Detection of Pathogenic Viruses in the Ambient Air in Seoul, Korea

Tae-Hee Han1 · Sang-Hun Park2 · Ju-Young Chung3 · Hyo-Won Jeong2 · Jihun Jung2 · Jae-In Lee2 · Young-Ok Hwang2 · Il-Young Kim2 · Jip-Ho Lee2 · Kweon Jung2

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Abstract
The possible transport of pathogenic microorganisms during Asian dust events could be an important concern for health workers; however, this is still uncertain owing to a lack of supporting evidence. The present study aimed to investigate the presence of pathogenic microorganisms in air samples collected during the Asian and non-Asian dust periods. Between March and September 2016, air samples were collected at three weather observation stations in Seoul using a high-volume air sampler. Multiplex PCR was performed using the Allplex™ respiratory and gastrointestinal panel assay kits to detect 46 microorganisms. RT-PCR was performed for kassievirus, Aichivirus, and human parechovirus (HPeV) detection. In total, 71 air samples were collected during the Asian (8 samples) and non-Asian (63 samples) dust periods. During an Asian dust event, only one human rhinovirus (HRV)-positive air sample was collected on April 23. During the non-Asian dust period, HRV, HPeV, norovirus (NoV), enteroaggregative \textit{Escherichia coli} (EAEC), enterotoxigenic \textit{E. coli} (ETEC), and \textit{Blastocystis hominis} were detected in four, two, one, one, one, and one air samples, respectively. Pathogenic viruses were mostly detected in ambient air samples during the non-Asian dust period, which suggests a possible air-borne transmission of viral pathogens; however, the role of Asian dust in epidemics caused by pathogenic viruses is unclear.

Keywords Air · Virus · Dust

Introduction
Asian dust is a meteorological phenomenon in which sand and particulate matter originating from the southeast of Mongolia and northwest of China are transported by the wind to Korea, Taiwan, Japan, and western USA. It is known to occur mostly in the spring, although it is sometimes observed throughout the year (Griffin 2007; Kim et al. 2006). Asian dust contains heavy metals (lead, mercury, or cadmium), air pollutants (NO$_3^-$, SO$_2^-$), and various microorganisms that could elicit harmful effects on human health. Increased microdust (PM10) and ultra-microdust (PM2.5) levels during the Asian dust period are associated with an increase in the daily mortality rates, cardiovascular diseases, cerebrovascular accidents, hospitalization by acute exacerbation of underlying pulmonary or allergic diseases (Kwon et al. 2002; Chen and Yang 2005; Kashima et al. 2017), and carcinogenic effects (Goldberg et al. 2017; Weichental et al. 2017).

The possible role of African dust events in transporting the viruses causing influenza or foot and mouth disease to European areas has been reported (Griffin et al. 2001). Recent studies regarding the detection of microorganisms in the ambient air during the Asian dust period were mostly focused on the composition of bacterial communities (Nishimura et al. 2010; Yamaguchi et al. 2016; Cha et al. 2016). Therefore, their role in transporting pathogenic viruses into the ambient air could not be clarified (Park et al. 2005; Chen et al. 2010). The purpose of the present study was to detect pathogenic viruses in the ambient air in Seoul during the Asian and non-Asian dust periods.
Materials and Methods

Air samples were collected at three meteorological observation stations located in the southern (Yangjae-dong, latitude: N37°27′, longitude: E127°1′), eastern (Gueui-dong, latitude: N37°31′, longitude: 127°05′), and northern (Jongro-dong, latitude: 37°36′, longitude: 126°59′) parts of Seoul.

Between March and September 2016, ambient air samples were collected at the flow rates of 6–8 L/min using polycarbonate (PC) filters (SKC Inc., Eighty-Four, PA) with the pore size of 0.4 µm for 4 h (Vereecken et al. 2010). The size of the particulate matter (> 0.3, > 0.5, > 0.7, > 1.0, > 2.0, and > 5.0 µm) was confirmed by a particle counter (ARTI HHPC-6 [HACH, Tokyo, Japan]). Information about Asian dust was obtained from the open access database of the Korean Meteorological association (http://web.kma.go.kr/info_open/public_data/guidepage). The particulate matter attached to membrane filters was separated by mixing them with 20 ml of phosphate buffered saline (PBS) and centrifuging at 3515×g. Then, supernatants were stored in a refrigerator at −80 °C.

RT-PCR was performed to detect respiratory viral pathogens [Influenza A (IF-A) and IF-B, respiratory syncytial virus (RSV)-A and RSV-B, human adenoviruses, human metapneumovirus (hMPV), human coronavirus (hCoV) 229E, hCoV NL63, hCoV OC43, parainfluenza-virus (PIV)-1, PIV-2, PIV-3, PIV-4, RV, human enterovirus, and human bocavirus] and bacterial respiratory pathogens ([Streptococcus pneumoniae, Haemophilus influenzae, Chlamydophila pneumoniae, Legionella pneumophila, Bordetella pertussis, Bordetella parapertussis, and Mycoplasma pneumoniae]) using the Allplex™ respiratory panel assay (Seegene, Korea).

The Allplex™ gastrointestinal panel assay (Seegene, Korea) was used to detect viral (rotavirus, NoV-I, NoV-II, astrovirus, enteric adeno- virus, sapovirus), bacterial [Salmonella spp., Shigella spp., Vibrio spp., Campylobacter spp., Clostridium difficile toxin B, Clostridium perfringens, Yersinia enterocolitica, E. coli O157:H7, enterohemorrhagic E. coli (stx1/2), enteropathogenic E. coli (eaeA), ETEC (lt/st), EAEC (aggR), Aeromonas spp.], and protozoan (Giardia lamblia, Cryptosporidium spp., Blastocystis hominis, Dientamoeba fragilis, Cyclospora cayetanensis) pathogens. Additionally, conventional RT-PCR was used to detect other gastrointestinal viral pathogens such as HPeV, klassevirus, NoV, and Aichivirus, as described previously (Han et al. 2011, 2014a, b).

Semi-nested PCR was performed to confirm the genotypes of HRV, NoV, GII, and HPeV using primers based on the VP4/VP2 (Han et al. 2009), capsid (Chung et al. 2010), and VP1 genes (Han et al. 2011). PCR products were purified with QIAquick (Qiagen), sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and subsequently resolved by an ABI 3730 XL autoanalyzer (Applied Biosystems). The resulting sequences were aligned by BioEdit v7.0 and a phylogenetic tree was constructed using MEGA 4.1 software. The finally obtained sequences were registered at the GenBank database as follows: HRV(MG204628-MG204632), HPeV(MG204633-204634), and NoV(MG198995).

Results

Between March and September 2016, 71 ambient air samples were collected in three meteorological stations located in Seoul (Yangjae-dong, Jayang-dong, and Pyeongchang-dong). Asian dust was observed for 6 days (March 6, March 8, April 22, April 23, April 24, and April 27). During the Asian dust period, ambient air samples were collected at Yangjae (six samples), Pyeongchang (one sample), and Jayang (one sample); 63 air samples were collected (Yangjae-26, Jayang-19, Pyeongchang-18) during the non-Asian dust period. The monthly distribution of the collected air samples was as follows: 20 samples were collected in March (6, 7, 8, 9, 10, 21, 22, and 30), 18 samples in April (5, 7, 9, 13, 19, 20, 22, 23, 24, and 30), 5 samples in May (7, 13, 28, and 29), 12 samples in June (9, 11, 21, and 23), 11 samples in July (12, 14, 21, and 28), 4 samples in August (2 and 4), and 1 sample in September (6). The daily average concentrations of PM10 were 52.1 and 44.8 µg/m³, whereas the daily average concentrations of PM2.5 were 51.4 and 44.5 µg/m³ in the Asian and non-Asian dust periods, respectively.

HRV was detected in ambient air samples collected during the non-Asian dust period on April 7 (Yangjae), June 9 (Yang Jae), June 23 (Jongro), and July 21 (Yangjae), 2016. However, only one air sample collected during the Asian dust period on April 23, 2016 (Table 1) was positive for HRV. Genotyping of HRV-positive samples was used to detect the presence of HRV-A and HRV-C in three and two samples, respectively (Fig. 1).

HPeV was detected in two air samples collected during the non-Asian dust period on March 9 (Yangjae) and March 10 (Yangjae), 2016; they were determined to be HPeV-1 (Fig. 2). A sample collected on June 21, 2016 (Yangjae) was positive for NoV, and it had the NoV GI-17 genotype (Fig. 3). RT-PCR was negative for the presence of klassevirus, Aichivirus, and NoV GIV in all samples collected during the Asian and non-Asian dust periods.

During the non-Asian dust period, EAEC and ETEC were detected in samples collected on March 10 (Yangjae) and March 30 (Jongro), respectively. B. hominis, one of the protozoan agents, was positive in a sample collected on April
The respiratory panel assay (Seegene, Korea) detecting seven kinds of respiratory bacterial pathogens was performed, and all samples were negative for the pathogens.

Discussion

The present study is the first to detect and genotype the viral pathogens HRV, HPeV, and NoV in ambient air samples collected in Seoul, Korea. Until now, the transmission of respiratory viruses by aerosol was mostly confirmed by studies on experimental animals, volunteers, and indoor samples collected from the hospital or office (Griffin 2007; Myatt et al. 2004), although HRV or influenza viruses have been previously detected in outdoor ambient air samples (Myatt et al. 2004).

The possible transmission of the hand, foot, and mouth disease virus to Europe by African dust was suggested (Griffin et al. 2001), but it was not confirmed in Korea during the Asian dust period (Park et al. 2005). In a recent study in Taiwan, the influenza virus was detected in ambient air samples during the Asian dust period (Chen et al. 2010; Myatt et al. 2004), which was not proven in a Korean study (Park et al. 2005). In the present study, the presence of HRV, one of the respiratory viral pathogens, was confirmed, for the first time, in ambient air samples in Seoul, although the presence of the influenza virus could not be detected. However, it is unlikely that Asian dust has an important role in epidemics caused by respiratory viral pathogens, because HRV was more frequently detected in non-Asian dust samples than in Asian dust samples.

The rate of positive detection of important respiratory viruses is known to have a close association with climatic variables such as temperature and humidity (du Prel et al. 2009). However, some limitations to this study exist, such as the short study period, small number of samples during the Asian dust period, and restricted sampling area, which should be considered with regard to the results of this study. It has also to be considered that this study could only show monitoring results of viruses in the ambient air, which could not conclude the role of Asian dust in spreading microbial agents. And it is difficult to prove the infectivity of detected viral sequences by biomolecular tests in the air.

Norovirus is one of the important viral pathogens causing acute gastroenteritis (AGE), which is mostly transmitted by the fecal to oral route, though it could also be transmitted by contact and airborne transmission (Nasaroff 2011; Nenonen et al. 2014; Bonifait et al. 2015). In the present study, NoV was detected for the first time in ambient air samples in Korea and had the genotype GII-17, which is presently prevalent worldwide (Chan et al. 2017).

Although HPeVs can be classified into several genotypes, HPeV-1 and HPeV-3 are the most clinically important genotypes. The HPeV-1 infection is mostly represented by a mild respiratory or gastrointestinal infection, but sometimes becomes clinically severe, leading to

Table 1  Detected microorganisms in the ambient air in Seoul

| Period          | Date of sampling | Sample location | Microorganisms detected |
|-----------------|------------------|-----------------|-------------------------|
| Asian dust      | 4/23/16          | Yangjae         | HRV-A                   |
| Non-Asian dust  | 4/7/16           | Yangjae         | HRV-A                   |
| Non-Asian dust  | 6/9/16           | Yangjae         | HRV-C                   |
| Non-Asian dust  | 6/23/16          | Jongro          | HPeV-1                  |
| Non-Asian dust  | 7/21/16          | Yangjae         | HPeV-C                  |
| Non-Asian dust  | 3/9/16           | Yangjae         | HPeV-1                  |
| Non-Asian dust  | 3/10/16          | Yangjae         | HPeV-1                  |
| Non-Asian dust  | 3/10/16          | Yangjae         | EAEC                    |
| Non-Asian dust  | 3/30/16          | Jongro          | ETEC                    |
| Non-Asian dust  | 4/5/16           | Yangjae         | B. hominis              |
| Non-Asian dust  | 6/21/16          | Yangjae         | NoV GII-17              |

Fig. 1  Phylogenetic analysis of the partial VP4/VP2 (−440 bp) gene of HRVs. The tree was constructed using the minimal evolution method with the Tamura–Nei model. The bootstrap values from 1000 replicates are shown on each branch. The strains in this study (MG204629-32) are shown in boldface.

5 (Yangjae). The respiratory panel assay (Seegene, Korea) detecting seven kinds of respiratory bacterial pathogens was performed, and all samples were negative for the pathogens.
sepsis-like illness and central nervous system infection in infants. HPeV-3 is considered to be one of the most important viral pathogens to be differentiated, especially in neonates and young infants with a sepsis-like illness (Han et al. 2013). The present study is the first to report the detection of HPeV-1 in ambient air samples during the non-Asian period. The study tried to detect other viral gastrointestinal pathogens such as Aichivirus, klassevirus, and NoV-GIV, which were proven to circulate in Korea in previous studies, but all ambient air samples showed negative results for these viruses in PCR.

The previous studies regarding bacteria in ambient air samples during the Asian dust period mostly focused on delineating the bacterial community using the metagenomic method, which showed different results depending on the region and sampling time (Yamaguchi et al. 2016; Cha et al. 2016). The present study aimed to investigate the presence of clinically important respiratory and gastrointestinal bacterial pathogens in ambient air samples using the multiplex RT-PCR panel assay, which was positive for EAEC and ETEC in two ambient air samples during the non-Asian dust period.

In conclusion, this study was the first to confirm the presence of pathogenic viruses, including HRV, NoV, and HPeV in ambient air in Korea. Most of these pathogenic viruses were detected in ambient air samples during the non-Asian dust period, which suggests that Asian dust might not play a major role in epidemics caused by viral pathogens, although further studies are needed to confirm these findings.

Fig. 2 Phylogenetic analysis of the partial VP1 (741-bp) gene of HPeVs. The tree was constructed using the minimal evolution method with the Tamura–Nei model. The bootstrap values from 1000 replicates are shown on each branch. The strains in this study (MG204633-4) are shown in boldface.
Fig. 3 Phylogenetic analysis of the partial capsid sequence (303 bp) of GI NoV. The tree was constructed using the minimal evolution method with the Tamura–Nei model. The bootstrap values from 1000 replicates are shown on each branch. The strain in this study (MG198995) is shown in boldface.

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