Sources and sinks: revisiting the criteria for identifying reservoirs for American cutaneous leishmaniasis

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Molecular-based approaches might be misleading for assessing the ‘potential importance’ of possible reservoirs for parasites unless used in conjunction with population dynamics and ecological studies that provide an understanding of the linkages between the enzootic transmission cycle (in wild animals) and that in domestic animals. Better understanding of ecological constraints on Leishmania transmission is needed to assess fully the passage of the parasite between its sandfly vector and different mammalian host species. We use a straightforward mathematical framework to illustrate that misuse of association patterns, as guidance for implementation of control measures, can in fact increase the endemism of leishmaniasis.

Sources and sinks in population ecology

In one of the most cited ecological papers ever, Pulliam [1] suggested that populations can be regulated in a patchy environment only if the subdivided population [the metapopulation (see Glossary)] exhibits ‘source–sink dynamics’. This occurs when some patches of habitat (sources) can sustain exponential growth, whereas others (sinks) do not provide the necessary conditions for (positive) population growth. Regulation of total population size occurs when the metapopulation reaches a stable size (defined as a stable population [the metapopulation] exhibiting ‘source–sink dynamics’; more specifically, we need to recognize that reservoirs are those species that sustain exponential growth, whereas sinks do not generate new infections by the vector. By contrast, incidental hosts lack such a dynamic feedback and should be considered as sinks for infection, therefore they cannot transmit the disease.

In Box 1, we illustrate how recent mathematical models can provide insights into the dynamic nature of a reservoir, and in Box 2, we show how results can be summarized using the concept of the basic reproductive number, \( R_0 \). In both cases, we use parameters generated using data from Las...
Rosas, Venezuela [7,8], where an outbreak of leishmaniasis in the mid-1970s was related to the movement of infected donkeys from an endemic area. An exhaustive examination of mammalian wildlife species from the region revealed no infection among all examined taxa that are commonly suggested as reservoirs for *Leishmania* parasites. Further studies in the region have supported the view that equines act as reservoirs for ACL [9,10] and provided evidence against dogs, the other host significantly infected at Las Rosas, acting as reservoirs [11].

A consensus in the recent ACL literature is that in order to understand the dynamics of transmission, we need to

**Box 1. Modeling American cutaneous leishmaniasis and the possible outcomes for zooprophylaxis**

Using the framework of source–sink dynamics, Chaves and Hernández [12] showed that the ability of this disease to become endemic depends on whether the disease persists (endemically) in its reservoir host (i.e. in the source). This result implies that only reservoir control would have an effect on the endemcity of the disease and is illustrated via simulations of a model. The model consists of five differential equations in which the number of infected organisms [donkeys (*R*, Equation I), vectors (*V*, Equation II), humans (*H*, Equation III) and dogs (*P*, Equation IV)] is tracked, and the density of sandflies (*SF*, Equation V) varies seasonally using the sinusoidal function (*E*(t), Equation VI):

\[
\begin{align*}
\frac{dR}{dt} &= \beta_{R}R(V - R) - \gamma_{R}R \\
\frac{dV}{dt} &= \beta_{V}R(SF(t) - V) - \mu_{V}V \\
\frac{dH}{dt} &= \beta_{H}V(A - H) - \gamma_{H}H \\
\frac{dP}{dt} &= \beta_{P}V(D - P) - \gamma_{P}P \\
\frac{dSF}{dt} &= E(t) - SF
\end{align*}
\]

\[E(t) = a + b \sin(2\pi t)\]  

The parameter \(\beta_i\) represents the rate of infectious contacts between vectors and individuals of species \(i\), \(\gamma_i\) is the recovery rate for individuals of species \(i\), and \(\mu\) is the mortality rate of infected sandflies. \(B, D\) and \(A\) represent the total numbers of individuals in each population (donkeys, dogs and humans, respectively), which are assumed to be constant in the model when no host culling is implemented. In this model, only donkeys are reservoirs (they have a transmission feedback with sandfly vectors) whereas humans and dogs are incidental hosts. (For other general assumptions of the model see Ref. [12].) Figure I shows the results of simulations with parameters based on data from Las Rosas, Venezuela [7]; as explained in detail in Ref. [8]. If we assume that values for \(\beta_i\) are an inverse function of the total number of hosts (irrespective of whether these are infected or uninfected reservoirs, or incidental), we can show that:

\[
\beta_i \propto \frac{1}{A + B + D}
\]

Equation 7 implies that zooprophylactic control measures are likely to increase the rate of contact between sandfly vectors and vertebrate hosts. As illustrated in Figure I Figure I, if the culled host is the reservoir, the result could be the eventual eradication of ACL. However, culling an incidental host could lead to increased *Leishmania* transmission to all the remaining hosts, thus causing an increased prevalence of the disease. This result indicates that the presence of incidental hosts could have a similar effect to that of increased biodiversity, the so-called ‘dilution-effect’ [45], with more host species making transmission to any one of them less likely. Examples illustrating the effect of host species removal have been documented for two different vector-borne diseases: (i) human outbreaks of sleeping sickness increased after cattle were killed by rinderpest [46]; and (ii) West Nile virus increased in humans when American robins migrated south [47].

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Figure I. Prevalence dynamics for American cutaneous leishmaniasis in a system with two incidental hosts, humans (dot-dashed) and dogs (dashed); a reservoir, the donkeys (dotted); and a sandfly vector (unbroken). (a) Dynamics based on parameters from Las Rosas, Venezuela. (b) Dynamics resulting from culling the reservoir (i.e. donkeys). (c) Dynamics resulting from culling an incidental host (i.e. dogs). Data modified from Chaves et al. [8].
Box 2. Reproduction number and the dilution effect in vector/multi-host systems

Consider the case for a vector-transmitted pathogen (e.g., ACL, West Nile virus or Lyme disease) with two host species that occur at abundance C and B. Each of these hosts is bitten at a rate determined by the background rate of vector biting \( b \) and its relative abundance in the community (e.g. for C, relative abundance = \( C/(C + B) \); note that, if each individual of species C is \( n \) times more attractive than an individual of species B, then the relative abundance that determines the proportion of bites received by C is \( nC/(nC + B) \)). We also assume that each host species has a relative efficiency as a host for the pathogen, which is determined by \( 1/(a + d + \delta) \), where \( a \) is the pathogen induced host mortality, \( d \) is the background host mortality rate and \( \delta \) is the rate at which the host recovers from infection. We designate this aggregate parameter \( c \) for species C and \( b \) for species B. When this figure has a small value, the host does not sustain infection with the pathogen for a long period of time and either dies or quickly recovers. We also assume a vector population density of \( M \) and that vectors live for a period of \( 1/\mu \) years.

We can then write a simple matrix expression (Equation I) for ‘next generation’ infections whenever an infected host is introduced into the population:

\[
\begin{pmatrix}
0 & \frac{Mb}{\mu(B + nC)} & \frac{MnC}{\mu(B + nC)} \\
\frac{bB}{B + nC} & 0 & 0 \\
\frac{cnC}{B + nC} & 0 & 0
\end{pmatrix}
\]

The basic reproductive number \( (R_0) \) for the pathogen is then given by the dominant eigenvalue of this matrix (Equation II) [12,28]:

\[
R_0 = \frac{1}{(B + nC)} \sqrt{\frac{Mb}{\mu} (bB^2 + cnC^2)}
\]

This can be shown to generalize for \( n \)-species of hosts to Equation III:

\[
R_0 = \frac{1}{\sum_{i=1}^{n} a_i A_i} \sqrt{\frac{Mb}{\mu} \left( \sum_{i=1}^{n} \left( \frac{a_i A_i}{v_i} \right)^2 \right)}
\]

Here, \( a_i \) is the relative attraction of species \( i \) to vectors, \( A_i \) is the abundance of species \( i \) and \( v_i \) is its ‘viability’ as a host \((1/(a_i + d_i + \delta_i))\). This expression can be used to examine the factors that contribute to the dilution effect. Cutaneous leishmaniasis provides a special case of this because a host might be bitten but not pass on the infection: its abundance and relative attraction to vectors will thus enter into the denominator but not the numerator, thus providing a more powerful dilution effect. This difference in the dilution effect is shown in Figure I. In all cases, when there is only one reservoir an increased biodiversity diminishes \( R_0 \). By contrast, when all species are reservoirs, \( R_0 \) can either diminish or increase.

![Figure I](image_url)

Figure I. Dilution effect as a function of the addition of species. Added species (compared with donkeys [8]) are 50% less viable and 50% less abundant (a); 50% more viable and 50% less abundant (b); 50% more viable and 50% more abundant (c); 50% more viable and 50% less abundant (d). Unbroken lines indicate the case where all species are reservoirs (sources), broken lines when only one species is a reservoir and the rest incidental hosts (sinks). When \( R_0 \) is below 1 (dotted line), the disease does not become established [28].
determine which species are the reservoirs for the Leishmania parasites [11–16], and that molecular techniques can provide definitive insights into this problem [17]. We posit that these insights are misleading unless used in conjunction with an ecological understanding of the dynamics of the different populations involved. In the following section, we review the criteria currently used by parasitologists.

Revisiting ‘reservoir criteria’ from an ecological perspective

Silva et al. [16] present five criteria that enable reservoirs of the Leishmania species causing ACL to be identified. These criteria can be misleading if used alone (see below) because they include conditions that apply equally well to incidental and reservoir hosts.

1) Overlap between the geographical and temporal distribution of vector and hosts

This occurs when species co-occur in time and space, without there necessarily being any biological interaction between them [18]. If surveys are conducted without comprehensive sampling of all potential host species, artificial patterns of association between incidental host species might appear, thus leading to the misclassification of incidental host species as reservoir species. Models for species co-occurrence are used in ecology to test the null hypothesis that such patterns are random (see, e.g., Ref. [19]).

2) Presence of the same parasite in ‘reservoirs’ and humans

Several incidental host species can have the same parasite as a result of being infected from the same source. The usefulness of this criterion is constrained by the need to test for transmission potential and non-random patterns of co-infection.

3) The maintenance of parasites in skin lesions and blood at densities high enough to infect vectors

This can also be misleading because transmission might not occur owing to social or behavioral constraints [6]. For example, either the peak in biting activity of sandflies might not correspond with the time when they contact a host species or, in the case of humans, skin lesions can be covered and thus block transmission. Thus, it is the dynamic feedback in transmission between the co-occurring host and vector species, rather than the presence of Leishmania parasites, that ultimately makes transmission possible. This needs to be demonstrated empirically.

4) Infection prevalence higher than 20%

Because ACL is a vector-transmitted disease and vector abundance changes seasonally [20–24], we can expect prevalence of Leishmania spp. infections to vary seasonally [13], above and below the threshold value of 20% (Box 3). In fact, the unquestioning use of prevalence in different hosts can be misleading when establishing risk factors. Prevalence values are also affected by measurement errors, which might vary as a function of monitoring efficacy of susceptible and infected individuals for each host species. This heterogeneity in measuring the true abundance of a host can lead to false correlations that obscure the real relationship between reservoirs and incidental hosts.

5) Survival of the host for long enough to guarantee parasite transmission

This is an important prerequisite, because a host cannot be a reservoir if it does not survive long enough to develop a sufficient pathogen load to infect vectors. Ultimately, this is the most important limiting factor for insect vectors to transmit a pathogen [25,26].

A related factor, which is not usually considered but which is equally important in determining the long-term persistence of a pathogen, is the average duration of the infection in a host [27]. Unless there are major differences in the attraction to susceptible vectors, an infectious host species with a longer average duration of infection is a more important reservoir species than a host species with a shorter infection period, because it produces more infections on average, given the existence of a dynamic feedback. For diseases with frequency-dependent transmission, those with a constant force of infection (rate of infection per susceptible host), a difference in infection period can be significant because the species with the longest infection times are those most likely to support the pathogen indefinitely. However, for diseases with density-dependent transmission, those where the force of infection is proportional to the density of a host species, host densities and the duration of infection could exhibit a trade-off. For example, the importance of two less-abundant species can be equivalent to a single high-density species, because population abundance partially compensates for low duration of infection [28–32].

The transmission of Leishmania parasites responsible for ACL is probably located midway along the spectrum of possibilities between frequency- and density-dependent transmission. Our inability to quantify this exactly underscores the need for a better understanding of the ecology of transmission – this cannot be achieved by simple examination of the host or parasite DNA [16,33,34].

All of the points above indicate that a formal definition of the importance of a host species as a reservoir should be approached from an ecological or population dynamic perspective. It needs to be recognized that initial, often arbitrary, divisions of primary and secondary reservoirs might vary from place to place (and at different times of the year) because of the dynamics of transmission, and that such roles (primary or secondary reservoirs) are the same as long as species are sources for infection. Thus, it is essential to develop criteria that explicitly and experimentally (when ethical) demonstrate the existence of the dynamic feedbacks necessary for transmission, in terms of the ability of one vertebrate host to transmit to another using an insect vector (see, e.g., Refs [35,36]).

The dynamics of multi-host pathogens can be extremely complex, especially if all of the interspecific interactions between species lead to significant changes in their relative abundances [28]. Complex dynamics are often nonlinear, which usually leads to paradoxical results when data are
Box 3. Spurious patterns of association in multi-host systems

Epidemiological studies of ACL are generally cross-sectional, i.e. they show results over limited periods of time [48,49]. To reproduce a survey of correlations between prevalence in several host species, we considered the model simulations from Las Rosas, Venezuela (see Figure I of Box 1) and randomly sampled 1000 time points from the dynamics. Interestingly, the highest correlation was between humans and dogs, the incidental hosts. This is true, even though samples are from a deterministic system. When white (Gaussian) noise is added linearly to such signals to introduce the problem of measurement error [50], the picture becomes even less clear. In the case of a zoonosis, such as leishmaniasis, measurement error might be a result of different efficiencies for sampling species associated with trap size, baits, etc. We see that, as measurement error increases, the correlation between cases in any two species becomes more variable, and the average strength of the association decreases, producing, in some cases, low correlations between the reservoirs and the incidental hosts (Figure I). According to criteria based exclusively on these correlations, these patterns might underestimate the importance of a species as a reservoir, because its prevalence is poorly associated with that of other species that are mere incidental hosts, and even worse, lead to the wrong identification of reservoir host species.

![Figure I](image_url)

**Figure I.** Prevalence correlation. (a) Shows values obtained from simulations with the deterministic system; (b,c,d) include measurement error, added to the output of the simulations by using identically and independently distributed Gaussian noise with a variance that produced a 10%, 25% and 50% coefficient of variation, respectively, in the measurements for dogs (D) and donkeys (R). Precise values from the deterministic system were used for humans (H) and vectors (V).

interpreted in a static way. As illustrated in Box 3, static interpretations can lead to control policies with disastrous outcomes (i.e. increasing the incidence of human infections), particularly when the focus of control is on a single species. Ultimately, the control of vector-transmitted diseases of humans and their domestic livestock will require bringing together field biologists with deep experience of the natural history of the hosts and parasite, geneticists who understand the risk of drug resistance [37], theoretical ecologists, and those who use new molecular tools. A successful example of this multi-disciplinary approach is being undertaken for Chagas’ disease in northwestern rural Argentina, where mathematical modeling [38], extensive studies on the ecology of vectors [39], studies on socio-economic conditions of human hosts [40], longitudinal studies on reservoirs [41], and studies on infection of vectors [42], have been carried out within a wide conceptual framework, relying on DNA tools when more conventional approaches are not sensitive or informative enough. However, more funding is needed to promote these multi-disciplinary initiatives – they are the only viable route to long-term sustained control and management.
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