Our findings add to the diversity of *Entamoeba* species found in humans. The incidence and effect of infection in infants by the newly recognized species *E. bangladeshi* await future epidemiologic studies.

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Autochthonous *Leishmania siamensis* in Horse, Florida, USA

To the Editor: *Leishmania siamensis*, a recently described species, was identified as the cause of autochthonous visceral leishmaniasis in 2 men in southern Thailand (*1*,*2*). Cutaneous leishmaniasis has been reported in horses in Europe and South America. Lesions in horses are solitary or multiple nodules that are often ulcerated and most commonly occur on the head, pinnae, legs, and neck. Other clinical signs are usually absent. In South America, biochemical characterization has identified *L. braziliensis* in horses (*3*). Leishmaniasis has been reported in horses in Puerto Rico (*4*), and equine leishmaniasis has been described, but no reports have been published, in the United States. *L. infantum* has been reported in equine cutaneous leishmaniasis in Europe (*5*). A report from central Europe recently identified an organism with 98% nucleotide identity over the ITS (internal transcribed spacer) 1 region to *L. siamensis* as the cause of cutaneous leishmaniasis in 4 horses (*6*). *L. siamensis* was also identified in a case of cutaneous bovine leishmaniasis in Switzerland (*7*).

In August 2011, a 10-year-old, 505-kg Morgan horse mare in Florida, USA, with no history of travel outside the eastern United States was evaluated at the University of Florida for an ulcerated mass in the left pinna. When, 6 months earlier, the owner had noticed the mass, it was ≈1 cm in diameter, firm, raised, and covered with hair. Three months later, the ear was unchanged, and the mare was successfully impregnated. Over the subsequent 2 months, the mass began to grow and ulcerate. At that time, veterinary consultation was obtained and a biopsy performed. Histologic

study showed that the dermis was hyperplastic and diffusely infiltrated with neutrophils, macrophages, and lymphocytes (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/18/9/12-0184-Techapp.pdf). Numerous intracytoplasmic protozoal organisms with a small nucleoid and smaller kinetoplast most consistent with *Leishmania* sp. were observed in macrophages. No treatment was pursued. After 45 days, the mare was seen at the University of Florida because of progression of the lesions. The mass on the internal aspect of the pinna was 6 cm × 3 cm and ulcerated, and 3 new firm 1 cm–diameter nodules were observed on the outer pinna of the same ear. Multiple soft, less-defined, 1 cm to 3 cm–diameter nodules were observed along both sides of the neck, shoulders, and withers. No other abnormalities were observed on physical examination or thoracic and abdominal ultrasound, and lymph nodes were not enlarged. Ultrasound confirmed a ≈90 day viable pregnancy. Complete blood count and plasma chemistry were within normal limits.

Tissue aspirates were taken of the multiple ear lesions and of the nodules along the neck and shoulder. From the ulcerated lesion, marked mixed, predominantly neutrophilic inflammation was seen, and rare neutrophils and macrophages contained intracellular protozoal organisms consistent with *Leishmania* sp. amastigotes (online Technical Appendix Figure 2). These organisms were 4–5 μm in diameter and round with pale basophilic cytoplasm. They had an eccentrically placed, basophilic, oval nucleus and a small, basophilic, rod-shaped kinetoplast oriented perpendicular to the long axis of the oval nucleus. No organisms were seen in any other aspirates.

Fresh tissue was submitted for PCR, which has been determined suitable for detecting Old World leishmaniasis in dogs (*8*). Results were negative. Given the clear clinical, cytologic, and histologic evidence for cutaneous leishmaniasis, additional consensus PCR was performed as described (*9*), targeting the ITS1 region. Direct sequencing was performed by using the BigDye Terminator Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on ABI 3130 automated DNA sequencers (Applied Biosystems) at the University of Florida Interdisciplinary Center for Biotechnology Research Sequencing Facilities (Gainesville, FL, USA). The resultant sequence was 310 bp after primers were edited out. Sequence alignment yielded a genotype with 99% identity to the first *L. siamensis* isolate (GenBank accession no. EF200012) and 100% identity to 2 more recently submitted sequences from human visceral leishmaniasis isolates from Thailand (GenBank accession nos. JQ001751 and JQ001752) (online Technical Appendix). The sequence was submitted to GenBank (accession no. JQ617283).

The mare delivered a stillborn foal at 350 days' gestation. Histopathology did not reveal any infectious organisms in the fetal tissues; however, the chorioallantois showed moderate villous atrophy, which was presumed to be the cause of fetal death. One month after foaling, the mare's cutaneous lesions were 90% resolved.

Because the mare in this report was born in the United States and had never left the country, this case appears to be autochthonous. Mode of transmission is unknown. Phlebotomine sand flies found in Florida include *Lutzomyia shannoni*, *Lu. cubensis*, *Lu. vexator*, and *Lu. cruciata*. *Lutzomyia* sp. are competent vectors of *Leishmania* spp. in other areas of the world. However, the vector for reported cases of *L. siamensis* in other regions has not been identified. Although leishmaniasis is infrequently diagnosed in any species in Florida, models have shown that with climate change, the range of sand flies and accompanying leishmaniasis in North America is expected to expand substantially (*10*). This report raises many avenues for further investigation: the prevalence of leishmaniasis in horses in the United States, understanding of the life cycle and vectors, and the zoonotic potential of this *Leishmania* species.

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Novel Vectors of Malaria Parasites in the Western Highlands of Kenya

To the Editor: The primary malaria control techniques, indoor application of residual insecticides and insecticide-treated bed nets, are used on the basis of previously assumed key characteristics of behaviors of vectors of malaria parasites, i.e., resting and feeding indoors (*1*). Any deviation from the typical activities of a species related to exophagy (feeding outdoors) and exophily (living and resting indoors) (*2*) or to population replacement, followed by increased outdoor biting or resting (*3*), may undermine malaria control efforts. Identification of mosquitoes that transmit malaria parasites has, for the most part, relied on the use of outdated morphologic keys (*4*,*5*) and, more recently, species-diagnostic PCR (*6*). Cryptic species or subpopulations that exhibit divergent behaviors (*7*) may be responsible for maintaining malaria parasite transmission, and without adequate discriminatory techniques, these vectors may be misidentified and their key behavioral differences overlooked.

We evaluated indoor and outdoor trapping methods for anopheline mosquitoes in Bigege village, in Kisii Central District in the highlands of western Kenya, which are prone to periodic malaria epidemics. During May–August 2010, we captured 422 female *Anopheles* spp. mosquitoes, primarily from indoor and outdoor light traps. Of these, we identified 161 (38.2%) as species previously described as vectors in the area (*An. gambiae* sensu latu, *An. funestus* s.l., or *An. coustani* [*8*]) by using the standard morphologic key for sub-Saharan species (*4*). We identified another 52 (12.3%) as species not associated with malaria parasite transmission (*1*), but 209 (49.5%) could not be definitively identified. We extracted DNA from 418 mosquitoes and analyzed it for sibling species of the *An. gambiae* complex by using a diagnostic PCR (*6*). Of the 418 DNA samples tested, 80 (19.1%) were identified as *An. arabiensis*; 2 specimens were identified as *An. gambiae* s.s. (0.4%) but the remaining 336 (80.3%) could not be identified by PCR because no amplification product was observed.

To identify these specimens further, we performed molecular characterization by sequencing the ribosomal second internal transcribed spacer (ITS2) and the mitochondrial CO1 loci. Of the 422 female *Anopheles* mosquito specimens, we sequenced DNA from 348, of which 74 (21.3%), 33 (9.5%), and 25 (7.2%) corresponded to GenBank sequences of *An. arabiensis*, *An. coustani*, and *An. funestus* mosquitoes, respectively. However, 216 (62.1%) could not be matched (<90% identity) to any of the 224 ITS2 or 164 CO1 published sequences of anopheline vectors or nonvectors. These 216 specimens could be grouped into several separate clades, distinct from known vectors in the area (Figure). Specimens were grouped by ITS2 sequence. These groups were ranked by abundance and arbitrarily named species A-J. Of the 348 sequenced DNA specimens, the most abundant group having identical but novel ITS2 and CO1 sequences (species A, n = 147, 42.2%) could not be matched definitively to a single species by using the morphologic key. The mosquitoes in this group were most frequently caught outdoors (132, 89.8%). For 64 of a total of 192 traps, collections were made every 2 hours between 6:30 PM and 6:30 AM for 64 nights. Of 30 specimens of species A from these collections, 22 (73.3%) were caught outdoors before 10:30 PM. Data we have collected on human sleeping patterns from this area suggest that a significant proportion of the population is still outdoors before 10:30 PM and therefore exposed to these vectors.

Five of 293 mosquitoes tested had ELISA results positive for *Plasmodium falciparum* sporozoites. All 5 had no previously published ITS2 or CO1 sequences, nor could they be identified by morphologic features. All were collected outdoors. Four of the 5 were in the sequence A group (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0283-Techapp.pdf), and 1 belonged to species I (Figure). The sporozoite

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Technical Appendix

Multiple alignment with Fast Fourier Transform of Leishmania siamensis ITS1 sequences. Nucleotides with differences are shown in red.

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Horse FL           ATTACA-CCAAAAAACATACAGG-TAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Bovine|GQ281282    ATTACA-CCAAAAAACATACAGGCTAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Horse|GQ281281     ATTACA-CCAAAAAACATACAGGCTAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Human|JQ001751     ATTACA-CCAAAAAACATACAGG-TAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Human|JQ001752     ATTACA-CCAAAAAACATACAGG-TAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Human|GQ293226     ATTACA-CCAAAAAACATACAGG-TAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Human|GQ226034     ATTACA-CCAAAAAACATACAGG-TAGAGA-GTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCG-ATATGCCTTTCCCACATACACAAACACAGCAATATATATGT
Human ref|EF200012 ATTACACCCAAAAAACATACAGG-TAGAGAGGTAGTAGAATACATCTACTCGGGGAGGCATGTTTTTTCCGTATATGCCTTTCCCACATACACAAACACAGCAATATATATGT

Horse FL           ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Bovine|GQ281282    ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Horse|GQ281281     ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Human|JQ001751     ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Human|JQ001752     ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Human|GQ293226     ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Human|GQ226034     ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT
Human ref|EF200012 ATATATATACGTATATTGCTATACCCAAAAACCATACCGTAAAAAGCAAAAAGGCCGGTCGACGCCAAATGCCGCGCGTATACAGTGGAAAAGTCCGTTCGTTACGGCTCTTT

Horse FL           CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGGCTTCCTATTTCGTTGAAGAACGCAGTA
Bovine|GQ281282    CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGGCTTCCTATTTCGTTGAAGAACGCAGTA
Horse|GQ281281     CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGGCTTCCTATTTCGTTGAAGAACGCAGTA
Human|JQ001751     CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGG----------------------------
Human|JQ001752     CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGG----------------------------
Human|GQ293226     CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGAA-TGACTTGG----------------------------
Human|GQ226034     CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGACTGACTTGG----------------------------
Human ref|EF200012 CTCTCTCGCGGGTGTGTGTGTGGATAACGGCTCACATAACGTGTCGCGATGGA-TGACTTGGCTTCCTATTTCGTTGAAGAACGCAGTA
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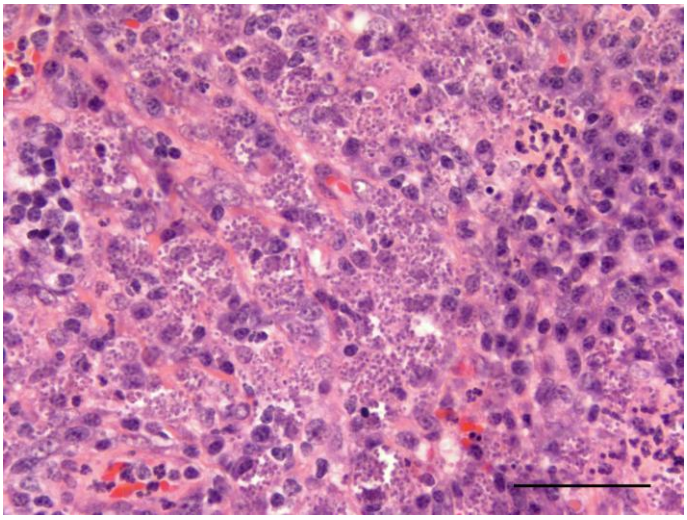

Figure 1. Histologic section of an ulcerated mass from a horse with cutaneous leishmaniasis. Macrophage cytoplasms contain myriad protozoa, each with a small nucleoid and a smaller kinetoplast. Original magnification ×50.

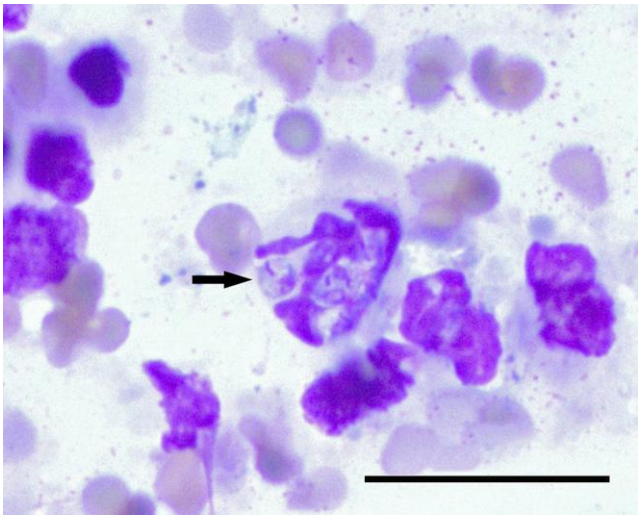

Figure 2. Fine needle aspirate of an ulcerated mass from a horse with cutaneous leishmaniasis exhibiting an intracellular amastigote (arrow). Original magnification ×100.