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LETTER

Molecular Epidemiology of Porcine Circovirus Type 3 Infection in Swine Herds in China

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Dear Editor,

Porcine circovirus type 3 (PCV3) is a new-emerging circovirus belonging to the genus *Circovirus* in the family *Circoviridae* in which PCV type 1 (PCV1) and PCV type 2 (PCV2) were well documented (Palinski *et al.* 2017). PCV1 is a cell culture-derived virus and is considered to be nonpathogenic for swine, whereas PCV2 is the primary etiological agent of porcine circovirus-associated diseases (PCVAD) that causes severe economic losses in the swine industry worldwide (Zhai *et al.* 2014). PCV3 was firstly reported in the United States of America in 2016 (Palinski *et al.* 2017). After that, PCV3 has been detected in Poland, South Korea, Italy, Brazil, United Kingdom, and China, and was reported to be associated with porcine dermatitis and nephropathy syndrome, congenital tremors, reproductive failure, and multi-systemic inflammation (Kwon *et al.* 2017; Li and Tian 2017; Palinski *et al.* 2017; Stadejek *et al.* 2017; Zheng *et al.* 2017). The first case of PCV3 in China was reported in Guangdong Province in March 2017 (Shen *et al.* 2017). After that, PCV3 cases were frequently reported in other provinces in South China such as Guangxi, Jiangxi, Fujian, and Hunan provinces (Fu *et al.* 2017). The status of PCV3 prevalence in Central and North China was rarely reported. To figure out current status of PCV3 prevalence, a national epidemiological survey on PCV3 was conducted in this study.

A total of 730 clinical tissue samples were collected from 60 pig farms in 17 provinces and 3 autonomous municipalities with different geographical distributions (South China: Fujian, Jiangsu, Sichuan, Zhejiang, Anhui, Hubei, Jiangxi, Guangdong and Shanghai; Central China: Shandong, Shanxi, Henan, Shaanxi, and Gansu; North China: Hebei, Jilin, Inner Mongolia, Beijing, Tianjin, and Liaoning) in China. The collected samples included lung (84), lymph node (75), tonsil (58), kidney (65), brain (56), spleen (82), heart (77), liver (34), and stillbirth (199). These samples were individually collected, stored and transported in ice boxes. All animal studies in this study were approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine with IACUC number 2018054. The tissue samples were homogenized and diluted with phosphate-buffered saline (PBS: 0.1 mol/L, pH 7.4) and then subjected to centrifuge at 10,000 rpm for 10 min at 4 °C. The supernatants were then utilized for DNA extraction (Tiangen, Beijing, China) following the manufacturer's instructions. PCV3 DNA detection using conventional PCR was performed as previously described with forward primer 5′-CCACAGAAGGCGCTATGTC-3′ and reverse primer 5′-CCGCATAAGGGTCGTCTTG-3′ (Palinski *et al.* 2017). The PCR reaction was performed as follows: 94 °C for 3 min, followed by 35 cycles of 94 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min.

One hundred and four out of 730 clinical samples were tested to be PCV3 positive using PCR and the positive rate was 14.25% (Table 1). As for the geographical distribution, the positive rates of PCV3 in South China (16.16% ± 6.04%) was significantly higher than North China (5.96% ± 3.84%) analyzed by One-way ANOVA using Sigmaplot 11 software (Systat Software Inc., San Jose, CA) ($P = 0.0098$). There was no statistically

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Table 1 Sample information and positive rates in different provinces or municipalities.

Geographical distribution	Province/ municipality	No. of farms	No. of samples	Sample positive rate (%)
South China	Fujian	3	44	22.73 (10/44)
	Jiangsu	2	28	17.86 (5/28)
	Sichuan	2	26	15.38 (4/21)
	Zhejiang	3	41	17.07 (7/41)
	Anhui	4	50	16.00 (8/50)
	Hubei	3	45	17.78 (8/45)
	Jiangxi	3	29	13.79 (4/29)
	Guangdong	3	38	26.32 (10/38)
	Shanghai	2	22	9.09 (2/22)
Central China	Shandong	5	77	15.58 (12/77)
	Shanxi	3	37	13.51 (5/37)
	Henan	4	55	16.36 (9/55)
	Shaanxi	3	40	5.00 (2/40)
	Gansu	4	54	14.81 (8/54)
North China	Hebei	4	32	9.38 (3/32)
	Jilin	2	19	5.26 (1/19)
	Inner Mongolia	5	43	9.30 (4/43)
	Liaoning	2	17	5.88 (1/17)
	Beijing	2	19	5.26 (1/19)
	Tianjin	1	14	0.00 (0/14)
	In total	60	730	14.25 (104/730)

significant difference between South China and Central China (13.05% ± 4.62%). Since the previously published literatures about the PCV3 epidemiological survey were mainly focused on provinces in South China, the results in this study for the first time indicated that PCV3 has widely spread to central and north parts of China and has nationwide distribution.

PCV3 has been reported to mainly infect lymphoid tissues, such as lung, lymph nodes, and tonsil (Fan *et al.* 2017; Fu *et al.* 2017). In this study, the primary PCV3-postive tissues were found to be lung, followed by lymph nodes, stillbirth, kidney, brain, tonsil, spleen, heart, and liver (Table 2). To quantity virus genome copy numbers in above different types of tissues, a commercial real-time PCR kit (Beijing Anheal Laboratories Co., Ltd. Beijing, China) was applied. Real-time PCR was performed using an ABI 7500 instrument (Applied Biosystems, Foster City, USA). The real-time PCR mixture contained 12.5 μL Premix Ex Taq (Takara, Dalian, China), 1 μL template DNA, 1 μL primers (1 μmol/L), 1 μL probe (0.5 μmol/L), and sterile water to bring the final volume to 25 μL. The amplification parameters were set as 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s, and 60 °C for 30 s. The means of Ct values of different types of tissues were

Table 2 Percentage of PCV3-positve tissues and Ct values of real-time PCR.

Tissue	% of PCV3+	Ct value
Lung	29.8 (25/84)	29.7 ± 4.4
Lymph nodes	21.3 (16/75)	30.1 ± 4.5
Stillbirth	14.07 (28/199)	29.5 ± 7.3
Kidney	13.8 (9/65)	27.2 ± 4.6
Brain	12.5 (7/56)	31.5 ± 3.6
Tonsil	12.1 (7/58)	30.5 ± 2.9
Spleen	7.3 (6/82)	32.5 ± 3.1
Heart	6.5 (5/77)	33.4 ± 2.8
Liver	2.9 (1/34)	35.8

The Ct values were expressed by mean ± SD.

used to reflect the relative amount of virus genome copy number according to the product manufacture's instruction. The highest genome copy numbers were found in kidney samples, followed by stillbirth, lung, lymph nodes, tonsil, brain, spleen, heart, and liver (Table 2). The mean Ct values of PCV3-negative samples were 38.7 ± 2.3.

To figure out potential multiple infections of PCV3-postive tissue samples, PCV3-postive samples were also used to detect other porcine viruses including PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV), porcine parvovirus (PPV), and classical swine fever virus (CSFV) using conventional PCR as previously described (Li *et al.* 2013, 2014, 2017; Lv *et al.* 2016). Consistent with previous reports (Fu *et al.* 2017), PCV2 and PRRSV were the primary viral pathogens in multiple infections. Thirty-five, 41, and 26 PCV3-positive clinical samples were found to be co-infected with PCV2, PRRSV, and PCV2/PRRSV, respectively. No co-infection with CSFV, PRV, and PPV was observed in this study. There were only two cases of singular PCV3 infection without other viruses detected in above PCV3-positive clinical samples. The above results were consisted with previous reports in which co-infections of PCV3 with other swine pathogens such as PCV2, PPRSV, and astrovirus were frequently detected and may aggravate the clinical signs (Phan *et al.* 2016; Fu *et al.* 2017).

To get complete PCV3 genome sequence of positive samples, two pairs of primers was used as previously described (Kwon *et al.* 2017). The complete genomes of six PCV3 isolates in this study were obtained and deposited in GenBank under the accession numbers from MF769806 to MF769811. To perform phylogenetic analysis, PCV3 genome sequences obtained in this study and from NCBI were aligned using Clustal W, and a phylogenetic tree was constructed using the maximum-likelihood method with

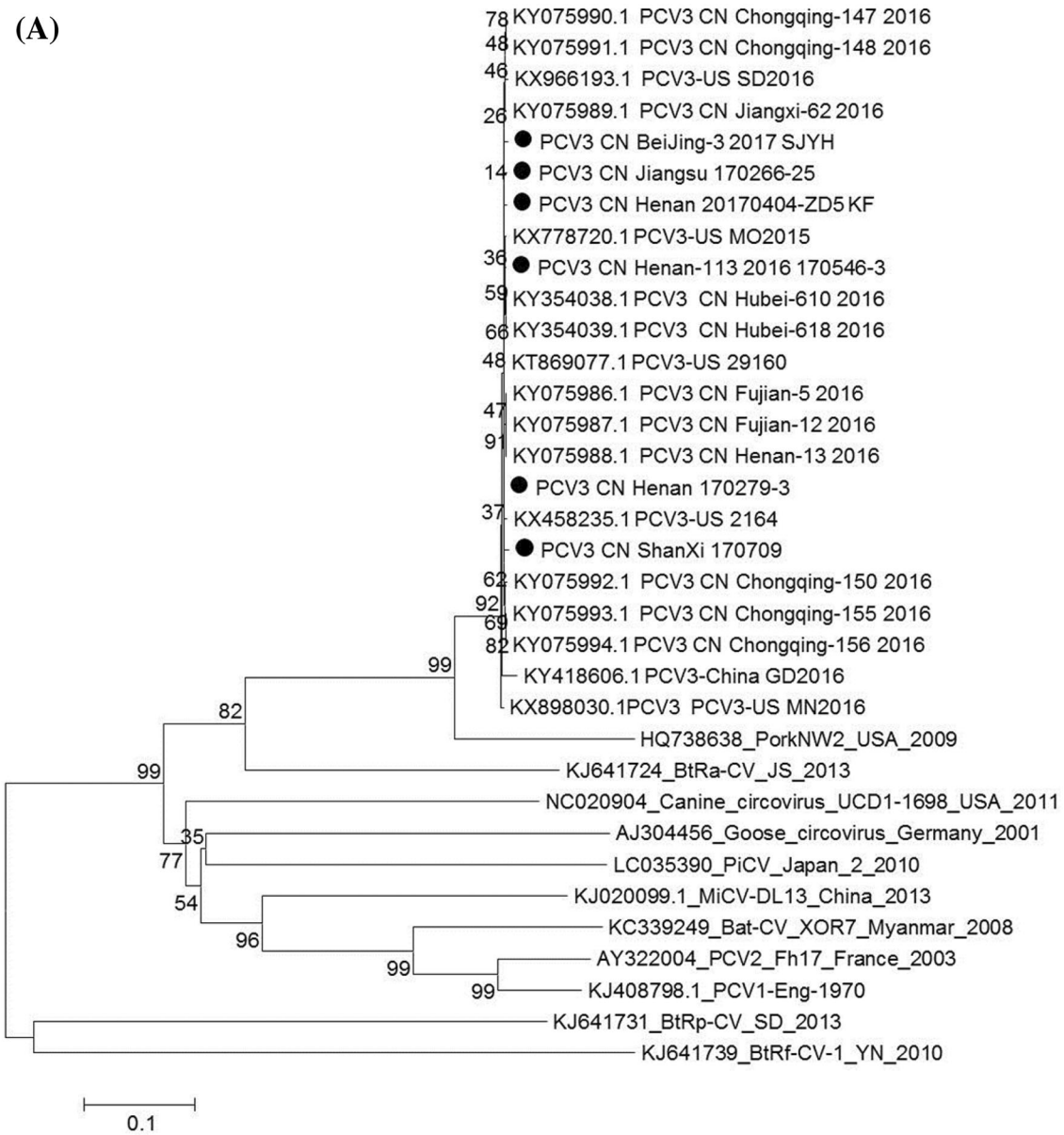

Fig. 1 continued

1000 bootstrap replicates in MEGA7.0 software (Kumar *et al.* 2016). A phylogenetic tree for full-length *Cap* gene was also constructed as described above.

Six PCV3 genomes were obtained from above PCV3-postive samples. Among of them, one PCV3-postive samples came from South China (Jiangsu Province), four came from Central China (Henan and Shanxi Province), and one came from North China (Beijing). The above six PCV3 genomes in this study were compared with other reported PCV3. As shown in Fig. 1A, six PCV3 isolates were clustered in different branches, and shared 98.9%–100% genome similarity with other reported strains. There were no distinct branches formed among all reported PCV3s according to geographical distribution and time of virus isolation. In Fu's study, PCV3 isolates were divided into PCV3a, PCV3b, and PCV3c clades according to two amino acid mutations (A24 V and R27 K) on *Cap* gene (Fu *et al.* 2017). The full-length of six PCV3 *Cap* genes were also analyzed by above amino acids and compared with other reported PCV3 strains. As shown in Fig. 1B, two PCV3 strains from Henan (Central China) were clustered into clade PCV3a, two PCV3 strains from Beijing (North China) and Jiangsu (South China) were clustered into clade PCV3c, and two PCV3 strains from Shanxi and Henan (Central

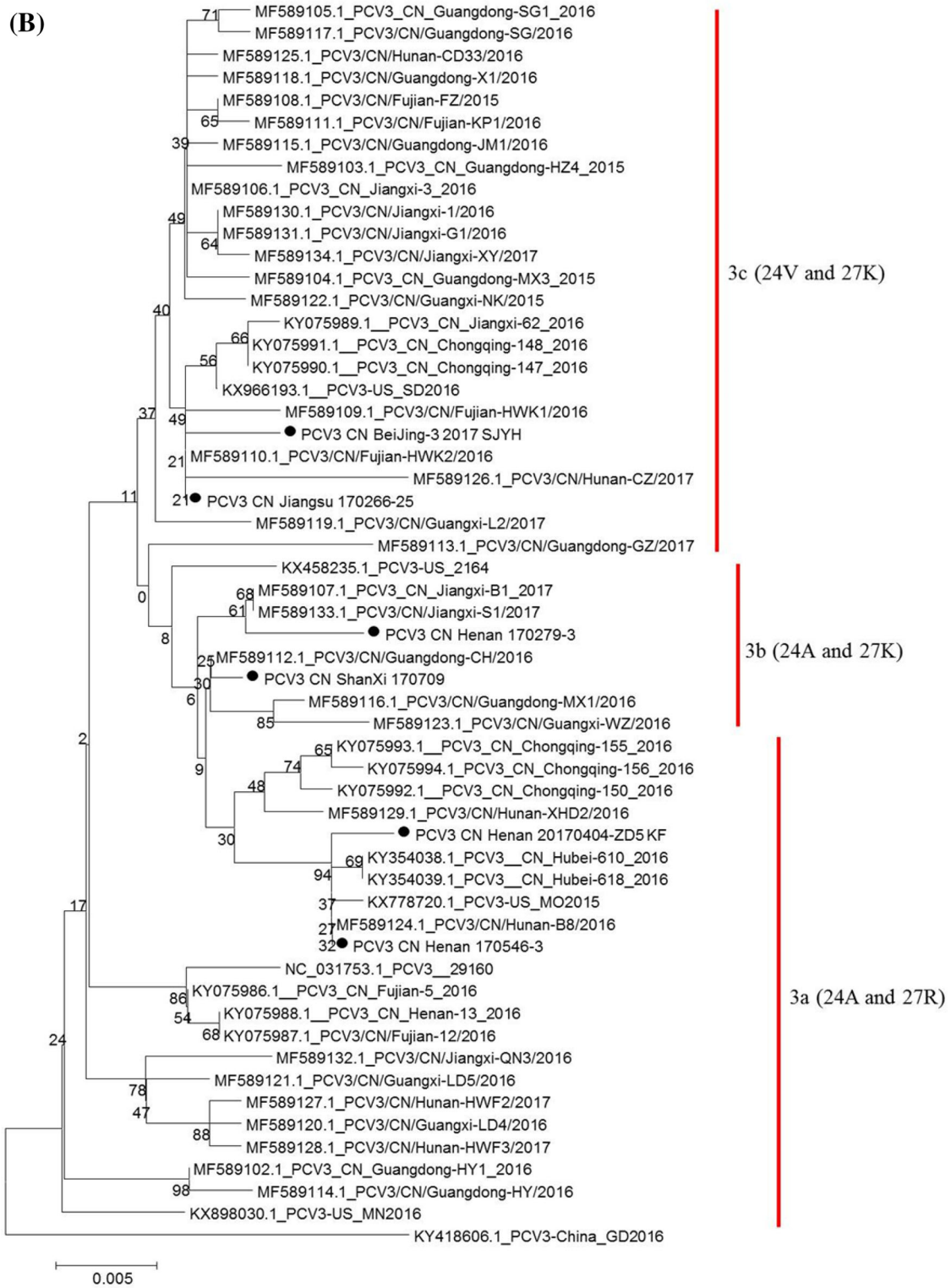

Fig. 1 Phylogenetic analysis of PCV3 strains. **A** Whole genome-based phylogenetic relationship of six PCV3 isolates in this study with other reported PCV3. **B** Full-length *Cap* gene-based phylogenetic relationship of six PCV3 isolates in this study with other reported PCV3. Dot indicates the PCV3 isolates in this study.

China) were clustered into clade PCV3b. Clade PCV3b were first identified in Fu's study and exclusively circulated in Guangdong, Guangxi and Jiangxi Province. In this study, clade PCV3b was found to extend into Central China (Henan and Jiangxi) and PCV3c into North China (Beijing). Therefore, we, for the first time, report that PCV3 has

geographically spread to Central and North China (Table 1).

So far, the pathogenicity of PCV3 has not been evaluated due to the failure of PCV3 isolation on cells. Also, singular PCV3 infection was rarely detected in clinical samples and several severe pathogenic porcine viruses such as PRRSV and PCV2 were found to be co-infected with PCV3. Therefore, studies about the pathogenicity of singular PCV3 infection and/or PCV3 co-infected with other important viral pathogens on pigs should be the priority to elucidate the significance of PCV3 on swine industry.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they do not have any conflict of interest.

Animal and Human Rights Statement This study was approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine with IACUC Number 2018054.

References

Fan S, Ku X, Chen F, Wang Y, Yu X, He Q (2017) Complete genome sequence of a novel porcine circovirus type 3 strain, PCV3/CN/Hubei-618/2016, isolated from China. Genome Announc 5(pii):e00100–e00117

Fu X, Fang B, Ma J, Liu Y, Bu D, Zhou P, Wang H, Jia K, Zhang G (2017) Insights into the epidemic characteristics and evolutionary history of the novel porcine circovirus type 3 in southern China. Transb Emerg Dis 65:e296–e303

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Kwon T, Yoo SJ, Park CK, Lyoo YS (2017) Prevalence of novel porcine circovirus 3 in Korean pig populations. Vet Microbiol 207:178–180

Li X, Tian K (2017) Porcine circovirus type 3: a threat to the pig industry? Vet Rec 181:659–660

Li X, Galliher-Beckley A, Huang H, Sun X, Shi J (2013) Peptide nanofiber hydrogel adjuvanted live virus vaccine enhances cross-protective immunity to porcine reproductive and respiratory syndrome virus. Vaccine 31:4508–4515

Li X, Galliher-Beckley A, Pappan L, Trible B, Kerrigan M, Beck A, Hesse R, Blecha F, Nietfeld JC, Rowland RR, Shi J (2014) Comparison of host immune responses to homologous and heterologous type II porcine reproductive and respiratory syndrome virus (PRRSV) challenge in vaccinated and unvaccinated pigs. Biomed Res Int 2014:416727

Li Y, Yan S, Li X, Yang Q, Guo L, Wang Y, Xiao Y, Tan F, Tian K (2017) From mouse to pig: is PRV vaccine safe across two species? Virus Res 236:44–49

Lv C, Yang Q, Gao X, Yao Y, Li X, Xiao Y, Tian K (2016) Complete genome sequence of a classical swine fever virus isolate belonging to new subgenotype 21d from Henan Province, Central China. Genome Announc 4(pii):e00093–e00116

Palinski R, Pineyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM (2017) A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 91(pii):e01879–e01916

Phan TG, Giannitti F, Rossow S, Marthaler D, Knutson TP, Li L, Deng X, Resende T, Vannucci F, Delwart E (2016) Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virol J 13:184

Shen H, Liu X, Zhang P, Wang L, Liu Y, Zhang L, Liang P, Song C (2017) Genome characterization of a porcine circovirus type 3 in South China. Transbound Emerg Dis 65:264–266

Stadejek T, Wozniak A, Milek D, Biernacka K (2017) First detection of porcine circovirus type 3 on commercial pig farms in Poland. Transbound Emerg Dis 64:1350–1353

Zhai SL, Chen SN, Xu ZH, Tang MH, Wang FG, Li XJ, Sun BB, Deng SF, Hu J, Lv DH, Wen XH, Yuan J, Luo ML, Wei WK (2014) Porcine circovirus type 2 in China: an update on and insights to its prevalence and control. Virol J 11:88

Zheng S, Wu X, Zhang L, Xin C, Liu Y, Shi J, Peng Z, Xu S, Fu F, Yu J, Sun W, Li J, Wang J (2017) The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province. Transbound Emerg Dis 64:1337–1341