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Investigation on host susceptibility of Tibetan pig to infection of porcine reproductive and respiratory syndrome virus through viral challenge study

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ABSTRACT

Previous reports showed that infection of porcine reproductive and respiratory syndrome virus (PRRSV) stimulated a variable host response and pig susceptibility to PRRSV was largely dependent on its genetic composition. In the present study, host susceptibility of Tibetan pig to PRRSV was compared with other two pig breeds, ZangMei black and Large White, by challenge of them with highly pathogenic PRRSV (HP-PRRSV). In the first challenge test, each eight piglets of the three breeds were inoculated with HP-PRRSV and clinical symptoms, viremia and animal mortality were examined up to 28 days post inoculation (DPI). In the secondary pathological study, each twelve piglets of the three breeds were challenged and three pigs of each breed were sacrificed on 4, 7, and 14 DPI for examination of gross damage and lung microscopic lesions. The results showed that no typical clinical signs such as cough, diarrhea and high fever were observed in challenged Tibetan pigs, which however all occurred in Large White accompanied with ~40% mortality (3/8). In addition, a significant low and short viremia was detected specifically in Tibetan pigs. Based on histopathological analysis of lung sections, a mild to moderate interstitial pneumonia in Tibetan pigs and a much severe pneumonia in Large White were identified on 7–14 DPI. In summary, the study demonstrated that three genetically different pig breeds exhibited a differential host susceptibility to HP-PRRSV and Tibetan pig was much less susceptible to the virus in the three tested pig breeds.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by respiratory problems in young pigs and late-term reproductive failure in sows, causing severe economic loss in pig production industry worldwide. The etiological agent of PRRS is an RNA virus referred as PRRS virus (PRRSV) identified in early 1990 s with two distinct genotypes, European genotype 1 and North American genotype 2 (Wensvoort et al., 1991; Benfield et al., 1992; Collins et al., 1992). In 2006, a highly pathogenic PRRSV variant (HP-PRRSV) of North American genotype that caused high fever, respiratory distress and high rates of morbidity and mortality in growing pigs emerged in several provinces of China (Li et al., 2007; Tian et al., 2007; Tong et al., 2007). Later HP-PRRSV was reported in Vietnam, Thailand, Laos, South Korea and some other countries of Asia (Feng et al., 2008; Metwally et al., 2010; An et al., 2011; Ni et al., 2012). Since the first outbreak of 2006, HP-PRRSV has been investigated intensively and it was recognized that HP-PRRSV is different substantially from the classic PRRSV in several aspects, such as the infection involved in pigs of all ages with much more severe disease and aberrant immune response (Xiao et al., 2010; Han et al., 2014), an expanded tissue tropism *in vivo* (Li et al., 2012) and a high occurrence of bacterial co-infection (Guo et al., 2013).

Although PRRS virus infects domestic and feral pigs of *Sus scrofa* naturally (OIE, 2008), the host susceptibility to the virus is variable in pig breeds (Lunney et al., 2011; Rowland et al., 2013). Pigs challenged with PRRSV displayed variable clinical signs (Petry et al., 2005; Vincent et al., 2006; Reiner et al., 2010), different gross or microscopic lung lesions (Halbur et al., 1998) and divergent expression of immune response genes (Petry et al., 2007; Arceo

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et al., 2013) according to pig breeding lines. The investigation on host susceptibility to highly pathogenic PRRSV showed that most of the commercial Western lean type pig breeds were susceptible to HP-PRRSV, but a variable host susceptibility to HP-PRRSV was observed in some Chinese indigenous pigs. Dapulian pig (DPL), for example, was reported to be resistant to HP-PRRSV infection (Jiang et al., 2013). The other two Chinese indigenous pigs, the hybrid wild boar (Wu et al., 2011) and Tongcheng pigs (Zhou et al., 2011), had either a lower frequency of infection in natural conditions or a mild lung lesion post challenge, though both pig breeds were susceptible to HP-PRRSV. These data suggested that genetic difference may play a role in swine resistance/tolerance to HP-PRRSV.

Tibetan pig is a unique swine breed descended recently from wild boars originally found on the Tibetan plateau. Living in cold and harsh environment on the plateau for long time, Tibetan pigs have undergone a specific selection to enrich the disease resistance-related gene categories in their genome (Li et al., 2013). Previous observation on host susceptibility to PRRSV showed that Tibetan pigs reared in the conservation farm did not exhibit any typical clinical symptoms of PRRS, even in HP-PRRSV pandemic and the natural transmission existed among different pig breeds on the same farm (unpublished data). However, the host resistance or susceptibility of Tibetan pig to PRRSV was not yet determined experimentally. In the present study, the host susceptibility of Tibetan pig to HP-PRRSV was evaluated by artificial inoculation of the virus. The experimental data on clinical features, microscopic lung lesions and viral load in blood post challenge suggest that Tibetan pig is less susceptible to HP-PRRSV in the three pig breeds.

2. Materials and Methods

2.1. Virus and animals

HP-PRRSV JXA1 isolate (GenBank: JN836553.1) was identified from an intensive pig farm in South-western China in 2009 (Tian et al., 2007; Zhou et al., 2012) and the virus used in the study was propagated in Marc-145 cells less than five passages.

Healthy 4–6 week piglets of three breeds including Tibetan pig (Tib), ZangMei black (ZM) and Large White (LW) were provided by the conservation farm of Tibetan pigs, Sichuan Animal Science Academy. ZangMei black® is a registered breed selected from the descendants of multiple crosses between Tibetan pigs and Meishan pigs. Virus challenge experiments were carried out in Laboratory Animal Center of West China Hospital of Sichuan University under the approval and supervision of IACUC/AEC. The piglets were demonstrated to be free of PRRSV, porcine circovirus type 2 (PCV2) and classical swine fever virus (CSFV) by PCR or RT-PCR before sending to the laboratory animal center and were housed in separate and air-conditioned rooms. Animals were fed and treated in accordance with the Guidelines of Animal Management of Sichuan University.

2.2. Experimental design

Animal test was composed with two parts. In the first challenge part, each eight piglets of Tibetan pig, ZangMei black and Large White were intramuscularly inoculated with 1 ml of 4×10^5 $TCID_{50}$/ml HP-PRRSV JXA1 isolate virus per piglet on day 0. Clinical signs, pig death and the rectal temperature were examined daily, body weight was measured weekly, and individual blood samples were collected on −1, 4, 7, 14, 21 and 28 days post inoculation (DPI). Pigs were euthanized at 28 DPI except the dead ones. In the secondary pathological study part, each twelve piglets of the three pig breeds were inoculated with the same dose of the virus as in the challenge test. On 4, 7 and 14 DPI, three pigs of each breed were sacrificed for sampling of lung tissue and gross pathology examination. The rest pigs were euthanized at 14 DPI. Control lung tissue was collected on day 0 from three sacrificed piglets of each breed, which were inoculated intramuscularly with 1 ml of Marc-145 cell culture supernatant on day −1.

2.3. Histopathological examination

Lung tissue samples were collected at 0, 4, 7 and 14 DPI from each lobe of left lung and immediately fixed in 10% neutral buffered formalin. The fixed lung were dehydrated, embedded in paraffin and sectioned into 4 μm. Four sections of each pig were stained with hematoxylin and eosin (H & E) for histopathological evaluation of lung lesions. One representative section was selected from each time point of three pigs and shown in Fig. 3.

2.4. Determination of viral load by quantitative RT-PCR

Total RNA was extracted from pig sera by using RNAiso Plus (TaKaRa, China) and retro-transcribed into cDNA with PrimeScript™ RT reagent Kit (TaKaRa, China). The real-time quantitative RT-PCR (RT-qPCR) was performed on Multicolor Real-Time PCR Detection System IQ5 (Bio-Rad, USA) using real-time PCR assay kit (SYBR® Premix EX Taq™ II, TaKaRa, China) and specific primers of Nsp2 (Table 1). A 10-fold dilution series of Nsp2-cloned plasmid were used to construct the standard curve for absolute quantification of viral copies in serum samples.

2.5. Quantitative detection of cytokine-gene expression

Blood total RNA was extracted and retro-transcribed to cDNA as described in Section 2.4 above. The specific primers for IFN-α, IFN-γ, TNF-α, IL-2, IL-10 and IL-4 (Table 1) were used to quantitatively characterize their expression in pig's blood at different time points post virus inoculation. The conditions for RT-qPCR were 95 °C for 3 min, followed by 40 cycles of 95 °C for 6 s, optimal annealing temperature (55–60 °C) for 9 s, and 72 °C for 10 s. TBP (TATA-box binding protein) was used as reference gene (Uddin et al., 2011) and the relative expression of the six cytokines was analyzed according to the $2^{-\Delta\Delta Ct}$ method (Liver and Schmittgen, 2001). The means of paired groups were analyzed by a paired Duncan's *t* -test using SPSS 19.0.

3. Results

3.1. Clinical features of PRRS-challenged piglets

After challenge, clinical signs of anorexia, coughing, sneezing and diarrhea began to appear in the affected LW and ZM piglets within 2–3 DPI and more severe symptoms such as increased respiratory rates, shivering and hyperspasmia exhibited within 6–8 DPI in the affected Large White but not in ZangMei black. Three from eight challenged LW piglets died on 9, 11 and 13 DPI, respectively. The challenged

Table 1
Primers used in RT-qPCR.

Gene	Forward primer	Reverse primer
Nsp2	ccggatggtgaattccaact	agcacgtacttggcgctac
IFN-γ	gaagaattggaaagaggagagtgac	tccatgctcctttgaatggc
IL-2	agctctggagggagtgctaa	tgtttcagatccctttagttcca
IL-10	atgggcgacttgttgctgac	cagggcagaaattgatgacag
IL-4	gctgccccagagaacacgac	aggttcctgtcaagtccgctc
TNF-α	cccacgttgtagccaatgtc	gaggtacagcccatctgtcg
IFN-α1	tcagctgcaatgccatctg	gaagggagagattctcctcat