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

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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.

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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 anti-

bodies (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/17/2/297-appT.htm](http://www.cdc.gov/EID/content/17/2/297-appT.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](http://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 encephalitis suggestive of

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

| Primer   | Sequence, 5' →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              |

\*S, sense; AS, antisense.

†Genbank accession no. NC\_009942.

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 during 1994–2006 (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.

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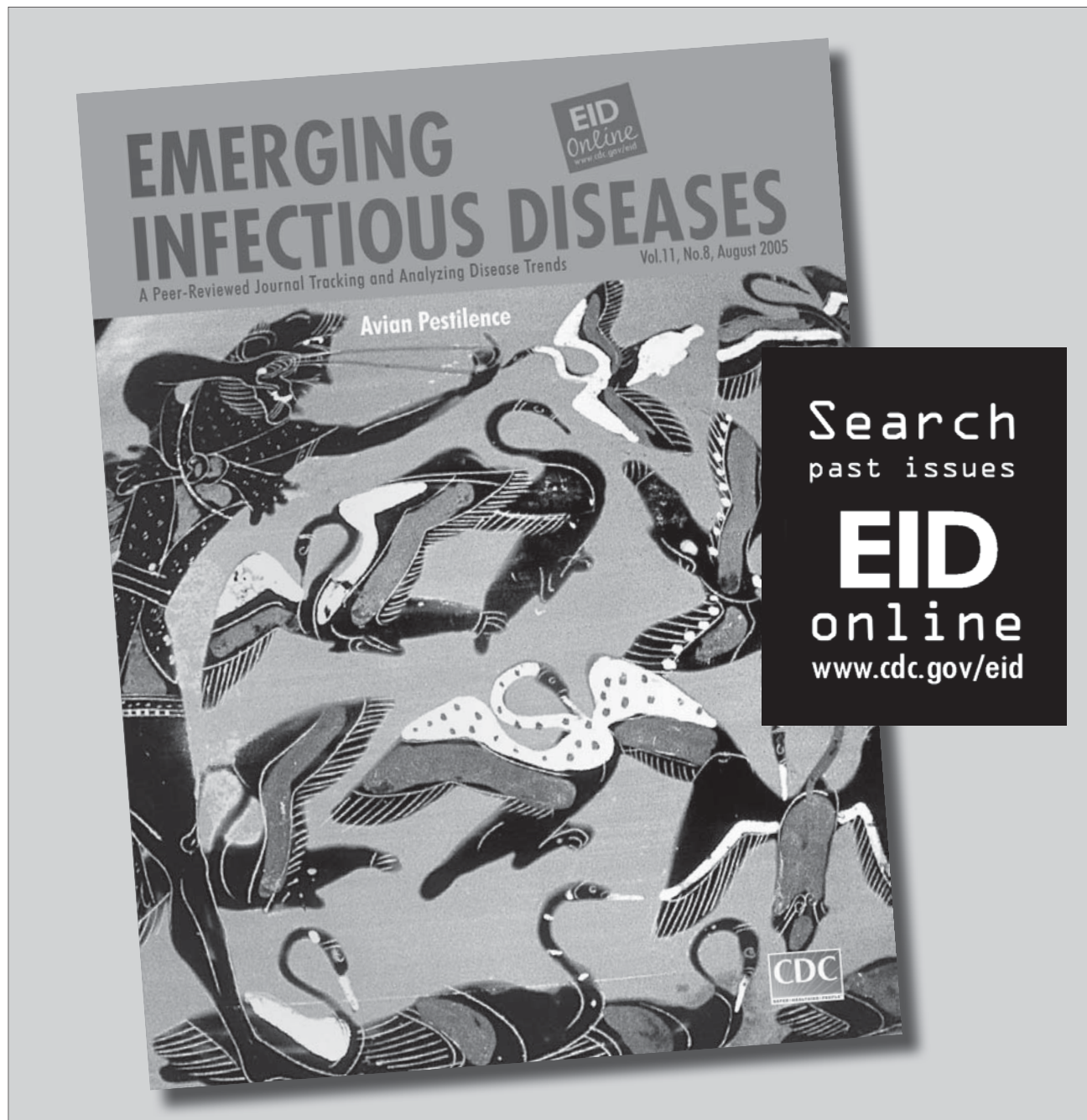
Dr Yeh is a researcher at the National Veterinary Research and Quarantine Service in South Korea. His main research interests are emerging and zoonotic infectious diseases, vector-borne pathogens, and *Lawsonia intracellularis*.

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## Technical Appendix

### Reverse Transcription–PCR Methods

Total RNA was extracted from 50 mg–100 mg of tissue by using the BioRobot M48 (QIAGEN, Valencia, CA, USA) and the MagAttract RNA Cell Mini M48 Kit (QIAGEN). Extracts were eluted into 100  $\mu$ L nuclease-free water. All samples were extracted and tested in duplicate. A West Nile virus (WNV) control was prepared by extracting RNA from a 100- $\mu$ L volume containing ten 50% tissue culture infective doses of WNV stock virus. Extracted RNA samples were denatured at 70°C for 10 min.

As reported by Johnson et al. (1), reverse transcription–PCR (RT-PCR) was performed by using a 1-step RT-PCR kit (QIAGEN) with 37.5 pmol/L of each of the 2 first-stage primers. Similarly, 2.0  $\mu$ L of RNase-free water was added to no-template controls that were incubated with diagnostic samples. Reaction tubes were incubated at 45°C for 45 min and 95°C for 11 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 45 s, and primer extension at 72°C for 60 s.

The nested RT-PCR cycle used similar conditions except for a 5-min primer extension period. For the nested reaction, 1.5  $\mu$ L of the first-stage amplification product was added to 48.5  $\mu$ L of a PCR mixture that contained 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 2.0 mmol/L MgCl<sub>2</sub>, 0.8 mmol/L dNTP pool, 1.0 unit Taq DNA polymerase (QIAGEN), and 37.5 pmol each of the nested primers. Reaction tubes were incubated for 11 min at 95°C, followed by 35 cycles of the cycling conditions described for the first stage. All incubation and amplification



procedures were performed by using a Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany).

After RT-PCR, amplification products (5  $\mu$ L) were analyzed by electrophoresis on a 3% agarose gel containing 0.5  $\mu$ g/mL ethidium bromide. A 248-bp product indicated that WNV RNA was in the original sample. Following the method of Lanciotti et al. (2), we used a 1-step RT-PCR kit with 5  $\mu$ L of RNA and 50 pmol/L of each primer in a 50- $\mu$ L reaction volume and the following cycling times and temperatures: 1 cycle at 45°C for 1 h and at 94°C for 3 min and 40 cycles at 94°C for 30 s, 55°C for 1 min, and 68°C for 3 min. After RT-PCR, amplification products (5  $\mu$ L) were analyzed by gel electrophoresis on a 3% agarose gel containing 0.5  $\mu$ g/mL ethidium bromide. A 408-bp product indicated that WNV RNA was in the original sample. RNA integrity was confirmed by RT amplification of  $\beta$ -actin mRNA in all samples.

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