NOTE *Bacteriology*

Prevalence of Swine Viral and Bacterial Pathogens in Rodents and Stray Cats Captured around Pig Farms in Korea

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(Received 28 December 2012/Accepted 12 July 2013/Published online in J-STAGE 26 July 2013)

ABSTRACT. In 2008, 102 rodents and 24 stray cats from the areas around 9 pig farms in northeast South Korea were used to determine the prevalence of the following selected swine pathogens: ten viral pathogens [porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), rotavirus, classical swine fever virus (CSFV), porcine circovirus type 2 (PCV2), encephalomyocarditis virus (EMCV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), pseudorabies virus (PRV) and Japanese encephalitis virus (JEV)] and four bacterial pathogens (*Brucella*, *Leptospira*, *Salmonella* and *Lawsonia intracellularis)*. In total, 1,260 tissue samples from 102 rodents and 24 stray cats were examined by specific PCR and RT-PCR assays, including tissue samples of the brain, tonsils, lungs, heart, liver, kidneys, spleen, small intestine, large intestine and mesenteric lymph nodes. The percentages of PCR-positive rodents for the porcine pathogens were as follows: 63.7% for *Leptospira*, 39.2% for *Brucella*, 6.8% for *Salmonella*, 15.7% for *L. intracellularis*, 14.7% for PCV2 and 3.9% for EMCV. The percentages of PCR-positive stray cats for the swine pathogens were as follows: 62.5% for *Leptospira,* 25% for *Brucella*, 12.5% for *Salmonella*, 12.5% for *L. intracellularis* and 4.2% for PEDV. These results may be helpful for developing control measures to prevent the spread of infectious diseases of pigs.

KEY WORDS: pathogens, prevalence, rodents, stray cats, swine farms.

doi: 10.1292/jvms.12-0568; *J. Vet. Med. Sci.* 75(12): 1647–1650, 2013

Rodents, cats and dogs around swine farms have been shown to be vectors or carriers of a number of swine pathogens [7, 8, 16, 18]. The contact and proximity of these animals with pigs may increase the likelihood of the co-transmission of infections [16]. Therefore, screening rodents and stray cats for swine pathogens is essential to determine the risk of microbial transmission on swine farms. However, such studies have rarely been performed in South Korea. To identify and assess the prevalence of swine pathogens carried by rodents and stray cats, we conducted a survey of the pathogens carried by rodents and stray cats in 2 provinces of South Korea. The assessed pathogens were 10 viral pathogens, including porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), rotavirus, classical swine fever virus (CSFV), porcine circovirus type 2 (PCV2), encephalomyocarditis virus (EMCV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), pseudorabies virus (PRV) and Japanese encephalitis virus (JEV) and 4 bacterial pathogens, including *Brucella* species (spp.), *Leptospira* spp., *Salmonella* spp. and *Lawsonia intracellularis*.

A total of 102 rodents and 24 cats were captured using wire traps placed around 9 selected pig farms in Gyeonggi and Gangwon provinces in South Korea. The traps were checked every day, and the bait was refreshed every second day for one-week periods in April and May in 2008. The collected animals were necropsied at the College of Veterinary Medicine of Kangwon National University. After gross examination, tissue samples of the brain, tonsils, lungs, heart, spleen, liver, kidneys, mesenteric lymph nodes and small and large intestines and were antiseptically collected and stored individually at −70°C until assayed. Total DNA and RNA were extracted from lysed tissue homogenates or bacterial cultures using commercial Genomic DNA Extraction kits (YGT300 and YGB300, RBC, Taipei, Taiwan) and a Viral RNA Extraction kit (iNtRON Biotechnology, Gyeonggi-do, Korea) according to the manufacturers' instructions. The DNA and RNA samples were stored at −70°C prior to use.

RT-PCR and PCR were conducted according to the protocols reported previously (Table 1). For the detection of PRRSV, JEV, CSFV, RV, TGEV, EMCV and PEDV, RT-PCR was performed using specific primer sets: ORF 7 F/R (637 bp), JEMF/R (619 bp), CSFV V324/326 (288 bp), rot3/5 (309 bp) and RNAs P1/P2 (859 bp), EMCV VP1 F/R (850 bp) and RNAs T1/T2 (651 bp), respectively [9, 14, 19, 21, 22, 31]. For PCV2, PRV and PPV DNA detection, PCR assays were conducted with the following specific primers: ORF 2 P1/P2 (494 bp), *gD* P3/P4 (217 bp) and VP2 P5/P6 (118 bp), respectively [2]. The genus-specific primers *BCSP*31 B4/B5 (223 bp) and *LipL*41F/R (408 bp) for *Brucella* and *Leptospira*, respectively, were also used for PCR [1, 28]. Finally, for *L. intracellularis* DNA detection, the 16S rDNA 878F/1050R specific primer (182 bp) was used [6].

The detection results of the pathogens in rodents and stray

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Table 1. Oligonucleotide primers used in the PCR detection of viral and bacterial pathogens causing porcine infectious diseases

Pathogen	Primer pairs and target region		Sequence	Products length (bp)	Reference
Leptospira spp.	*LipL41*	F	5'-GGCTATCTCCGTTGCACTCTTTG-3'	408	[28]
		R	5'-ATCGCCGACATTCTTTCTACACG-3'		
Brucella spp.	*BCSP31*	B4	5'-TGGCTCGGTTGCCAATATCAA-3'	223	[1]
		B5	5'-CGCGCTTGCCTTTCAAGGTCTG-3'		
Lawsonia intracellularis	16s rDNA	878F	5'-TAACGCGTTAAGCACC-3'	182	[6]
		1050R	5'-GTCTTGAGGCTCCCCGAAAGGCACCTCTTAATC-3'		
EMCV	VP1	F	5'-CACGGATATTGTAGTCCTGGT-3'	850	[19]
		R	5'-CGCACCTTCGGATATACTGTC-3'		
PEDV	RNAs	T1	5'-GGGCGCCTGTATAGAGTTTA-3'	651	[14]
		T2	5'-AGACCACCAAGAATGTGTCC-3'		
TGEV	RNAs	P1	5'-GATGGCGACCAGATAGAAGT-3'	859	[14]
		P2	5'-GCAATAGGGTTGCTTGTACC-3'		
Rotavirus	rot3/5	P3	5'-GGCTTTAAAAGAGAGAATTTC-3'	309	[9]
		P5	5'-GGTCACATCATACAATTCTAA-3'		
CSFV	V324/V326	F	5'-GGTGAGAGCAAGCCTCGCAAAGACAG-3'	288	[22]
		R	5'-CCCTACCTCACGGAATGGGGCAAAG-3'		
PRRSV	ORF 7	F	5'-GCCCCTGCCCAICACG-3'	637	[21]
		R	5'-TCGCCCTAATTGAATAGGTGA-3'		
PPV	VP2	P5	5'-GCAGTACCAATTCATCTTCT-3'	118	[2]
		P6	5'-TGGTCTCCTTCTGTGGTAGG-3'		
JEV	JEM	F	5'-CACGGAAGAGATGGGGCT-3'	619	[31]
		R	5'-GTCGACGCCCGCTTGAAGCT'-3'		
PCV2	ORF 2	P1	5'-ATCATGTGGCTCGCAAGCTT-3'	494	[2]
		P2	5'-TCCTTCTAGCACCAAGTACA-3'		
PRV	gD	P3	5'-ATGCCCWTAGTAGGACTAGCA-3'	217	[2]
		P4	5'-TCAACTCCATGTGCCATGTAC-3'		

Table 2. The prevalence of viral and bacterial pathogens causing porcine infectious diseases in rodents and stray cats in northeast areas of South Korea

Pathogen[b)]	Rodents No. positive / No. sample tested (%)	Stray cats No. positive / No. sample tested (%)	Total Prevalence (%)
Leptospira spp.	65/102 (63.7)	15/24 (62.5)	80/126 (63.5)
Brucella spp.	40/102 (39.2)	6/24 (25)	46/126 (36.5)
Salmonella spp. [a)]	7/102 (6.8)	3/24 (12.5)	10/126 (8.0)
Lawsonia intracellularis	16/102 (15.7)	3/24 (12.5)	19/126 (15.1)
EMCV	4/102 (3.9)	0/24 (0)	4/126 (3.2)
PEDV	0/102 (0)	1/24 (4.2)	1/126 (0.8)
TGEV	0/102 (0)	0/24 (0)	0/126 (0)
Rotavirus	0/102 (0)	0/24 (0)	0/126 (0)
CSFV	0/102 (0)	0/24 (0)	0/126 (0)
PRRSV	0/102 (0)	0/24 (0)	0/126 (0)
PPV	0/102 (0)	0/24 (0)	0/126 (0)
JEV	0/102 (0)	0/24 (0)	0/126 (0)
PCV2	15/102 (14.7)	0/24 (0)	15/126 (11.9)
PRV	0/102 (0)	0/24 (0)	0/126 (0)

a) Standard culture method was used to isolate *Salmonella* species. b) For detection of viral pathogens, the tissue samples of each rodent or cat including the brain, tonsil, lung, heart, liver, kidney, spleen, small intestine, large intestine and mesenteric lymph node were examined individually for each pathogenic agent. In regard to bacterial pathogens, the specific target tissues were selected to detect the presence of *Brucella* (spleen, liver and kidney), *Leptospira* (kidney), *Salmonella* (liver, spleen, small intestine and large intestine) and *Lawsonia intracellularis* (small intestine and large intestine).

cats are shown in Table 2. Isolation of *Salmonella* was attempted by pooling homogenized tissue samples from the liver, spleen, small and large intestine and isolates were identified using the API 20E test kit (bioMerieux, Durham, France). *Salmonella* was recovered from 10 of 126 samples (8%), including 6.8% of rodent samples and 12.5% of stray cat samples. Regarding the important causes of diarrhea in pigs, *L. intracellularis* was detected in intestinal samples from 15.1% of rodents and stray cats combined (15.7 and 12.5%, respectively). A high prevalence of *L. intracellularis* infection in herds and individual pigs (20 to 69% of the pigs tested) has previously been reported in South Korea, which suggests that the presence of *L. intracellularis* in rodents and stray cats is the likely source of infection of pigs [13, 17]. Fecal shedding of *L. intracellularis* can continue for up to several weeks in rodents and mice after inoculation [5]. Therefore, rodents may pose a risk as carriers.

The high prevalence of *Leptospira* and *Brucella* in rodents and stray cats captured around swine farms was surprising; 63.5% of all samples were positive for *Leptospira,* and 36.5% were PCR positive for *Brucella*. Because of the unexpectedly high detection rate, 8 to 10 of the PCR amplicons of both pathogens were sequenced. The sequencing analysis showed 99% similarity to the *Leptospira interrogans* serovars Pomona and Canicola and *Brucella* genus sequences deposited in GenBank (data not shown). *Leptospira* and *Brucella* are important zoonotic pathogens, and in South Korea, the prevalence of both pathogens in domestic animals and humans is relatively high [10, 12, 30]. Brucellosis has not been reported on swine farms in Korea. However, *Leptospira* has been detected in several pigs with reproductive problems [11, 12]. In the present study, rodents showed a higher infection rate than that reported previously, in which the average frequency of infection by *Leptospira* was 12.6% [4]. Because the time periods and the locations of the swine farms differ between the previous and present studies, direct comparison is difficult.

It has been reported that the PCR assay used for *Brucella* detection in this study also detects closely related bacterial species, such as *Oligella ureolytica* and *Ochrobactrum* anthropi [20, 24]. Because it is unknown whether such pathogens are prevalent in rodents and cats, false-positive results due to those pathogens cannot be excluded. Regardless, the relatively high prevalence of *Leptospira* and *Brucella* infections in rodents and stray cats observed in this study suggests that both species likely pose a significant risk to humans and have an economic impact on swine farms due to possible reproductive loss and illness.

Regarding the viral pathogens, TGEV, rotavirus, CSFV, PRRSV, PPV, PRV and JEV were not detected by PCR. Remarkably, four (3.9%) rodents tested positive for EMCV, and the virus was found in the lungs, heart, liver, kidneys and spleen (data not shown). Previous studies have reported that rodent extermination and prevention is an important factor in EMCV control. Rodents are already known as natural hosts of EMCV, and the infection of pigs by the ingestion of infected rodent feces or rodent carcasses has been reported [25–27]. Although the speed of transmission among rodents is slower than that observed in pigs, infected rodents shed virus in their feces for a long period of time. This implies that EMCV can persist in the rodent population by animal-to-animal virus transmission alone, which makes the rodent population a potential reservoir for EMCV in commercial pig farms. However, PEDV was not detected in any of the examined rodent tissues, although it was discovered in one cat (4.2%, 1/24). Interestingly, the virus was not found in the viscera where PEDV is normally located and was instead found only in the tonsils. PEDV has frequently been detected in pigs in Korea and has become one of the most important viral enteric diseases [3]. Despite vaccination programs, the loss caused by PEDV infection is continuous and serious in Korea. It has been reported previously that human and cat sera react positively in the PEDV ELISA [29]. Therefore, it is possible that cats may play a role in PEDV transmission on swine farms.

In the present study, PCR detected PCV2 in several rodent organs with an overall prevalence of 14.7% (15/102), including the tonsils, lymph nodes, spleen, lungs, liver, heart and kidneys. Our findings are in agreement with previous experimental studies in mice, which have demonstrated that viral replication occurs in mice following PCV2 inoculation. Inoculated mice develop lesions that are similar to those observed in pigs [15, 23]. It is possible that PCV2-infected rodents may replicate viruses *in vivo* and ultimately become continuous viral carriers. In this study, the animals were collected in the spring (April and May), which is the appropriate season for rodent activity, and virus transmission is most likely to occur during the spring. Therefore, rodent extermination may be beneficial and could potentially reduce viral transmission on swine farms.

To our knowledge, this is the first study demonstrating the prevalence of bacterial and viral pathogens in multiple organs in rodents and stray cats in South Korea. The results obtained show that the rodents and stray cats captured around pig farms carried *Brucella*, *Leptospira* spp., *L. intracellularis*, PCV2, EMCV and PEDV. Rodents, whether they are reservoirs or carriers of these pathogens, can spread pathogenic bacteria and viruses between locations, directly to farm animals and indirectly to humans. The negative results for TGEV, rotavirus, CSFV, PRRSV, PPV, PRV and JEV and the very low prevalence of *Salmonella* spp. may be related to the low prevalence of these pathogens in Korean farm animals. In conclusion, the risk for the transmission of pathogens by rodents should be seriously considered when choosing hygiene barriers on farms, and thus, rodents have to be eliminated by active measures to prevent the infection of pigs by disease agents present in the murine population.

ACKNOWLEDGMENT. This study was supported by funding from the Veterinary Science Technical Development projects of the NVRQS, South Korea.

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