

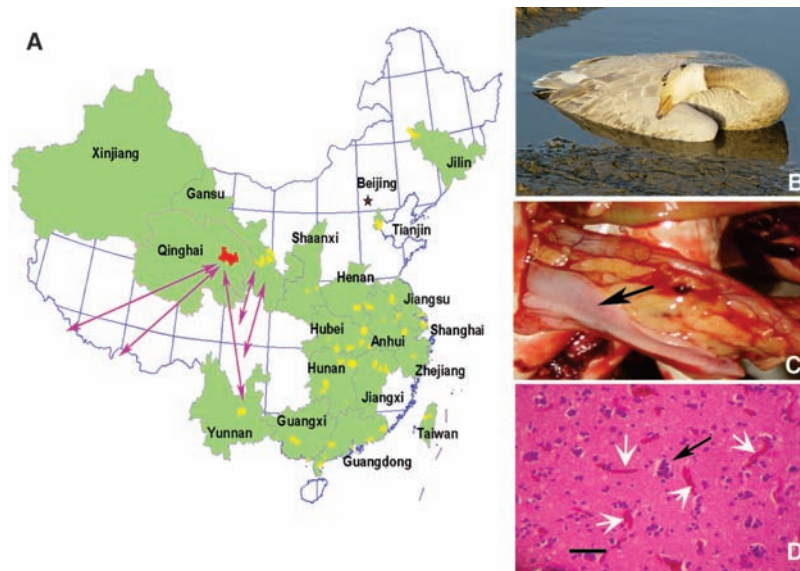
## Highly Pathogenic H5N1 Influenza Virus Infection in Migratory Birds

J. Liu,<sup>1\*</sup> H. Xiao,<sup>2,4\*</sup> F. Lei,<sup>3\*</sup> Q. Zhu,<sup>5</sup> K. Qin,<sup>1</sup> X.-w. Zhang,<sup>6</sup> X.-l. Zhang,<sup>1</sup> D. Zhao,<sup>1</sup> G. Wang,<sup>2,4</sup> Y. Feng,<sup>2,4</sup> J. Ma,<sup>2</sup> W. Liu,<sup>2</sup> J. Wang,<sup>6</sup> G. F. Gao<sup>2,†</sup>

Avian influenza virus (AIV) involving at least three subtypes, H5, H7, and H9, has emerged as an important pathogen in the poultry industry and is of major current global health concern (1). The first case report of chicken-to-human transmission was in Hong Kong in 1997 (2); since 2003, H5N1, a highly pathogenic AIV, has emerged in 10 Asian countries, including Thailand, Vietnam, and China (Fig. 1), and has claimed at least 53 human lives. Until recently, migratory waterfowl seemed to be exempt from widespread infection, although sporadic cases were recorded (3). Here we describe an outbreak of highly pathogenic H5N1 infection among waterfowl in Lake Qinghaihu, Gangcha County, Qinghai Province, in western China (Fig. 1).

On 4 May 2005, a few birds were found dead on Bird Island, and by the end of June more than a thousand birds were affected. This lake is one of the most important breeding locations for migratory birds that overwinter in Southeast Asia, Tibet, and India (Fig. 1). Several species were infected, including the bar-headed goose (*Anser indicus*), great black-headed gull (*Larus ichthyaetus*), and brown-headed gull (*Larus brunnicephalus*). Two key symptoms were noticed: abnormal neurological signs (tremor and opisthotonus) and diarrhea. Among the gross lesions, pancreatic necrosis was obvious and was confirmed by tissue section where extensive areas of lytic necrosis were seen, consistent with pathology observed in domestic geese and ducks infected with H5N1 AIV (3). Brain sections revealed glial cell infiltration, perivascular cuffing, and congestion in the blood vessels. Serological tests (4) from one bar-headed goose and one brown-headed gull confirmed the presence of high-titer antibody against H5N1 AIV.

Several H5N1 viruses were isolated from the viscera, brain, and swabs of the oropharynx and cloaca of sick and dead birds. Four of the isolates from different bird species were com-



**Fig. 1.** (A) The reported H5N1 AIV prevalence sites during the 2004 outbreak in China are highlighted in yellow (8). Arrows indicate the migratory routes of the bar-headed goose (A. *indicus*) to Lake Qinghaihu. (B) A sick bar-headed goose showing typical opisthotonus before dying. (C) Bar-headed goose pancreas with pin-point necrotic lesions (arrow). (D) Microscopic lesions in bar-headed goose brain, showing congestion in the blood vessels (white arrows) and glial cell infiltration (black arrow). Hematoxylin and eosin  $\times 25$  (scale bar, 50  $\mu\text{m}$ ).

pletely sequenced (4) and appeared to be closely related. None of the GenBank sequence data for known H5N1 AIV genomes completely matched our sequences, implying the viruses are reassortants. Five of the eight genomic segments (M, PA, PB1, PB2, and NS) were closely related to a Hong Kong 2004 isolate (A/peregrine falcon/HK/D0028/04) (3). We observed several characteristics in our four isolates: first, the sequence PQGERRRKKR/G, denoting multiple basic amino acids at the cleavage site of the hemagglutinin; second, a virulence island in the PB2 gene, E627K, first seen in Hong Kong in 1997 (5); and third, a deletion of 20 amino acids in neuramidase (amino acid positions 49 to 69), also associated with high virulence.

To test virulence, mice and chickens were infected with the BhGoose/QH/1/05 (4) isolate.

All eight infected chickens died within 20 hours, and seven of eight infected mice died within 72 hours; the last died 96 hours post-infection. Earlier isolates taken from ducks in China were less virulent in mice and chickens (6). Hence we speculate that viruses might be emerging from reassortants that originate in birds overwintering in southeast Asia (7).

The occurrence of highly pathogenic H5N1 AIV infection in migrant waterfowl indicates that this virus has the potential to be a global threat: Lake Qinghaihu is a breeding center for migrant birds that congregate from southeast Asia, Siberia, Australia, and New Zealand.

### References and Notes

- R. J. Webby, R. G. Webster, *Science* **302**, 1519 (2003).
- K. Subbarao et al., *Science* **279**, 393 (1998).
- K. S. Li et al., *Nature* **430**, 209 (2004).
- Materials and methods are available as supporting material on Science Online.
- M. Hatta et al., *Science* **293**, 1840 (2001).
- H. Chen et al., *Proc. Natl. Acad. Sci. USA* **101**, 10452 (2004).
- T. H. Cheng et al., *Fauna Sinica: Aves* (Science Press, Beijing, 1979), vol. 2.
- Available at [www.china.com.cn/chinese/zhuanti/qlg/483177.htm](http://www.china.com.cn/chinese/zhuanti/qlg/483177.htm)
- Supported by the Ministry of Science and Technology, PR China [grant nos. 2004BA519A29, 2004BA519A11, 2004BA519A10, and 2004BA519A50; National Basic Research Program (973) of China 2005CB523000], the Chinese Academy of Sciences (The President Fund and CAS Information Special grant no. INF105-SDB-3-A2), the State Forestry Administration of China, and the National Natural Sciences Foundation of China (grant nos. 30471282 and 30228025). Sequence data derived from this study were deposited in GenBank with accession no. DQ100542-DQ100573.

### Supporting Online Material

[www.sciencemag.org/cgi/content/full/1115273/DC1](http://www.sciencemag.org/cgi/content/full/1115273/DC1)  
Materials and Methods  
Figs. S1 and S2  
References and Notes

25 May 2005; accepted 29 June 2005  
Published online 6 July 2005;  
10.1126/science.1115273  
Include this information when citing this paper.

<sup>1</sup>College of Veterinary Medicine, China Agricultural University, Beijing 100094, China. <sup>2</sup>Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China. <sup>3</sup>Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. <sup>4</sup>Graduate School, Chinese Academy of Sciences, Beijing, China. <sup>5</sup>Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing 100071, China. <sup>6</sup>Beijing Genomics Institute, Chinese Academy of Sciences, Beijing 101300, China.

\*These authors contributed equally to this work.  
†To whom correspondence should be addressed.  
E-mail: gaof@im.ac.cn (G.F.G.); jhl@cau.edu.cn (J.L.)