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Small Animal Diseases

Outbreak of leishmaniasis caused by *Leishmania enriettii* in guinea pigs (*Cavia porcellus*)[1]

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ABSTRACT.- Ribeiro C., Koubiach K.N., Faccini L.S., Teixeira M.C., Schüür F.A., Thomaz-Soccol V., Barros C.S.L. & Coelho A.C.B. 2023. **Outbreak of leishmaniasis caused by *Leishmania enriettii* in guinea pigs (*Cavia porcellus*).** *Pesquisa Veterinária Brasileira 43:e07241, 2023.* Faculdade de Veterinária, Centro Univesitátio Ritter dos Reis, Av. Manoel Elias 2001, Porto Alegre, RS 91240-261, Brazil. E-mail: annaccarolina@hotmail.com

We describe an outbreak of leishmaniasis in seven guinea pigs (*Cavia porcellus*) in which nodular ulcerated skin lesions of varying sizes were observed in the nasal cavity, upper lip, pinnae, vulva, and periarticular region of the limbs. Cytologic exam of collected samples of the lesions in the auricle of one of the animals revealed macrophages containing parasitophorous vacuoles of approximately 4.0μm in diameter in their cytoplasm with morphology suggestive of *Leishmania* sp. Although skin lesions spontaneously regressed in two of the Guinea pigs, only one survived. All six animals that died were necropsied. Grossly, all animals showed bloody nodular cutaneous lesions with crusts. One of the guinea pigs had distended dark red and firm lungs. Histopathology of the skin lesions revealed histiocytic interstitial acanthotic dermatitis associated with a myriad of Leishmania organisms within macrophages cytoplasm. In the lung, the lesions were characteristic of broncho-interstitial pneumonia with focal infiltrates of neutrophils, epithelioid macrophages, and multinucleated giant cells containing 2μm basophilic amastigotes with morphology compatible with *Leishmania* spp. A focal granulomatous lesion ,associated with the causal agent in the lung is a novel description of leishmaniasis in guinea pigs caused by *L. enriettii.* The polymerase chain reaction (PCR) technique with mini-exon primer performed in samples of lesions from two affected guinea pigs was positive and equal to the reference strain, identifying *Leishmania enriettii*. The cytological, macroscopic, and histological lesions associated with the PCR technique allowed the diagnosis of leishmaniasis and the identification of the specie *L. enriettii.*

INDEX TERMS: *Leishmania enriettii*, guinea pig, *Cavia porcellus*, leishmaniasis.

RESUMO.- [**Surto de leishmaniose causada por *Leishmania enriettii* em cobaias (*Cavia porcellus*)**]. Descrevemos um surto de leishmaniose em sete cobaias (*Cavia porcellus*), com lesões cutâneas nodulares ulceradas de tamanhos variados observadas na cavidade nasal, lábio superior, pavilhões auriculares, vulva e região periarticular dos membros. No exame citológico foram encontrados macrófagos contendo vacúolos parasitóforos no citoplasma de aproximadamente 4.0μm em diâmetro com morfologia sugestiva de *Leishmania* sp. Apesar de regressão espontânea das lesões cutâneas terem ocorrido em duas das sete cobaias, apenas um sobreviveu. Seis dos sete animais afetados morreram e foram necropsiados. Macroscopicamente, todos os animais apresentaram lesões cutâneas nodulares, crostosas e sanguinolentas. Uma das cobaias tinha pulmões vermelho-escuros, distendidos e firmes. A histopatologia das lesões cutâneas revelou dermatite acantótica intersticial histiocítica associada a miríades de organismos de *Leishmania* no citoplasma de macrófagos. Nos

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pulmões as lesões eram características de pneumonia broncointersticial com infiltrado focal de neutrófilos, eosinófilos, macrófagos epitelioides e células gigantes multinucleadas contendo amastigotas basofílicos de 2µm com morfologia compatível com *Leishmania* spp. Lesões granulomatosas focais associadas ao agente no pulmão são um achado inédito na leishmaniose causada por *L. enriettii* em cobaias. A técnica de reação em cadeia da polimerase (PCR) com primer mini-exon realizada em amostras de lesões de duas cobaias afetadas foi positiva, identificando *Leishmania enriettii*. Os aspectos macroscópicos, citológicos, e histológicos associados à técnica da (PCR), permitiram o diagnóstico da leishmaniose e a identificação da espécie *L. enriettii*.

TERMOS DE INDEXAÇÃO: *Leishmania enriettii*, cobaia, *Cavia porcellus*, leishmaniose.

INTRODUCTION

Leishmaniases are diseases caused by protozoans from the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). Clinically and epidemiologically, there are three forms of leishmaniasis, each species specific: visceral, cutaneous, and mucocutaneous (Ecco et al. 2000, Fighera et al. 2003, McAdam et al. 2015). The protozoan is transmitted by sandfly vectors (Diptera: Phlebotominae) when females take a blood meal from the vertebrate host (Paranaiba et al. 2017).

Mucocutaneous leishmaniasis affects several animal species, including humans (McAdam et al. 2015). Guinea pigs (*Cavia porcellus*) are affected by a non-zoonotic mucocutaneous presentation of leishmaniasis caused by the protozoan *Leishmania enriettii* (Medina 1946, Muniz & Medina 1948, Lainson 1997). This disease was described 75 years ago in southern Brazil (Medina 1946). Although a recent review paper on leishmaniasis caused by *L. enriettii* was published (Paranaiba et al. 2017), the disease is still poorly understood and clinically mistaken by other conditions, such as neoplastic lesions (Fighera et al. 2003).

This study aims to evaluate the clinical, epidemiological, pathological, and molecular aspects of an outbreak of leishmaniasis in guinea pigs caused by *L. enriettii*.

MATERIALS AND METHODS

Seven guinea pigs were affected, five males and two females, aged between eight months and three years (Table 1). Clinical data came from on-site visits done by the authors to the household where the outbreak occurred and by the owners, whom the authors asked to monitor the guinea pigs. All the guinea pigs that died did so spontaneously.

We performed cytology on material sampled by fine-needle aspiration from ulcerated skin lesions of the pinna and limbs from Guinea pig 4 in slides stained by a commercial Romanowsky *stain* (Panótico Rápido, LB, Laborclin, Brazil).

Fragments of several organs from necropsied Guinea pigs 1, 2, 4-7 were harvested, fixed in 10% buffered formalin, routinely processed for histopathology, and stained with hematoxylin and eosin (HE). Tissue samples from five guinea pigs (1,4-7) revealed unfit for histological examination due to advanced *postmortem* changes.

Punch biopsy for the polymerase chain reaction (PCR) for identification of the protozoan taken from the lesions of the pinna and muzzle of Guinea pigs 4 and 5 and processed according to a technique already described (Marfurt et al. 2003).

RESULTS

We found leishmaniasis in five male and two female guinea pigs with ages ranging between eight months and three years (Table 1, raised as pets in the same household, located in the municipality of Viamão (30°4′51″ S, 51°1′22″ W), Rio Grande do Sul, Southern Brazil. The guinea pigs were outdoors in a space bounded by fences, which limited their contact with other animals, such as dogs, cats, rabbits, and domestic chickens that also lived or had access to the household. None of the other animals in this home were affected. Guinea pigs fed at will on fruits, vegetables, and legumes leftovers, commercial pelleted feed for rabbits, and water.

The owner observed the appearance of lesions two months before submission and noted they evolved quickly and in a similar manner across all animals. During clinical examination, the affected guinea pigs had identical skin lesions that consisted of ulcerated, bloody, and crusty nodules, being grossly similar in all animals, with variations only in the number (focal or multifocal lesions), size, and in the affected anatomical region (Table 1). The most frequent sites of injuries were the mucocutaneous junction of the snout and the nasal cavity (Fig.1) and the pinnae (Fig.2), and the limbs (Fig.3). Aside from mucocutaneous lesions, guinea pigs didn't present any other clinical signs and remained clinically stable. All animals died spontaneously and quickly. The number and aspect of lesions were unrelated to the age or sex of the guinea pigs. Guinea pigs 3 and 7 showed spontaneous regression of the lesions, and one of them (Guinea pig 3) remains alive so far with only a small

Table 1. Outbreak of leishmaniose by *Leishmania enriettii*. Data on seven affected guinea pigs and distribution of mucocutaneosus lesions

ID	Sex	Age	Distribution of lesions
1	M	10 months	Mucocutaneous junction of the muzzle and nasal cavity, and both forelimbs
2[a]	M	1 year	Mucocutaneous junction of the muzzle, both pinnae, and the four limbs
3[b]	M	1 year	Right pinna
4[c,d]	M	2 years	Mucocutaneous junction of the muzzle and nasal cavity, both pinnae, and the four limbs
5[c]	M	3 years	Mucocutaneous junction of the muzzle and nasal cavity, the upper lip, and the four limbs
6	F	8 months	Mucocutaneous junction of the muzzle and nasal cavity, both pinnae, the four limbs, and vulva
7[e]	F	2 years	

ID = identification of guinea pig, M = male, F = female, [a] macroscopic and histopathological lesions in the lung, [b] survived, [c] material from lesions sampled for polymerase chain reaction, [d] material from lesions sampled for cytology, [e] regression of lesions, no lesions at necropsy.

lesion in the dorsal aspect of the right pinna; over five months, this lesion significantly regressed and is approximately half of the initial lesion. Lesions of Guinea pig 7 regressed utterly, and it survived for six months, then died unexpectedly.

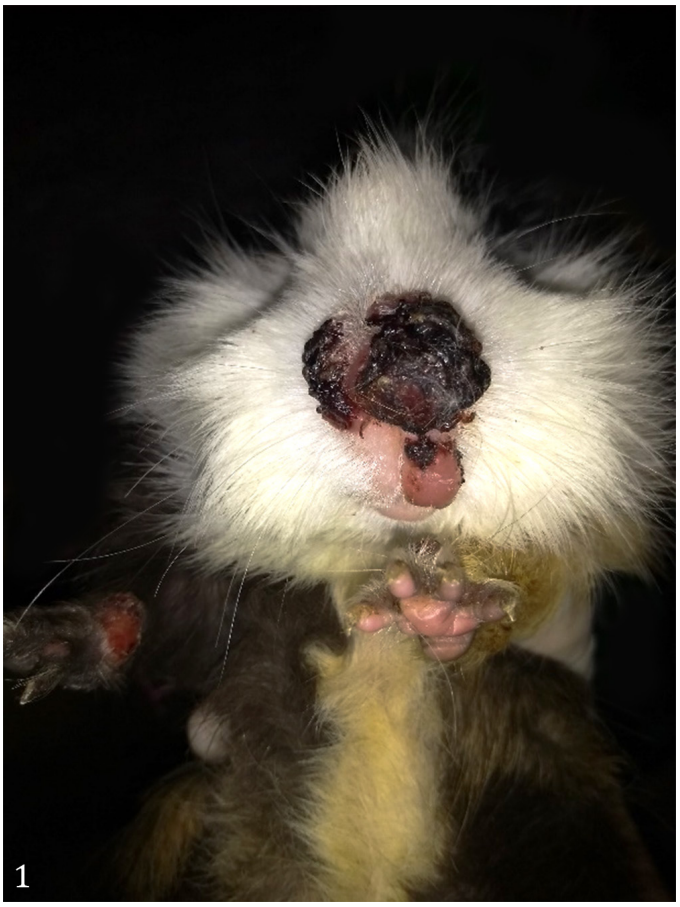

Fig.1. Crusted and ulcerated nodule on the muzzle and upper lip. The nodule also affecting the mucocutaneous junction and the anterior mucosa of the nasal cavity. Leishmaniasis (*Leishmania enriettii*), *Cavia porcellus* 5.

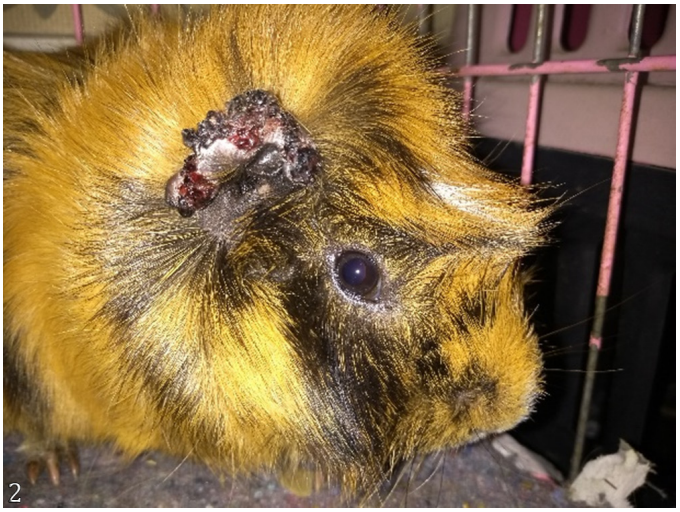

Fig.2. A crusted, ulcerated and bleeding mass partially destroys the right pinna. Leishmaniasis (*Leishmania enriettii*), *Cavia porcellus* 4.

Cytology displayed numerous macrophages with abundant pink foamy cytoplasm filled with oval parasitophorous vacuoles containing amastigotes approximately 3-4μm in diameter and round to oval nuclei (Fig.4).

DNA amplification for *Leishmania* isolated in guinea pigs and biopsy samples was similar to the reference strains of *L. enriettii* with approximately 350 base pairs (bp) in length. For *L. infantum*, the band was 435 bp, and for *L. amazonensis* and *L. braziliensis* were 283 and 226 bp, respectively. In the he polymerase chain reaction (PCR) technique with mini-exon primer, both samples were positive and equal to the reference strain, identifying *L. enriettii*.

The necropsy lesions were similar in five of the six necropsied guinea pigs, except for Guinea pig 7, where cutaneous lesions had regressed. The nodules were of varying sizes. The lesions have thick crusts consisting of coagulated blood and adhere to the subepidermal tissue. The cut surface of the nodules was white and firm. The most extensive lesion (3.5x2.5cm)

Fig.3. An ulcerated and bleeding nodule partially in the distal portion of the thoracic limb. Leishmaniasis *(Leishmania enriettii), Cavia porcellus* 6.

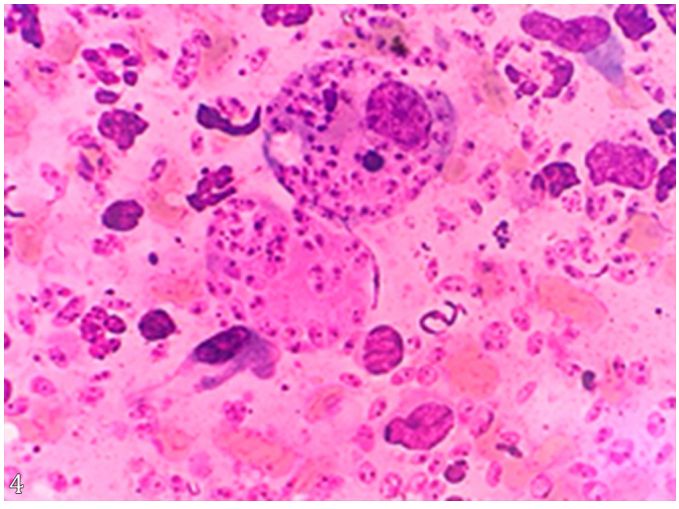

Fig.4. Foamy macrophages contain numerous oval amastigotes approximately 4.0μm in diameter in their cytoplasm from ulcerated skin lesions of the pinna and limbs. Cytology. Leishmaniasis (*Leishmania enriettii*), *Cavia porcellus* 4. Quick Panoptic, obj.40x.

was in the muzzle. In Guinea pig 2, the lungs were dark-red, non-collapsing, and firm (Fig.5).

The superficial dermis of Guinea pig 2 had moderate e focally extensive chronic dermatitis, fibrosis, and acanthosis. There was marked diffuse lamellar orthokeratotic hyperkereratosis.

Pulmonary histology of Guinea pig 2 showed neutrophilic, eosinophilic, histiocytic, diffuse, and moderate broncho-interstitial pneumonia. The alveolar *septa* were thickened with mild neutrophil and macrophage infiltrate; there was type II pneumocyte hyperplasia. Abundant neutrophils and foamy macrophages occasionally filled the alveolar lumen. In multifocal areas, there were granulomatous nodules with epithelioid macrophages, which sometimes formed multinucleated giant cells (MGC). Macrophages and MGC contained intracytoplasmic basophilic amastigotes approximately 2µm in diameter with morphology compatible with *Leishmania* sp. (Fig.6). Amastigotes were also in focal areas with marked eosinophilic infiltrate.

DISCUSSION

The epidemiological data, the clinical picture, the macroscopic lesions, and the histopathology observed in the guinea pigs of the present outbreak bear a close similarity to those described by several authors for the mucocutaneous leishmaniasis by *Leishmania enriettii* (Medina 1946, Muniz & Medina 1948, Paraense 1952, 1953, Bray et al. 1969, Bryceson et al. 1974, Kanan 1975a, 1975b, Machado et al. 1994, Lainson 1997, Ecco et al. 2000, Fighera et al. 2003, Paranaiba et al. 2017). Also, the agent was confirmed as *L. enriettii* by PCR.

In the mucocutaneous cases of leishmaniasis by *L. enriettii* in guinea pigs previously reported, the anatomical sites that most frequently presented lesions were the pinnae, muzzle, and mucocutaneous junction of the nasal cavity and periocular region (Paraense 1953, Kanan 1975b, Thomaz-Soccol et al. 1996).

In the outbreak reported here, the pinnae, muzzle, and mucocutaneous junction of the nasal cavity were the most affected sites. The evolution and gross aspect of the mucocutaneous lesions did not seem to be correlated to the degree of infection, as seen by Animal 2, which had discrete lesions while showing marked pulmonary changes associated with *L. enrietti* amastigotes. Our findings led us to avoid the denomination "mucocutaneous" that is used in the literature to designate the condition (Kanan 1975a, 1975b, Ecco et al. 2000, Fighera et al. 2003), since a visceral lesion co-existed. In animal leishmaniasis, the separation of the condition into visceral, cutaneous, and mucocutaneous forms, seems sometimes arbitrary, and perhaps the use of these denominations should be replaced by the more comprehensive and uncompromising term "leishmaniasis". The finding of a granulomatous focal lesion in association with the causative agent in the lung of one guinea pig is a novelty in reported cases of *L. enriettii* caused by cases of leishmaniasis in guinea pigs.

L. enriettii inoculated intravenously into guinea pigs induces, within five days, nodules in the nostrils, nasal septum, scrotum, vulva, and limbs (Kanan et al. 1975b).

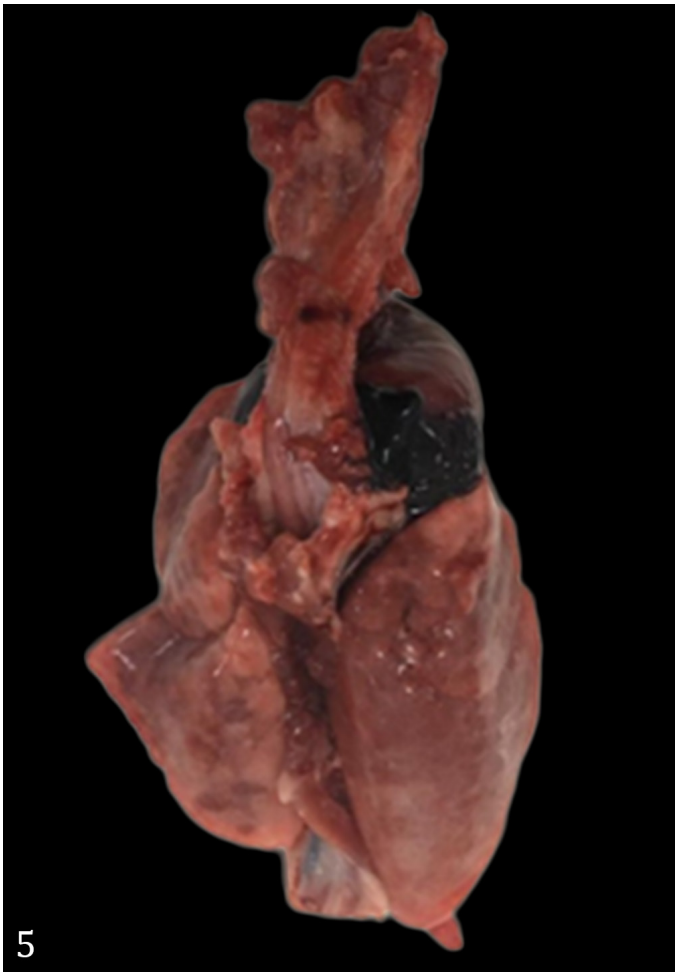

Fig.5. The lungs were dark-red, non-collapsing, and firm. Gross lesions in the lung. Leishmaniasis (*Leishmania enriettii*), *Cavia porcellus* 2.

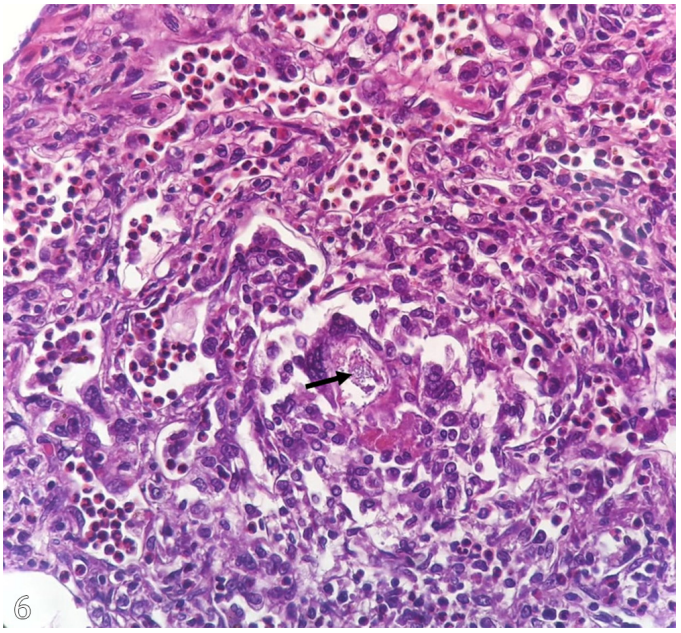

Fig.6. The alveolar septa are thickened by mononuclear inflammatory infiltrate; epithelioid macrophages, sometimes arranged as multinucleated giant cells, contain intracytoplasmic basophilic organisms (arrow) approximately 2µm with morphology compatible with *Leishmania* sp. Leishmaniasis (*Leishmania enriettii*), histopathology of the lung, *Cavia porcellus* 2. HE, obj.40x.

The findings indicate a hematogenic distribution of the infection, and immunosuppression is a known risk factor for visceral leishmaniasis. The inoculation of *L. enriettii* in wild guinea pigs (*Cavia aperea*) intravenously produces similar lesions (Lainson 1997). This may also be the mechanism of dissemination of the agent to the lung. Previous reports note severe pneumonia during necropsy; however, the etiological agent is not described (Kanan et al. 1975b, Fighera et al. 2003). In the current outbreak, pneumonia was present during necropsy, and none of the animals showed clinical respiratory signs.

The first documented mucocutaneous case of leishmaniasis in *guinea pigs* caused by *L. enriettii* is from southern Brazil (Medina 1946). The causative agent demonstrates 100% infectivity to guinea pigs and no zoonotic potential (Medina 1946, Muniz & Medina 1948, Machado et al. 1994, Lainson 1997). After almost 70 years since this documentation, cases of *L. enrietti* in captive red kangaroos (*Macropus rufus*) and humans were reported from Australia and Africa, respectively. Phylogenetic analysis of the protozoa allotted them as new members of the *L. enrietti* complex, a new subgenus of the *Leishmania spp.* Parasites (Dougall et al. 2011, Pothirat et al. 2014, Kwakye-Nuako et al. 2015). These studies demonstrate the vast geographic spread of the *L. enrietti* complex and its capability to affect different vertebrate hosts, including humans. The mucocutaneous lesions described in kangaroos and humans had a similar gross presentation of ulceration and crusts as those seen in the *Cavia porcellus* of this report.

CONCLUSION

The cytological, gross, and histological findings associated with the PCR results allowed for the leishmaniasis diagnosis in *Cavia porcellus,* with *Leishmania enriettii* being identified as the agent. Due to the presence of both cutaneous and pulmonary lesions, the best denomination for the disease is leishmaniasis in guinea pigs and should be considered as a differential diagnosis for pneumonia in this species.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Bray R.S., Ellis D.S. & Bird R.G. 1969. The fine structure of *Leishmania enriettii*. Trans. R. Soc. Trop. Med. Hyg. 63(1):10-11. <PMid:5789062>

Bryceson A.D.M., Brayt R.S. & Dumonde D.C. 1974. Experimental cutaneous leishmaniasis. IV. Selective suppression of cell-mediated immunity during the response of guinea-pigs to infection with *Leishmania enrietti*. Clin. Exp. Immunol. 16(2):189-202. <PMid:4619877>

Dougall A.M., Alexander B., Holt D.C., Harris T., Sultan A.H., Bates P.A., Rose K. & Walton S.F. 2011. Evidence incriminating midges (Diptera: Ceratopogonidae) as potential vectors of *Leishmania* in Australia. Int. J. Parasitol. 41(5):571-579. <https://dx.doi.org/10.1016/j.ijpara.2010.12.008> <PMid:21251914>

Ecco R., Langohr I.M., Schossler J.E.W., Barros S.S. & Barros C.S.L. 2000. Cutaneous Leishmaniasis in guinea pigs (*Cavia porcellus*). Ciência Rural 30(3):525-528. <https://dx.doi.org/10.1590/S0103-84782000000300027>

Fighera R.A., Souza T.M., Schmidt C.A. & Barros C.S.L. 2003. Leishmaniose mucocutânea em cobaios (*Cavia porcellus*). MEDVEP, Revta Cient. Med. Vet. 1(3):177-182.

Kanan M.W. 1975a. The fine structure of *Leishmania enriettii* in the guinea-pig. Int. J. Parasitol. 5(6):651-657. <https://dx.doi.org/10.1016/0020-7519(75)90065-x> <PMid:1201899>

Kanan M.W. 1975b. Mucocutaneous leishmaniasis in guinea-pigs inoculated intravenousl with *Leishmania enriettii*: preliminary report. Br. J. Dermatol. 92(6):663-673. <https://dx.doi.org/10.1111/j.1365-2133.1975.tb03147.x> <PMid:1182080>

Kwakye-Nuako G., Mosore M.-T., Duplessis C., Bates M.D., Puplampu N., Mensah-Attipoe I., Desewu K., Afegbe G., Asmah R.H., Jamjoom M.B., Ayeh-Kumi P.F., Boakye D.A. & Bates P.A. 2015. First isolation of a new species of *Leishmania* responsible for human cutaneous leishmaniasis in Ghana and classification in the *Leishmania enriettii* complex. Int. J. Parasitol. 45(11):679-684. <https://dx.doi.org/10.1016/j.ijpara.2015.05.001> <PMid:26099650>

Lainson R. 1997. On *Leishmania enriettii* and other enigmatic *Leishmania* species of the neotropics. Mem. Inst. Oswaldo Cruz 92(3):377-387. <https://dx.doi.org/10.1590/s0074-02761997000300014> <PMid:9332605>

Machado M.I., Milder R.V., Pacheco R.S., Silva M., Braga R.R. & Lainson R. 1994. Naturally acquired infections with *Leishmania enriettii* Muniz & Medina 1948 in guinea-pigs from São Paulo, Brazil. Parasitology 109(Pt 2):135-138. <https://dx.doi.org/10.1017/s0031182000076241> <PMid:8084659>

Marfurt J., Nasereddin A., Niederwieser I., Jaffe C.L., Beck H.-P. & Felger I. 2003 Identification and differentiation of *Leishmania* species in clinical samples by PCR amplification of the miniexon sequence and subsequent restriction fragment length polymorphism analysis. J. Clin. Microbiol. 41(7):3147-3153. <https://dx.doi.org/10.1128/JCM.41.7.3147-3153.2003> <PMid:12843055>

McAdam A.J., Milner D.A. & Sharpe A.H. 2015. Infectious disease, p.341-402. In: Kumar V., Abbas A.K. & Aster J.C. (Eds), Robbins and Cotran Pathologicc Basis of Disease. 9th ed. Elsevier, Philadelphia.

Medina H. 1946. Estudos sobre leishmaniose. I. Primeiros casos de leishmaniose espontânea observados em cobáios. Arq. Biol. Tecnol. 1:39-74. <https://dx.doi.org/10.1590/S1516-89132001000500003>

Muniz J. & Medina H. 1948. Leishmaniose tegumentar do cobaio (*Leishmania enrietti*). Arq. Biol. Tecnol. 3(2):13-35.

Paraense W.L. 1952. Infection of the nasal mucosa in guinea-pig leishmaniasis. Anais Acad. Bras. Ciênc. 24:307-310.

Paraense W.L.1953. The spread of *Leishmania enriettii* through the body of the guinea-pigs. Trans. R. Soc. Trop. Med. Hyg. 47(6):556-560. <https://dx.doi.org/10.1016/s0035-9203(53)80008-8> <PMid:13113665>

Paranaiba L.F., Pinheiro L.J., Torrecilhas A.C., Macedo D.H., Menezes-Neto A., Tafuri W.L. & Soares R.P. 2017. *Leishmania enriettii* (Muniz & Medina, 1948): A highly diverse parasite is here to stay. PloS Pathog. 13(5):e1006303. <https://dx.doi.org/10.1371/journal.ppat.1006303> <PMid:28542526>

Pothirat T., Tantiworawit A., Chaiwarith R., Jariyapan N., Wannasan A., Siriyasatien P., Supparatpinyo K., Bates M.D., Kwakye-Nuako G. & Bates P.A. 2014. First isolation of *Leishmania* from northern Thailand: case report, identification as *Leishmania martiniquensis* and phylogenetic position within the *Leishmania enriettii* complex. PLoS Neg. Trop. Dis. 8(12):e3339. <https://dx.doi.org/10.1371/journal.pntd.0003339> <PMid:25474647>

Thomaz-Soccol V., Pratlong F., Langue R., Castro E., Luz E. & Dedet J.P. 1996. New isolation of *Leishmania enriettii* Muniz & Medina 1948 in Parana state, Brazil, 50 years after the first description, and isoenzymatic polymorphism of the *L. enriettii* taxon. Ann. Trop. Med. Parasitol. 90(5):491-495. <https://dx.doi.org/10.1080/00034983.1996.11813074> <PMid:8915125>