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NOTE

Bacteriology

Genetic variants and phylogenetic analysis of *Haemophilus parasuis* (HPS) *OMPP2* detected in Sichuan, China from 2013 to 2015

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ABSTRACT. To investigate the genetic variation in *Haemophilus parasuis* (HPS) in Sichuan, China, 11 isolates were analyzed based on the outer membrane protein P2 (*OMPP2*) sequence. Sequence analysis showed that the 11 isolates shared 93.0 to 100% nucleotide homology with 15 reference strains, and the consistency between the 26 strains was 89.0%. The isolates of HPS-1, 2, 4, 5, 6, 7, 8, 10 and 11 had a 69-base deletion from 770 base pairs (bp) to 850 bp, which was infrequent in China. The phylogenetic tree showed that HPS-3 and HPS-8 had closer relationships with European and Japanese strains, but shared 98.7% nucleotide homology with the SW114 Japanese strain.

KEY WORDS: *haemophilus parasuis*, outer membrane protein, sequence analysis

Haemophilus parasuis (HPS) is a commensal colonizer of the porcine upper respiratory tract, and it causes high morbidity and mortality in all age groups of pigs [1, 4]. HPS has at least 15 confirmed serotypes and others that are non-typeable. The main serotypes in China are 4, 5, 13, 12 and 14 [2]. The outer membrane is an important structure of gram-negative bacteria, and *OMPP2*, which was confirmed to be a virulence factor of HPS, is a member of the porin family [5, 7]. *OMPP2* is diverse and easy to mutate and the *OMPP2* genes could be divided into 2 genotypes, which were named as genetic type-I and type-II [3]. Recently, *OMPP2* was found to induce proinflammatory cytokine mRNA expression in porcine alveolar macrophages, suggesting that *OMPP2* may play an important role in the pathogenesis of disease caused by HPS [6]. In summary, the sequence analysis of HPS *OMPP2* may provide new information regarding gene mutations that could be useful for vaccine research, pathogenesis, and heritable variation of HPS.

The 11 HPS isolates were provided by the Animal Quarantine Laboratory, Sichuan Agricultural University (Table 1). A pair of primers was designed based on the published GenBank sequence (FJ685756.1). The sense primer was 5′-CGGGGTACCATGAAAAAAACACTAG-3′ and the antisense primer was 5′-CGCGGATCCTTACCATAATACAC-3′. PCR was performed in a 25-μl mixture containing 12.5 μl of 2× Master Mix, 2 μl of 10 μM sense and antisense primers, 2 μl template, and 6.5 μl ddH_2O. The PCR was carried out as follows: 94°C for 5 min, followed by 30 cycles of denaturing at 94°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 1 min, and a final elongation was performed at 72°C for 10 min. The *OMPP2* recombinant plasmid was confirmed by DNA sequencing (Life Technology Inc., Shanghai, China). Sequences of the 11 isolates were analyzed, by comparing them with those of 15 reference strains using DNASTAR software and the Clustal W algorithm of MegAlign.

The HPS *OMPP2* was amplified and a 1,071-bp fragment was separated via agarose gel electrophoresis. The results of the analysis showed that the 26 sequences, including 11 isolates and serotypes 1–15 of the reference strains, shared 93.0 to 100.0%, 86.3 to 100.0% and 89.00% nucleotide sequence homology, amino acid homology, and nucleotide sequence consistency, respectively. HPS-2, 3, 4, 5 and 8 had the highest homology with Japanese strains SW140 and SW114, with nucleotide and amino acid sequence homology at 98.7 to 99.2% and 96.5 to 97.8%, respectively. HPS-1, 7, 9 and 10 had the highest homology with the 84-22113 U.S.A. strain, with greater than 99.0% nucleotide and amino acid sequence homology. Sequence analysis showed two main differences between isolates and reference strains. In the 430 to 490-bp region of HPS *OMPP2*, HPS-1, 6, 7, 9, 10 and 11 had a 31-bp deletion, but HPS-2, 4 and 5 were only missing 25 bases. Furthermore, in the 770 to 850-bp region of HPS *OMPP2*,

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Table 1. HPS strain summary

Name	Location	Year	Isolation site	Serovar	GenBank
HPS-1	Suining	2013	Lung	4	KU375454
HPS-2	Pujiang	2013	Lung	4	KU508597
HPS-3	Suining	2013	Lung	5	KU508598
HPS-4	Nanchong	2014	Lung	10	KU508599
HPS-5	Mianyang	2014	Lung	Unknown	KU508600
HPS-6	Guangan	2014	Lung	Unknown	KU508601
HPS-7	Meishan	2014	Lung	4	KU508602
HPS-8	Suining	2014	Lung	Unknown	KU508603
HPS-9	Pujiang	2014	Joint	12	KU508604
HPS-10	Xichang	2015	Joint	13	KU508605
HPS-11	Pixian	2015	Joint	Unknown	KU508606

Table 2. *OMPP2* nucleotide and amino acid sequence homology across strains

	HPS-1	HPS-2	HPS-3	HPS-4	HPS-5	HPS-6	HPS-7	HPS-8	HPS-9	HPS-10	HPS-11	No.4	SH0165	SW114	SW124	SW140	84-15995	84-17957	84-22113	131	174	C5	D74	F042	H425	H465
HPS-1		96.2	93.8	96	96	97.6	99.9	93.8	99.6	99.7	97.9	98.1	99	93.6	97.3	96.8	98.1	97.5	97.9	94.1	99	94	93.3	97.2	99.7	95.9
HPS-2	91.1		93.5	99.3	99.3	97.8	96.3	93.5	96	96.1	97.9	95.9	96.2	93.2	97.7	99.2	97	97.6	97.9	93.4	96.4	93.3	93	97.3	96.5	94.1
HPS-3	89.7	88.3		93.3	94	94	93.9	99.8	93.7	93.8	93.9	95.2	93.1	98.7	93.4	94	94	93.8	93.9	97.6	94.3	98.2	97.8	93.5	94.1	97.2
HPS-4	90.8	98.6	88		99.1	97.6	96.1	93.3	95.8	95.9	97.7	95.7	96	93	97.5	99	96.9	97.4	97.7	93.2	96.2	93.1	92.8	97.1	96.3	93.9
HPS-5	90.3	98.1	89.4	97.8		98	96.1	94	95.8	95.9	97.7	95.9	96	93.7	97.5	99.2	96.9	97.4	97.7	93.7	96.2	93.6	93.4	97.1	96.3	94.4
HPS-6	94.4	94.4	89.4	94.2	94.7		97.7	94	97.4	97.5	99.7	97.6	97.4	93.8	98.7	97.9	98.6	99.4	99.7	94.1	97.4	94	93.5	99.1	97.9	95
HPS-7	99.7	91.4	89.9	91.1	90.5	94.7		93.9	99.7	99.8	98	98.1	99.1	93.7	97.4	96.9	98.1	97.6	98	94.2	99.1	94.1	93.4	97.3	99.8	96
HPS-8	89.7	88.3	99.5	88	89.4	89.4	89.9		93.7	93.8	93.9	95.2	93.1	98.7	93.4	94	94	93.8	93.9	97.6	94.3	98.2	97.8	93.5	94.1	97.2
HPS-9	99.4	91.1	89.7	90.8	90.3	94.4	99.7	89.7		99.5	97.7	97.9	98.8	93.4	97.1	96.7	97.9	97.3	97.7	93.9	98.8	93.9	93.1	97	99.5	95.7
HPS-10	99.7	91.4	89.9	91.1	90.5	94.7	100	89.9	99.7		97.8	98	98.9	93.5	97.2	96.8	98	97.4	97.8	94	98.9	93.9	93.2	97.1	99.6	95.8
HPS-11	95.3	94.7	89.1	94.4	93.9	99.2	95.5	89.1	95.3	95.5		97.7	97.7	93.7	99	97.8	98.9	99.6	100	94.2	97.7	94.1	93.4	99.4	98.1	95.1
No.4	96.7	91.1	91.6	90.8	90.8	95.3	96.9	91.6	96.7	96.9	95.5		97.6	94.9	97.8	96.4	97.9	97.3	97.7	95.4	98.3	95.3	94.8	97	98.3	95.6
SH0165	97.8	91.6	89.3	91.4	90.8	94.2	98.1	89.3	97.8	98.1	95	95.5		92.7	97.4	96.7	98.1	97.5	97.7	93.4	98.3	93.2	92.5	97.2	99.1	95.1
SW114	89.1	87.2	96.5	86.9	88.3	88.8	89.4	96.5	89.1	89.4	88.5	91.1	88.7		93.1	93.7	93.8	93.5	93.7	97.8	94	98.8	98.9	93.2	93.8	97.1
SW124	94.4	94.7	89.4	94.4	93.9	97.2	94.7	89.4	94.4	94.7	98.1	95.5	95	88.8		97.4	98.4	99	99	93.7	97.3	93.6	93	98.7	97.6	94.7
SW140	92.5	98.1	89.7	97.8	97.8	94.7	92.8	89.7	92.5	92.8	94.4	92.5	92.5	88.5	93.9		97.5	97.5	97.8	93.7	97	93.6	93.4	97.2	97.1	94.4
84-15995	95.5	92.8	89.9	92.5	91.9	96.9	95.8	89.9	95.5	95.8	97.8	95.8	95.5	89.4	96.9	94.2		99.1	98.9	94.3	97.9	94.2	93.5	98.8	98.3	95.2
84-17957	94.2	93.9	88.8	93.6	93.1	98.1	94.4	88.8	94.2	94.4	98.9	94.4	94.4	88.3	98.1	93.6	98.3		99.6	94	97.3	93.9	93.2	99.5	97.8	94.9
84-22113	95.3	94.7	89.1	94.4	93.9	99.2	95.5	89.1	95.3	95.5	100	95.5	95	88.5	98.1	94.4	97.8	98.9		94.2	97.7	94.1	93.4	99.4	98.1	95.1
131	90.2	88	94.9	87.7	88.5	89.4	90.5	94.9	90.2	90.5	89.6	92.2	89.8	95.5	89.9	88.8	90.5	89.4	89.6		94.4	98.2	97.9	93.9	94.4	96.6
174	98.1	91.6	90.2	91.4	90.8	94.4	98.3	90.2	98.1	98.3	95.3	96.9	96.7	89.7	94.7	93	95.5	94.2	95.3	90.8		94.3	93.8	97	99.3	95.5
C5	90.2	88	95.7	87.7	88.5	89.4	90.5	95.7	90.2	90.5	89.6	92.2	89.8	97.7	89.9	88.8	90.5	89.4	89.6	95.9	90.8		98.6	93.9	94.3	97.3
D74	88.8	86.6	94.7	86.3	87.7	88.3	89.1	94.7	88.8	89.1	88	90.8	88.4	97.7	88.5	88	88.8	87.7	88	95.7	89.4	97.2		92.9	93.6	96.6
F042	94.2	93.9	88.8	93.6	93.1	98.1	94.4	88.8	94.2	94.4	98.9	94.4	94.4	88.3	98.1	93.6	98.3	99.4	98.9	89.4	94.2	89.4	87.7		97.5	94.8
H425	99.2	91.9	90.2	91.6	91.1	95.3	99.4	90.2	99.2	99.4	96.1	97.2	98.1	89.7	95	93.3	96.4	95	96.1	90.8	98.6	90.8	89.4	95		96.1
H465	93.8	88.7	94.6	88.4	89.2	91.2	94.1	94.6	93.8	94.1	91.5	93.2	93.6	94.9	91.5	89.5	92.1	90.9	91.5	93.6	93.2	94.4	93.9	90.9	94.3	

Homologous comparison of nucleotide sequences on the right upper and homologous comparison of amino acid sequences on the bottom left.

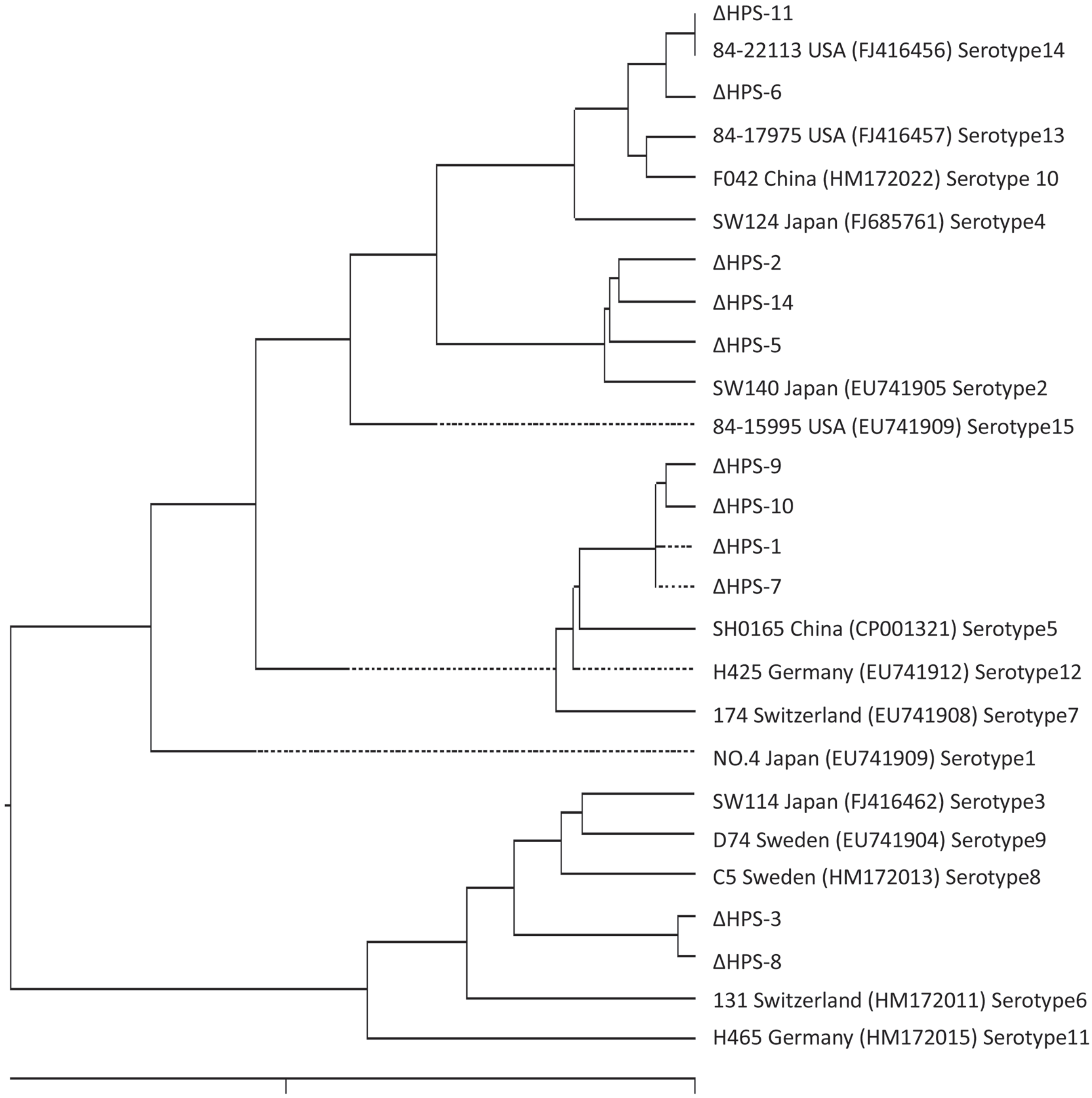

Fig. 1. Phylogenetic tree analysis of HPS based on *OMPP2* nucleotide sequences.

the other 9 strains had a 69-bp deletion, with the exception of HPS-3 and 8, which had deletions similar to those in strains 174, 84-15995, 84-17975, 84-22113, F042, H425, NO.4, SW124 and SW140 *OMPP2* (Table 2).

Phylogenetic tree (Fig. 1) analysis revealed clustering between HPS-6 and HPS-11 and they were closest to a cluster containing U.S.A. strain 84-22113, HPS-2, HPS-4, HPS-5, and Japanese strain SW140. HPS-1, 7, 9 and 10 were clustered with the classic Chinese strain SH0165. HPS-3 and HPS-8 were in a single cluster, in a branch with C5, Swedish strain D74, and Japanese strain SW114.

OMPP2 nucleotide sequence homology showed highest homology (98.7%) between the 2013 and 2014 Suining Sichuan province strains HPS-3 and HPS-8 with SW114. The nucleotide sequence consistency reached to 99.83% between these strains, with only two single base differences found in *OMPP2*. Therefore, we speculated that HPS-3 and HPS-8 were most likely introduced from Japan and the Suining Sichuan province may have persistent infection of these strains.

In general, 11 isolates showed significant differences in the *OMPP2* sequence, especially in the 770 to 850-bp region, where 9 isolates had a 69-bp deletion. This deletion was important for the virulence of HPS. Therefore, since strong HPS virulence may be prevalent in the Sichuan area, pig farms should work toward the prevention of HPS infections and be aware of the potential crisis associated with HPS.

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