficiency, inadequate management and parasitic infestation. Dehydration and extravasations of fluid could be also contributed to increase the rate of deaths (Burgess 1971 and St. George 1988). During outbreaks 2000 and 2001 two arthropod vectors were observed on animal during this outbreak. The observed vectors were Mosquitoes, culicoids and Tabanus flies that play an important role in the transmission of the disease. This observation is agreed with (St. George 1986., St. George 1996., Sayed et al., 2001., Hamoda et al., 2002 and Yerhum 2003). , data presented in table (28) showed clearly the percentage of positive sera samples either by SPA or VNT in BEF virus outbreaks at 2001 more than those BEF virus outbreak at 2000.The percentage of positive sera by SPA in Beni-Suef Governorate was (64.8%, 71.9%) in BEFV outbreaks at 2000 and 2001 respectively. The same picture found by VNT is nearly to that by SPA the react positive sera by VNT were (53.8%, 63.33%) in Beni-Suef Governorate during BEF virus outbreaks at 2000 but it was (66.3 %, 68.5%) in El-Fayoum Governorate during the same outbreaks. This finding mean that positive sera samples tested by SPA or VNT higher in BEF outbreak 2001 than BEF virus outbreak at 2000 due to previously infected animal in the same area with BEF virus this finding agree with (Yeruham et al., 2003, and Sayed et al., 2002)

We noticed that SPA agglutination test gave nearly the same result of VNT and SPA agglutination test has advantage lies its rapidity, sensitivity, easily performed and screening large quantities of serum samples at short time, and can detect antibody in early infection low titer. Several researchers were used this technique for detection of several viral disease (Kessaler et al., 1975., Tracy and Cather 1986., Madbouly 1987 and Khausto et al., 1988). But still consider that VNT is the test of choice in most diagnostic laboratories for its easily application, not need purified viral antigen and easily to read, also it difficult interpret end point and canít detected early infection low antibody titer

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