Journal of Integrative Agriculture 2017, 16(9): 2047–2054

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Evaluation of an attenuated vaccine candidate based on the genotype C of bovine parainfluenza virus type 3 in albino guinea pigs

MA Lei*, ZHU Yuan-mao*, YANG Ting, XUE Fei

Division of Livestock Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, P.R.China

Abstract

Bovine parainfluenza virus type 3 (BPIV3) is considered as one of the most important respiratory tract pathogens of both young and adult cattle, and widespread among cattle in the world. BPIV3 was first reported in China in 2008 and four strains of BPIV3 were isolated from Shandong Province, known as genotype C (BPIV3c). Pathogen investigations had shown that BPIV3c infection was very common among cattle in China. To date, BPIV3 can be classified into genotypes A, B and C based on genetic and phylogenetic analysis. Serological survey also demonstrates that BPIV3 infection is widespread in China, however, there is still no available vaccine for BPIV3 prevention in China nowadays. In the present study, the BPIV3c strain SD0835 was continuously passaged on Madin-Darby bovine kidney (MDBK) cells for hundreds of times, and the pathogenicity of passage 209 was reduced in guinea pigs. The passage 209 of BPIV3c strain SD0835 was used as a live vaccine candidate to immunize the guinea pigs. The vaccination results revealed that two vaccinations could induce excellent serum neutralizing antibody responses as well as proliferation of T lymphocytes. The vaccinated guinea pigs were well protected against challenge with a low passage of BPIV3c strain SD0835. Additionally, the percentages of $CD4^+$ and $CD8^+$ T cell subsets of animals in vaccinated group increased after immunization; T cell subsets on day 2 after challenge in both groups decreased, and the decline of $CD4^+$ and $CD8^+$ T cell subsets levels of four guinea pigs in vaccinated group was relatively moderate, comparing with that of the control group. These data support further testing of the attenuated virus as an effective candidate vaccine.

Keywords: bovine parainfluenza virus type 3, attenuated vaccine, genotype C, guinea pig

1. Introduction

Bovine parainfluenza virus type 3 (BPIV3) is recognized as one of the most important respiratory tract pathogens of both young and adult cattle and has been involved in the bovine respiratory disease syndrome (BRDC) development in cattle (Autio *et al*. 2007; Snowder *et al*. 2007). Recently, a high seropositivity rate for BPIV3 was detected by virus neutralization test in cattle in three provinces of Northern China and reached to 91%, which implicated that a very high

Received 17 February, 2017 Accepted 2 May, 2017
MA Lei, E-mail: nihaowsml2008@163.com; Correspondence XUE Fei, Tel: +86-451-51051740, Fax: +86-451-51997166, E-mail: fxue@hvri.ac.cn
* These authors contributed equally to this study.

doi: 10.1016/S2095-3119(17)61661-7

level of BPIV3 infection occurred in cattle in China (Huo *et al*. 2012). BRDC is commonly referred to as "Shipping fever". The classic clinical signs are nasal and ocular discharges, coughing, anorexia, pyrexia, dyspnea and sometimes diarrhea. BRDC is still a major health problem of cattle worldwide, and it is responsible for major economic losses in both beef and dairy industries (Babcock *et al*. 2010).

BPIV3 is distributed worldwide among cattle. It was reported that four of seven isolates of BPIV3 from Australia were genetically different from other three Australian isolates and the previously reported BPIV3 strains and classified as genotype B (BPIV3b), and the other three Australian isolates and the previously reported strains of BPIV3 were classified as genotype A (BPIV3a) in 2008 (Horwood *et al*. 2008). However, there was no report about the detection and isolation of BPIV3 in China before 2008. BPIV3 was first detected from nasal swabs collected from cattle with respiratory disease in Shandong Province of China and was successfully isolated using Madin-Darby bovine kidney (MDBK) cell cultures in 2008 (Zhu *et al*. 2011). Analysis on the genetic characteristics of the Chinese isolates of BPIV3 revealed that these strains were significantly different from the genotypes A and B of BPIV3, and were classified as genotype C (BPIV3c). Shortly after that report, the isolation of BPIV3c from cattle was reported in many countries, including Argentina, Korea, Japan, and the USA (Maidana *et al*. 2012; Oem *et al*. 2013; Konishi *et al*. 2014; Neill *et al*. 2015). Further investigations for pathogens showed that BPIV3c infections were very popular among cattle in China.

After the first isolation of BPIV3c strain SD0835, studies on pathogenesis of SD0835 were carried out in Balb/c mice and guinea pigs (Dong *et al*. 2012; Shi *et al*. 2014). The results showed that SD0835 was more pathogenic to albino guinea pigs than Balb/c mice and could cause a few clinical signs and gross pneumonic lesions in guinea pigs, which were similar to those observed in calves experimentally infected with BPIV3 field isolate (Bryson *et al*. 1979). These findings implicated that the guinea pig was a relatively ideal laboratory animal infection model to study pathogenesis and evaluate the effects of BPIV3 vaccines. Meanwhile the Chinese BPIV3c strain SD0835 was serially passaged in MDBK cells for about two hundreds of times to prepare an attenuated BPIV3c vaccine candidate. And the virulence of SD0835 passage 209 was found to be reduced in guinea pigs (data not shown). So far no commercial BPIV3 vaccines are available for cattle in China. And it is necessary to develop effective vaccines for prevention of BPIV3 infection in cattle in China. The present study is attempted to evaluate the efficacy of the serially passaged BPIV3 strain SD0835 (passage 209) as an attenuated vaccine candidate in guinea pigs.

2. Materials and methods

2.1. Cells and virus

MDBK cells were propagated in minimum essential medium (MEM, GIBCO) supplemented with 10% fetal bovine serum (FBS) (BIOCHROM AG, Germany) and kept at 37°C. The Chinese strain SD0835 of BPIV3c described previously (Zhu *et al*. 2011) was passaged serially in MDBK cell monolayers to prepare an attenuated BPIV3 strain. And BPIV3-F209 (passage 209) with a titer of 1 mL $10^{8.3}$ $TCID_{50}$ (50% tissue culture infective doses) was used as an attenuated BPIV3 vaccine strain candidate.

2.2. Animals and vaccination procedure

A total of 14 specific pathogen free (SPF), female albino guinea pigs (weighing about 200 g at the beginning of the experiment) were purchased from a commercial breeder, fed standard chow and water *ad libitum*, and housed under the controlled conditions in individually ventilated cages. The animals were randomly divided into two groups, and each group had seven guinea pigs. All experimental animal procedures were approved by the Office of Laboratory Animal Management of Heilongjiang Province, China.

The $TCID_{50}$ of BPIV3-F209 was adjusted to $10^{5.3}$ $TCID_{50}$ mL^{-1} by adding appropriate volume of MEM. Each guinea pig in vaccinated group was injected with 200 μL of the diluted virus suspension by intramuscular route into its foreleg and repeated after 3 weeks. The control animals were injected with the same volume of supernatant from the uninfected MDBK cells and repeated after 3 weeks.

2.3. Challenge

Three weeks after the second vaccination, all animals were challenged with a low passage (passage 2) of BPIV3 strain SD0835. All guinea pigs were anesthetized with 10% chloral hydrate by intraperitoneal injection and inoculated intranasally with $2\times10^{7.0}$ $TCID_{50}$ mL^{-1} in 200 μL volume of the low passage of SD0835.

2.4. Evaluation of clinical signs after vaccination and challenge and pathological examinations after challenge

Clinical monitoring of guinea pigs was performed throughout the study. Observations were made regarding the guinea pig's activity level, mental alertness, body condition, and clinical signs of respiratory disease. Rectal temperatures were recorded daily lasting for 1 week after the first and