Domestic Mammals as Reservoirs for *Leishmania donovani* on the Indian Subcontinent: Possibility and Consequences on Elimination

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Abstract

Leishmania donovani is the causative agent of historically anthroponotic visceral leishmaniasis (VL) on the Indian subcontinent (ISC). *L. donovani* is transmitted by the sand fly species *Phlebotomus argentipes*. Our collaborative group and others have shown that sand flies trapped outside in endemic villages have fed on cattle and dogs in addition to people. Domestic animals are reservoirs for *L. donovani* complex spp., particularly *L. infantum*, in other endemic areas. Multiple studies using quantitative PCR or serological detection methods have demonstrated that goats, cattle, rats and dogs were diagnostically positive for *L. donovani* infection or exposure in eastern Africa, Bangladesh, Nepal and India. There is a limited understanding of the extent to which *L. donovani* infection of domestic animals drives transmission to other animals or humans on the ISC. Evidence from other vector-borne disease elimination strategies indicated that emerging infections in domestic species hindered eradication. The predominant lesson learned from these other situations is that non-human reservoirs must be identified, controlled and/or prevented. Massive efforts are underway for VL elimination on the Indian subcontinent. Despite these herculean efforts, residual VL incidence persists. The specter of an animal reservoir complicating elimination efforts haunts the final push toward full VL control. Better understanding of *L. donovani* transmission on the Indian subcontinent and rigorous consideration of how non-human reservoirs alter VL ecology are critical to sustain elimination goals.

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Summary

Leishmania donovani is the causative agent of historically anthroponotic visceral leishmaniasis (VL) on the Indian subcontinent (ISC). *L. donovani* is transmitted by the sand fly species*Phlebotomus argentipes* . Our collaborative group and others have shown that sand flies trapped outside in endemic villages have fed on cattle and dogs in addition to people. Domestic animals are reservoirs for *L. donovani* complex spp., particularly *L. infantum* , in other endemic areas. Multiple studies using quantitative PCR or serological detection methods have demonstrated that goats, cattle, rats and dogs were diagnostically positive for *L. donovani* infection or exposure in eastern Africa, Bangladesh, Nepal and India. There is a limited understanding of the extent to which *L. donovani*infection of domestic animals drives transmission to other animals or humans on the ISC. Evidence from other vector-borne disease elimination strategies indicated that emerging infections in domestic species hindered eradication. The predominant lesson learned from these other situations is that non-human reservoirs must be identified, controlled and/or prevented. Massive efforts are underway for VL elimination on the Indian subcontinent. Despite these herculean efforts, residual VL incidence persists. The specter of an animal reservoir complicating elimination efforts haunts the final push toward full VL control. Better understanding of *L. donovani* transmission on the Indian subcontinent and rigorous consideration of how non-human reservoirs alter VL ecology are critical to sustain elimination goals.

Introduction

Leishmaniases are a spectrum of sand fly-borne infectious diseases of humans spanning from localized cutaneous lesions to systemic disease. The causative agents of Leishmaniases are more than 20 species of the protozoan parasite genus *Leishmania* (*Trypanosomatida* :*Trypanosomatidae*) (Burza et al., 2018, Espinosa et al., 2018).*Leishmania* spp. are often characterized as "Old World"; found in Eurasia, Africa and the Mediterranean basin, or "New World"; found in the Americas, based on their geographical distribution (McMahon-Pratt and Alexander, 2004, Reithinger et al., 2007). Old World Visceral Leishmaniasis (VL) is caused by *L. donovani* and *L. infantum* . New World VL is primarily caused by *L. infantum*(Marcili et al., 2014, Shaw, 2006, Monge-Maillo and López-Vélez, 2013). The epidemiology of leishmaniasis is based on ecological interactions between human and reservoir systems, parasite and sand fly species, regional characteristics of endemic areas, present and past susceptibility of human and animal populations and parasite and human behavior (Ready, 2008, Oryan and Akbari, 2016, Patz et al., 2000, Ready, 2013, Lukeš et al., 2007). The *Leishmania* life cycle is directly associated with ecological variables in rural or peri-domestic environments that shelter sand fly niches and reservoir hosts in conjunction with human living areas (Daszak et al., 2001). Leishmaniases are found across tropical, equatorial areas, with warm and rainy climates abetting reproduction of *Phlebotomines* (Fischer et al., 2011). Human activities such as migration, deforestation, unorganized urbanization or shifts in infection vulnerability caused by immunosuppression and malnutrition are reflected in significant increases in human susceptibility to leishmaniasis (Alvar et al., 2008, Desjeux, 2004, Thakur, 2000).

Anthroponotic spread of *Leishmania* and elimination strategy of VL on the ISC.

The World Health Organization listed leishmaniasis in category I, Emerging or Uncontrolled Diseases. Although leishmaniasis is endemic in over 98 countries and territories, eco-epidemiological hotspots for visceral leishmaniasis (VL) occur in East Africa, the Indian sub-continent (ISC), and Brazil. More than 50% of global VL incidence has been reported in India, Nepal and Bangladesh (Burza et al., 2018, WHO, 2020). VL is caused by *L. donovani* complex spp. protozoan parasites and manifest epidemiologically by two types of etiologies**(Figure 1)** : Anthroponotic visceral leishmaniasis (AVL), where parasites are transmitted by sand fly bites from human to human and occurs mainly on the ISC and in East Africa; and zoonotic visceral leishmaniasis (ZVL), which is transmitted from infected animals to humans, with domestic dogs as the primary reservoir host and occurring in Mediterranean countries, the Middle East, Asia, and South America**(Figure 2)** (Desjeux, 2004, Esch and Petersen, 2013). Despite these geographic separations, parasites within the *L. donovani*complex have a high level of genetic similarity, estimated to be greater than 99% (Franssen et al., 2020). This indicates that there are not likely to be DNA-encoded reasons for host-preference differences between*L. donovani* and *L. infantum* . Instead, the*Leishmania* life cycle is directly impacted by ecological variables in rural or peridomicile environments that shelter sand fly niches and reservoir hosts in proximity to humans. *Leishmania donovani* infection on the ISC has traditionally been recognized as an anthroponotic disease with a continuous cycle of transmission in and around human dwelling inhabited by vector sand flies.(Stauch et al., 2011, Malaviya et al., 2011). Active VL patients are thought to be the primary reservoir for AVL on the ISC **(Figure 3)** , based on the clustering of cases around households with past VL history (Barnett et al., 2005, Bern et al., 2005, Das et al., 2016).

National programs to eliminate VL were initiated by the governments of India, Bangladesh, and Nepal in 2005 as a major public health problem on the ISC. The goal of these programs was to reduce the incidence of VL at the sub-district or district level to less than 1 VL case per 10,000 population per annum by the 2015 (WHO, 2005). A key strategy of the VL elimination agenda has been systematic Indoor Residual Spraying (IRS), targeting sand flies feeding on people within their homes, as*Phlebotomus argentipes* is the only recognized *L. donovani*insect vector on the ISC. This serves as one of the prongs in a three-pronged approach; in addition to early active case identification, and comprehensive treatment of clinical cases to eliminate VL (Huda et al., 2012, Mondal et al., 2009). Although IRS campaigns have made considerable progress in reducing VL in India, Nepal, and Bangladesh, they fell short of the 2015 elimination target and are not on track to meet the extended deadline set for 2020 (Programme, 2017, Rijal et al., 2019). It is possible that these shortcomings are influenced by zoonotic sources of transmission, which to date have not been considered in intervention strategies on the ISC.

Challenges of elimination and possible infection sources on the ISC.

An assumption behind the elimination campaign is that only symptomatic human VL and post kala-azar dermal leishmaniasis (PKDL) cases are infectious to *P. argentipes* on the ISC (Figure 3). However, this hypothesis has not been completely evaluated, and is challenged by the possible existence of latent, asymptomatic human reservoirs of the parasite and potential animal reservoirs (Stauch et al., 2011, Molina et al., 2020, Singh et al., 2014). Asymptomatic VL cases are much more common than clinical cases, as shown in highly endemic villages in India and Nepal (Ostyn et al., 2011, Gidwani et al., 2011). Because of this high frequency, the role of asymptomatic individuals in VL transmission should be assessed to effectively identify human reservoirs in South Asia (Bern et al., 2010). Additionally, co-infection with HIV is a significant threat to VL management. Molina *et al* . used xenodiagnosis as a tool for evaluating VL infection in HIV+ patients and concluded that early-stage asymptomatic VL patients with HIV infection were infectious to sand flies (Molina et al., 2020). Further, transmission studies suggest PKDL patients are a significant parasite source for sand flies, particularly in immunocompromised people (Kamhawi and Serafim, 2017). PKDL is a form of VL sequelae where papular or macular dermal lesions occur that can be extensively parasitized and/or arise in response to immune reactions against persistent dermal parasites in treated VL patients (Zijlstra et al., 2017, Mukhopadhyay et al., 2014). As another reservoir of infection, PKDL patients represent a major health risk for disease resurgence in that skin parasite load is a strong predictor of positive xenodiagnosis

(Mondal et al., 2019, Molina et al., 2017) (Figure 3).

Risk factors for VL on the ISC include migration, proximities of households to animal shelters, and crowded conditions (Custodio et al., 2012, Bern et al., 2010, Ranjan et al., 2005). Livestock density in India is regarded as a threat variable for increasing human VL incidence (Barnett et al., 2005). The phenomenon of multiple frustrated feeding attempts seen by *Leishmania* -infected sand flies means a vector may feed on several nearby hosts including livestock (Bern et al., 2005, Courtenay et al., 2017). Indeed, the presence of cattle has been shown to attract or sustain higher sand fly densities, thus indirectly contributing to human infection through human-sand fly exposure. The prevalence of outdoor *P. argentipes* combined with the inclination of ISC residents to sleep outdoors during peak sand fly season correlate with an increased risk of VL (Perry et al., 2013, Picado et al., 2012).

In Chad, epizootics in domestic animals aggravated Guinea worm eradication efforts and indicated that dogs may have been a source of worms acquired by humans (Thiele et al., 2018, Eberhard et al., 2014). The presence of an animal reservoir raised the question of whether these animals were the culprits hampering eradication efforts (Galán-Puchades, 2017, Callaway, 2016). While it is widely known that *L. donovani*infection is transmitted between people, however, there has been little attention paid to the possibility of infections of domestic animals on the ISC despite genetically similar *Leishmania* species having a known zoonotic transmission ecology, (Esteva et al., 2017, Quinnell and Courtenay, 2009). Mathematical models are an important tool to understand disease transmission dynamics and assess elimination viability. Nevertheless, most models are limited by data gaps of critical biological parameters such as duration of immunity, infectiousness of different disease stages, comorbidities leading to recrudescent disease and transmission etc. (Esteva et al., 2017, Coffeng et al., 2019). A recent xenodiagnosis study on asymptomatic VL subjects in the Mediterranean basin (Molina et al., 2020) and India (Singh et al., 2020b) indicated serologically positive asymptomatic people were not the cause for ongoing transmission **(Figure 3)** , suggesting that there could be an alternative non-human host sustaining transmission. These models conclude that xenodiagnosis experiments and more clinical data are necessary to predict the role of asymptomatic infection, PKDL, and domestic animals as infection reservoirs on the ISC (Tiwary et al., 2017, Le Rutte et al., 2016, Singh et al., 2020a).

Role for domestic animals in the current ecology of disease.

Reservoir hosts are an integral part of the *Leishmania* parasite life cycle. The pathogen – vector – reservoir relationship is complex and reveals regional and temporal variation (Raymond et al., 2003). Several studies suggest that wild, domestic and synanthropic mammals including rock hyraxes, rats, foxes, pigs, cats can be infected and dogs have been identified as reservoir host for *Leishmania* (Akhoundi et al., 2016, Palatnik-de-Sousa and Day, 2011, Rohousova et al., 2015, Dereure et al., 2000). A reservoir species is characterized as one or more epidemiologically bound populations where a pathogen can be permanently established as an infection and from which an infecting organism is transmitted to the target population (Haydon et al., 2002). Identification of mammalian *Leishmania* reservoirs has occurred by demonstrating that the mammal could harbor parasites in its blood (parasitemia) through culture and more recently through PCR/qPCR, while in many cases, their role as a reservoir has not been confirmed**(Table 1)** . Mammalian reservoirs are required for transmission of zoonotic and rural/sylvatic infections to sustain the life cycle of multiple *Leishmania*species (Esteva et al., 2017). VL is assumed to have only an anthroponotic transmission cycle on the ISC since there is still no direct evidence of non-human mammalian hosts infectious to the sand fly vector (Stauch et al., 2011, Singh et al., 2010). *Phlebotomus argentipes* , the principle vector of VL in India, aggregates in and around animal housing, which offers a favorable, stable micro-climate near a blood meal and may alter disease dynamics by altering vector/host contact rates (Keeling, 1999). Several research studies indicate that IRS has altered *P. argentipes* feeding behavior from endophilic to exophilic and sand flies are most likely to feed on outdoor livestock in areas where IRS occurs (Cameron et al., 2016). Alternative blood sources for exophilic *P. argentipes* may include cattle, goats, dogs, or rodents (Poche et al., 2018) **(Figure 1).** Importantly, PCR evidence of *L. donovani* parasitemia has been found in cattle, buffalo, and goats (Bhattarai et al., 2010).

The previous conclusion that there is no animal reservoirs for L. donovani on the ISC is brought into

question by observations of anti-*Leishmania* specific antibodies and *Leishmania*quantitative PCR positivity detected from domestic animal blood**(Table 1)** . Dogs have been incriminated as a bridge between the sylvatic cycle of *Leishmania* and humans. Investigators believe dogs were responsible for the zoonotic cycle of*Leishmania* (Courtenay et al., 2002b, Courtenay et al., 2002a). In Brazil dogs are the primary reservoir host for *L. infantum* (Esch et al., 2012), a visceralizing *Leishmania* species genetically very similar to *L. donovani* (Courtenay et al., 1994, Blackwell et al., 2009, Akhavan et al., 2010). Canine *Leishmania* infection in VL-endemic areas of Bangladesh and Sudan. Another recent study in India found *L. donovani* DNA in the blood of local dogs, goats and cows, indicating that these are potential animal reservoirs of VL**(Table 1)** . Rodents and dogs are both classical reservoir species for other *L. donovani* complex spp. Other less traditional reservoirs caused outbreaks of *L. infantum* within certain foci (Molina et al., 2012, Ruiz-Fons et al., 2013). Importantly, natural zoonotic infections with *L. donovani* have been identified in dogs, other domestic animals and rodents in other areas outside the ISC**(Figure 2)** . A study of the emergence of VL in Middle Eastern and Mediterranean countries indicated that high prevalence of infected dogs, in the presence of a competent vector species, led to the onset of human disease (Hamarsheh et al., 2012, Nasereddin et al., 2005). Besides being the primary reservoir for zoonotic VL in many endemic regions, the presence of infected dogs was a critical risk factor predisposing humans to infection in these locations (Gavgani et al., 2002). The epidemiology of vector-mediated transmission from dogs and other non-traditional reservoir species on the ISC requires closer inspection to evaluate their potential role. The totality of *L. donovani* reservoirs needs to be more widely understood to protect *L. donovani*elimination goals (Cameron et al., 2016).

Conclusion

To effectively control VL on the ISC, a more global understanding of its epidemiology and regional transmission dynamics is needed. Management of potential domestic and sylvatic zoonoses would involve implementation of a more complex elimination strategy than exists under the current framework, which considers humans as the only reservoir of ISC *L. donovani* . Previously only considered anthroponotic, *L. donovani*has recently been shown to have non-human mammalian hosts by molecular and serological studies in many countries. As domestic mammals are a common documented source of sand fly blood meals, the contributions of livestock as parasite hosts or reservoirs for *L. donovani* remains an open question for additional investigation. Xenodiagnosis is the most practical way to assess transmissibility of parasites from a potential host to sand flies, and can confirm the role of these populations in the emerging non-human ecology of *L. donovani* . These host-transmission insights are critical to reinforce models forecasting VL outbreaks on the ISC and worldwide.

This article should serve as a catalyst for epidemiologists, entomologists, veterinarians, physicians, parasitologists and immunologists to come together and evaluate critical interactions between sand flies, mammalian hosts and *L. donovani* leading to transmission on the ISC. The main question is not how domestic animals become infected with *L. donovani* but rather: to what extent does*L. donovani* circulate in domestic species after being fed upon by infected *P. argentipes* ? Instead of performing costly active surveillance to look for the lack of clinical VL cases, we should instead consider how can we best monitor transmission (or lack thereof) within the whole potential *L. donovani* ecosystem. Infection outcome is determined by parasite virulence as well as by host genetic predisposition and immune/health status of the host. Infection can be controlled by removing exposure to the parasite through insecticides or reducing the parasite burden through chemo and/or immunotherapy (Baneth et al., 2008). Surveillance is complicated by the long incubation period of *L . donovani* in mammals, leading to periods of latency, when infection can be difficult to detect to prevent transmission. Interdisciplinary collaboration and broadly based ecological surveillance of all potential *P. argentipes* blood meal sources and xenodiagnosis studies will be required for some time to rule out zoonotic contributions and achieve *L. donovani* transmission eradication goals on the Indian Subcontinent.

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Conflict of interest

The authors declare no conflict of interest

Tables

Table 1. Identification of *L. donovani* infection in domestic animals through molecular and serological methods.

Country	Location	Detection Method	Potential Reservoir Sp
India	**Bihar**	PCR, rk39 ICT	Goat, Rat, Cow
	Thiruvananthapuram	PCR	Dog
	Himachal Pradesh	rk 39 ICT	Dog
Nepal	**Dharan**	PCR	Buffalo, Cow, Goat
	Kathmandu	qPCR	Dog
Bangladesh	**Mymensingh**	rk39 ICT, PCR, qPCR	Dog
	Mymensingh	DAT, ELISA, rk39 ICT	Domestic Cattle, Goat
Sri Lanka	**Matale**	rk39 ICT, Microscopy	Dog
Ethiopia	**Alduba, Dimeka, Kafta Humera**	qPCR, PCR, DAT, NNN culture	Rodents
	Addis Zemen, Kafta Humera, Sheraro	PCR, ELISA, DAT, rk 39 ICT	Dog, Cow, Goat, Sheep
Sudan	**Bandigueo, Urn Salala**	DAT, ELISA	Donkey, Cow, Goat, Rat
	Blue Nile and Gedaref State	PCR, DAT	Dog
	Barbar El Fugara	NNN culture, IFAT	Dog

Abbreviations: rK39= recombinant *Leishmania* K39 protein, PCR= Polymerase chain reaction; ICT= Immunochromatographic test; qPCR= quantitative polymerase chain reaction; DAT= Direct agglutination test; ELISA= Enzyme-linked immunosorbent assay; NNN= Novy-MacNeal-Nicolle medium; IFAT= Immunofluorescence antibody test

Figure Legends

Figure 1. Life cycles of zoonotic vs anthroponotic spread of *Leishmania* spp . Animals depicted have been documented as a blood source in *Leishmania* positive sand flies.

Figure 2. Distribution of *L. donovani* complex spp. and different transmission ecologies. AVL= anthroponotic VL, ZVL= zoonotic VL.

Figure 3. The skin as a predominant source for *Leishmania* parasites in different clinical settings. Sand flies use their serrated proboscis to probe the skin for dermal blood vessels creating a pool of blood from which to feed. Asymptomatic *L. donovani* infection provides a setting with productive immunity and a relatively low parasite load in the skin and blood. During symptomatic VL, high parasitemia and patches of highly parasitized macrophages in the skin lead to a highly increased chance of sand fly infection during feeding. PKDL is associated with an immune response against *Leishmania* parasites in the skin and therefore is characterized by a significant parasite burden within dermal macrophages. After treatment for VL, parasitemia may diminish before parasites in the skin are eradicated. However, patients with both forms of PKDL have been found to be readily infectious to sand fly vectors. Nodular/papular PKDL patients, shown to have significantly increased parasite burden in the skin compared to macular PKDL, are more infectious to sand flies than macular PKDL patients.

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