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Emergence of Porcine Deltacoronavirus from Korean Domestic Farm, 2015

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Introduction: Two field strains (HKU15-44 and HKU15-155) of porcine deltacoronavirus (PDCoV) were firstly reported from fecal samples of swine in Hong Kong in 2012 (1). On February 2014, from cases showing clinical symptoms with severe diarrhea, PDCoV was identified in the United States (2). The viruses have nearly 99% identity to the above Hong Kong strains (1,2).

In April 2014, in another Asian country, PDCoV (KOR/KNU14-04/2014) was detected from diarrheic piglets in South Korea. The complete genomic sequence of KOR/KNU14-04/2014 was 98.8~99.8% identity with other PDCoV strains circulating China and USA (3). In this study, we further report the presence and genetic characterization of PDCoV from cases showing symptoms of diarrhea in Korean swine farms.

Materials and Methods: In this study, feces samples (n=681) collected from January 2013 to March 2015 were screened for the presence of porcine deltacoronavirus (PDCoV). Total RNA was extracted by using Trizol LS(Invitrogen, USA) following the manufacturer's instructions. The RNA was then converted into cDNA with the use of random hexamers and commercial RNA to cDNA EcoDry Premix kit (Clontech, Japan) following the manufacturer's protocol. For complete sequencing of the S and N genes of PDCoV, we followed the protocol described in the previous study (2). PDCoV positive samples were amplified with primer sets (PDCoV-SF2, PDCoV-SR2 and PDCoV-NF1, PDCoV-NR1). The specific PCR bands were purified by QIA quick Gel Extraction Kit (Qiagen,Germany), cloned utilizing TA cloning kit (Topcloner TA kit; Enzynomics,Korea), and subsequently transformed into competent *Escherichia coli* cells (DH5α). The purified recombinant plasmidswere sequenced by Macrogen Inc (Seoul, Korea). The genetic relationship of two PDCoV strains (SL2, SL5) with others coronaviruses was inferred from a codon-based alignment of 33 sequences of the complete S gene and 33 sequences of the complete N gene. The phylogenetic tree was reconstructed by the maximum likelihood model with 1000 bootstrap replicates implemented in fast tree program.

Results: The results of RT-PCR screening on a relatively large collection of feces samples (n = 681) from January 2013 to March 2015 did not reveal the presence of PDCoV until the end of 2014. However, on March 2015 year, the first PDCoV positive samples (SL2,SL5) were detected from SL swine farm in Gyeongbuk province. The phylogenetic trees based on the complete S gene and N gene showed that SL2 and SL5 clustered to the branch of PDCoV, related closer to PDCoV circulating in the United States and China rather than the previous PDCoV reported in South Korea (KNU14-04_ KM820765).

Conclusions: In this study, screening tests for the presence of PDCoV was done on 681 fecal samples of pigs, spanning January 2013 to March 2015, and covered all of 9 provinces of South Korea. However, by both of RT-PCR targeting S and N genes, samples collected until the end of 2014 were all negative for nucleic acid of PDCoV. It was on March 2015, the first PDCoV positive samples were detected in a 600- scale sow farm (SL farm) in Gyeongbuk province. For genetic characterization PDCoV strains detected from SL farm(SL2, SL5), the complete sequences of S gene (3,483 nucleotides) and N gene (1,029nucleotides) were obtained. The phylogenetic trees based on the S gene and N gene showed that SL2 and SL5 clustered to the branch of PDCoV. It was interesting to note that, both of SL2 and SL5 were closest to PDCoV circulating in the United States and China rather than the previous PDCoV reported in South Korea (KNU14-04_ KM820765). It was thus suggested that the emerging PDCoVs in South Korea might differentially introduced into pig farms.

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Simultaneous hemagglutinin subtyping andpathotyping of influenza A (H5N8) virus directly from clinical samples using adiagnostic microarray

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Introduction: Development of rapid diagnostic techniqueof HPAI virus will allow a more rapid response, in order to reduce the spreadof the disease and minimize economical loss of poultry industry. In the presentstudy, we developed a low-density microarray for the rapid detection andidentification of avian influenza virus hemagglutinin subtypes H5, H7 and H9. This microarray was also designed to detect HPAI by using the cleavage sitesequences of the HA gene. We evaluated this diagnostic microarray

usingclinical samples of HPAI H5N8 clade 2.3.4.6 viruses that caused the SouthKorean outbreaks during 2014 and 2015

Materials and Methods: A total of 55 probes were chosen usingin-house programs for the selection of HA subtype and pathotype-specificprobes. RT-PCR of HA, and M genes was performed for subtyping and pathotypingusing diagnostic microarray. The reverse primers for the HA, and M genes werelabeled with Cy-3 at the 5′ end. The Cy3-labeled RT-PCR products were thenhybridized to the microarray.

Results: All subtypes and pathotypes were correctlydetermined and were found to be identical to the nucleotide sequencing results.The assay can be completed in less than 6 h and can be performed using clinicalsamples without egg-propagation.

Conclusions: This diagnostic microarray has enormouspotential for the rapid subtyping and pathotyping of clinical samples for thedetection of HPAI viruses.

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Prevalence and pathogenic potential of *Campylobacter* isolates from wild birds in Korea

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Introduction: *Campylobacter* is a major cause of foodborne diarrheal illness in humans and the most common bacteria causing gastroenteritis worldwide (1). *Campylobacter* species can be isolated from a variety of sources, including wild and other domestic animals, food, and the environment; therefore, it poses a risk to public health. Wild birds function as effective disseminators of *Campylobacter* through fecal contamination of raw materials, food plants, or final products directly or indirectly. Despite the argument that the level of human infection caused by wild birds is too low to present a major health hazard to humans, human infection from wild birds is occasional but endless. Another important route of human infection is wild birds that mediate environmental contamination of surface water by droppings of *Campylobacter*. Wild birds have been identified as reservoirs for pathogenic *Campylobacter* and transmission to agricultural animals. Understanding the epidemiology of *Campylobacter* species in wild birds appears to be an essential part of the puzzle. However, little is known about the prevalence of these organisms in wild Asian birds. Thus, to address the lack of information and to gain more insight into the role of migratory birds as reservoirs or transporters of *Campylobacter*, we determined the occurrence of *Campylobacter jejuni* and *C. coli* isolated from migratory birds in Asia via Korea as well as determined the pathogenic potential of these *Campylobacter* isolates.

Materials and Methods: Isolation and identification of *Campylobacter* species.

This study was carried out from April to December 2012, and samples were collected from eight orders of live wild birds. Cloacal swab (one per individual) was placed in BBL™ CultureSwab™ containers (Difco, Detroit, MI, USA) and transported on ice to the laboratory within 24 h where they were pre-enriched in Bolton broth (Oxoid Ltd., Basingstoke, England) supplemented with cefoperazone, vancomycin, trimethoprim, and cycloheximide (Oxoid) for 24 h at 42°C in a microaerophilic environment of 10% CO_2, 5% O_2, and 85% N_2, and then were streaked onto modified Charcoal Cefoperazone Deoxycholate agar (Oxoid, UK). Presumptive *Campylobacter* colonies on the plates were further cultured on 5% sheep blood agar plates, the isolates were identified to the genus level by amplifying the 16S rRNA gene specific for *Campylobacter*.

Detection of virulence genes.

PCR was used to examine the presence of following virulence-associated genes in: *flaA, ciaB, cadF, wlaN, pldA, virB11, dnaJ, racR, cdtA, cdtB* and *cdtC*. The amplification was performed as described previously (2). The size of the obtained amplicon was verified using a 1.5% gel electrophoresis and visualized with UV trans-illumination after RedSafe™ (iNtRON Inc., Sungnam, Korea) staining.

Results: A total of 1,517 individual birds of various species captured throughout South Korea were tested for *Campylobacter* spp. The overall prevalence of *Campylobacter* spp. in all wild birds in this study was 6%. A total of 96 *Campylobacter* spp. were isolated from 23 bird species, including 68 isolates of *C. jejuni*, 16 of *C. coli*, and 13 unidentified species. A total of 53 isolates of *C. jejuni* and seven *C. coli* were examined for the presence of the virulence genes. The amplicons of *cadF* were detected in all of the *C. jejuni* isolates from wild bird; *cadF, flaA, cdtB* and *cdtC* were detected in all of the *C. coli* isolates. Noteworthy is the detection of the *wlaN* gene carried by *C. jejuni* isolated from wild bird with the prevalence of 9.4%

Conclusions: The goal of this study was to establish general information on the prevalence of *Campylobacter* in wild Korean birds. To gain insight to the pathogenicity of *C. jejuni and C. coli* isolates from wild birds, we characterized the isolates for the presence of major virulence-associated genes, such as cytotoxin expression, adherence, invasion and colonization. Furthermore, *wlaN* involved in the expression of ganglioside mimics in Guillain–Barre´syndrome was also identified in *C. jejuni* isolates from wild birds. *C. jejuni and C. coli* isolates from wild birds coupled with the occurrence of known virulence markers, suggests that wild birds in Korea shed *Campylobacter* spp. in their feces that are potentially pathogenic to humans. Wild birds are particularly relevant to the potential spread of pathogens because of their movement between human living areas,