

First Case of Human Ehrlichiosis in Mexico

To the Editor: Ehrlichiosis is a zoonotic disease transmitted to humans through the bite of infected ticks (1). The first recognized human ehrlichial infection, Sennetsu fever, was described in Japan in 1954 (2). The first case of human ehrlichiosis in the United States was recognized in 1986 and was reported in 1987 (3). The disease is caused by intracellular gram-negative bacteria of the *Ehrlichia* genus. The bacteria can be found in the monocytes and granulocytes of peripheral blood. Human monocytic ehrlichiosis is caused by *E. chaffeensis*, and human granulocytic ehrlichiosis is caused by *E. equi* or *E. phagocytophila*, which was first recognized in 1994 (4). Most cases occur between April and September, and the reservoirs are field animals such as rodents, deer, and dogs. The clinical spectrum of the disease is similar to that of other febrile illnesses; without adequate and timely treatment, approximately 5% of the patients die (5).

In the United States, more than 400 cases of serologically confirmed *E. chaffeensis* infection have been documented since 1996 (6). No cases have been reported in Mexico.

In February 1997, we evaluated a 41-year-old male patient from Merida. The patient had been exposed to ticks during activity in a rural area 1 week before the onset of illness. Clinical manifestations included frequent hyperthermia, rash, myalgia, headache, anorexia, fatigue, and cough. Physical examination showed bilateral cervical lymphadenopathy, and a chest radiograph showed an interstitial bilateral infiltrate. Hematic cytometry showed thrombocytopenia of $134 \times 10^3/\mu\text{L}$ and 3200 leukocytes (1440 neutrophils/ μL). Hepatic transaminases were elevated, with an aspartate aminotransferase: 92 U/L (normal: 22 U/L), alanine aminotransferase: 48 U/L (normal: 18 U/L), gamma-glutamyltranspeptidase: 278 U/L (normal: 28 U/L); and globulins: 4.8 g/dL with a polyclonal pattern. No antibodies against rickettsia, dengue virus, B-19 parvovirus, or HIV were detected. A serum sample gave a positive reaction by indirect immunofluorescence assay against *E. chaffeensis* at titers of 1:64 on week 2 and 1:128 on week 3. No infected monocytes or granulocytes were observed in peripheral blood. Remission of the

clinical manifestations began on week 4 and was completed on week 6.

This case indicates the existence of human ehrlichiosis in Yucatan, Mexico. Reactivity to *E. chaffeensis* suggests human monocytic ehrlichiosis; however, as antibody testing was not performed with *E. phagocytophila* or *E. equi*, the possibility of human granulocytic ehrlichiosis cannot be excluded. In any event, case reports indicate the need for deliberate search for cases. Dengue is endemic in this area of Mexico, and ehrlichiosis should be considered as a differential diagnosis.

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HIV-1 Subtype F in Single and Dual Infections in Puerto Rico: A Potential Sentinel Site for Monitoring Novel Genetic HIV Variants in North America

To the Editor: Although international efforts to systematically collect, characterize, and classify HIV isolates from around the world have increased considerably, data on HIV-1 genetic variations in Puerto Rico are limited. This island (population 3.7 million) has one of the highest

AIDS incidence rates in the United States (53.3 cases per 100,000) (1). To evaluate the potential for a multiple subtype distribution pattern in Puerto Rico, we analyzed genetic variations between HIV-1 strains isolated from peripheral blood mononuclear cells of 63 asymptomatic HIV-infected female commercial sex workers from 12 communities. These participants were part of 290 female commercial sex workers followed in a larger cross-sectional study of risk behavior (2).

HIV-1 subtypes F (n = 4) and B (n = 44) strains were identified in persons infected with a single viral subtype with a molecular screening assay based on restriction fragment length polymorphism (RFLP) analysis and with DNA sequencing of the viral protease gene-prot (3). The remaining 15 specimens were classified by RFLP as potential dual infections. Further cloning and sequencing of prot from three of these specimens confirmed one dual infection involving subtypes F and B viruses and identified two infections caused by genetically distinct quasispecies of subtype B variants.

In further detailed pairwise analysis of HIV-1 prot genes, a small nucleotide divergence of 0.3% (0.0 to 1.1) within subtype F contrasted with a typical value of 6.3% (5.1 to 7.8) for the intrasubtype distance within subtype B prot sequences (4). The 99% similarity between prot subtype F Puerto Rican sequences suggested an epidemiologic link or a recent introduction of subtype F in Puerto Rico. Comparative sequence analysis of the C2-V3 env is useful in establishing the time that elapsed from infection on the basis of an annual nucleotide divergence of 0.5% to 1% in this region (5). Such analysis has been used to study the epidemiologic link between cases (4,6). Thus, we compared env sequences from two of five persons infected with prot subtype F strains. This analysis provided several observations. Env nucleotide divergence of 13.2% did not support a direct epidemiologic link between these strains. Furthermore, the relatively high intrasubtype diversity between env sequences suggested that evolution from a common progenitor would have taken a minimum of approximately 13 years. Phylogenetic analysis classified these two env sequences as subtype B, indicating that at least some of Puerto Rican prot subtype F viruses represent

HIV-1 mosaics involving closely related prot F and significantly divergent env B sequences. Overall, discrepancy in both subtype assignment and nucleotide diversities within prot and env regions may indicate that distinct F/B mosaics circulating in Puerto Rico were likely the result of recombination between highly homogeneous subtype F of relatively recent arrival and divergent resident subtype B viruses.

HIV-1 infections with subtype F strains including B/F mosaics have been reported in Brazil (3,7). To evaluate a potential HIV-1 linkage between Brazil and Puerto Rico, a comparative phylogenetic analysis was done on subtype F viral prot sequences from these countries. This analysis documented that HIV-1 subtype F strains in Puerto Rico are distinct from both Brazilian and Romanian viruses. Furthermore, our results show that genetic analysis of prot allows tracking of subtype F viruses of different origin. Recently, by this approach, HIV-1 prot subtype F of Puerto Rican origin and F prot/B env mosaic were identified in HIV-1-infected persons in New York city (8). Observation of HIV-1 subtype F strains in Puerto Rico together with the recent report describing the first cases of such infections in New York indicates the potential for further emergence of subtype F on the North American continent. The presence of a complex distribution pattern of subtype F infections in Puerto Rico has serious implications for the evaluation and development of HIV diagnostics and vaccines.

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The nucleotide HIV-1 sequences obtained in this study were submitted to GenBank; their accession numbers are AF096813-AF096833.

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Paratyphoid Fever in India: An Emerging Problem

To the Editor: Enteric fever is a major public health problem in India, accounting for more than 300,000 cases per year, *Salmonella typhi* is the most common etiologic agent (1), but *Salmonella paratyphi A*, the other causative agent, causes more asymptomatic infections than *S. typhi*. According to earlier reports from India, *S. paratyphi A* was implicated as a causative agent in 3%-17% of enteric fever cases (2). However, a large community-based study in an urban slum of Delhi during October 1995 to October 1996 found that *S. paratyphi A* caused approximately 20%-25% of the cases of enteric fever in this region (3). An outbreak of enteric fever due to a single *S. paratyphi A* strain in an urban residential area was reported in 1996 from New Delhi, where contaminated water was

implicated as the probable source (4,5). This outbreak prompted a retrospective analysis of the laboratory records of the All India Institute of Medical Sciences, New Delhi, over a 5-year period (1994-1998) to study the change, if any, in the etiology of enteric fever in North India.

We evaluated all blood culture records from the institute's clinical bacteriology laboratory for April to October (the months with the highest number of enteric fever cases) each year. Records were from patients residing in New Delhi and the surrounding areas of North India. The blood was collected by a phlebotomist in the outpatient department or by a resident doctor in hospital wards. Blood cultures were carried out by standard laboratory technique (6). Five ml of blood was added to 50 ml of brain heart infusion broth (Hi-Media Laboratory, India) under aseptic conditions. Bacterial identification was accomplished by standard microbiologic protocol (6). Susceptibility to antibiotics (amoxycillin, chloramphenicol, cotrimoxazole, gentamicin, ciprofloxacin, and ceftriaxone) was tested by the comparative disk diffusion method (Stokes method) (7). Chi-square for trend was calculated, and the p value was determined.

The total number of blood cultures performed for enteric fever cases (10,109 in 1994, 12,092 in 1995, 17,652 in 1996, 15,997 in 1997, and 17,012 in 1998) did not change significantly over this period. The isolation of *S. typhi* changed little (Chi-square = 2.367; p = 0.123; statistically not significant). However, the proportion of *S. paratyphi A* isolates rose from 6.5% in 1994 to 44.9% in 1998 (Chi-square = 22.20; p <0.001; statistically significant). The proportion of *S. paratyphi A* isolations in enteric fever cases from 1994 to 1998 was 6.5%, 21.2%, 50.5%, 30.7%, and 44.9%, respectively. Even excluding the strains from the 1996 outbreak (4), we found that the proportion of *S. paratyphi A* in enteric fever cases increased compared with *S. typhi* (Chi-square = 30.528; p <0.001). With our catchment area, case definition of enteric fever, and laboratory methods remaining the same during this period, it appears that the etiology of enteric fever in North India is changing significantly.

The age-wise distribution of *S. typhi* and *S. paratyphi A* showed that *S. typhi* was a significant isolate from children < 5 years of age, while this distribution was not observed for *S. paratyphi A*, which involved those > 5 years