

ties in Asia. CDAD is estimated to cost the healthcare system in the United States \$3.2 billion annually (8). With longer hospitalization for persons after they test positive for CDT, as seen in our study, rising CDAD rates could increase hospital occupancy and result in excess healthcare expenditures.

CDAD in Asia is an emerging challenge that needs to be recognized. Its control will ultimately depend on priority being given to epidemiologic surveillance, infection control, and stewardship of antimicrobial agents.

**Poh Lian Lim,
Timothy M.S. Barkham,
Li Min Ling,
Frederico Dimatatac,
Tamuno Alfred,
and Brenda Ang**

Author affiliations: Tan Tock Seng Hospital, Singapore (P.L. Lim, T.M.S. Barkham, L.M. Ling, F. Dimatatac, B. Ang); and Clinical Trials & Epidemiology Research Unit, Singapore (T. Alfred)

DOI: 10.3201/eid1409.070043

References

- Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med*. 2002;346:334–9. DOI: 10.1056/NEJMc0111603
- McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353:2433–41. DOI: 10.1056/NEJMoa051590
- Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*. 2005;353:2442–9. DOI: 10.1056/NEJMoa051639
- McCusker ME, Harris AD, Perencevich E, Roghmann M-C. Fluoroquinolone use and *Clostridium difficile*-associated diarrhea. *Emerg Infect Dis*. 2003;9:730–3.
- Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *CMAJ*. 2004;171:33–8. DOI: 10.1503/cmaj.1040876
- Cunningham R, Dale B, Undy B, Gaunt N. Proton pump inhibitors as a risk factor for *Clostridium difficile* diarrhea. *J Hosp Infect*. 2003;54:243–5. DOI: 10.1016/S0195-6701(03)00088-4
- Alfa MJ, Du T, Beda G. Survey of incidence of *Clostridium difficile* infection in Canadian hospitals and diagnostic approaches. *J Clin Microbiol*. 1998;36:2076–80.
- O'Brien JA, Lahue BJ, Caro JJ, Davidson DM. The emerging infectious challenge of *Clostridium difficile*-associated disease in Massachusetts hospitals: clinical and economic consequences. *Infect Control Hosp Epidemiol*. 2007;28:1219–27. DOI: 10.1086/522676

Address for correspondence: Poh Lian Lim, Department of Infectious Diseases, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433; email: pllim@post.harvard.edu

West Nile Virus in Golden Eagles, Spain, 2007

To the Editor: Although West Nile virus (WNV) has not been isolated in Spain, several recent studies provide evidence for its circulation in this country (1–5). We report isolation of WNV in Spain from 2 golden eagles (*Aquila chrysaetos*).

A captive-bred 2-year-old male golden eagle (GE-1) was released into the wild in central Spain. The bird's location was monitored daily by telemetry, and it remained within a radius of 100 km from its original release point. On September 15, 2007 (1 month after release), it was found moribund and was moved to a rehabilitation and captive breeding center for endangered raptors. Upon admission, the bird was in fair condition but debilitated and aggressive. It then became increasingly disorientated, showed a head tilt, and died 5 days

after admission, despite intensive supportive care and treatment for secondary infections.

Eleven days after admission of GE-1, an adult male golden eagle (GE-2) and an adult female Bonelli's eagle (*Hieraetus fasciatus* [BE-1]) living in pairs (with a golden eagle and a Bonelli's eagle, respectively) in enclosures were found disorientated, debilitated, and with impaired vision. Both birds were placed in isolation and received intensive supportive care; they slowly recovered. The respective pair of each bird (GE-3 and BE-2, respectively) remained asymptomatic. A magpie (MP-1) that had entered the golden eagle enclosure 5 days before admission of GE-1 was also placed in isolation, but remained healthy. After necropsy of GE-1, tissue samples (brain, kidney, and spleen) from this bird and oropharyngeal swabs from GE-2, BE-1, and MP-1 (obtained at day 11 after admission of GE-1) were subjected to virologic analysis.

Avian influenza and Newcastle disease were excluded by reverse transcription-PCR (RT-PCR) (6,7) of oropharyngeal and cloacal swabs from GE-1, GE-2, BE-1, and MP-1. Real-time RT-PCR specific for WNV (8) was conducted with brain, kidney, and spleen tissue homogenates from GE-1 and oropharyngeal swabs from GE-2, BE-1, and MP-1. All samples except that from MP-1 yielded specific WNV genome amplification products, which were confirmed after amplification and sequencing by using a previously described method (9).

Serum samples from clinically affected eagles (GE-1, GE-2, and BE-1), the magpie (MP-1), and the healthy Bonelli's eagle (BE-2) contained WNV-neutralizing antibodies detected by a virus neutralization test performed as described (4,5). A serum sample from GE-3 (asymptomatic) remained negative up to 74 days after admission of GE-1. Specificity of the neutralization test was assessed

by titration in parallel against a second, cross-reacting flavivirus (Usutu virus). Results showed that the highest titers were always obtained against homologous virus (WNV).

Virus isolation was conducted by placing filter-sterilized, clarified tissue homogenates (brain, kidney and spleen) from GE-1 and oropharyngeal swab eluate from GE-2 onto monolayers of BSR (baby hamster kidney) cells and Vero cells. The remaining 2 samples (oropharyngeal swabs from BE-1 and MP-1) were negative for virus. Isolates were identified by using real-time and conventional RT-PCR (8,9). WNV-specific cDNAs from the nonstructural protein 5-coding region of the genome (171 nt) were amplified by RT-PCR (9) from brain tissue of GE-1 (sample GE-1b), oropharyngeal swab of BE-1 (sample BE-1o), and first-passage infection supernatant of oropharyngeal swab from GE-2 (sample GE-2o). These samples were

subjected to molecular analysis. Nucleotide sequences from the 3 samples were identical, except at 1 nt position in BE-1o (GenBank accession nos. EU486169 for GE-1b, EU486170 for GE-2o, and EU486171 for BE-1o). Phylogenetic analysis matched these isolates most closely with recent western Mediterranean WNV isolates within lineage 1a (Figure).

WNV was detected in 3 eagles of 2 species. The birds with the index and secondary cases had no direct contact. Transmission could have occurred through mosquito bites. The 2-year-old golden eagle died as a result of infection, and the 2 remaining infected eagles recovered. The 3 ill birds were potentially more susceptible because of stress (GE-1) or age (GE-2 and BE-1 were older birds). Serologic analysis detected WNV-specific antibodies in the affected birds and some contacts. Nucleotide sequence analysis showed high genetic identity among these new

isolates, which cluster within lineage 1a of WNV.

Although information on WNV in Spain is scarce, its detection and relationship to the death of a raptor in the wild are of concern because many species of eagles, including the Spanish imperial eagle (*A. adalberti*), are endangered species. We recently found evidence of WNV infection in several Spanish imperial eagles sampled during 2001–2005 (5). Studies are ongoing to further characterize genetic and biologic properties of the new WNV isolates described to identify their genetic relationships with other WNV strains and to clarify the epidemiology of WNV in the study region.

Acknowledgments

We thank the personnel of the Centro de Estudios de Rapaces Ibéricas for their efforts in this study; Vanessa Rodríguez for laboratory assistance; Montserrat Agüero for helpful discussions; and the Junta de Comunidades de Castilla–La Mancha for support. This study is a contribution to the epidemiologic network of rehabilitation centers in Castilla–La Mancha and the Red de Vigilancia Sanitaria de Castilla–La Mancha network in Castilla–La Mancha.

This study was supported in part by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (OT01-002).

**Miguel Angel Jiménez-Clavero,
Elena Sotelo, Jovita Fernández-
Pinero, Francisco Llorente,
Juan Manuel Blanco,
Julia Rodríguez-Ramos,
Elisa Pérez-Ramirez,
and Ursula Höfle**

Author affiliations: Centro de Investigación en Sanidad Animal, Valdeolmos, Spain (M.A. Jiménez-Clavero, E. Sotelo, J. Fernández-Pinero, F. Llorente); Centro de Estudios de Rapaces Ibéricas, Sevilleja de la Jara, Spain (J.M. Blanco, J. Rodríguez-Ramos, U. Höfle); and Instituto de Investigación en Recursos Cinegéticos, Ciudad

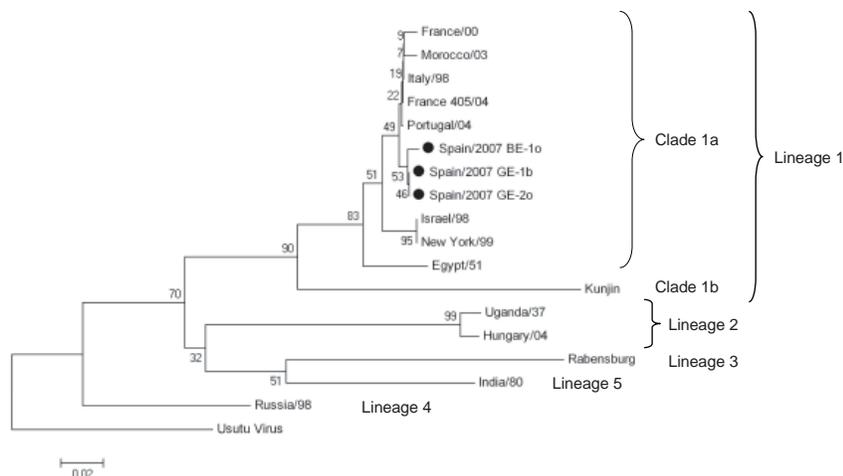


Figure. Phylogenetic tree of 18 partial nonstructural protein 5 West Nile virus nucleotide sequences (171 nt for each isolate, except 126 nt available for the Portugal/04 isolate) constructed with MEGA version 4 software (www.megasoftware.net). The optimal tree was inferred by using the neighbor-joining method. The percentage of successful bootstrap replicates ($N = 1,000$) is indicated at nodes. Evolutionary distances were computed with the Kimura 2-parameter method (with gamma correction). All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons. Branch lengths are proportional to the number of nucleotide changes (genetic distances). Scale bar shows number of base substitutions per site. Isolates sequenced in this study are indicated by solid circles. GenBank accession nos. are as follows: France/00 (AY268132), Morocco/03 (AY701413), Italy/98 (AF404757), France 405/04 (DQ786572), Portugal/04 (AJ965630), Israel/98 (AF481864), New York/99 (DQ211652), Egypt/51 (AF260968), Kunjin (D00246), Uganda/37 (M12294), Hungary/04 (DQ116961), Rabensburg (AY765264), India/80 (DQ256376), Russia/98 (AY277251), and Usutu virus (NC_006551) (outgroup).

Real, Spain (E. Perez-Ramirez, U. Höfle)

DOI: 10.3201/eid1409.080190

References

1. Bofill D, Domingo C, Cardeñoso N, Zaragoza J, de Ory F, Minguell S, et al. Human West Nile virus infection, Catalonia, Spain. *Emerg Infect Dis.* 2006;12:1163–4.
2. Kaptoul D, Viladrich PF, Domingo C, Niubó J, Martínez-Yélamos S, de Ory F, et al. West Nile virus in Spain: reports of the first diagnosed case (in Spain) in a human with aseptic meningitis. *Scand J Infect Dis.* 2007;39:70–93. DOI: 10.1080/00365540600740553
3. Figuerola J, Sorriquer R, Rojo G, Gómez-Tejedor C, Jiménez-Clavero MA. Seroconversion in wild birds and local circulation of West Nile virus, Spain. *Emerg Infect Dis.* 2007;13:1915–7.
4. Figuerola J, Jiménez-Clavero MA, Rojo G, Gómez-Tejedor C, Sorriquer R. Prevalence of West Nile virus neutralizing antibodies in colonial aquatic birds in southern Spain. *Avian Pathol.* 2007;36:209–12. DOI: 10.1080/03079450701332329
5. Höfle U, Blanco JM, Crespo E, Naranjo V, Jiménez-Clavero MA, Sanchez A, et al. West Nile virus in the endangered Spanish imperial eagle. *Vet Microbiol.* 2008;129:171–8. DOI: 10.1016/j.vetmic.2007.11.006
6. Ward CL, Dempsey MH, Ring CJ, Kempson RE, Zhang L, Gor D, et al. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. *J Clin Virol.* 2004;29:179–88. DOI: 10.1016/S1386-6532(03)00122-7
7. Creelan JL, Graham DA, McCullough SJ. Detection and differentiation of pathogenicity of avian paramyxovirus serotype 1 from field cases using one-step reverse transcriptase polymerase chain reaction. *Avian Pathol.* 2002;31:493–9. DOI: 10.1080/0307945021000005860
8. Jiménez-Clavero MA, Agüero M, Rojo G, Gómez-Tejedor C. A new fluorogenic real-time RT-PCR assay for detection of lineage 1 and lineage 2 West Nile viruses. *J Vet Diagn Invest.* 2006;18:459–62.
9. Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J Clin Microbiol.* 2001;39:1922–7. DOI: 10.1128/JCM.39.5.1922-1927.2001

Address for correspondence: Ursula Höfle, Instituto de Investigación en Recursos Cinegéticos, Consejo Superior de Investigaciones Científicas, Universidad de Castilla–La Mancha, Junta de Comunidades de Castilla–La Mancha, Ciudad Real, Spain; email: ursula.hofle@uclm.es

Merkel Cell Polyomavirus and Merkel Cell Carcinoma, France

To the Editor: Merkel cell carcinoma (MCC) is a primary cutaneous neuroendocrine tumor. This aggressive skin cancer is uncommon but increasing in frequency. During 1986–2001, incidence rate tripled; average annual increase was 8% (1). MCC shares epidemiologic features with Kaposi sarcoma, a malignant tumor associated with human herpesvirus 8 infection (2). In particular, MCC affects predominantly immunocompromised patients such as organ transplant recipients (3,4), patients with B-cell lymphoid tumors (5), and patients with AIDS (6). This similarity between MCC and Kaposi sarcoma may support the hypothesis of an infectious origin of MCC.

A new polyomavirus, provisionally named Merkel cell polyomavirus (MCPyV), has been recently identified in tumor tissue from patients with MCC. Furthermore, clonal integration of viral DNA within the tumor genome was observed in most of the cases (7). To assess the implication of MCPyV in MCC, we tested tumor biopsy samples collected from 9 patients with MCC. Patient median age was 65 years, and 2 patients were immunocompromised (patient 1 had a lymphoma, which was treated with rituximab; patient 7 had psoriatic rheumatism, which was treated with corticosteroids and meth-

otrexate). As controls, biopsy samples from 15 patients with diverse proliferative or inflammatory skin or mucosa lesions were tested (Table).

DNA was extracted from fresh tissue samples by using the QIAamp DNA Mini Kit (QIAGEN, Courtaboeuf, France) according to the manufacturer's instructions. Paraffin was removed from previously formaldehyde-fixed, paraffin-embedded biopsy samples with xylene, and the samples were rehydrated with decreasing concentrations of ethanol. The extracts were tested for MCPyV DNA by PCR using 3 sets of primers initially described by Feng et al. (7) to target the predicted T-antigen (LT1 and LT3 primer pairs) and the viral capsid (VP1 primer pair) coding regions. Extracted DNA (5 µL) was added to 45 µL of the reaction mixture, which contained 5 µL 10× PCR buffer, 10 µL 5× Q-solution (QIAGEN), 2.5 mmol/L MgCl₂, 200 µmol/L each dNTP, 2.5 units Taq DNA polymerase (QIAGEN), and 15 pmol of each primer. Touchdown PCR conditions were as follows: 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s; annealing at 61°C (10 cycles), 59°C (10 cycles), and 57°C (15 cycles) for 30 s; extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. Amplification products were subjected to electrophoresis in a 2% agarose, 1× Tris-borate-EDTA gel stained with ethidium bromide and examined under UV light. The sizes of the fragments amplified with the LT1, LT3, and VP1 primers pairs were 439, 308, and 351 bp, respectively. A negative control was included in each experiment; positive samples were confirmed by analyzing a second stored sample aliquot, and the amplified fragments were sequenced by using the same primers used for the amplification. The sequences were submitted to GenBank under accession numbers AM992895–AM992906. Total DNA level in sample extracts was measured by using the LightCycler control DNA kit targeting the β-globin gene (Roche