Current Status of Smallpox Vaccine

To the Editor: The possible use of smallpox virus as a weapon by terrorists has stimulated growing international concern and led to a recent review by the World Health Organization of the global availability of smallpox vaccine. This review found approximately 60 million doses worldwide, with little current vaccine manufacture, although limited vaccine seed remains available (1). Ongoing discussions in the United States suggest that the national stockpile should contain at least 40 million doses to be held in reserve for emergency use, including in case of a terrorist release of smallpox virus (O'Toole, this issue, pp. 540-6).

The current U.S. stockpile contains approximately 15.4 million doses of vaccinia vaccine (Dryvax) made from the New York City Board of Health strain of vaccinia and was produced by Wyeth Laboratories in 13 separate lots. The vaccine is lyophylized in glass vials with rubber stoppers and sealed with a metal band. When rehydrated, each vial contains 100 doses and has a potency of at least 10^8 plaque-forming units (pfu)/ml. Some vials of the vaccine stockpile have shown elevated moisture levels and thus failed routine quality control testing; however, the vaccine in these vials remains potent, and the failed lots have not been discarded.

The diluent used to rehydrate the vaccine contains brilliant green, which makes the vaccine easier to visualize when administered with bifurcated needles. Over time, the brilliant green has deteriorated, and most of the available diluent does not pass quality control. Discussions are under way with Wyeth to begin production of sufficient new diluent for the entire stockpile.

The vaccine is administered by superficial inoculation (scarification) with a bifurcated needle. Fewer than 1 million bifurcated needles are held as part of the stockpile. As with the diluent, Wyeth has been requested to produce additional bifurcated needles.

Vaccinia virus produces adverse reactions in a small percentage of vaccinated persons. Adverse reactions are treated with vaccinia immune globulin (VIG), currently only available from Baxter Healthcare Corporation (5,400 vials of VIG in stock). Each vial contains 5 ml of VIG; the recommended dose for postvaccine complications is 0.6 ml per kg of body weight. This volume is sufficient to treat adverse reactions in approximately 675 adults. Further, the entire stockpile of VIG has been placed on hold while the cause of a slight pink discoloration is investigated. Until the cause of the discoloration is determined or another approved supply of VIG is obtained, no vaccinia vaccine is being released. While unknown, the rate of adverse reactions in today's population is likely to be greater than seen during the global eradication campaign because of recent increases in the number of immunocompromised persons. The Department of Defense has recently contracted the processing of new lots of VIG (to be administered intravenously rather than by the intramuscular route like existing VIG stocks); however, maintaining adequate stocks of VIG will remain a challenge.

In the event of release of smallpox virus, persons at high risk and persons exposed but not yet showing clinical illness would be vaccinated immediately. Intensive case detection and vaccination of contacts and other persons at risk would follow. All vaccine, including lots retained after failed quality control tests, would be made available for emergency use. Previous studies have found that more than 90% of susceptible persons respond to vaccinia virus with a titer of 10^7 pocks/ml (2). In an emergency, consideration would be given to diluting the existing vaccine as much as 10-fold, so that each vial could conceivably contain 1,000 doses of vaccine, rather than the current 100 doses. The present vaccine container is sufficiently large to accommodate the added diluent. The absence of sufficient quantities of VIG to protect against adverse reactions during a mass immunization campaign would necessitate careful screening of those receiving the vaccine; some persons with adverse reactions would likely go untreated.

While the intentional release of smallpox virus would represent a global emergency, the existing national stockpile could be effectively used to limit the spread of disease and buy time while the pharmaceutical industry begins emergency vaccine production.

James W. LeDuc and John Becher
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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West Nile Fever in Czechland

To the Editor: After heavy rains in July 1997, extensive floods occurred along the Morava River, Czech Republic. Populations of *Aedes* mosquitoes increased rapidly in the flooded areas, prompting surveillance for mosquito-borne virus infections in the Breclav area, South Moravia. We collected 11,334 female mosquitoes (9,100 *Aedes vexans,* 917 *Ae. cinereus,* 11 *Ae. cantans,* 1,074 *Ae. sticticus*, and 232 *Culex p. pipiens*) from July through September 1997 and tested them for virus in 117 monospecific pools by intracranial inoculation of suckling mice. Seven virus isolates were obtained and identified by complement-fixation and neutralization tests. Six isolates (five from *Ae. vexans*, one from *Ae. cinereus*) were identified as the bunyavirus Tahyna, California serogroup, and one (strain 97-103 from 57 *C. p. pipiens* collected at Lanzhot, 48°40'N, 16°56'E, on September 17) was identified as the flavivirus West Nile (1). A crossed comparison of 97-103 and topotype Eg-101 (2) West Nile virus strains and their antisera (prepared in mice by three intraperitoneal doses at weekly intervals) by plaque reduction neutralization (PRN) on XTC-2 cells (3,4) showed their antigenic relationships: reciprocal titers of homologous/heterologous sera were 512/512 in Eg-101 and 512/64 in 97-103. Strain 97-103 has lower virulence than Eg-101 in that it does not kill adult ICR mice and may represent a subtype of West Nile virus.

Blood samples were obtained from 619 persons seeking treatment at hospital and outpatient clinics in the Breclav area from June 23 through September 29, 1997. Sera were inactivated at 56°C for 30 minutes, diluted 1:8, and assayed by PRN for antibodies against *c.* 30 plaque-forming units (PFU) per well of West Nile virus strains Eg-101 and 97-103. All sera causing 90% reduction of PFU at 1:8 dilution were titrated, and the highest serum dilution showing 50% PFU reduction was regarded as the titer. Antibodies neutralizing West Nile virus were detected in 13 (2.1%) persons: 2.8% of 179 male and 1.8% of 440 female. Persons with detectable West Nile virus antibody were questioned about their health history during the previous 5 years, and their medical records were reviewed; none recalled having had tickborne encephalitis (Central-European encephalitis [CEE] virus is the only other flavivirus present in Czechland) or having been vaccinated against CEE or yellow fever virus. Titers of PRN antibodies to CEEV were all below 16. Two of the seropositive persons had traveled abroad during the last 5 years: one to Croatia in 1996, and one to South Australia during 1951 to 1994.

Paired serum samples were obtained from 72 of the 619 persons examined. A significant increase (≥4 times) in antibody titer against West Nile virus between the first (acute-phase) and second (convalescent-phase) samples was detected four times: in 2 of 41 young persons (≤16 years of age) and in 2 of 31 adults (>16 years of age). Among the four seroconverting persons, only the two children had clinical symptoms compatible with West Nile fever. A 9-year-old boy had fever (39°C) for 4 days, sore throat, headache, muscle ache, pronounced fatigue, and nausea lasting approximately 6 days, with recovery after 13 days. Neutralizing antibodies to West Nile virus, Eg-101 and 97-103, were 64 and 32 on July 22 and 512 and 256 on August 4, respectively. A 9-year-old girl had fever (38°C-39°C) for 3 days, sore throat, headache, muscle ache, pronounced fatigue, nausea, vomiting, maculopapular rash (including flushed face), and slightly enlarged inguinal lymph nodes. The illness lasted approximately 7 days, with complete recovery after 17 days. Neutralizing antibodies to West Nile virus, Eg-101 and 97-103, were 64 and 32 on August 6 and 256 and 128 on August 20, respectively. Of the remaining nine seropositive persons lacking paired serum samples, one had severe headache, muscle ache, prolonged fatigue, nausea, pain on eye movement, maculopapular rash, and insomnia in summer of 1997. Two other persons had had "summer fever" (sore throat and lymphadenitis; headache with pain on eye movement) in 1997. The other persons who seroconverted did not report any substantial illness. In total, clinical symptoms in five persons are compatible with West Nile fever.

These are the first reported human cases of West Nile fever in Central Europe (5); an extensive outbreak occurred in Romania in 1996, with approximately 500 patients hospitalized and a 4% to 8% fatality rate (6,7). West Nile virus should be viewed as a potential agent of local sporadic cases, clusters, or outbreaks, even in temperate Europe. Environmental factors (including human activities) that enhance vector population densities (heavy rains followed by floods, irrigation, higher than usual temperatures due to global warming) might produce an increased incidence of West Nile fever and other new or reemerging mosquito-borne diseases. Surveillance for West Nile fever should monitor population density and infection rate of principal vectors, antibodies in vertebrates and exposed human groups, and routine diagnosis of human infections.

Zdenek Hubálek, Jirí Halouzka, and Zina Juricová
Institute of Vertebrate Biology, Academy of Sciences, Brno, Czech Republic

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Ofloxacin-Resistant *Vibrio cholerae* O139 in Hong Kong

To the Editor: Unexpected outbreaks of cholera occurred in many areas of the world in 1997-98, partly because of weather changes associated with the El Niño phenomenon (1). Outbreaks caused by antibiotic-resistant *Vibrio cholerae* O1 and O139 have been documented in the Indian subcontinent (2-4), Africa (5), and Ukraine (6).

In Hong Kong, nonduplicate bacterial strains of *V. cholerae* O1 and O139 isolated from patients and environmental sources and received in the Public Health Laboratory between January 1, 1993, and June 30, 1998, were identified by conventional biochemical tests (7,8) and API 20E (bioMerieux, France); serotyped by slide agglutination with polyvalent O1 and mono-specific Inaba and Ogawa antisera (Murex, Dartford, United Kingdom); and checked with O139 antiserum (Denka Seiken, Tokyo, Japan). Biotyped and antibiotic susceptibilities were determined by the Kirby-Bauer disk-diffusion assay (8-10). Antibiotics tested included chloramphenicol and tetracycline (from 1993 to 1996) and ofloxacin (added in routine testing from 1997). *V. cholerae* isolates available for further study were tested with the standard broth microdilution method (11) to measure minimum inhibitory concentrations (MICs) of susceptibilities to chloramphenicol, tetracycline, and ofloxacin.

No antibiotic resistance was seen in *V. cholerae* isolates in testing conducted from 1969 to 1995. The first *V. cholerae* isolate with reduced susceptibility to chloramphenicol but sensitive to tetracycline was encountered in Hong Kong in 1996. This O1 El Tor Ogawa strain was imported from Nepal. Since then, more O1 strains were isolated that exhibited reduced antibiotic susceptibilities to chloramphenicol and tetracycline but not to ofloxacin (12). In May 1998, seven *V. cholerae* O139 strains were isolated that displayed patterns of antibiotic susceptibilities strikingly different from those of O1 isolates; the former were all sensitive to tetracycline but showed reduced susceptibilities to chloramphenicol and ofloxacin. All *V. cholerae* O1 strains tested have been susceptible to ofloxacin; O1 isolates falling into intermediate categories for chloramphenicol and tetracycline susceptibilities (31% and 27.6%, respectively) were common.

The first isolate of *V. cholerae* O139 in Hong Kong came from the imported case of a patient who had traveled to other provinces of China (13,14). Isolation of O139 continued sporadically since then, with six cases between 1993 and the

1st quarter of 1998. In May 1998, a cluster of seven imported cases of *V. cholerae* O139 were reported with strains isolated from seven persons who became ill with severe diarrhea after visiting Zhuhai in Guangdong Province, China. Of 13 *V. cholerae* O139 isolates tested, 7 showed intermediate resistance to chloramphenicol and high-level resistance to ofloxacin (MIC 16 µg/ml) but no resistance to tetracycline (MIC 50s and MIC 90s were 0.25 µg/ml). This is the first evidence of a quinolone-resistant strain of *V. cholerae* O139 in Hong Kong. Of the O1 isolates, none were resistant to chloramphenicol and ofloxacin, but six were resistant to tetracycline (MIC 50s and MIC 90s were 0.25 µg/ml and 8 µg/ml, respectively).

Although all O1 isolates were sensitive to chloramphenicol, there was only a twofold difference in MIC90 to chloramphenicol between O1 and O139 isolates. MIC90s of ofloxacin for O139 were nearly 10 times higher than those for O1 strains.

The novel appearance of O139 resistant to ofloxacin with MICs of 16 µg/ml from Guangdong Province, China, was of special concern. Preliminary results using pulsed-field gel electrophoresis analysis of chromosomal DNA showed that these ofloxacin-resistant O139 strains had identical fingerprint patterns and probably belonged to the clone that had caused severe diarrheal disease in the region. Two previous surveys of *V. cholerae* antibiotic susceptibilities had not described any ofloxacin-resistant O139 strains (15,16). The potential for rapid spread of these strains threatens cholera prevention and control efforts that may still rely on chemotherapy.

Different antimicrobial resistance patterns of *V. cholerae* O1 and O139 were noted. Among the resistant O1 isolates, four were local, one was from other provinces of China, and one was from Thailand. All the resistant O139 isolates were imported from Guangdong Province, China. Antibiotic resistance was found in strains from local isolates and from neighboring countries. The unique patterns of antimicrobial resistance for the O1 and O139 isolates suggest different mechanisms of resistance. As quinolones are used heavily in this region to treat cholera and other enteric diseases, selective pressure could encourage emergence of ofloxacin resistance. Prudent use of antibiotics should be exercised during antimicrobial therapy and prophylaxis for cholera and other enteric diseases to decrease the selection of more resistant clones in our locality.

Kai Man Kam, Kit Yee Luey, Tze Leung Cheung, Kwan Yee Ho, Kwok Hang Mak, and Paul Thian Aun Saw
Department of Health, Hong Kong SAR Government

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Plant Pathology and Public Health

> The day will come when the sign of the plant pathologist will stand forth in the street alongside that of the physician and surgeon. . . . For what will it profit us if all the ills and diseases of the human race be banished and we then face starvation because of diseases and pests in our food (1).

To the Editor: Every year plant diseases affect human society, resulting in inadequate nutrition and economic loss. The potato famine in the mid-1800s is the best-known example of a fungal plant pathogen's effect on history (2-4); *Phytopthora infestans* has recently reemerged in the Americas (5). Among the silent problems that have enormous effects on human society each year are crop infections by geminiviruses and tomato spotted wilt virus (6). These plant viruses are transmitted by whiteflies, leafhoppers, or thrips to hundreds of species of plants. They cause diseases of crops and ornamental plants around the world.

More obvious problems include ergotism, caused by the alkaloids produced by the fungus *Claviceps purpurea*. Ergotism was associated with the growth of rye, particularly in cool climates that cannot support wheat, and was implicated in the aberrant human behavior responsible at least in part for the Salem witch trials and St. Anthony's fire (2,7). In the last 5 years, a new plant disease, sorghum ergot (*Claviceps africana*), has spread north from Brazil into the United States. This fungus also causes disease in Australia, a sudden change from its known occurrence in Africa (8). Sorghum is the fifth most important cereal crop in the world, with approximately 45 million hectares under cultivation for food, beverages, feed, and fodder (8). Ergot alkaloid toxicity has not yet been demonstrated, but potential nutritional and economic losses could have substantial impact on public health.

With our increased awareness of the fragility of the environment, including the quality of our drinking water, opportunities may exist for physicians to interact with plant pathologists. Concern is growing about the use of *Burkholderia cepacia*, a bacterial phytopathogen, for the biologic control of seedling diseases (9). Although *B. cepacia* is effective for the biologic control of fungal diseases in the agricultural environment (10), this bacterium could contaminate the public water supply and subsequently influence the health of the immunosuppressed or persons with cystic fibrosis (9-11). This risk exemplifies the need to integrate plant health measures with human and veterinary health guidelines.

Plant pathology and public health also intersect with post-harvest fungal infections of seed and grain, particularly *Aspergillus flavus* and *Fusarium moniliforme* (2), which produce aflatoxin and fumonisin, respectively. During the past 2 drought years in Texas, aflatoxin in contaminated corn and peanuts has become a public health problem. In 1998, more than 50 pet dogs died of aflatoxicosis, perhaps by eating aflatoxin B1-contaminated corn used in dog food (12).

Although the veterinary and medical communities are well aware of the risks associated with plant pathogens when they enter the animal or human food supply, more routine interactions with plant pathologists could benefit public health. For example, plant pathologists can often predict impending plant disease outbreaks. This information can be used by epidemiologists to sound a warning about impending food shortages or poor food quality, particularly in developing countries. Plant pathologists are also developing new types of resistance in host plants and alternative strategies for managing plant diseases. These measures should improve food quality and reduce the negative public health impact associated with plant diseases.

Karen-Beth G. Scholthof
Texas A&M University, College Station, Texas, USA

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Pet-Associated Zoonoses

To the Editor: We read with interest the article by Grant and Olsen on preventing zoonotic diseases in immunocompromised persons (1). We completely agree with the benefits of communication between physicians and veterinarians. However, we want to emphasize that pet-associated illnesses are not limited to the immunocompromised; pregnant women and young infants should be included in this high-risk category. Our recently published survey (2) reaffirms the need for education of the general public, parents, and—to a lesser extent—pediatricians regarding pet-associated hazards.

Leslie L. Barton,* Rodrigo G. Villar,† and Megan Connick‡
*University of Arizona Health Sciences Center, Tucson, Arizona, USA; †Indian Health Service, Gallup, New Mexico, USA; and ‡Washington University School of Medicine, St. Louis, Missouri, USA

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