Outbreak of Porcine Epidemic Diarrhea in Piglets in Gansu Province, China

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ABSTRACT

Background: Porcine epidemic diarrhea virus (PEDV) is a member of the genus Coronavirus and family Coronaviridae. The virus is the aetiological agent of porcine epidemic diarrhea (PED), and detected for the first time in 1978 in England. PED causes acute enteritis and severe diarrheal disease with high mortality, particularly in suckling piglets; massive economic losses in many locations resulted. The severe acute diarrhea outbreak associated with high morbidity and mortality. By now, PEDV cases confirmed by laboratory testing have been reported from a lot of countries (southern China, Thailand, Vietnam, Korea and Japan). Gansu is located in the northwest of China; and few case of PEDV infection has been detected. And previous research has shown that the immunity induced by the commercial Korean vaccine do not provide cross-protection to the recent Chinese PEDV strain. So the virus may as a variant PEDV emerging in Gansu. The study was aimed at detecting the presence of piglets positive to PEDV in Gansu province of China.

Materials, Methods & Results: A total of 120 samples were collected from piglets from different farms in Gansu province, China from March 2011 to December 2012. The clinical symptoms and anatomic pathological changes of diarrheal piglets were observed. The total RNA was extracted from intestinal contents of diarrheal suckling piglets with a commercial High Pure Viral RNA Kit. Real-time RT-PCR was used to investigate enteropathogen. The reverse transcription was prepared in total of 25 µL volume according to the manufacturers’ instructions with the following component volumes per well: 8 µL total RNA, nuclease-free water 2.5 µL, RT-PCR reaction liquid 12.5 µL, multiplex RT-PCR enzyme mix 1.0 µL, and fluorescence probe 1.0 µL. The reaction was performed at 48°C 10 min, 95°C 10 min, 95°C 15 s, 60°C 45 s. Real-time PCR was performed using ABI PRISM 7500 real-time PCR machine. Samples with threshold cycles (Cts) of 35 cycles were considered positive, and those with Cts < 35 were considered negative. Quality control for the PCR reaction included nuclease-free water as negative amplification control in addition to a positive amplification control provided by the manufacturer. The diarrheal piglets showed severe watery diarrhea, dehydration with milk curd vomitus, mild hemorrhage, undigested curdled milk in the stomach, and thin-walled intestines with severe mucosal atrophy and foamy fluid. The Real-time RT-PCR test identified 87 positive piglets - 18 in Jiayuguan (18% of all tested piglets), 12 in Wuwei (10% of all tested piglets), 11 in Shuangta (9.2% of all tested piglets), 30 in Jingning (25% of all tested piglets) and 16 in Tianshui (13.3% of all tested piglets).

Discussion: Relatively higher number of positive piglets in the Gansu Province, Northwest China indicates that this is the region where PEDV has entered the country. And the outbreak of PEDV in Gansu caused severe diarrheal disease in piglets; heavy economic losses in many farms resulted (mortality was 60-85%). Given that the infection has been so far reported only from southern China, our study implies the potential of PEDV to cover new territories. Coronavirus is a significant risk pathogen in humans and animals. Animal Coronavirus is a very serious risk to sustained and healthy development of animal husbandry. Therefore, effective biosecurity control and strict sanitary measures are key components of PED prevention and control. Meanwhile the potential mechanism of PEDV interacts with the host antiviral innate system and the molecular mechanisms that regulate the host innate antiviral immune responses should be further studied.

Keywords: porcine epidemic diarrhea, PEDV, Real-time RT-PCR, epidemiology, Gansu.
INTRODUCTION

Porcine epidemic diarrhea virus (PEDV) is a member of the genus Coronavirus and family Coronaviridae. The virus is the aetiological agent of porcine epidemic diarrhea (PED), and detected for the first time in 1978 in England [3]. PED causes acute enteritis and severe diarrheal disease with high mortality, particularly in suckling piglets; massive economic losses in many locations resulted [2,6,7]. It has been reported that a severe acute diarrhea outbreak associated with high morbidity (80-100%) and mortality (50-91%) was observed in suckling piglets in Thailand and Guangdong, China [1,4,5]. By now, PEDV cases confirmed by laboratory testing have been reported from a lot of countries (southern China, Thailand, Vietnam, Korea and Japan). Gansu is located in the northwest of China; and few case of PEDV infection has been detected. And previous research has shown that the immunity induced by the commercial Korean vaccine do not provide cross-protection to the recent Chinese PEDV strain [1]. So the virus may as a variant PEDV emerging in Gansu. The study was aimed at detecting the presence of piglets positive to PEDV in Gansu province of China.

MATERIALS AND METHODS

A total of 120 samples (intestine and stool) were collected from piglets from different farms that had diarrhea in Jiayuguan (n = 20), Wuwei (n = 18), Shuangta (n = 15), Jingning (n = 45) and Tianshui (n = 22) in Gansu province, China from March 2011 to December 2012 (Figure 1). The clinical symptoms and anatomic pathological changes of diarrheal piglets were observed.

The total RNA was extracted from intestinal contents of diarrheal suckling piglets with a commercial High Pure Viral RNA Kit1 according to the manufacturer’s protocol. The reverse transcription was prepared in total of 25 µL volume according to the manufacturers’ instructions with the following component volumes per well: 8 µL total RNA, nuclease-free water 2.5 µL, RT-PCR reaction liquid 12.5 µL, multiplex RT-PCR enzyme mix 1.0 µL, and fluorescence probe 1.0 µL. The reaction was performed at 48°C 10 min, 95°C 10 min, 95°C 15 s, 60°C 45 s. Real-time PCR was performed using ABI PRISM 7500 real-time PCR machine2. Samples with threshold cycles (Cts) of 35 cycles were considered positive, and those with Cts < 35 were considered negative. Quality control for the PCR reaction included nuclease-free water as negative amplification control in addition to a positive amplification control provided by the manufacturer.

Figure 1. Location of the situation of PED in Gansu province, China, 2011-2012 (sandybrown ellipse).
RESULTS

The diarrheal piglets showed severe watery diarrhea, dehydration with milk curd vomitus, mild hemorrhage, undigested curdled milk in the stomach, and thin-walled intestines with severe mucosal atrophy and foamy fluid (Figure 2).

The Real-time RT-PCR test identified 87 positive piglets (Figure 3) - 18 in Jiayuguan (18% of all tested piglets), 12 in Wuwei (10% of all tested piglets), 11 in Shuangta (9.2% of all tested piglets), 30 in Jingning (25% of all tested piglets) and 16 in Tianshui (13.3% of all tested piglets).

Figure 2. The piglets diarrhea of Gansu China. Severe watery diarrhea (a and b), dead piglets (c), undigested curdled milk in the stomach (d), milk curd vomitus (e), and thin-walled intest (f).

Figure 3. Part of dynamic curve of real-time fluorescence PCR of positive RNA samples.
DISCUSSION

So, relatively higher number of positive piglets in the Gansu Province, Northwest China indicates that this is the region where PEDV has entered the country. And the outbreak of PEDV in Gansu caused severe diarrheal disease in piglets; heavy economic losses in many farms resulted (mortality was 60-85%). Given that the infection has been so far reported only from southern China [7], our study implies the potential of PEDV to cover new territories.

Coronavirus is a significant risk pathogen in humans and animals. Animal Coronavirus is a very serious risk to sustained and healthy development of animal husbandry [8]. Therefore, effective biosecurity control and strict sanitary measures are key components of PED prevention and control. Meanwhile the potential mechanism of PEDV interacts with the host antiviral innate system and the molecular mechanisms that regulate the host innate antiviral immune responses should be further studied.

CONCLUSION

Real-time RT-PCR was used to investigate enteropathogen from diarrhea samples from local pig farms with severe diarrhea in piglets in the province of Gansu in China. The results demonstrate that the enteropathogen is porcine epidemic diarrhea virus. A better efficiency of pork production in the province of Gansu in China might be achieved by the improvement of surveillance, prevention, and control programs of infectious diarrhea in young pigs, particularly porcine epidemic diarrhea virus.

REFERENCES