# Surveillance for West Nile Virus in Dead Wild Birds, South Korea, 2005–2008

## Jung-Yong Yeh, Hyun-Ju Kim, Jin-Ju Nah, Hang Lee, Young-Jun Kim, Jin-San Moon, In-Soo Cho, In-Soo Choi, Chang-Seon Song, and Joong-Bok Lee

To investigate the possibility of West Nile virus (WNV) introduction into South Korea, the National Veterinary Research and Quarantine Service has conducted nationwide surveillance of WNV activity in dead wild birds since 2005. Surveillance conducted during 2005–2008 found no evidence of WNV activity.

Wild birds are considered the principal hosts of West Nile virus (WNV). In the United States, surveillance of birds for WNV is used to quickly detect outbreaks and take action against its spread. The sampling of sick or dead birds can indicate WNV in a region before human and equine cases occur (1). This approach is considered the most effective method for detecting WNV in a specific region. During 1999, mass deaths among wild birds indicated the emergence and rapid spread of WNV in North America.

Although WNV has not yet been detected in South Korea, the perceived threat of its arrival has been highlighted by reports of WNV infection in a dead cinereous vulture (*Aegypius monachus*) in the Vladivostok region of Russia, which is adjacent to the Korean peninsula (2), and in several samples from cinereous vultures and cattle egrets (*Bubulcus ibis*) in the Russian Far Eastern Region during 2002–2004 (3). A variety of migratory birds, such as Mandarin ducks (*Aix galericulata*), cinereous vultures, bean geese (*Anser fabalis*), and white-fronted geese (*Anser albifrons*), fly from Russia to South Korea during the winter for the breeding season (4–6). Furthermore, Saito et al. recently reported that test results on several migrating birds captured in Japan were positive for flavivirus antibodies (7). This finding suggests that the threat of WNV in South Korea is increasing because many migratory birds share flyways over South Korea and Japan (8). Therefore, spread of the virus by migratory birds from WNV-infected areas, such as Russia, into uninfected hosts throughout the Korean peninsula is likely.

## The Study

A wide variety of bird species from all regions of South Korea were tested, and particular attention was paid to susceptible species and birds with neurologic signs. Carcasses of wild birds submitted to the Conservation Genome Resource Bank for Korean Wildlife, Seoul National University, Seoul, South Korea, were used for this study. The study also included samples from dead wild birds submitted to the Animal Disease Diagnostic Center of the National Veterinary Research and Quarantine Service of the Ministry of Food, Agriculture, Forestry and Fisheries of South Korea.

Investigation focused on the presumed peak period of mosquito vector activity (April-October) and included samples from dead wild birds. A total of 715 wild birds (belonging to 72 species) from all regions of South Korea were found dead and were examined during 2005-2008. All carcasses underwent postmortem examination, during which samples were obtained for diagnosis. In 2005, a total of 51 samples were tested; 167 samples were tested in 2006, 239 in 2007, and 258 in 2008. Taxonomic families of the collected birds and their migratory status are shown in the online Appendix Table (www.cdc.gov/EID/content/17/2/297appT.htm). Samples from Ae. monachus, A. fabalis, and A. albifrons birds, which are known to migrate from the Russian Eastern Region to South Korea (4,5), were included. Samples of dead wild birds such as Corvidae spp. and raptors (Accipitridae and Strigidae spp.), which have been identified as potential sources of WNV for resident birds (9,10), were also included.

Carcasses were subjected to necropsy, and brains and kidneys were obtained. Organs were homogenized in phosphate-buffered saline (10% suspension) and centrifuged. Ten 50% tissue culture infectious doses of a stock WNV were used as a control for antigen detection. WNV RNA in samples was investigated by reverse transcription–PCR with primers (Table). Information on the RNA extraction and the reverse transcription–PCR used is available in the online Technical Appendix (www.cdc.gov/EID/ content/17/2/297-Techapp.pdf).

During 2005–2008, we analyzed 1,309 organ samples (639 brain and 670 kidney) from dead birds for WNV RNA. WNV was not detected in these samples. Diagnostic examination of wild birds as a part of the nationwide surveillance has not detected patterns or clusters of birds with evidence of neurologic disease or viral encephalitides suggestive of

DOI: 10.3201/eid1702.100551

Author affiliations: National Veterinary Research and Quarantine Service, Gyeonggi-do, South Korea (J.-Y. Yeh, H-J. Kim, J.-J. Nah, J.-S. Moon, I.-S. Cho); Seoul National University, Seoul, South Korea (H. Lee, Y.-J. Kim); and Konkuk University, Seoul (I.-S. Choi, C.-S.Song, J.-B. Lee)

#### DISPATCHES

Primer	Sequence, 5' $\rightarrow$ 3'	Orientation*	Genome position†	Product size, bp
WN233	TTGTGTTGGCTCTCTTGGCGTTCTT	S	233	408
WN640	CAGCCGACAGCACTGGACATTCATA	AS	640	408
AmWN1401	ACCAACTACTGTGGAGTC	S	1401	445
AmWN1845	TTCCATCTTCACTCTACACT	AS	1845	445
AmWN1485	GCCTTCATACACACTAAAG	S (nested PCR)	1485	248
AmWN1732	CCAATGCTATCACAGACT	AS (nested PCR)	1732	248

Table. Oligonucleotide primers used for reverse transcription-PCR of West Nile virus in dead wild birds, South Korea, 2005–2008

WNV infection. Several cases of mass die-offs among wild birds were the result of chemical poisoning (11).

### Conclusions

Our surveillance of wild birds conducted during 2005– 2008 supports the hypothesis that WNV has not reached South Korea and corroborates findings of previous reports. In a study conducted at the National Institute of Health, Korea Centers for Disease Control and Prevention, 2,275 pools of mosquitoes were tested for WNV RNA; results for all samples obtained during 2006–2008 were negative (12). The study reported that 27 cerebrospinal fluid samples and 57 serum specimens obtained from patients who were suspected of having Japanese encephalitis and dengue fever were also negative for WNV. In another surveillance study of mosquitos and crows in Japan, a country near South Korea, no WNV RNA was detected. This study included mosquitoes obtained in a park in Tokyo during 2002–2006 and 329 captured or dead crows obtained during1994-2006 (13). In addition, antibodies against WNV antibodies were not detected in 18 crows sampled during 1995-2003. The first human WNV infection in Japan was confirmed in a person who returned from the United States in 2005 (14). However, no indigenous human or equine cases have been reported.

Although our surveillance found no evidence of WNV in South Korea, WNV could be introduced into this country in the near future. Moreover, several species of mosquitoes with the ability to transmit WNV have been identified in South Korea. Turell et al. reported that mosquitoes captured in Paju County, Gyeonggi Province, South Korea, were highly susceptible to WNV infection when they fed on viremic chickens (15).

Introduction of WNV into South Korea would undoubtedly become a major public health problem. An outbreak similar to the one that occurred in New York during 1999 could result in the disease becoming endemic to the country. Continued surveillance of dead wild birds is essential to enable prompt detection of WNV. Additionally, WNV surveillance programs in South Korea should continue to examine cases of viral encephalitis in horses and mass deaths among birds. Temperature increases caused by climate change should also be taken into account, and vigilant monitoring of emerging arboviruses, in addition to WNV, will be required. Finally, increased cooperation between the government and other agencies, such as wildlife conservation organizations and horse-racing authorities, is needed for early detection of WNV disease and development of effective veterinary and public health strategies.

This study was supported by a grant from the National Veterinary Research and Quarantine Service, Republic of Korea.

Dr Yeh is a researcher at the National Veterinary Research and Quarantine Service in South Korea. His main research interests are emerging and zoonotic infectious diseases, vector-borne pathogens, and *Lawsonia intracellularis*.

#### References

- Eidson M, Kramer L, Stone W, Hagiwara Y, Schmit K. Dead bird surveillance as an early warning system for West Nile virus. Emerg Infect Dis. 2001;7:631–5. DOI: 10.3201/eid0704.010405
- Loktev VB. West Nile virus, vulture—Russia (VLADIVOSTOK): ProMED-MAIL; 2004 [cited 2010 Dec 21]. http://www.promed mail.org
- Ternovoĭ VA, Protopopova EV, Surmach SG, Gazetdinov MV, Zolotykh SI, Shestopalov AM, et al. The genotyping of the West Nile virus in birds in the Far Eastern Region of Russia in 2002–2004 [in Russian]. Mol Gen Mikrobiol Virusol. 2006; 4:30–5.
- 4. Jin S-D, Baek U-K. Research on wintering of *Aegypius monachus* in Korea. Mun Hwa Jae. 2009;42:62–71.
- Kim J, Park J, Yoo B, Rhee D. The migration route and monitoring of the migratory birds in Korea. In: Wildlife Biology. Inchon (South Korea): National Institute of Environmental Research; 2002. Report 24:153–64.
- Moores N, Park J-G, Kim A. The birds Korea checklist: 2009 [cited 2010 Dec 21]. http://www.birdskorea.org
- Saito M, Osa Y, Asakawa M. Antibodies to flaviviruses in wild ducks captured in Hokkaido, Japan: risk assessment of invasive flaviviruses. Vector Borne Zoonotic Dis. 2009;9:253–8. DOI: 10.1089/ vbz.2008.0111
- Lee W-S, Gu T-H, Park J-Y. A field guide to the birds of Korea. Seoul (South Korea): LG Foundation; 2005.
- Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis. 2003;9:311– 22.
- Nemeth N, Gould D, Bowen R, Komar N. Natural and experimental West Nile virus infection in five raptor species. J Wildl Dis. 2006;42:1–13.

### Surveillance for WNV in Dead Wild Birds

- 11. Genome Resource Bank for Korean Wildlife. 2009 [cited 2010 Dec 22]. http://www.cgrb.org/index\_e.htm
- Han M-G, Lee H-I, Lee C-S, Lee W-G, Jeong Y-E, Cho J-E, et al. Surveillance of West Nile viruses from 2006 to 2008, Korea: no evidence of infection. The Microbiological Society of Korea's International Symposium. May 28–30, 2009. Jeju Island, Seoul (South Korea): The Microbiological Society of Korea; 2009. p. 237.
- Tabei Y, Hasegawa M, Iwasaki N, Okazaki T, Yoshida Y, Yano K. Surveillance of mosquitoes and crows for West Nile virus in the Tokyo metropolitan area. Jpn J Infect Dis. 2007;60:413–6.
- Koizumi K, Nakajima Y, Matsuzaki M, Koido N, Ohsone Y, Lim CK, et al. First report of West Nile fever in Japan [in Japanese]. Kansenshogaku Zasshi. 2006;80:56–7.
- Turell MJ, Mores CN, Dohm DJ, Lee WJ, Kim HC, Klein TA. Laboratory transmission of Japanese encephalitis, West Nile, and Getah viruses by mosquitoes (Diptera: Culicidae) collected near Camp Greaves, Gyeonggi Province, Republic of Korea 2003. J Med Entomol. 2006;43:1076–81. DOI: 10.1603/0022-2585(2006) 43[1076:LTOJEW]2.0.CO;2

Address for correspondence: Jung-Yong Yeh, National Veterinary Research and Quarantine Service, Anyang 430-824, South Korea; email: yeh02@nvrqs.go.kr

