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Case Report

Combined treatment with topical fluconazole microemulsion for Canine leishmaniasis: Case report

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Abstract

Canine leishmaniosis (CanL) is a zoonosis mainly caused by *Leishmania infantum*, (New World synonym *L. chagasi*) and occasionally by *L. Braziliensis*. CanL, also known as Canine Visceral Leishmaniosis, is a multisystemic disease with several clinical signs including poor body condition, generalized muscular atrophy, lymphadenomegaly and excessive skin ulcers and scaling. Fluconazole (FLZ) is an antifungal agent that inhibits a key enzyme for the production of ergosterol, the main sterol in membranes of fungi and parasites. We report a case of CanL in one dog with persistent cutaneous manifestations after early amelioration of systemic signs after usual treatment with allopurinol. A mongrel six-year-old female dog admitted in a veterinary clinic in the city of Posadas, Misiones, Argentina, was diagnosed with CanL after cytological examination of skin, lymph nodes and bone marrow, and treated with allopurinol as initial systemic treatment. Later a microemulsion with FLZ as active pharmaceutical ingredient was indicated for topical aplication on skin lesions. The dog resolved lesions after combined treatment. This study provides baseline data about the efficacy of FLZ microemulsion on skin lesions of CanL. Although further work is needed, this semisolid dosage form could be useful for the local treatment of CanL in the New World.

1. Introduction

Leishmaniasis, a zoonosis caused by *Leishmania* parasites, is an ancient disease that has recently been classified as an emerging pathology worldwide. This neglected disease has turned into a public health problem since environmental changes and other factors are favouring its expansion; there is also concern about treatment failure and emerging resistance to antileishmanial drugs [1,2].

Phlebotominae sand flies are the biological vectors of this protozoal disease. Although small forest rodents have been considered the main reservoir for the parasites, there is concern about domestic dog becoming an important reservoir for human visceral leishmaniasis caused by *L. infantum*; urbanization, deforestation and inadequate vector control contribute to this threaten. The number of infected dogs in South America is estimated in millions, rates of infection are particularly high in Venezuela and Brazil. In recent years the disease showed an increasing spreading tendency to the South of the continent involving countries like Paraguay and Argentina [3,4]. There is some evidence that a high prevalence of canine infection is associated with high risk of human disease in this region, where a number of *Leishmania* species have been isolated from dogs. In most cases canine leishmaniosis (CanL) is caused by *L. infantum* (syn. *L. chagasi*) and *L. braziliensis.* In neotropical regions of South America canine tegumentary leishmaniosis, a clinical form with single cutaneous or mucosal lesions, is in general associated with *L. braziliensis* infection, although other species have also been reported [5,6] CanL is characterized by a long asymptomatic incubation period after which dogs may remain asymptomatic, develop a mild illness or a symptomatic disease. Diffuse alopecia, dry seborrhea with silverwhite, skin scaling, ulcers in footpads and ears, eczema in nose or ears, mucosal ulcers, poor body condition and lymphadenopathy are some of the main signs. However, lymphadenomegaly, exfoliative dermatitis and weight loss appear as the most relevant signs of CanL on which veterinarians based their suspicion of infection. [7,8]

Several drugs are used for the therapy of CanL, however in most cases treatments do not eliminate the infection but they are able to relieve clinical signs. Most treated animals cure clinically but they might relapse. Combined therapy, usually allopurinol and meglumine antimoniate or miltefosine is needed for dogs that do not respond well to first-line medicine. Other treatments include Amphotericin B, pentamidine, aminosidine (paramomycin), ketoconazole, and metronidazole with spiramycin or with enrofloxacin. Effectiveness of second line drugs still needs investigation [9,10]

Fluconazole (FLZ) is an antifungal that reaches high concentration in the skin due to great affinity to keratin in the stratum corneum. However, the use of FLZ as topical treatment is not a clinical practice [11,12]. Since FLZ inhibits the production of ergosterol, the main sterol in membranes of fungi and parasites, oral FLZ has been reported for the treatment of human cutaneous leishmaniasis caused by *L. major* [13-15]. FLZ has been used in veterinary practice for canine nasal aspergillosis/penicilliosis and Cryptococcosis [16,17]

Herein we report a case of CanL in one dog with resistant tegumentary lesions treated with combined therapy of oral allopurinol and FLZ- ME applied topically. Microemulsions (ME) have outstanding properties for topical application and better FLZ penetration and retention within the skin was observed when comparing a ME with other topical vehicles such as creams or gels. [18] In vitro assay with FLZ-ME showed inhibitory activity against *L braziliensis* promastigotes. [19]

2. Case Report

A mongrel six-year-old female dog (weight 8 kg), was admitted in a veterinary clinic ("Veterinaria del Oeste"; city of Posadas, Misiones, Argentina) with regular general condition. Physical examination revealed deteriorated body condition, lymphadenopathy and multiple ulcerated lesions in nasal and ocular regions, and the forelimbs (Table 1**a**; Figure 1**a, b**).

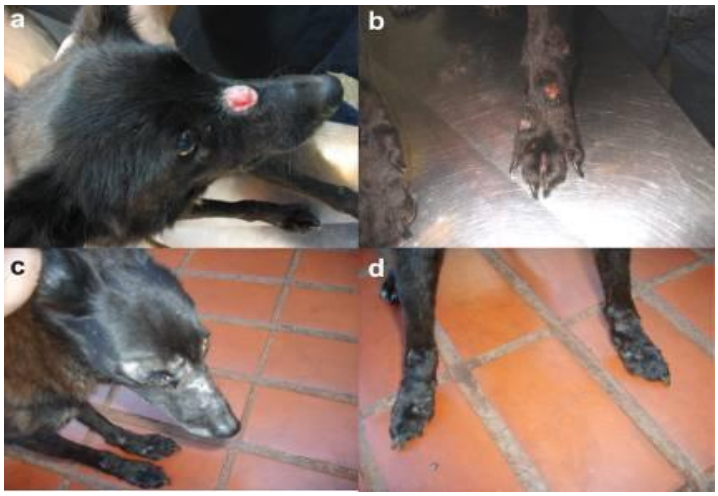

Figure 1: a,b lesions before local treatment; c,d after first course of combined treatment with local fluconazole

Table 1: Lesion localization and description

No. of lesion	Localization	a- Before the application of local treatment			b- After 3 weeks of combined treatment		
		Size (cm)	Type of lesion	OI	Size (cm)	Type of lesion	OI
1	Dorsum of nose	3	Ulcer	No	1	Hyperkeratosis	No
2	Dorsum left carpal región	1	Crusted wound	yes	1	Crusted	No
3	Dorsum left metacarpian medial región	0.5	Crusted wound	yes	---	Crusted	No
4	Side of right eye	0.5	Ulcer	No	0,5	Crusted	No
5	Side of left eye	1	Crusted wound	yes	---	Hyperkeratosis	No

OI: overinfection; --- no lesion

Cytological examination was made on samples taken by fine needle aspiration of popliteal lymph nodes and cutaneous lesions. Samples were stained with a rapid stain technique (Tinción 15, Biopur Diagnostics) and were observed at 100x (by immersión) with optic microscope (Wesco Bio VU 2300). Lymph node revealed heterogeneous cell population, naked nuclei, small and medium lymphocytes, lymphoblasts, some mitotic figures, polymorphonuclear neutrophils (PMN) and eosinophils, mononuclear macrophages (MN) and plasma cells. Free bacteria and free microorganisms round to oval, with a core and basophils kinetoplast compatible with *Leishmania sp*. amastigotes. Cutaneous lesions curettage revealed hemorrhagic background, sheets and strands of keratin, isolated squamous epithelial cells, PMN and eosinophils in varying degrees of degeneration, MN lymphocytes and free bacteria. Presence of free amastigotes of *Leishmania sp*. and basophils yeast microorganisms compatible with *Malassezia sp.* were also observed.

Serological evaluation with an immunocromatographic method based in the detection of specific antibodys against rk 39 antigen was made (Kalazar Detect,Lab.In Bios) and Leishmania infantum was confirmed as the etiologic agent.

The diagnostic was visceral leishmaniosis with lymphadenitis, presence of reactive lymph node and overinfection with malassezia. The dog was initially prescribed allopurinol 75 mg twice a day and after two weeks of treatment a FLZ-ME was added twice a day topically over the lesions. Topical formulation was prepared according to composition selected and discussed in a previous work. [18]

After 3 weeks of combined treatment some of the lesions disappeared or reduced and ulcers were healed (Table 1**b**; Figure 1**c,d**) No adverse effects were observed. Local treatment was suspended after 6 weeks. After two years of initial cure of ulcers, a little relapse was observed despite the maintenance of systemic administration of Allopurinol. Local treatment was applied again and after 5 weeks lesions were healed (Figure 2).

Figure 2: Dog after the second year follow-up and second treatment with local FLZ microemulsion

International ethical standards were considered and Approval has been obtained from the Ethical Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires (050313-2 EXP-FYB 0747495/2012 and 170511-1)

3. Discussion

In the case presented the dog had an early amelioration of systemic manifestations such as lymphadenomegaly and biochemical alterations, but cutaneous lesions needed a more intensive treatment. We observed that concomitant use of topical FLZ gave additional

support for the cure of lesions and diminished disease progression. The clinical cure reported herein is relevant as topical therapy lead drug to the target site with less adverse effects. Combined therapy in the case of dogs would enable a better control of resistant cutaneous manifestations found in many clinical cases and it might also reduce the need for systemic therapy. This last fact is encouraging considering that pentavalent antimonials and miltefosine are not always available for use in dogs and as mentioned allopurinol is not completely effective for the control of some lesions. Moreover it is important to consider the emerging resistance to pentavalent antimonials and there is also evidence of low sensitivity to these drugs in the case of dogs. A key point is that CanL is not a homogenous disease; a wide spectrum of clinical manifestations and different degrees of severity can occur, so it is essential to consider a different therapeutic approach for each clinical stage. [20]

Early treatment of cutaneous lesions might help to prevent disease progression. Also, topical FLZ therapy is useful to avoid co-infections with *Malassezia spp.* very common in dogs. Topical treatments help therapy compliance which is a major issue since it is critical that infected dogs receive treatment to reduce parasite load. This case is encouraging; further work and experience with more animals is needed to assess the efficacy of FLZ for the local treatment of CanL.

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References

[1] Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous Leishmaniasis. *Lancet Infect Dis* 2007; 7 (9) 581-596.

[2] Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004; 27 (5):305-18.´

[3] Salomon OD, Sinagra A, Nevot MC, Barberian G, Paulin P, Estevez JO, *et al*. First visceral leishmaniasis focus in Argentina. *Mem Inst Oswaldo Cruz, Rio de Janeiro* (2008). *103* (1): 109-111.

[4] Gómez N, Estevez O, Gisbert MA, Blanco A, Castillo V, Wolberg A. Leishmaniosis Visceral en los Caninos y Felinos: Actualización. *Vet. Arg.* 2011; 28: 282.

[5] Solano-Gallego L, Koutinas A, Miro´ G, Cardoso L, Pennisi MG, Ferrer L, *et al*. Review. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. *Vet Parasitol* 2009; 165: 1–18.

[6] Urbano J, Sanchez-Moreno ME, Ovalle CE, Rosales MJ, Camargo YC, Gutierrez-Sanchez R, *et al*. Characterization of cutaneous isolates of *Leishmania* in Colombia by isoenzyme typing and kDNA restriction analysis. *Rev Ibero-Latinoam Parasitol* 2011; 70 (1): 16-24.

[7] Cavalcanti A, Lobo R, Cupolillo E, Bustamante F, Porrozzi R. Canine cutaneous leishmaniasis caused by neotropical Leishmania infantum despite of systemic disease: A case report. *Parasitol Int* 2012. doi:10.1016/j.parint.2012.05.002.

[8] Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L. Review Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part one. *Trends Parasitol* 2008; 24 (7) 324-330. doi:10.1016/j.pt.2008.04.001

[9] Miro´ G, Cardoso L, Pennisi MG, Oliva G, Baneth G. Review Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part two. *Trends Parasitol* 2008; 24 (8) 371-377.

[10] Solano-Gallego L, Miró G, Koutinas A, Cardozo L, Pennisi MG, Ferrer L, *et al*. Leish Vet guidelines for the practical management of canine leishmaniosis. *Parasite Vector* 2011; 4:86.

[11] Wildfeuer A, Faergemann J, Laufen H, Pfaff G, Zimmermann T, Seidl HP, *et al*. Bioavailability of fluconazole in the skin after oral medication. *Mycoses* 1994; 37 (3-4):127-130.

[12] Faergemann J. Pharmacokinetics of fluconazole in skin and nails. *J Am Acad Dermatol* 1999; 40 (6 Pt 2):S14-20.

[13] Laffitte E, Genton B, Panizzon RG. Cutaneous leishmaniasis caused by *Leishmania tropica*: treatment with oral fluconazole. *Dermatology* 2005; 210:249–251.

[14] Baron S, Laube S, Raafat F, Moss C. Cutaneous leishmaniasis in a Kosovan child treated with oral fluconazole. *Clin Exp Dermatol* 2004; 29:546–547.

[15] Alrajhi AA, Ibrahim EA, De Vol EB, Khairat M, Faris RM, Maguire JH. Fluconazole for the treatment of cutaneous leishmaniasis caused by *Leishmania major*. *N Engl J Med* 2002; 346:891–895.

[16] Sharp NJH, Harvey CE, O'Brien JA. Treatment of canine nasal aspergillosis/ penicilliosis with fluconazole. *J Small Anim Pract* 1991; 32 (10) 513–516.

[17] Tiches D, Vite CH, Dayrell-Hart B, Steinberg SA, Gross S, Lexa FA. A case of canine central nervous system cryptococcosis: management with fluconazole. *J Am Anim Hosp Assoc* 1998; 34 (2) 145-151.

[18] Salerno C, Carlucci A. M., Bregni C. Study of in vitro drug release and percutaneous absorption of fluconazole from topical dosage forms. *AAPS Pharm SciTech* 2010; 11(2): 986-993.

[19] Salerno C, Carlucci AM, Gorzalczany S, Bregni C. *In vitro* Inhibition of *Leishmania braziliensis* Promastigotes Growth by a Fluconazole Microemulsion. *J Mol Pharm Org Process Res* 2014; 2: 115. doi: 10.4172/2329-9053.1000115

[20] Ferrer L, Baneth G, Bourdeau P, Koutias A, Miró G, Pennisi M.G. *et al*. Response to the letter: Some remarks about the LeishVet directions for the treatment of canine leishmaniosis. Letter to the Editor. *Vet Parasitol* 2010; 169: 418–420.

