

Studies on Bovine Ephemeral Fever virus

Abuelyazeed A. EL-Sheikh,

Department of Virology, Faculty of
Veterinary Medicine Zagazig University

Abstract

Bovine ephemeral fever is an acute, arthropod-borne disease of cattle. The disease characterized by sudden onset of fever, stiffness, disinclination to movement accompanied by lameness, high morbidity and very low mortality. Recovery usually occurs within three to four days of the onset of clinical signs. In this study, the viral isolation, antigen detection as well as antibodies demonstration after natural infection were studied. The results showed that the virus was isolated in the third and fourth days of infection. Antigen was detected by immunofluorescent technique from the first day to the tenth of infection. Antibodies against the virus were detected by ELISA from the sixth days to the fourteenth days of infection. The level of antibodies increased gradually and reach the maximum at the tenth day of infection.

Introduction

Bovine ephemeral fever (BEF) is a viral disease of cattle and buffaloes besides subclinical involvement of a variety of ruminant species. The clinical severity of the disease is not apparent and the mortality is low. However, it can cause significant economic impact through reduced milk production in dairy herds, loss of condition in beef cattle and loss of draught animals at the time of harvest. Available evidence indicates clinical signs of BEF, which include bi-phasic fever, anorexia, muscle stiffness, ocular and nasal discharge, ruminal stasis and recumbency, are due primarily to a vascular inflammatory response. Recovery usually occurs within three to four days, hence the term ephemeral. The causative virus is transmitted by haematophagous insects that appear to be borne on the wind, allowing rapid spread of the disease.

Bovine ephemeral fever virus has been classified as the type species of the genus Ephemerovirus in the Rhabdoviridae (1). (2). BEFV is a single stranded, negative sense RNA virus. The complete virus particle is bullet shaped, enveloped and consists of helical nucleocapsid. The most reliable

methods for viral detection are fluorescent antibody technique, ELISA, immunoperoxidase, neutralization test and RT-PCR Mellor, 2001. Specific fluorescence indicating the presence of BEF viral antigen could be detected at the time of peak clinical response in individual cells in the lungs, spleen and lymph nodes as well as neutrophils. Before and after the peak fever some fluorescence was seen in cells which appeared to be reticular cells in the lymph nodes. Viral isolation in mice could be made from blood, lungs, spleen and lymph nodes over a period of no more than 3 days. It is postulated that viral growth takes place mainly in the reticuloendothelial cells in the lungs, spleen and lymph nodes and not in vascular endothelium or lymphoid cells. An antibody rise to BEF virus did not necessarily indicate recent BEF virus infection, and should be considered of diagnostic value only when taken in conjunction with clinical signs of disease.

In Egypt, the disease was first described by Rabagliati, 1924. In summer 1991, a typical form of the disease has been recorded in different governorates in lower Egypt Hassan et al., 1991. A second outbreak of BEF occurred in summer 2000, where it included several governorates in lower and upper Egypt and characterized by 50% morbidity and 2.5% mortality, Zaghawa, 2000. This work was conducted to define the antibodies response of cattle to natural infection with ephemeral fever infection along with viral isolation and antigen detection.

Materials and methods

Samples. Blood samples were collected from naturally infected herd at Gharbia governorate. The blood samples were collected in the first, second, third, fourth, fifth, sixth, tenth, twelve, and fourteen days of infection. Blood was collected from three cows and pooled for each day. On each day, two blood samples were collected, one with anticoagulant for virus isolation and antigen detection and second without anticoagulant for antibodies detection. For virus isolation and antigen detection, the buffy coat was obtained from the samples and washed three times with phosphate buffered saline.

BEF virus and antisera. They were kindly supplied by rabies department, at vaccine institute, Abbasia, Cairo.

FITC IgG conjugate. Rabbit anti-bovine FITC IgG conjugate was commercially purchased from Sigma.

Horse raddish peroxidase. Rabbit antibovine horse raddish peroxidase IgG conjugate was commercially purchased from Sigma.

Isolation of BVDV on MDBK: Propagation of field samples on Madin-Darby bovine kidney cells (MDBK) was carried out according to (Jewett et al., 1990). The cells were provided by virology department, faculty of veterinary medicine, Zagazig university. It was grown with modified Eagle's minimum essential medium (EMEM) with Earl's balanced salt solution and L. glutamine without sodium bicarbonate. The medium was supplemented with 2% new born calf serum and antibiotic mixture and antimycotic. The collected samples were inoculated to MDBK and propagated for three blind passages. The inoculated plates were examined daily for recording the cytopathic changes in the cells.

Detection of BEFV antigen in collected samples by indirect fluorescent antibody technique: The technique was done according to. Buffy coat swabs were made on glass slides and fixation was done by immersing the slides into Petri dish containing acetone 100 % for 10 minutes then dried at room temp. BEFV antiserum was added to the slides, and incubated at 37 °C for 30 minutes in humidified atmosphere. After incubation, the slides were washed three times with PBS for 5 minutes. The rabbit anti-bovine conjugate with FITC (Sigma) was diluted at 1:200 with PBS and added to the slides and incubated at 37 °C for 60 minutes in humidified atmosphere. After washing as above, slides were examined under fluorescence microscope after mounted in a pre-warmed mounting buffer.

Identification of BEF antibodies in the collected serum by indirect

ELISA:

The test was carried out according to. A blocking ELISA for detecting antibodies to bovine ephemeral fever virus (BEFV) in cattle is used. In this test, plates were coated overnight by BEFV at 4 C. After absorption of antigen, the plates were saturated with a blocking buffer for 30 minutes at room temp. Serum samples were tested in duplicates. Serum samples were diluted in PBS 0.05% tween and added by 50 ul /well, then was incubated 1 hr at 37°C. After incubation, the serum was decanted and the plates were washed three times with PBS 0.05% tween (200 ul/ well). The conjugate (rabbit anti-bovine peroxidase) diluted to 1/300 was added by 50 ul/ well and incubated 1

hr at 37°C. The plates were washed three times with PBS tween 0.05% and IP substrate (3-amino-9-ethyl-carbazole and 0.2% urea hydrogen peroxide in 95% ethyl alcohol) was added by 50 ul/ well. The plates were incubated 10 minutes at room temperature, washed by double distilled water then dried at room temperature and the result was recorded.

4-Results

Results of BEFV isolation from collected samples on MDBK cell line:

Trials to isolate the causative virus in cell culture were unsuccessful in the first passage. In the second and third passages, the virus was isolated only from samples collected in the third and fourth days of infection and not from other samples. The virus induces cells degeneration. The CPE was clear between the 3rd and 5th days post inoculation, especially in 3rd passages, see fig 1 and table 1.

Results of IF technique for detection of BEFV antigen:

IF technique detected the viral antigen in buffy coat sample collected from the first day of infection till the tenth day of infection. Samples collected from the twelve and fourteen days of infection showed negative results. Table 1 and Fig (2) shows the result of IF test.

Results of ELISA test for detection of BEFV antibodies:

Positive antibodies against BEFV were detected in the serum samples collected at days sixth, tenth, twelve and fourteenth. The results showed that antibodies against the virus were increased gradually from the sixth days of infection and reach the maximum level at the twelve day of infection. Table (2) and fig 3 summarized results of ELISA test for detection of BEFV antibodies in the samples collected from infected cattle.

Discussion

Bovine ephemeral fever (BEF), a vector-borne disease of cattle, is caused by the Ephemerovirus of the family rhabdoviridae. In this work, the relationship between viral infection and antibodies response was studied.

Samples were collected from blood where the maximum titer of the virus is present.

Virus was isolated at third and fourth days of infection. This result disagree with .. who mentioned that viraemia was demonstrated on either the first or

second day of clinical disease and lasted for at most 48 hours. However, virus could be detected in blood and during viremic stage and virus is difficult to be isolated.

Virus antigen was detected by IF from the first day of infection to the tenth days of infection. This result agree with

Antibodies were detected by ELISA starting from the sixth day of infection and reach the maximum at twelve day and decrease again at fourteenth day. Evidence is presented that subclinical infections with other arboviruses may limit an ephemeral fever epidemic by providing temporary protection by interference. Antibodies response was monitored during clinical disease by .. NT. Low levels of neutralising antibody could be detected within one or two days after the cessation of viraemia. Natural BEFV infection induces a strong neutralizing antibody response and infection usually induces durable immunity. The greater simplicity and sensitivity of the test when compared with the VN test makes it the preferred test for the diagnosis and monitoring of clinical bovine ephemeral fever. The sensitivity of the B/ELISA was compared with the virus neutralisation (VN) test using a total of 380 sera from cattle.

Table 1 : the results of virus isolation and antigen detection by IF

Sample number	Days of collection	Result of Virus isolation	Result of Ag detection by IF
1	First	-ve	+ve
2	Second	-ve	+ve
3	Third	+ve	+ve
4	Fourth	+ve	+ve
5	Fifth	-ve	+ve
6	Sixth	-ve	+ve
7	Tenth	-ve	+ve
8	Twelve	-ve	-ve
9	Fourteen	-ve	-ve

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