Transplacental Transmission of a North American Isolate of *Leishmania infantum* in an Experimentally Infected Beagle

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summer (June) showed no clinical signs of disease, while histologic examination of the intestines showed only a minimal coccidian infection. This indicates that as fish grow older and, presumably, increasingly more immunocompetent, or as water temperatures increase and stabilize, the fish may be able to eradicate the parasites or become more resistant to infection, reinfection, or pathologic effects (Steinhagen et al., 1998; Paperna, 1999). This is fortunate, because no chemotherapeutics are approved by the U.S. Food and Drug Administration for treatment of piscine coccidiosis (Center for Veterinary Medicine, 2000). Piscine coccidiosis is also not considered a notifiable or significant disease by the World Organization for Animal Health (Office International des Epizooties, 2003), and thus, development of treatments for this disease has received limited attention. Therefore, prevention of clinical coccidiosis currently depends on maintaining a proper environment, providing optimal nutrition, and reducing stress among fish populations.

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Transplacental Transmission of a North American Isolate of *Leishmania infantum* in an Experimentally Infected Beagle

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ABSTRACT: *Leishmania infantum*, an etiologic agent of zoonotic visceral leishmaniasis, is widespread among foxhounds in the United States. Although sand flies are widely distributed throughout the United States, epidemiological data do not support a major role for sand flies in the transmission of *L. infantum* in foxhounds in this country. Congenital transmission of human visceral leishmaniasis is reported in humans and might also occur in dogs. We have previously isolated *L. infantum* from Virginia foxhounds and used this isolate (LIVT-1) to experimentally infect beagles. Four female beagles, chronically infected with LIVT-1, were bred to a male beagle chronically infected with *L. infantum chagasi*. One beagle was able to maintain her pregnancy, and 4 puppies were delivered by cesarean section. One puppy was malformed and autolytic at delivery, and tissues were not collected or analyzed. The remaining puppies were killed at the time of cesarean section, and selected tissues were collected for parasite culture and PCR. Promastigotes were not cultured from tissues in any of the puppies. *Leishmania* sp. DNA was detectable by PCR in liver, bone marrow, and heart from all 3 puppies and in the spleen, lymph node, kidney, and placenta in 2 puppies. Placental tissue from the dam was PCR negative. This is the first report of maternal transmission of a North American isolate of *L. infantum* from an experimentally infected dog.

Leishmaniasis is a vectorborne disease caused by infection with *Leishmania* spp.; these parasites are usually transmitted by phlebotomine sand flies. Studies have suggested that transmission might also occur infrequently by sexual contact (Catone et al., 2003), blood transfusions (Owens et al., 2001; Giger et al., 2002), and direct contact (Lainson and Bray, 1964; Nuwayri-Salti and Khansa, 1985). Rare cases of congenital transmission have been documented in humans (Low and Cooke, 1926; Nyakundi et al., 1988; Yadav et al., 1989; Eltoum et al., 1992; Meinecke et al., 1999). Similarly, it has been suggested that maternal transmission can occasionally occur in dogs (Mancianti and Sozzi, 1995; Masucci et al., 2003).

Leishmania infantum, an etiologic agent of human visceral leishmaniasis, is widespread among foxhounds in the United States (Rosypal et al., 2003). Sand flies are present throughout much of the country, particularly in the southeast (Young and Perkins, 1984). However, epidemiological data do not support a role for sand flies in the transmission of *L. infantum*. This suggests that an alternative mode of transmission could maintain infections in U.S. foxhounds. The risk of zoonotic spread from infected dogs to humans is currently unknown for the United States. In this study, we determined whether vertical transmission can occur in dogs infected with a North American isolate of *L. infantum*.

Intact female beagles (n = 4) were experimentally infected with promastigotes of the LIVT-1 strain of *L. infantum* originally isolated from a naturally infected foxhound from Virginia (Rosypal et al., 2003). All of the dogs had clinical signs compatible with canine leishmaniasis. The parasite had been reisolated from the beagles by culture of lymph node and bone marrow aspirates, and it was detectable by polymerase chain reaction (PCR) conducted on bone marrow (A. Rosypal, unpubl. obs.). The female dogs were bred to a male beagle chronically infected with *L. infantum chagasi* both by natural service and by artificial insemination. Before breeding, semen was collected from the male dog and placed in *Leishmania* sp. culture media (30% v/v fetal bovine serum, 1% penicillin/streptomycin, 2% human urine, in Grace's Insect Media) at 25 C.

TABLE I. PCR results from selected tissues taken at necropsy from puppies delivered from a beagle experimentally infected with a North American isolate of *Leishmania infantum.*

	PCR results*		
Tissue	Puppy 1	Puppy 2	Puppy 3
Liver	+	+	+
Bone marrow	+	+	+
Spleen	+	+	−
Heart	+	+	+
Lymph node	+	−	+
Kidney	−	+	+
Placenta	+	+	−

* +, positive; −, negative.

One of the 4 dogs became pregnant and was able to sustain the pregnancy, but the other 3 dogs either did not conceive or did not maintain their pregnancies. For this reason, the following data were collected from a single female dog that sustained her pregnancy. Four puppies were delivered by cesarean section on day 60 of gestation (normal gestation period = 63 days) to preclude the possibility of transvaginal transmission during natural birth. Puppies were either unable to breathe at birth or were killed immediately following delivery. One puppy was nonviable and deformed at the time of delivery and tissues were not collected. Portions of liver, femoral bone marrow, spleen, heart, lymph node, kidney, and placental tissues were collected from the remaining 3 puppies for parasite culture and PCR.

DNA was extracted from tissue samples with a commercial kit (DNA Mini Kit, Quiagen®, Valencia, California). For each 50-μl reaction, 1 μl of DNA was added to 45 μl of Platinum® PCR Supermix (Invitrogen™ Life Technologies, Carlsbad, California) in a 0.5-ml thin-walled microcentrifuge tube. To the reaction tube, 2 μl of primers 13A and 13B were added; these primers amplify a conserved minicircle region of kinetoplast DNA from all species of *Leishmania* (Rodgers et. al., 1990).

Optimal PCR amplification conditions consisted of initial denaturation at 95 C for 2 min; 38 cycles of denaturation at 94 C for 30 sec, annealing at 62 C for 30 sec, and extension at 68 C for 30 sec; and a final extension at 72 C for 10 min. PCR product (1 μl) was used as template DNA in a second 50-μl reaction and was subjected to the same cycling conditions described. PCR products were electrophoresed on a 2% agarose gel and with size markers to detect the 116-bp PCR product. DNA extracted from LIVT-1 promastigotes was used a positive control, and a negative control without DNA was included. To determine the sensitivity of the PCR assay, DNA was extracted from a known number of LIVT-1 promastigotes. Tenfold serial dilutions of DNA were made and subjected to PCR. DNA detection limit was <1 organism.

Promastigotes were not cultured from any puppy tissues. Parasites were not isolated from the sire's semen culture. The expected 116-bp product was amplified by PCR in liver, bone marrow, and heart tissue from all 3 puppies (Table I). *Leishmania* sp. DNA was also detectable in spleen, lymph node, kidney, and placental tissues from 2 puppies. No PCR product was detectable in testes collected at necropsy from the sire (data not shown). *Leishmania* sp. DNA was demonstrable by PCR in uterine tissue collected at necropsy from the dam (data not shown). Placental tissue from the dam was PCR negative.

This is the first report of maternal transmission of a strain of *L. infantum* from North America. The most likely route of transmission was across the placenta. Puppies were delivered by cesarean section, which eliminated the possibility of transmission through microscopic lesions in the birth canal, and *Leishmania* sp. was detected by PCR in placenta and uterine tissue from the dam.

Leishmania sp. parasites circulate in blood. The placental blood supply is in close proximity to the maternal circulation, and parasites might pass into fetal circulation. This mode of transmission has been previously reported in humans (Low and Cooke, 1926) and in experimentally infected mice (Nuwayri-Salti and Khansa, 1985). Paternal transmission was excluded because no parasites were cultured from semen and there was no detectable *Leishmania* sp. DNA in testes tissue tested by PCR.

Pregnancy has a systemic effect that biases the immune system toward a Th2 immune response that protects the fetoplacental unit while simultaneously increasing susceptibility to intracellular pathogens (Wegmann et al., 1993). It has been previously shown that Th1 cytokines can be harmful to the placenta and might compromise fetal survival (Wegmann et al., 1993; Raghupathay, 1997). Resistance to leishmaniasis is associated with a strong Th1 type response marked by increased secretion of interferon-γ, tumor necrosis factor, and interleukin 2 compared with symptomatic dogs (Pinelli et al., 1994; Santos-Gomes et al., 2002). Moreover, dogs with clinical disease develop a strong humoral response (Nieto et al., 1999) and a simultaneous lack of an appropriate cell-mediated immune response, resulting in disease progression (Pinelli et al., 1994). Humoral immunity is driven by a Th2 immune response and the production of cytokines by type 2 helper T cells.

The dogs used in this study were clinically symptomatic with high anti-*Leishmania* sp. antibody responses (data not shown), so they were already biased toward a Th2 immune response. In addition to generalized immunosuppression required during pregnancy to prevent immune reactions directed against foreign antigens on the fetus, a shift to a Th2- from a Th1-type response during clinical disease could increase both the severity of disease and the chance of congenital transmission of canine leishmaniasis. Previous work in experimentally infected mice demonstrated increased susceptibility to leishmaniasis during pregnancy (Krishnan, Guilbert, Russell et al., 1996).

In this study, 3 of 4 beagles that were bred were unable to conceive or maintain their pregnancies. Similarly, in a murine model, Krishnan, Guilbert, Wegmann et al. (1996) found that *Leishmania major* infection was associated with increased frequencies of fetal resorption and implantation failure in pregnant mice. Although mice and dogs have different types of chorioallantoic placentas (Loke, 1982), the various numbers of placental layers and types of placental attachments found between species might play a role in the probability of congenital transmission of leishmaniasis.

Zoonotic visceral leishmaniasis is caused by both *L. infantum* and *Leishmania chagasi* (Mauricio et al., 1999). The finding that a North American isolate of *L. infantum* is transmitted vertically is in contrast to previous work that inferred maternal transmission of *L. chagasi* does not occur in dogs, even though some placental tissue was PCR positive (Andrade et al., 2002). Rare cases of vertical transmission of *L. infantum* from the Mediterranean Basin have been previously described in humans and in dogs (Mancianti and Sozzi, 1995; Meinecke et al., 1999; Masucci et al., 2003). *Leishmania infantum* MON-1 is the most common zymodeme causing canine leishmaniasis in southern Europe and is the same type identified in infected foxhounds in the United States (Gaskin et al., 2002). It is possible that the strain of *L. infantum* infecting U.S. foxhounds has the unique ability to routinely cross the placenta. This mode of transmission could be responsible for maintaining the infections in this group of dogs. Additional research with larger numbers of dogs experimentally or naturally infected with the North American strain of *L. infantum* need to be conducted to determine the efficiency of maternal transmission and parasite survival in transplacentally infected puppies.

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The Finding of *Echinostoma* (Trematoda: Digenea) and Hookworm Eggs in Coprolites Collected From a Brazilian Mummified Body Dated 600–1,200 Years Before Present

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ABSTRACT: The identification of parasites from ancient cultures expands our list of parasites infective to extant humans. A partially mummified human body from the archeological site of Lapa do Boquete, Minas Gerais State, Brazil, was recently discovered. It was interred between 600 and 1,200 yr ago. Dietary analysis showed that the mummified body was from a society that had a mixed subsistence of agriculture and gathering of wild foods. Coprolites from the body contained numerous helminth eggs. The eggs were identified as those of *Echinostoma* sp. and hookworm. Hookworm infection in pre-Columbian populations is already established, but this is the first evidence of *Echinostoma* sp. eggs found in human coprolites. The diagnosis of a true infection, as opposed to false parasitism, is discussed. The possibility of *Echinostoma ilocanum* infection is discussed, as this is a common species found in humans in the Asiatic region, which could have been introduced in South America in the pre-Columbian period. Alternative possibilities are also considered, including indigenous Brazilian *Echinostoma* species.

One of the most significant contributions of archeology to parasitology is the documentation of parasite species infective to ancient humans that are not known from the present clinical literature. In some cases, false parasitism is implicated, such as the find of *Cryptocotyle lingua* eggs in an Alaskan Yupik mummy (Zimmerman, 1998). False parasitism occurs when parasite eggs are passed in the feces of a subject who is not infected with the parasite. In other cases, real infection is implicated, such as the discovery of acanthocephalan eggs in archeological sites of the Great Basin of North America (Fry, 1970). Diagnosing infection from the archeological record is only possible when the physical remains analyzed are of human origin and when the dietary practices of the human population are known (Reinhard et al., 1987; Reinhard, 1988). If these 2 criteria are met, then the possibility of confusing false parasitism with true infection can be reduced.

Archeologists recently excavated the cave, Lapa do Boquete. This site is situated in the Peruaçu River Valley of northern Minas Gerais State, Brazil. The region is characterized by cerrado vegetation com-