Veterinary Microbiology 173 (2014) 241–248

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Relationships of bovine ephemeral fever epizootics to population immunity and virus variation

Lu-Jen Ting, Ming-Shiuh Lee, Shu-Hwae Lee, Hsiang-Jung Tsai, Fan Lee *

Animal Health Research Institute, 376 Chung-Cheng Road, Tamsui District, New Taipei City 25158, Taiwan

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 21 July 2014
Accepted 22 July 2014

Keywords:
Bovine ephemeral fever virus
Epizootic
Population immunity
Genetic variation
Taiwan

ABSTRACT

Bovine ephemeral fever is an arthropod-borne bovine viral disease caused by infection with bovine ephemeral fever virus which belongs to genus *Ephemerovirus* within the family *Rhabdoviridae*. In this study, serological data and virological information about the disease and the virus, spanning from 2001 to 2013, were employed to analyze the relationships of bovine ephemeral fever epizootics to population immunity and virus variation. National and regional surveillance data indicated that 2 of the 3 major epizootics and 87% regional outbreaks were associated with lower neutralizing antibody titers and immunity coverage, reflecting the importance of population immunity for the control of bovine ephemeral fever. Phylogenetic analysis and sequence comparison demonstrated that Taiwanese bovine ephemeral fever viruses were >96.0% and >97.6% similar to the East Asian isolates in nucleotide and amino acid sequences, respectively. These analyses supported that the Taiwanese viruses shared the same gene pool with the strains of the other East Asian countries, mainly Japan.

1. Introduction

Bovine ephemeral fever (BEF), or three-day sickness, is an arthropod-borne viral disease that affects bovine and water buffalo (Nandi and Negi, 1999). The disease is characterized by a high fever of up to 42 °C, decrease or loss of appetite, depression, nasal and ocular discharges, excessive salivation, sudden decrease in milk production, respiratory distress, lameness and stiffness. Without appropriate medical care, affected animals may die within 12 h (St. George et al., 1984; Young and Spradbrow, 1990; Uren et al., 1992).

The etiological agent of BEF is the bovine ephemeral fever virus (BEFV), a member of the genus *Ephemerovirus* within the family *Rhabdoviridae*. The virion of BEFV is enveloped and bullet-shaped, and possesses a single-stranded, negative-sense RNA genome with a length of approximately 14.9 kb (Della-Porta and Brown, 1979). The genome encodes five structural proteins: surface glycoprotein, nucleoprotein, matrix protein, phosphoprotein, and RNA polymerase (Walker et al., 1991).

Among the BEFV structural proteins, the 81-kDa surface glycoprotein is the major neutralizing and protective antigen (Cybinski et al., 1990), and four neutralizing epitopes, G1, G2, G3, and G4, on this protein are recognized. The G1 site is located on amino acid residues Y^{487} to K^{503}. The G2 site is located on L^{169} to I^{187}. The G3 site is located on three distant domains: Q^{48} to I^{63}, F^{217} to P^{231}, and I^{262} to Q^{271} (Kongsuwan et al., 1998). The location of the tentative G4 epitope has remained unclear.

Bovine ephemeral fever was first recognized in Taiwan in 1967 (Wang et al., 2001), in China in 1955 (Zheng and Qiu, 2012), and in Japan in 1950s (Kato et al., 2009). In

* Corresponding author. Tel.: +886 2 26212111x516; fax: +886 2 26225345.
E-mail address: fanlee@mail.nvri.gov.tw (F. Lee).

http://dx.doi.org/10.1016/j.vetmic.2014.07.021
0378-1135/

Taiwan, the BEF epizootics from 1967 to 2002 have been reported (Hsieh et al., 2005), and two additional epizootics broke out in 2007 and 2012–2013. Control of BEF mainly depends on vaccinating cattle to establish acquired immunity and decreasing the number of dipteral vectors to block the route of transmission. Commercially available inactivated BEF vaccines have been applied to the field; meanwhile, BEF outbreaks still occur in Taiwan almost every year and epizootics every a few years (Chiu and Lu, 1987; Liao et al., 1998; Wang et al., 2001; Hsieh et al., 2005), suggesting ineffective control of the disease. It was proposed that the frequent BEF reemergence in Taiwan was associated with inadequate herd immunity in the cattle population.

The purpose of the present study was to use serological and virological information to analyze the possible factors affecting BEF protective immunity in the cattle population of Taiwan. Immunity coverage, phylogenetic relationships of field isolates, and variation of the isolates were analyzed.

2. Materials and methods

2.1. Serological surveillance between 2001 and 2013

To monitor population immunity of a given administrative district, bovine serum samples from cattle vaccinated with commercially available BEF inactivated vaccine were taken by local animal disease diagnosis centers in April and October of each year. Thirty cattle were sampled at each farm in general, and 4438 ± 595 samples were submitted annually from 16 counties/cities to the Division of Epidemiology, Animal Health Research Institute. The samples were heated at 56 °C for 30 min to remove activities of complements and the neutralizing antibody titers of the samples were measured by a virus neutralization test (VNT).

2.2. VNT

The VNT was performed in accordance with the procedure described by Gaffar Elamin and Spradbrow (1978) and adapted to a format of 96-well microtiter plate. Each heat-inactivated bovine serum sample was twofold serially diluted in 96-well microtiter plate, followed by addition of a virus suspension containing 100 $TCID_{50}$/well of BEFV 2001-YL-Taiwan strain. After a one-hour incubation at 37 °C, BHK-21 baby hamster cell line was added and then incubated at 37 °C in a humid chamber with 5% CO_2 for four days. The neutralizing antibody titer was expressed as the $\log_2$ of the reciprocal serum dilution that protected cells from the cytopathic effect.

2.3. Virus isolation

Lungs, spleens or blood from the diseased cattle, submitted by local animal disease diagnostic centers, were subjected to virus isolation. The lung or spleen tissue was emulsified with serum-free minimal essential medium supplemented with penicillin (500 U/ml) and streptomycin (500 μg/ml) to make a 10% w/v suspension. The blood cells were washed three times with phosphate-buffered saline, and freeze-thawed before inoculation. Each of the emulsified tissue suspension and thawed blood cells was inoculated onto BHK-21 cells. The inoculated cells were cultivated for five to seven days and observed daily. Whenever the cytopathic effect was observed, the supernatant of the culture was subjected to RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) for virus identification. Two additional blind passages were performed if the cytopathic effect was not observed.

2.4. Extraction of nucleic acids

Total nucleic acids within 200 μl of the supernatant of the cell culture were extracted using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Mannheim, Germany). The extract was dissolved in 100 μl of elution buffer and stored at −20 °C until use.

2.5. RT-PCR and sequencing

A primer pair, forward primer gpF1 (5′-ATGTTCAAGGTCCTAATAATTACC-3′) and reverse primer gpR1872 (5′-TTAATGATCAAAGAATCTATC-3′), was designed on a basis of published sequences of BEFV surface glycoprotein gene.

To amplify the gene of the isolated BEFV, RT-PCR was carried out in a 25 μl reaction mixture composed of 2.5 μl of 10× reaction buffer (Invitrogen, Life Technologies, Carlsbad, California, USA), 1.0 μl of each primer (2.5 μM/μl), 2.5 μl of dNTPs (2.5 mM/μl each), 0.2 μl of AMV reverse transcriptase (9 U/μl; Promega, Madison, Wisconsin, USA), 0.3 μl of ribonuclease inhibitor (40 U/μl; Promega), 0.5 μl of Taq DNA polymerase (5 U/μl; Invitrogen), 16.0 μl of ribonuclease-free distilled water, and 1.0 μl of extracted nucleic acids. The reaction was carried out utilizing the 96-Well GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies, Grand Island, New York, USA) with the following protocol: 42 °C for 40 min, 95 °C for 2 min, 38 cycles of 95 °C for 60 s, 55 °C for 60 s, 72 °C for 90 s, and a final extension of 72 °C for 5 min.

The RT-PCR product was analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized by ultraviolet transillumination. To determine its nucleotide sequence, the RT-PCR product with expected length was sequenced with the 3700XL DNA analyzer (Applied Biosystems, Life Technologies, Carlsbad, California, USA) by a commercial sequencing service (Mission Biotect, Taipei, Taiwan).

2.6. Phylogenetic analysis and sequence comparison

Nucleotide sequences of the full-length surface glycoprotein gene of the Taiwanese BEFVs isolated from 1984 to 2012 and 18 published sequences of Australian, Chinese, Israeli, Japanese, and Turkish BEFV isolates (Supplementary Table 1) were phylogenetically analyzed. Phylogenentic relationships between the sequences were analyzed by the neighbor-joining method with the software Molecular Evolutionary Genetics Analysis version 5, or MEGA 5 (Tamura et al., 2011).