post-chikungunya stage in the epidemic areas. Clinical vigilance is recommended to identify patients with unfavorable outcomes 3 months after disease onset and for those in whom post-chikungunya chronic inflammatory rheumatism develops and who require specific treatment. Detailed guidelines for diagnosis and treatment of these patients with chronic rheumatoid arthritis are needed.

References


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Molecular Detection of Ehrlichia chaffeensis in Humans, Costa Rica

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To the Editor: Human monocytic ehrlichiosis (HME), a tickborne zoonosis caused by the rickettsial pathogen Ehrlichia chaffeensis (Rickettsiales: Anaplasmataceae), is considered an emerging pathogen in the United States and, increasingly, in many countries around the world (1). In Costa Rica, past reports of human cases of ehrlichiosis were diagnosed solely by clinical evaluation and cytomorphology (2,3); recent studies have detected E. canis in dogs and their ectoparasites (4,5). However, molecular detection of natural Ehrlichia infection detected in humans in Costa Rica has not been reported.

In a small rural area of Zarcero, province of Alajuela, north central region of Costa Rica, blood samples were drawn from 20 patients who had histories of tick bites and nonspecific symptoms of fatigue, arthralgia, and myalgia beginning ≥1 year before sampling. The samples were referred for Ehrlichia molecular analysis. In addition, blood samples were drawn from 2 patients of 2 health care clinics in the Alajuela province districts of San Carlos and Alajuela who had clinical signs compatible with recent ehrlichiosis; the samples were sent for confirmation by PCR. All anticoagulated samples were transported within 4 hours to the laboratory for processing. No serologic assays were performed; cytomorphologic estimation and laboratory data were provided from the local health facilities, mostly generated 1 year before this molecular analysis.

DNA was isolated the same day of sampling from whole blood (200 μL) by using the QIAamp Blood Kit (QIAGEN, Santa Clarita, CA, USA) according to the manufacturer’s instructions. Purified DNA from each blood sample was quantified by spectrophotometry, yielding 20–32 ng/μL of DNA. Nested PCR assays were performed as described (6,7). To avoid DNA contamination, first PCR, second PCR, and electrophoresis were performed in separate rooms, following strict rules of pipetting and cleaning, and repeated ≥3 times. In addition, endpoint PCR for the variable-length PCR target gene was performed on samples that were positive in the nested assay, according to Paddock et al. (8). For DNA sequencing, PCR reactions were performed, and products were separated by agarose gel electrophoresis. A nested PCR mixture containing water and 1 containing unrelated Brucella abortus DNA were used as negative controls in every assay. As an internal
control, the 22 samples were assayed for the β-globin gene (TaKaRa Bio/Clontech, Mountain View, CA, USA). All samples were positive for the β-globin gene by PCR with primers PC04 and GH20. Fragments (bands) were excised from electrophoretic gels by using sterile scalpels. These fragments were then placed in a PCR mixture and used as a template. PCR mixtures were pooled and purified by using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer’s instructions. DNA was sequenced at Macrogen Inc. (Seoul, South Korea). The sequences obtained were compared to those previously deposited in GenBank. The arthropod vector or vectors, and vertebrate reservoir or reservoirs of E. chaffeensis in Costa Rica are unknown, and further ecologic studies are required to determine these aspects of human monocytic ehrlichiosis in Central America. Epidemiologic and ecologic surveys are needed to trace and control the dissemination of this public health threat.

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**References**


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Disseminated Mycobacterium tuberculosis in Imported Sooty Mangabey, Thailand

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To the Editor: Tuberculosis caused by bacteria of the Mycobacterium tuberculosis complex affects humans and various species of captive and free-living wildlife (1). In addition, M. tuberculosis has been used experimentally in many different species of Old World monkeys as part of the attempt to establish a suitable model for human tuberculosis (2). We report a case of disseminated tuberculosis caused by M. tuberculosis Spoligotype International Type (SIT) 52 in a recently imported sooty mangabey (Cercopithecus atys) from South Africa to Thailand.

A juvenile male sooty mangabey was imported from South Africa to Thailand in September 2009. Within 1 week, while in quarantine, convulsion and salivation developed in the mangabey, and it died suddenly. This animal, along with another mangabey and 4 mustached guenons (Cercopithecus cephus), was imported from its native Africa to Thailand for the pet trade. Complete histories of the second mangabey and the mustached guenons were not available.

A complete necropsy of the dead sooty mangabey was conducted, and full histopathologic and microbiological analysis was performed. At necropsy, the mangabey was emaciated, with no subcutaneous and abdominal fat tissues. Disseminated granulomas (up to 2 cm) were observed throughout the carcass, including the lungs, liver, spleen, kidneys, multiple lymph nodes (hilar, mediastinal, mesenteric, splenic, hepatic, renal, and pancreatic), and the ileum. The lung was also multifocally adhered to the thoracic wall and pleural diaphragm.

Histologically, the granulomas in all tissues examined demonstrated similar histopathologic features, characterized by a central core of caseous necrosis and surrounded by an unorganized rim of mixed inflammatory cells, including neutrophils, lymphocytes, plasma cells, and epithelioid macrophages. Numerous acid-fast bacilli were present in the cytoplasm of the epithelioid macrophages and in the necrotic area of all tissues. Acid-fast bacilli were isolated from countries in Africa (3–8). In the mangabey reported here, fulminant tuberculosis was diagnosed within 1 week after it arrived in Thailand, during the 21-day quarantine period. The granulomas were morphologically similar to the histopathologic description of tuberculosis lesions of experimentally infected cynomolgus macaques (Macaca fascicularis), which demonstrated lesions as early as 3 weeks after infection, with a gradual increase in severity (2). Previously, East African–Indian lineage (9) and Beijing spoligotype (SIT 1) accounted for most M. tuberculosis isolates in Thailand (10). In nonhuman primates in Thailand, M. tuberculosis complex had been detected at rates of up to 50% (5 positive samples from 10 test samples) by PCR from buccal swabs in long-tailed macaque (Macaca fascicularis) (1). M. tuberculosis belonging to SIT 52 observed in this case has been primarily isolated from countries in Africa (9). Only 1 case of

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