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Bovine ephemeral fever: a review

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Abstract

Bovine ephemeral fever is a viral disease of cattle and buffaloes besides subclinical involvement of a variety of ruminant species. The subtropical and temperate regions of Africa, Asia and Australia have experienced the major epidemic of the bovine ephemeral fever but the occurrence in the tropics can not be overlooked. Although the substantial role played by the vectors viz., mosquitoes and culicoides in bovine ephemeral fever perpetuation and dissemination, other vector involvement if any should be extensively studied. The clinical severity of the disease is not apparent and the mortality is low. However, high morbidity, enormous economic losses in terms of significant reduction in production, disruption of national and international trade and finally a variety of complications resulting from the disease have drawn appreciable attention from the researchers around the world to resolve the unsolved questions in this area. In this review, detailed informations of all the aspects of the disease has been provided in a simple, lucid and easily understandable manner.

Keywords: Bovine ephemeral fever (BEF); Three days' sickness; Vero; Hamster kidney; Hamster lung; *Aedes albopictus*; Monkey kidney stable line (MS); Culicoides; Culex; Anopheles; Kimberley virus

Résumé

La fièvre éphémère bovine est une maladie virale des bovins et des buffles présentant des complications infracliniques chez plusieurs espèces de ruminants. Les régions subtropicales et tempérées de l'Afrique, de l'Asie et de L'Australie ont connu une épidémie majeure de fièvre éphémère bovine mais l'évènement de la maladie dans les tropiques ne peut pas être oublié. A côté du rôle subtantiel joué par les vecteurs tels que moustiques et culex dans la transmission et la dissémination de la fièvre éphémère bovine, d'autres vecteurs devraient intervenir. La sévérité clinique de la maladie n'est pas apparente et la mortalité est faible. Cependant, une haute morbidité, d'énormes pertes économiques en terme de réduction significative de production, interruption de commerce nationale et internationale et

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finalement une variété de complications provenant de la maladie ont attiré l'attention des chercheurs à travers le monde afin de résoudre les questions non résolues dans cette région. Dans cette étude, des informations détaillées de tous les aspects de la maladie sont fournis.

Mots-cléf: Fièvre éphémère bovine; Maladie de trois jours; Vero; Rein de hamster; Poumon de hamster; Lignée de rein de singe; Virus Kimberlley

1. Introduction

Bovine ephemeral fever is a viral disease of cattle, *Bos taurus*, *Bos indicus*, and *Bos javanicus* and water buffalo *Bubalus bubalis*, although BEF virus subclinically infect a greater range of ruminant species. The disease has a variety of names including 'three days sickness', stiff sickness, dengu fever of cattle, bovine epizootic fever and lazy man's disease. However, the name of bovine ephemeral fever is most commonly used and is very apt. The disease in cattle is characterized by acute febrile reaction, stiffness, lameness 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 is major economic losses due to drop in production in dairy herds and reduction in condition of prime animals or disruption of stock movement and disruption of markets. It is the prime time to create the substantial awareness both in individuals and industry owners about the epidemiology, transmission, prevention and control of the disease to avoid the enormous economic losses.

2. History and distribution

The origin of ephemeral fever is obscure. The first reports of the ephemeral fever were probably in mid-nineteenth century when the disease was first noticed in East Africa [1] and subsequently in Rhodesia [2], Kenya [3], South Africa [4], Indonesia [5], India [6], Egypt [7], Palestine [8], Australia [9] and in 1949 in Japan [10]. 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. The geographical distribution of ephemeral fever is considerable and spans the tropical regions of Africa, Australia and Asia with extensions into the subtropics and some temperate regions. The disease has never been reported in Western hemisphere, North and South America. The serological evidence indicated that Newzealand and Pacific Islands are free from the disease. At present the disease is enzootic in South Africa, India, Japan and parts of Australia.

3. Etiology

Ephemeral fever is caused by BEF virus, a single stranded negative sense RNA virus under rhabdoviridae family [11]. Electron microscopic study reveals that the bullet shaped BEF virus has a fringe of fine surface projections and measures about 80×120–140 nm. Although most of the BEF virus are bullet shaped, south African strains are mostly conical but closely related to bullet shaped Asian and Australian strains serologically.

4. Physicochemical properties

The BEF virus contains RNA and is sensitive to diethylether and sodium deoxycholate suggesting the presence of lipid containing envelope. Citrated whole blood from BEF affected cattle remain 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 h at 37°C [12].

5. Molecular and biochemical characterization

BEF virus structurally resembles other mammalian rhabdoviruses. Virions are bullet shaped and contain five structural proteins: L (Mr = 180 kDa), G (Mr = 81 kDa), N (Mr = 52 kDa), M1 (Mr = 43 kDa) and M2 (Mr = 29 kDa) [13]. As for rabies virus and VSV, the BEF virus membrane glycoprotein (G) can be removed from the virions 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 [14]. Two potential peptidase cleavage sites can be identified in the BEF virus G protein corresponding to lysine residue at position +13 and +18. A stretch of 16 hydrophobic amino acids at residues 539–554 appears to constitute the transmembrane domain of the BEF virus G protein. This region is bounded by basic residues (R and K) which are characteristic of other rhabdovirus transmembrane segments. The conformational dependent as well as conformational independent neutralizing and non-neutralizing antigenic determinants are present on the G protein of BEF virus [15, 16]. The G protein presents type specific and neutralizing antigenic sites and corresponds to the spike glycoprotein of other rhabdoviruses. Six neutralization sites have been identified by competitive binding of G protein Mabs.

In addition to the virion 81 kDa G protein, a 90 kDa non-structural glycoprotein (Gns) is synthesized in BEF virus infected cells. It is synthesized in similar abundance to the G protein but has not been detected in virions. The Gns protein has the structural characteristics of a rhabdovirus glycoprotein including a signal domain, hydrophobic transmembrane and 8 potential *N*-glycosylation sites.

The signal domain comprises a primarily hydrophobic segments at the N terminus which appears to terminate at arginine (residue 15). The function of the Gns protein is not clearly known. Indeed, as the Gns protein shared amino acid sequence homology and general structural characteristics with other rhabdovirus G protein, it might expected to have a similar function [14].

A 3789 nucleotides of the BEF virus genome located 1.65 kb downstream of the N gene after cloning and sequencing revealed two long ORF. The first ORF encodes G protein and second ORF situated between G and L genes encodes Gns protein [14]. Two related glycoproteins (G and Gns) encoded in the BEF virus were expressed from the recombinant vaccinia virus. The recombinant G protein (79 kDa) appears slightly smaller than the native G protein but reacted with Mabs directed against all defined neutralizing antigenic sites (G1, G2, G3a, G3b and G4). The recombinant Gns protein (mol. mass 90 kDa) was identical in size to the native Gns protein and failed to react with anti-G protein MAb or polyclonal antibodies but the G and Gns proteins were localized intracellularly in the ER/Golgi complex and at the cell surface only G protein is associated with budding and mature virus particle but not Gns protein. Both rabbit and cattle vaccinated with r^{vv}-G developed high level of antibodies which neutralized BEF virus in either mammalian or insect cells. Contrastingly, r^{vv}-Gns vaccinated rabbits and cattle failed to produce neutralizing antibodies and after challenge BEF virus was isolated from 2/3rd of the vaccinated cattle [17].

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 [18]. The BEF N protein gene cloned in *E. coli* was expressed as glutathione-*S*-transferase fusion protein. An analysis of amino acid sequence relationship with other rhabdovirus *N* proteins reinforces the view that BEF virus is more closely related to vesiculo virus than to rabies virus. However, an antigenic analysis also demonstrate direct links with rabies virus suggesting that reported serological crossreactions between BEF virus related viruses and lyssaviruses are determined by conserved structural elements that do not reflect the overall relationship of the viruses. The *N* protein of BEF is a structural component which has multiple functions in nucleocapsid assembly and the regulation of transcription and replication [19].

6. Isolation and growth of BEF virus

The successful growth of BEF virus was achieved by inoculating leucocyte from a cow with clinical disease intracerebrally into suckling mice 1–3 days old [20], suckling hamster and rats [21]. Virulent strains become stabilized after 6 passages causing paralysis and death 2–3 days post inoculation leading to loss of pathogenicity for calves [22].

BEF virus grows very poorly or not at all in cells or bovine origin. It grows very well in BHK-21 cells inoculated with mouse brain or bovine leucocyte

suspension. It also grows in bovine kidney, hamster lung, Vero and *Aedes albopictus* cell lines and hamster lung tissue culture. Not all BEF strains produce CPE and the presence of virus is generally demonstrated by immunofluorescence [23]. The monkey kidney stable line (MS) show a cytopathic effect characterized by rounding of cells, granular appearance of the cytoplasm followed by detachment from the glass after 48 h incubation. The pinpoint plaques developed in MS cells 2–4 days post inoculation and reached 1–1.5 nm diameter by the 8–10th day [24].

BEF virus can multiply in mosquito species after the intrathoracic injection of mouse adapted virus or by feeding blood virus mixtures. The multiplication of the virus has been shown in two species of culicoides, *C. brevitarsis* and *C. marksi* and in one species of mosquito, *Culex annulirostris*, 6–8 days post infection [25].

7. Host affected

Besides cattle in Asia, water buffalo are susceptible to ephemeral fever [26] but in Australia they are refractory to experimental infection. Subclinical infection in Australian water buffalo is common as judged by serological survey [22]. The role of wild life as reservoir host is still obscure. In Kenya, the antibody to BEF virus is demonstrated in African buffalo, water buck, wildebeest and hartebeest [27]. Domestic and feral red deer in Australia have a high prevalence of neutralizing antibody [23]. Sheep and other domestic animals are not susceptible though passaging of BEF virus through sheep experimentally has become possible.

8. Arthropod vector

The epidemiological evidence strongly suggested that a flying insect vector is responsible for the transmission of the disease before the virus was isolated from any insect. BEF virus has been isolated from various species of culicoides and mosquitoes. In Africa, BEF virus was first isolated from a mixed pool of culicoides species [27]. The virus in Zimbabwe was isolated from *C. coarctatus* and *C. imocola* by Blackburn et al. in 1985 and in Australia from *C. brevitarsis* [28]. The BEF virus has also been isolated from mosquito species *Anopheles bancrofti* and *Culicine* species. No species has yet been proven as a vector for BEF virus transmission and the role of insect in mechanical transmission of disease is yet to be confirmed.

9. Ecology

Bovine ephemeral fever is most prevalent during rainy seasons when the insects are numerous and the spread is influenced by the wind movement. The virus is

associated with the leucocytes fractions of blood and the disease is easily transmitted by inoculating iv blood taken from diseased animals showing febrile reactions to susceptible cattle. Mechanical transmission through insect vector or by direct contact does not occur and the virus does not persist much beyond the 4th day after subsidence of the fever. Animals once infected confer lifelong immunity although a second bouts of illness two or three weeks apart is not uncommon [29, 30].

10. Pathogenesis

The pathogenesis of the disease is complex and the release of lymphokines mediate the host inflammatory process resulting the final outcome of the disease. The virus does not cause wide spread tissue damage. In every case there is early neutrophilia with an abnormal level of immature neutrophil in the circulation. There is significant drop in plasma calcium and elevated level of plasma fibrinogen. Affected animals respond well to non-steroid anti-inflammatory drugs and calcium infusion. There is development of solid immunity in animals following infection and repeat clinical episode usually involve the newborn or young calves since the previous outbreak [31–34].

11. Clinical signs

BEF virus infection of cattle can be expressed in a wide spectrum from imperceptible clinical signs to death. The host response to infection and the environment heavily influence the severity and final outcome of the disease. The disease is more severe in adult cattle than in young animal; in fat animals than in lean animals, in heavy bulls than in light steers, in high lactating cattle than in dry cows. If affected cattle are without shade and water, they may suffer from severe dehydration.

The natural case of BEF is characterized by sudden onset of fever (41–42°C), lameness along with listlessness, inappetence and a starring coat followed by lachrymation, serous oral and nasal discharges, joint pain and general stiffness. The fever in BEF is biphasic, triphasic or occasionally multiphasic. The first phase body temperature is always lower than in later phase. Ruminal function may cease resulting constipation. Lactation may decline suddenly or completely in dairy cows. In most of the cases, milk production returns normal progressively with recovery but level is always lower than preillness level [35]. In most of the cases, there is temporary or permanent paralysis of all the four limbs. The paralysed cattle maintain sternal recumbency but in later stage they assume lateral recumbency. There is salivation and difficulty in swallowing. Bloat is a variable phenomenon. The progressive loss of reflexes, coma and death result within 1–4

days after paralysis. Complete recovery occur in 95–97% cases whether the clinical signs are mild or severe.

Complications usually occur and are manifested by pneumonia, mastitis, hind quarter paralysis, abnormal gait, abortion in late pregnancy and temporary (upto 6 months) infertility in bulls. Pulmonary and subcutaneous emphysema are rare occurrences. All kind of changes are not found in one outbreak or a series of outbreaks. Climatic stress determines the severity of the clinical signs. Dairy cows in early and midlactation are more likely to die than dry cows [36].

12. Pathology

BEF is an inflammatory disease, serofibrinous polysinovitis, polyarthritis, polytendovaginitis, cellulitis and focal necrosis of skeletal muscles are the common pathological lesions. The lungs may show fatty oedema and lymph nodes are edematous. The lesions in the upper cervical region of spinal cord has also been reported by Hill and Schultz [36].

Microscopically, there is neutrophilia, leucocytosis and high fibrinogen level. A steady reduction in erythrocyte number in the early part of the infection followed by a larger fall which corresponded to hemosiderosis of lymph node and spleen. Lesions have been described in venules and capillaries in tendon sheath, synovial membranes, muscle, fascia and endothelium, perivascular neutrophilic infiltration, focal or complete necrosis of vessel walls, thrombosis and perivascular fibrosis [37].

13. Diagnosis

Ephemeral fever is usually diagnosed from history and clinical signs. A diagnosis can be made from the sudden onset of febrile reactions lasting for 2–5 days with spontaneous recovery. The seasonal occurrence and symptoms of oropharyngeal secretions, joint pains and stiffness are of value. However, a confirmatory diagnosis can be obtained by isolation of BEF virus from blood taken into heparin or EDTA anti-coagulant during fever or by demonstration of rising titre of neutralizing and complement fixing antibodies in paired sera collected during illness and two or three weeks later [38]. The virus can be isolated by inoculating blood from clinically affected cattle to susceptible or unweaned mice. Serological diagnosis can be complicated by the previous infection of antigenically related virus such as Kimberley virus. Kimberley virus infection is subclinical and causes the development of low titre of serum neutralizing antibodies to BEF virus without conferring any protection against BEF virus. A prior infection with Kimberley virus sensitizes the cattle so that a secondary instead of primary antibody response occurs on first exposure to BEF virus. Tomori et al. in 1974 [39] have succeeded in producing clinical signs similar to BEF in cattle injected to Kotonkan virus.

14. Control and prevention

Without the knowledge of insect vector and the incubation time following the bite of insects, control of vectors will be of little success. Attempts can be made to alleviate the symptoms of the affected cattle through medication and to prevent infection by vaccination.

15. Treatment

Rational treatment will be of very effective if applied early in BEF cases. First, the affected cattle should be provided complete rest during acute illness and convalescence. Second, symptomatic treatment with anti-inflammatory drug for instance, salicylates or phenylbutazone have been found to be beneficial. Various antibiotics can be used to check the secondary bacterial infections. The intravenous or subcutaneous administration of calcium borogluconate has been found to be beneficial to some but not all cattle. Isotonic fluids have been used iv to treat dehydration. During acute illness, no medicine should be given orally to avoid inhalation pneumonia due to inability of the animal to swallow [23].

16. Vaccines

A single attack of BEF virus conferring life long immunity to cattle encouraged the researchers to develop a vaccine. A killed vaccine developed by Vanselow et al. in 1985 [40] is useful for 6 months protection against BEF or can be administered as an antigenic booster to cattle previously given live attenuated BEF virus vaccine.

The rapid loss of virulence for cattle of BEF virus was reported by Van der Westhuizen in 1967 [41], when passaged in mice. Surprisingly, the loss of immunogenicity along with virulence occurs at the 8th passage level. The immunogenicity can be enhanced by mixing mice vaccine with adjuvant [42–44]. Since BEF can be grown in tissue culture, this system is much more practical than mice for producing vaccine. The adjuvants which are used to enhance the antigenicity of live attenuated vaccines are Freund's incomplete adjuvant (south Africa), and aluminum hydroxide and Quil A (Australia). In Japan, aluminum phosphate gel is used with the killed vaccine but not with the live vaccine. The practical difficulties with the adjuvant mixed with live vaccine are a proportion of viruses got inactivated and the test if viability of BEF virus in cell culture is quite impossible as the adjuvants are toxic to cell culture. If the modified live vaccine strains are not replicated in the host, then development of a efficacious killed vaccine would be a desirable target.

17. Economic effects

The economic effect of BEF is severe and range from mortality to effects on both trade within and between countries.

Mortality expressed as percentage of a whole herd usually counts less as BEF virus affects the most productive numbers of the herd- the mature cows, bulls and working oxen. A persistent body weight loss presumably in fat and muscle is insignificant and pre-illness body weight is regained two weeks after recovery [45]. The loss of milk production ranges from 34–95% with an average of 46%. The milk yield did not reach to pre-illness level on convalescence. There may be some loss by abortion in the females [46] and males may suffer from temporary infertility [47]. Lastly, BEF virus poses a severe threat both on national and international trade of animals. Many countries require cattle and buffaloes free from BEF neutralizing antibodies to be imported from a country where the disease is prevalent. It is a costly affair to keep the bulls whose semen is to be exported in insect proof area and to monitor the evidence of BEF virus infection continuously [48].

18. Future trend in BEF virus research

Ephemeral fever is a disease not completely understood. The virus has been characterized to a fair degree but its relation to other rhabdoviruses needs to be studied. The isolates of BEF virus from different parts of the world indicated that they are antigenically similar although Australian isolates from insects demonstrated some differences. A precise biochemical characterization of all isolates provide the basis of virulent or avirulent property of the virus. Furthermore the function of the Gns and the protein expressed by 1.6 kb region containing several long open reading frames lie between the Gns gene and the L gene consensus sequences need to be elucidated.

The persistence of the BEF virus between the epidemics requires an indepth study in epidemic areas viz. tropics of Africa, Asia and Australia. It is proved that both mosquito and culicoides can support the BEF virus growth but the potential range of insect vector remain to be identified. Until the epidemiology is clearly understood, the economic loss of the cattle industry due to BEF virus is difficult to estimate. A cheap, efficacious killed vaccine should be kept ready to combat the outbreak situations for a short period of time.

However, future research should be directed towards the evolvement of a potent live attenuated, recombinant or DNA vaccine which may be a suitable alternative to current vaccines [49]. Knowledge of different kinds of vectors in various parts of the world and the behavior of BEF virus in the vectors would help for the novel and effective management and control of the disease.

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