Bovine ephemeral fever (Three day sickness): A Review with special reference to the Egyptian situation

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SUMMARY

Bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus, which causes a disabling febrile infection of cattle and water buffalo. The disease is common in tropical and subtropical regions of Africa, Asia, Australia and the Middle East and is of major economic importance to dairy and grassing industries. BEF virus is classified as the type species of the genus Ephemerovirus that also includes, Adelaide River virus, Berrimah virus, Kimberley, Malakal and Puchong viruses. Serological cross reactions were detected between viruses of the BEFV serogroup and rabies related viruses. The antigenic relationship between rabies virus and bovine ephemeral fever virus (BEFV) was applied for rapid diagnosis of BEFV infection using rabies antiserum and for possible protection against BEF after vaccination with killed rabies vaccine. Some strains of the virus stimulate the immune response to produce protective antibody for only 6 months. Problems with conventional vaccines stem from lack of potency of inactivated vaccines require more antigenic mass than it has been possible to achieve economically, and attenuated virus vaccine suffer from a loss in immunogenicity linked with the attenuation process. Further studies are needed toward the evolvement of a potent live attenuated, recombinant or DNA vaccine which may be a suitable alternative to current vaccines.

INTRODUCTION

Bovine ephemeral fever is an infectious disease of cattle characterized by inflammation of mesodermal tissues as achieved by muscular shivering, stiffness, lameness, enlargement of the peripheral lymph nodes (Radostits et al., 2000). Bovine ephemeral fever has a variety of names including three-day sickness, stiff sickness and dengue fever of cattle, bovine epizootic fever and lazy man s disease (Nandi and Negi, 1999). The disease in cattle is accompanied by febrile reaction and spontaneous recovery in three days. The morbidity may be high but the mortality is low. It mainly occurs in subtropical and temperate regions of Africa, Asia and Australia. The disease has major economic significance, as there are major economic losses due to drop in

milk production in diary herds and reduction in condition of prime animals or disruption of stock movements and disruption of markets (Walker,2005) . Recent outbreaks recorded increase morbidity and morality rates as in Saudi Arabia during 1990 and 1996 as well as in Egypt 2000 and 2001.The sever clinical manifestations and economic losses during the last time create the substantial awareness both in individual and industry owners about the epidemiology, transmission, prevention and control of the disease to avoid the enormous economic losses.

2. History and geographical distribution:

Bovine ephemeral fever was first recorded in mid-nineteenth century when the disease was first noticed in East Africa (Schweinfurth, 1867), subscequently in Rhodesia (Bevan, 1907), Kenya (Kennedy, 1915), South Africa (Theiler, 1906), Indonesia (Merkens, (1919), India (Meadows, 1919), Egypt (Rabagliati, 1924), Palestine (Rosen, 1931), Japan (Inaba, 1963) and Saudi Arabia (Lane, 1983). The disease may be epidemic in much of Africa and Southern Asia since antiquity but the development of more intensive cattle industry has enhanced its rapid spread to wide area. Bovine ephemeral fever is considered endemic in tropical regions of Africa, Australia, and Asia.

In brief infection with BEF virus has been occurred in most of Africa, so it has been not reported from all countries. Known epidemic areas are the Middle East Iran, Afghanistan, Pakistan India, Burma, and Japan to Indonesia and Australia to the southeast. The disease has never been reported in Western hemisphere, North and South America. Serological evidence indicated that New Zealand and Pacific Islands are free from the disease (St.George, 1988).

A retrospective epidemiological investigation of an outbreak of bovine ephemeral fever in 1991 affecting dairy cattle herds on the Mediterranean coastal plain was carried out by Yeruham and Baverman (2005), the work summerises the following:

From August to October 1991 bovine ephemeral fever (BEF) occurred sporadically in two localities in Israel. The morbidity and mortality rates reached 2.6% and 0.1%, respectively. Only 12/50 dairy cattle herds were clinically infected with BEF in the dairy community. The total morbidity rate reached 0.8%. The lowest morbidity rate was recorded in young heifers (5.5%) and the highest in adult cows (75%). Only heifers over the age of three months were clinically affected. The spread of the disease apparently followed the local prevailing night winds, which blow from east to west, i.e., from the land toward the sea. The morbidity period lasted 61 days. The low incidence and morbidity rates were possibly due to the low virulence of the virus strain involved in the 1991 epidemic. Retrospective analysis indicates that vectors - apparently mosquitoes - infected with BEF virus could have been overwintering.

3. Bovine ephemeral fever in Egypt:

The first detailed account of BEF was written in French by Piot (1896). His discretion of his experiences with an ephemeral fever epidemic in 1895 in Egypt shows very clearly that the disease was the same 100 years ago. The disease was described clinically by Rabagliati, (1924), as three days fever or stiffness in cattle.

During July-August 1991 clinical manifestations appeared in 5 dairy cattle farms in 4 provinces in Egypt. BEF virus antigen was detected in leukocytes of infected animals using the indirect immnufluorescence test (Hassan et al., 1991). BEF virus was isolated from the same outbreak by inoculation of baby mice and BHK21 Cell culture (Soheir, 1994).

A sever outbreak of BEF was observed in Egypt in summer 2000. Mortality rates ranged between 2-10% with fever, stiffness, lameness, dyspnea, abortion, subcutaneous emphysema and recumbancy. Milk yield was reduced to about 25% during the peak of the outbreak. BEF virus was detected in leukocytes from animals showing clinical symptoms using immunofluorescence and immunoperoxidase techniques (Zaghawa et al., 2000 and Hassan, 2000).

In comparison to this outbreak a relatively mild outbreak occurred during summer 2001. The BEF virus was confirmed by both immunofluorescence and immunoperoxdase tests in the leukocytes of clinically diseased animals (Zaghawa et al, 2002) as well as by the PCR (Khalil et al, 2001). Moreover, El-Shamy (2003) reported that FA, ELISA and NT were sensitive tools for identification of BEFV.

Sahar Saber et al. (2004) detected antibodies to BEFV in cattle sera from Domietta, Dakahlia and El-fayoum Governorates. The prevalence of antibodies was 26.03% , 14% and 72.72% at Domietta , Dakahlia and El-fayoum Governorates, respectively. Moreover, Comparative clinical and epidemiological studies on bovine ephemeral fever virus in Sakha farm , Kafr El-Sheikh was done (Al-Gaabary et al. 2005). The Comparative studies of rabies and bovine ephemeral fever virus was carried out with respect to their replication in different lab. Animals and tissue cultures (El-Habbak, 2005).

4. Etiology:

Bovine ephemeral fever is caused by an arthropod-borne, formally unclassified rhabdovirus with known 4 stereotypes: DDP63, CSIRO368, DDP61 and FUK-11 (Gard et al, 1984 and Kaneko et al., 1986). Recently BEF virus is classified as the type species of the genus Ephemerovirus that also includes, Adelaide River virus, Berrimah virus, Kimberley, Malakal and Puchong viruses (Wunner et al., 1995). BEF virus is single stranded negative sense RNA virus. It bullet-shaped with fringe of fine surface projections and measures 80X120-140nm (Delta-Porta and Brown, 1979).

4.1. Physiochemical properties of BEF virus:

The bovine ephemeral fever virus contains RNA and is sensitive to diethyl ether and sodium deoxycholate suggesting the presence of lipid containing envelop. Citrated whole blood from BEF affected cattle remains infective at 4 C. There is loss of infectivity of BEF virus at low pH (2.5) or high pH (12.0) within 10 minutes. The virus is inactivated within 10 minutes at 56 C and 18 hours at 37 C (St. George, 1981).

El-Shamy (2003) studied the physico-chemical properties of BEFV through its exposure to different temperature, different pH values and different numbers of freezing-thawing cycles.as well as its sensitivity to formaline and binary ethylene emine (BEI). Treatment of BEFV with BEI and formaline result in inactivation of virus within 6 hours.

4.2. Molecular and biochemical characterization of BEF virus:

BEF virus has the structural characteristics of a rhabdovirus with a bullet or cone shaped morphology (Murphy et al., 1972), 42S ss RNA genome (Delta-Porta and Brown, 1979), a lipid envelop and 5 virion proteins: L (with an Mr of 180 K), G (81 K), N (52 K), M1 (43 K) and M2 (29 K) (Walker et al., 1991).
As for rabies virus and vesicular stomatitis, the BEF virus membrane glycoprotein (G) can be removed from the virion by treatment with non-ionic detergents. The amino acid sequence of virion G protein revealed a signal domain, a central hydrophobic core and a polar domain, approaching the peptidase cleavage site (Walker et al., 1992). Two potential peptidase cleavage sites cab be identified in the BEF virus G protein corresponding to lysine residue at position +13 and +18. Transmembrane domain of the BEF virus consists of a stretch of 16 hydrophobic amino acids at residues 539-554. This region is bounded by basic residues (R and K), which are characteristic for othe rhabdovirus transmembrane segment.The conformational dependent neutralizing and non-neutralizing antigenic determinants are present on the G protein of BEF virus (Cybinski et al., 1992). The G protein presents type specific and neutralizing antigenic sites and corresponds to the spike glycoprotein of other rhabdoviruses. Competitive binding of G protein maps has identified six neutralization sites.The non-structural glycoprotein (Gns) is synthesized in BEF virus infected cells as G protein but not detected in virions. This Gns protein consists of signal domain, hydrophobic transmembrane and potential N-glycosylayion sites as other rhabdoviurses. The Gns shared amino acid sequence homology and general structural characteristics with other rhabdovirus G protein (Walker et al., 1992).The sequence of the RNA genome of bovine ephemeral fever virus (BEFV) was determined from the start of the L (polymerase) gene to the end of the untranslated 5' trailer sequence, completing the sequence of the 14900 nucleotide (nt) genome.The 6470 nt L gene encodes a single long ORF of 2144 amino acids with a deduced molecular weight of 249766 Da. The 70 nt BEFV 5' trailer region displays partial terminal complementarily with the 3' leader sequence and contains a 26 nt direct repeat of the U-rich domain of

the 3' leader region. The BEFV L protein contains all characteristic sequence motifs of amino acid blocks I-VI, conserved among RNA polymerase proteins of single-stranded (-) RNA viruses, separated by regions of lower homology (Dhillon et el., 2000).

The N protein of the BEF virus is phosphorylated and remains associated with nucleocapsid after detergent disruption of the virions in high concentration of salt but is released by treatment with RNase A. This N protein of BEF virus is a structural component, which has multiple functions in nucleocapsid assembly and regulation of transcription and replication (Walker et al., 1994).

Wang et al (2001) analysed the glycoprotein gene of Taiwanese isolates of BEFV between 1983 and 1999 and they showed homology, with no alteration of 4 amino acids in antigenic sites G1, G3b and G3c. Phylogenetic analysis of Taianese isolates of BEFV revealed 2 distinct clusters, the 1983-1989 and 1996- 2002. Both were distinct from 2 Japanese strains and Australian BB7721 strain.

Hsieh et al. (2005) discussed the relationship between seven epizootics of BEFV in Taiwan and possible variant BEF viruses. They found that the glycoprotein gene of the isolates from 1999, 2001 and 2002 had 99.2 – 99.9% homology without consistent amino acid variations in the neutralization sites. They also mentioned that the ourrence of frequent small epizootics implies the dominance of BEFV over host immunity, but not a variant virus. phylogenetic analysis of Taiwanese isolates revealed 2 distinct clusters, the 1983-1989 and 1996-2002 isolates.

4.3. Antigenic relationship with other rhabdoviruses:

Bovine ephemeral fever virus is serologically related to rabies virus serotypes and the related viruses Adelaide, Obodhiang and Kotonkan (Callisher et al., 1989). However, the Adelaide River virus and BEF virus genomes are far more complex than those of other known rhabdoviruses, containing 4-6 long open reading frames (ORFs) between the G and L genes (Wang et al., 1994). Bovine ephemeral fever virus was considered as one strain of rabies virus (Sewel and Brocklesby, 1990).

Serological cross reactions were detected between viruses of the BEFV serogroup and rabies related viruses, suggesting that these viruses form a large complex within the lyssavirus genus (Callisher et al., 1989a) Polyclonal mouse ascetic fluids derived from BEF virus, rabies virus and other several related viruses were also known to cross react in immunoblots with purified preparations of rabies virus N proteins (Walker et al., 1994).

Recently the antigenic relationship between rabies and BEF viruses was studied with the molecular biology and described that alignment of the glycoprotein of rabies virus and BEF virus revealed similarities in the location of the neutralizing epitopes and extensive conservation of cysteine residues,

suggesting that basic elements of the folded structure of these glycoproteins are preserved (Kongsuwan et al., 1998).

4.4. Application of the antigenic relationship between rabies virus and BEFV:

Protection against rabies virus conferred by immunizing mice with other rhabdoviruses and preliminary evidence of molecular similarities between bovine ephemeral fever-related viruses and rabies-related viruses was recorded by Callisher et al., 1989b).

The application of the antigenic relationship between rabies virus and BEFV to study the humeral and cell mediated immune response of calves after vaccination with inactivated rabies vaccine was described by Zaghawa et al. (2001). They recorded that the resulted antibodies against homologous (rabies) virus and the heterologous (BEF) virus as well as the cell- mediated immune response suggested a possible protection of calves against BEFV when vaccinated with killed rabies vaccine. They added that further investigations are needed to confirm the level of protection by applying the challenge test with virulent BEFV on the vaccinated cattle with killed rabies vaccine.

4.4.2. Diagnosis of BEF using Rabies virus antiserum:

The antigenic relationship between rabies virus and bovine ephemeral fever virus (BEFV) was applied for rapid diagnosis of BEFV infection among cattle in Egypt during summer 2001. The specific anti-serum to rabies virus was used for rapid diagnosis of BEFV by immunofluorescence (IF) and immunoperoxidase (IP) techniques in leukocyte and blood films of naturally infected cattle as well as impression smears of brain of inoculated mice (Zaghawa et al., 2002).

IF and IP tests showed a clear reaction with both rabies and BEFVanti-sera, the degree of reactivity was more intense in case of BEFV anti-serum and quite sufficient clear in case of rabies virus antiserum. They concluded that Rabies virus anti-serum can be used for diagnosis of BEF by IF and IP and this application is a good tool for diagnosis of BEF when a sudden outbreak occurs and there is no available BEFV anti-serum. Gehan et al. (2004) applied avidin biotin complex immunoperoxidase and immunofluorescent techniques for diagnosis of bovine ephemeral fever.

5. Epidemiology:

5.1. Method of transmission and sources of infection:

Transmission of BEF occurs through insect vectors as sand fly (Ceratopogonidae family), mosquitoes as Aedes spp., Culex annulirostris, Anopheles bancroftii and A. annulipes and the sand fly Culicoides brevitarsis (Standfast and Miller, 1985). The source of infection is the affected animal with clinical disease.

The disease now occurs annually in areas where it used to occur only one each decade, probably because of establishment of the virus in a new vector (Uren et al 1987).
Spread is largely independent of cattle movement, and transmission does not occur through contact with infected animals or their saliva or ocular discharge. The disease is not spread through semen, nor is intrauterine administration of the virus a suitable route of transmission (Radostitis et al., 2000).

5.2. Environment risk factors:
The disease occurs in the summer months and spread depends largely on the insect vector population and the force direction of prevailing winds. When a strong wind prevails the vectors may be carried over large tracts of lands or water. Cyclical development of the virus in
insect vector is suspected. The disease tends to disappear for long periods to return in epizootic form when the resistance of the population is diminished. Reoccurrence depends primarily on suitable environmental conditions for increase and dissemination of insect vector (Radostitis et al., 2000).

During periods of quiescence the disease is still present but the morbidity is reputed to be very low. However in many enzootic areas the degree of surveillance is less than intense, and clinical cases may occur without being observed. The reason why an epizootic develops out of an enzootic herd, or area population, may be due to change in virulence, or in the vector population (St.George, 1988).

5.3 Animal risk factors:
All cattle, which have not suffered from an attack, are susceptible. Calves under 6 months are rarely affected (maternal immunity?)
and the disease is mostly seen in cattle less than 2 years of age (Uren, 1989). In Egypt the outbreak of summer 2000 had another picture in which many calves under 6 months had affected besides dairy cattle over 2 years old which are highly producers are severely affected (Zaghawa et al., 2000, Yeruham et al. 2002 and Hosein et al. 2004).
Neither clinical reaction nor any development of antibodies in sheep and pigs inoculated with the virus of BEF were recorded (Snowdon, 1970).
Buffaloes become infected naturally but the clinical disease has notbeen confirmed in this animal in Australia (Young, 1979). In Egypt a mild clinical disease was observed in buffalo with transient fever, anorexia, reduction in milk yield and stiffness. Some few cases developed subcutaneous and lung emphysema (personal observation, Zaghawa, 2001 not published). This observation is coincide with that of Bai et al. (1989) who recorded that buffaloes are susceptible to experimental infection.
5.4. Immunity after natural infection:
Cattle are usually immune for one or two years after natural infection though there have been many exceptions to this as recorded in the Egyptian outbreak during summer 2001. Milder strains of the virus

stimulate the immune response to produce protective antibody for only 6 months (Hungerford et al., 1990). In outbreak of earlier years it was thought that cattle once affected remained immune for the rest of their life.

5.5. Economic importance:

The economic effect of BEF is sever and is usually due to mortality rates 1-10%, abortion, culling due to infertility, decrease in milk production and the effect of both trade within and between countries.

1% mortality rate expressed as a percentage of the whole herd is often given but 2-10 was recorded during the Egyptian outbreak 2000 (personal observation not published).

There is a very temporary loss of weight due to dehydration, which is restored immediately after convalescence. Pre-illness body weight is regained 2 weeks after recovery.

The loss in milk production is the essential factor of the disease as it causes about 46% in natural disease (Davis et al., 1984). Most cows did not return to pre-illness production levels on convalescence. Loss of milk production is similar in both dairy and beef cows and that a suckling calf will lose weight.

Abortion of cows in late stage of pregnancy is another factor for economic losses, however the virus does not appear to cross the placenta (Parsonson and Snowdon, 1974).

Some losses, which are hardly to qualify, are temporary infertility in bulls and effect on trade (ST.George, 1977). Other economic losses are the costs of treatment and costs for application of control programs as insect control and vaccination if found.

6. Pathogenesis:

The pathogenesis of the disease is difficult to understand, but it is clear that pathophysiological effects of host inflammatory response are involved in the expression of disease. There is an early neutrophilia with an abnormal level of immature neutrophil olymorphonuclear cells in the circulation (lift shift). There is a rise in plasma fibrinogen and a significant drop in plasma calcium (Fenner et al. 1993).

After an incubation period of 2-10 days a viral septicemia develops with localization and inflammation in mesodermal tissues particularly joints, lymph nodes and muscles. Increased respiration rate, dyspnea, limb stiffness and pain are characteristic to this febrile stage, which lasts for 2 days. Vascular endothelium and lymphoid cells is not the site of virus multiplication as the virus grows principally in the reticuloendothelial cells in the lungs, spleen, and lymph nodes.

A biphasic fever with peaks 12-24 hours apart was observed after intravenous injection of the virus. Increased vascular permeability with serofibrinous inflammation of serous and synovial cavities was recorded (Young and Spradbrow, 1990). The virus can be detected in the serosal and synovial

fluids and in the mesothelial cells of synovial membrane and epicardium and in neutrophils in the fluids Young and Spradbrow, 1985).
The study of ephemeral fever in cattle has defined a range of hematological and biochemical changes in blood, which are characteristic of an inflammatory response. One of the clinical signs
of ephemeral fever, a temporary paralysis reversible by treatment with calcium borogluconate, is similar to that in milk fever (parturient paresis), a disease of multiparous dairy cows. It is postulated that an inflammatory event occurs in the periparturient period of multiparous cows, which partially accounts for the falls in plasma calcium. This can precipitate a paralysis and other hypocalcaemic signs similar to that seen in acute ephemeral fever *(St.George, 1995 and Hassan, 2000).*

7. Clinical signs:
The disease is sudden in onset. The animal shows a very high temperature (40-42 C) or more, which is biphasic, triphasic or multiphasic (Nandi and Negi, 1999). Shivers show muscle trembling,
appears obviously sick, slobbers at the mouth, passes mucous from the nose and eye, may grind the teeth and is of its food. Lactation is suppressed.
After about 12 hours the animal becomes staggering and may go down being partially or completely paralyzed. The lymph nodes are swollen.
After a period varied from 2 to 5 days (usually about 3 days) the nimal recovers if allowed to lie quietly in some shady, protected spot.
The recumbent period (sternal and later lateral) is usually 8-24 hours, which may extend to 7 days. Shivering occurs in about half of the cases. Food and water are refused due to painful swallowing is painful. Respiratory rate may be heard in the increased breathing.
Affected animals commonly stand with their necks extended, looking very dejected. Rumination ceases for about 2 days. Some cases are constipated and some cause diarrhea.
There is lameness, which commonly develops on the second day and persists for two days in about half the cases. Some become recumbent and a few remain down for as long as a week. Old cases become excitable and this suggests central nervous system involvement. Rare
cases exhibit persistent paralysis.
A few cases exhibits subcutaneous swelling around the sub-mandibular region where it is emphysematous and some around limbs where it is edematous (Hill and Scultz, 1977). The development of subcutaneous and pulmonary emphysema is possibly due to nutritional deficiency of selenium (Odiawo, 1989.
Bulls and fat cows are severely affected than other cattle. Calves suffer less than adult cattle. Pregnant cows abort during fever (Beviridge, 1986).
In some cases there is a peculiar hardening of the udder, which is sharply defined and angular, just as though the udder contained some large angular foreign body. This symptom may persist for months after the disappearance of other symptoms; usually affect only one quarter,
which yield normal milk (Hungerford et al., 1990).

The clinical symptoms observed in the outbreak in Saudi Arabia during 1996 were sudden onset of disease over the dairy farms with fever (41.5 C), anorexia and labored breathing accompanied by dilatation of nostrils; the mucous membrane of the nares and conjunctiva are congested. The animals showed lameness and were reluctant to move. The morbidity rate was 59% and the mortality rate was less than 1% (Abu Elzein et al., 1997).
During the Egyptian outbreak 2000 the most observed clinical signs were fever, stiffness and lameness, dyspnea, abortion, subcutaneous emphysema, recumbency. Milk yield was reduced to about 25% during the peak of the outbreak (Zaghawa et al., 2000 and Hassan, 2000).
Complications usually occur and manifested by pneumonia, mastitis, hind quarter paralysis, abnormal gait, abortion in late stage of pregnancy and temporary (up to 6 months) infertility in bulls (Nandi and Negi, 1999).

8. Post-mortem lesions:
The commonest lesion is edema of the lymph nodes throughout the body.
Increased fluid in the peritoneal, pleural and pericardial cavities is noted and may contain fibrin with serofibrinous inflammation, edema and patchy congestion.
Synovial sacs also contain increased fluid with fibrinous flocculation and neutrophils, which persist some days after the fever, subside with degenerative changes in the synovial membranes.
Emphysema of the lung and sometimes areas of consolidation occur (Hungerford et al., 1990 and Zaghawa et al. 2000 and Hassan, 2000).
Congestion of the abomasal mucosa, nasal cavities, the small intestine and kidneys.
Persistent recumbent animals showed degenerative changes in the spinal cord similar to those produced by physical compression (Radostitis et al., 2000)
9. Histopathology:
Histopathologically, endothelial hypoplasia and perivascular infiltration with neutrophils is seen in the synovial membranes and tendon sheaths (Coombs,1978).

10. Clinical pathology:
The erythrogram showed a sever anemia represented by highly significant decrease in RBCs, PCV % and Hb content. The leukogram showed leukocytosis (shift to the left) with neutrophilia and
lymphopenia. The analysis of serum major elements showed significant hypocalcaemia, hypophosphtemia and hypomagnesemia (Hassan, 2000 and Attia &Selim,2000). BEFV was found to induce apoptosis (China et al. 2004).

11. Diagnosis:
Bovine ephemeral fever is usually diagnosed from history and clinical signs. A diagnosis can be made from the sudden onset of febrile reaction lasting for 2-5 days with spontaneous recovery. The seasonal occurrence and the

symptoms of oropharyngeal secretions, joint pain and stiffness are of value (Nani and Negi,1999).

11.1. Virus isolation:

Virus isolation can be achieved by inoculating leukocyte from clinically diseased animal intracerebrally into suckling mice 1-3 days old (Van der Westhuizen, 1967), suckling hamster and rats (Inaba etal.,1968). Virulent strains become stabilized after 6 passages causing paralysis and death 2-3 days post inoculation leading to toss of pthogenicity in calves (St.George,1977).

BEF virus grows very poorly or not at all in cells of bovine origin.

It grows very well in BHK-21 cells inoculated with mouse brain or bovine leukocyte suspension. It also grows in hamster lung, VERO and Aedes cell lines as well as the monkey kidney stable lines (MS).

The cytopathogenic effect characterized by rounding of cells, granular appearance of the cytoplasm followed by detachment from the glass 48 hours post-inoculation. The pinpoint plaques develop in MS cells 2-4 days post-inoculation and reached 1-1.5nm diameter by the 8-10 days
(Buxton and Fraser,1975).

Not all BEF strains produce cytopathogenic effect and the presence of virus is generally demonstrated by immunofluorescence (St Georgwe,1988).

11.2. Detection of BEF virus antigen by immunoflourescence and immnuoperoxidease tests:

BEF virus antigen can be detected in blood and leukocyte films prepared from feverish animals by IF and IP tests. Confirmation of BEF virus antigen in impression smears of brain of experimentally infected suckling mice and inoculated tissue culture with the suspected material is achieved by IF and IP tests.

The application of IF and IP was proved to be simple, rapid , sensitive and specific tests for detection of BEFV antigens in blood and buffy coat films, impression smears of infected mice as well as the inoculated tissue culture with suspected material of BEF virus.

Furthermore, IP staining can be considered as a field test in the presence of diagnostic reagents (Zaghawa et al.,2002).

11.3. Polymerase chain reaction (PCR):

The serological identification of rhabdoviruses considered a field diagnostic problem due to the antigenic relationship between the members of this group (Callisher et al.,1989). Reverse transcriptase polymerase chain reaction (RT-PCR) has been developed with many advantages as it is possible to detect as little as 2 fragment of viral RNA from infected tissue by ethidium bromide staining after 30 cycle of PCR (WU et al.,1992). There is no need for virus replication, moreover, RT-PCR is not time consuming since all procedures involved take about 6 hours to be completed (Davis and Boyle, 1990).

The application of RT-PCR on leukocytes and brain tissue of infected mice with the suspected material of BEF virus yielded a clear single band on agarose gel stained with ethidium bromide. The amplified DNA fragment corresponds to 500 base pair (bp). PCR confirm the diagnosis

of BEF outbreak which is sensitive specific and of value for rapid diagnosis (Khalil et al.,2001). RAPID was developed to detect and quantify the G protein-encoding gene of BEFV. This new technique uses a nested PCR and magnetic bead-basaed DNA probing assay. The diagnostic sensitivity of RAPID assay, real-time RT-PCR and conventional RT-PCR for detection of 34 clinical blood samples suspected to have BEFV infections were 72.73 , 36.36 and 18.18%, respectively (Lee ands Chang, 2004 and Hsieh et al. 2005). The results indicated that the RAPID was more sensitive and specific than the conventional RT-PCR and real-time RT-PCR assays for detection of BEFV. 12. Treatment:

Symptomatic treatment with anti-inflammatory drug has been found to be beneficial. Various antibiotic can be used to chick the secondary bacterial infection Treatment with phenyl butazone in combination with calcium gluconate reverses a number of the symptoms. The intravenous administration of calcium can now be considered a justifiable addition to the treatment regimen together with prolonged phenyl-butazone therapy (Uren,1989).

Finadyne at 22.5 mg/kg intravenous, one dose daily for three days, e.g. 20 ml for 450 kg cow or bull.

Aspirin (acetyl-salicylic acid) 60-120 g for mature cattle, 100-250 mg for calves.

Corticosteriods such as dexamethasone are helpful but may cause abortion in late pregnancy.

Rapid recoveries in 24-36 hours have followed use of Tylan at 11 mg per kg body weight.

Intravenous electrolyte infusion may be of value in some cases.

Avoid drenching at all cases due to drenching pneumonia. Make the animal comfortable in a shed place with careful nursing Ketoprofen is a safe and effective drug for the treatment of locomotor

symptoms of bovine ephemeral fever, but has no effect on the duration of clinical respiratory abnormalities (Fenwick and Daniel, 1997).

Treatment of animals with calcium, magnesium and phosphorus overcome the downer position caused by the infection with bovine ephemeral fever (Hassan et al.,2000).

13. Vaccination:

The vaccination against BEF is still a scientific and field question, which has no decisive answer. There are no commercially available vaccines, which have been extensively tested in the field. Killed vaccines are generally considered to have low efficacy and attenuated viruses need to be used in an adjuvant base and 2 injections are required (Vanselow, 1985).

Attenuated vaccines are expensive to produce, have a short shelf life, and breakdowns are recorded after their use. The worry about their use in the possibility of their transmission by insect from vaccinated animals to the general population, and subsequent interference with a test and eradication program. There is, too, the possibility that they may assume full virulence while undergoing natural passage (Radostits et al.,2000).

The rapid loss of virulence for cattle of BEF virus was reported (Van der Westhuizen, 1967). The loss of virulence together with immunogenicity occurs at the 8th passage. The immunogenicity of the life vaccine can be enhanced by mixing with Freunds incomplete adjuvant (South Africa), aluminum hydroxide and Quil A (Australia)(Vanselow et al.(1995). They found that a vaccine regim of two consective vaccinations with attenuated virus combined with the adjuvant Qual A provided excellent protection against BEFV for at least 12 months, whereas one vaccination with Quil A vaccine or two vaccinations with vaccine containing trye adjuvant aluminium hydroxide gel did not provide significant protection.

The practical difficulties with the adjuvant mixed with live vaccine are a proportion of viruses got inactivated and the test of viability of BEF virus in cell culture is quite impossible as the adjuvant are toxic to cell culture *(Nandi and Negi, 1999).*

Problems with conventional vaccines stem from lack of potency of inactivated vaccines require more antigenic mass than it has been possible to achieve economically, and attenuated virus vaccine suffer from a loss in immunogenicity linked with the attenuation process *(Murphy et al.,1999).*

A Japanese BEF killed vaccine is used for short term protection or as an antigenic booster for cattle previously vaccinated with the life attenuated vaccine *(Inaba et al., 1974).* Recent field application proved the inability of BEF killed vaccine to protect animals against natural infection as recorded in Egypt during 2001 outbreak *(Zaghawa, 2001* personal observation, not published) and in Taiwan *(Wang et al.,2001)* .

The lack of potency of inactivated BEFV vaccine and the loss of immunogenicity of the attenuated vaccine, essentiate a rapid solution of a heterologous vaccine to control the repeated outbreak of BEFV.

The antigenic relationship between rabies and BEFV was applied by immunizing cattle with inactivated rabies vaccine in a trial for protection against BEF. Antibodies against both homologous (rabies) virus and the hetrologous (BEF) virus was titrated. The resulted humeral and the cell-mediated immune response suggested a possible protection of calves against BEFV when vaccinated with Defensor (killed rabies vaccine). It is clear that further investigations are needed to confirm the level of protection by applying the challenge test with virulent BEFV on the vaccinated cattle with killed rabies vaccine *(Zaghawa et al.,2001).*

Fahmy (2004) vaccinated calves with BEF-BEI inactivated and adjuvenated with MontanideISA25. The humoral immunity to BEFV was determined by SNT and ELISA. The SNT titers were ranged from 1.2 – 2.1 log10 between 2^{nd} to 36^{th} weeks post-vaccination. The ELISA titers were ranged from 1.85 – 2.95 log10 between 2^{nd} to 40^{th} weeks post-vaccination.

14. Future research:

The mechanism of persistence of BEF virus in the interepizootic periods needs studies in depth in endemic areas. It is clear that Culicoides and mosquitoes support virus growth, however the potential vectors in much of the range of ephemeral fever remain to be

identified.
The application of biotechnology for the identification of different serotypes of BEF virus including the virulent and a virulent strains is a point of interest. Further studies are needed toward the evolvement of a potent live attenuated, recombinant or DNA vaccine which may be a suitable alternative to current vaccines. The application of the antigenic relationship between BEF virus and BEF-like viruses a well as rabies needs further investigation to see the possible protection with heterologous vaccination.

REFERENCES

Abu Elzein, E.M.E.; Gameel, A.A.; Al Afaleq, I.A.I.; Guindi, O.A.I. and Bukhari, A. (1997): Bovine ephemeral fever in Saudi Arabia. Veterinary Record, 140:630-631

AL-Gaabary, N.M.; Osman, S.A.and Foad,F. (2005): Comparative clinical and epidemiological studies on bovine ephemeral fever virus in Sakha farm, Kafr .El-Sheikh, Egypt. Assiut Vet. Med. J. Vol. 51 (104): 76-84.

Attia, H. and Selim, H. (2000): Clinical hematological anf biochemical studies on bovine ephemeral fever. Proceeding of the 9th Sci. Cong. 2000, Fac. Vet. Med., Assiut Uni., Egypt,: 292-300.

Bai ,WB, Zhang ZG, Jiang CL (1989): Clinical and pathological responses of water buffalo (Bubalus bubalis) to experimental infection with bovine ephemeral fever virus--Chinese strain. Aust Vet. J. Nov;66(11):373-4

Bevan, LEW (1907): Preliminary report on the so called "Stiff sickness" or "Three day sickness of cattle in Rodesia. J.Comp. Path., 20: 104-113.

Beveridge, I.B. (1986): Animal health in Australia, 2nd ed., Dept.Primary Indust Vol. 1: 18-24

Buxton,A. and Fraser,G. (1977): Animal microbiology. Blackwell Sceintific publication, Oxford, :569-570

Calisher, C.H.; Karabatsos, N.; Zeller, H.; Digoutte, J.P.;Tesh, R.B.; Shope, R.E.; Travassos Da Rosa, A.P.A. and St. George, T.D. (1989): Antigenic relationships among rhabdoviruses from vertebrate and haematophagous arthropods. Intervirology, 49: 241-257.

Calisher, C.H.; Karabatsos, N.; Thompson,L and Smith,J.S. (1989): Protection against rabies virus conferred by immunizing mice with other rhabdoviruses and preliminary evidence of molecular similarities between bovine ephemeral fever-related viruses and rabies-related viruses. In Arbovirus research in Australia. Proceedings 5th Symposium, August 28-September1, 1989, Birshane, Australia Coombs, G.P. (1978): Bovine ephemeral fever. Proc. US. Ainm. Health. Assoc., 82:29-35

Chang, C.J. ; Shih, W.L.; Yu, F.L.; Laio, m.H. and Liu, H.J. (2004): Apoptosis induced by bovine ephemeral fever virus. J.Virol. Methods. 122: 165-170.

Cybinski DH, Davis SS, Zakrzewski H. (1992): Antigenic variation of the bovine ephemeral fever virus glycoprotein. Arch Virol;124(3-4):211-24

Davis SS, Gibson DS, Clark R. (1984): The effect of bovine ephemeral fever on milk production. Aust Vet J Apr;61(4):128

Davis, V.S. and J.A. Boyle (1990): Adapting the polymerase chain reaction to a double-stranded RNA genome. Analytical Biochem. 189: 30-34.

Della-Porta AJ, Brown F. (1979): The physico-chemical characterization of bovine ephemeral fever virus as a member of the family Rhabdoviridae. J Gen Virol 1979 Jul;44(1):99-112

Dhillon J, Cowley JA, Wang Y, Walker PJ. (2000): RNA polymerase (L) gene and genome terminal sequences of ephemeroviruses bovine ephemeral fever virus and Adelaide River virus indicate a close relationship to vesiculoviruses. Virus Res;70(1-2):87-95.

El-Habbak, A.S.E. (2005): Comparative studies on rabies and bovine ephemeral fever viruses. M.V.Sc. thesis (Virology), Fac. Vet. Med., Zagazig University, Banhana branch.

El-Shamy, A.M.M. (2003): Studies on bovine three day sickness virus. M.V.Sc. Thesis (Virology), Fac. Vet. Med., Suez Canal University.

Fayoumy, A.F. M.(2004): Trials for preparing combined vaccines against some microbial diseases in bovine. M.V.Sc. Thesis (Bacteriology , immunology and Mycology), Fac. Vet. Med., Suez Canal University.

Fenner, F.J.;Gibbs,E.P.L.; Murphy, F.A.;Rott, R.Studert,M.J. and White D.O.(1993): Bovine ephemeral fever. In Intervirology, 2nd ed. Academic Press, California.

Fenwick DC, Daniel RC. (1997): Evaluation of the effect of ketoprofen on experimentally induced ephemeral fever in dairy heifers. Aust Vet J;75(3):221-223

Gard GP, Cybinski DH, Zakrzewski H. (1984): The isolation of a fourth bovine ephemeral fever group virus. Aust Vet J Oct;61(10):332

Gehan,G. Shehab; Abd El-Ghaffar, S.k.H. and Abd El-Rahim, I.H.A. (2005): Application of avidin biotin complex immunoperoxidase and immunofluorescent techniques for diagnosis of bovine . Vet. Med. J. Med. Vol. 52 (1): 135-147.

Hassan, H. Y. (2000): An outbreak of bovine ephemeral fever in Egypt during 2000. 1. Clinical and epidemiological investigations. 9th Sc.
Cong. 2000, Fac. of Vet. Med., Assiut Uni., Egypt. p: 346-353.

Hassan, H.B.; El.Danaf, N.B.; Hafez, M.A.M.; Regab, A.M. and fathia, M.M.C. (1991): Clinico-diagnostic studies on bovine ephemeral fever with detection of its virus for the first time in Egypt. **First sci. Congress, Egyptian Soci. for cattle Diseases, 1-3 december,1991:** 77-85.

Hill, H.M.W. and Schultz, K. (1977): Atoxia and paralysis associated with bovine ephemeral fever infection. Aust. Vet. J., 53: 217-221.

Hungerford, T.G. (1990): Diseases of lifestock 9th ed. McGraw-Hill Book Co. Sydney.

Hosein, H.I.; Magda, M.S.; Azzam, R.A. and El-Bably, M.A. (2004): Situation of bovine ephemeral fever virus (BEF) in Egypt. SCVMJ, VII(1): 242-246.

Hsieh, Y.C.; Chen, S.H.; Chou, C.S.; gsiao, H.W.; Chen, S.Z.; lee, Y.Y and Liu, H.J. (2005): Development of a reliable assay protocol for identification of diseases (RAPID)-bioactive amplification with probing (BAp) for detection of bovine ephemeral fever virus. J. Virol. Methods, 18. Inaba, Y. (1963): Bovine ephemeral fever. Jap. Agric. Res. Q. 3: 36-42
Inaba, Y., Tanaka,Y., Sato. K.,Ito, H.Omeri,T. and Matumoto, M. (1968): Bovine epehemeral fever. I.Propagation of the virus in suckling hamster, mouse and rat and hamster kidney BHK-21-W-12

cell. Jpn.J. Microbiolo.,12:457-469 Inaba Y, Kurogi H, Takahashi A, Sato K, Omori T. (1974): Vaccination of cattle against bovine ephemeral fever with live attenuated virus followed by killed virus. Arch Gesamte Virusforsch;44(2):121-32

Kaneko N, Inaba Y, Akashi H, Miura Y, Shorthose J, Kurashige K. (1986): Isolation of a new bovine ephemeral fever group virus. Aust Vet J Jan;63(1):29

Khalil, S.A.; Khadr, A.M.; Zaghawa, A. and Akela, M.A. (2001): Application of PCR and immunoperoxidase for diagnosis of bovine ephemeral fever virus in Egypt during summer 2000. Proceeding of the 6th sci. Cong.,Egyptian Society for cattleDiseases, 4 – 6 November, 2001, Assiut, Egypt. P, 135 – 140.

Kongsuwan, K.; D.H. Cybinski; J. Copper and P.J. walker (1998): Location of neutralizing epitopes on the G protein of bovine ephemeral fever rhabdovirus. Journal of general Virology. 79: 2573-2581

Kennedy, W. (1915): Ephemeral fever or three day sickness in cattle.

Vet. J., 71: 126-128 Lane, R.P. (1983): Fauna of Saudi Arabia.5, 529. Cited after Abu Elzein, **E.M.E.; Gameel, A.A.; Al Afaleq, I.A.I.; Guindi, O.A.I. and Bukhari, A. (1997):** Bovine ephemeral fever in Saudi Arabia. Veterinary Record, 140:630-631

Lee, Y.G.and Chang, K.S. (2004): Application of A flow type qurtz crystal microbalance immunosensor for real time determination of cattle bovine ephemeral fever virus in liquid. Talanta 65: 1335-1342.

Meadows, D. (1919): Notes on an ephemeral fever in Indian cattle resembling South African "Three days sickness". Vet. J. 75: 48-50

Merkins,J. (1919): Ned-indische Bl diergeresk, 31: 48-50 Murphy FA, Taylor WP, Mims CA, **Whitfield SG. (1972):** Bovine ephemeral fever virus in cell culture and mice. Arch Gesamte Virusforsch 38(2):234-49

urphy, F.A. ; Gibbs, E.P.J.; Horzinek, M.C. and Studdert,M.J. (1999): Veterinary Virology. Third Edition. Copyright by Academic Press. Nandi, S. and Negi, B.S. (1999):

Bovine ephemeral fever: a review. Comparative Immunology , Microbiology and Infectious Diseases, 22: 81-91.

Odiawo GO. (1989): The relationship between selenium deficiency and the development of pulmonary and subcutaneous emphysema in bovine ephemeral fever virus-infected cattle. Onderstepoort J Vet Res Jun;56(2):123-5

Parsonson, I.M. and Snowdon, A. (1974): Bovine ephemeral fever virus: Excretion in the semen of infected bulls and attemps to infect female cattle by the intrauterine inoculation of virus. Aust. Vet. J. 50: 335-337.

Piot, J. B. (1896): Epizootic Dengue Fever of cattle in Egypt. Prix National Academy of Medicine, France Rabagliati, D.S. (1924): Three days fever or stiffness in cattle. Vet. Rec. 4: 503-504.

Radostits, O.M. ; Gay, C.C. Blood, D.C. ; and Hinchcliff, K.W. (2000): 9th ed

Rosen, S.G. (1931): Ephemeral fever "three days of cattle in Palestine. Vet. J. , 87: 244-246.

Sahar saber, M.M.(2004): Epizootological studies on ephemeral fever in cattle. Ph.D. Thesis (Infectious Diseases) , Cairo University.

Schweinfurth, G. (1867): The heart of Africa. Low and Searle, London, pp 280-281

Sewel, M.M.A. and Brocklesby (1990): Hand bock of animal diseases in the tropics, 4th Edition, Bailliere Tindall, London.

Snowdon WA. (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 Jun;46(6):258-66

Soheir, M. Banoub (1994): Ephemeral fever (Three day sickness) in closed milking cow farm at Sharquia. Proceeding of the 6th Sci. Cong., 20-22 Nov., Fac.Vet. Med., Assiut, Egypt.: 558-563

St. George, T.D., Standfast,H.A.; Chrisie,D.G.; Knott, S.G.; and Morgen, I.R. (1977): The epizootology of bovine ephemeral fever in Australia and Ppua New Guinea. Aust.V.J53:17-28

St. George, T.D. (1981): Ephemeral fever. IN E.J. Gibbs,editor, Virus diseases of food animals, Vol.11. Acvademic press. London: 541-564.

St. George, T.D. (1988): Bovine ephemeral fever: A review. Trop. Anim. Health Prod., 20: 194-202.

St George TD, Murphy GM, Burren B, Uren MF. (1995): Studies on the pathogenesis of bovine ephemeral fever. IV: A comparison with the inflammatory events in milk fever of cattle. Vet.Microbiol.;46(1-3):131-42

Standfast, H.A. and Miller, M.J. (1985): Veterinary viral diseases, ed.A.J. Delta-Porta, p. 394. New york; Academic press Theiler, A. (1906-1907): Rep.Govt.Vet.Bact.Transvaal: 22-23

Tzipori, S. (1975): British Veterinary Journal. 131: 720- 726 Uren MF, St George TD, **Kirkland PD, Stranger RS, Murray MD. (1987):** Epidemiology of bovine ephemeral fever in Australia 1981-1985. Aust J Biol Sci;40(2):125-36

Uren MF. (1989): Bovine ephemeral fever. Aust Vet J Aug;66(8): 233-6

Uren MF, Walker PJ, Zakrzewski H, St George TD, Byrne KA. (1994): Effective vaccination of cattle using the virion G protein of bovine ephemeral fever virus as an antigen. Vaccine ;12(9):845-850

Vanselow BA, Abetz I, Trenfield K. (1985): A bovine ephemeral fever vaccine incorporating adjuvant Quil A: a comparative study using adjuvants Quil A, aluminium hydroxide gel and dextran sulphate. Vet Rec Jul 13;117(2):37-43

Vanselow, B.A.; Walthall, J.C. and Abetz, I..(1995): field trials of ephemeral fever vaccines. Vet. Microbiol. 46 (1-3): 117 – 130.

Van der Westhuizen B. (1967): Studies on bovine ephemeral fever. I. Isolation and preliminary characterization of a virus from natural and experimentally produced cases of bovine ephemeral fever. Onderstepoort J Vet Res Apr;34(1):29-40

Walker PJ. (2005): Bovine ephemeral fever in Australia and the world. Curr Top Microbiol Immunol. 2005;292:57-80.

Walker PJ, Byrne KA, Cybinski DH, Doolan DL, Wang YH. (1991): Proteins of bovine ephemeral fever virus. J Gen Virol Jan;72 (Pt1):67-74

Walker, P.J.; Byrene, K.A.; Riding, G.A.; Cowley, J.A.; Wang, Y and McWiliam, S. (1992): The genome of bovine ephemeral fever rhabdovirus contains two related glycoprotein genes. Virology, 191: 49- 61.

Walker PJ, Wang Y, Cowley JA, McWilliam SM, Prehaud CJ. (1994): Structural and antigenic analysis of the nucleoprotein of bovine ephemeral fever rhabdovirus. J Gen Virol ;75 (Pt 8):1889-1899.

Wang, Y.; McWilliam,S.M.; Cowley, J.A.and Walker, P.J. (1994): Complex genome organization in the Gns-L intergenic region of Aeldiade River rhabdovirus. Intervirology 203: 63-72.

Wang FI, Hsu AM, Huang KJ. (2001): Bovine ephemeral fever in Taiwan. J Vet. Diagn. Invest. ;13(6):462-7

Wu ,C.C ; T.L. Lin ; H.G. Zhang ; V.S. Davis and J.A. Boyle (1992): Molecular detection of infectious bursal disease virus by polymerase chain reaction. Avian diseases, 36:221-226.

Wunner, W.H.; Calisher, C.H.; Dietzgen, R.G. ; Jackson, A.O.; Kitamjima, E.W.; Lafon, M,F.; leong, L.C.; Nichol, S.T.; Peter, D.; Smith. J.S. and Walker, P.J. (1995): Rhabdoviridae. In virus Taxonomy. Sixth Report of the International Committee on Taxonomy of viruses, pp; 288 – 293. Edited by F.A. Murphy, C.M.Fauquet, D.H.L. Bishop, S.A.Ghabrial, A.W. Jarvis, G.P. Marrtelli, M.A. Mayo and M.D. Summers. Vienna and New York: Springer-Verlag.

Yeruham, I.; Berverman,Y.; Yadin, H.; Van Ham.M. Chai, D.; Tiomkin, D and Frank,D. (2002): Epidemiological investigations of outbreak of bovine ephemeral fever virus in Isreal. The Veterinary record, Jul 27, 117-121

Young PL. (1979): Infection of water buffalo (Bubalus bubalis) with bovine ephemeral fever virus. Aust Vet. J. ;55(7):349-50

Young, P.L. and Spradbrow, P.B. (1980): The role of neutrophiles in bovine ephemeral fever virus infection of cattle. Journal of infectious Diseases, 142: 50-55.

Young PL, Spradbrow PB. (1985): Transmission of virus from serosal fluids and demonstration of antigen in neutrophils and mesothelial cells of cattle infected with bovine ephemeral fever virus. Veterinary microbiology, 10: 199-202

Young PL, Spradbrow PB. (1990): Clinical response of cattle to experimental infection with bovine ephemeral fever virus. Vet. Rec., 27: 86-88

Zaghawa, A.; Akela, M.A.; Khadr, A.M. and Hassan H.Y.(2000): An outbreak of bovine ephemeral fever in Egypt during 2000. 1. Clinical and epidemiological investigations. 9th Sc. Cong. 2000, Fac. of Vet. Med., Assiut Uni., Egypt. p: 346-353.

Yeruham I, Gur Y, Braverman Y (2005): Retrospective epidemiological investigation of an outbreak of bovine ephemeral fever in 1991 affecting dairy cattle herds on the Mediterranean coastal plain. Vet J. 2005 Nov 25.

Zaghawa, A. Hassan,H.Y.;Khalil,S.;Waer,M.;khodier,M.;and Reda,I.M.(2001): Immune response of calves to inactivated rabies vaccine and its possible protection against Bovine ephemeral fever. 6th Sci. Cong Egyptian Socity for cattle Diseases, 4-6 Nov. 2001, Assiut, Egypt.

Zaghawa A.; , Khalil, S.A. ; Hassan H.Y and Al-Gaabary, M.H. (2002): Diagnostic value of the antigenic relationship between Bovine ephemeral fever and Rabies viruses. 2nd Scientifif Vet. Med. Congress, Fac. Vet. Med. Sadat City, Menoufia University, 23-24 April,2002.