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Experimental infection with bovine ephemeral fever virus and analysis of its antibody response cattle

F.Y. Zheng *, Q.W. Chen, Z. Li, X.W. Gong, J.D. Wang, H. Yin *

State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 1 Xujiaping, Yanchangbao, Lanzhou 730046, Gansu, China

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ABSTRACT

Bovine ephemeral fever (BEF) is an arthropod-borne viral disease that occurs throughout mainland China. LS11 obtained in the 2011 BEF epidemic was a wild strain, and its virulence and antibody response have never been studied in China. Therefore, the issues were investigated in this work. Experimental cattle were intravenously infected with different doses of BEF virus, and some non-infected cattle were simultaneously monitored. Blood and serum samples were collected from all animals over the course of our study. Infected cattle were challenged for a second time with BEF virus to determine protective period of the antibodies. BEF virus was detected in blood samples from infected cattle, but not in monitored cattle. The neutralizing antibodies (nAbs) against BEFV were easier to be detected and persisted for longer periods in cattle infected with higher doses of BEFV than in those infected with lower doses. When the titer of nAbs was equal to 5 or 6, re-infected cattle still could mount a challenge against BEFV. However, after 3 or 6 months, when nAbs were no longer apparent, re-infected cattle displayed typical symptoms of BEF. Our findings indicated that vaccination should be performed once the titer of nAb decreased to 5 or 6.

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1. Introduction

Bovine ephemeral fever (BEF), which is also known as three-day sickness, is an arthropod vector-borne viral disease of cattle and buffalo. BEF occurs in Australia, Asia and Africa and can have significant adverse economic impacts. Affected livestock show a decrease in the quantity and quality of their milk, with increased incidences of abortion, lameness and paralysis (Walker, 2005). The etiological agent of this disease is bovine ephemeral fever virus (BEFV) (Wunner et al., 1995; Hsieh et al., 2005).

Live attenuated and inactivated vaccines against BEF virus have been in use for many years, and the occurrence of BEF has been reduced over time (Tzipory and Spradbrow, 1973a, 1973b; Vanselow et al., 1985, 1995; Yan et al., 2000; Aziz-Boaron et al., 2013, 2014). However, vaccination strategies have never truly controlled the spread of BEF virus. In particular, the incidence of BEF outbreaks in mainland China, Taiwan, Israel and Turkey has increased in recent years. Many dairy cows have died in mainland China as the prevalence of BEF has increased over time (Aziz-Boaron et al., 2012; Zheng and Qiu, 2012; Tonbak et al., 2013; Ting et al., 2014).

BEF was first observed in mainland China in 1955, and its presence was confirmed in 1976 (Bai et al., 1991). BEF was identified in Taiwan in 1967 (Wang et al., 2001), and in Japan in the 1950s (Kato et al., 2009). According to a large-scale serological survey, BEF is prevalent throughout China (Li et al., 2015). Several serious outbreaks have been reported in Zhejiang, Henan, Jiangxi and Gansu provinces, and the affected animals consisted of dairy and beef cattle. Epidemics of BEF in Central China appear to occur every 5–7 years. Outbreaks on a small scale take place in Southern China almost every 2–3 years (Zheng and Qiu, 2012).

The inactivated BEF vaccine derived from the JB76H strain has been in use across mainland China since 1995 (Yan et al., 2000). Presently, the vaccine has been applied to a low number of herd, therefore the risk of BEF outbreaks and epizootics remains. Because the next outbreak cannot been accurately predicted after a BEF epidemic and anti-BEFV antibodies remain only approximately six months after vaccination (Jin, 2001), it is very difficult to ensure adequate cattle herd immunity between two BEF outbreaks. Cattle farmers need to know the exact inoculation time well in advance, so that they can vaccinate susceptible cattle populations to effectively prevent against BEF.

BEF virus LS11 was a wild virulent strain that was obtained during the 2011 BEF epidemic in Luoyang city of Henan Province (Zheng and Qiu, 2012). Virulence of the strain and its antibody response have never been studied in China. In the current study, we experimentally

* Corresponding authors.
E-mail addresses: zfycaas@126.com (F.Y. Zheng), yinhong@caas.cn (H. Yin).

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inoculated cattle with BEFV LS11 strain and analyzed the antibody response. The aim of these experiments was to determine the virulence of LS11 strain and the protective period of the antibodies.

2. Material and methods

2.1. Virus and experimental cattle

The LS11 strain of BEF virus was derived from blood samples of infected dairy cattle during the 2011 BEF epidemic, which occurred in Luoyang, Henan Province (Zheng and Qiu, 2012). Virus-containing blood samples were mixed with an anticoagulant and stored at −80 °C until required.

21 cattle (18-months-old) were used in animal experiments. The animals must be free for BEF virus and its antibodies. Thus, blood and sera samples of the cattle were collected prior to experiment. The presence of BEF virus was determined from the blood samples by reverse transcription polymerase chain reaction (RT-PCR) assay (Zheng et al., 2011), and anti-BEF virus antibodies were detected from the sera with micro-neutralization test (MNT) (Bai and Yan, 2002). The results indicated that BEF virus and its antibodies were absent in the experimental animals.

In addition, we must indicate that the experiment animals were treated well and protected from unnecessary pain and suffering, according to Institutional Animal Care and Use Committee (IACUC) for ethical considerations.

2.2. Determination of BEF virus titer

The titer of BEF virus LS11 strain in blood sample was determined using median lethal dose (LD50) in suckling mice, because newborn mice has a long history of use for BEF virus isolation (Van der Westhuizen, 1967). The blood containing-BEF virus was serially diluted ten-fold, and four dilutions including10^0, 10^{-1}, 10^{-2} and 10^{-3} were obtained. For each diluted sample, eight newborn mice (1–3 days old) were infected at dosage of 0.01 ml/mouse by intracerebral injection. The deadly mice during 5–10 days after infection were counted. LD50 was determined according to Reed–Meunch method. By calculation with Reed–Meunch method, BEF virus LS11 strain LD50 in suckling mice was $10^{-2.5}$/0.01 ml.

2.3. BEFV virus infection and sample collection

BEF virus infection was performed on 20 July 2013, when arthropod vector insects were very active. We randomly divided nine of the animals into three groups (Groups 1–3), with each group containing three cows. The animals in groups 1 (number A, B and C), 2 (number D, E and F) and 3 (number G, H and J) were intravenously infected with the blood including LS11 strain at doses of 2 ml/animal, 4 ml/animal and 8 ml/animal, respectively. The remaining 12 animals were used as sentinel cattle. Six of the twelve sentinel cows were housed with the experimentally infected cattle. The remaining six sentinel cattle were housed together in an adjacent stall, but separated from BEF virus-infected animals. For all the infected cattle and sentinel animals, serum and blood samples were collected every 24 h, over 15 days. After this 15-day period, serum samples were collected every month for a further 18 months. That is, 15 blood and 33 serum samples were collected from each animal.

2.4. Virus isolation

The isolation of BEF virus was carried out in the brains of suckling mice (1–3 days old) as previously reported (Zheng et al., 2009a, 2009b). Blood samples collected from the 9 infected animals and 12 sentinel cattle were inoculated into the brains of suckling mice, and then subjected to seven passages in suckling mice. Successful infection was confirmed if the suckling mice showed clinical signs.

2.5. RT-PCR assay

The presence of BEF virus was determined using RT-PCR as described previously (Zheng et al., 2011). RT-PCR assay had high sensitivity and specificity, and the limit of detection for the assay was 200 copies (Zheng et al., 2011). BEF virus RNA was extracted from blood and insect samples using a MiniBEST Viral RNA/DNA extraction kit (Takara, Dalian, China). Extracted RNA was converted into cDNA and then subjected to PCR using a PrimeScript™ One Step RT-PCR Kit. The thermal cycling conditions used have been described previously, with the addition of an initial step at 50 °C for 30 min.

2.6. Detection of antibodies against BEF virus using MNT and indirect enzyme-linked immunoassay (ELISA)

All serum samples derived from the infected animals and sentinel cattle were examined for the presence of antibodies against BEF virus using previously described MNT assay (Bai and Yan, 2002). Serum samples were diluted (1:2 to 1:4) with cell growth medium in a total volume of 25 μl/well, with each dilution performed in duplicate. Then, 100 $TCID_{50}$ BEF virus was added to each well with 25 μl/well and the mixtures were incubated at 37 °C for 60 min, following the addition of 3×10^4 BHK-21 cells to each well. Cultures were maintained for 5 days at 37 °C in 5% carbon dioxide. A cytopathic effect (CPE) was observed during 3–5 days. Samples were considered positive when cells in two wells (1:4 dilution) exhibited no CPE, or else the serum was negative. If the cells in only one of the two wells exhibited CPE, the serum was considered ambiguous.

The titer of neutralizing antibodies (nAbs) against BEF virus in positive serum samples was also determined. Each serum sample was serially diluted two-fold up to 1:64 in 96-well microtiter plates. The nAb titer was determined according to the method of Reed–Meunch (Bai and Yan, 2002).

Sera were also assessed using an indirect ELISA kit to detect antibodies against BEF virus (Li et al., 2015; Zheng et al., 2009a, 2009b). Serum samples were diluted (1:10), then dispensed (100 μl) into microtiter plate wells coated with antigen, and incubated at 37 °C for 30 min. Wells were then washed three times with PBS containing 0.01% Tween 20 (PBST), and 100 μl of diluted goat-anti-cattle horseradish peroxidase (HRP)-conjugated IgG was added to each well. Plates were incubated at 37 °C for 30 min, and washed three times with PBST. Substrate solution (100 μl) was added to each well and plates were incubated at 37 °C for 10 min in the dark. Color development reactions were stopped by adding 100 μl of termination solution. The absorbance (OD) of each well at 450 nm was then measured. Reference controls included positive, negative, and blank samples. The Ab% values were calculated using the following formula: $OD_{sample} - OD_{negative\ control} / OD_{positive\ control} - OD_{negative\ control}$. A serum sample was considered positive if Ab% was ≥0.3, and while a sample was considered negative if Ab% was ≤0.22. An Ab% value of 0.22–0.3 indicated a borderline sample.

The samples with discrepant results deriving from MNT and the indirect ELISA kit were tested again with MNT assay. The right results were accepted according to the MNT.

2.7. Determination of the protective period of BEF virus nAbs

When the BEF virus nAb titer was equal to 4 or 5, one of the three animals in Groups 1, 2 and 3 was re-infected with 400 LD50 BEFV LS11 strain. The remaining two animals in each group were re-infected when the nAb had decreased after three months and six months. Blood samples were collected from all cattle at 1–10 days post-infection (dpi), and the presence of BEF virus in the samples was determined virus isolation. We determined that the animals were

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