大韓獸醫學會誌(2007) 第47卷 第4號
Korean J Vet Res(2007) 47(4) : 425~428

Hemagglutinating encephalomyelitis virus infection in Korean suckling pigs

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(Accepted: September 28, 2007)

Abstract : From January to June 2006, 54 suckling pigs had been submitted in virology lab., College of Veterinary Medicine, Seoul National University. All pigs had suffered from various symptoms such as respiratory sign, enteric signs, neurologic signs, etc. Among 54 pigs, 24 pigs (44.4%) were positive for porcine hemagglutinating encephalomyelitis virus (HEV) through reverse transcription–nested polymerase chain reaction. According to this result, HEV infections seemed to be prevalent and widespread in Korean swine farms, and the infection is associated with respiratory signs and neurologic signs more than enteric signs. The HEV positive pigs showing respiratory signs were co-infected with viruses such as PRRSV, and PCV2, or bacteria such as *Pasteurella* spp. The single infection may subclinically have an influence on outbreak of other respiratory pathogens in suckling pigs.

Key words : clinical signs, porcine hemagglutinating encephalomyelitis virus, suckling pigs

Porcine hemagglutinating encephalomyelitis virus (HEV) is a member of the genus Coronavirus, family Coronaviridae, order Nidovirales. HEV causes encephalomyelitis, or vomiting and wasting disease (VWD) in suckling piglets and occasionally at the onset of the disease, respiratory signs such as sneezing, coughing, or upper respiratory embarrassment are observed. When piglets under 3 weeks-old are infected with HEV, their mortality rate is 100%, but pigs more than 3 weeks-old show no obvious clinical signs [10].

In Canada in 1962, Greig *et al.* isolated a previously unrecognized viral pathogen of swine from the brain of suckling pigs with encephalomyelitis [3]. The virus responsible for this disease was called hemagglutinating encephalomyelitis virus because of its hemagglutinating properties; it was later classified as a coronavirus [2, 12]. In 1969, an antigenically similar, if not identical, virus was isolated in England from suckling pigs showing anorexia, depression, and vomiting, but without signs clearly associated with encephalomyelitis [1]. Animals that did not die but remained stunted during the growth; the condition was therefore called VWD. Mengeling and Cutlip were later able to experimentally reproduce both of the major forms of the disease (i.e., the clinically apparent ence-phalomyelitis and VWD) using the same field isolates [9].

Although epizootiologic studies have revealed that infection of swine with HEV is prevalent, naturally occurring disease is uncommon. Neonatal pigs are usually protected by passively acquired colostral antibody, and they subsequently develop an age-related resistance to the potential clinical effects of the virus. HEV has been observed in pig-raising countries around the world, but in Korea, there has been no report about HEV infection [10].

From January to June 2006, 54 suckling pigs had been submitted in virology lab., College of Veterinary Medicine, Seoul National University. Viral RNA was extracted from 10% tissue homogenates using TRIzol according to the manufacturer's instructions. For reverse transcription, 10 μl of extracted RNA and 10 pmol of external reverse primer (5'- AATCTGGTGCCACTGAAGATTGG-3'; Bioneer, Korea) were mixed. The mixture was denatured by heating to 95 and was immediately placed on ice. The remaining reagents, which consisted of 5×buffer (50 mM

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Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$), 10 mM DTT, 0.3 mM of each dNTP, and M-MLV reverse transcriptase in final volume of 20 μl, were added. The mixture was incubated at 37°C for 60 min. The previously published pairs of specific primer for detection of HEV were used. S2-F1 (5'-GTTACAGCAAAGGTTAGTCCTGG-3'; external forward primer; Bioneer, Korea), S2-R1 (described above, external reverse primer, Bioneer, Korea), N-S2-F2 (5'- TGGATGTTCACTGGTAGTAGC-3'; internal forward primer; Bioneer, Korea), N-S2-R1 (5'-GGTTGGGTGTCGATGTGTTCAGC-3'; internal reverse primer; Bioneer, Korea) were used for reverse transcription-nested polymerase chain reaction (RT-nPCR) of spike gene [13]. In RT-PCR, 2 μl of cDNA was mixed with a reaction mixture containing 10 pmol each of S2-F1 and S2-R1, 10×buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, enhancer solution), 0.3 mM dNTP, 2.5 mM $MgCl_2$, and 2.5 U Taq polymerase. Diethyl pyrocarbonate-treated water was added to make up a total volume of 25 μl. The PCR was performed at 94°C for 2 min, followed by 25 cycles of 94°C 30 sec, 65°C 30 sec, 68°C 45 sec, and a final extension at 72°C for 7 min, and then held at 4°C. RT-nPCR was performed on 2 μl of each amplification product by using N-S2-F2 and N-S2-R1. The RT-nPCR was performed at 94 30 sec, 65 30 sec, 72 30 sec, and a final extension at 72 for 7 min. The RT-nPCR products were analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide. The size of amplified products was 282 bp. DNA sequencing was performed through automatic DNA sequencing methods.

Among 54 pigs, 24 pigs (44.4%) were positive for porcine HEV through RT-nPCR. The nucleotide homology was 95~100% for HEV 67N strain.

Geographically, HEV positive pigs were from all parts of country, which are each 6 cases from Kyonggi-, Jeolla-, and Gyeongsang-province, 5 cases from Chungcheong-province, and each 1 case from Gangwon- and Jeju-province, respectively.

At necropsy, 16 HEV positive pigs had empty stomach presenting anorexia, and 9 had lung lesions such as pulmonary edema, consolidation, interstitial pneumonia. 4 had some lesions in digestive organs like

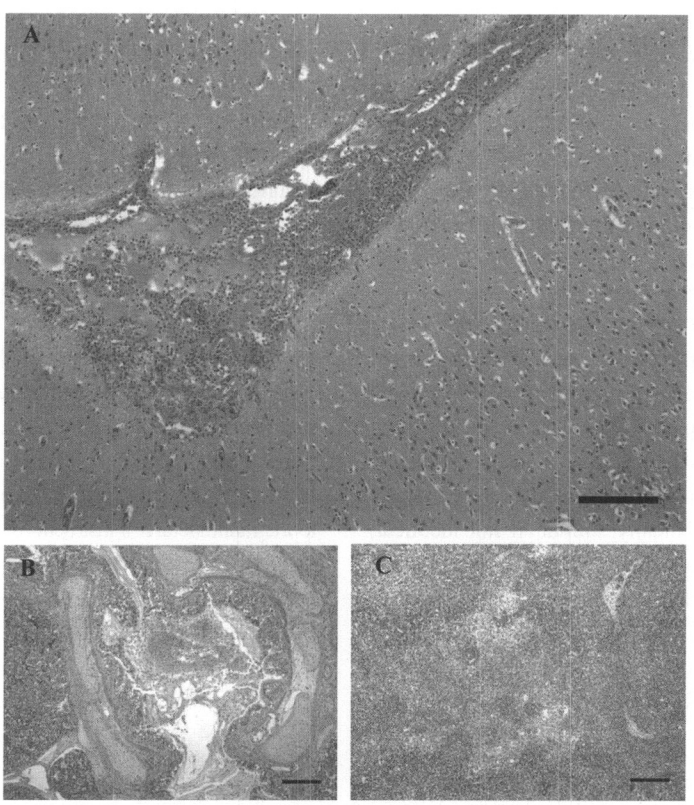

Fig. 1. Histological finding of HEV positive pigs: severe subacute diffuse lymphoplasmacytic menigoencephalitis (A), severe subacute diffuse lymphoplamacytic bronchointerstitial pneumonia (B), lymphoid follicle depletion (C) (Bars = 200 μm).

enlargement of mesenteric lymph node, intestinal congestion, thinning of intestine wall.

Histologically, most cases did not represent any lesions in brains or spinal cords, but only 2 cases represented some lesion such as diffuse lymphoplasmacytic meningoencephalitis (Fig. 1A). Other histological lesions were severe diffuse bronchointerstitial pneumonia (Fig. 1B), lymphoid follicular depletion (Fig. 1C), moderate acute suppurative tonsillitis, villous atrophy and fusion.

The results were classified according to clinical signs as follow, excluding miscellaneous cases such as sudden death, dermatitis, arthritis, etc; respiratory signs, neurologic signs, and enteric signs. The category of respiratory signs includes abdominal respiration, coughing, and dyspnea. For those, detections of porcine respiratory and reproductive syndrome virus (PRRSV), porcine circovirus-2 (PCV2), and *Pasteurella* spp. [6, 7] were performed additionally. The category of neurologic signs includes posterior paresis, prostration, recumbency, and hyperesthesia. For those, detections of Aujeszky's disease virus (ADV) and *Streptococcus* spp. were performed additionally. The category of enteric signs includes diarrhea and vomiting. For those, detections of porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), *E.coli, Salmonella* spp., *and Clostridium* spp. were performed additionally [5]. The results according to clinical signs are represented in Table 1.

According to this result, HEV infections seemed to be prevalent and widespread in Korean swine farms, and the infection is associated with respiratory signs and neurologic signs more than enteric signs. But, against the categories of neurologic and enteric signs, the category of respiratory signs did not represent any single infections of HEV.

Several observations indicate that HEV is able to replicate in the upper respiratory tract with or without producing clinical signs. Mengeling *et al.*, Pensaert *et al.*, and Hirahara *et al.* recorded the isolation of HEV from the nasal cavity, trachea, and lungs of diseased and healthy pigs [4, 8, 11]. Respiratory signs such as sneezing and nasal secretion were produced after intranasal or oral inoculation of conventional pigs at the age of 50 days but not after subcutaneous inoculation and not in intranasally inoculated 70-day-old animals.

Table 1. Detection of HEV and other pathogens according to clinical signs (excluding miscellaneous cases such as sudden death, dermatitis, arthritis, etc)

Category	Remarkable clinical signs	HEV	PRRSV	PCV2	*Pasteurella spp.*			No. of cases
Respiratory signs	Abdominal respiration, coughing, dyspnea	+	-	-	+			1
		+	-	+	-			3
		+	+	+	-			3
		-	+	+	-			2
		-	+	+	+			1
	Total	7/10	6/10	9/10	2/10			10
	Remarkable clinical signs	HEV	ADV	*Streptococcus spp.*				No. of cases
Neurologic signs	Posterior paresis, prostration, recumbency, hypersthesia	+	-	-				5
		+	-	+				2
		-	-	+				1
		-	-	-				5
	Total	7/13	1/13	3/13				13
	Remarkable clinical signs	HEV	PEDV	TGEV	*E.coli*	*Salmonella spp.*	*Clostridium spp.*	No. of cases
Enteric signs	Diarrhea, vomiting	+	-	-	+	-	-	4
		+	-	-	+	-	+	2
		-	+	-	+	+	-	2
		-	-	-	+	+	-	1
		-	-	-	+	-	-	7
	Total	6/16	2/10*	0/10*	16/16	3/16	2/16	16

*Tests for PEDV, TGEV were accomplished in 10 samples

Hirahara *et al.* obtained only sneezing and nasal secretion in 10-day-old, colostrum-deprived piglets upon intranasal inoculation; intracerebral inoculation resulted in nervous signs in only two of seven animals. The virus is excreted for 8~10 days in oronasal secretions. The mode of transmission occurs through nasal secretions. Most infections under field circumstances have a subclinical course [4]. In the same way, the prevalence of HEV infection in Korea was very high, but it might not mean all of the infections cause clinical diseases. In that positive for HEV in the pigs showing respiratory signs were co-infected with viruses such as PRRSV, and PCV2, or bacteria such as *Pasteurella* spp., the single infection seems to have subclinical influence on outbreak of respiratory disease in suckling pigs.

Acknowledgment

This work was supported by a grant (Code # 20070401034009) from BioGreen 21 Program, Rural Development Administration, Korea.

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