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References

1. Fasanella A, van Ert M, Altamura SA, Garofolo G, Buonavoglia C, Leori G, et al. Molecular diversity of *Bacillus anthracis* isolates in Italy. *J Clin Microbiol*. 2005;43:3398–401.
2. Fasanella A, Losito S, Trotta T, Adone R, Massa S, Ciuchini F, et al. Detection of anthrax vaccine virulence factors by polymerase chain reaction. *Vaccine*. 2001;19:4214–8.
3. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J Bacteriol*. 2000;182:2928–36.
4. Fasanella A, Fabiano MP, Garofolo G, Losito S. Preliminary studies on recombinant protective antigen (rPA) vaccine against anthrax for veterinary use. In: Abstracts of the International Veterinary Vaccines and Diagnostic Conference. Oslo, Norway; Jun 25–29, 2006. Oslo: University of Oslo; 2006. p. 69.

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Invasive Freshwater Snail, China

To the Editor: *Pomacea canaliculata*, an invasive freshwater snail native to South America, was first introduced as a food to Taiwan in 1979 and then to Mainland China in 1981 (1). It adapted well to the environment, particularly to the southern parts of the Mainland, spreading rapidly to more than 10 provinces (Figure) and causing tremendous damage to agriculture and the ecosystem (1,2). Thousands of hectares of rice, vegetables, and other crops in these provinces were destroyed (2).

Even more alarming were the multiple outbreaks of a severe brain disease (angiostrongyliasis) in Taiwan that were linked to *P. canaliculata* (3,4). Angiostrongyliasis is caused by *Angiostrongylus cantonensis*, a lung nematode of wild rodents, commonly known as the rat lungworm. In Mainland China, epidemiologic evidence also indicates that *P. canaliculata*, because of its high susceptibility to *A. cantonensis*, is becoming the most important natural intermediate host for this parasite (5). Previously, other terrestrial snails like *Achatina fulicaria*, and some species of slugs such as *Philomycus bilineatus* were regarded as the major intermediate hosts for *A. cantonensis* (6). Epidemiologic survey results from 1997 to 1999 demonstrated that 20.8%–69.4% of *P. canaliculata* were infected with *A. cantonensis* in some regions of Guangdong, Zhejiang, and Fujian Provinces (5). Even in provinces where the snail is not found, a high incidence and prevalence of infection occur because of its widespread distribution, high susceptibility to *A. cantonensis*, and growing popularity as a food. In 1997, 2002, and 2002, ingestion of raw or undercooked *P. canaliculata* meat led to 3

outbreaks of angiostrongyliasis infecting >100 patients (6,7). A 2006 outbreak in Beijing infected 131 persons (8). Based on the biologic characteristics of *P. canaliculata*, blocking its life cycle is one of the most effective methods to limit the outbreak of angiostrongyliasis. However, the current widespread distribution of *P. canaliculata* in China and the lack of a highly effective control method make the disease extremely difficult to eliminate (9). More outbreaks associated with ingestion of this snail will likely occur if food safety rules are not strictly enforced. Citizens must also be educated to avoid eating raw, undercooked snail meat or raw vegetables from regions that may be contaminated with infective mucous trails deposited by these snails (10).

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Figure. Distribution of *Pomacea canaliculata* in China. The dark triangles indicate the regions where angiostrongyliasis outbreaks were reported due to ingestion of raw or undercooked *P. canaliculata* snails.

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References

1. Xu HG, Qiang S. Inventory invasive alien species in China. Beijing: Chinese Environmental Science Press; 2004.
2. Zhou XC. Risk analysis of invasive *Pomacea canaliculata*. *Jian Yan Jian Yi Ke Xue*. 2004;14:37-9.
3. Tsai HC, Liu YC, Kunin CM, Lai PH, Lee SS, Chen YS, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* associated with eating raw snails: correlation of brain magnetic resonance imaging scans with clinical findings. *Am J Trop Med Hyg*. 2003;68:281-5.
4. Tsai HC, Liu YC, Kunin CM, Lee SS, Chen YS, Lin HH, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: report of 17 cases. *Am J Med*. 2001;111:109-14.
5. Lin W, Wang XT. Epidemiological survey of angiostrongyliasis in Mainland China. *Chin J Zoonoses*. 2004;20:1004-5.
6. Hollingsworth RG, Cowie RH. Apple snails as disease vectors. In: Joshi RC, Sebastian LC. Global advances in ecology and management of gloden apple snails. Nueva Ecija: Philippine Rice Institute; 2006. p. 121-32
7. Chen XG, Li H, Lun ZR. Angiostrongyliasis, mainland China. *Emerg Infect Dis*. 2005;11:1645-7.
8. The number of the case of angiostrongyliasis caused by eating *P. canaliculata* reached 131 in Beijing. [cited 2007 Jan 21]. Available from http://news3.xinhuanet.com/fortune//2006-09/12/content_5078790.htm
9. Cowie RH. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: Barker GM. Molluscus as crop pests. Wallingford (UK): CABI Publishing; 2002. p. 145-92
10. Tsai HC, Lee SS, Huang CK, Yen CM, Chen ER, Liu YC. Outbreak of eosinophilic meningitis associated with drinking raw vegetable juice in southern Taiwan. *Am J Trop Med Hyg*. 2004;71:222-6.

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**Possible Avian
Influenza (H5N1)
from Migratory
Bird, Egypt**

To the Editor: Wild migratory birds are reservoirs for low pathogenic avian influenza (LPAI) viruses (1), but their role in transmitting highly pathogenic avian influenza (HPAI) viruses is hotly debated and unclear (2-4). Beginning in July 2005, a clade of HPAI (H5N1) viruses rapidly expanded from an apparent focus in western People's Republic of China and spread to the Middle East, Africa, and Europe (5). Genetic analysis of HPAI virus isolates from dead wild birds along major flyways indicated that the strains were closely related to the Qinghai H5N1 A/bar-headed goose/Qinghai/65/2005 virus (clade II) (GenBank accession no. DQ095622). In addition to transmission to domestic poultry, HPAI (H5N1)-infected mute swans have been implicated in direct transmission to humans in Azerbaijan (6).

The US Naval Medical Research Unit No. 3 and the Ministry of Environment of Egypt have collaborated since 2003 in obtaining samples from migratory birds to detect circulating influenza viruses. During the 2005-06 migratory birds season, 1,304 migratory birds were sampled from either live bird markets or cage birds trapped by fishermen in Port Said, Damietta, Fayoum, Arish, and Sharm el Sheikh (online Appendix Figure, panel A, available from www.cdc.gov/EID/content/13/7/1120-appG.htm).

A total of 203 cloacal swab samples were positive for influenza A virus matrix gene when tested by real-time PCR, and 2 were also positive for the hemagglutinin 5 (H5) gene by using specific primers (7). Of the 2 migratory birds positive for the H5 gene, the first was a common teal (*Anas crecca*) captured in the Nile Delta region of Damietta in October 2005 (online Appendix Figure, panel A). Sequenc-

ing of the H5 gene showed that this virus was an LPAI most closely related to strain A/mallard/Bavaria/1/2005(H5N2) (GenBank accession no. DQ387854 (2)).

In January 2006, an influenza A H5 virus (weak positive result) was detected in another common teal (trapped in a cage by a fisherman) sampled from the Damietta region in December 2005 (online Appendix Figure, panel A). The low viral load, coupled with the failure to isolate the virus, precluded the laboratory from conducting sequence analysis at the time on the basis of insufficient template material. After the outbreak of influenza A (H5N1) in poultry and humans in Egypt in February 2006, additional retrospective attempts to concentrate RNA were used to assess potential introduction scenarios. After multiple RNA extractions were conducted and the RNA was concentrated, this specimen was found to be positive for the neuraminidase 1 (N1) gene by real-time PCR.

The hemagglutinin gene from both teal strains was sequenced ($\approx 1,596$ bp). Sequences were aligned with other influenza A (H5N1) strains from Egypt (9 from humans, 5 from chickens). Twenty other strains with high similarity and from different locations were selected by using a GenBank search algorithm and included in the alignment. A phylogenetic analysis was conducted by using the Kimura 2-parameter model. The LPAI H5 virus strain was used as an outgroup in a neighbor-joining phylogenetic tree. Bootstrap analysis with 500 replicates of sequence data was also conducted by using MEGA 3.1 software (8).

Phylogenetic analysis showed clustering of the HPAI (H5N1) strains collected from 1 geographic region (country) (online Appendix Figure, panel B). All HPAI (H5N1) strains from Egypt from humans or chickens analyzed clustered with a bootstrap support value of 98%. Furthermore, the A/Teal/Egypt/14051-NAMRU3/2006