

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

1. Nasci RS. Movement of chikungunya virus into the Western Hemisphere. *Emerg Infect Dis*. 2014;20:1394–5. <http://dx.doi.org/10.3201/eid2008.140333>
2. Mathew AJ, Goyal V, George E, Thekkemuriyil DV, Jayakumar B, Chopra A, et al. Rheumatic-musculoskeletal pain and disorders in a naive group of individuals 15 months following a chikungunya viral epidemic in south India: a population based observational study. *Int J Clin Pract*. 2011;65:1306–12. <http://dx.doi.org/10.1111/j.1742-1241.2011.02792.x>
3. Chopra A, Anuradha V, Ghorpade R, Saluja M. Acute chikungunya and persistent musculoskeletal pain following the 2006 Indian epidemic: a 2-year prospective rural community study. *Epidemiol Infect*. 2012;140:842–50. <http://dx.doi.org/10.1017/S0950268811001300>
4. Ribéra A, Degasne I, Jaffar Bandjee MC, Gasque P. Chronic rheumatic manifestations following chikungunya virus infection: clinical description and therapeutic considerations [in French]. *Med Trop (Mars)*. 2012;72:83–5.
5. Bouquillard E, Combe B. A report of 21 cases of rheumatoid arthritis following chikungunya fever. A mean follow-up of two years. *Joint Bone Spine*. 2009;76:654–7. <http://dx.doi.org/10.1016/j.jbspin.2009.08.005>
6. Brighton SW, Simson IW. A destructive arthropathy following chikungunya virus arthritis: a possible association. *Clin Rheumatol*. 1984;3:253–8. <http://dx.doi.org/10.1007/BF02030766>
7. Ganu MA, Ganu AS. Post-chikungunya chronic arthritis—our experience with DMARDs over two year follow up. *J Assoc Physicians India*. 2011;59:83–6.
8. Essackjee K, Goorah S, Ramchurn SK, Cheeneebash J, Walker-Bone K. Prevalence of and risk factors for chronic arthralgia and rheumatoid-like polyarthritis more than 2 years after infection with chikungunya virus. *Postgrad Med J*. 2013;89:440–7. <http://dx.doi.org/10.1136/postgradmedj-2012-131477>
9. Marimoutou C, Vivier E, Oliver M, Boutin J-P, Simon F. Morbidity and impaired quality of life 30 months after chikungunya infection: comparative cohort of infected and uninfected French military policemen in Reunion Island. *Medicine (Baltimore)*. 2012;91:212–9. <http://dx.doi.org/10.1097/MD.0b013e318260b604>
10. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis Rheum*. 2008;59:762–84. <http://dx.doi.org/10.1002/art.23721>

Address for correspondence: Maud Foissac, Maladies Infectieuses et Tropicales, Hôpital Jacques Puel, Ave de l' Hôpital 12027, Rodez CEDEX 9, France; email: maudpikmin@yahoo.fr

Molecular Detection of *Ehrlichia chaffeensis* in Humans, Costa Rica

Norman Rojas, Daniela Castillo, Priscilla Marin

Author affiliation: Universidad de Costa Rica, San Jose, Costa Rica

DOI: <http://dx.doi.org/10.3201/eid2103.131759>

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 μ L) 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/ μ L 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

Table. Clinical manifestations in patients and laboratory findings of *Ehrlichia* PCR-positive and negative blood samples, Costa Rica

Characteristics	Patient group, no. (%)	
Clinical findings at first examination	PCR-positive, n = 5	PCR-negative, n = 17
History of tick bite	5 (100)	12 (70.6)
Fever	2 (40)	1 (6)
Rash	4 (80)	11 (64.7)
General arthralgia	5 (100)	11 (64.7)
Headache	4 (80)	7 (41.2)
Detection of morulae	1 (20)	12 (70.6)
Positive response to doxycycline	1 (20)	8 (47.1)
Laboratory findings	Range (reference value)*	
Leukocytes	5.3–7.9 × 10 ⁹ cells/L (5–10 × 10 ⁹ cells/L)	6.0–8.9 × 10 ⁹ cells/L (5–10 × 10 ⁹ cells/L)
Platelets	190–299 × 10 ⁹ cells/L (150–450 × 10 ⁹ cells/L)	217–329 × 10 ⁹ cells/L (150–450 × 10 ⁹ cells/L)
Hemoglobin	12.9–14.9 (12.5–17 g/dL)	12.7–16.1 (12.5–17 g/dL)
Aspartate aminotransferase	27–39 U/L (13–39 U/L)	26–36 U/L (13–39 U/L)
Alanine transaminase	21–49 U/L (7–52 U/L)	25–41 U/L (7–52 U/L)

*Laboratory values at time of sampling for this study.

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.

Three of the 20 human blood samples from Zarcero that were tested, and the 2 samples from patients of different locations, were positive for 16S rRNA gene fragment of 390 bp and showed 99%–100% identity to the *E. chaffeensis* strain Arkansas gene (GenBank accession no. NR074500.1). These fragments were sequenced and deposited in GenBank, under accession numbers CR1 San Carlos (KF888343), CR2 Alajuela (KF888344), CR3 Zarcero (KF888345), CR4 Zarcero (KF888346), and CR5 Zarcero (KF888347). Amplified bands for 2 of the 5 samples positive by nested PCR (CR1 and CR4) were identical to those specific for the 5 repeats of variable-length PCR target gene (459 bp).

A summary of clinical manifestations and results of DNA analysis is shown in the Table. The major symptoms reported by most patients were rash, predominantly macular in extremities; general arthralgia and myalgia; and headache. Comparison of clinical and laboratory findings of patients with PCR-positive and -negative results showed no clear differences, possibly related to the length of time between the acute phase of illness and the sampling for molecular analysis, which was usually 1 year. Nevertheless, it is noteworthy that the patients' samples harbored detectable *Ehrlichia* DNA 1 year after the acute phase, which was similar to findings in recent reports (9). Equally, related to the 2 patients evaluated in this study who had a recent

history of infection (CR1 and CR2), no major physical or biochemical alterations were observed, suggesting that disease manifestation was mild or that samples were taken during the convalescent phase. The incongruent high detection of intracellular morulae and low PCR results point out the necessity of improving techniques for early diagnosis of this disease, particularly in primary health care clinics. Clinicians and public health authorities should be aware of the presence of this pathogen in the region and should include molecular tools in the diagnosis of this zoonosis.

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.

Acknowledgement

We thank Ricardo Gutiérrez for his assistance in the sequence analysis.

This work was supported by a Fondos del Sistema grant from the Consejo Nacional de Rectores.

Dr. Rojas is a professor of Bacteriology at the School of Microbiology, University of Costa Rica. His research interests include molecular biology, diagnostics, and epidemiology of human and zoonotic bacteria, especially *Brucella*, methicillin-resistant *Staphylococcus aureus*, and tickborne pathogens.

References

- Ismail N, Bloch KC, McBride JW. Human ehrlichiosis and anaplasmosis. Clin Lab Med. 2010;30:261–92. <http://dx.doi.org/10.1016/j.cll.2009.10.004>
- Hernández-de Mezerville V, Padilla-Cuadra JI. Choque séptico por ehrlichiosis. Acta Med Costarric. 2007;49:118–20 [cited 2013 Nov 25]. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0001-60022007000200011&lng=en&nrm=iso&tlng=es

3. Rojas-Solano JR, Villalobos-Vindas J. Ehrlichiosis granulocitotrópica humana. *Acta Med Costarric*. 2007;49:121–3 [cited 2013 Nov 25]. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0001-60022007000200012&lng=en&nrm=iso
4. Rojas A, Rojas D, Montenegro V, Gutiérrez R, Yasur-Landau D, Baneth G. Vector-borne pathogens in dogs from Costa Rica: first molecular description of *Babesia vogeli* and *Hepatozoon canis* infections with a high prevalence of monocytic ehrlichiosis and the manifestations of co-infection. *Vet Parasitol*. 2014;199:121–8. <http://dx.doi.org/10.1016/j.vetpar.2013.10.027>
5. Romero LE, Meneses AI, Salazar L, Jiménez M, Romero JJ, Aguiar DM, et al. First isolation and molecular characterization of *Ehrlichia canis* in Costa Rica, Central America. *Res Vet Sci*. 2011;91:95–7. <http://dx.doi.org/10.1016/j.rvsc.2010.07.021>
6. Dawson JE, Stallknecht DE, Howerth EW, Warner C, Biggie K, Davidson WR, et al. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to infection with *Ehrlichia chaffeensis*, the etiologic agent of human ehrlichiosis. *J Clin Microbiol*. 1994;32:2725–8.
7. Kocan AA, Levesque GC, Whitworth LC, Murphy GL, Ewing SA, Barker RW. Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerg Infect Dis*. 2000;6:477–80. <http://dx.doi.org/10.3201/eid0605.000505>
8. Paddock CD, Sumner JW, Shore GM, Bartley DC, Elie RC, McQuade JG, et al. Isolation and characterization of *Ehrlichia chaffeensis* strains from patients with fatal ehrlichiosis. *J Clin Microbiol*. 1997;35:2496–502.
9. Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, et al. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. *Parasit Vectors*. 2014;7:298–305. <http://dx.doi.org/10.1186/1756-3305-7-298>

Address for correspondence: Norman Rojas, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, 2060 San Jose, Costa Rica; email: norman.rojas@ucr.ac.cr

Disseminated *Mycobacterium tuberculosis* in Imported Sooty Mangabey, Thailand

Sawang Kesdangsakonwut,
Angkana Sommanustweechai,
Angkana Chairasert

Author affiliations: Chulalongkorn University, Bangkok, Thailand (S. Kesdangsakonwut); Ministry of Public Health, Muang Nonthaburi, Thailand (A. Sommanustweechai); Mahidol University, Bangkok (A. Chairasert)

DOI: <http://dx.doi.org/10.3201/eid2103.131785>

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 (*Cercocebus 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 and classified as *M. tuberculosis* on the basis of 1-tube multiplex PCR (3) and sequencing of 16S rRNA gene results. Spoligotyping revealed that the *M. tuberculosis* isolate belonged to SIT 52.

The international wildlife trade had been reported to be a major source of imported zoonoses, particularly tuberculosis, in nonhuman primates (4–8). 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