

Cutaneous leishmaniasis in horses in Costa Rica. Study of 5 cases

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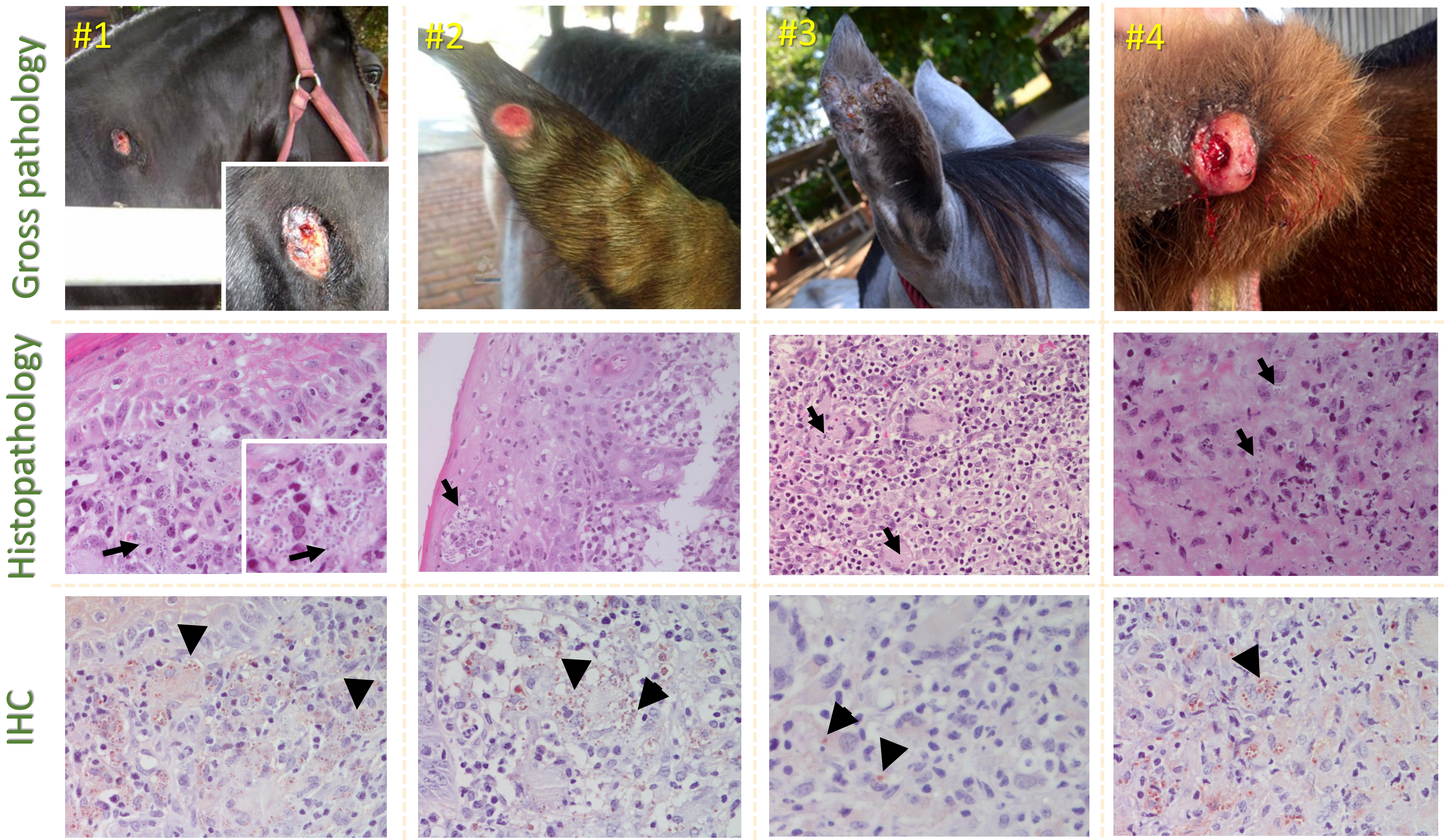
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Abstract

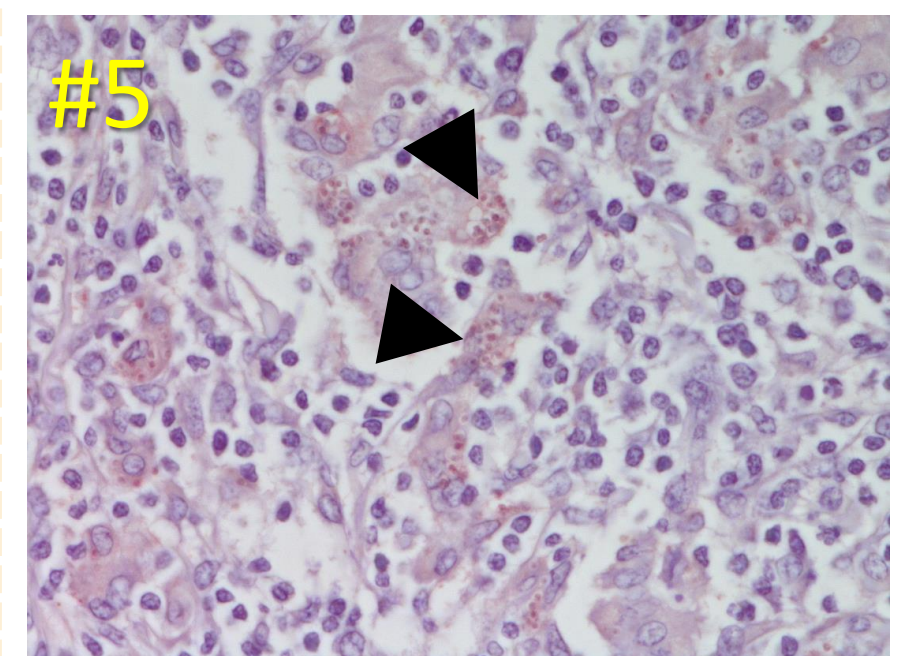
Leishmaniasis is one of the most prevalent parasitic public health problems worldwide. In the present study, 5 cases of equine cutaneous leishmaniosis in Costa Rica are described. The clinical features and gross pathology of the were typical of a recurrent chronic alopecic and ulcerative lesion within the skin from ears or head and neck areas. A histopathological examination of biopsied lesions pointed out the possibility of *Leishmania* spp. infection with the presence of a granulomatous inflammatory reaction in the dermis with multinucleated giant cells macrophages, lymphocytes and few neutrophils and eosinophils. Necrotic areas were occasionally observed. To rule out some differential diagnoses among them mycotic, an immunohistochemical detection of *Leishmania* spp. was conducted in paraffin tissue section using a rabbit polyclonal antibody raised against *Leishmania donovani*, *L. infantum* and *L. amazonensis* and a established protocol for diagnostics. Strong positive reaction was observed within the cytoplasm of macrophages and MNGCs where amastigotes where clearly identified. Tissue sections were obtained for laser-capture microdissection followed by PCR and sequence analysis to identify the species of *Leishmania* spp. This report shows the importance of *Leishmania* spp. as a causative agent of equine cutaneous disease in the new world and the importance as a possible emerging pathogen. Leishmaniasis is the second leading parasitic casuse of death in people, after malaria, making it a top priority for the WHO, and equines may have a role in the epedemiology of the disease.

Cases. Clinical signs and pathology



Gross lesions were non-healing skin wounds in the pinna or neck skin. At histopathology, various stages of necrosis and infiltration of dermis by mixed inflammatory cells, including multinucleated giant cells were observed. Granulomas in the dermis were also present in some sections. Multiple ovoid amastigotes within histiocytes and in between collagen fibres were identified (arrows), ranging from 2 to 4 μ m in diameter. Amastigotes had a small round nucleus surrounded by a clear halo. These findings suggested that cutaneous leishmaniosis was amongst differential diagnosis based on histology analysis.

Immunoreactivity to *Leishmania* spp. amastigotes was observed in macrophages, within the inflammatory cell infiltrate and in macrophages within granulomas as well as of extracellular stages (arrowheads). The distribution of immunostaining was variable, and correlated with the inflammatory response.



Discussion

In Costa Rica, cutaneous leishmaniosis in humans is common in rural areas, but can also be acquired in semi-urban and urban areas. It also takes place in rainforests and arid areas. This disease is present in the southern USA, Mexico, Central and South America, with Brazil and Peru reporting the majority of cases. The etiological agent most frequently involved in cutaneous leishmaniosis is *L. (Viannia) panamensis*, although *L. (V.) braziliensis* has been reported; the parasite reservoir hosts known are the sloths *Bradypus griseus* and *Cholepus hoffmani*; *L. panamensis* has been isolated in a species of wild mouse named *Heteromys desmarestianus*. The horse may be a carrier/reservoir of the disease and many cases can be misdiagnosed as possible histoplasmosis, other fungal infections, etc. The retrieval of good quality protozoan DNA from the formalin-fixed samples has proven to be difficult and new laser-capture microdissected lesions are being analysed using a combination of FFPE-nucleic acid extraction kits combined with complex specific PCR and sequence analysis. This report shows the importance of *Leishmania* spp. infection in horses in the new world and the impact as a possible emerging zoonotic pathogen.