An Outbreak of Gastroenteritis in Japan due to *Escherichia coli* O166

To the Editor: Enteroaggregative *Escherichia coli* (EAggEC) heat-stable enterotoxin 1 (EAST1) was originally found as an enterotoxin of EaggEC (1). Recently, Yamamoto et al. (2) reported that the *EAST1* gene, or its variants, were present not only in EAggEC but in other diarrheagenic *E. coli,* including some enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC). Hedberg et al. (3) found that an outbreak of gastrointestinal illness in 1991 had been caused by EAST1-producing *E. coli* that possessed the EPEC gene locus for enterocyte effacement. We propose that *E. coli* producing EAST1 but possessing no other identifiable pathogenic properties may compose either a new group of diarrhea-associated *E. coli* or a new subgroup of ETEC.

In an outbreak of gastroenteritis on July 23, 1996, in Osaka, Japan, 54 of 91 persons attending a meeting held in an office building on July 22, 1996, became ill. The patients did not eat any common foods except the lunch served at the office. Symptoms were diarrhea in 52 (96%); abdominal pain in 32 (59%); nausea in 8 (15%); fever in 8 (15%); and vomiting in 5 (10%). The mean incubation period was 17 hours.

Stool specimens of 33 patients were examined, and *E. coli* O166 with an unidentifiable H antigen were isolated from 29 specimens. Laboratory tests for other bacterial pathogens and viruses were negative. The isolates showed the same DNA banding pattern in pulsed-field gel electrophoresis after treatment with the restriction enzymes *Xba* I or *Not* I.

The *E. coli* O166 organisms did not adhere to HEp-2 cells in a localized, diffuse, or enteroaggregative manner and did not give mannose-resistant hemagglutination of human or bovine red blood cells. Although the organisms were further analyzed for expression of known ETEC colonization factors by a dot-blot assay using specific monoclonal antibodies, they did not express CFA/I, CS1, CS2, CS3, CS4, CS5, CS6, CS7, CS17, PCFO159, PCFO166, or CFA/III. In polymerase chain reaction (PCR) tests, the bacteria did not have coding genes for verocytotoxin of enterohemorrhagic *E. coli,* heat-labile, or heat-stable enterotoxin of ETEC, attachment and effacement (*eae*A) of EPEC, or invasion (*inv*E) of enteroinvasive *E. coli.* Consequently, they are not assigned to any of the recognized diarrheagenic groups of *E. coli*: EPEC, ETEC, enterohemorrhagic *E. coli,* enteroinvasive *E. coli*, EAggEC, and diffusely adhering *E. coli*. According to the PCR method of Yamamoto et al. (2), however, the organisms possessed the *EAST1* gene.

To our knowledge, this is the first report of an outbreak caused by EAST1-producing *E. coli* that did not have other well-characterized virulence genes. We believe that these strains should be assigned to a new subgroup of ETEC. Such strains would not be detected in most current surveys for diarrheagenic *E. coli*, as tests for EAST1 are rarely included. The role of EAST1 in pathogenicity has been controversial. We propose that diarrheal specimens be examined for EAST1-producing *E. coli* so that the distribution of these organisms worldwide can be determined.

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Vibrio cholerae Outbreak in Italy

To the Editor: On 16 June, the microbiology unit of the Hospital of Lodi communicated to the local public health unit that *Vibrio cholerae* had been isolated and identified by standard biochemical tests in stool samples of an outpatient whose clinical data were unknown. On the same day, we contacted and interviewed the patient to investigate risk factors for cholera. The patient reported abdominal pain starting on 6 June and then severe diarrhea (10 to 12 stools per day) until 13 June; on that day the patient went to his general practitioner, who gave him loperamide and suggested a coproculture. The patient never traveled to cholera-endemic areas; did not eat raw mussels, uncooked fish, or vegetables of uncertain origin or from cholera-infected areas; and did not swim in rivers or lakes. The patient reported that he ate a seafood salad in the canteen of his work place on 5 June and that three out of the four persons who ate the same kind of salad also had abdominal symptoms. Subsequently, the Istituto Superiore di Sanità (ISS) in Rome confirmed isolation of toxinogenic *V. cholerae* O1, biotype El Tor, serotype Ogawa, from stools from the index patient.

The canteen where the index patient had eaten the seafood salad was 1 of 17 supplied by a single cooking center that used a precooked, frozen, ready-to-eat product including shrimps, scallops, mussels, hen clams, cuttlefishes, and squid. Each product was cooked and frozen in the country of origin and mixed in Italy by an importer who packaged the seafood salad. Tracking the products around the world was difficult, but we learned that at least some had come from Far East countries where cholera is endemic. Approximately 125 servings of the same food were distributed within our local public health area (Azienda Sanitaria Locale) and more than 400 in other areas.

We performed an epidemiologic case-control investigation beginning 18 June involving 454 persons (94 who had eaten the seafood salad and 360 controls who had eaten in the same canteen any food except seafood salad); 37 (39%) of the persons who had eaten the seafood salad had had at least one episode of diarrhea or other relevant gastrointestinal symptoms, as compared to one (0.3%) of those who had not eaten it. We did not find symptomatic patients. The corresponding odds ratio was 233 (95% confidence interval, 97 to 560). No symptomatic person had to be hospitalized because of symptoms or required intravenous treatment; three or more loose or watery stools during a 24-hour period were reported in 24 cases. We performed coprocultures (using TCBS medium) between 23 June and 3 July of all 94 persons who had eaten the seafood salad. One positive coproculture for *V. cholerae* O1 Ogawa (same strain) was identified on 25 June; the isolation was subsequently confirmed by the ISS. This second patient with a positive culture worked in a factory in a different town. She had severe diarrhea on 6, 7, and 8 June. Her family doctor gave her rifaximin but did not ask for a coproculture, but a specimen was obtained on 23 June. She did not report risk factors for cholera infection, except having eaten seafood salad on June 5 in the canteen of her work place. The delay between exposure to *V. cholerae* and the coprocultures was longer than 1 week (median delay 26 days, range 19 to 31), and it is therefore not surprising that others who had eaten the seafood salad did not have positive results. Both the culture-positive index case-patient and the woman were recultured three more times; negative results were obtained.

The identification of this cholera outbreak is a sentinel episode confirming (1,2) that, if not adequately monitored, food preparation and distribution can cause serious infectious diseases in industrialized countries.

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Shiga Toxin–Producing *Escherichia coli* O157:H7 in Japan

To the Editor: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 infection, which can cause hemolytic uremic syndrome and death, is a global public health concern. Patients younger than 5 years of age are at high risk for hemolytic uremic syndrome (1) and shed the organism longer than adults (2). The public health importance of this symptomatic shedding in transmission among preschool children is well established (3); however, that of symptom-free shedding in adults is unknown. We report here that the rate of symptom-free STEC O157:H7 shedding is higher in adults 30 to 49 years of age than in others.

STEC infections have been notifiable in Japan since August 1996. When STEC is found in the feces of patients in schools, families, and hospitals, local health centers and public health institutes must test (generally using MacConkey sorbitol agar with cefixime-potassium tellurite medium) for the pathogen in stool specimens of contacts of the patients. The pathogen is also sought twice a month in the stool specimens of food-handlers. All isolates from culture-positive patients are collected by Japan's National Institute of Infectious Diseases.

In 1997, 1,412 STEC O157:H7 human isolates were examined for subtyping of Shiga toxin genes *stx1* and *stx2* by polymerase chain reaction, for genotyping by *Xba*I-digested pulsed-field gel electrophoresis (PFGE) (4,5), and for their relationship with symptoms; 1,381 isolates (from culture-positive persons with well-characterized clinical status) were further analyzed. The rates by age group among STEC O157:H7-shedding persons reporting one or more symptoms (vs. culture-positive persons without symptoms) were as follows: 82% (475 of 576) younger than 10 years old; 81% (145 of 178), 10 to 19 years; 63% (98 of 156), 20 to 29 years; 25% (32 of 128), 30 to 39 years; 34% (34 of 100), 40 to 49 years; 54% (57 of 106), 50 to 59 years; 56% (38 of 68), 60 to 69 years; 68% (47 of 69), older than 70 years. Culture-positive persons under 20 years of age, especially children under 10 years of age, were more likely to have symptoms than other age groups. Intermediate rates of symptom-free persons with positive stool cultures occurred in young adults (20 to 29 years of age) and the elderly (70 years of age), while the highest rates of stool-positive, symptom-free persons were adults, especially those between 30 and 49 years of age. In terms of pathogen virulence, we did not find significant differences in the distribution of *stx* subtypes and PFGE genotypes between strains shed by symptomatic and asymptomatic persons. These results suggest that the rate of symptom-free STEC O157:H7 shedding may be associated with age rather than organism-related factors. Possible age-related host factors that could influence the presence of STEC O157:H7 in the stools of symptom-free persons include qualitative and quantitative differences in intestinal cross-reactive antibodies against STEC O157:H7, intestinal bacterial flora, or the sensitivity to Stx toxins between children and adults. Further investigations will be required to determine the relative importance of these and other host factors. Our finding of a high rate of asymptomatic shedding in adults may suggest the potential for secondary transmission of the bacteria from symptom-free STEC O157:H7-shedding adults to healthy children.

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Streptococcus pyogenes Erythromycin Resistance in Italy

To the Editor: *Streptococcus pyogenes* resistance to erythromycin began to emerge as a serious problem worldwide in the early 1990s. In some areas in Italy, 30% to 40% of strains have beome resistant (1-3). Throughout Italy, the use of macrolides, particularly the newest ones (azithromycin and clarithromycin), has increased in the treatment of infections caused by Group A streptococci. This therapeutic approach is contrary to current guidelines, which recommend using betalactam antibiotics as first-choice therapy and reserving macrolides only for patients allergic to betalactams.

In 1997 in Finland, a decrease was observed in the use of macrolide antibiotics in ambulatory patients from 2.40 defined daily doses per 1,000 inhabitants in 1991 to 1.38 in 1992. Subsequently, the maintenance of doses at 1.28 to 1.74 defined daily doses resulted in a substantial decrease in the percentage of group A streptococcal resistance to erythromycin, reported as 16.5% in 1992, 19% in 1993, 15.6% in 1994, 10% in 1995, and 8.6% in 1996 (4). These data prompted us to evaluate such phenomena in our geographic area, the urban area of Genoa, Italy (approximately 120,000 inhabitants).

From January 1991 to June 1998, 311 (6.1%) of 5,117 strains of *S. pyogenes* throat swabs from patients with pharyngotonsillitis were isolated. We observed a higher number of group A streptococci isolates from throat swabs starting in 1996 than we had in 1991 to 1995 (chi-square = 35.653, $p < 0.0001$). All isolates were tested for susceptibility to penicillin and erythromycin by standard susceptibility tests (broth microdilution) as recommended by the National Committee for Clinical Laboratory Standards. All isolates were susceptible to penicillin. From 1991 to 1996, the percentage of *S. pyogenes* resistant or with intermediate resistance to erythromycin increased from 0% to 50% (1992, 6%; 1993, 13%; 1994, 14%; 1995, 24%; 1996, 50%). In 1997 and the first half of 1998, resistance to erythromycin decreased to 39% and 34%, respectively. The number of resistant strains before 1996 was significantly lower than from 1996 to 1998 (chi-square = 50.386, $p < 0.0001$). Analysis of antibiotic consumption in our district showed an increase in the use of macrolides (erythromycin and the new compounds clarithromycin and azithromycin) from 0.445 defined daily dose per 1,000 inhabitants in 1994 to 1.140 in 1996. In 1997 and in the first half of 1998, consumption decreased to 0.9 and 0.8, respectively; we observed a correlation between the number of resistant isolates and the defined daily dose increase (correlation [R^2] = 0.795, $p = 0.0153$).

S. pyogenes resistance to erythromycin rose from 6% to 50% in only 4 years and then rapidly decreased from 50% to 34% in an 18-month period, corresponding to a 57% decrease in defined daily dose (from 1.41 in 1996 to 0.8 in the first half of 1998). Our data suggest that *S. pyogenes* resistance to erythromycin is associated with frequency of macrolide use.

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Estimated Incidence of *Clostridium difficile* Infection

To the Editor: Since the publication of our article Increasing hospitalization and death, possibly due to *Clostridium difficile* diarrheal disease (1), we have received several requests to estimate the incidence of *C. difficile* infection. Our original study included only hospitalized patients treated at the Lovelace Medical Center from 1993 to 1996, and no information on the incidence of *C. difficile* infection. In response to these requests, we used inpatient and outpatient medical claims for the Lovelace managed care population to calculate incidence rates. We searched medical claims for the Lovelace Health Plan/Senior Plan (LHP) to identify patients who had a *C. difficile* diagnosis between January 1, 1993, and December 31, 1997. LHP members are residents of New Mexico, most residing in or near Albuquerque.

LHP had approximately 713,000 person-years of enrollment from 1993 to 1997. We identified 104 members with a *C. difficile* diagnosis on a claims record during this period. This group includes most of the patients in our original study. Most patients (62.5%) were identified exclusively from inpatient records; another 15.4% had both an inpatient and an outpatient record with a *C. difficile* diagnosis; and 22.1% had only an outpatient *C. difficile* diagnosis. We calculated an age-adjusted rate of infection (adjusted to the 1990 U.S. population), for each year and for the 5-year period. The incidence of *C. difficile* infection for all members during 1993 to 1997 was 14.8 cases per 100,000 person-years of enrollment. The patients rates for male and female were essentially the same (14.4 vs. 15.5, respectively). The rates increased dramatically with age. For persons ages 0-4, the age-adjusted rate per 100,000 person-years of enrollment (number of cases) was 5.3 (2); for 5-14, 2.7 (3), for 15-24, 2.2 (2); for 24-34, 6.4 (6); for 35-44, 9.2 (12); for 45-54, 15.7 (17); 55-64, 16.8 (10); 65-74, 38.5 (19); and 75+, 98.9 (33). The overall average rate of infection was 15.4; there were 104 cases.

The rate of infection may have declined since 1993 in this population. The 1993 rate was 24.5 per 100,000 person-years of enrollment, declining to 11.1 in 1997 (1993, 24.4; 1994, 19.1; 1995, 9.9; 1996, 12.3; and 1997, 11.1).

Our method for estimating rates has some limitations. We did not examine laboratory records to confirm the diagnosis. In addition, some laboratory-confirmed infections may not have resulted in a claims record with a *C. difficile* diagnosis. The Lovelace managed-care population is an insured, generally healthy population that may not have the characteristics of patients in other health care delivery settings or, because of its geographic restriction, the characteristics of the general U.S. population. Nevertheless, these estimates provide a basis for determining the magnitude of the public health problem of *C. difficile* infection. Additional surveillance studies are needed to better estimate the incidence of infection and to determine whether the incidence has declined during recent years.

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Diphtheria in Eastern Nepal

To the Editor: Diphtheria, caused by *Corynebacterium diphtheriae*, was a major childhood killer until the advent of the Expanded Program on Immunization when diphtheria, pertussis, and tetanus (DPT) vaccination was greatly increased; diphtheria gradually declined in many countries. We report two cases of diphtheria identified at the B.P. Koirala Institute of Health Sciences Hospital, Dharan, Nepal.

During April 1996, a 6-year-old patient had fever (for 5 days), difficulty in swallowing and breathing, and change of voice (for 4 days). Throat examination showed a grayish-white membrane over the right tonsil, uvula, and adjacent soft palate. The membrane could not be removed, and the larynx could not be examined. Swabs were taken from the membrane area and sent to the laboratory, where smears were made and stained by Gram and Albert stains. Gram-stained smears showed a large number of gram-positive bacilli with the appearance of Chinese letters, and Albert stain showed bacilli with numerous metachromatic granules. A diagnosis of faucial diphtheria, with a strong possibility of laryngeal diphtheria, was made. The patient was treated with parenteral penicillin and diphtheria antitoxin. His condition improved after 6 days of therapy.

In December 1996, a 9-year-old patient sought treatment for chronic pain and discharge in the left ear. On examination, he had mucopurulent discharge, antral perforation, and mastoid tenderness. Throat examination showed bilateral tonsilitis. A provisional diagnosis of acute mastoiditis associated with acute septic tonsillitis was made. Throat swabs were collected and sent to the laboratory; smear findings showed typical organisms morphologically resembling *C. diphtheriae*. Culture done on 10% defibrinated sheep blood agar and Loefflers serum slope grew colonies consistent with *C. diphtheriae*. In addition to local antibiotic to the ear, the patient was given parenteral penicillin, gentamicin, and metronidazole. Because the patient had no features of systemic toxicity, no antidiphtheria serum was administered. The patient became well and was discharged on day 4.

In the first case, a throat culture could not be done because the patient had already received local antiseptic paint. However, the diagnosis was clinically consistent with classic diphtheria with features of toxicity. In the second case, diphtheria was suspected only after bacteriologic examination. Unlike patient 1, patient 2 had no evident features of systemic toxicity. Hence the isolate could be nontoxigenic. Localized diphtheria due to nontoxigenic *C. diphtheriae* is known to occur (1).

The two patients did not give a complete history of immunization and may not have been vaccinated (or may have been partially vaccinated) with DPT. On the Indian subcontinent, DPT vaccination coverage is reported to be 80%. However, it may not be so in all areas, and immunization may have decreased to approximately 50% in certain areas of Southeast Asia (2). This may also be true in certain areas of eastern Nepal. An immunization status survey done in midwestern Nepal from 1989 to 1990 showed that DPT coverage was unsatisfactory (3). Lack of sustained immunization may even result in outbreaks. The recent epidemics of diphtheria in the Ukraine, Russian Federation, and other countries of the former Soviet Union are examples of resurgence due to ineffectively maintained immunization programs (4,5).

Diphtheria, still occasionally seen in many Southeast Asian countries including India and Nepal, is thought to be declining in these areas. However, accurate data have not been recently available, particularly from Nepal, because reporting is infrequent, laboratory confirmation is not available, and the extent of carriers is not clearly known (2).

These two cases show the persistence of diphtheria in a population in Nepal immunized with DPT and underscore the need for careful surveillance, laboratory documentation of clinical diphtheria, and increased immunization of children in this area.

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Commercial Use of *Burkholderia cepacia*

To the Editor: In their review of the potential threat to human health by the commercial use of *Burkholderia cepacia*, Holmes et al. (1) focus on the biopesticidal uses of this bacterium in agriculture. By virtue of its ability to antagonize a number of soilborne plant pathogens, *B. cepacia* is an attractive natural alternative to currently used chemical pesticides, such as captan, mancozeb, and metalaxyl. The replacement of these highly toxic agents, which are among the mainstays of crop protection chemicals, by safer products is a laudable goal. However, despite being nonpathogenic to healthy humans (and thus classified as a Biosafety Level 1 species), *B. cepacia* can cause life-threatening pulmonary infection in persons with cystic fibrosis. Holmes et al. call for a moratorium on the use of *B. cepacia* in agriculture until more is known about risks from such use.

Perhaps of greater concern than agricultural use is *B. cepacia*'s use as a bioremedial agent. Holmes et al. only briefly refer to the capacity of this species to degrade chlorinated aromatic substrates such as those found in certain pesticides and herbicides. By virtue of its extraordinary metabolic versatility, *B. cepacia* can use such compounds as nutrient carbon energy sources. In addition, some strains produce enzymes capable of degrading nonnutritive substrates, such as trichloroethylene (TCE), a major ground water contaminant used in the dry cleaning industry and in degreasing solvents.

The degree to which *B. cepacia* is being used in bioremediation products is unknown; however, the species has been used extensively to degrade ground water TCE contamination in at least one large U.S. city. A number of environment-friendly bioremediation products containing only naturally occurring, nonpathogenic bacteria are being marketed for use in drain opening and grease eradication systems. Because their formulations are proprietary, it is not known if these products contain *B. cepacia*; however, franchises that distribute such totally natural, noncorrosive, nontoxic products specifically target fast-food restaurants, photo processing facilities, and hospital radiology departments.

In the United States, the biopesticidal use of microorganisms such as *B. cepacia* is regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act; however, the use of naturally occurring, nonpathogenic bacteria as bioremedial agents is essentially unregulated. Only new microorganisms (i.e., intergeneric or formed by combining genetic material from organisms in different genera) are regulated by EPA under the Toxic Substances Control Act (TSCA) (2). Ironically, TSCA regulations provide a strong disincentive to the development of safer microbiologic alternatives for use in bioremediation. For example, although the genetic elements responsible for TCE degradation by *B. cepacia* have been cloned, their recombination into another nonpathogenic bacterial host (e.g., *Escherichia coli*) would constitute a new microorganism, the licensure of which would be considered prohibitively time-consuming and expensive by many companies.

In Canada, biopesticidal uses of microorganisms are regulated by the Pest Management Regulatory Agency of Health Canada, under the Pest Control Products Act (PCPA); bioremedial uses are regulated by Environment Canada under the Canadian Environmental Protection Act (CEPA) (3). Both naturally occurring and genetically engineered microorganisms are strictly controlled under these acts. However, accurate species identification is the cornerstone of all notification of products under the Canadian regulations. This presents a further dilemma. At least five genomovars (discrete species) constitute what has recently been designated the "*B. cepacia* complex" (4). Insofar as the taxonomy of this group is poorly defined, there are no conventional taxonomic designations to distinguish pathogenic from nonpathogenic species. At present, it appears that all five *B. cepacia* genomovars are capable of causing infections in vulnerable persons (4).

Because the epidemiology of *B. cepacia* complex infection in humans is incompletely understood, the threat posed by the inclusion of this species in biopesticides and bioremedial products is difficult to quantify. However, we agree with Holmes et al. that such use should be approached with considerable caution. In a broader context, the commercial use of *B. cepacia* illustrates our incomplete understanding of nonpathogenic bacteria and their potential to cause human disease. Regulations governing the use of microorganisms in industry must constantly adapt to keep pace with the emergence of infections due to nonpathogens and limit risk to human health.

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Human Rabies in Israel

To the Editor: Rabies, a major zoonotic disease in the Middle East, has two main epidemiologic forms: urban and sylvatic. The last case of human rabies in Israel was in the Golan Heights in 1971 (1). Twenty-five years later, in 1996, rabies was reported in a 20-year-old soldier, and then two cases were documented in 1997.

The first case-patient, a soldier in the Golan Heights, was bitten on the lip by an unidentified animal while sleeping. The wound was cleansed and sutured; clinical signs started 39 days later with high fever and headache. The patient was admitted to an emergency room with hallucinations, difficulty in swallowing, and generalized weakness, and rabies was considered in the differential diagnosis; 3 days later the patient became comatose. Samples of saliva, serum, and cerebrospinal fluid; skin biopsy tissue; and corneal impressions were sent to the Pasteur Institute, Paris, France. Eight days after clinical signs developed, rabies was diagnosed by the Kimron Institute by heminested reverse transcription-polymerase chain reaction (hnRT-PCR) on the patient's saliva (2). The RT step was performed with the specific primer 113 (5'-GTAGGATGATATATGGG-'3 at 1013-1030), followed by PCR with the 509 (5'-GAGAAAGA ACTTCAAGA-'3 at 1156-1173) and 304 (5'-GAGT CACTCGAATATGTC-'3 at 1513-1533) primers. The hn-PCR was performed with the 509 and 105 (5'-TTCTTATGAGTCACTCGAATA TGTCTTGTTTAG-'3 at 1393-1426) primers (3). The PCR results were confirmed by the Pasteur Institute 3 days later, and the patient died 35 days after clinical symptoms appeared.

The second case-patient was a 7-year-old girl admitted to the hospital unconscious. Two months before admission, she had been scratched while sleeping by an unidentified animal. On the second hospital day, generalized convulsions and gasping occurred. During the following days, brain stem function progressively deteriorated. Rabies was diagnosed by hnRT-PCR on the saliva sample, and the diagnosis was confirmed by the Centers for Disease Control and Prevention (CDC). The patient died despite supportive care.

The third case-patient, a 58-year-old man with fever, headache, and sore throat, was diagnosed as having pharyngitis and received an oral antibiotic. The patient had been bitten 3 months earlier while sleeping. On admission, the lumbar puncture, computerized tomography scan, and electroencephalogram were normal. On the third hospital day, he had respiratory arrest; during orotracheal intubation, acute laryngospasm with copious amounts of salivation occurred. Rabies was suspected, and viral RNA in the saliva was detected by hnRT-PCR. One day later the patient died.

We injected antemortem saliva and postmortem brain tissue from these patients into suckling mice intracerebrally. Virus was isolated from saliva samples of case-patients 1 and 3 but not from the sample of case-patient 2. Rabies virus antigen in the brain tissue was confirmed by direct immunofluorescence assay, and viral RNA was detected by RT-PCR.

For genetic analysis, we used brain samples from the three case-patients and from animals that died of rabies near the location of the case-patients to amplify and sequence a 328-bp (264 bp from the 3' of the N gene and 64 bp of the 3' NS-N region) fragment. On the basis of homologic results of nucleotide sequences in the three case-patients and in virus isolates from animals in the same regions, we concluded that a reservoir for rabies in foxes is responsible for infection of all three humans.

The three human isolates were tested with a panel of 19 anti-N protein monoclonal antibodies (CDC, Atlanta, GA, USA) and compared with those of rabies isolates from the geographic vicinity of the human cases. Isolates from case-patients 1 and 3 belonged to variant 1 (MAb C18 negative) and were similar to virus isolates from 10 foxes, one jackal, and four cattle in the same regions. Isolates from case-patient 2 belonged to antigenic variant 2 (MAbs C2, C7, C12, C13, C18 negative) and were similar to isolates from four foxes, one dog, and one cow in the vicinity of the second case-patient.

Early antemortem diagnosis of virus in an infected human is very important. Checking for virus in saliva eliminates the difficulty of tissue sampling from humans with suspected cases of rabies, and the sensitivity of hnRT-PCR makes it the technique of choice for detecting limited amounts of virus. Previous work showed that a 200-bp region of the N gene had only one nucleotide difference between them (4). Moreover, two samples from a region in western Mexico, isolated 30 years apart, were identical in sequence (4). Incorporation of the reference strains Pasteur and SAD B19 into our phylogenetic tree indicated that the three human viruses we isolated belong to lyssavirus genotype 1.

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Emerging Infections and Disease Emergence

To the Editor: Emerging infections have been defined as diseases whose incidence in humans has increased within the last 2 decades or threatens to increase in the near future (l). This definition, with minor variations, has continued to be used, although occasional debate erupts over whether one disease or another is truly emerging. Use of the term "emerging" has facilitated communication about the changed pattern of infectious diseases in recent years. While the study of infectious organisms and clinical training about emerging infections are manifestly necessary, they are not a sufficient foundation for understanding the process of disease emergence. I would propose, specifically, that we distinguish between emerging diseases—the study of specific infections that are changing—and the study of disease emergence.

Studies of emerging infections typically rely on disease, organismic, or syndromic approaches. Meetings on emerging infections typically cover newly recognized or characterized organisms or diseases and update information about the recognition, diagnosis, treatment, prevention, and control of these infections. This ongoing education is essential for practicing clinicians, who finished their formal training before AIDS, Lyme disease, ehrlichiosis, *Helicobacter pylori* infection, cryptosporidiosis, cyclosporiasis, and many other infections were described. These meetings also help clinicians learn how to fit new information into their existing knowledge base: What is the probability that a person with rash and fever has ehrlichiosis and that a person with fever and pulmonary infiltrates has hantavirus pulmonary syndrome?

By contrast, understanding the process of disease emergence involves studying the origins and ecology of emerging infections. Many disciplines relevant to disease emergence lie outside traditional infectious disease training and research and include evolutionary biology, demography, population dynamics, ecology, vector biology, climatology, epidemiology, genetics, veterinary medicine, and behavioral sciences (2). Infectious diseases of animals and plants have both a direct and indirect impact on human health. The study of infectious diseases in other species may provide important insights into understanding the process of disease emergence in humans. The study is also relevant to understanding the species-to-species spread of organisms.

Tools used to study and understand disease emergence include mathematical modeling, geographic information systems, remote sensing, molecular methods to study the genetic relatedness of organisms, and molecular phylogeny. Paleobiology, paleoecology, and studies that allow the reconstruction of past events may help inform future research and policy.

A major challenge is to reach people with relevant skills, knowledge, and experience and develop a coherent framework to advance the understanding of the process of disease emergence. No one institution, organization, or country can itself prevent or manage emerging infectious diseases.

In the study of emerging infections we focus on the organism, the patient, and the human population. The study of disease emergence must be at the systems level and must look at ecosystems, evolutionary biology, and populations of parasites and hosts, whatever their species. A primary goal should be to identify conditions or combinations or sequences of events that herald a changed pattern of infections so that preventive strategies can be used.

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Malaria Control in South America

To the Editor: The article by Roberts et al. regarding DDT use and malaria in South America (1) correctly observes that health policy makers have shifted the emphasis of malaria control programs from vector control to case detection and treatment and that malaria control has been woefully underfunded in recent years. However, their conclusions that increased malaria is due to decreased spraying of homes with DDT and that DDT is still needed for malaria control do not withstand close scrutiny.

The authors did not mention several factors influencing malaria increase in recent decades, including growing antimalarial-drug resistance, the deterioration of public health systems responsible for malaria control, and large-scale migration to areas at high risk for malaria (e.g., almost all Brazilian malaria cases occur in the Amazon region) (2,3). Extradomiciliary malaria transmission, poor housing conditions, and human behavior in frontier areas such as the Amazon region limit the usefulness of insecticides. Thus, the deduction of causality between less house spraying with DDT and increased malaria incidence under those circumstances is questionable.

Roberts et al. have not actually linked increased malaria with eliminating DDT use but rather with eliminating house spraying altogether, without implementing effective alternatives. Malaria's recent decline in Brazil is due to a strategy that combines health education, aggressive case detection and treatment, and environmental management to eliminate *Anopheles* breeding sites (C. Catão Prates, unpub. data). A similar strategy has sharply reduced malaria incidence and deaths in Colombia (W. Rojas, unpub. data). In Mexico, use of two synthetic pyrethroid insecticides (deltamethrin and lambda cyhalothrin) for bed-net treatment and house spraying is controlling malaria at a much lower cost than the use of the alternative insecticides tried earlier and mentioned by Roberts et al. (4). Far from being pursued "without meaningful debate," the reduction and phaseout of DDT and other persistent organic pollutants is the subject of a 3-year United Nations–facilitated global negotiation process begun in June 1998.

Roberts et al. assert that DDT applied indoors does not move easily from the application site; however, a mass balance model indicates that 60% to 80% of the DDT ends up outdoors within 6 months (K. Feltmate, A model and assessment of the fate and exposure of DDT following indoor application [bachelor's thesis]. Ontario: Trent University; 1998). From there, DDT can be transported long distances in air, waterborne sediments, and biota, accumulating in humans and other nontarget species (5). Meanwhile, residents of sprayed houses accumulate high, persistent body levels of DDT through skin contact and food contaminated with DDT from air and dust (6).

Long considered a probable human carcinogen, DDT also is associated with reduced lactation, premature births, absorbed fetuses, and lower birth weights (7-9). In addition, recent animal research has raised the possibility that exposure of human fetuses or infants to DDT may cause permanent behavioral changes and impairment of body systems (10-12).

Synthetic pyrethroid insecticides used on bed nets or for house spraying against malaria-infected mosquitoes seem safer for human health than DDT because humans and other mammals possess the ability to hydrolyze the pyrethroids rapidly and excrete them from the body (13-14). Nevertheless, DDT and pyrethroids share known health risks, notably endocrine disruption, and the possible transgenerational consequences of chronic human exposure to pyrethroids have not yet been studied (10,15-16). Optimal protection of human health requires the development of integrated malaria control strategies that eliminate or reduce routine insecticide use by taking maximum advantage of environmental management, biological controls, and other nonchemical vector control measures (17).

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Malaria Control in South America—Response to P.C. Matteson

To the Editor: Dr. Matteson, whose letter relies heavily on unpublished information and nonrefereed publications, states that growing drug resistance has contributed to increasing malaria. While drug resistance is important, when DDT use declined below effective levels (1), the proportion of *Plasmodium falciparum* infections (including infections with resistant strains) compared with *P. vivax* infections (no resistance) did not progressively increase (2). Moreover, malaria has increased in Central America, where drug resistance is unknown (3-6). As for attributing increasing malaria to deteriorating public health systems, the changes imposed on developing countries (in organizational structures of malaria control programs and prohibiting DDT [1,7]) correlate with increasing malaria rates (1).

Dr. Matteson states that large-scale migration explains why almost all Brazilian malaria cases occur in the Amazon Basin. However, DDT cleared malaria from the more populated and temperate southern regions of the country (8, unpublished report: U.S. Agency for International Development review in 1973-74 of Brazil's malaria eradication program). When DDT was in full use (pre-1980), large increases in malaria did not accompany population movement (1). With the 1970s' colonization program of the Basin came malaria problems, but not large population-based malaria increases. DDT prevented that (1,9-11). However, since DDT has been eliminated, persistent urban malaria is again becoming a problem (12-16).

Other factors (biting behavior, housing conditions, and human behavior), which Dr. Matteson attributes to increasing malaria, have always thwarted interdiction of malaria transmission in the Amazon Basin (17;18; an unpublished report: U.S. Agency for International Development review in 1973-74 of the malaria eradication program in Brazil) and are no more important today than they were before.

A UN-facilitated global negotiation process cited as a meaningful debate for malaria control is an effort to provide a legally binding agreement for global elimination of DDT and other persistent organic pollutants, not an open forum for debate of DDT use for malaria control.

Dr. Matteson claims that DDT is associated with reduced lactation. In the United States, where DDT has been banned for 26 years, mothers who stay home breast-feed for an average of 25.1 weeks—mothers who work parttime, for 22.5 weeks (19). In Belize, mothers in urban areas, where DDT is not used for malaria control, breast-feed less than 38.4 weeks—mothers in rural areas with lifetime exposures to DDT breast-feed more than 57.2 weeks (20).

The World Wildlife Fund's mass balance model of DDT sprayed in houses used to refute our assessment that DDT does not readily move away from sprayed houses also mentions that "There are few...data against which to validate the results of this...model, although actual data...should not be difficult to obtain." (21). Studies of DDT use in agriculture show that most DDT settles where it is applied (22).

Studies have shown no meaningful population-based adverse health effects from DDT use, despite more than 50 years' exposure, and evidence argues forcefully that DDT does not cause breast cancer (23).

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On the Etiology of Tropical Epidemic Neuropathies

To the Editor: In a recent report of an epidemic of optic neuropathy in Dar es Salaam, Tanzania (1), Dolin et al. state that the disease is clinically identical to one of the forms of epidemic neuropathy found in Cuba between 1991 and

1993 (2). Cases of peripheral neuropathy have been part of both epidemics (1,2). Both epidemics occurred in nutritionally deficient populations (1,3).

Dolin et al. state that the cause of the Tanzanian epidemic is unknown and probably difficult to establish; however, we believe findings from the Cuban epidemic could be used to study the etiology of this and other tropical epidemic neuropathies.

In Cuba, several research groups isolated and characterized an enterovirus in the cerebrospinal fluid (CSF) of epidemic neuropathy patients (4,5). Enterovirus sequences were found in CSF of 40 (36%) of 111 epidemic neuropathy patients versus 1 (8%) of 12 control surgical patients ($p < 0.01$, chi-square test with 2 x 2 contingency tables) (5). Recently, this enterovirus has been shown to form quasispecies, which could account for altered biologic properties (de la Fuente et al., submitted for pub.). We thus propose that epidemic neuropathy has a nutroviral etiology: Nutritional deficits and stress make the population more likely to become ill after infection with enterovirus quasispecies with altered biologic properties.

The relationship between the host's nutritional status and virus evolution could be key in understanding the cause of epidemic neuropathy, the Tanzanian epidemic of optic neuropathy, and other tropical epidemic neuropathies. Etiologic factors must be identified before appropriate intervention and treatment strategies can be implemented.

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Risk for Ebola Virus Infection in Côte d'Ivoire

To the Editor: In Taï National Park, Côte d'Ivoire, where a new strain of Ebola virus was isolated (1), the World Health Organization is conducting a project to identify the reservoir of the virus and evaluate the risk for its emergence in local populations. In March 1998, we conducted qualitative and quantitative surveys of the villagers' awareness of and risk for Ebola infection. In four villages close to Taï National Park (4 km to 10 km), we carried out structured interviews with 150 villagers and in-depth interviews with 17 villagers and three traditional healers.

Of the 150 villagers participating in the structured interviews, 18.0% had heard of Ebola (90.7% had heard of yellow fever). Of those aware of Ebola, 96.3% thought it life-threatening; 65.4% of them thought it preventable. When ill, 81.2% of the respondents generally relied on traditional healers or herbal medicine. During in-depth interviews traditional healers discussed their treatment practices. In one treatment, an incision is made on the skin and medicinal herbs are applied to the incision. Such traditional practices were implicated in the spread of Ebola virus in Gabon, where a traditional healer and his assistant (who were infected with Ebola virus) were suspected of spreading the virus to their patients through an unsterilized blade (1). The same practices would seem to pose a risk for virus transmission in Côte d'Ivoire.

Even though officially Taï National Park is protected from human activities to preserve its natural ecology, 84.0% of the 150 respondents to our survey often hunted or farmed in the park, 62.2% had encountered chimpanzees, and 53.3% had eaten chimpanzee meat. According to the in-depth interviews, chimpanzee meat is available at bush meat markets and is thought safe for eating, even though primates infected with Ebola virus have been linked with human cases (2,3).

Our survey results show that, even though no large-scale Ebola outbreaks have occurred in this area, villagers living near the park are at particularly high risk for infection because they are not aware of Ebola and do not know that their local customs and behavior may be putting them at risk. To prevent future Ebola epidemics in Africa, information, education, and communication (IEC) programs should be established (3). Moreover, further sociocultural studies on perceptions and behavior should be conducted in addition to exploring the nature of the virus and its cycle in the wild (2,4,5).

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