CellPress REVIEWS

Science & Society

Culling Dogs for Zoonotic Visceral Leishmaniasis Control: The Wind of Change

Filipe Dantas-Torres,[1,2]
Guadalupe Miró,[3]
Dwight D. Bowman,[4]
Luigi Gradoni,[5] and
Domenico Otranto[2,*]

Visceral leishmaniasis, caused by *Leishmania infantum*, is a zoonosis, and culling seropositive dogs has been recommended to control the disease in some endemic countries. However, no scientific evidence supports the effectiveness of this strategy to reduce the incidence of visceral leishmaniasis. Economic and ethical issues concerning dog culling are discussed.

Zoonotic Visceral Leishmaniasis: A Persistent Threat to Impoverished Populations

Zoonotic visceral leishmaniasis (ZVL), caused by *L. infantum*, is a neglected human disease prevalent in tropical, subtropical, and temperate regions of the globe. The disease is transmitted by phlebotomine sand flies and is present in countries of Central and South America, the Mediterranean region, the Middle East, and Central Asia [1]. In the Americas, most of the cases are reported from Brazil, where 4200–6300 new cases are estimated to occur every year [1]. The disease affects mainly poor people and may be fatal even if treated. Dogs are the primary domestic reservoir of *L. infantum* [2]. Most of the infected dogs present subclinical infection, whereas a susceptible fraction may suffer from severe clinical disease. While healthy infected dogs serve as a marginal parasite source to vectors, sick dogs are highly infectious owing to their poor cellular immune response and moderate to very high parasite load [3].

Canine leishmaniosis (CanL) is widespread in both the Old and New World [2]. In Europe, CanL is highly prevalent in the Mediterranean region, but in the past decade it has spread northwards [2]. In South America, CanL is expanding southwards up to northern Argentina, southern Brazil, Paraguay, and Uruguay [2,4]. Reasons for the CanL expansion may be related to the uncontrolled movement of dogs, the establishment of permanent phlebotomine populations in previously free areas, as well as to the inefficiency of current control strategies. In this regard, culling of seropositive dogs has long been recommended as a control strategy in several countries, as a means to decrease the prevalence of infection in dogs and, thus, the incidence of ZVL [1]. Nonetheless, systematic reviews of studies evaluating the effectiveness of this strategy have concluded that available scientific information is insufficient to support its use. In Brazil, thousands of dogs are culled every year and, in spite of that, the prevalence of canine infections is still high in several endemic foci. With dengue fever, leishmaniasis is considered the principal example of failure of infectious disease control, and the need for changing core strategies has been emphasized [2,5,6].

While many questions surrounding dog culling and possible reasons for its failure in controlling ZVL have been addressed elsewhere [5], this control measure is still applied indiscriminately in some endemic countries. We provide a current critical appraisal of dog culling in the Old and New World, with emphasis on Brazil, where this strategy has been in place for decades. Myths and facts regarding the management of CanL are discussed in the light of the recent scientific evidence.

Culling Dogs for ZVL Control

Culling of animal reservoirs has been a common practice since ancient times to control diseases of zoonotic concern, including rabies. The impact of this measure on disease transmission depends on several variables, including biology and virulence of the pathogen, direct versus vector transmission, burden of infected animals versus those culled, epidemic versus endemic situations, and the presence of wild reservoirs.

Culling of seropositive dogs has long been adopted in countries where ZVL is endemic (Table 1). One of the first countries to adopt this strategy was China, in association with vector-control measures [5]. While ZVL is now regarded as under control, there is no robust evidence to confirm that dog culling played a significant role in the decrease of human cases in China, where outbreaks of ZVL are still sporadically reported. Though as early as 1984 the World Health Organization leishmaniasis guidelines stated that 'there are few communities where indiscriminate dog control is acceptable', dog culling is still (or was until recently) recommended in countries of Central Asia, Caucasia, and the Balkans, as well as in some countries of South America [1]. For example, dog culling as a measure to control ZVL has been recommended by law since 1963 in Brazil, with a rough estimate of millions of dog lives being terminated in the past ∼60 years, including seropositive dogs, seronegative dogs spontaneously given by their owners, and free-ranging dogs. An example comes from Belo Horizonte (Minas Gerais, southeastern Brazil). During 1993–1997, a total of 415 683 dogs were serologically

Table 1. Countries Endemic for Zoonotic Visceral Leishmaniasis (ZVL), and the Practice of Infected Dog Culling as a Control Measure. Information Based on Endemic Country Profiles (2009–2011) reported by Alvar *et al*. [1] and Partially Updated (2014–2016) by the World Health Organization[vii]

Dog culling	Region and countries	General comments
Recommended officially	South America: Argentina, Brazil, Colombia, Uruguay, and Venezuela. Mediterranean region: Morocco, Tunisia, Middle East and Central Asia: Armenia, Azerbaijan, Iran, Iraq, Syria, Uzbekistan	In Brazil, dog culling is no longer the only option and dog owners may now decide to treat their dogs with miltefosine. Likewise, dog culling is no longer officially recommended in Argentina and Uruguay
Not recommended officially, but eventually unofficially done in practice	South America: Paraguay Mediterranean region: Albania, Algeria Middle East and Central Asia: Afghanistan, Georgia, China, Kazakhstan, Saudi Arabia, Syria, Tajikistan	In some countries, dog culling is not officially recommended, but is performed. For instance, there is no formal control program in Paraguay, but seropositive dogs are often eliminated. As another example, positive dogs may be euthanised in the USA, but only if owners agree

screened in this municipality and 15 117 were positive [7]. The authors estimated that 12 924 false-positive dogs were eliminated whereas 2003 false-negative dogs were not. In 1994–1997, 169 cases of human visceral leishmaniasis (with 17 deaths) were diagnosed in the same municipality [7].

Dog population management, through capture and culling of free-ranging dogs, has been a common practice of zoonosis-control centres in Brazil. However, this strategy is gradually being replaced with capture and spay/neuter, which is now approved by law in this country[i].

In the past decade, it became clear that culling dogs, whether privately owned or free-ranging (Box 1), failed to control ZVL in Brazil. While some trials have suggested a decrease in the incidence of infection in humans [6], none of them have demonstrated the impact of this measure on the incidence of clinical cases.

The failure of this strategy has been attributed to several possible factors. In developing countries, free-ranging dogs play a major role as a *L. infantum* reservoir because they are not reachable by control measures [8]. Other animal reservoirs may be present in endemic areas, even in urban centres. Examples are represented by hares in south Madrid, Spain [9]. Furthermore, a recent study confirmed that *L. infantum*-infected Brazilian patients are also infectious to sand flies, especially those who are ill and coinfected with HIV [10]. So, even if privately owned infected dogs may be systematically eliminated, there will always be potential alternative reservoirs. Another aspect is that culled dogs are rapidly replaced by young dogs more susceptible to the infection. In addition, the time delay between sample collection, testing, removal of seropositive animals, and euthanasia (about 80–180 days) may represent a major hindrance to the implementation of the control strategy. Newly infected dogs may remain undetected for several months, until a new survey is performed in that area (incubation time, on average, is 6 months). The limited infrastructures in public health laboratories, and scarce human resources, further complicate the serological diagnosis and potentially increase the timespan between sample collection, testing, and subsequent decisions.

The limitation of serological tests in terms of sensitivity and specificity is also a major problem in endemic areas where different *Leishmania* and *Trypanosoma* species

Box 1. From Indiscriminate to Selective Culling

Until recently, the dog-culling strategy was applied indiscriminately to all seropositive dogs in Brazil, regardless of whether they were sick or subclinically infected, only exposed, or truly infected. Nowadays, owners have the right to treat their dogs. So, the dog-culling strategy is becoming selective, as it has been for a long time in other countries where CanL is endemic. For instance, seropositive dogs may be eliminated in the USA, but only if owners agree [1], whereas in Europe euthanasia is generally reserved to severely sick dogs, as a means to avoid prolonged suffering. The question is what should be done with unowned infected dogs. In Italy, for instance unowned dogs are usually serologically screened before entering into a municipal kennel and, if positive and sick, their treatment is covered by the municipality. From a practical viewpoint, the adoption of such a policy in developing countries is unrealistic, mainly for economic constraints. Also, putting several infected dogs in a kennel could represent a public health risk if sand flies are not controlled and if noninfected dogs living within and near the kennel are not permanently protected against sand flies with topical insecticides. Under the scenarios of economic crisis and political upheaval [8], this possibility sounds utopic in some regions, such as in Latin America, though animalist associations from developed countries are inverting this trend by relocating and promoting the adoption of sick animals from endemic areas to nonendemic areas.

occur. For instance, dogs alleged to be positive for *L. infantum*, can be infected by *L. braziliensis* (a pathogen for which a dog's reservoir role has not been demonstrated) or *Trypanosoma caninum* (unknown zoonotic role) instead, being wrongly eliminated due to serological cross-reactions. While the diagnostic procedures used in the reservoir control program have changed recently in Brazil [11], cross-reactions may still occur, leading to the unnecessary elimination of false-positive dogs. In a similar fashion, another concern is the lack of agreement between tests use by the public authorities and tests commercially available in Brazil, with potential consequences for the management of infected dogs [11].

Managing CanL: Myths and Facts

In CanL-endemic areas, accurate diagnosis is important to differentiate 'past exposure' from 'current infection', or 'clinical' from 'subclinical' infection, ultimately to guide decisions regarding the clinical management of dogs. Samples from suspected animals should be assessed by quantitative serology and parasitological or molecular tests. A definitive diagnosis of CanL can only be issued if all available lines of evidence are interpreted together. Diagnosing subclinical infection in dogs, often seronegative, may be troublesome due to inherent limitations of available serological and parasitological assays. This particular issue led the Ministry of Health of Brazil to change the diagnostic criteria, by adopting a rapid immunochromatograhic test (TR DPP® CVL, Bio-Manguinhos) as the official screening test, and an ELISA (EIE LVC, Bio-Manguinhos) as the confirmatory test. This reduced the number of false positives and, thus, the number of dogs wrongly eliminated [11,12].

Leishmania prevention in non-infected dogs should actually be the main control

Table 2. Strategies for the Control, Prevention, and/or Treatment of *Leishmania infantum* Infection in Dogs

Strategy	Principle and aims	Pros	Cons
Dog culling	Dogs are serologically tested and, if positive, culled. The aim is to reduce the number of infected dogs and ultimately the risk of ZVL in humans	Infectious dogs that are not protected with repellents are removed from transmission areas	There is no bona fide scientific evidence showing the effectiveness of this strategy. This strategy can be questioned from an ethical perspective, especially when seropositive, healthy dogs are culled
Vector control with topical pyrethroids	Healthy and sick dogs living in (or visiting) an endemic area are treated with repellents to reduce their exposure to sand fly bites	Dogs are protected against sand fly bites and therefore there is a reduction in the risk of *L. infantum* transmission by infectious dogs to other dogs as well as other susceptible hosts, including humans	Difficulties in applying spot-on solutions on a monthly basis, due to lack of owner compliance. Collars may be longer lasting, but collar loss may discourage owners from buying another collar, especially in low-income countries
Vaccination	Healthy and seronegative dogs living in endemic areas are vaccinated to reduce the risk of disease development	Vaccinated dogs are at a lower risk of developing clinical disease and, if infected after vaccination, may be less infectious to sand fly vectors	Most of available vaccines in endemic areas are non-DIVA vaccines and serological results may be misinterpreting. The efficacy of available vaccines is below 90%
Treatment	Infected, sick dogs are treated to cure clinical disease	In addition to curing clinical disease, the treatment may reduce the parasite load and, consequently, the infectiousness of treated dogs to sand flies	Clinical relapses are common. Depending on clinical stage, the prognosis may be poor. The use of drugs currently used for treating leishmaniasis in humans could theoretically lead to the selection of drug-resistant parasites, but in practice this risk is low considering that only a very small proportion of infected dogs are treated in endemic areas
Immunomodulation	Sick dogs maybe treated with immunomodulators to strengthen their cellular immune response	Immunomodulation helps dogs to mount a strong, cell-mediated immune response, which is protective	There is limited information from randomized controlled clinical trials supporting the use of immunomodulators in dogs at different disease stages

strategy (Table 2). As demonstrated by laboratory and field studies, the most efficacious and effective way to reduce the risk of dog infection is the protection against sand fly bites by topical pyrethroids, such as deltamethrin, permethrin, and flumethrin [13,14]. Public health authorities and veterinary practitioners should promote their use as the first-line prevention strategy, as they are also recommended by guidelines prepared by expert groups such as LeishVet[ii], Canine Leishmaniosis Working Group (CLWG)[iii], Tropical Council for the Control of Animal Parasites (TroCCAP)[iv] and the European Scientific Counsel Companion Animal Parasites (ESCCAP)[v]. Improving environmental and housing conditions may also help to reduce vector populations around and inside human houses.

Although the available vaccines do not prevent the establishment of infection, their use can contribute to lower parasite loads in otherwise susceptible animals, which may eventually help to limit the transmission from dogs to other dogs and to humans [14]. The large-scale use of vaccines in endemic areas, however, can be problematic because the conventional diagnostic tests are unable to discriminate between antibodies elicited by vaccination and those produced upon infection (Table 2). The development of DIVA (differentiating between infected and vaccinated animals) vaccines, should be encouraged. Furthermore, it is important to emphasize that vaccination should not replace the use of topical pyrethroids.

Infected dogs should also be protected with topical pyrethroids to avoid the risk of transmission to other dogs and people. If an infected dog is also sick, treatment should be recommended using appropriate protocols available for each clinical stage[ii]. Treating sick dogs with leishmanicidal and leishmaniostatic drugs may result in clinical recovery and in the reduction/abrogation of infectiousness for several months, as assessed experimentally [15].

The Wind of Change

The effectiveness of the dog culling strategy has been increasingly debated, as it is evident that it has failed to control ZVL. This strategy has been considered scientifically unsound and unethical [5]. Indeed, unless dogs are screened (and eventually eliminated) monthly, there will always be newly infected dogs if sand flies and other animal reservoirs are present. Also, culling programs are unrealistic in large endemic areas, owing to the limited laboratory infrastructures, scarce human resources, and economic constraints. It is unfortunate that such an ineffective control strategy has been in place for so many years in some countries, like Brazil and China, as this resulted in the unnecessary death of millions of dogs, with a minor (if any) impact on ZVL. The good news is that there is a wind of change. Many countries where dog culling has been recommended for years have replaced, or are on the way to replacing, this strategy with more effective and humane options. In South America, for instance, dog culling was initially implemented in Argentina and Uruguay, but now it is no longer recommended. Similarly, the mandatory culling of seropositive dogs is no longer applied in Brazil, since miltefosine is now marketed for the treatment of CanL in this country[vi]. The accession or the negotiation before the accession to the European Union of endemic Balkan countries, traditionally recommending culling of CanL cases, has resulted in the adherence of those countries to ethical principles which refuse a dog-culling strategy for ZVL control. Improving the general health of dogs, vaccination, and immunomodulation may also reduce the risk of disease development, although these measures should be used on an individual basis, that is, their adoption should rely on the veterinary practitioner's recommendation and owner's decision. As far as ZVL is concerned, we believe that the only way to reduce the burden of this and other tropical diseases in developing countries is by promoting better quality of life (better nutrition and housing conditions, access to basic sanitization, and so on) and increased access to basic healthcare (i.e., rapid diagnosis and early treatment) in endemic areas.

Acknowledgments

Thanks to members of the Companion Vector-Borne Disease World Forum (CVBD World Forum), LeishVet and Brasileish for the discussions about leishmaniasis control strategies, including dog culling. This article was developed in support of the CVBD World Forum consensus statement relative to leishmaniasis control in dogs. This consensus statement should be published soon.

Resources

[i] www.planalto.gov.br/ccivil_03/_ato2015-2018/2017/Lei/L13426.htm
[ii] www.leishvet.org
[iii] www.gruppoleishmania.org/en
[iv] www.troccap.org
[v] www.esccap.org
[vi] www.sbmt.org.br/portal/wp-content/uploads/2016/09/nota-tecnica.pdf
[vii] www.who.int/leishmaniasis/burden/endemic-priority-alphabetical/en/

[1]Department of Immunology, Instituto Aggeu Magalhães (Fiocruz-PE), Pernambuco, Brazil
[2]Department of Veterinary Medicine, Università degli Studi di Bari, Bari, Italy
[3]Veterinary Faculty, Animal Health Department, Universidad Complutense de Madrid, Madrid, Spain
[4]Department of Microbiology and Immunology, Cornell University, Ithaca, New York, USA
[5]Unit of Vector-borne Diseases, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

*Correspondence: domenico.otranto@uniba.it (D. Otranto).
https://doi.org/10.1016/j.pt.2018.11.005

References

1. Alvar, J. *et al.* (2012) Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* 7, e35671
2. Dantas-Torres, F. *et al.* (2012) Canine leishmaniosis in the Old and New Worlds: unveiled similarities and differences. *Trends Parasitol.* 28, 531–538
3. Courtenay, O. *et al.* (2014) Heterogeneities in *Leishmania infantum* infection: using skin parasite burdens to identify highly infectious dogs. *PLoS Negl. Trop. Dis.* 8, e2583
4. Satragno, D. *et al.* (2017) Autochthonous outbreak and expansion of canine visceral leishmaniasis, Uruguay. *Emerg. Infect. Dis.* 23, 536–538

5. Costa, C.H. (2011) How effective is dog culling in controlling zoonotic visceral leishmaniasis? A critical evaluation of the science, politics and ethics behind this public health policy. *Rev. Soc. Bras. Med. Trop.* 44, 232–242
6. Werneck, G.L. *et al.* (2014) Effectiveness of insecticide spraying and culling of dogs on the incidence of *Leishmania infantum* infection in humans: a cluster randomized trial in Teresina, Brazil. *PLoS Negl. Trop. Dis.* 8, e3172
7. Alves, W.A. and Bevilacqua, P.D. (2004) Quality of diagnosis of canine visceral leishmaniasis in epidemiological surveys: an epidemic in Belo Horizonte, Minas Gerais, Brazil, 1993–1997. *Cad. Saude Publica* 20, 259–265
8. Otranto, D. *et al.* (2017) Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. *Trends Parasitol.* 33, 813–825
9. Maia, C. *et al.* (2018) Parasite biology: the reservoir hosts. In *The Leishmaniases: Old Neglected Tropical Diseases* (Bruschi, F. and Gradoni, L., eds), pp. 79–106, Springer
10. Ferreira, G.R. *et al.* (2018) Human competence to transmit *Leishmania infantum* to *Lutzomyia longipalpis* and the influence of human immunodeficiency virus infection. *Am. J. Trop. Med. Hyg.* 98, 126–133
11. Dantas-Torres, F. *et al.* (2018) Level of agreement between two commercially available rapid serological tests and the official screening test used to detect *Leishmania* seropositive dogs in Brazil. *Vet. J.* 234, 102–104
12. Fraga, D.B. *et al.* (2016) The rapid test based on *Leishmania infantum* chimeric rK28 protein improves the diagnosis of canine visceral leishmaniasis by reducing the detection of false-positive dogs. *PLoS Negl. Trop. Dis.* 10, e0004333
13. Travi, B.L. *et al.* (2018) Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. *PLoS Negl. Trop. Dis.* 12, e0006082
14. Miró, G. *et al.* (2017) Novel areas for prevention and control of canine leishmaniosis. *Trends Parasitol.* 33, 718–730
15. Miró, G. *et al.* (2011) Infectivity to phlebotomus perniciosus of dogs naturally parasitized with *Leishmania infantum* after different treatments. *Parasit. Vectors* 4, 52

Letter

Plasmodium: Yet More Don'ts

Miles B. Markus[1,2,*]

I agree with Geoffrey McFadden that terminological communication in regard to *Plasmodium* and malaria needs to be more accurate [1]. His advice is especially pertinent given that despite his 'lecture' [2] on why there is no such noun as '*Plasmodia*' (except in particular biological circumstances related to the nonitalicized word 'plasmodia' [2]), this linguistic delusion still crops up in publications. By contrast, it should be noted that use of the adjective 'plasmodial' is entirely in order, and even desirable. The malariological acceptability or otherwise of the two words should not be confused – as has happened unfortunately, albeit rarely. McFadden advises us to 'eschew lazy language' [1]. Accordingly, I draw attention here to some additional traps for the unwary.

Mosquitoes

'Anopheline' is both an adjective ('anopheline mosquito') and a noun. Don't use an upper case 'A', except at the beginning of a sentence; and do not italicize the word as if it is a Latinized genus name.

We Don't 'Treat' Hypnozoites

It is not unusual to come across 'treat' or variations thereof in reference to hypnozoites, as in this hypothetical sentence: 'Primaquine was administered to treat hypnozoites in the liver'. The hypnozoites are not ill, however. There is nothing wrong with them, so they do not require treatment. They need to be 'eliminated', 'inactivated', or 'killed', not 'treated'.
For that matter, we do not 'treat *Plasmodium vivax*'. It is '*Plasmodium vivax* malaria' that is treated.

'Infection' Happens Once

I have occasionally seen 'infection' used to describe a malarial recrudescence or relapse [3]. This is incorrect. A person can become 'infected' only once. In the absence of superinfection or reinfection taking place, subsequent bouts of parasitemia or clinical attacks should not be called 'infections' because the patient is already infected. We can, though, speak of a 'recrudescing infection' or a 'recrudescent infection'. Likewise, 'relapsing infection' and 'relapsed infection' are correct, but 'relapse infection' is not. The difference is subtle.

Use Correct Parasite Stage Names

The terms 'bradyzoite' and 'tachyzoite' cannot be applied to *Plasmodium*, as has been done inadvertently. They refer exclusively to bradyzoic merozoites and tachyzoic merozoites of tissue cyst-forming coccidia such as *Toxoplasma* [4]. The correct plasmodial terms are 'bradysporozoite' and 'tachysporozoite' [5]. Bradysporozoites inoculated into a primate host by a mosquito are thought to become hypnozoites [6].

'Malaria' Is Not the Causative Organism

'Malarias' makes sense if it refers specifically to the infections caused by different parasite species, because 'malaria' is the name for a disease, as McFadden emphasizes [1]. This plural usage is uncommon, though, and is perhaps not to be recommended. However, when 'malarias' is used as shorthand for parasites *per se* (instead of for the disease), we have a semantic problem. Here is a quotation from the preface to a well-known book [7]: 'the fact that lower primates . . . harbor malarias infective to man, and which produce disease in him, is a relatively new concept'. The disease name 'malaria' has often been stretched like this to cover the causative organism(s) as well, and certainly subsequent to the publication of the cited book [7]. But it is not correct, and frequent repetition in the literature does not make it correct. In the example provided above, 'harbor malarias infective to man' should have read, for instance: 'harbor plasmodial parasites infective to man'. Alternative phrasing that would also have been suitable (and perhaps this is what the authors actually meant) is: 'harbor malaria parasite species that can infect man'.

As an aside, whereas we can get away with 'malaria parasite' as an abbreviation for 'malaria-causing parasite' (the previous sentence and the titles of two books serve as examples [8,9]), that is about as far as we can go without becoming unscientifically imprecise. See McFadden's explanation as to why various other non-disease-associated words should not be used in conjunction with 'malaria' [1].