Current opinion

Vaccination against canine leishmaniasis in Brazil

Filipe Dantas-Torres [a,*], Fábio dos Santos Nogueira [b], Ingrid Menz [c], Paulo Tabanez [d], Sydnei Magno da Silva [e], Vitor Márcio Ribeiro [f], Guadalupe Miró [g], Luís Cardoso [h], Christine Petersen [i], Gad Baneth [j], Gaetano Oliva [k], Laia Solano-Gallego [l], Lluís Ferrer [l], Maria Grazia Pennisi [m], Patrick Bourdeau [n], Carla Maia [o], Domenico Otranto [p], Luigi Gradoni [q], Orin Courtenay [r], Carlos Henrique Nery Costa [s]

[a]*Department of Immunology, Aggeu Magalhães Institute, Oswaldo Cruz Foundation, Recife, Brazil*

[b]*Fundação Educacional de Andradina, São Paulo, Andradina, Brazil*

[c]*Self-employed Veterinarian, Campinas, Brazil*

[d]*Self-employed Veterinarian, Brasília, Brazil*

[e]*Department of Immunology, Microbiology and Parasitology, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Brazil*

[f]*Veterinary School, Pontifical Catholic University of Minas Gerais, Betim, Brazil*

[g]*Department of Animal Health, Veterinary Faculty, Universidad Complutense de Madrid, Madrid, Spain*

[h]*Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*

[i]*Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, United States*

[j]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel*

[k]*Department of Veterinary Medicine and Food Production, University of Naples Federico II, Naples, Italy*

[l]*Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain*

[m]*Department of Veterinary Sciences, University of Messina, Polo Universitario Annunziata, Messina, Italy*

[n]*Veterinary School of Nantes ONIRIS, University of Nantes, LUNAM, Nantes, France.*

[o]*Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa (UNL), Lisbon, Portugal*

[p]*Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

[q]*Unit of Vector-borne Diseases, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

[r]*Zeeman Institute, and School of Life Sciences, University of Warwick, Coventry, United Kingdom*

[s]*Department of Community Medicine, Federal University of Piauí, Teresina, Brazil*

* Corresponding author at: Av. Prof. Moraes Rego s/n, 50740465 Recife, Pernambuco, Brazil. Tel.: +55 81 21237826.

E-mail address: filipe.dantas@cpqam.fiocruz.br (F. Dantas-Torres).

ABSTRACT

Prevention of canine *Leishmania infantum* infection is critical to management of visceral leishmaniasis in people living in endemic areas of Brazil. A bill (PL 1738/11), currently under consideration, proposes to establish a national vaccination policy against canine leishmaniasis in Brazil. However, there is no solid scientific evidence supporting the idea that this could reduce transmission from infected vaccinated dogs to sand flies to a level that would significantly reduce the risk of *L. infantum* infection or visceral leishmaniasis in humans. Thus, we advocate that insecticide-impregnated collars should be made mandatory for public health purposes and that vaccines are applied on a case-by-case optional basis for individual dog protection.

Keywords: *Leishmania*, zoonosis, prevention, vaccination, topical insecticides

1. Introduction

Brazil is one of the largest foci of human visceral leishmaniasis (VL) caused by *Leishmania infantum*, with an annual incidence ranging from 3455 to 4456 cases during 2013–2017 (Ministério da Saúde, 2019). Dogs have been serologically screened and culled as part of the national VL control programme, which also includes indoor residual spraying insecticides and human VL treatment (Ministério da Saúde, 2014). However, this strategy has not apparently led to reductions in either the incidence of human VL, nor the infection prevalence in dogs, though statistically powered trials to test these intervention measures are generally lacking (Romero and Boelaert, 2010; Rocha et al., 2018).

Tools to prevent *L. infantum* infection or canine leishmaniasis (CanL) in dogs have been licensed in Brazil, including topical spot-on insecticides and insecticide-impregnated collars, and vaccines. Public reaction to culling pet dogs has also catalysed recent legislation to now allow veterinarians to treat infected seropositive dogs with miltefosine as an alternative to euthanasia (Ministério da Agricultura, Pecuária e Abastecimento and Ministério da Saúde, 2016).

In pursuit of an effective method to reduce VL transmission, a bill (PL 1738/11) to introduce obligatory annual canine vaccination is currently under Brazilian government-level examination (Câmera dos Deputados, 2019). According to the bill, vaccination will be mandatory in areas of moderate (annual average of ≥2.4 to 4.4 human VL cases in the past five years) and intense (≥4.4 human VL cases per year) transmission, but not in areas of sporadic transmission (≥0.1 to <2.4 human VL cases per year).

Originally proposed in 2011 to prevent CanL, the bill was accepted in 2018 by the Committee on Social Security and Family and by the Committee on Agriculture, Livestock, Supply and Rural Development. It is now under analysis by the Committee

on Finance and Taxation (as of 16th August 2019). If accepted, the bill then will be assessed for constitutional, legal, juridical and legislative regulations by the Committee on Constitution, Justice and Citizenship, and scrutinised by the plenary of the Chamber of Deputies, and/or voted on by the Brazilian Federal Senate.

With such an important decision on the national VL control policy being imminent, the aim of this paper is to provide a review of the scientific evidence supporting the proposed vaccination strategy in light of alternative intervention methods, and in so doing to provide the authors' informed expert opinion on the bill PL 1738/11.

2. Licensed CanL vaccines

The vaccine Leishmune® (Zoetis), was licensed in 2003, but the requirements for research, development, production, evaluation, registration, license renewal, commercialization, and use of CanL vaccines were amended in 2007 (Ministério da Agricultura, Pecuária e Abastecimento and Ministério da Saúde, 2007), and this vaccine was withdrawn from the market in 2014. According to a technical note of the Ministry of Agriculture, Livestock and Food Supply, Leishmune® did not completely satisfied the requirements for phase III studies (Ministério da Agricultura, Pecuária e Abastecimento, 2014). Another vaccine, Leish-Tec® (Ceva Animal Health), was licensed in 2007 and currently is the only CanL vaccine commercially available in Brazil.

An effective CanL vaccine should induce strong and long-lasting proinflammatory (Th1-dominated) immune response in dogs in order to either (i) prevent the establishment of an initial infection, or (ii) control its progression towards severe disease and (iii) promote the abrogation of *Leishmania* transmissibility by vaccinated dogs if they get infected (Gradoni, 2015).

The best-case scenario (i) is difficult to achieve by current anti-protozoan vaccines, despite there is evidence from the field that in endemic areas a proportion of dogs repeatedly exposed to sand flies potentially infected by *L. infantum*, never manifest evidence of infection (i.e. parasite demonstration by microscopy/culture or DNA amplification from target tissues), while presenting low antibody titres. The strong refractoriness to infection of these "resistant" dogs might be the result of a particular immunogenetic background (Soutter et al., 2019) or of natural booster doses determined by events of defective *L. infantum* transmission by the vector, as recently seen in a hamster-sand fly laboratory model (Gradoni et al., 2019).

As for the (ii) scenario, an effective vaccine could represent an important tool for veterinary care at individual level for dogs exposed to risk of *L. infantum* infection. A vaccine-mediated Th1-type immune response will impair parasite multiplication and dissemination. Increased parasite burden and dissemination are associated with pathologic immunoglobulin production and immune complex formation in dogs.

On the other hand, scenario (iii), theoretically associated with very good clinical efficacy of the vaccine, is of key importance as a public health intervention outcome. Dogs are the most important source of *L. infantum* infection to sand fly vectors (Quinnell and Courtenay, 2009). Canine infectiousness, which can only be ascertained by xenodiagnosis using colonized sand flies, is generally believed to be correlated with disease progression (Courtenay et al., 2002, 2014), although subclinically infected dogs (elsewhere defined as "asymptomatic") were shown to exhibit various degrees of infectiousness. Unfortunately, CanL studies suffer from a lack of consistency in the definition of subclinical dogs, which may have brought to contradictive conclusions (Dantas-Torres et al., 2014).

Table 1 summarizes the main features of available CanL vaccines, by focusing on the above scenarios. Leish-Tec® is currently the only vaccine available in Brazil. Other two vaccines are commercially available in Europe, CaniLeish® (Virbac Animal Health) and LetiFend® (Laboratorios LETI) licensed by the European Medicine Agency in 2011 and 2017, respectively. Importantly, these vaccines have not been tested for efficacy or effectiveness against human VL.

3. Can currently licensed CanL vaccines reduce the risk of infection or VL in humans?

A study with CaniLeish® revealed that significantly fewer of the sand flies which fed on the vaccinated dogs were infected when compared to those which fed on the control dogs (Bongiorno et al., 2013). A previous study conducted in Brazil reported low transmission rates to sand flies among dogs vaccinated with either Leishmune® or Leish-Tec® (Fernandes et al., 2014), but a more recent study showed no statistically significant difference in the general comparison between Leish-Tec®-vaccinated and placebo dogs (Regina-Silva et al., 2016). Vaccination does partially protect dogs against development of severe clinical signs (Gradoni, 2015), which are correlated with infectiousness to sand flies (Courtenay et al., 2014) and therefore could have some impact on population-level transmission, but theoretically only if dogs disproportionately contributing to onward transmission are identified and vaccinated.

Mathematical models have suggested that canine vaccination could have a limited to no effect on the infection incidence in humans, as compared with insecticide-impregnated collars (Sevá et al., 2016; Shimozako et al., 2017; Gomez et al., 2018). Other simulation studies assessed possible additive effects of Leishmune® or Leish-Tec® vaccination to dog culling in controlling human VL, the former based on data from

Araçatuba (São Paulo) and Belo Horizonte (Minas Gerais), south-eastern Brazil (Palatnik-de-Sousa et al., 2009). While this study concluded that Leishmune® vaccination could increase the efficacy of culling against human VL incidence (Palatnik-de-Sousa et al., 2009), the Leish-Tec® study suggested that it probably would not have any additional impact on dog infection rates to protect humans in high-risk areas (Grimaldi et al., 2017).

In summary, there is no current scientific evidence that canine vaccination significantly reduces the infectiousness of infected vaccinated dogs. And although there are no robustly designed community-level field studies to evaluate canine vaccination efficacy or effectiveness against human infection or VL disease incidence (Romero and Boelaert, 2010), the existing data suggest that current CanL vaccines need improvement to warrant a national canine vaccination policy as a public health intervention.

4. Can insecticide-impregnated collars protect dogs from *L. infantum* infection and reduce the risk of human infection and VL?

Three brands of insecticide-impregnated collars to protect dogs against sand fly bites are available in Brazil, Scalibor® ProtectorBand (MSD Animal Health), and Leevre® (Ourofino Animal Health), both of which containing 4% deltamethrin, and Seresto® (Bayer Animal Health), which contains 10% imidacloprid and 4.5% flumethrin. The collars are designed to reduce the number of sand flies feeding on treated animals and to increases sand fly mortality (Lucientes, 1999; Halbig et al., 2000; David et al., 2001; Alves et al., 2015). Considering that the extrinsic incubation period of *L. infantum* in the vector is 5–7 days to reach the infective form, these effects reduce the likelihood of a collared dog acquiring infection and being a source of *Leishmania* parasites for onward

198 transmission. In this way collars are expected to reduce the number of infectious bites
199 on humans.
200 Both Scalibor® and Seresto® are efficacious in reducing incident infections in
201 individual dogs, evidenced by reductions in seroconversion, detection of parasite DNA,
202 parasite culture or cytology. From the 10 studies of variable design, Scalibor® provides
203 a median 53.5% (IQR: 49.1%–80.4%; range: 42.4%–100%) protection against canine
204 seroconversion incidence as tested across endemic regions including Brazil (Oliveira-
205 Lima et al., 2002; Camargo-Neves et al., 2004; Coura-Vital et al., 2018; Kazimoto et
206 al., 2018; Lopes et al., 2018), North Africa (Aoun et al., 2008), and Middle East
207 (Gavgani et al., 2002). Of these, the five Brazilian studies report a median 48.3% (IQR:
208 48.0–53.0%; range: 42.4–69.7%) protective effect against *L. infantum* infection in dogs.
209 In one followed-up study of 3,742 seronegative Brazilian dogs, the efficacy of these
210 collars against infection was 48% estimated by intention-to-treat analysis that included
211 all recruited dogs, irrespective of collar losses and other non-protocol events (Coura-
212 Vital et al., 2018). The equivalent efficacy estimate by per-protocol analysis which
213 included only dogs wearing collars continuously and adhering to the study protocol,
214 increased to 63% (Coura-Vital et al., 2018).
215 Seresto®, tested less extensively, and exclusively in Italian sheltered dogs, provided a
216 median level of protection of 93.4% (IQR: 90.9–96.7%; range: 88.3–100%) (Otranto et
217 al., 2013; Brianti et al., 2014, 2016), which is relatively higher than Scalibor®, as
218 substantiated by one comparative study of the two collars randomized between dogs.
219 That study showed Seresto® to prevent 88.3% incident canine infections compared to
220 61.8% by Scalibor® (Brianti et al., 2016). Moreover, Seresto® provide 8 months of
221 protection against sand flies, whereas for Scalibor® is labelled for 4 months in Brazil
222 and 5 months in Europe, though a recent laboratory study demonstrated a sustained anti-

223 feeding efficacy of ≥94% for 12 months against *Phlebotomus perniciosus* (Paulin et al.,
224 2018). As a follow up consequence of this study, the Ministry of Health of Italy
225 authorized the extension of the label recommendation of Scalibor® for 12 months
226 (Ministero della Salute, 2018). This extended recommendation is also valid in other
227 European countries, such as Portugal and Spain (MSD Animal Health, 2019a, 2019b).
228 From the public health perspective, only two studies have evaluated the protective
229 effect of the community-wide application of Scalibor® in dogs on the incidence of *L.*
230 *infantum* infection and clinical VL cases in humans, in this case children who are the
231 high-risk group. Both studies were cluster randomized trial designs involving
232 community-wide distribution of Scalibor® in hyperendemic villages in Northwest Iran.
233 In the first study, the authors estimated that the odds of seroconversion was reduced by
234 43% (95% CLs: 10%, 63%) in ≤10-year-old children (the high-risk group), and by 54%
235 (95% CLs: 30%, 70%) in dogs (Gavgani et al., 2002). The second study was an
236 effectiveness trial against clinical VL in the same infant age group conducted in 80
237 randomly assigned villages, where collars were fitted to dogs prior to four consecutive
238 transmission seasons. That trial was designed by researchers but implemented by the
239 local Ministry of Health. At the end of the follow-up period, the relative risk of infantile
240 VL was 50% (95% CI: 30–82%), with a 48% reduction in the absolute number of
241 clinical infantile VL cases (Courtenay et al., 2019).
242 In addition to the epidemiological outcomes in dogs and humans, Scalibor® has been
243 reported to reduce also domestic sand fly vector densities (Silva et al., 2018), and sand
244 fly infection prevalence with *L. infantum* (Kazimoto et al., 2018); both studies were
245 conducted in Brazil.
246 We found no peer-reviewed scientific publication on the efficacy of Leevre® in the
247 international literature. According to a study report available online (Ourofino, 2000),

this collar works for 6 months, with the repellent efficacy against *Lutzomyia longipalpis*, ranging from 81% to 93% and the insecticidal efficacy ranging from 71 to 100%.

4.1. Intervention objectives

The majority of the collar studies achieved the reported levels of protection within 1–2 transmission seasons, or years, of intervention. However, it is important to recognise that most studies have collared and monitored outcomes in individual dogs, representing the degree of protection to be expected by pet owners purchasing and fitting collars to their owned dogs (e.g. household-level protection). For public health objectives, by contrast, community-wide collar coverage is required so that the remaining population benefits from the consequential reductions in transmission (i.e. analogous to providing herd immunity by community vaccination). One key knowledge gap is the minimum coverage threshold (percent of total dogs collared) required in any given transmission intensity setting. For example, in the effectiveness trial in Iran, the mean annual Scalibor® coverage per village was 87% (95% CI: 84.2–89.0%, range: 65.7–100%), however changes in human VL incidence attributed to the intervention did not prove to relate to collar coverage, or indeed any other demographic measure in the studied villages (Courtenay et al., 2019). Moreover, field studies generally indicate that collars have been more efficacious in areas where transmission is seasonal (e.g. Italy), as compared to areas where the transmission occurs all year round (e.g. Brazil) (Otranto and Dantas-Torres, 2013).

5. Summary guidelines for preventing *Leishmania infantum* infection in dogs

The LeishVet association has published guidelines for the management of CanL (Solano-Gallego et al., 2011; Miró et al., 2017), with recommendations to help the veterinary clinician to better understand, diagnose, treat and prevent infection and disease. LeishVet has been involved in many meetings and discussions on this topic with veterinarians, human medical professionals, public health regulators from endemic and non-endemic countries, the pharmaceutical industry and organizations concerned with the hazard of zoonotic VL. The Brasileish group has also been involved in the organisation of scientific meetings and guidelines for the management of CanL in Latin America. Moreover, members of this group have been involved in advisory meetings on CanL and human VL, organized by public health authorities, including the Pan American Health Organisation and the Ministry of Health of Brazil. In the following lines, some major points from the LeishVet and Brasileish guidelines for preventing *L. infantum* infection in dogs are summarized:

- The main way to avoid *L. infantum* infection is to use topically applied pyrethroids (i.e. permethrin, deltamethrin or flumethrin) with proven activity against female sand flies. These products are available in spot-on formulations or in collars and reduce the risk of new infections in non-infected dogs and the biting of sand flies on already infected dogs.
- Currently available vaccines do not prevent the establishment of infection and may allow maintenance of an infected but clinically healthy status in some dogs. The decision to vaccinate should be based upon individual benefit/risk to the dog, age, breed, life-style or use, habitat, reproductive status, and owner compliance.
- Immune modulators assessed to date in CanL include domperidone and some dietary nucleotides in combination with an active hexose correlated compound.

Domperidone has proven preventative efficacy and dietary nucleotides have been suggested to reduce disease progression in *L. infantum*-infected dogs, but more studies are needed to evaluate the real efficacy of both drugs. In particular, it is important to assess whether infected dogs treated with these immune modulators may serve as a source of *L. infantum* to sand flies (Travi and Miró, 2018).

- Other measures to prevent sand fly bites include: keeping dogs indoors from dusk to dawn; reducing microhabitats favourable to sand fly breeding in the vicinity of the house and in other locations where dogs spend time; and indoor house-spraying with residual insecticides.

6. Concluding remarks

Controlling CanL in Brazil is not an easy enterprise, owing to the inherent complexities involved in its transmission cycles in urban and rural settings. For decades, the public health authorities have attempted to reduce the incidence of VL through the mass elimination of seropositive dogs, with no apparent success. The available scientific data support the community-wide use of insecticide-impregnated collars, rather than vaccination, to reduce the risk of infection in dogs and humans (Gavgani et al., 2002; Otranto et al., 2013; Brianti et al., 2014, 2016; Paulin et al., 2018; Courtenay et al., 2019). This conclusion is supported by others. In 2015, the European Commission requested the scientific opinion of the European Food Safety Authority about CanL, with the objective of mitigating the probability of introduction of the infection into free areas in the European Union through movements of infected dogs. The Animal Health and Welfare Panel conducted systematic reviews to evaluate the efficacy of vaccines, topically applied insecticides and prophylactic medication. The panel members along with members of a working group on CanL (which includes some of the co-authors of

the present paper: GB, PB, LG and LSG) concluded that topically applied insecticides were the most effective mitigation measure to reduce the probability of introduction and establishment of CanL in free areas (EFSA Panel on Animal Health and Welfare, 2015).

The global expense of vaccination (i.e. three initial doses plus annual booster vaccination, cold chain, and a range of consumables) and chemotherapeutic treatments are much higher than applying insecticide-impregnated collars (e.g. two collars per year for a collar labelled for 6 months of protection). Currently available CanL vaccines are recommended for use only in seronegative and healthy dogs. So, the costs of pre-testing add to the cost of vaccination. By contrast, the dog's infection and health status has little, if any, influence on the efficacy of insecticide-impregnated collars.

CanL affects disproportionally dogs living in low-income areas in Brazil, as it happens in most endemic foci in Latin America. Consequently, many dog owners living in the most affected areas cannot handle the costs of preventive measures. Hence, public health authorities in Brazil play a pivotal role in delivering health education for dog owners and promoting tangible actions that could help preventing *L. infantum* infection in dogs. Furthermore, even if privately-owned dogs are protected, stray dogs will keep playing a role as reservoirs of *L. infantum* and thus a critical role in control campaigns.

Concluding, we agree generally with the actions proposed by the bill 1738/11, but we strongly suggest to replace the mandatory vaccination of dogs, with the community-wide application of insecticide-impregnated collars. While available vaccines can be recommended on a case-by-case basis, they should not replace the use of insecticide-impregnated collars because infected vaccinated dogs may still serve as a source of infection to the vectors, which may potentially transmit the parasites to naïve hosts.

Acknowledgments

FDT is the recipient of a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 313118/2018-3). CM has the support of the Portuguese Ministry of Education and Science (via Fundação para a Ciência e a Tecnologia, I.P.), through an Investigator Starting Grant (IF/01302/2015).

Conflict of interest

Some of the authors (FDT, FSN, IM, PT, VMR, GM, LC, CP, GB, GO, LSG, LF, MGP, PB, CM, DO, LG, and OC) have been involved in studies, acted as consultants and/or participated in meetings sponsored by various companies (Bayer Animal Health, Boehringer Ingelheim, Ceva Animal Health, Elanco, IDEXX Laboratories, Laboratorios LETI, Merck Sharp and Dohme, Virbac Animal Health, Zoetis, Bioiberica, and/or Ecuphar) with products to prevent, diagnose and/or treat canine leishmaniasis in the past decade. These companies had no influence on the authors' decision to prepare and publish the present paper.

References

Alves, F.S., Oliveira, R.D., Aguiar, G.R., Moura, A.C.J., Leme, F.O.P., Araujo, R.B., Costa-Val, A.P., 2015. Antifeeding and short-term insecticidal effects against *Lutzomya longipalpis* in dogs treated with permethrin or deltamethrin. Rev. Bras. Ci. Vet. 22, 71–76.

Aoun, K., Chouihi, E., Boufaden, I., Mahmoud, R., Bouratbine, A., Bedoui, K., 2008. Efficacy of deltamethrin-impregnated collars Scalibor in the prevention of canine leishmaniasis in the area of Tunis. Arch. Inst. Pasteur Tunis. 85, 63–68.

Bongiorno, G., Paparcone, R., Foglia Manzillo, V., Oliva, G., Cuisinier, A.M., Gradoni, L., 2013. Vaccination with LiESP/QA-21 (CaniLeish®) reduces the intensity of

372 infection in *Phlebotomus perniciosus* fed on *Leishmania infantum* infected dogs - a
373 preliminary xenodiagnosis study. Vet. Parasitol. 197, 691–695.
374 Brianti, E., Gaglio, G., Napoli, E., Falsone, L., Prudente, C., Solari Basano, F., Latrofa,
375 M.S., Tarallo, V.D., Dantas-Torres, F., Capelli, G., Stanneck, D., Giannetto, S.,
376 Otranto, D., 2014. Efficacy of a slow-release imidacloprid (10%)/flumethrin (4.5%)
377 collar for the prevention of canine leishmaniosis. Parasit. Vectors. 7, 327.
378 Brianti, E., Napoli, E., Gaglio, G., Falsone, L., Giannetto, S., Solari Basano, F., Nazzari,
379 R., Latrofa, M.S., Annoscia, G., Tarallo, V.D., Stanneck, D., Dantas-Torres, F.,
380 Otranto, D., 2016. Field evaluation of two different treatment approaches and their
381 ability to control fleas and prevent canine leishmaniosis in a highly endemic area.
382 PLoS Negl. Trop. Dis. 10, e0004987.
383 Camargo-Neves, V., Rodas, L.A.C., Pauliquévis Jr., C., 2004. Avaliação da efetividade
384 da utilização de coleiras impregnadas com deltametrina a 4% para o controle da
385 leishmaniose visceral americana no estado de Sao Paulo: resultados preliminares.
386 BEPA 1, 7–14.
387 Câmara dos Deputados, 2019. PL 1738/2011. Brasília: Câmara dos Deputados.
388 Available from:
389 https://www.camara.leg.br/proposicoesWeb/fichadetramitacao?idProposicao=51084
390 1 [cited 2019 July 10].
391 Cotrina, J.F., Iniesta, V., Monroy, I., Baz, V., Hugnet, C., Marañon, F., Fabra, M.,
392 Gómez-Nieto, L.C., Alonso, C., 2018. A large-scale field randomized trial
393 demonstrates safety and efficacy of the vaccine LetiFend® against canine
394 leishmaniosis. Vaccine 36, 1972–1982.
395 Coura-Vital, W., Leal, G.G.A., Marques, L.A., Pinheiro, A.D.C., Carneiro, M., Reis,
396 A.B., 2018. Effectiveness of deltamethrin-impregnated dog collars on the incidence

of canine infection by *Leishmania infantum*: A large scale intervention study in an endemic area in Brazil. PLoS One. 13, e0208613.

Courtenay, O., Bazmani, A., Parvizi, P., Ready, P.D., Cameron, M.M., 2019. Insecticide-impregnated dog collars reduce infantile clinical visceral leishmaniasis under operational conditions in NW Iran: A community-wide cluster randomised trial. PLoS Negl. Trop. Dis. 13, e0007193.

Courtenay, O., Carson, C., Calvo-Bado, L., Garcez, L.M., Quinnell, R.J., 2014. Heterogeneities in *Leishmania infantum* infection: using skin parasite burdens to identify highly infectious dogs. PLoS Negl. Trop. Dis. 8, e2583.

Courtenay, O., Quinnell, R.J., Garcez, L.M., Shaw, J.J., Dye, C., 2002. Infectiousness in a cohort of Brazilian dogs: why culling fails to control visceral leishmaniasis in areas of high transmission. J. Infect. Dis. 186, 1314–1320.

Dantas-Torres, F., Baneth, G., Miró, G., Cardoso, L., Oliva, G., Solano-Gallego, L., Bourdeau, P., Otranto, D., 2014. Further thoughts on "Asymptomatic dogs are highly competent to transmit *Leishmania* (*Leishmania*) *infantum chagasi* to the natural vector". Vet. Parasitol. 204, 443–444.

David, J.R., Stamm, L.M., Bezerra, H.S., Souza, R.N., Killick-Kendrick, R., Lima, J.W., 2001. Deltamethrin-impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. Mem. Inst. Oswaldo Cruz. 96, 839–847.

EFSA Panel on Animal Health and Welfare, 2015. Scientific opinion on canine leishmaniosis. EFSA Journal 13, 4075.

Fernandes, C.B., Junior, J.T., de Jesus, C., Souza, B.M., Larangeira, D.F., Fraga, D.B., Tavares Veras, P.S., Barrouin-Melo, S.M., 2014. Comparison of two commercial vaccines against visceral leishmaniasis in dogs from endemic areas: IgG, and

422 subclasses, parasitism, and parasite transmission by xenodiagnosis. Vaccine. 32,
423 1287–1295.
424 Gavgani, A.S., Hodjati, M.H., Mohite, H., Davies, C.R., 2002. Effect of insecticide-
425 impregnated dog collars on incidence of zoonotic visceral leishmaniasis in Iranian
426 children: a matched-cluster randomised trial. Lancet 360, 374–379.
427 Gomez, S.A., Chapman, L.A.C., Dilger, E., Courtenay, O., Picado, A., 2018. Estimating
428 the efficacy of community-wide use of systemic insecticides in dogs to control
429 zoonotic visceral leishmaniasis: A modelling study in a Brazilian scenario. PLoS
430 Negl. Trop. Dis. 12, e0006797.
431 Gradoni, L., 2015. Canine *Leishmania* vaccines: still a long way to go. Vet. Parasitol.
432 208, 94–100.
433 Gradoni, L., Bongiorno, G., Foglia Manzillo, V., Gizzarelli, M., Oliva, G., 2019. A
434 hamster model of defective sand-fly transmission may explain the occurrence of
435 canine *Leishmania* seroreactors without evidence of infection in endemic areas of
436 visceral leishmaniasis. Proceedings of the 10th International Symposium on
437 Phlebotomine Sandflies, San Cristòbal, Galàpagos Islands, Ecuador.
438 Grimaldi Jr, G., Teva, A., Santos, C.B., Santos, F.N., Pinto, I.D., Fux, B., Leite, G.R.,
439 Falqueto, A., 2017. Field trial of efficacy of the Leish-tec® vaccine against canine
440 leishmaniasis caused by *Leishmania infantum* in an endemic area with high
441 transmission rates. PLoS One 12, e0185438.
442 Halbig, P., Hodjati, M.H., Mazloumi-Gavgani, A.S., Mohite, H., Davies, C.R., 2000.
443 Further evidence that deltamethrin-impregnated collars protect domestic dogs from
444 sandfly bites. Med. Vet. Entomol. 14, 223–226.
445 Kazimoto, T.A., Amora, S.S.A., Figueiredo, F.B., Magalhães, J.M.E., Freitas, Y.B.N.,
446 Sousa, M.L.R., Melo, A.E.C.S., Campos, M.P., Alves, N.D., Werneck, G.L., 2018.

Impact of 4% deltamethrin-impregnated dog collars on the prevalence and incidence of canine visceral leishmaniasis. Vector Borne Zoonotic Dis. 18, 356–363.

Lopes, E.G., Sevá, A.P., Ferreira, F., Nunes, C.M., Keid, L.B., Hiramoto, R.M., Ferreira, H.L., Oliveira, T.M.F.S., Ovallos, F.G., Galati, E.A.B., Villegas, T.J., Bortoletto, D.V., Valadas, S.Y.O.B., Soares, R.M., 2018. Vaccine effectiveness and use of collar impregnated with insecticide for reducing incidence of *Leishmania* infection in dogs in an endemic region for visceral leishmaniasis, in Brazil. Epidemiol. Infect. 146, 401–406.

Lucientes, J., 1999. Laboratory observations on the protection of dogs from the bites of *Phlebotomus perniciosus* with Scalibor® Protectorbands: preliminary results. In: Killick-Kendrick, R., ed. Proceedings of the Canine Leishmaniasis Forum, Barcelona, Spain. Wiesbaden: Hoechst Roussel Vet, p. 92–4.

Ministério da Agricultura, Pecuária e Abastecimento, 2014. Nota Técnica N° 038/2014/DFIP/SDA. Brasília: Ministério da Agricultura, Pecuária e Abastecimento. Available from: http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-pecuarios/produtos-veterinarios/arquivos-comunicacoes-e-instrucoes-tecnicas/nota-tecnica-dfip-38-14-leishmune.pdf [cited 2019 May 6].

Ministério da Agricultura, Pecuária e Abastecimento, Ministério da Saúde, 2007. Instrução Normativa Interministerial M31/2007. Brasília: Ministério da Agricultura, Pecuária e Abastecimento, Ministério da Saúde. Available from: http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do%3Fmethod=visualizarAtoPortalMapa%26chave=815005048 [cited 2019 May 6].

Ministério da Agricultura, Pecuária e Abastecimento, Ministério da Saúde, 2016. Nota Técnica Nº 11/2016/CPV/DFIP/SDA/GM/MAPA. Brasília: Ministério da Agricultura, Pecuária e Abastecimento, Ministério da Saúde. Available from:

http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-pecuarios/produtos-veterinarios/legislacao-1/notas-tecnicas/nota-tecnica-no-11-2016-cpv-dfip-sda-gm-mapa-de-1-09-2016.pdf/view [cited 2019 May 6].

Ministério da Saúde, 2014. Manual de vigilância e controle da leishmaniose visceral. Brasília: Ministério da Saúde. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_controle_leishmaniose_visceral_1edicao.pdf [cited 2019 May 1].

Ministério da Saúde, 2019. Leishmaniose visceral - casos confirmados notificados no Sistema de Informação de Agravos de Notificação. Brasília: Ministério da Saúde. Available from: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/leishvi/bases/leishvbrnet.def [cited 2019 June 10].

Ministero della Salute, 2018. Modifica dell'autorizzazione all'immissione in commercio del medicinale per uso veterinario «Scalibor Protectorband 48 cm e 65 cm collare antiparassitario per cani. Rom: Ministero della Salute. Available from: https://www.gazzettaufficiale.it/eli/gu/2018/11/17/268/sg/pdf [cited 2019 June 12].

Miró, G., Petersen, C., Cardoso, L., Bourdeau, P., Baneth, G., Solano-Gallego, L., Pennisi, M.G., Ferrer, L., Oliva, G., 2017. Novel areas for prevention and control of canine leishmaniosis. Trends Parasitol. 33, 718–730.

Moreno, J., Vouldoukis, I., Schreiber, P., Martin, V., McGahie, D., Gueguen, S., et al., 2014. Primary vaccination with the LiESP/QA-21 vaccine (CaniLeish) produces a cell-mediated immune response which is still present 1 year later. Vet. Immunol. Immunopathol. 158, 199–207.

MSD Animal Health, 2019a. O que é a Scalibor? Available from: http://www.scalibor.pt/scalibor/qu-est-ce-que-scalibor [cited 2019 December 29].

MSD Animal Health, 2019b. Scalibor® 12 meses. Available from: https://www.scalibor.es/collar-scalibor/scalibor-durante-12-meses-flebotomo [cited 2019 December 29].

Oliva, G., Nieto, J., Foglia Manzillo, V., Cappiello, S., Fiorentino, E., Di Muccio, T., Scalone, A., Moreno, J., Chicharro, C., Carrillo, E., Butaud, T., Guegand, L., Martin, V., Cuisinier, A.M., McGahie, D., Gueguen, S., Cañavate, C., Gradoni, L., 2014. A randomised, double-blind, controlled efficacy trial of the LiESP/QA-21 vaccine in naïve dogs exposed to two *Leishmania infantum* transmission seasons. PLoS Negl. Trop. Dis. 8, e3213.

Oliveira-Lima, J.W., Nonato de Souza, R., Teixeira, M.J., Pompeu, M., Killick-Kendrick, R., David, J.R., 2002. Preliminary results of a field trial to evaluate deltamethrin-impregnated collars for the control of canine leishmaniasis in northeast Brazil. In: Killick-Kendrick, R., ed. Canine leishmaniasis: moving towards a solution. Proceedings of the Second International Canine Leishmaniasis Forum, Seville, Spain. Boxmeer: Intervet International, p. 7–14.

Otranto, D., Dantas-Torres, F., 2013. The prevention of canine leishmaniasis and its impact on public health. Trends Parasitol. 29, 339–345.

Otranto, D., Dantas-Torres, F., de Caprariis, D., Di Paola, G., Tarallo, V.D., Latrofa, M.S., Lia, R.P., Annoscia, G., Breitshwerdt, E.B., Cantacessi, C., Capelli, G., Stanneck, D., 2013. Prevention of canine leishmaniosis in a hyper-endemic area using a combination of 10% imidacloprid/4.5% flumethrin. PLoS One 8, e56374.

Ourofino, 2000. Avaliação da eficácia da coleira LEEVRE, no controle do flebotomíneos *Lutzomyia longipalpis*, em condições experimentais, em cães. São Paulo: Ourofino. Available from: https://s3-sa-east-1.amazonaws.com/vetsmart-

521 contents/Documents/DC/Ourofino/Relatorio_Estudo_Avaliacao_Leevre_Flebotomin
522 eos_Caes.pdf [cited 2019 May 6].
523 Palatnik-de-Sousa, C.B., Silva-Antunes, I., Morgado, A.A., Menz, I., Palatnik, M.,
524 Lavor, C., 2009. Decrease of the incidence of human and canine visceral
525 leishmaniasis after dog vaccination with Leishmune in Brazilian endemic areas.
526 Vaccine 27, 3505–3512.
527 Paulin, S., Frénais, R., Thomas, E., Baldwin, P.M., 2018. Laboratory assessment of the
528 anti-feeding effect for up to 12 months of a slow release deltamethrin collar
529 (Scalibor®) against the sand fly *Phlebotomus perniciosus* in dogs. Parasit. Vectors.
530 11, 529.
531 Quinnell, R.J., Courtenay, O., 2009. Transmission, reservoir hosts and control of
532 zoonotic visceral leishmaniasis. Parasitology 136, 1915–1934.
533 Regina-Silva, S., Feres, A.M., França-Silva, J.C., Dias, E.S., Michalsky, E.M., de
534 Andrade, H.M, Coelho, E.A., Ribeiro, G.M., Fernandes, A.P., Machado-Coelho,
535 G.L., 2016. Field randomized trial to evaluate the efficacy of the Leish-Tec® vaccine
536 against canine visceral leishmaniasis in an endemic area of Brazil. Vaccine 34,
537 2233–2239.
538 Rocha, I.C.M., Santos, L.H.M., Coura-Vital, W., da Cunha, G.M.R., Magalhães,
539 F.D.C., da Silva, T.A.M., Morais, M.H.F., Oliveira, E., Reis, I.A., Carneiro, M.,
540 2018. Effectiveness of the Brazilian Visceral Leishmaniasis Surveillance and Control
541 Programme in reducing the prevalence and incidence of *Leishmania infantum*
542 infection. Parasit. Vectors 11, 586.
543 Romero, G.A., Boelaert, M., 2010. Control of visceral leishmaniasis in Latin America-a
544 systematic review. PLoS Negl. Trop. Dis. 4, e584.

Sevá, A.P., Ovallos, F.G., Amaku, M., Carrillo, E., Moreno, J., Galati, E.A., Lopes, E.G., Soares, R.M., Ferreira, F., 2016. Canine-based strategies for prevention and control of visceral leishmaniasis in Brazil. PLoS One 11, e0160058.

Shimozako, H.J., Wu, J., Massad, E., 2017. The preventive control of zoonotic visceral leishmaniasis: efficacy and economic evaluation. Comput. Math. Methods Med. 2017, 4797051.

Silva, R.A.E., Andrade, A.J., Quint, B.B., Raffoul, G.E.S., Werneck, G.L., Rangel, E.F., Romero, G.A.S., 2018. Effectiveness of dog collars impregnated with 4% deltamethrin in controlling visceral leishmaniasis in *Lutzomyia longipalpis* (Diptera: Psychodidade: Phlebotominae) populations. Mem. Inst. Oswaldo Cruz 113, e170377.

Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., The LeishVet Group, 2011. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit. Vectors 4, 86.

Soutter, F., Solano-Gallego, L., Attipa, C., Gradoni, L., Fiorentino, E., Foglia Manzillo, V., Oliva, G., Tasker, S., Helps, C., Catchpole, B., 2019. An investigation of polymorphisms in innate and adaptive immune response genes in canine leishmaniosis. Vet. Parasitol. 269, 34–41.

Travi, B.L., Miró, G., 2018. Use of domperidone in canine visceral leishmaniasis: gaps in veterinary knowledge and epidemiological implications. Mem. Inst. Oswaldo Cruz. 113, e180301.