Vet. Pathol. 33:731–734 (1996)

Cutaneous Leishmaniasis in Two Horses

J. A. Ramos-Vara, B. Ortiz-Santiago, J. Segalès, and R. W. Dunstan

Abstract. This report contains the clinical, histologic, immunohistochemical, and electron microscopic findings in two cases of equine cutaneous leishmaniasis. Nodular, sometimes crusty or ulcerated lesions were confined to the pinna and adjacent neck in both animals. The dermal inflammatory infiltrate was lymphohistiocytic in horse No. 1 and pyogranulomatous with formation of tuberculoid granulomas in horse No. 2. Numerous *Leishmania* organisms were found within macrophages in both animals. There was moderate to intense and specific reaction by immunoperoxidase using a polyclonal antiserum against *Leishmania* in both horses. *Leishmania* amastigotes were also revealed by electron microscopy. This is the first report of equine cutaneous leishmaniasis recognized in North America and Puerto Rico. Leishmaniasis should be considered in the differential diagnosis of cutaneous nodular diseases in the horse.

Key words: Electron microscopy; histopathology; horses; immunohistochemistry; leishmaniasis; skin.

Horse No. 1 was a 4-year-old Paso Fino colt that had a 4-month history of ulcerated and crusty papules and nodules of the right pinna that progressed to involve the skin of the right maxilla and neck (Fig. 1). This horse was born in Colombia and at the age of 8 months was shipped to the United States. After quarantine in Florida, it was shipped to Puerto Rico. Physical examination revealed coalescent, superficial, and occasionally ulcerated lesions. Horse No. 2 was a 2.5-year-old Paso Fino colt; he and his dam were born in Puerto Rico and had never left the island. This horse had ulcerated and nonulcerated 5–10-mm-diameter nodular lesions on the left pinna that eventually involved the right pinna and the neck and shoulder.

Samples of the lesions were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE) and Giemsa methods. Additional sections were stained by a peroxidase–antiperoxidase (PAP) method using a modification of a previously reported technique.[9] The primary antiserum, a rabbit polyclonal antiserum against *Leishmania infantum* amastigotes, was incubated overnight at 4 C. The bridge antiserum (goat anti-rabbit, Nordic Laboratories, Tilburg, The Netherlands) was applied for 1 hour at room temperature followed by a 1-hour incubation at room temperature with PAP complex. Skin from a naturally infected dog with numerous *Leishmania* amastigotes was the positive control, normal rabbit serum served as the negative control, and diaminobenzidine was the chromogen. Samples for electron microscopy were postfixed in osmium tetroxide, dehydrated, and embedded in a mixture of epon–araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Sections were examined with a Phillips 301 electron microscope at 60 kV.

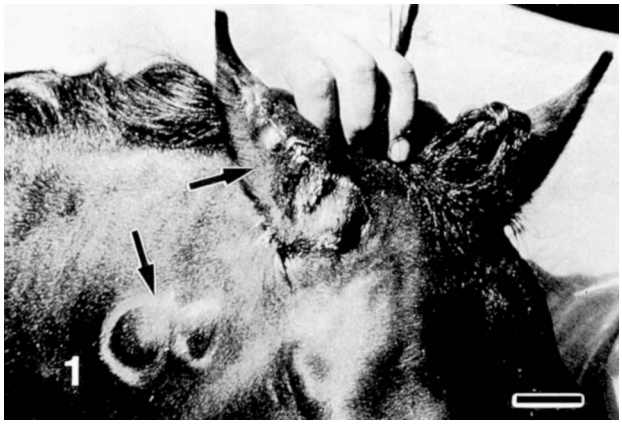

Fig. 1. Skin; horse No. 1. Ulcerated and nonulcerated nodules (arrows) on the neck and pinna. Bar = 7 cm.

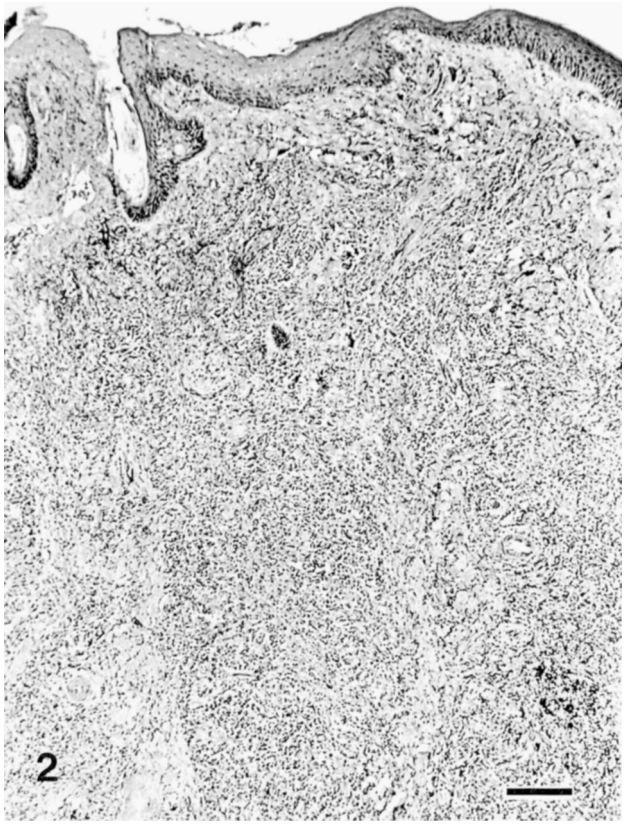

Fig. 2. Skin; horse No. 1. Nodular to diffuse lymphohistiocytic infiltrate. HE. Bar = 200 μm.

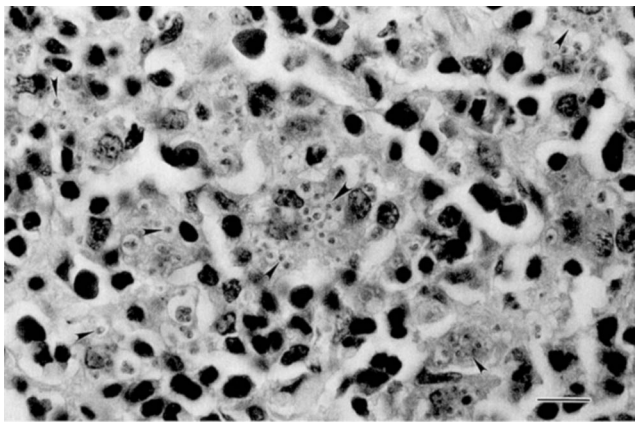

Fig. 3. Skin, dermis; horse No. 1. Numerous macrophages have intracytoplasmic protozoa organisms (arrowheads). Lymphocytes and plasma cells are also observed. HE. Bar = 30 μm.

Histologically, the cutaneous lesions of horse No. 1 had a multinodular to diffuse lymphohistiocytic inflammatory reaction involving the dermis and superficial subcutis (Fig. 2). The inflammation was associated with zones of ulceration and adnexal effacement with peripheral granulation tissue. Within the cytoplasm of macrophages were many protozoal organisms. These organisms were ovoid and 2–4 μm in diameter, with a round nucleus surrounded by a clear halo (Fig. 3). Kinetoplasts were frequently noted with Giemsa stain. Horse No. 2 had areas of diffuse dermal infiltrate composed of neutrophils, macrophages, multinucleate giant macrophages, and rare eosinophils. Numerous protozoal organisms were found within phagocytic cells. The nonulcerated lesions of horse No. 2 had multinodular dermal tuberculoid granulomas (Fig. 4) characterized by a central area of liquefactive necrosis surrounded by a thick band of neutrophils mixed with macrophages and multinucleate giant macrophages (Fig. 5). Protozoal organisms were less common in the nodular lesions and were located either free or within macrophages adjacent to necrotic regions. A tentative diagnosis of leishmaniasis in both animals was confirmed by immunohistochemical and ultrastructural evaluation. Immunohistochemical staining for *Leishmania* revealed a moderate to strong reaction for the organisms in both animals (Fig. 6A). Substitution of the primary antiserum by a nonimmune rabbit serum revealed no immunostaining (Fig. 6B).

Electron microscopic examination of the affected tissues in both horses revealed one or more (up to 35 per cell in horse No. 2) protozoal organisms within intracytoplasmic vacuoles in macrophages; in horse No. 2, organisms also were found in multinucleate giant cells and rarely within neutrophils (Fig. 7). These organisms were ovoid with a nuclear envelope, kinetoplast, single nonemerging flagellum with its base in front of the nucleus, flagellar pocket, pellicular microtubules, and numerous electron-dense and electron-lucent vacuoles (Fig. 8). The morphology was typical of *Leishmania* amastigotes.[12]

After diagnosis, horse No. 1 was treated with intravenous sodium stibogluconate (Pentostam, 100 mg/ml), 6 ml/day for 10 days, with treatment repeated once 30 days following the initial 10-day treatment. The lesions resolved completely after the second round of treatment. When this animal died 2 years later of an unrelated condition, there was no clinical evidence of the disease. A necropsy was not performed. Horse No. 2 was not treated and is still alive.

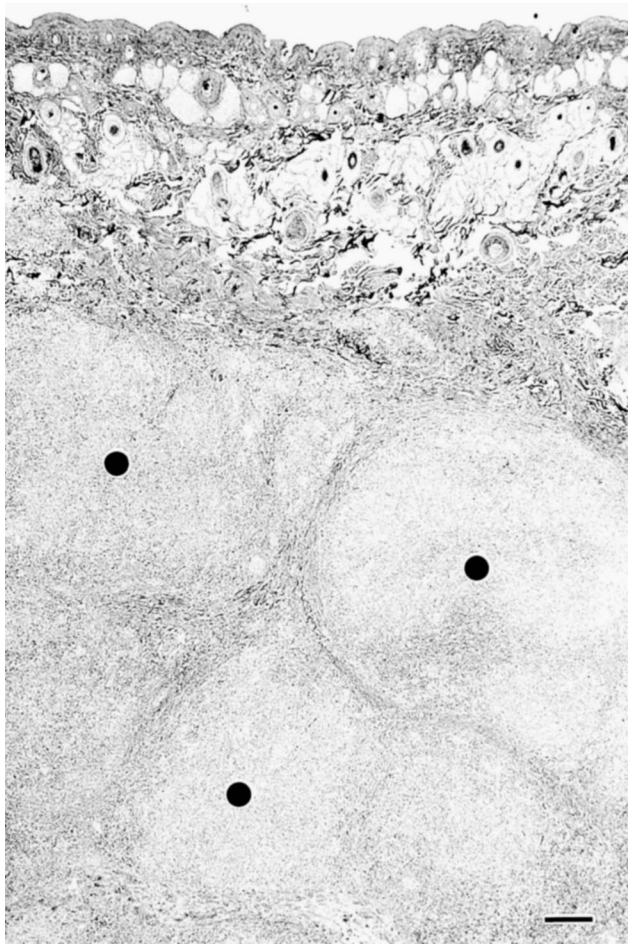

Fig. 4. Skin, dermis; horse No. 2. Multiple granulomas are seen deep in the dermis (circles). HE. Bar = 400 μm.

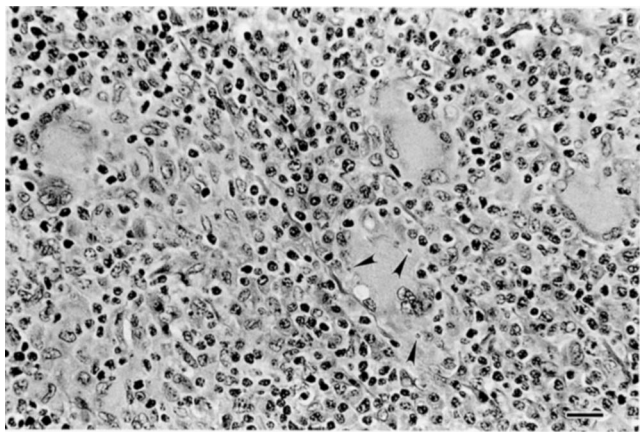

Fig. 5. Skin, dermis; horse No. 2. The inflammatory infiltrate contains numerous lymphocytes, macrophages, plasma cells, and fewer multinucleate giant cells. Note protozoan organisms within giant cells (arrowheads). HE. Bar = 40 μm.

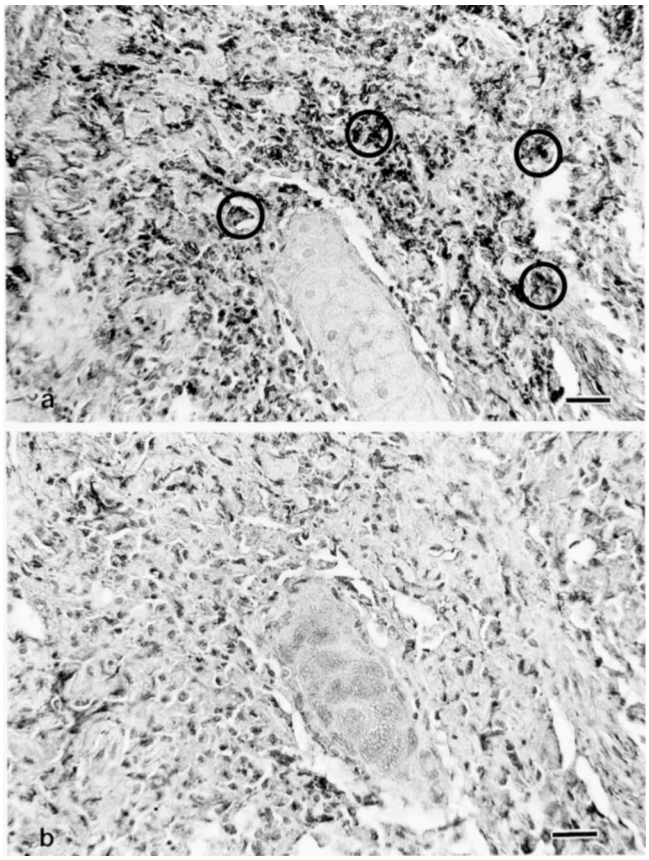

Fig. 6. Skin, dermis. **Fig. 6A.** Horse No. 1. Macrophages depict an intense immunoreaction for *Leishmania* that is revealed as a dark brown precipitate (circles). **Fig. 6B.** Negative control. Absence of reaction. Immunoperoxidase, diaminobenzidine chromogen. Bar = 25 μm.

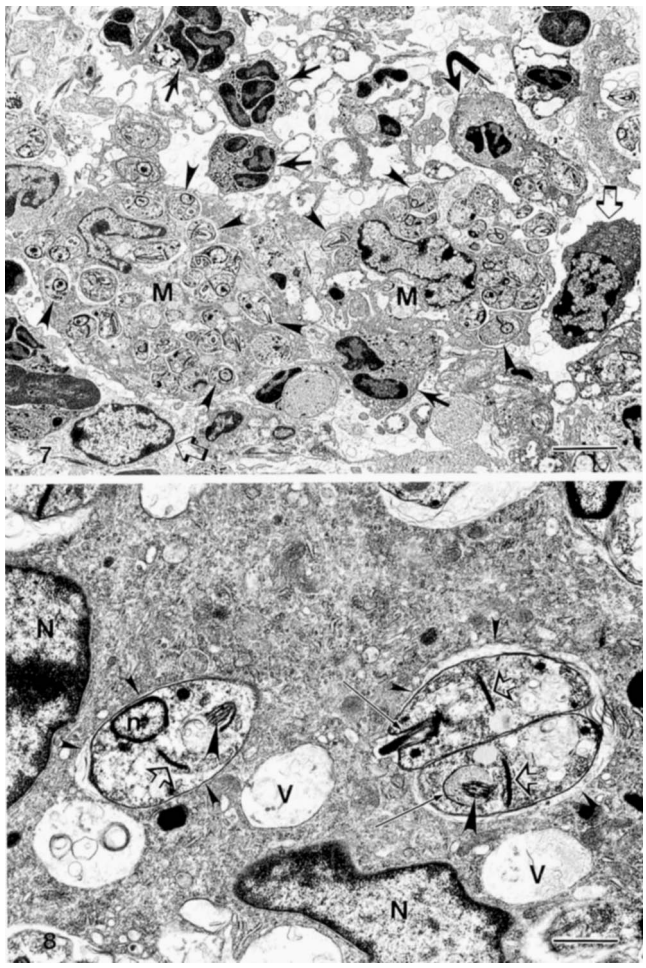

Fig. 7. Electron micrograph. Skin, dermis; horse No. 2. The dermal pyogranulomatous infiltrate contains neutrophils (arrows), plasmacytoid cells (open arrows), macrophages, and multinucleate giant cells (M). Numerous amastigotes of *Leishmania* (arrowheads) are within parasitophorous vacuoles of giant cells. One neutrophil (curved arrow) is being phagocytized by a macrophage that also contains *Leishmania* organisms. Uranil acetate and lead citrate. Bar = 7.5 μm.

Fig. 8. Electron micrograph. Skin, dermis; horse No. 2. Three *Leishmania* amastigotes are seen within parasitophorous vacuoles (small arrowheads) of a multinucleate giant cell. Note Kinetoplast (open arrows), flagellum (large arrowheads), flagellar pocket (long arrow), nucleus of the giant cell (N), nucleus of *Leishmania* (n), and vacuoles (V). Uranyl acetate and lead citrate. Bar = 1.6 μm.

Cutaneous leishmaniasis in horses, mules, and donkeys has been diagnosed in several South American countries.[15] It was described for the first time in a horse from Argentina with ulcerated cutaneous and ocular lesions.[10] Since then, this disease has been recognized in Venezuela[1,3,7] and Brazil.[2,5,8,11,15,16] In cases where biochemical characterization has been performed, *Leishmania braziliensis* has been identified.[5,11,16] All reported cases in equids have had skin involvement. The location of cutaneous lesions in horse Nos. 1 and 2 is in accordance with other descriptions, where the most common sites were the head (ears, eyes, muzzle),[1,8,11] scrotum, legs,[1,4,7,11] neck, and penis.[1,7] The large number of parasites as noted in the histologic sections from these two horses has also been described[4] but is not a consistent finding.[1,10] In horse No. 2, the presence of tuberculoid granulomas in some lesions might indicate a delayed immune-mediated response to *Leishmania*.

In all mammalian species affected, the mode of transmission of leishmaniasis is believed to be by various species of sand flies; however, some researchers failed to isolate organisms from sand flies in endemic areas.[1,11] The sand fly *Lutzomia cayennensis* (subspecies *viequesensis* and *puertoricensis*) has been detected in Puerto Rico,[17] although it feeds mainly on poikilothermic animals (e.g., lizards). Sand flies have not been studied in detail in Puerto Rico, and other species could exist with a feeding preference for mammals (A. Morrison, CDC San Juan, Puerto Rico, personal communication). Although horse No. 1 developed lesions while in Puerto Rico, it likely became infected in Colombia. Clinical signs in canine leishmaniasis can develop several months or years after the initial infection, depending on host im-

munocompetence, and incubation times from several months to 7 years have been described.[14]

Lesions in horse No. 1 resolved with antimony therapy. In a previous report,[5] antimony was also successfully used to treat a horse with cutaneous leishmaniasis. After the second series of treatments, lesions had healed and *Leishmania* organisms could not be identified by histologic assessment or culture. This horse was pregnant and gave birth to a normal, unaffected foal.

Horse No. 2 was born in Puerto Rico and never left this island, which indicates that it was infected in Puerto Rico. Epidemiologic studies are in progress to determine the vector for *Leishmania* as well as possible zoonotic implications.

A review of the literature failed to identify a confirmed case of equine leishmaniasis in North America. However, the disease has been recognized with some frequency in dogs in Mexico and in endemic foci in more temperate regions of the United States.[6,13] Clinicians should be aware that leishmaniasis can and does occur in horses. Although it is a rare disease, leishmaniasis should be considered in the differential diagnosis of any papulonodular and/or ulcerated lesion in the horse, especially when such lesions occur on the head and ears and do not respond to antibiotic or antifungal therapy.

Acknowledgements

We appreciate the help provided by Dr. M. Domingo, Autonomous University of Barcelona, Spain, with immunohistochemical techniques and by Ralph Common and Carol Ayala with photography and electron microscopic techniques. We also thank Dr. A. Morrison, Centers for Disease Control, San Juan, Puerto Rico, for the information about sand flies in this island.

References

1 Aguilar CM, Fernández E, De Fernández R, Deane LM: Study of an outbreak of cutaneous leishmaniasis in Venezuela. The role of domestic animals. Mem Inst Oswaldo Cruz **79**:181–195, 1984

2 Aguilar CM, Rangel EF, Deane LM: Cutaneous leishmaniasis is frequent in equines from an endemic area in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz **81**:471–472, 1986

3 Aguilar CM, Rangel EF, García L, Fernández E, Momen H, Grimaldi G, De Vargas Z: Zoonotic cutaneous leishmaniasis due to *Leishmania* (*viannia*) *braziliensis* associated with domestic animals in Venezuela and Brazil. Mem Inst Oswaldo Cruz **84**:19–28, 1989

4 Barbosa-Santos EG, Marzochi MC, Urtado W, Queirós F, Chicarino J: Immunotherapy and chemotherapy of mucocutaneous and disseminated cutaneous leishmaniasis in a horse in Brazil. J Trop Med Hyg **45**(Suppl): 119, 1991

5 Barbosa-Santos EGO, Marzochi MCA, Urtado W, Queirós F, Chicarino J, Pacheco RS: Leishmaniasis disseminated by *Leishmania braziliensis* in a mare (*Equus caballus*): immunotherapy and chemotherapy assays. Mem Inst Oswaldo Cruz **89**:217–220, 1994

6 Binhazim AA, Chapman WL, Latimer KS, Styles M, Comer K: Canine leishmaniasis caused by *Leishmania leishmania infantum* in two Labrador Retrievers. J Vet Diagn Invest **4**:299–305, 1992

7 Bonfante-Garrido R, Meléndez E, Torres R, Morillo N, Arredondo C, Urdaneta I: Enzootic equine cutaneous leishmaniasis in Venezuela. Trans R Soc Trop Med Hyg **75**:471, 1981

8 Falqueto A, Varejão JBM, Sessa PA: Cutaneous leishmaniasis in a horse (*Equus caballus*) from endemic area in the state of Espirito Santo, Brazil. Mem Inst Oswaldo Cruz **82**:443, 1987

9 Ferrer L, Rabanal R, Domingo M, Ramos JA, Fondevila D: Identification of leishmania amastigotes in canine tissues by immunoperoxidase staining. Res Vet Sci **44**: 194–196, 1988

10 Mazza S: Leishmaniosis cutánea en el caballo y nueva observación de la misma en el perro. Bol Inst Clin Quirur **3**:462–464, 1927

11 Oliveira-Neto MP, Pirmez C, Rangel E, Schubach A, Grimaldi G Jr: An outbreak of American cutaneous leishmaniasis (*Leishmania braziliensis*) in a periurban area of Rio de Janeiro city, Brazil: clinical and epidemiological studies. Mem Inst Oswaldo Cruz **83**:427–435, 1988

12 Scholtyseck E: Fine Structure of Parasitic Protozoa, pp. 44–47. Springer-Verlag, Berlin, Germany, 1979

13 Sellon RK, Menard MM, Meuten DJ, Lengerich EJ, Steurer FJ, Breitschwerdt EB: Endemic visceral leishmaniasis in a dog from Texas. J Vet Intern Med **7**:16–19, 1993

14 Slappendel RJ: Canine leishmaniasis. A review based on 95 cases in The Netherlands. Vet Q **10**:1–16, 1988

15 Vexenat JA, Barretto AC, Rosa ACO, Sales CC, Magalhaes AV: Infecçâo natural de *Equus asinus* por *Leishmania braziliensis braziliensis*—Bahia, Brasil. Mem Inst Oswaldo Cruz **81**:237–238, 1986

16 Yoshida ELA, Correa FMA, Marques SA, Stolf HO, Dillon NL, Momen H, Grimaldi G Jr: Human, canine and equine (*Equus caballus*) leishmaniasis due to *Leishmania braziliensis* (*L. braziliensis braziliensis*) in the South-West region of Sâo Paulo state, Brazil. Mem Inst Oswaldo Cruz **85**:133–134, 1990

17 Young DG, Duncan MA: Guide to the identification and geographic distribution of *Lutzomya* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem Am Entomol Inst **54**:644–646, 1994

Request reprints from Dr. José A. Ramos-Vara, Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (USA).