Transmission of the bacterium occurs primarily through bites from arthropods, including the dog tick (*Dermacentor variabilis*), the wood tick (*D. andersoni*), the lone star tick (*Amblyomma americanum*), and the deer fly (*Chrysops* spp.). In addition, contact with infected animals, most commonly rabbits, wild rodents, and cats, is another common route of transmission to humans (1,6).

Tularemia occurs in various animal species. Lagomorphs, rodents, and sheep are most susceptible; infected animals are frequently found dead or moribund. Carnivores are less susceptible; however, feline tularemia occurs sporadically, and human infections associated with bites and scratches from infected cats have been recognized (7). In addition to arthropod bites, contact with infected dead rabbits or their tissues appears to be the most common source of human infection. A wide variety of case reports have been published describing unique incidences of rabbit-human transmission, including a lawn mower aerosolizing rabbit nests along with their occupants (8), consumption of undercooked rabbit meat (9), and contact with a "lucky" rabbit's foot (10).

The purpose of this report is to alert veterinarians, veterinary laboratory personnel, and public health officials that rabbit tularemia can be easily overlooked on gross examination in animals displaying lesions of hepatic coccidiosis, a common disease of the wild rabbit. Therefore, all rabbits submitted for postmortem examinations should be regarded as potentially infected with tularemia, particularly during seasons when vectors are active.

## Dae Young Kim, Thomas J. Reilly, Susan K. Schommer, and Sean T. Spagnoli

Author affiliation: University of Missouri, Columbia, Missouri, USA

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Address for correspondence: Dae Young Kim, Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, 1600 E Rollins St, Columbia, MO 65211, USA; email: kimdy@missouri.edu



# Imported Leishmaniasis in Dogs, US Military Bases, Japan

To the Editor: Leishmaniasis is found in canids in ≈50 of the 88 countries where leishmaniases are found in humans (1). In Japan, 2 cases of imported canine leishmaniasis have been documented in dogs from Spain (2,3). We report 2 cases of leishmaniasis in dogs in which dermatitis developed mainly on the face. Leishmaniasis was diagnosed from results of a serologic rk39 test, followed by PCR of skin lesion specimens for the Leishmania spp.-specific small subunit (SSU) rRNA gene. Because the dogs had lived on a US military base in Sicily, Italy, for 3 years before their owners were transfered to Japan, the animals were likely infected with L. infantum in Italy.

Animal 1 was a 6-year-old female dog that had lived in Sicily for 3 years, since 2003, and had been brought to Japan in September 2006. While she lived in Italy, she had exhibited alopecic, pruritic, and crusty skin lesions, mainly around the face and on the forearms and hind legs.

In November 2006, the dog was brought to the US Army Veterinary Command's Zama Veterinary Treatment Facility with dermatitis (online Appendix Figure, panel A, www.cdc. gov/EID/content/16/12/2017-appF. htm) and additional signs of kidney failure. A serum specimen was positive by the rk39 dipstick test for diagnosis of visceral leishmaniasis (Kalazar Detect; InBios, Seattle, WA, USA). A skin punch biopsy specimen was obtained for cultures and PCR for the parasites in December 2006. Cultures of 4 skin specimens were all negative, probably because of cool transportation of the samples for 1.5 days before the cultures were started. The dog's condition was treated with ketoconazole and then allopurinol. The skin conditions initially improved, but the lesions did not completely resolve (online Apendix Figure, panels B–D). In May 2008, the dog was humanely killed because of central vestibular disease with unknown cause. A necropsy was not performed.

Animal 2 was a 12-year-old male dog that had also lived in Sicily for 3 years since 2000, and was brought to Yokosuka Base in Japan in 2003. In January 2004, the dog was positive for visceral leishmaniasis by the rk39 test; no particular clinical signs were observed.

In March 2007, the dog was referred to Zama Veterinary Treatment Facility with pruritic alopecia on the dorsum and head, and a skin punch biopsy specimen was obtained for histopathologic evaluation. The presence of amastigotes of *Leishmania* species within areas of dermal inflammation

was confirmed at the Armed Forces Institute of Pathology (Washington, DC, USA). In April 2007, a second skin punch biopsy specimen was obtained for PCR.

PCR was performed for the Leishmania-specific SSU rRNA gene (4). For primary PCR, primers R221 (5'-GGTTCCTTTCCTGATTTACG-3') and R332 (5'-GGCCGGTAAAGGCC GAATAG-3') were used. For nested PCR, primers R223 (5'-TCCCA TCGCAACCTCGGTT-3') and R333 (5'-AAAGCGGGCGCGGTG CTG-3') were used. In the primary reaction, the expected PCR products of ≈603 bp were detected in 2 of 4 skin DNA specimens from patient 1 and 1 of 5 skin DNA specimens from patient 2 (Figure, panel A, lanes 2, 3, 9). In the nested reaction, the expected PCR products of ≈359 bp were seen in all 4 specimens from patient 1 and in 4

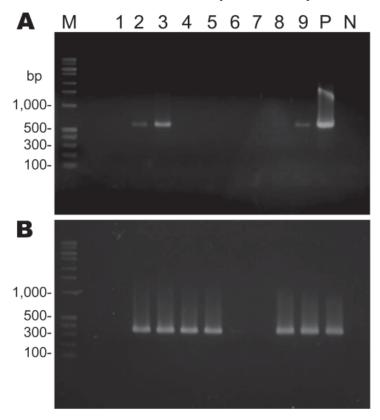


Figure. PCR amplification of the *Leishmania* spp.–specific small subunit rRNA gene from skin biopsy specimens from infected dogs, Japan. DNA samples (100–200 ng) were subjected to primary PCR (A), followed by nested PCR (B). Lanes 1–4, skin DNA samples from patient 1; lanes 5–9, skin DNA samples from patient 2; M, DNA molecular marker; P, positive control; N, negative control.

of 5 specimens from patient 2 (Figure, panel B, lanes 1–4, and 5, 6, 8, 9); some bands were faint. The nucleotide sequences (288 bp) of the nested PCR product of patient 1 were 100% identical to those of patient 2 and sequences of the SSU rRNA gene of *L. infantum* (IPT1 strain, used as a positive control), *L. infantum* (M81429), *L. donovani* (M80295), and *L. chagasi* (M81430).

Global warming, which causes changes in the distribution of the sand fly vectors, and human-produced risk factors, such as travel, migration, and urbanization, may increase the incidence of leishmaniasis (5). Military mobility and operations are also a major risk factor for leishmaniasis in humans and canids (6). In Japan, of >300 kala-azar (visceral leishmaniasis) patients reported, 218 were soldiers who returned from the People's Republic of China before and after World War II (7). In the present study, 2 dogs infected with L. infantum had been brought to Japan from Italy by US military families.

Dog-to-dog transmission by direct contact with contaminated blood through biting may explain the recent outbreaks of leishmaniasis in foxhounds in North America (8). In Japan, although no sandfly species that could transmit leishmania have been reported (7), direct dog-to-dog transmission of leishmaniasis can occur. Babesia gibsoni infection is prevalent among fighting dogs in Japan, likely because of the transmission of infected erythrocytes through biting (9). Greater sharing of information and of diagnostic procedures is required in Japan because few medical and veterinary practitioners have experience with leishmaniasis patients.

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### Yuta Kawamura, Isao Yoshikawa, and Ken Katakura

Author affiliations: Hokkaido University Graduate School of Veterinary Medicine, Sapporo, Hokkaido, Japan (Y. Kawamura, K. Katakura); and US Army Japan District Veterinary Command–Zama Branch Kangawa, Japan (I. Yoshikawa)

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Address for correspondence: Ken Katakura, Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku Sapporo 060-0818, Hokkaido, Japan; email: kenkata@vetmed.hokudai.ac.jp

## Serologic Evidence of Pandemic (H1N1) 2009 Infection in Dogs, Italy

To the Editor: Until recently, the general consensus has been that dogs are poorly susceptible to natural infection with influenza A viruses; however, since the recent upsurge of influenza A circulating subtypes H5N1 and H1N1 viruses, cases of natural infection in dogs have apparently increased. Thus, the role of these animals is being reconsidered in the transmission and spread of influenza viruses (*1*–*3*).

In April 2009, the most recent of the human influenza A pandemics, pandemic (H1N1) 2009, was detected in Mexico. The virus rapidly spread worldwide, within weeks of its first isolation. To date, pandemic (H1N1) 2009 has primarily infected humans, although transmission from infected humans to other animals, including pigs, turkeys, ferrets, cats, and dogs has been reported (4,5).

In Italy (population ≈58 million), the first human cases of pandemic (H1N1) 2009 were reported in May 2009; confirmed cases peaked during the second week of November 2009 (week 46) (6). As of May 9, 2010, Italy had recorded an estimated 5,582,000 cases of pandemic (H1N1) 2009. In Italy as well, the population has ≈7 mil-

lion companion dogs and  $\approx 7.5$  million cats (7). Because of the close contact between persons and their companion animals, we initiated this serologic study to determine whether evidence of pandemic (H1N1) 2009 transmission could be found in companion animals in Italy.

We tested serum specimens from dogs (n = 964) and cats (n =97), originally submitted to the Istituto Zooprofilattico Sperimentale delle Venezie in Legnaro, Italy, from October through December 2009 (weeks 41-53), for assessment of rabies vaccine efficacy. An average of 70 samples were tested per week; the highest number of samples (n = 106) was tested for week 51 and the lowest (n = 25) for week 53. Testing for antibody to influenza A nucleoprotein was performed by using a commercially available competitive ELISA (cELISA) (ID Screen Influenza A Antibody Competition Assay; ID Vet, Montpellier, France), according to the manufacturer's instructions. Previous work from our laboratory has assigned a sensitivity of 93.98% and specificity of 98.71% to this cELISA for the testing of canine serum samples (8). In total, 29 serum specimens tested at a 1:10 dilution, all from dogs, were positive after a second confirmatory screening. None of the 97 feline serum samples were positive by cELISA.

The cELISA-positive serum specimens were then treated with receptordestroying enzyme (RDE; Sigma-Aldrich, St. Louis, MO, USA) (1 part serum: 3 parts RDE) for 16 h at 37°C, followed by heat inactivation at 56°C for 30 min. We then tested the specimens by the hemagglutination inhibition (HI) test against the pandemic virus A/Verona/Italy/2810/2009 (H1N1), A/swine/Italy/711/2006 (H1N1), and H3N8 (A/canine/Florida/2004) by using 0.5% chicken erythrocytes and standard methods (9). Seven serum samples (nos. 4410, 4438, 4444, 4460, 4517, 4520, 4681) were positive by HI