studies of this species. Our main concern about their work is the inaccurate picture that is presented of the cholera epidemic in Brazil. Some of the statements made in the final paragraphs are in disagreement with the official epidemiologic records and the characteristics of the *Vibrio* bacteria that occurred in Brazil during the 1990s epidemic (2).

In 1991, the seventh cholera pandemic reached South America by the Pacific coast, spreading to Brazil in the same year (3). In Brazil, the first cholera cases were reported in the Amazon region bordering Peru; within a few months a large number of cholera cases were recorded in states facing the Atlantic Ocean in the northeastern region (2). According to the official figures of the Brazilian Ministry of Health (2), 168,598 cases of cholera caused by a *V. cholerae* O1 El Tor strain occurred in Brazil from 1991 to 2001. Of these, 155,363 (92.1%) occurred in the northeastern area of the country, with 2,037 deaths. From 2001 to 2003, the number of confirmed cases was 4,756, 734, and 7, respectively.

Sarkar et al. (1) indicate that 60,000 cases occurred from 1991 to 2001 in Rio de Janeiro, a city localized in the southeastern region; the official records report only 349 cases. The statement that "since 1993, no cholera cases caused by O1 have been reported" is also perplexing. From 1994 to 2001, the official records report 68,583 cases of cholera in Brazil (51,324 of these in 1994, the second major year of cholera incidence). Are the authors suggesting that this number of cases was caused by non-O1 *V. cholerae*? The official records state that the cholera epidemic in Brazil was caused by an El Tor O1 strain (4,5).

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## Fluoroquinolone-resistant *Salmonella* Paratyphi A

**To the Editor:** Fluoroquinolones have been the drug of choice for treating typhoid and paratyphoid fever since the beginning of the 1990s. Multidrug-resistant strains began to prevail in disease-endemic areas, and former first-line antimicrobial drugs, such as chloramphenicol, were sometimes ineffective (1). In recent years, however, strains with decreased susceptibility to quinolones have emerged, and clinical treatment failure is a serious concern (2–5).

An 87-year-old woman was referred from a local clinic to Yokohama Municipal Citizen's Hospital in July 2002 because *Salmonella enterica* serovar Paratyphi A was detected in her urine. She had no subjective symptoms such as pain on urination or urinary urgency, and her temperature was normal. She had never had paratyphoid fever, and she had not traveled abroad. No other person in the community had paratyphoid. Before being admitted to the hospital, she had experienced frequent episodes of urinary tract infection and had been empirically treated each time with oral antimicrobial drugs, including ciprofloxacin. She had been given a dose of 600 mg/day for 7 days, 25 times in the last 4 years.

The patient did not display any abnormal findings on physical examination. *S.* Paratyphi A was not detected in the urine but was confirmed in the stool; therefore, the previous report of bacteriuria could have been due to contamination of a urine sample with feces. An ultrasound showed a polyp and multiple stones in her gallbladder. A carrier state was suspected. Bile was obtained by duodenal aspiration and was positive for *S.* Paratyphi A. The patient was considered to be an asymptomatic cholecystic carrier of *S.* Paratyphi A.

On disk diffusion susceptibility testing, the isolate was resistant to nalidixic acid (NA) and to ofloxacin. The MIC of ofloxacin was as high as 256 μg/mL, and the MIC of ciprofloxacin was128 μg/mL (Table). An open cholecystectomy was performed for treatment of the polyp, the stones, and the highly quinolone-resistant bacteria. A routine perioperative intravenous antimicrobial agent, cefmetazole, was administered as surgical prophylaxis. The polyp was malignant, and the operation was

Table. MICs of antimicrobial agents for the isolate of *Salmonella enterica* serovar Paratyphi A*

| Antimicrobial agent | MIC (μg/mL) | |
|---|---|---|
| | Etest | Broth microdilution |
| Ampicillin | 4 | ND |
| Chloramphenicol | 16 | ND |
| Gentamicin | 0.06 | ND |
| Kanamycin | 1 | ND |
| Streptomycin | 0.75 | ND |
| Sulfamethoxazole/trimethoprim | 0.5 | ND |
| Tetracycline | 8 | ND |
| Cefoperazon | 1.5 | ND |
| Cefotaxime | 0.38 | ND |
| Ceftriaxone | 0.19 | ND |
| Imipenem | 0.19 | ND |
| Aztreonam | 0.125 | ND |
| Fosfomycin | 64 | ND |
| Nalidixic acid | >256 | ND |
| Norfloxacin | >256 | 1024 |
| Ofloxacin | >32 | 256 |
| Sparfloxacin | >32 | 256 |
| Ciprofloxacin | >32 | 128 |
| Levofloxacin | ND | 128 |
| Tosufloxacin | ND | 128 |

*ND, no data.

curative. The patient resumed normal activities, and had no further fecal excretion of *S.* Paratyphi A.

Polymerase chain reaction (PCR) amplification and DNA sequencing were conducted to detect mutations responsible for the fluoroquinolone resistance. Nucleotide sequences of *gyrA*, *gyrB*, *parC*, and *parE* genes were investigated. The primers used for PCR amplification and DNA sequencing have been previously described (6,7). An ABI Prism dye terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems, Foster City, CA) and an automated sequencer (311A; Perkin-Elmer, Applied Biosystems) were used. The isolated strain possessed triple point mutations. The first 2 mutations were in the *gyrA* gene, which encodes DNA gyrase, at codon 83 (TCC to TTC), which substitutes phenylalanine for serine, and at codon 87 (GAC to AAC), which substitutes asparagine for aspartic acid. The third mutation was in the *parC* gene, which encodes DNA topoisomerase IV, at codon 84 (GAA to AAA), which substitutes lysine for glutamic acid. No mutations were found in *gyrB* and *par E*.

*S.* Typhi and Paratyphi A with decreased susceptibility to fluoroquinolones emerged on the Indian subcontinent, Southeast Asia, and Central Asia in the mid-1990s (2–5). On disk diffusion testing, these strains were NA-resistant, and susceptible to ofloxacin or ciprofloxacin; however, the MICs of ciprofloxacin increased to 0.25–4 μg/mL, 10- to 100-fold higher than the usual NA–susceptible strains (5,8,9). NA-resistant strains of *S.* Typhi have 1 point mutation at the target site of quinolones, DNA gyrase, in the quinolone resistance–determining region of *gyrA*, either at codon 83 or codon 87 (2,3). Several epidemics of typhoid and paratyphoid fever caused by NA-resistant strains with clinical failure of quinolone treatment have been reported (4,5).

An experimental attempt had been previously made to induce the production of strains with high-level fluoroquinolone resistance by culturing strains of *S.* Typhi and Paratyphi A in ciprofloxacin-supplemented medium (7). One of these in vitro–induced, high-level resistant strains of *S.* Paratyphi A had triple mutations in the *gyrA* gene at codons 83 and 87 and in the *parC* gene at codon 84, which are the same triple mutations as those seen in the current in vivo case.

Full fluoroquinolone resistance has already emerged in the community in nontyphoid *Salmonella* species. In a clinical isolate of *S. enterica* serovar Typhimurium, the MIC of ciprofloxacin was 32 μg/mL, and mutations in both *gyrA* and *gyrB* were noted (10).

Our findings strongly suggest that high-level quinolone resistance was induced through the long-term carrier state of *S.* Paratyphi A under selective pressure of frequent quinolone administration. The resistance is associated with the 2 mutation sites in *gyrA* and 1 site in *parC*, and multiple point mutations are likely related to the acquisition of full resistance. Physicians should be aware of the emergence of *S.* Typhi and Paratyphi A, as well as nontyphoid *Salmonella* species, which are highly quinolone-resistant.

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## Pygmy Populations Seronegative for Marburg Virus

**To the Editor:** A serosurvey was conducted in Durba, a mining village near Watsa, northeastern Democratic Republic of Congo, the epicenter of Marburg hemorrhagic fever (MHF) outbreaks in 1994 and 1998–2000 (1–3). In this survey, Bausch et al. found a prevalence of anti-Marburg immunoglobulin (Ig) G of 0.35% (2 of 565) in the nonmining population, but a prevalence of 3.75% (13 of 347) in miners. Mine work was an independent risk factor for seropositivity for anti-Marburg IgG (1). Given that widespread secondary transmission could not be documented in the seropositive miners, primary transmission from the unknown reservoir likely occurred in the mines where rodent, shrew, bat, and other fauna were abundant. No evidence of Marburg virus (MBGV) infection was found in samples from small mammals, amphibians, and arthropods collected in and around Gorumbwa mine (R. Swanepoel, pers. comm.); the origin of the MHF outbreak remained unknown.

We hypothesized that the MBGV reservoir's habitat might not be limited to gold mines around Durba, but may exist in caves or forests in the wider Watsa area. As hunter-gatherers, pygmies enter caves for shelter and are in frequent contact with wild animals and body fluids of butchered game. Earlier studies found that pygmies were seropositive for filoviruses significantly more often than subsistence farmers (for filoviruses [4,5], for Ebola but not Marburg [6]). We conducted a seroprevalence study to verify whether pygmies living in the Watsa area constitute another population at risk for primary transmission of MBGV.

The Watsa area's population (≈180,000) includes 4,000 pygmies living predominantly in its southern parts (1). The pygmies live seminomadically in the forest, occasionally leaving to exchange goods with the sedentary Bantu population.

We invited the pygmy population to meet with our study representatives at sites 50–90 km from Durba. Three hundred persons volunteered during a 5-day period. After informed verbal consent was obtained, the study participants were interviewed, and a blood sample was taken from each volunteer. For operational reasons, we excluded children <10 years old. According to local customs, men received small quantities of salt and soap and women received an item of second-hand clothing as an appreciation for their efforts. Ethical clearance was obtained by the ethics committee of the Institute of Tropical Medicine in Antwerp and the representative of the Ministry of Health in Watsa.

The study questionnaire was similar to one used in the Durba 1999 survey; we did not maintain a recall period of 1 year for exposures related to medical treatment, as this did not appear to be a meaningful time span for the pygmies. Procedures for collecting and handling blood samples were similar to the Durba survey, and the same laboratory tests were applied. Serum samples were considered positive only if they were positive for Marburg IgG in both enzyme-linked immunosorbent assay and indirect immunofluorescence assay (IFA) (1).

The study participants originated from 39 different settlements. Their median age was 30 years (range 10–75; q1 20, q3 40); half of them were males. Most study participants reported activities (hunting 60%, entering caves 98%) and contacts with wild animals (rodents 79%, bats 78%, monkeys or apes 99%) thought to be risk factors for the primary transmission of filoviruses. Whenever noticeable differences existed between the sexes, men tended to be exposed more frequently than women, often significantly so. Pygmies were significantly more exposed to wild animals than the nonmining general population; the difference was particularly large concerning contact with bats (Table). From one fourth to one third of study participants reported a direct or potential contact with someone with a febrile hemorrhagic syndrome. Women were more frequently exposed to these risk factors for secondary transmission in the household or community than men, sometimes significantly so; pygmies were less exposed to these risk factors than the nonmining general population (Table). Almost all study participants had been exposed at least once in their