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From mouse to pig: Is PRV vaccine safe across two species?

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ABSTRACT

Pseudorabies (PR) is an economically important viral disease of pigs which can infect numerous species of mammals including rodents. Commercial PR vaccines have been widely used worldwide to control and eradicate this disease. However, some PRV vaccines such as Bartha-K61 were occasionally reported to be lethal to mice. Since mice are commonly found in pig farms, the safety issue of PRV live vaccine across different species was never addressed. In this study, PRV vaccine strain Bartha-K61 was *in vivo* propagated in mice for five passages. The mortality of mice ranged from 80%-100% at each passage of PRV infection. The fifth passage of PRV was used to infect piglets to test its virulence on this species. The infected piglets clinically behaved normally and survived by the end of study (terminated at 10 days post-infection). Histopathologically, there was infiltration of eosinophile granulocyte in tonsil and lung and no other changes were observed in other organs of infected pigs. Immunohistochemistry staining results showed that PRV antigen was only found in lung sample of one piglet. Therefore, the above results suggested there was no safety concern of Bartha-K61 PRV vaccine on pigs after the vaccine virus was passaged in mice for 5 times. The result of this study may suggest that mice may play a minimal role in the derivation of PRV vaccine-like field viruses that are believed to cause disease in young pigs.

1. Introduction

Pseudoraibies (PR) is an economically important viral disease of pigs worldwide. The causative agent of this disease is pseudorabies virus (PRV), which belong to genus *Varicellovirus* of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae* (Wittmann and Rziha, 1989). Pig is the natural host of PRV and is more sensitive to virus infection. The infected pig shows central nervous system disorders and respiratory symptoms with high mortality. The modified live PRV DIVA (differentiating infected from vaccinated animals) vaccine with nonessential gE gene deletion has been widely used worldwide to control the disease (Wang et al., 2014). Combined with some commercial serological diagnostic kits such as PRV gE-antibody detection ELISA kit, some North American and European countries have successfully eradicated PRV from domestic pig population (Muller et al., 2011). However, PR still remains as one of the most important pig viral pathogens that seriously threatened local pig industry in China. Since 2011, the outbreaks of PRV variants in Bartha-K61 vaccinated pig herds have been constantly reported, and the origin of these variant viruses remains to be addressed (An et al., 2013; Luo et al., 2014).

PRV can infect many species of mammals including ruminants, carnivores, and rodents. The virulence-determined genes of PRV may vary according to different species (Mettenleiter, 1996). The vaccine strain PRV Bartha-K61 has been successfully attenuated by extensively passaging on porcine kidney cells, chicken embryos and embryo fibroblast cells and proved to be safe on pigs. However, Bartha-K61was reported to be lethal to other species such as mice, rabbits, and goats since the intact wild-type TK gene remained in vaccine virus genome may work as virulence-determined gene on these animal species (Brittle et al., 2004; Kim et al., 1999; Luo et al., 2014). Due to heavy density of pigs and poor biosecurity measurements in Chinese pig farms, the rodents such as mice can be found everywhere and may play an important role in transmission and/or evolution of PRV since mice can be infected and replicate the virus serving as a potential source of infection for pigs. Therefore, in this study, we explored the safety issue of Bartha-K61 PRV vaccines on pigs after several rounds of virus passaging *in vivo* in mice.

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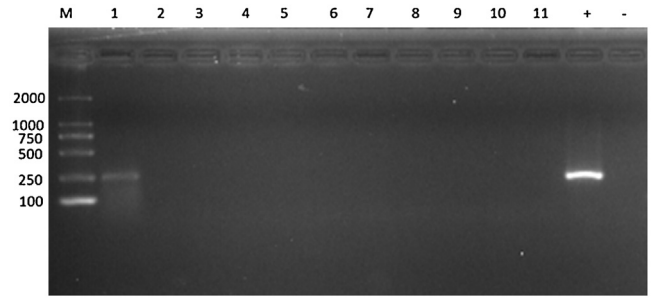

Fig. 1. Viral DNA detection in different organs of mice by using PCR. Total DNA was extracted from brain (1), heart (2), liver (3), spleen (4), lung (5), kidney (6), bladder (7), ovarian ducts (8), adrenal gland (9), mandibular lymph node (10), and inguinal lymph node (11) of infected mice and was used as template for PCR amplification. "+" and "−" indicate the positive and negative control. "M" indicates DNA ladder DL2000.

2. Materials and methods

2.1. Virus and cells

PRV Bartha-K61vaccine was purchased from Merial Animal Health (Lyon, France). Propagation of PRV strains was performed by utilizing PK15 pig kidney cells as previously described (Zhang et al., 2015a).

2.2. Experimental infection of mice

Twenty 6-week-old specific-pathogen-free (SPF) BALB/c mice were randomly divided into two groups. Mice in group 1 were intranasally inoculated with 50 μl 1×10^5 $TCID_{50}$/mL of PRV Bartha-K61. Mice in group 2 were injected with DMEM served as uninfected controls. Clinical signs of mice were observed daily after viral infection. The moribund and survived mice (experiment was terminated at 10dpi) were humanely euthanized and the organs including brain, heart, liver, kidneys, lung, spleen, ovaries, urinary bladder, and lymph nodes were used for virus detection by PCR. Brain samples were pooled and homogenized for virus titration on PK-15 cells. For the next round of mice infection, 50 μl 1×10^5 $TCID_{50}$/mL virus from pooled supernatant of Bartha-K61-infected brain homogenate was used. Twenty mice were used for each of another two rounds of virus infection. Thirty-five and 80 mice were used for the 4th and 5th rounds of virus infection in which 10 mice were used as uninfected control (Table 1).

Table 1
Mortality of mice after PRV infection.

Round	Number of mouse died at							In total	Virus titer ($TCID_{50}$/ml)
	5dpi	6dpi	7dpi	8dpi	9dpi	10dpi	11dpi		
1st	0	0	2	6	0	0	0	8/10	$10^{5.41}$
2nd	0	0	0	0	4	0	4	8/10	$10^{5.88}$
3rd	8	0	0	0	2	0	0	10/10	$10^{5.2}$
4th	1	4	11	5	4	0	0	25/25	$10^{5.29}$
5th	6	29	19	13	3	0	0	70/70	$10^{5.57}$

2.3. Experimental infection of piglets

Fifteen two-week-old piglets were randomly divided into three groups. Pigs in group 1 and 2 were intranasally inoculated with 2 ml of 1×10^5 $TCID_{50}$ Bartha-K61 vaccine or mice-origin Bartha-K61 (mBartha) brain homogenates. Piglets in group 3 were inoculated with DMEM serving as uninfected controls. After virus infection, rectal temperature and clinical signs were recorded on a daily basis. All piglets were euthanized by the end of study which was terminated at 10dpi. The above mice and pig animal trials were approved by the Animal Care and Ethics Committee of China National Research Center for Veterinary Medicine.

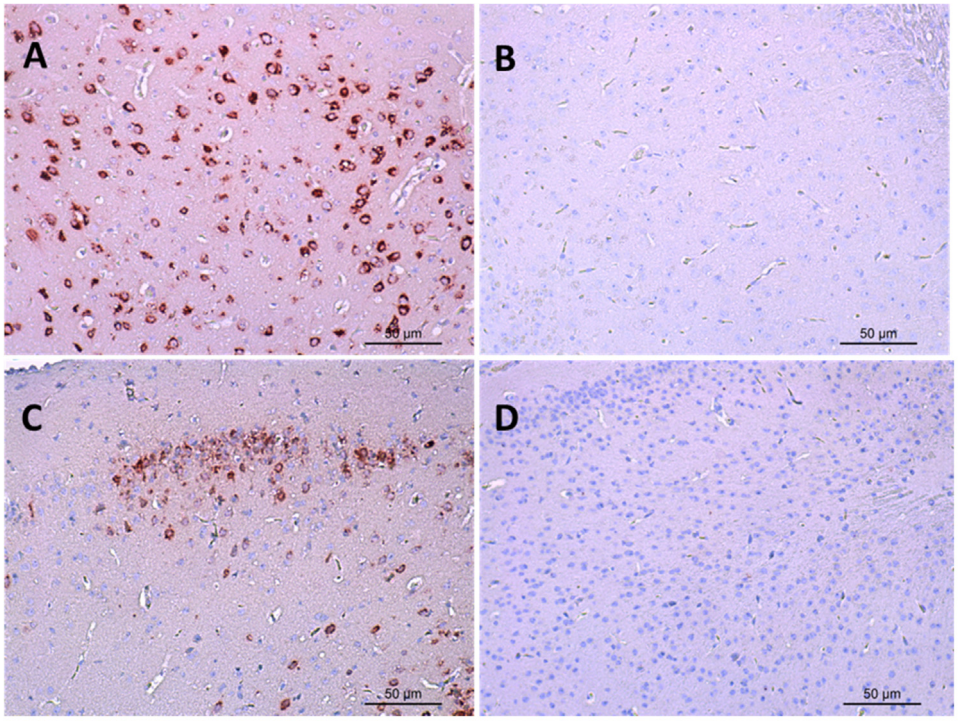

Fig. 2. Viral Antigen detection in different organs of mice by using IHC. Positive staining in brain samples of PRV-infected mice (A, C). Brain samples of non-infected mice (B, D).