

Examining the reservoir potential of animal species for *Leishmania infantum* infection

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Abstract Leishmaniasis is a parasitic disease transmitted by sandfly *Phlebotomus* spp. and is seen in tropical and subtropical countries in which an estimated 12 million persons are infected. Among various types of leishmaniasis, zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* is an important amphixenosis shared by human and other animals. Although identifying the natural reservoir host would help better understand the transmission dynamics of *Leishmania* spp., little effort has been made to quantitatively clarify the dynamics involving the reservoir host of ZVL. The present study investigated the reservoir potential of four wild animals in maintaining ZVL, using prevalence data from Latin American countries in Amazons and examining the role of crab-eating fox, spiny rat, common opossum and black rat in maintaining the transmission. Reflecting frequent reinfections, a susceptible-infected-susceptible model was employed, enabling us to estimate model parameters from endemic prevalence data. The next generation matrix of the multi-host system was computed, permitting us to theoretically examine the reservoir potential of each animal species. Our estimates indicated that there is no unique reservoir host consisting of single animal species. Crab eating fox was considered to play an important role in maintaining *L. infantum* transmission, but this was the case only in combination with other hosts. The present study indicates that animal species other than canine play important roles in maintaining transmission of *Leishmania infantum*, which is different from conventional wisdom that centered on the importance of canine only. Greater

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sample size with additional entomological and genetic insights into inter-specific contact would be required to implement more explicit assessments.

Keywords Basic reproduction number · Reservoir · Epidemiology · Mathematical model

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1 Introduction

Leishmaniasis is a parasitic amphi xenosis caused by *Leishmania* spp. that belongs to *Trypanosomatidae*. The disease is transmitted by sandfly *Phlebotomus* spp. and classified as visceral and cutaneous leishmaniasis [1–10]. Leishmaniasis is prevalent in tropical and subtropical 88 countries, and an estimated 12 million persons are infected. The population at risk is as large as 350 million. Visceral leishmaniasis takes several years before the infected individual develops symptoms. Among the cases of visceral leishmaniasis, hypertrophy of spleen and liver is observed. The patient may often naturally recover, but if it is left untreated, severe cases could result in death within two years of illness onset [1–10]. Among various types of leishmaniasis, zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* is an important amphi xenosis that is shared by both human and other animal species. Historically, ZVL was observed in Asia, Middle East and Europe. It was then geographically spread to Latin America, and in the present day, it is mainly seen in India, Nepal, Bangladesh, Sudan and Brazil [1].

The reservoir host of an infectious disease is the animal species that can maintain transmission, and thus, allow persistence of the disease. Such animal species can act as the source of disease for humans. If the reservoir host is identified, public health experts could consider potential countermeasures against the infection. Moreover, one can better understand the mechanisms of transmission dynamics [10, 13]. In mathematical sense, one can identify the reservoir host using various approaches such as by analyzing epidemiological datasets [10, 12–14]. For instance, Nishiura et al. [10] have defined the reservoir host by examining the next generation matrix of a multi-host system. In a population with four different animal species, a 4-by-4 next generation matrix $K = \{R_{ij}\}$ is defined, i.e.,

$$K = \begin{pmatrix} R_{11} & R_{12} & R_{13} & R_{14} \\ R_{21} & R_{22} & R_{23} & R_{24} \\ R_{31} & R_{32} & R_{33} & R_{34} \\ R_{41} & R_{42} & R_{43} & R_{44} \end{pmatrix}, \quad (1)$$

characterizing secondary transmissions through interactions within and between four animal species, where R_{ij} gives the average number of secondary cases in host i caused by a single primary case in host j in a fully susceptible population. The basic reproduction number of this system is given by the dominant eigenvalue of K [11]. To understand the role of host 1, one has to consider two hypothetical settings, i.e.,

when the population consists of host 1 alone, and when the host 1 does not exist in the population. With regard to the former condition, the secondary transmission is described by R_{11} only, and if $R_{11} > 1$, it indicates that the host type 1 can maintain transmission on its own. Such host is referred to as the **maintenance host**. As for the latter condition, the population consists of remaining 3 species, and the next generation matrix of such hypothetical population K' reads

$$K' = \begin{pmatrix} R_{22} & R_{23} & R_{24} \\ R_{32} & R_{33} & R_{34} \\ R_{42} & R_{43} & R_{44} \end{pmatrix}. \quad (2)$$

If the dominant eigenvalue of K' is greater than 1, it indicates that the transmission could be maintained even in the absence of host type 1. If the dominant eigenvalue of K' is smaller than 1, the host type 1 is regarded as an **essential host** for maintaining transmission. We regard the host species that can act as both maintenance and essential host as the reservoir host [10].

The reservoir host of ZVL has yet to be explicitly identified. Without understanding the reservoir potential of various animal species, it is difficult to consider possible countermeasures against animals for the prevention of human infection. The purpose of the present study is to quantitatively assess the reservoir dynamics of *L. infantum* infection in wildlife through the analysis of empirical data. Since publications of ZVL in wildlife setting are commonly and consistently seen in Amazons, Brazil and its surrounding areas, the present study focuses on published evidence from northwestern part of Latin America.

2 Materials and methods

While visceral leishmaniasis has been seen in various countries in tropical and sub-tropical zones, the present study focused on Amazon area from which particularly high incidence in humans has been reported. Since the infectious disease spreads across borders, not only Brazil but also Colombia and Venezuela are also included as the subject of our study. From these countries, wild animals have been sampled and researchers isolated *L. infantum*. Figure 1 shows the observed prevalence data (i.e. proportion positive based on cross sectional surveys) of four animal species, namely, crab-eating fox (*Cerdocyon thous*), spiny rat (*Trichomys apereoides*), common opossum (*Didelphis marsupialis*) and black rat (*Rattus rattus*) [1, 15]. Along with the sample positives, the confidence intervals are also given, reflecting sample size for each species. The present study focuses on prevalence surveys that used polymerase chain reaction (PCR) method, because the prevalence of four species was commonly examined using PCR and the datasets based on other laboratory methods (e.g. parasitemia) were not consistently available across all species.

In Fig. 1, the confidence intervals are wide, because the prevalence surveys rested on small number of samples. Nevertheless, at first sight of the figure, one can observe that crab-eating fox yields the highest prevalence. A quick thought in relation to the reservoir dynamics may be that crab-eating fox plays an important role in maintain-

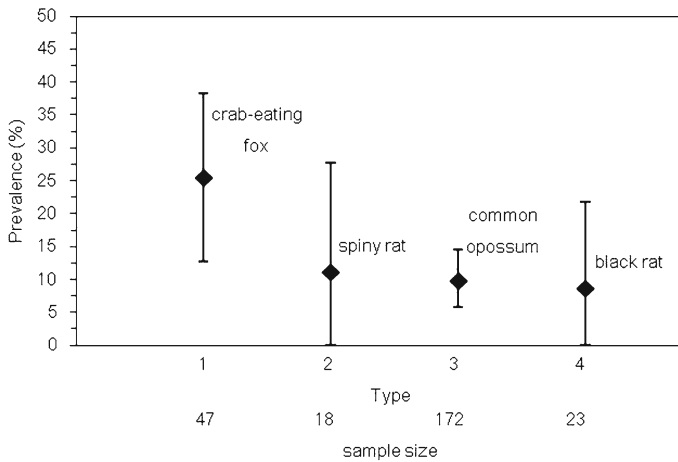


Fig. 1 Observed prevalence data of leishmaniasis by different animal species. From an animal species with the highest prevalence, we label the species in ascending order. The *whiskers* represent confidence intervals derived from binomial distribution

ing transmission of *L. infantum*. However, it is more fruitful to explicitly identify the reservoir host based on objective analysis and thoroughly examine the reservoir potential of other animal species. To achieve an explicit and objective analysis, the present study employs a simple epidemiological model.

From experimental studies, it has been known that these wild animals do not often reveal symptoms and experience frequent re-infections [1, 16, 17]. Such evidence helps us to assume that all individuals never die of the infection and acquire immunity, and thus, to adopt an susceptible-infectious-susceptible (SIS) model [18–24]. Let x_i and y_i be proportions susceptible and infectious of species i , respectively, we have

$$\begin{cases} \frac{dx_i}{dt} = -\lambda_i x_i + \gamma_i y_i + \mu_i y_i, \\ \frac{dy_i}{dt} = \lambda_i x_i - \gamma_i y_i - \mu_i y_i, \end{cases} \quad (3)$$

where γ_i is the recovery rate of species i , μ_i the natural death rate of i , and λ_i is the force of infection of species i described by

$$\lambda_i(\beta_{ij}) = \sum_{j=1}^4 \beta_{ij} y_j, \quad (4)$$

where β_{ij} is the transmission rate from species j to i . The natural death rate is assumed as identical to the natural birth rate, and both x and y equally contribute to the natural birth event. Accordingly, in the first subequation of system (3), the demographic term is $\mu_i y_i$, because $\mu(x_i + y_i) - \mu_i x_i = \mu_i y_i$. The mean infectious period (or recovery rate) and the natural death rate are assumed known and can be extracted from literature. Assumed infectious periods and life expectancies are shown in Table 1 [25–31].

Table 1 Average life-expectancy at birth and mean infectious period of *Leishmania infantum* among four animal species

Type(s)	Species	$1/\mu_i$ Life expectancy at birth (days)	$1/\gamma_i$ Mean infectious period	References
1	Crab-eating fox	1143	105	[25,26]
2	Spiny rat	1278	90	[27,28]
3	Common opossum	1095	24	[29,30]
4	Black rat	548	90	[28,31]

Latin names of types 1–4 are *Cerdocyon thous*, *Trichomys apereoides*, *Didelphis marsupialis* and *Rattus rattus*, respectively

Matrix B_1, B_2, B_3 (assortative mixing)

$$B_i = \begin{pmatrix} \alpha_1\alpha_1(\theta + (1-\theta)n_1) & \alpha_1\alpha_2(1-\theta)n_1 & \alpha_1\alpha_3(1-\theta)n_1 & \alpha_1\alpha_4(1-\theta)n_1 \\ \alpha_2\alpha_1(1-\theta)n_2 & \alpha_2\alpha_2(\theta + (1-\theta)n_2) & \alpha_2\alpha_3(1-\theta)n_2 & \alpha_2\alpha_4(1-\theta)n_2 \\ \alpha_3\alpha_1(1-\theta)n_3 & \alpha_3\alpha_2(1-\theta)n_3 & \alpha_3\alpha_3(\theta + (1-\theta)n_3) & \alpha_3\alpha_4(1-\theta)n_3 \\ \alpha_4\alpha_1(1-\theta)n_4 & \alpha_4\alpha_2(1-\theta)n_4 & \alpha_4\alpha_3(1-\theta)n_4 & \alpha_4\alpha_4(\theta + (1-\theta)n_4) \end{pmatrix} \quad \theta = \begin{cases} 0.9 & (i = 1) \\ 0.7 & (i = 2) \\ 0.5 & (i = 3) \end{cases}$$

Matrix B_4 (WAIFW)

Matrix B_5 (WAIFW)

$$B_4 = \begin{pmatrix} \beta_1 & \beta_2 & \beta_3 & \beta_4 \\ \beta_2 & \beta_2 & \beta_3 & \beta_4 \\ \beta_3 & \beta_3 & \beta_3 & \beta_4 \\ \beta_4 & \beta_4 & \beta_4 & \beta_4 \end{pmatrix} \quad B_5 = \begin{pmatrix} \beta_1 & \beta_4 & \beta_4 & \beta_4 \\ \beta_4 & \beta_2 & \beta_4 & \beta_4 \\ \beta_4 & \beta_4 & \beta_3 & \beta_4 \\ \beta_4 & \beta_4 & \beta_4 & \beta_4 \end{pmatrix}$$

Fig. 2 Five different contact matrixes for leishmaniasis in wild animals. Matrix B_1 assumes that the contact rates between different types of animals are smaller than contacts within the same species. The within-group contact is weighted by θ that can be interpreted as the proportion of contacts that are spent for within-group mixing. B_3 assumes that half of contacts are spent for between-group mixing and B_2 is intended to be between B_1 and B_3 . B_4 assumes that the contact rates of animal types that yielded higher prevalence than others would be more influential than others. B_5 assumes that within-group mixing tends to be much higher than between-group mixing and that the between-group mixing is approximated by the parameter for type 4

Doing so, unknown parameters are only β_{ij} and the estimates would help determine the next generation matrix of the multi-host system for *L. infantum* infection. Nevertheless, while we have 4 data inputs from empirical observation (Fig. 1) and assumes a stationary state [19,21,23], the matrix of β_{ij} is 4×4 and the degree of freedom is insufficient. For this reason, we construct plausible contact matrices using only 4 parameters. Constructing multiple contact matrices, we address the uncertainty with respect to unobserved contact, as was also similarly practiced elsewhere [10,12,33].

Figure 2 shows the assumed five patterns of contact matrices. In principle, it is natural to assume that the transmission rate within the same species is greater than those occurring between species [10–12,32,33]. Matrices B_1 , B_2 and B_3 are referred to as the formulation that rests on assortative mixing assumption. The parameter θ is interpreted as the proportion of contacts spent for within group mixing. For B_1 , B_2 and B_3 , we assume θ at 0.9, 0.7 and 0.5, respectively. n_i stands for the relative population size among all four species. Since we never have an access to such data, $n_i = 0.25$

for any i for mathematical convenience. Matrices B_4 and B_5 qualitatively intends to capture the underlying transmission mechanism by allocating four parameters in sixteen entries [and such matrices are referred to as the matrices that describe who acquire infection from whom (WAIFW)]. Matrix B_4 assumes that the contact rates of animal types that yielded lower prevalence would be influential on others. Matrix B_5 assumes that the contacts between different animal species are very infrequent and non-diagonal elements are compensated by the contact rate of species with the lowest prevalence.

Maximum likelihood method was employed to infer unknown parameters of contact matrix. From the SIS model (4), it is evident that the prevalence at stationary state is given by

$$y_i^* = \frac{\lambda_i(\beta_{ij})}{\lambda_i(\beta_{ij}) + \gamma_i + \mu_i} \quad (5)$$

Let k_i be the sample size of species i among which m_i were positive. The likelihood function to estimate β_{ij} is

$$L(\beta_{ij}; \mathbf{k}, \mathbf{m}) = \prod_{i=1}^4 \binom{k_i}{m_i} \left(\frac{\lambda_i(\beta_{ij})}{\lambda_i(\beta_{ij}) + \gamma_i + \mu_i} \right)^{m_i} \left(\frac{\gamma_i + \mu_i}{\lambda_i(\beta_{ij}) + \gamma_i + \mu_i} \right)^{k_i - m_i} \quad (6)$$

Minimizing the negative logarithm of (6), parameters β_{ij} are estimated, and subsequently, the next generation matrix $\mathbf{K} = \{R_{ij}\}$ is quantified as $R_{ij} = \beta_{ij}/(\gamma_j + \mu_j)$ [11, 34, 35].

Let \mathbf{P}_i be the projection matrix on type i , i.e., $p_{ii} = 1$, and $p_{ij} = 0$ for all other entries. The host-specific reproduction number U_i [10] is given by the dominant eigenvalue of the matrix that includes only the type(s) of interest, i.e.,

$$U_i = \rho(\mathbf{P}_i \mathbf{K}), \quad (7)$$

where $\rho(\cdot)$ denotes the largest eigenvalue. It should be noted that the species i in this context can be either single animal species or a combination of multiple animal species. Whereas Funk et al. [13] discussed the next generation matrix \mathbf{K} by explicitly separating the vector species from others, the present study simplifies the model and considers the R_{ij} as the average number of secondary cases in animal species i caused by a single infected animal in j ‘through sandfly bites’. This simplification was conducted because there aren’t known multiple vector species for leishmaniasis. Let \mathbf{I} be identity matrix. The host-excluded reproduction number Q_i is given by the dominant eigenvalue of the matrix that excludes only the type(s) of interest, i.e.,

$$Q_i = \rho((\mathbf{I} - \mathbf{P}_i) \mathbf{K}). \quad (8)$$

For a species or a combination of species, those satisfying $U_i > 1$ is referred to as the maintenance host as it indicates that the presence of species can allow transmission to be maintained. Similarly, those satisfying $Q_i < 1$ is referred to as the essential host, because the transmission cannot be continued in the absence of that (those) host(s). The minimum set of hosts that satisfy both $U_i > 1$ and $Q_i < 1$ is defined as the

reservoir community [10,13]. If the condition is satisfied by a single species, such host is referred to as the unique reservoir host. Since the sampling distributions of U_i and Q_i are unclear, the 95 percent confidence intervals (CI) were derived from bootstrapping method.

As mentioned above, we assumed that the natural death rate and recovery rate are known and fixed (Table 1). Whereas the natural death rate may not have a large impact on quantitative fate of the reservoir identification exercise, it is fruitful to examine the sensitivity of U_i and Q_i to the recovery rate γ_i . Thus, we varied the recovery rate by multiplying 0.50, 0.75, 1.25 and 1.50 to the baseline value and examined how U_i and Q_i changes. For each set of assigned value, we implemented the maximum likelihood estimation and calculated the dominant eigenvalue of the next generation matrix.

3 Results

Quantifying the next generation matrix, the dominant eigenvalue yields the basic reproduction number R_0 , interpreted as the average number of secondary cases caused by a typical primary case, in the presence of all four species. The maximum likelihood estimates of R_0 for matrices B_1, B_2, B_3, B_4 and B_5 were 1.32, 1.35, 1.43, 1.77 and 1.36, respectively, reflecting that the assumed stationary state is an endemic equilibrium.

Table 2 shows maximum likelihood estimates of the host-specific reproduction number U_i , while Table 3 shows maximum likelihood estimates of the host-excluded reproduction number Q_i . U_i for type 1 (crab-eating fox) was greater than 1 for matrices B_1, B_2, B_3 and B_5 , but not for B_4 . Moreover, even though type 1 was both maintenance and essential hosts using B_3 and B_5 , it was not the case for B_1 and B_2 . Thus, the reservoir potential of type 1 was not consistent across matrices, and moreover, type 1 was not regarded as the reservoir for more than half of the assumed matrices. Type 3 satisfied $U_i > 1$ using B_1 , but otherwise $U_i > 1$ was not observed for any other types of animal on their own.

As for combinations of host, a combination of types 1 and 2 as well as 1 and 4 was regarded as both maintenance and essential hosts using matrices B_2, B_3 and B_5 . Another combination, types 1 and 3 was also shown to be the reservoir community except for matrix B_1 . Thus, only the combination of types 1 and 3 satisfied to be the reservoir community when matrix B_4 was used, presumably due to a mathematical reason that the third column of B_4 is calculated to be too small due to shorter infectious period of common opossum compared with other animal species.

When B_1 (highly assortative contact matrix) was employed, all single type except for types 2 and 4 as well as all combinations of host satisfied $U_i > 1$ due partly to the assumed extent of independence in transmission dynamics from other animal species. Nevertheless, all of them did not satisfy $Q_i < 1$ (Table 3) indicating that the essential host does not exist for the combinations (of up to two animal species) we examined.

Combination of three species can be inspected from Tables 2 and 3 by conversely reading U_i and Q_i for single type of host. Namely, if we would like to know U_i of a host combination of 1, 2 and 3, we should look at Q_i for type 4. It is clear from the table that combinations of types 1, 2 and 3 and types 1, 3 and 4 satisfied both $U_i > 1$ and $Q_i < 1$ for all different types of matrices. Nevertheless, 95 % CI in Tables 2 and 3

Table 2 The host-specific reproduction number U of *Leishmania infantum*

Type(s)	B_1 $\theta = 0.9$	B_2 $\theta = 0.7$	B_3 $\theta = 0.5$	B_4	B_5
1	1.29 (0.63, 2.44)	1.19 (0.56, 2.31)	1.08 (0.48, 2.17)	0.87 (0.08, 2.06)	1.09 (0.21, 2.30)
2	0.99 (0.13, 3.70)	0.74 (0.06, 3.18)	0.52 (0.03, 2.65)	0.08(0.00, 0.94)	0.38 (0.00, 3.32)
3	1.04 (0.60, 1.69)	0.90 (0.49, 1.51)	0.75 (0.38, 1.33)	0.23 (0.13, 0.37)	0.87 (0.15, 1.59)
4	0.93 (0.12, 3.42)	0.64 (0.05, 2.80)	0.41 (0.02, 2.20)	0.17 (0.03, 0.59)	0.17 (0.03, 0.59)
1 and 2	1.30 (0.63, 3.70)	1.21 (0.56, 3.26)	1.12 (0.48, 2.97)	0.88 (0.08, 2.72)	1.15 (0.21, 3.70)
1 and 3	1.31 (0.65, 2.46)	1.29 (0.63, 2.45)	1.32 (0.61, 2.55)	1.53 (0.62, 2.95)	1.22 (0.22, 2.76)
1 and 4	1.30 (0.63, 3.43)	1.21 (0.56, 2.92)	1.11 (0.48, 2.70)	0.93 (0.10, 2.37)	1.14 (0.21, 2.57)
2 and 3	1.07 (0.60, 3.71)	0.99 (0.49, 3.27)	0.90 (0.38, 3.75)	0.96 (0.52, 2.00)	0.93 (0.15, 3.53)
2 and 4	1.00 (0.13, 3.73)	0.77 (0.07, 3.35)	0.57 (0.03, 2.97)	0.32 (0.05, 1.43)	0.49 (0.05, 3.46)
3 and 4	1.04 (0.60, 3.42)	0.91 (0.49, 2.81)	0.76 (0.38, 2.24)	0.26 (0.13, 0.68)	0.87 (0.17, 1.62)

Types 1–4 are *Cerdocyon thous*, *Trichomys apereoides*, *Didelphis marsupialis* and *Rattus rattus*, respectively. If $U > 1$, the corresponding estimate is given in bold letters. Numbers in parentheses represent the 95 % confidence intervals

Table 3 The host-excluded reproduction number Q of *Leishmania infantum*

Type(s)	B_1 $\theta = 0.9$	B_2 $\theta = 0.7$	B_3 $\theta = 0.5$	B_4	B_5
1	1.08 (0.60, 3.74)	1.00 (0.49, 3.43)	0.91 (0.38, 3.23)	0.99 (0.52, 2.23)	0.95 (0.18, 3.67)
2	1.31 (0.65, 3.42)	1.31 (0.63, 2.97)	1.34 (0.61, 2.92)	1.56 (0.62, 3.15)	1.26 (0.23, 2.97)
3	1.30 (0.63, 3.74)	1.23 (0.56, 3.47)	1.16 (0.48, 3.38)	0.96 (0.10, 3.09)	1.22 (0.22, 3.95)
4	1.31 (0.65, 3.71)	1.34 (0.63, 3.39)	1.40 (0.61, 3.42)	1.73 (0.78, 3.84)	1.31 (0.23, 4.04)
1 and 2	1.04 (0.60, 3.42)	0.91 (0.49, 2.81)	0.76 (0.38, 2.24)	0.26 (0.13, 0.68)	0.87 (0.17, 1.62)
1 and 3	1.00 (0.13, 3.73)	0.77 (0.07, 3.35)	0.57 (0.03, 2.97)	0.32 (0.05, 1.43)	0.49 (0.05, 3.46)
1 and 4	1.07 (0.60, 3.71)	0.99 (0.49, 3.27)	0.90 (0.38, 3.75)	0.96 (0.52, 2.00)	0.93 (0.15, 3.53)
2 and 3	1.30 (0.63, 3.43)	1.21 (0.56, 2.92)	1.11 (0.48, 2.70)	0.93 (0.10, 2.37)	1.14 (0.21, 2.57)
2 and 4	1.31 (0.65, 2.46)	1.29 (0.63, 2.45)	1.32 (0.61, 2.55)	1.53 (0.62, 2.95)	1.22 (0.22, 2.76)
3 and 4	1.30 (0.63, 3.70)	1.21 (0.56, 3.26)	1.12 (0.48, 2.97)	0.88 (0.08, 2.72)	1.15 (0.21, 3.70)

If $Q < 1$, the corresponding estimate is given in bold letters. Numbers in parentheses represent the 95 % confidence intervals

were very wide, ranging from below to above the value of 1, not allowing us to fully judge the reservoir potential.

Results from sensitivity analysis are shown in Fig. 3. Except for type 1 (Fig. 3a), the results of $U_i > 1$ or $U_i < 1$ did not greatly vary by substantially varying the infectious period γ_i plus and minus 50 % of its original value. Similarly, except for type 1 (Fig. 3b), we did not observe any host with $Q_i < 1$ by varying infectious period. Different reservoir dynamics were observed for type 1 mainly for assortative mixing, especially using B_1 , perhaps because of high sensitivity of U_i and Q_i to diagonal element of the next generation matrix, and thus, to the infectious period of

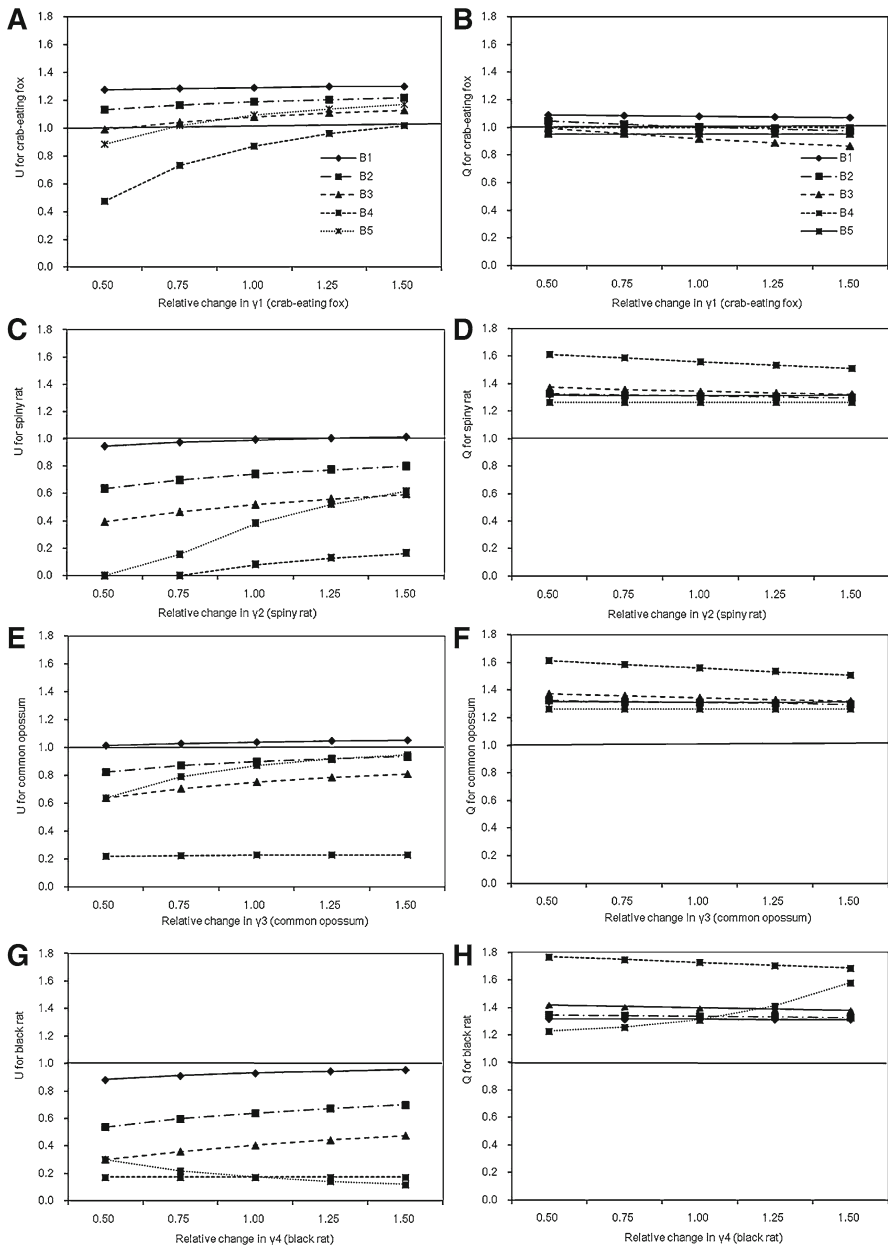


Fig. 3 Sensitivity of the reservoir potential to infectious period of animals. The *vertical axes* represent the reproduction numbers that allow us to assess the reservoir potential, while *horizontal axes* represent relative change in the rate of recovery for each animal species. Panels **a**, **c**, **e** and **g** examines the sensitivity of the host-specific reproduction number, while **b**, **d**, **f** and **h** show the results of the host-excluded reproduction number. In each panel, the *horizontal solid line* represents the threshold value at which the reproduction number takes the value 1

the corresponding host type. Except for highly assortative matrices, the identification of the reservoir was not sensitively influenced by the mean infectious period.

4 Discussion

The present study examined the reservoir potential of four animal species, i.e., crab-eating fox, spiny rat, common opossum and black rat, in allowing persistence of *L. infantum* transmission. While crab-eating fox yielded the highest prevalence among four species, it was not consistently regarded as the unique reservoir host across different contact matrices. All other types did not satisfy $U_i > 1$ consistently. As for the combination of two types, i.e., types 1 and 2, 1 and 3 and 1 and 4, both $U_i > 1$ and $Q_i < 1$ were satisfied for a part of assumed matrices, and as for the combination of three types, types 1, 2 and 3 and types 1, 3 and 4 satisfied both $U_i > 1$ and $Q_i < 1$ for all matrices. However, there has been no strong biological or ecological indication that type 1 (crab-eating fox) has some unique interaction with other animal species, especially in maintaining transmission of *L. infantum*. Moreover, $Q_i > 1$ was consistently the case using B_1 which assumes that 90 % of contacts are spent for within-group mixing. Since $Q_i > 1$ indicates that the remaining types of host could maintain transmission, the combinatory dynamics involving type 1 is not regarded as essential. All these results yield new insights into the reservoir dynamics of *Leishmania* spp.: previously, only canine was thought to act as the reservoir host of leishmaniasis as has been seen with *L. donovani* [1–5]. The present study is the first to explicitly indicate that animal species other than canine are likely to play important roles in maintaining transmission of *L. infantum*. Monitoring infections in animal species other than canine is deemed important to prevent human infection.

Matrix B_1 adopts highly assortative mixing, and one could imagine that this matrix might accurately reflect the most realistic situation of multi-host transmission dynamics of *L. infantum* in wild life. It depends on the detailed behavior of sandflies (*Phlebotomus* spp.), but it is likely that different types of animals do not frequently interact from each other through common sandflies. As our analysis revealed, either single species or combination of two or more hosts allow transmission to be maintained. However, they are not regarded as essential. Strictly adhering to our definition of the reservoir host, it is likely that all types 1–4 act as the reservoir community as a single group. $R_0 > 1$ is in line with $U_i > 1$ for four animals in combination, and the possible absence of transmission without four species is consistent with $Q_i < 1$.

It should be noted that all uncertainty bounds (i.e. 95 % CI) in Tables 2 and 3 were very wide, ranging from below to above the value of 1. These did not enable us to more precisely interpret our findings, and it does indicate that the sample size should be increased in future studies.

If we have an opportunity to further examine the reservoir dynamics of *Leishmania* spp., there are number of points that could considerably improve our understanding and should be kept in our mind. First, an important source of uncertainty was seen in the assumed contact matrices that capture within- and between-host mixing through sandflies. One could survey the host preference of *Phlebotomus* spp. by exploring the biting behavior (e.g. how many are biting crab-eating fox and how many for others)

and investigating their host specificities (e.g. how often inter-species transmission could occur by sharing an identical sandfly). It is anticipated that the share rate of sandfly is high within the same host type and low between different types, but we have yet to understand the plausible quantitative value. Second, not necessarily directly surveying these characteristics through entomologic investigations, but also one could examine genetic or molecular characteristics of *Leishmania* spp. For instance, one could implement phylogenetic analysis to accurately capture the route of transmission and evolution, thereby permitting us to track the inter-specific transmission in an explicit manner [18–24, 36–38]. Such analysis could also shed light on our assumption of SIS model (e.g. if there is immune reaction through frequent re-infections and if there is an indication of evolution of the pathogen) and analysis of genetic data does not force us to adopt a stationary state assumption [18–24].

Enumerating limitations, we have noted that the following points are regarded as the weakness of the present study: (1) limited sample size, (2) unknown mixing pattern, (3) SIS-type assumption and (4) stationary state assumptions. In addition, it is unlikely, but there is a possibility that some important animals have not been covered by empirical studies and we have missed their contribution to the transmission dynamics. Thus, not only the number of samples and genetic data, but an ecological survey with broader research perspectives would be required in the future.

Despite these limitations, we stress out that our study has shown that animal species other than canine play important role in maintaining the transmission of *L. infantum*, which is different from the conventional wisdom that tended to focus on canine only. Future research studies should be conducted to clarify the extent of inter-specific interactions. Developing an indirect estimation method of such mixing pattern is one of our ongoing studies.

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References

1. Quinell, R.J., Courtenay, O.: Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* **136**, 1915–1934 (2009)
2. Ready, P.D.: Epidemiology of visceral leishmania. *J. Clin. Epidemiol.* **6**, 147–154 (2014)
3. Costa, A.P., Ferreira, S.J.I.G., Fournier, R.S., Lopes, M.G., Ramirez, D., Acosta, C.L., Lima, J.T.R., Labruna, M.B., Gennari, S.M., Marcili, A.: Survey of *Leishmania infantum chagasi* in wild and domestic animals in urban area and Atlantic rainforest fragment in Northeast, Brazil. *J. Biodivers. Bioprospect. Dev.* **2**, 1–5 (2014)
4. Fisa, R., Gallego, M., Castillejo, S., Aisa, M.J., Serra, T., Riera, C., Carrio, J., Gallego, J., Portus, M.: Epidemiology of canine leishmaniosis in Catalonia (Spain). The example of the Priorat focus. *Vet. Parasitol.* **83**, 87–97 (1999)

5. Jimenez, M., Gonzalez, E., Martin-Martin, I., Hernandez, S., Molina, R.: Could wild rabbits (*Oryctolagus cuniculus*) be reservoirs for *Leishmania infantum* in the focus of Madrid, Spain? *Vet. Parasitol.* **202**, 296–300 (2014)
6. Morales, M.A., Cruz, I., Rubio, J.M., Chicharro, C., Canavate, C., Laguna, F., Alvar, J.: Relapses versus reinfections in patients coinfectd with *Leishmania infantum* and human immunodeficiency virus type 1. *J. Infect. Dis.* **185**, 1533–1537 (2002)
7. Molina, R., Jimenez, M.I., Cruz, I., Iriso, A., Martin-Martin, I., Sevillano, O., Melero, S., Bernal, J.: The hare (*Lepus granatensis*) as potential sylvatic reservoir of *Leishmania infantum* in Spain. *Vet. Parasitol.* **190**, 268–271 (2012)
8. Rio, L., Chitimia, L., Victoriano, I., Rua, P., Gerrikagoitia, X., Barral, M., Munoz-Garcia, C.I., Goyena, E., Garcia-Martinez, D., Fisa, R., Riera, C., Murcia, L., Segovia, M., Berriatua, E.: Evidence for widespread *Leishmania infantum* infection among wild carnivores in *L. infantum* periendemic northern Spain. *Prev. Vet. Med.* **113**, 430–435 (2014)
9. Talmi-Frank, D., Jaffe, C.L., Nasereddin, A., Baneth, G.: *Leishmania tropica* experimental infection in the rat using luciferase-transfected parasites. *Vet. Parasitol.* **187**, 57–62 (2012)
10. Nishiura, H., Hoyer, B., Klaassen, M., Bauer, S., Heesterbeek, H.: How to find natural reservoir hosts from endemic prevalence in multi-host population. A case study of influenza in waterfowl. *Epidemics* **1**, 118–128 (2009)
11. Diekmann, O., Heesterbeek, J.A., Roberts, M.G.: The construction of next-generation matrices for compartmental epidemic models. *J. R. Soc. Interface* **7**, 873–885 (2010)
12. Nishiura, H., Cook, A.R., Cowling, B.J.: Assortativity and the probability of epidemic extinction: a case study of pandemic influenza A (H1N1-2009). *Interdiscip. Perspect. Infect. Dis.* **2011**, 194507 (2011)
13. Funk, S., Nishiura, H., Heesterbeek, H., Edmunds, W.J., Checchi, F.: Identifying transmission cycles at the human–animal interface: the role of animal reservoirs in maintaining gambiense Human African Trypanosomiasis. *PLoS Comput. Biol.* **9**, e1002855 (2013)
14. Viana, M., Mancy, R., Biek, R., Cleaveland, S., Cross, P.C., Lloyd-Smith, J.M., Haydon, D.T.: Assembling evidence for identifying reservoirs of infection. *Trends Ecol. Evol.* **29**, 270–279 (2014)
15. Herrera, L., Xavier, S.C., Viegas, C., Martinez, C., Cotias, P.M., Carrasco, H., Urdaneta-Morales, S., Jansen, A.M.: Trypanosoma cruzi in a caviomorph rodent: parasitological and pathological features of the experimental infection of *Trichomys apereoides* (Rodentia, Echimyidae). *Exp. Parasitol.* **107**, 78–88 (2004)
16. Laurenti, M.C., Rossi, C.N., Matta, V.L.R., Tomokane, T.Y., Corbett, C.E.P., Secundino, N.F.C., Pimenta, P.F.P., Marcondes, M.: Asymptomatic dogs are highly competent to transmit *Leishmania (Leishmania) infantum chagasi* to the natural vector. *Vet. Parasitol.* **196**, 296–300 (2013)
17. Borja-Cabrera, G.P., Santos, F.N., Santos, F.B., Trivellato, F.A., Kawasaki, J.K., Costa, A.C., Castro, T., Nogueira, F.S., Moreira, M.A.B., Luvizotto, M.C.R., Palatnik, M., Sousa, C.B.P.: Immunotherapy with the saponin enriched-Leishmune^R vaccine versus immunochemotherapy in dogs with natural canine visceral leishmaniasis. *Vaccine* **28**, 597–603 (2010)
18. Guo, H., Li, Y.M., Shuai, Z.: Global stability of the endemic equilibrium of multigroup SIR epidemic models. *Can. Appl. Math. Q.* **14**, 259–284 (2006)
19. Rodrigues, P., Gomes, M.G.M., Rebelo, C.: Drug resistance in tuberculosis—a reinfection model. *Theor. Popul. Biol.* **71**, 196–212 (2007)
20. Rodrigues, P., Margheri, A., Rebelo, C., Gomes, M.G.M.: Heterogeneity in susceptibility to infection can explain high reinfection rates. *J. Theor. Biol.* **259**, 280–290 (2009)
21. Gomes, M.G.M., White, L.J., Medley, G.F.: Infection, reinfection, and vaccination under suboptimal immune protection: epidemiological perspectives. *J. Theor. Biol.* **228**, 539–549 (2004)
22. Gomes, M.G.M., Franco, A.O., Gomes, M.C., Medley, G.F.: The reinfection threshold promotes variability in tuberculosis epidemiology and vaccine efficacy. *Proc. R. Soc. Lond. Ser. B* **271**, 617–623 (2004)
23. Gokaydin, D., Oliveira-Martins, J.B., Gordo, I., Gomes, M.G.M.: The reinfection threshold regulates pathogen diversity: the case of influenza. *J. R. Soc. Interface* **4**, 137–142 (2007)
24. Gomes, M.G.M., White, L.J., Medley, G.F.: The reinfection threshold. *J. Theor. Biol.* **236**, 111–113 (2005)
25. Courtenay, O., Quinell, R.J., Garcez, L.M.: Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission. *Parasitology* **125**, 407–414 (2002)

26. Courtenay, O., Quinell, R.J., Garcez, L.M., Shaw, J.J., Dye, C.: 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 (2002)
27. Oliveira, F.S., Pirmez, C., Pires, M.Q., Brazil, R.P., Pacheco, R.P.: PCR-based diagnosis for detection of *Leishmania* in skin and blood of rodents from an endemic area of cutaneous and visceral leishmaniasis in Brazil. *Vet. Parasitol.* **129**, 219–227 (2005)
28. Alderton, D.: *Rodents of the World*. Blandford Press, New York (1996)
29. Grabiec, M., Turlejski, K., Djavadian, R.L.: The partial 5-HT_{1A} receptor agonist buspirone enhances neurogenesis in the opossum (*Monodelphis domestica*). *Eur. Neuropsychopharmacol.* **19**, 431–439 (2009)
30. White, M.R., Chapman, W.L., Hanson, W.L., Greene, C.E.: Experimental visceral leishmaniasis in the opossum. *Vet. Pathol.* **26**, 314–321 (1989)
31. Svobodova, M., Votycka, J., Nicolas, L., Volf, P.: *Leishmania tropica* in the black rat (*Rattus rattus*): persistence and transmission from asymptomatic host to sand fly vector *Phlebotomus sergenti*. *Microbes Infect.* **5**, 361–364 (2003)
32. Ejima, K., Aihara, K., Nishiura, H.: On the use of chance-adjusted agreement statistic to measure the assortative transmission of infectious diseases. *Comput. Appl. Math.* **32**, 303–313 (2013)
33. Edmunds, W.J., O'callaghan, C.J., Nokes, D.J.: Who mixes with whom? A method to determine the contact patterns of adults that may lead to the spread of airborne infections. *Proc. R. Soc. Lond. Ser. B* **264**, 949–957 (1997)
34. Leung, K.Y., Kretzschmar, M., Diekmann, O.: SI infection on a dynamic partnership network: characterization of R₀. *J. Math. Biol.* **71**, 1–56 (2014)
35. Farrington, C.P., Whitaker, H.J.: Estimation of effective reproduction numbers for infectious diseases using serological survey data. *Biostatistics* **4**, 621–632 (2003)
36. Lembo, T., Hampson, K., Haydon, D.T., Craft, M., Dobson, A., Dushoff, J., Ernest, E., Hoare, R., Kaare, M., Mlengeya, T., Mentzel, C., Cleaveland, S.: Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. *J. Appl. Ecol.* **45**, 1246–1257 (2008)
37. Vanderburg, S., Rubach, M.P., Halliday, J.E., Cleaveland, S., Reddy, E.A., Crump, J.A.: Epidemiology of *Coxiella burnetii* infection in Africa: a OneHealth systematic review. *PLoS Neglected Trop. Dis.* **8**, e2787 (2014)
38. Haydon, D.T., Cleaveland, S., Taylor, L.H., Laurenson, M.K.: Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg. Infect. Dis.* **8**, 1468–1473 (2002)