Safety of inoculation of bovine parainfluenza virus 3 as potential vaccine vector in pigs

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Abstract Bovine parainfluenza virus 3 (BPIV3) is one of the most important respiratory pathogens in cattle. One BPIV3, named NM09, was isolated from cattle suffering from severe respiratory diseases in 2009. BPIV3 is a potential recombinant vaccine vector. To investigate whether NM09 can infect pigs and determine BPIV3 defense in these animals, BPIV3 antibody-free pigs were inoculated intramuscularly with the BPIV3 NM09 strain in a continuous passage. Clinical signs were observed each day after inoculation. Viral nucleic acid was detected in nasal and anal secretions. Results showed that virus-inoculated pigs displayed few observable clinical signs related to respiratory diseases. The antibody was identified, but the virus could not be detected in the second continuous passage in pigs. Thus, BPIV3 is a potential vaccine vector for genetic engineering.

Keywords Bovine parainfluenza virus · Potential vaccine vector · Safety

Bovine parainfluenza virus 3 (BPIV3), which belongs to the family *Paramyxoviridae*, is an enveloped and non-segmented negative-strand RNA virus. BPIV3 infection in cattle can gradually cause many clinical signs, such as coughing, pyrexia, anorexia, dyspnea, and nasal and ocular discharges. BPIV3 contains six genes, namely, N, P, L, F,

M, and A. These genes encode nucleocapsid protein (NP), phosphoprotein, large polymerase proteins, matrix, fusion, and attachment [1]. BPIV3 viruses can be classified as genotypes A, B, and C [5, 7, 13] based on phylogenetic analysis.

In various animals, the virulence of parainfluenza virus is weak because of the nature of host range restriction. Therefore, attenuated parainfluenza virus can be a carrier of expressing heterologous virus protective antigen. This virus maintains the balance between reduced virulence and immunogenicity within a specific immune group.

BPIV3 may also be a recombinant vaccine vector [1]. As a carrier, parainfluenza virus can efficiently participate in cell proliferation. Simultaneously, parainfluenza virus can effectively infect through the nasal cavity to induce local IgA and IgG antibody immune response, as well as the cell-mediated protective immune response. Vaccine inoculation can be simple, safe, and rapid in the nasal cavity during disease outbreak or epidemic. Nasal vaccine for viral infection and spread through the respiratory tract is particularly effective. As a carrier, parainfluenza virus only proliferates in the cytoplasm and does not integrate into the host cell genome. Thus, it will not result in cell transformation. The genome of parainfluenza virus is composed of multiple overlapping genes with a single unit of different mRNA expression patterns. Therefore, this virus constitutes an adjustable genome structure, which is convenient for exogenous gene insertion and maintenance of stability of the exogenous gene [10].

Previous research showed that the attenuated phenotypes, namely, recombinant BPIV3 and bovine/human PIV3, can protect hamsters completely against hPIV3 [3]. The function of BPIV3 as a human virus vaccine vector was explored by introducing the RSV attachment (G) and fusion (F) genes into the BPIV3 RNA genome. BPIV3

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recombines the RSV G and F proteins, which allow hamsters to generate RSV neutralizing and PIV3 hemagglutinin inhibition serum antibodies, thereby protecting the animals against attacks of virus RSV and human PIV3 [4]. A chimeric bovine-human parainfluenza virus type 3 vaccines rB/HPIV3 could induce significantly higher titers of hemagglutination inhibition (HAI) antibodies against HPIV3 [6]. In future, BPIV3 will be as a recombinant carrier to prevent a variety of pig disease. The infectivity of BPIV3 in pigs should be determined in consideration of future recombinant vaccine for pigs.

One BPIV3, named NM09, was isolated in 2009, and this strain can cause the bovine respiratory disease complex (BRDC). The phylogenetic analysis demonstrated that this NM09 isolate belonged to BPIV3 genotype A [12]. Recently, a recombinant BPIV3 expressing enhanced green fluorescent protein (rBPIV3-EGFP) was found to infect the epithelial cells in the nasal turbinates and trachea of hamsters [8]. To investigate whether NM09 can infect pigs and determine BPIV3 defense in these animals, we conducted a continuous passage of the NM09 strain in pigs. Infectivity was confirmed by pathogenic nucleic acid detection. Safety was demonstrated by temperature, respiratory symptoms, clinical signs, and histopathological changes.

Twelve healthy and BPIV3 antibody-free pigs, four to 5 weeks of age, were used in this study. All the animals were also tested for *porcine reproductive and respiratory syndrome virus* (PRRSV), *pseudorabies virus* (PRV), *porcine circovirus 2* (PCV2), and *classical swine fever virus* (CSFV) infections by RT-PCR. The pigs were bred in biological safety level 2 (BSL2) facilities at the Jilin Veterinary Biologicals 2–4 days before BPIV3 inoculation. Six animals each were used for first- and second-passage inoculations. The six piglets were randomly divided into two groups: three were inoculated with BPIV3, and others were used as controls. We inoculated BPIV3 antibody-free pigs with BPIV3 NM09 strain, of which the titer was $10^{5.0}$ 50 % tissue culture infective dose (TCID₅₀). Vaccination was performed intramuscularly (i.m., 1 ml) and intranasally (i.n., 1 ml). Nasal and anal swabs were obtained at each day post-inoculation (dpi) for RT-PCR. Serum was collected to detect the antibody against BPIV3 using an ELISA kit. Temperature was monitored at each dpi. Positive nasal and anal swabs were mixed for second-passage inoculation. Finally, the animal tissues were fixed for conventional histopathology and H&E staining.

RT-PCR for inoculated samples from first-passage pigs showed that many NP genes for BPIV3 were amplified using primers specific to the unique gene fragments. By contrast, no NP genes were detected from second-passage pigs (Fig. 1). The results indicated that the virus could not be detected in the second passage but only in the

first-passage pigs (Table 1). Except at 5–11 dpi, the viral nucleic acid was detected in pigs inoculated with BPIV3 NM09. The antibody was detected at 7 dpi until the end of the experiment. The antibody titer was up to 8. After inoculation, the animals did not show any severe respiratory symptoms, except mild depression for the first 2 days. The temperature was normal. The mean body mass was almost equal to that of the controls, and the difference was not significant. The histopathological results showed that the virus could not cause tissue damage to animals (data not shown).

Two strains of swine PIV3, namely, 81-19252 and 92-7783, were isolated from pig brains in a study in the United States. These viruses may be derived from BPIV3 [9]. Although these two swine PIV3 strains do not cause pig diseases, they can be possibly transferred from cattle to pigs. These strains are variants of BPIV3. In the current study, BPIV3 NM09 was artificially inoculated in pigs. The virus could replicate in pigs and be excreted from the body. However, progeny viruses did not seem infectious. Moreover, the viral gene was not detected in the second-passage inoculation pigs.

Fig. 1 Results of clinical temperature and viral nucleic acids by RT-PCR. **a** Temperature of pigs infected with BPIV3 NM09 and mock pigs during first-passage inoculation. **b** RT-PCR detection of inoculated pigs with BPIV3 NM09 at 1–14 days of inoculation. Samples from three pigs each day were lined together. Detection information is also shown in Table 1. (+) positive control. M: DNA Marker DL2000 (Takara)

Table 1 Detection of BPIV3 in inoculated pigs by RT-PCR and antibody titer in serum of pigs

Days post-infection Samples		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Antigen detection															
Nasal swab	First-passage inoculation	2/3	1/3	2/3	2/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	2/3
Anal swab		2/3	3/3	2/3	3/3	3/3	1/3	2/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3
Nasal swab	Second-passage inoculation	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Anal swab		0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Antibody titer															
	First-passage inoculation	0	0	0	0	0	0	2	4	4	8	8	8	16	16
	Second-passage inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Although several paramyxoviruses have been isolated from pigs in the United States and other countries, almost none of these viruses are extremely virulent and infectious across species [2]. ISU92-inoculated pigs exhibited mild clinical illness with symptoms including mild diarrhea and rectal temperatures slightly elevated at 2 and 3 dpi. Our comprehensive studies revealed that a similar phenomenon occurred in NM09-inoculated pigs. This finding indicated that NM09 was a potential host-specific change vector. Previous research suggested that host-specific amino acids of F, HN, P, and L proteins contain amino acid mutations between BPIV3 and swine PIV3 [9]. The corresponding amino acids are specific for swine PIV3, which may show changes for adapting to the new host. In the NM09 genes, the host-specific amino acids exhibited few changes. Only some amino acids were different, such as those of P protein (K/T271F, P/S473L) and L protein (S/T1355N). Nevertheless, BPIV3 NM09 could infect pigs and replicate in these animals, thereby introducing high levels of the antibody. This feature is beneficial for a potential vaccine vector.

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