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Short communication

The prevalence of porcine teschovirus in the pig population in northeast of China

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A B S T R A C T

The prevalence of porcine teschovirus (PTV) in swine herds in northeast China was investigated. In 2008–2009, 1384 samples of pig sera were collected from 42 farms in Shandong, Hebei, Heilongjiang, and Jilin provinces and in Tianjin City and were tested for specific antibody against PTV-8 by immunofluorescence assay. All 42 pig herds were positive for antibodies against PTV-8, and 61.3% of the serum samples were PTV-8 positive. During the survey, one PTV strain was isolated and named Fuyu/2009; phylogenetic analysis showed that the PTV Fuyu/2009 belongs to the PTV-8 serotype. The serological results indicate that most if not all pig herds in northeast of China have been exposed to PTV. RT-PCR performed on 114 clinical samples indicated a possible association between PTV and disease. According to genotyping based on partial VP1 sequences, four serotypes (PTV-2, -4, -6, and -8) were identified in northeast of China; sequence data also provided evidence of natural recombination between PTV serotypes and indicated that homologous recombination may be a driving force in PTV evolution. The role of PTV in disease remains inconclusive. The current results together with published results indicate that the prevalence of PTV is increasing among swine herds in northeast of China.

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1. Introduction

Porcine teschovirus (PTV) belongs to the family Picornaviridae and the genus Teschovirus. Two PTV strains (the Teschen and Talfan strains) were first isolated during outbreaks of polioencephalomyelitis in Europe in 1929 and 1957, respectively (Trefny, 1930; Harding et al., 1957). PTV was classified previously within the genus Enterovirus and was known as porcine enterovirus (PEV) but has been reclassified as PTV with 13 subtypes (PTV-1 to PTV-13) based on detailed genetic analyses (Zell et al., 2001).

As categorized by the Office International Epizooties, PTV is now a List B Pathogen and is now recognized as a worldwide pathogen of pigs. Among domestic animals, the pig is considered the most infected commonly and severely by PTVs. PTV causes various clinical symptoms such as neurological disorders (e.g., polioencephalomyelitis), reproductive disorders (Dunne et al., 1965), diarrhea (Izawa et al., 1962), and pneumonia (Meyer et al., 1966). Because PTV is persistent, it can survive on pig farms for a long time, and this persistence increases the probability of mixed infections with other pathogens such as PRRSV and CSFV. Mixed infections could lead to more serious diseases of pigs and greater economic loss than single infections. In 2007, RT-PCR had been used to detect PTV infection in numerous fever cases among swine in the Jiangsu region of China (Zhu, 2008); the PTV-positive rate was 47.5%, indicating that PTV infection is widespread among pigs in that region of China.

Because of a lack of serological data, the occurrence of PTV in pigs in China is difficult to assess. The current study had the following aims: to assess the occurrence of PTV in pigs in China based on the routine diagnosis and molecular epidemiology; A variety of assays were used. An indirect immunofluorescence assay was used to detect PTV-specific antibody. A multiplex RT-PCR (mRT-PCR) assay was used for simultaneous detection of multiple detect multiple viral infections of swine including those caused by PTV. A real-time reverse transcription polymerase chain reaction (RT-PCR) assay based on TaqMan probes was used to detect all types of PTVs.

2. Materials and methods

2.1. Survey

From September 2008 to September 2009, a total of 1384 sera samples were collected from 42 swine herds in northern China. Each sample was from one pig (age 20 days to 6 months), and from

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Table 1
Detection by immunofluorescence assay of PTV in sera samples collected from 42 swine herds in China.

Herd no.	No. of sera samples	No. of PTV-positive samples	% Positive
1	10	9	90.0
2	46	26	56.5
3	45	25	55.6
4	48	26	54.2
5	50	22	44.0
6	45	24	53.3
7	50	24	48.0
8	20	15	75.0
9	4	3	75.0
10	9	6	66.7
11	40	29	72.5
12	200	98	49.0
13	32	19	59.4
14	24	16	66.7
15	40	18	45.0
16	28	24	85.7
17	24	15	62.5
18	87	48	55.2
19	20	6	30.0
20	20	6	30.0
21	30	9	30.0
22	30	21	70.0
23	20	14	70.0
24	20	11	55.0
25	20	13	65.0
26	30	7	23.3
27	30	24	80.0
28	30	9	30.0
29	20	8	40.0
30	56	48	85.7
31	60	57	95.0
32	14	12	85.7
33	8	5	62.5
34	35	31	88.6
35	35	32	91.4
36	8	7	87.5
37	25	22	88.0
38	29	26	89.7
39	10	8	80.0
40	18	15	83.3
41	4	3	75.0
42	10	8	80.0
Total no. and mean %	1384	849	61.3

4 to 200 samples were obtained from each herd (Table 1). The herds were located in four provinces (Shandong, Hebei, Heilongjiang, and Jilin) and also in Tianjin City. The pigs were selected randomly for sampling. The collected sera were stored at −20 °C in the laboratory.

A total of 205 clinical samples including of brains, spleens, hearts, and livers were also collected. Of these, 114 were affected from commercial herds by porcine high fever syndrome in Heilongjian, Jilin, and Henan provinces. The other 91 clinical samples were collected from 12 commercial herds of nursery and fattening pigs in Henan, Heilongjiang, and Guangxi provinces; these pigs were 2 and 3 months old and showed symptoms of diarrhea, respiratory distress, and death.

2.2. Virus strain and cells

PRRSV HB strain (North American genotype, accession number EF112446), PTV-8 Jilin/2003 strain (accession number GQ293092), PCV type II 020 strain, PPV BQ strain (accession number EU790641), and PRVLXB strain were all obtained from the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences. PCV2, PPV, and PRV were propagated in PCV l-free PK-15 cells (Pogranichniy et al., 2002), which were provided kindly by the Veterinary Diagnostic Laboratory of Iowa State University (Ames, IA, USA). PRRSV was propagated in Marc-145 cells, which were obtained from the Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences. CSFV attenuated vaccine was purchased from Fuzhou Da Bei Nong Biotechnology (Fu zhou, China). PTV-8 was obtained from State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences. And was propagated in PK-15 cells derived from porcine kidney. PK-15 cells were maintained in Dulbecco's Modified Eagle Media (DMEM) containing 10% fetal bovine serum (Invitrogen).

2.3. Preparation of cells for the immunofluorescence assay (IFA)

PK-15 cells were cultured in 6-well cell culture plates and were inoculated with PTV-8 when they had grown into a monolayer. When the cytopathic effect (CPE) was approximately 75%, the cell culture was fixed in 50% acetone/50% methanol for 15 min at room temperature and permeabilized with 0.1% Triton X-100 for 10 min at room temperature. The fixed cells were washed three times with PBS, 3 min each time, and dried at room temperature. The cell culture plates that were now coated with PTV-8 were then used for IFA as described in the next section. Similar plates without PTV-8 were used as controls.

2.4. IFA

IFA was carried out using the fixed 6-well cell culture plates described in the previous section. The sera samples collected in the survey were diluted 1:20 in PBS (pH 7.2). The cell culture plates were equilibrated to room temperature, and 200 μl of the diluted serum was placed in one well. A positive control serum and a negative control serum, which were prepared and conserved in State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, were diluted 1:20 and were also added to the wells of each plate. The plates were incubated in a humidified chamber at 37 °C for 30 min, washed with PBS, and then soaked for 10 min in PBS and blotted. Fuorescein isothiocyanate-labeled goat anti-swine IgG (200 μl of a 1:200 dilution) was added to each well. The plates were incubated at 37 °C for 30 min and then washed as before. Each plate was dried before one drop of mounting fluid and a cover slip were added. Plates were examined immediately for fluorescence using a Zeiss fluorescent microscope at 400 nm.

2.5. mRT-PCR and TaqMan RT-PCR

A multiplex RT-PCR (mRT-PCR) assay was used for simultaneous detection of multiple viral infections of swine including PTV (Liu et al., 2011a). mRT-PCR was applied to 114 clinical samples.

A pair of primers and a TaqMan probe targeting the conserved highly sequence of the 5′-untranslated region (5′-UTR) of one to 13 serotypes of PTV were designed. A real-time reverse transcription polymerase chain reaction (RT-PCR) based on TaqMan probes was used to detect all types of PTVs (Zhang et al., 2013). RT-PCR was applied to 138 clinical samples.

3. Results

3.1. Serological survey

Among the 1384 sera samples, 849 (61.3%) reacted with PTV-8 in the IFA (1:20). All 42 swine herds were PTV-seropositive. The percentage of PTV-seropositive animals in the herds ranged from 23.3% to 95.0% (Table 1) and was greater than 40% in most herds (Fig. 1).

In one herd, sera samples were collected from the same 10 piglets at age 4, 8, 12, and 16 weeks. The percentage of pigs in which

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