

BOVINE EPHEMERAL FEVER (THREE DAY SICKNESS)

A Review

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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 evolvment 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 dairy herds and reduction in condition of prime animals or disruption of stock movements and disruption of markets. Recent outbreaks recorded increase morbidity and mortality 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), subsequently 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).

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 immunofluorescence 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 immunoperoxidase tests in the leukocytes of clinically diseased animals (Zaghawa et al, 2002) as well as by the PCR (Khalil et al,2001).

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).

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 other 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 8 potential N-glycosylation sites as other rhabdoviruses. 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 complementarity 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 al., 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).

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 were 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:

4.4.1. Possible protection against BEFV after vaccination with killed rabies vaccine:

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 BEFV anti-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.

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 once 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).

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 not been 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 coincides 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 polymorphonuclear cells in the circulation (left 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 animal 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, hypophosphatemia and hypomagnesemia (Hassan, 2000 and Attia & Selim, 2000).

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 et al., 1968). Virulent strains become stabilized after 6 passages causing paralysis and death 2-3 days post inoculation leading to loss of pathogenicity 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 1-1.5µm 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. George, 1988).

11.2. Detection of BEF virus antigen by immunofluorescence and immunoperoxidase 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)

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.

Corticosteroids 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, 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 live vaccine can be enhanced by mixing with Freund's incomplete adjuvant (South Africa), aluminum hydroxide and Quil A (Australia). 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 live 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 heterologous (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).

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 as well as rabies needs further investigation to see the possible protection with heterologous vaccination.

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