Autochthonous Outbreak and Expansion of Canine Visceral Leishmaniasis, Uruguay

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We report an outbreak of canine visceral leishmaniasis in Uruguay. Blood specimens from 11/45 dogs tested positive for *Leishmania* spp. Specimens of *Lutzomyia longipalpis* sand flies were captured; typing revealed *Leishmania infantum*. Our findings document an expansion of visceral leishmaniasis to southern South America and risk for vectorborne transmission to humans.

Visceral leishmaniasis (VL) is a zoonotic disease caused by flagellated protozoa of the genus *Leishmania* and transmitted by sand flies belonging to the Phlebotominae subfamily; those of the *Lutzomia longipalpis* species are the main vectors. VL affects humans and canids; canids are identified as the main reservoir of the parasite (*1*). This zoonosis has been endemic in northeastern Brazil for several centuries, but it has been recently expanding to southern areas of the South American continent (*2*–*4*). In 2010, the presence of the vector *L. longipalpis* sand flies was recorded for the first time in Uruguay (*5*); the right environmental conditions, the presence of competent sand fly vectors, and the constant appearance of new cases of canine and human leishmaniasis in border countries have made Uruguay susceptible to VL transmission (*5*).

In 2015, we performed a house-by-house survey in Arenitas Blancas (31°25.000′S, 58°00.066′W) in Salto, Uruguay. We included 49 dogs in the survey. Whole-blood samples from 11 (22%) tested positive for *Leishmania* spp. with 2 different diagnostic kits, TR DPP (Bio-Manguinhos, Rio de Janeiro, Brazil) and Speed Leish K (Virbac, Carros, France), both of which detect antibodies raised against *Leishmania* antigens in whole blood, plasma, or serum by immunochromatographic methods. Among the dogs whose specimens tested positive, 8 showed the common clinical signs of skin lesions, fever, weight loss, and eye lesions; 3 were asymptomatic. Dogs whose specimens tested positive came from 9 different houses in the same neighborhood (Figure, panel A); of these, 2 dogs had never traveled outside their residence, and in 2 other cases, both dam and offspring were infected. Three dogs came from breeding kennels, and the rest were born in Arenitas Blancas.

We performed lymph node biopsies and bone marrow aspiration in dogs whose specimens tested positive; we also confirmed infection by direct observation of amastigotes in stained slide smears of aspirates. After extracting DNA from tissue samples by using the Quick-DNA Universal kit (Zymo Research, Irvine, California, USA), we performed PCR and sequencing of the ribosomal internal transcribed spacer 1 (*6*) to achieve typing of *Leishmania* spp. at the species level. We aligned and analyzed the sequences by using MAFFT software (*7*); the neighbor-joining phylogenetic tree obtained from the analysis showed that sequences identified from our samples group together with sequences belonging to *L. infantum* reference strains that we sequenced, as well as with sequences obtained from GenBank (Figure, panel B). Accession numbers and percentage of identity of the sequences obtained from GenBank are *L. infantum*, KM677146.1 and KC477100.1 (100%); *L. donovani*, HM130608.1 and HQ830358.1 (99%); *L. amazonensis*, DQ182536.1 (86%); *L. guyanensis*, DQ182541.1 (81%); and *L. braziliensis*, DQ182537.1 (81%).

To verify that the complete domestic cycle of *Leishmania* spp. was taking place in the affected area, we placed CDC Miniature Light Traps (John W. Hock Company, Gainesville, FL, USA) in domiciles in which affected dogs had been found. All sampling was peridomestic and consisted of 13 traps placed overnight on 3 different nights; sampling resulted in collection of 3 sand flies, 1 male and 2 female. Using observational analysis, we identified the collected samples as *L. longipalpis*; this result was confirmed by PCR with species-specific primer LiCac (*8*,*9*). Furthermore, we performed PCR amplification with *Leishmania*-specific primers AJS1 and DeB8 (*8*) using sand fly DNA as a template. A PCR product of 300 bp from one of the sand flies was amplified and sequenced and showed *Leishmania* DNA in the vector (data not shown).

In summary, we describe an autochthonous outbreak of canine VL in Uruguay. The reported cases represent the expansion of VL to southern areas of the continent; the evidence shows that *L. infantum* is the parasite responsible for the outbreak in both canine hosts and a sand fly vector. The presence of competent vectors in the area constitutes a risk for the human population. Further work is needed to implement effective measures to control the extension of cases. It is also mandatory to improve surveillance of the vector and expand surveillance to other wild and domestic potential hosts. Finally, efforts should be made to prevent new cases of human VL in Uruguay.

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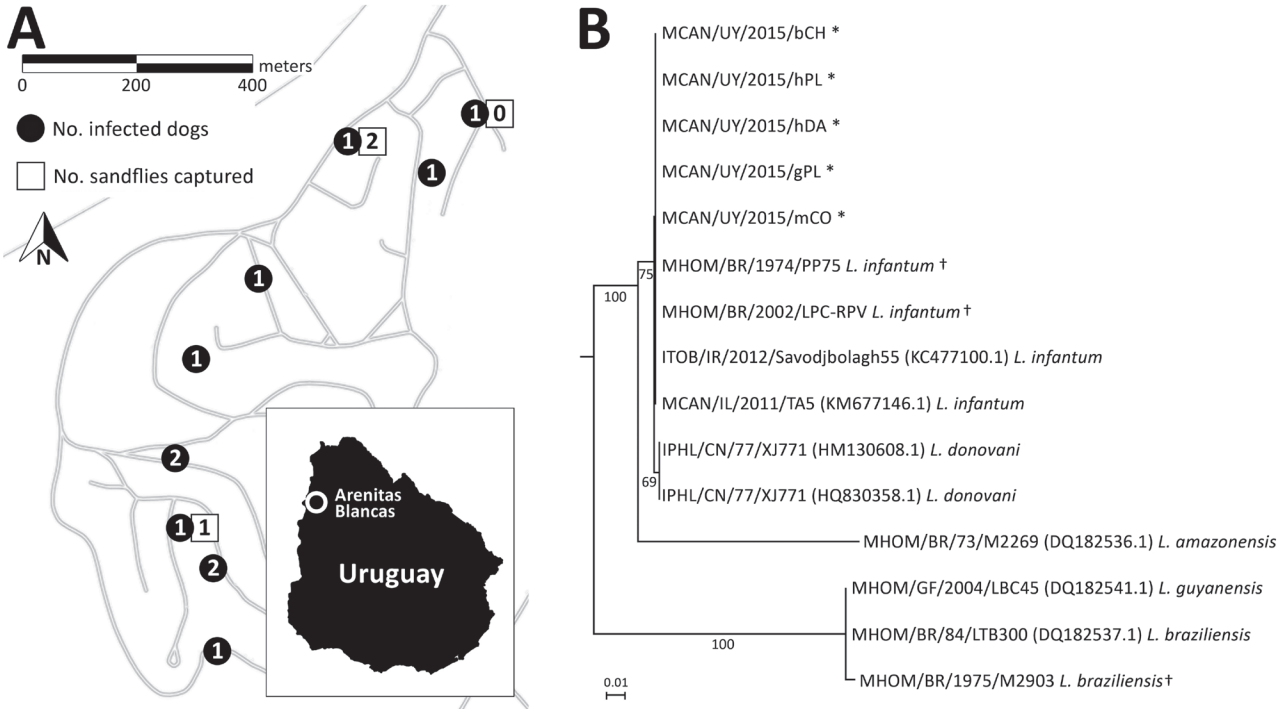

Figure. Survey of *Leishmania* spp. infection in dogs in Arenitas Blancas, Salto, Uruguay. A) Surveyed area in the locality of Arenitas Blancas in Salto, Uruguay. White squares represent the location of *Lutzomia longipalpis* sand fly captures, and black circles represent domiciles in which infected dogs were found; numbers indicate number of *Leishmania* spp.–infected sand flies or dogs at that location. B) Neighbor-joining phylogenetic tree obtained from the analysis of *Leishmania* internal transcribed spacer 1 sequences from tissue samples of infected dogs. Bootstrap values are represented at the nodes of major branches. Scale bar indicates nucleotide substitutions per site. *Sequences obtained from infected dog samples. †Reference strains sequenced by the authors.

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Worldwide Endemicity of a Multidrug-Resistant *Staphylococcus capitis* Clone Involved in Neonatal Sepsis

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A multidrug-resistant *Staphylococcus capitis* clone, NRCS-A, has been isolated from neonatal intensive care units in 17 countries throughout the world. *S. capitis* NRCS-A prevalence is high in some neonatal intensive care units in France. These data highlight the worldwide endemicity and epidemiologic relevance of this multidrug-resistant, coagulase-negative staphylococci clone.

Preterm birth is the world's leading cause of death before 5 years of age (*1*). Neonatal sepsis, mostly due to coagulase-negative staphylococci, occurs frequently in neonatal intensive care units, especially in very low birthweight preterm infants (*2*). Cases and series of neonatal sepsis involving *Staphylococcus capitis* have been reported in different countries (*3*) and were initially considered unrelated epidemic bursts. More recently, we detected a single multidrug-resistant clone of *S. capitis*, designated as the NRCS-A clone and characterized by a specific pulsed-field gel electrophoresis (PFGE) pattern, in several neonatal intensive care units (NICUs) in France, Belgium, the United Kingdom, and Australia (*4,5*). The clonality of the strains was confirmed by PFGE, multilocus sequence typing–like analysis, and whole-genome sequencing. We also showed that all NRCS-A isolates exhibited a decreased susceptibility to all of the antimicrobial agents frequently used in NICUs, namely β-lactams, aminoglycosides, and vancomycin (*5*). Furthermore, a recent study showed that *S. capitis* NRCS-A–associated sepsis constitutes an independent risk factor for severe illness in neonates (*6*).

We suspected that the initial report of NRCS-A dissemination in NICUs from 4 distant countries was only the tip of the iceberg and that the spread of NRCS-A strains was much wider than expected. To determine the extent of NRCS-A dissemination, we asked microbiologic laboratories worldwide to send us methicillin-resistant *S. capitis* strains isolated from blood cultures of neonates. These isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and subjected to PFGE using the *Sma*I restriction enzyme as previously described (*7*). NRCS-A's characteristic PFGE pattern was found for 154 strains isolated between 1994 and 2015 in 34 NICUs from 17 countries: Australia, Belgium, Brazil, Canada, Czech Republic, Denmark, Finland, France, Germany, the Netherlands, New Zealand, Norway, South Korea, Switzerland, Taiwan, the United Kingdom, and the United States.

Retrospective, laboratory-based epidemiologic investigations to estimate the prevalence of NRCS-A strains in NICUs could not be performed on the same worldwide scale, so we conducted such a study in France. Results collected from 47 of the 57 NICUs in France during 2014 indicated that only 4 NICUs were free of NRCS-A. In the 43 other NICUs, NRCS-A strains accounted for up to 46% of all cases of positive cultures of blood from neonates (median 13%, interquartile range 10%–20%) and represented 19% of all coagulase-negative staphylococci strains isolated from the blood cultures of neonates.

Taken together, these data unquestionably demonstrate the unusual worldwide endemicity of the multidrug-resistant NRCS-A clone in NICUs. In addition, the epidemiologic data from France highlight the propensity of NRCS-A to invade and settle in most NICUs on a national scale. Once endemic in a NICU, NRCS-A strains expose infected neonates to a risk of therapeutic failure because treatment of neonatal sepsis involving methicillin-resistant coagulase-negative staphylococci is usually based on vancomycin and aminoglycosides, to which NRCS-A isolates are not susceptible (*3–5*).

A thorough investigation of the determinants of the worldwide spread of NRCS-A is urgently needed to unravel the dissemination routes and reservoirs of this multidrug-resistant clone and to succeed in managing and controlling its diffusion. The risk of vancomycin treatment failure warrants an investigation of alternate antimicrobial stewardship strategies, in particular linezolid, daptomycin, and ceftarolin, to treat NRCS-A–associated neonatal sepsis.