

ARTICLE **OPEN**

Health-seeking behaviour, diagnostics and transmission dynamics in the control of visceral leishmaniasis in the Indian subcontinent

Graham F. Medley¹, T. Déirdre Hollingsworth^{2,3}, Piero L. Olliaro^{4,5} & Emily R. Adams^{2,6}

Countries in the Indian subcontinent have committed to reducing the incidence of kala-azar, a clinical manifestation of visceral leishmaniasis, to below 1 in 10,000 by 2020. We address the role of timing of use and accuracy of diagnostics in kala-azar control and elimination. We use empirical data on health-seeking behaviour and health-system performance from the Indian state of Bihar, Bangladesh and Nepal to parameterize a mathematical model. Diagnosis of cases is key to case management, control and surveillance. Treatment of cases prevents onward transmission, and we show that the differences in time to diagnosis in these three settings explain the observed differences in incidence. Shortening the time from health-care seeking to diagnosis is likely to lead to dramatic reductions in incidence in Bihar, bringing the incidence down to the levels seen in Bangladesh and Nepal. The results emphasize the importance of maintaining population and health-system awareness, particularly as transmission and disease incidence decline. We explore the possibility of diagnosing patients before the onset of clinical kala-azar (before 14 days fever), and show that this could have a marked impact on incidence, even for a moderately sensitive test. However, limited specificity (that results in false positives) is a major barrier to such a strategy. Diagnostic tests of high specificity used at an early stage of active infection, even if sensitivity is only moderate, could have a key role in the control of kala-azar, and prevent its resurgence when paired with the passive health-care system and tests of high sensitivity, such as the test for rK39 antibody response.

Nature 528, S102–S108 (3 December 2015), DOI: 10.1038/nature16042

This article has not been written or reviewed by *Nature* editors. *Nature* accepts no responsibility for the accuracy of the information provided.

The protozoan parasite *Leishmania* is transmitted by the bite of an infected sand fly. It disproportionately affects the poorest communities in endemic countries, and has an associated global mortality of 200,000–400,000 per year¹. An elimination campaign has been running in the Indian subcontinent (India, Nepal, Bangladesh, Bhutan and Thailand) since 2005. Three elimination time frames currently exist; the first, ending in 2015, is to establish progress that has been made; the second is the elimination of visceral leishmaniasis as a public health problem by 2017 (committed to by Indian subcontinent governments and visceral leishmaniasis programme managers); the third, as part of the London declaration on neglected tropical diseases, is to eliminate visceral leishmaniasis as a public health problem by 2020 (defined as less than 1 case of kala-azar in 10,000 people in endemic areas, at the block (India) and upazila (sub-district; Bangladesh) level in the Indian subcontinent). In this Article we use the term kala-azar to define the clinical disease and manifestations of the infection caused by visceral leishmaniasis.

Considerable progress has been made towards the target of less than 1 case in 10,000 by implementing novel case-detection strategies, rapid diagnostic testing and vector control activities. At present, Nepal has achieved elimination for two consecutive years and Bangladesh has reached the elimination targets in all upazilas (World Health Organization (WHO), personal communication). However, India has not yet reached these low levels and the

latest data are more than 1 case per 10,000 people in endemic districts. This higher rate of incidence is thought to be due to a combination of differences in underlying transmission, pre-elimination campaign endemicity, health systems, diagnosis rates and the use and success of vector control programmes². However, the relative contribution of these different factors has yet to be quantified and will be a crucial determinant of the success of the expansion of control programmes.

The case-defining conditions for kala-azar in the context of the elimination programme in the Indian subcontinent are prolonged fever of more than 2 weeks, splenomegaly and a positive rK39 test. The rK39 test is an antibody-based detection, immunochromatographic test that has been shown to have high sensitivity (around 97%) when combined with clinical symptoms³ (Table 1). There is, however, an inherent delay in receiving treatment because clinical definition of a suspect case of kala-azar requires at least 2 weeks of fever. This definition is used partly because of the low specificity of the rK39 test to identify infection rather than exposure, and to differentiate kala-azar from other fever-causing aetiologies. For the control of kala-azar, it has been proposed that a diagnostic test that detects active infection rather than the immune response to infection could be used⁴. Testing earlier could identify more patients and interrupt transmission by early treatment. However, the specificity of such a test would have to be high to avoid false positives, as many of the patients tested will present with non-specific symptoms. Consequently,

¹Global Health and Development, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK. ²WIDER, School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK. ³Warwick Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK. ⁴UNICEF/UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases (TDR), World Health Organization, Geneva CH-1211, Switzerland. ⁵Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK. ⁶Research Centre for Drugs and Diagnostics, Parasitology Department, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK. Correspondence should be addressed to: E. R. A. e-mail: emily.adams@lstmed.ac.uk.

Table 1 | Current diagnostic tests with sensitivity and specificity data taken from the Indian subcontinent where possible.

Diagnostic test	Sensitivity	Specificity
rK39 RDT [†]	97.0% (95% CI = 90.0–99.5)	90.2% (95% CI = 76.1–97.7)
DAT [‡]	97.1% (95% CI = 94.9–98.4)	95.7% (95% CI = 88.1–98.5)
Parasitology spleen ^{‡‡}	>95%	100%
Parasitology bone marrow ^{‡‡}	60–85%	100%
Antigen (KAtex) ^{‡‡}	68–100%	98%
PCR [†]	92.3% (95% CI = 88.4–94.9)	63.3 (95% CI = 53.9–71.8)

CI, confidence interval; DAT, direct agglutination test; PCR, polymerase chain reaction; RDT, rapid diagnostic test

the target product profile (TPP) of a diagnostic test that could identify less severe or even asymptomatic cases has not yet been defined. We considered the effect of such an intervention and the role of the specificity.

Within villages, cases of kala-azar tend to be clustered around index cases, suggesting that the drivers of local epidemics are individuals with kala-azar^{5,6}. Most infected people are asymptomatic, but may still contribute to transmission at low levels⁷. Patients with post-kala-azar dermal leishmaniasis (PKDL) are known to harbour parasites, often in the dermal region, and are assumed to be infectious to sand flies⁸. The relative role of people with kala-azar, asymptomatic individuals and people with PKDL in sustaining transmission has not been measured directly and is unknown^{6,9}. Some of this uncertainty will hopefully be addressed in the coming years, but at present more indirect methods are required to understand the dynamics of transmission and control. If kala-azar cases contribute to most of transmission, then early diagnosis and treatment is likely to be a highly effective intervention.

Very few modelling studies have been undertaken to help disentangle the interactions between individual, population and system processes for visceral leishmaniasis. This is partly because of a lack of quantitative information on the natural history of the disease¹⁰. The approach we present is to use a single model to compare information from three different endemic areas. From this we can infer the rates of progression through different clinical states, largely based on the fact that health seeking and health care have different outcomes in these areas. We then extend this core health-seeking model to account for transmission, using a simple, parsimonious framework to investigate the impact of changes in diagnosis on transmission dynamics.

We consider two interventions: reducing diagnostic delays in individuals who already fulfil the kala-azar definition, and introducing novel diagnostics to enable diagnosis of those who do not, or are yet to, fulfil the clinical definition of 14 days of fever and splenomegaly. We consider the dynamic consequences of these interventions, and highlight the potential for rebound epidemics as population (herd) immunity is curtailed. Finally, we consider the profile of a diagnostic required for diagnosis and treatment prior to full kala-azar, and emphasise that specificity, rather than sensitivity, is the limiting factor.

METHODS

Empirical data. The data that inform the model are from studies on self-reported time from symptoms to health seeking and eventual diagnosis of kala-azar (Fig. 1a). In Nepal and Bihar, 92 patients with kala-azar who had experienced 103 kala-azar episodes were interviewed. Patients waited for 30 days (95% confidence interval (CI) = 18–42) in Nepal before seeking health care, 3.75 times longer than in Bihar where patients waited 8 days (95% CI = 4–12). Conversely, the lag time from seeking health care to receiving a kala-azar diagnosis was 90 days (95% CI = 68–113) in Bihar compared with 25 days (95% CI = 13–38) in Nepal. The time span between diagnosis and treatment was short in both countries. In Bangladesh, a 2007 cross-sectional study in Godagari Upzila, Rajshahi, Bangladesh by the International Centre for Diarrhoeal Disease Research, of the knowledge of, attitude to, and practice surrounding kala-azar and its treatment by communities and health providers, also screened for kala-azar by rK39 dipstick test individuals who had had fever for more than 2 weeks. Around 5,000 households were surveyed, of these, 500 randomly selected household heads were interviewed, and indicated that it took 4 days

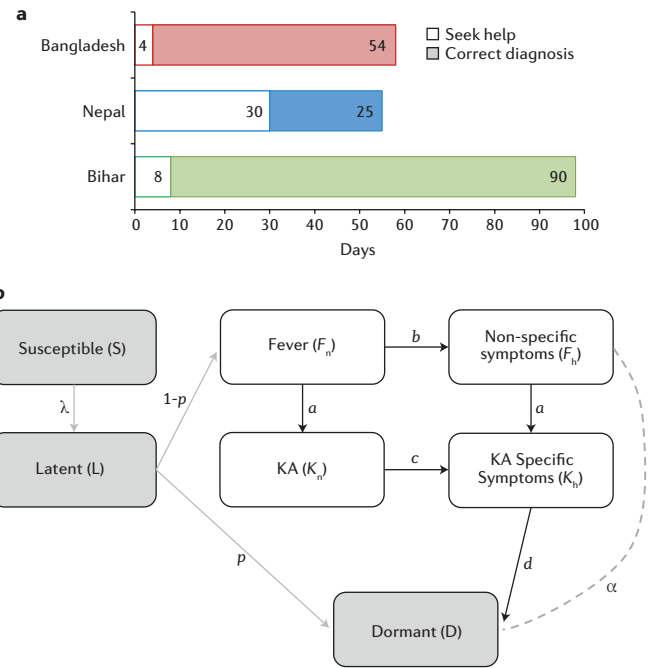


Figure 1 | Data and model on delays in diagnosis. **a**, Time from onset of symptoms (defined as fever) to health-seeking and then diagnosis, for Bihar, Nepal¹⁴ and Bangladesh (D. Mondal, personal communication). **b**, Flow diagram of model. Open boxes show the behaviour model with progression post-infection through fever and full kala-azar (KA) (vertical flow), and from non-health-seeking to health-seeking (horizontal flow) behaviour. The shaded boxes and grey arrows indicate the extra states required for the transmission model.

for people to seek medical help after onset of fever and 54 days until a correct diagnosis. The cumulative effect of these delays means that in Bihar patients are diagnosed 98 days after symptoms start, whereas in Bangladesh it is 58 days and in Nepal it is 55 days.

Mathematical models. We initially developed a model without transmission that mirrors the available data on the retrospective cohort of patients who have been diagnosed with kala-azar (Fig. 1b). The model includes two basic transitions: progression of disease from the point of developing fever to diagnosis, and progression to health-seeking behaviour, giving four possible states: non-health-seeking fever (F_n), health-seeking fever (F_{nr}), non-health-seeking kala-azar (K_n) and health-seeking kala-azar (K_{nr}). Note that we are using ‘fever’ to denote non-specific symptoms (the patient recognises the start of the illness that leads to a kala-azar diagnosis, but the symptoms would have been insufficiently specific for diagnosis of kala-azar at that time). In the model, given a passive health-care system and the absence of better diagnostics, only individuals who are health seeking and have clinical kala-azar can be diagnosed. We include a single parameter for disease progression (transitions from F to K , duration denoted $1/a$, where a is the rate of progression), thereby assuming that kala-azar does not have different pathology in different countries. We include three parameters that are determined by the health-seeking and diagnostic patterns and are location specific — two parameters for the onset of health seeking (F_n to F_{nr} and K_n to K_{nr} rates, denoted b and c , respectively) and one parameter for diagnosis (K_{nr} to D , denoted d). The equations are given in the Supplementary Information.

The model was parameterized to each locale. Each locale has two observations: the time from onset to health-seeking behaviour (entry into F_{nr} or K_{nr}), and the time from health seeking to diagnosis (K_{nr} to D). These periods were expressed as functions of the four model parameters. Unique values of the parameters cannot be estimated, so we generated parameter sets of a , b , c and d that reproduce the observed times. We assumed that individuals with full kala-azar are more likely to seek health care than those with non-specific symptoms ($c \geq b$). A grid of all possible integer values for durations in each

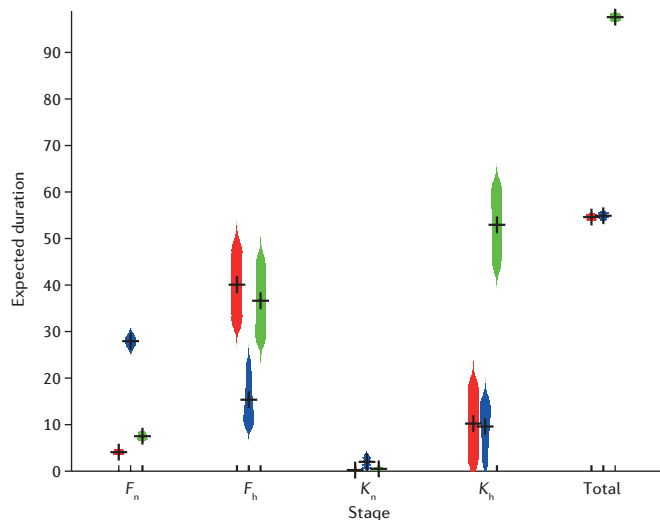


Figure 2 | Expected time in each stage of the model in each setting. The estimated duration in each stage for the combined parameter sets from each locale. From left to right, F_n , the expected time before health seeking or kala-azar (duration of time in fever, non-health-seeking state); F_h , time spent health-seeking with fever before kala-azar development; K_n , duration spent with kala-azar before health seeking; K_h , duration spent health seeking with kala-azar; total, duration spent between onset and diagnosis. Within each column, the localities are Bangladesh (red), Nepal (blue) and Bihar (green). Note that there are correlations within the parameter sets so that the total time from onset to diagnosis is constant (final column). The violin plots indicate the variability in the parameter values, and the crosses mark the mean.

state was compared with the observed outcomes. Parameter combinations that reproduce the observed times were retained, thus generating locale-specific combinations of parameters that produced results consistent with the data. The values of the parameters within the sets were correlated. For example, to reproduce the observed time to diagnosis, a shorter period spent health seeking is combined with a longer time waiting for diagnosis. Therefore, the parameters were retained as groups of appropriate parameters rather than ranges of possible values.

The model was then extended to include transmission dynamics (Fig. 1b). The extended model includes an incidence of infection and a latent class to account for delays between infection and onset of symptoms. Most latently infected individuals recover directly to the dormant stage without progressing to symptoms leading to kala-azar¹¹. The instantaneous rate of infection is calculated as the weighted sum of the force of infection from the various potentially infectious states: latent, fever, kala-azar and dormant. The equations, including analytical solution for the basic reproduction number, R_0 , and equilibrium state, are given in the Supplementary Information. The basic reproduction number, R_0 , is defined as the average number of onward infections caused by a single infectious individual in a wholly susceptible population throughout the infectious period of that individual. R_0 is a combination of the number of infections generated in each infection stage, which in turn is determined by the duration and infectiousness of the stage. For an endemic disease such as visceral leishmaniasis, populations are not wholly susceptible, and the number of onward transmissions will be reduced by potential contacts that are already infected or immune.

The duration of the latent stage, relative infectiousness of different stages and R_0 cannot be measured with current diagnostics and available epidemiological data. Consequently, we chose values that are consistent with current understanding¹⁰, that reproduce patterns consistent with general observations¹², and so that the equilibrium state of the model in the three settings was consistent with relative epidemiological patterns. All of the biological parameters were the same across all three settings, so differences between settings were due to the differences in health-seeking behaviour and health-care response (see Supplementary Information).

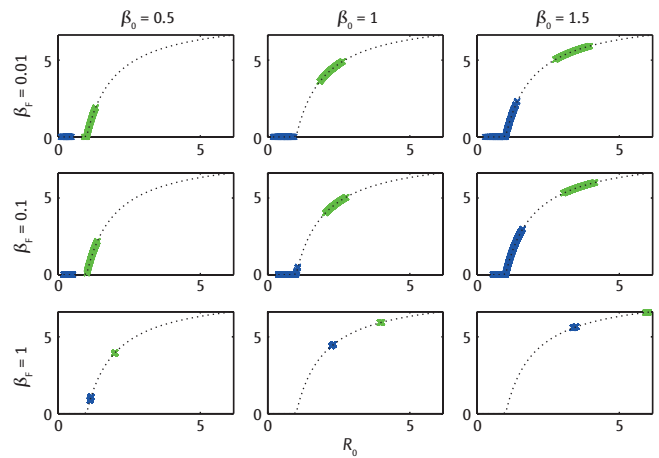


Figure 3 | Impact of diagnosis delay on transmission. Sensitivity analysis of the effect of different overall transmissibility, β_0 (columns), and the relative infectiousness of individuals with non-specific symptoms, β_f (rows). Within each panel the expected equilibrium incidence (cases per 10,000 per year) is plotted against the basic reproduction number, R_0 (dotted line). Each green point corresponds to one of the health-seeking, diagnosis and progression parameter sets for Bihar; blue are for Nepal. The points for Bangladesh are masked by those for Nepal.

We then used the model to examine two potential interventions. First, we considered the impact of reducing the time to diagnosis in Bihar to that estimated for Nepal and Bangladesh. Second, we examined the impact of a diagnostic and treatment intervention applied during the pre-kala-azar fever stage. We show results both for the average incidence at equilibrium, which may take many years to be reached, and for the average number of diagnoses over the first 5 years from introduction, to demonstrate the short-term effects. The impact of a diagnostic test is determined by both the sensitivity and specificity of each test, and how it is applied, for example a single test per patient or multiple testing. We include a testing rate, τ , so that the average interval between tests is $1/\tau$, and the proportion of individuals correctly diagnosed before the onset of kala-azar is $\tau S_E / (\tau S_E + a)$, where S_E is the per-test sensitivity. To account for specificity, we assume that there is a background rate of 200 additional cases of fever owing to other infections and not related to visceral leishmaniasis, and calculate the rate at which false positives arise as a function of the specificity per test, S_p , and the rate of testing. We present equilibrium results, and the numbers of true and false positives that are expected to arise over 5 years that follow the introduction of such an intervention.

RESULTS

Estimated pathways to diagnosis. The parameter sets that are consistent with the data are shown in Figure 2. There are 526 unique parameter combinations (a, b, c, d) for Bihar, 2,320 for Nepal and 312 for Bangladesh. The time between fever onset and progression to clinical kala-azar is 33 days $< a < 55$ days. In Nepal, individuals develop the disease faster than they seek health care, so that the typical path for an individual is fever to kala-azar to health seeking to diagnosis, with most individuals diagnosed and treated within the first few weeks of symptoms. In Bihar and Bangladesh, health seeking starts much earlier, but diagnosis is slower so that individuals enter the health system, but then remain in the symptomatic state without treatment. The major difference between Nepal and the other two regions is that kala-azar cases in Nepal most frequently first present to health-care services as clinical kala-azar, whereas in Bihar and Bangladesh people first present with non-specific symptoms. Consequently, in Bihar, patients are likely to be diagnosed and treated for more common infections (such as bacterial infection) owing to presentation of non-specific symptoms. There is a risk that treatment failure, rather than misdiagnosis, is blamed, leading to repeat treatments for the wrong diagnosis. In addition, many patients present to unqualified practitioners or the private health-care system, delaying correct diagnosis¹³.

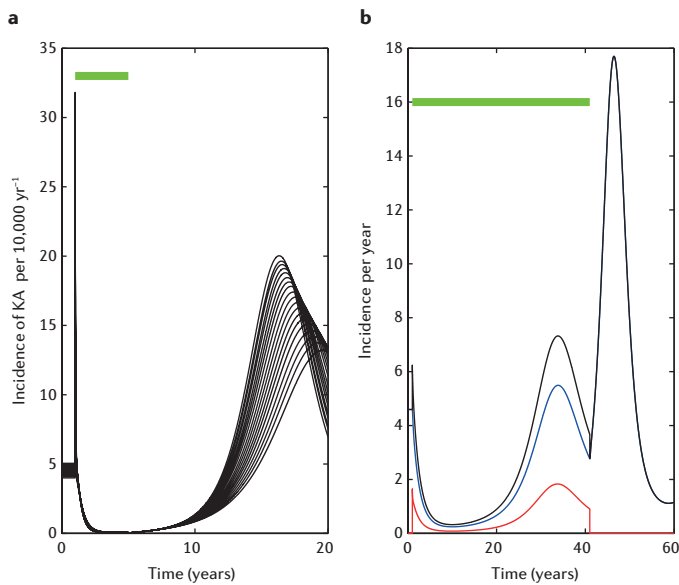


Figure 4 | The impact of interventions on transmission dynamics. **a**, The impact of reducing the time to diagnosis in Bihar to 10 days plus 14 days fever; the green bar indicates the duration of intervention. There is an initial increase in diagnoses of kala-azar (KA, black lines), which then leads to a reduction in underlying incidence and incidence of kala-azar. The intervention is lifted at 5 years, leading to a resurgence of cases and a large outbreak several years later. The different lines are for different parameter sets. **b**, Alternatively, the impact of introducing a diagnostic for febrile cases such that 30% of cases do not progress to kala-azar. This increases the number of diagnoses during the fever stage (red line) and decreases the number diagnosed with kala-azar (blue); the total diagnoses are shown in black. In this case, as the intervention does not lead to elimination, an epidemic occurs while the intervention is in place (green bar). When the intervention is lifted there is a further resurgence of cases.

Transmission dynamics. As with all transmission models, incidence of diagnosis increases non-linearly with the basic reproduction number, R_0 , for all values of the transmission parameters (Fig. 3). At equilibrium, as long as individuals with kala-azar provide most infection to vectors, the transmission potential is higher in Bihar than Nepal (the average number of onward transmissions per infected individual, R_0 , will be different). Only when transmission rates are high (Fig. 3), and therefore the number of onward infections, R_0 , is also high, are the incidences of disease in all settings similar. At this level of transmission, the diagnostic differences are masked by the high infectiousness of latent, fever and dormant cases and variation between parameter sets and settings is obliterated. In reality, the transmission dynamics are not at equilibrium, and incidences are reducing in all locations due, at least in part, to reductions in R_0 . Consequently, differences in incidence between settings can be explained by the differences in diagnostic delays that are consistent with the observed durations (Fig. 2). This suggests that variability in health-care seeking and diagnostic delays between different settings, and the resulting distribution of times with non-specific and specific symptoms are likely to have an impact on transmission patterns, particularly if there is differential infectivity at these different stages of infection¹⁴.

Dynamic consequences of interventions. Owing to a lack of further information, we set the relative transmission parameters to be those in the central panel of Figure 3 (see Table 1 for parameter values). At these values, the equilibrium for Nepal and Bangladesh is low, and elimination has been achieved for some parameter sets, and the equilibrium for Bihar is between 4 and 5 cases per 10,000 people per year.

We consider two interventions that shorten the period of high infectiousness and transmission potential. The impact of these interventions on the dynamics of diagnoses is similar (Fig. 4). First, we switched the delay in diagnosis seen in Bihar (43–63 days) to the average delay in Nepal (10 days, added to the 14 days of fever to become a clinical suspect; Fig. 4a). A reduction in time

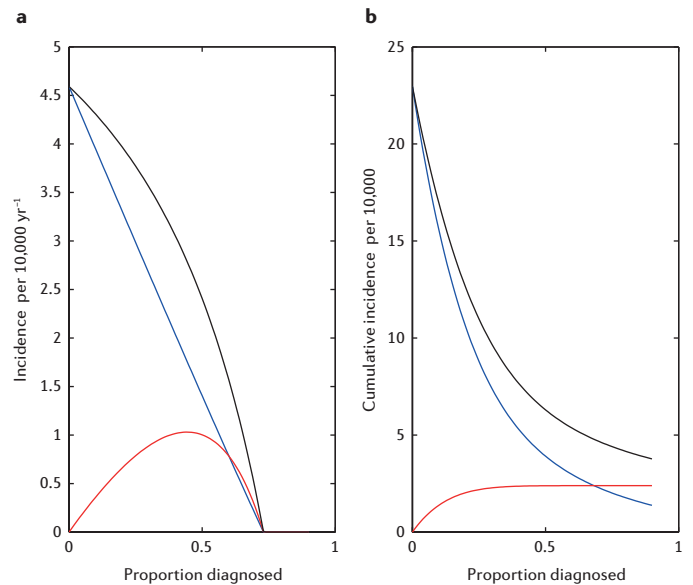


Figure 5 | The impact of a diagnostic among those with non-specific symptoms (fever). **a**, The equilibrium incidence of diagnosed cases (achieved after many decades) with different proportions diagnosed before full kala-azar; total incidence of diagnosed cases (black), those that are diagnosed with less specific symptoms (fever, red) and full kala-azar (blue). A 75% reduction is enough for infection elimination, 55% is enough for elimination as a public health problem (<1 case per 10,000 per year). **b**, The cumulative incidence over a shorter time scale of 5 years with different proportions diagnosed before kala-azar. Note the diminishing returns — halving the total number of cases of kala-azar can be achieved by preventing only 25% of cases from progressing.

to diagnosis shows an initial large peak in cases, as the cases that are ‘waiting’ to be diagnosed are found. This leads to a rapid reduction in transmission, which then leads to a decline in incidence. For a setting with an incidence of 5 per 10,000 people per year, elimination would be achieved with this change in the current model. Whether or not elimination would be achieved in reality depends on, among other things, the details of transmission from asymptomatic infections and spatial heterogeneities. If diagnostic delay is returned to its previous length after 4 years, then there is a rebound epidemic, but this is much slower and occurs over several years. Note that the predicted patterns are similar for all the parameter sets fitted to the Bihar situation.

Second, we introduce the diagnosis and treatment of patients while they are health seeking with non-specific febrile symptoms, before they develop kala-azar (before they have passed 14 days of fever and have splenomegaly; Fig. 4b). The dynamic response is similar to the dynamics of reducing diagnostic delays for those who have kala-azar, but without the immediate diagnostic spike. The modelled intervention (30% of kala-azar cases are diagnosed during non-specific fever) is insufficient to eliminate infection. Consequently, there is an epidemic while the intervention is in place, owing to the build-up of individuals with increased susceptibility to infection who were previously protected by the reduction in transmission. If the intervention is kept in place, then incidence eventually returns to a low level. When the intervention is removed, the second epidemic is a consequence of the increase in transmission owing to the longer infectious period when screening of fever cases is stopped.

In both interventions, the supply of full clinical kala-azar diagnoses is curtailed, transmission is reduced and there is a reduction of population (herd) immunity that leads to a bounce back of cases if the intervention is stopped. The speed at which the subsequent epidemic occurs is dependent on the success of the intervention — better curtailment of transmission results in a longer period to the next epidemic. The slow build-up means that there will be no obvious link between lengthening diagnosis delay and incidence, with clear implications for monitoring efficiency of diagnosis and treatment. In particular, if this intervention were implemented, then potentially there would be a reduction in clinical awareness of visceral leishmaniasis, resulting in a

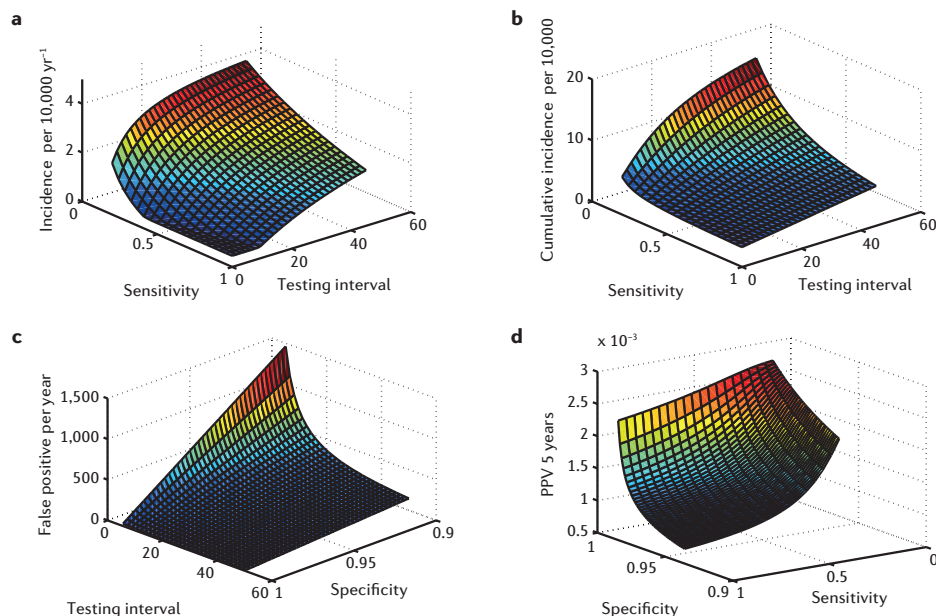


Figure 6 | The interaction between sensitivity and specificity. **a**, The equilibrium incidence of kala-azar cases as a function of the sensitivity per test and the testing interval. Elimination is only achieved for frequently applied tests (under 15 days), but can be achieved with relatively low sensitivity. **b**, As in **a** but the cumulative incidence is plotted over 5 years from the start of the intervention. This tends to reduce the importance of frequent testing for highly sensitive tests. **c**, The equilibrium incidence of false positives, assuming 200 non-visceral leishmaniasis fever cases as a function of testing interval and specificity. The relationship with specificity is linear. Note the juxtaposition of the highest numbers of false positives (short testing interval, low specificity) with the lowest numbers of true positives (short testing interval, high sensitivity in **a**). **d**, The proportion of positives that are true-positive positive predictive values, calculated as the average over 5 years after introduction of testing, as a function of sensitivity and specificity. The testing interval is fixed at 14 days (people seeking health care provide an opportunity for testing once every 2 weeks on average), and there are 200 people with non-visceral leishmaniasis fever who are health seeking. The shape is dominated by specificity owing to the numerical scales of **b** and **c**.

lengthening of diagnostic delays. We suggest that although reducing diagnosis delay through special efforts is likely to be an effective means to short-term reduction in cases, it is, depending on the epidemiological setting (baseline prevalence and biting rates), unlikely to be a sustainable route to long-term elimination. The quantitative details depend on specific parameter values and model structure, but this general pattern will be observed if those with kala-azar contribute most of the infection to vectors and onward transmission, and if there is sufficient (concomitant) immunity to kala-azar.

Impact of sensitivity of a novel diagnostic. We also consider the consequences of differing diagnostic sensitivities for diagnosis during the non-specific symptom phase in terms of its impact on equilibrium and short-term incidence of diagnoses (Fig. 5). Increased sensitivity will result in a larger proportion of cases being diagnosed earlier. At equilibrium, as the proportion of cases diagnosed increases, the incidence of diagnosis during the fever stage increases, but total diagnoses fall owing to reduced transmission (Fig. 5a). Elimination of transmission is possible even with relatively moderate sensitivity. If most transmission is from kala-azar cases, then halving the numbers progressing to this state will halve R_0 . However, achieving equilibrium incidence in such models takes many decades and is mainly of theoretical interest. Consequently, we also consider the impact on the numbers of cases expected over the 5 years that follow the introduction of the intervention (Fig. 5b). Clearly, the dramatic fall in cases (Fig. 4b) occurs for low values of sensitivity, and over the short-term there is little extra to be gained from diagnosing more than 25–30% of cases before kala-azar.

Profile of the diagnostic. We profiled the diagnostic required for early case detection in terms of both sensitivity and specificity (Fig. 6). The size of the false-positive problem is determined by the frequency of fever cases (visceral leishmaniasis and non-visceral leishmaniasis) being tested for kala-azar, the specificity of the test and the proportion of fever cases that are due to visceral leishmaniasis. We, therefore, modelled a scenario in which there is a background rate of non-visceral leishmaniasis cases that present for diagnosis, and,

to account for the possibility of multiple testing owing to the long duration of the fever stage for kala-azar (a known phenomenon¹⁵), we include a frequency of testing for health-seeking kala-azar cases with fever. If each person who seeks health care presents once, then the proportion of true cases diagnosed is the sensitivity (Fig. 5). However, if people are tested at multiple consultations, then there are multiple opportunities for a correct, true-positive result, and a lack in sensitivity can be overcome by more frequent testing (Fig. 6a,b). However, as the number of testing occasions increases, so the rate at which false positives are found increases, and increasing specificity linearly decreases the false positives (Fig. 6c). Holding the test frequency constant, the average positive predictive value (proportion of positives that are true positives) is shown in Fig. 6d as a function of sensitivity and specificity. There are likely to be many hundreds to thousands of false positives for every true positive identified unless the diagnostic, or diagnostic combination, is more than 99% specific. This problem is amplified by a decreasing prevalence of true infection, as witnessed in any elimination setting.

DISCUSSION

Our principal conclusion is that earlier diagnosis and prompt therapy have the potential to reduce ongoing transmission to elimination or near-elimination levels. This is indeed one of the pillars of the current elimination campaign, and, although this is likely to have already affected transmission in Nepal and Bangladesh, there is a large potential gain in Bihar, given that the diagnostic delays in this area are longer. We have also highlighted that curtailing transmission is likely to decrease population immunity in the long-term, so that there is a potential for large epidemics if vigilance is not maintained and diagnostic delays are allowed to increase. If diagnostic delays lengthen either the stage of health-care seeking or the ability of the system to recognise kala-azar, then subsequent epidemics are predicted to have a long lead time, and will not be immediately recognized. We have shown that the introduction of novel diagnostics on non-specific fever cases (before full kala-azar) can be effective even if sensitivity is relatively low, but that their introduction is prevented at present by less than ideal specificity, given the issues of delivery, safety and

cost of treatments. The paucity of data available to fit more complex models means that our results rely as much on understanding of infectious-disease epidemiology as on our simple model. Nonetheless, we believe that these conclusions are supported by current understanding, and, if nothing else, are valid, strong hypotheses.

We studied two mechanisms by which earlier diagnosis could occur in highly endemic settings, such as Bihar. The first is improving the health system to reduce delays in treatment. At present, differences in the time to diagnosis between countries are assumed to be because of differing time spent in the private health-care system where knowledge of kala-azar is relatively poor. Potentially, patients present with less-specific symptoms and enter a different diagnostic algorithm, and kala-azar is only suspected later. A study in Bihar using accredited social health activists (community health workers in India) to identify and refer suspected kala-azar cases showed that the time from onset of fever to seeking treatment and diagnosis at peripheral health facilities could be reduced to 32–50 days in total¹⁶. Our modelling reinforces the observation that reducing the time to diagnosis is an effective intervention, but as prevalence decreases it will be more difficult to maintain the necessary knowledge and infrastructure to sustain this. Intriguingly, the efficiency of the diagnostic process may have a natural equilibrium, depending on the frequency of diagnosis. We have also shown that resurgence in cases will occur after several years if transmission is reduced and not halted, and that the peak of the resurgence may well be higher than pre-intervention. Typically, local kala-azar outbreaks occur regularly — 3 peaks have occurred in India 14–15 years apart over the past 40 years¹⁷.

The second mechanism to reduce the time to diagnosis in highly endemic areas such as Bihar is to identify active infection before kala-azar onset. Specificity becomes important when designing a test that targets early case detection (before 14 days of fever). Only a test with very high specificity will allow patients to be treated, given the limited range of treatments available (toxicity, cost, administration or adherence, depending on the treatment). Nonetheless, if specificity is high enough to consider treating patients before they have 14 days of fever and become a clinical suspect, then patients could be tested and treated much earlier than is possible with the current diagnostic algorithm. This would eliminate a pool of patients that transmit visceral leishmaniasis to their local community, thereby substantially reducing future cases. Our analysis demonstrates that sensitivity of early testing for visceral leishmaniasis is not the main problem. Even a moderately sensitive test (30%) can have dramatic affect on kala-azar transmission alongside the current testing algorithm of passive surveillance with rK39. The challenge to testing earlier in the course of the disease, with the intention of treating, is avoiding a large number of false positives. However, as prevalence decreases, the positive predictive value of all tests will fall as more patients are false positives than true positives, but the resultant gain in sustainable elimination (if such a test were to be implemented) is significant.

Limitations of this model and data gaps

There are few well-conducted studies to describe the natural history of visceral leishmaniasis, which is multifactorial¹⁸, and hence the risks for an infected individual to become diseased are not quantified. Owing to the localized epidemics that are seen surrounding index cases it is reasonable to assume that patients with kala-azar are the main reservoir of infection for sand flies. It is also thought that those with PKDL and asymptomatic individuals or those with a dormant infection can also be infectious to sand flies, but the evidence base for this relies on anecdotal studies. Only one experimental study⁸ has been published whereby sand flies were allowed to feed on four people with PKDL. Of the 400 fed flies, 104 became infected. In our analysis we have assumed that asymptomatic individuals and those with PKDL are, on average across the population, considerably less infectious than symptomatic cases^{6,9}. Should asymptomatic individuals be more infectious than anticipated, the relative importance of kala-azar cases to R_0 will decrease. This means that the period of infectiousness will increase, reducing the probability of elimination and necessitating lower overall transmission rates to maintain the levels of incidence in the 1–5 cases per 10,000 people per year. If people with kala-azar are less infectious than we have assumed, the impact of reduction in diagnostic delays will be reduced, but the effect of early, novel diagnostics will be enhanced (results not shown). Of course, diagnostics for asymptomatic cases and people with

PKDL may be essential for elimination if these groups are shown to be involved in transmission of disease and maintain long-term infections.

Unfortunately, without more detailed data, the models we have described are only able to highlight the qualitative patterns, although they are robust across a large range of biologically plausible parameters. The health-seeking behaviour data are informative about ranges of parameters, including the duration of non-specific symptoms, a parameter that has not been previously estimated. The data would be greatly improved by systematically sampling a population in which incidence was also estimated.

In addition to the uncertainties on the human side of transmission, we also know little of sand-fly behaviour, life expectancy and range. Clearly, these vector dynamics will have an important role in the transmission cycle and in the design of effective interventions against transmission.

Diagnostics of the future

Diagnostics play a crucial part in the control and elimination of kala-azar and are a research priority¹⁹. We argue that one avenue to revolutionize the current control algorithm is to develop a highly specific test of active infection (whether symptomatic or asymptomatic), even if limited in sensitivity. There is currently no suitable antigen test capable of detecting active kala-azar. The only commercially available product, KAtex, has challenges in utility and sub-optimal sensitivity, although we would argue that low sensitivity should not necessarily be considered a barrier to implementation. Simplified molecular diagnostic tools that can be adapted for field situations are under development, including loop-mediated isothermal amplification²⁰. These tests, along with standard polymerase chain reaction, are able to detect circulating DNA in the blood of individuals who are actively infected. Studies show that molecular tests are very sensitive, and although they have low specificity, it may be that these are infections that are below the limit of detection of the reference standard, and therefore in fact true positives⁴. Diagnostics that are able to detect asymptomatic infection and PKDL may also be important, depending on the relative role of transmission in these groups.

Given the uncertainty in clinical progression of kala-azar and diagnostic performance, there is clearly a chance that some, possibly much, of the morbidity and mortality caused by *Leishmania* infection is being misclassified. The process of diagnosis of kala-azar provides both the clinical information for treatment, as well as the data on which surveillance is built. Consequently, it is inevitable that 100% of cases of kala-azar are diagnosed and treated, regardless of the performance of the health-care system or the health-seeking behaviour of the population. Ideally, surveillance would include information that is independent of clinical diagnosis. This is particularly needed as kala-azar becomes rarer, and it is likely that both clinical and patient awareness wanes. A diagnostic tool that enables population surveillance of infection and disease, independent of clinical diagnosis, is a crucial step in achieving, enforcing and demonstrating elimination.

1. Alvar, J. et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE* **7**, e35671 (2012).
2. Mondal, D. et al. Visceral leishmaniasis elimination programme in India, Bangladesh, and Nepal: reshaping the case finding/case management strategy. *PLoS Negl. Trop. Dis.* **3**, e355 (2009).
3. Boelaert, M. et al. Rapid tests for the diagnosis of visceral leishmaniasis in patients with suspected disease. *Cochrane Database System. Rev.* **6**, CD009135 (2014).
4. de Ruiter, C. M. et al. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. *J. Clin. Microbiol.* **52**, 3147–3155 (2014).
5. Bern, C., Courtenay, O. & Alvar, J. Of cattle, sand flies and men: a systematic review of risk factor analyses for South Asian visceral leishmaniasis and implications for elimination. *PLoS Negl. Trop. Dis.* **4**, e599 (2010).
6. Stauch, A. et al. Model-based investigations of different vector-related intervention strategies to eliminate visceral leishmaniasis on the Indian subcontinent. *PLoS Negl. Trop. Dis.* **8**, e2810 (2014).
7. Das, S., Matlashewski, G., Bhunia, G. S., Kesari, S. & Das, P. Asymptomatic *Leishmania* infections in northern India: a threat for the elimination programme? *Trans. Royal Soc. Trop. Med. Hyg.* **108**, 679–684 (2014).
8. Addy, M. & Nandy, A. Ten years of kala-azar in west Bengal. Part I. Did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? *Bull. World Health Organ.* **70**, 341–346 (1992).
9. Stauch, A. et al. Visceral leishmaniasis in the Indian subcontinent: modelling epidemiology and control. *PLoS Negl. Trop. Dis.* **5**, e1405 (2011).
10. Rock, K. S. et al. Uniting mathematics and biology for control of visceral leishmaniasis. *Trends Parasitol.* **31**, 251–259 (2015).

11. Hasker, E. et al. Strong association between serological status and probability of progression to clinical visceral leishmaniasis in prospective cohort studies in India and Nepal. *PLoS Negl. Trop. Dis.* **8**, e2657 (2014).
12. Dye, C. & Wolpert, D. M. Earthquakes, influenza and cycles of Indian kala-azar. *Trans. R. Soc. Trop. Med. Hyg.* **82**, 843–850 (1988).
13. Hasker, E. et al. Management of visceral leishmaniasis in rural primary health care services in Bihar, India. *Trop. Med. Int. Health* **15** (Suppl 2), 55–62 (2010).
14. Fraser, C., Riley, S., Anderson, R. M. & Ferguson, N. M. Factors that make an infectious disease outbreak controllable. *Proc. Natl Acad. Sci. USA* **101**, 6146–6151 (2004).
15. Boettcher, J. P. et al. Visceral leishmaniasis diagnosis and reporting delays as an obstacle to timely response actions in Nepal and India. *BMC Infect. Dis.* **15**, 43 (2015).
16. Das, V. N. et al. Impact of ASHA training on active case detection of visceral leishmaniasis in Bihar, India. *PLoS Negl. Trop. Dis.* **8**, e2774 (2014).
17. Muniaraj, M. The lost hope of elimination of kala-azar (visceral leishmaniasis) by 2010 and cyclic occurrence of its outbreak in India, blame falls on vector control practices or co-infection with human immunodeficiency virus or therapeutic modalities? *Trop. Parasitol.* **4**, 10–19 (2014).
18. McCall, L. I., Zhang, W. W. & Matlashewski, G. Determinants for the development of visceral leishmaniasis disease. *PLoS Pathogens* **9**, e1003053 (2013).
19. Matlashewski, G. et al. Research priorities for elimination of visceral leishmaniasis. *Lancet Global Health* **2**, e683–e684 (2014).
20. Adams, E. R., Schoone, G. J., Ageed, A. F., Safi, S. E. & Schallig, H. D. Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of *Leishmania* parasites in clinical samples. *Am. J. Trop. Med. Hyg.* **82**, 591–596 (2010).
21. Chappuis, F., Rijal, S., Soto, A., Menten, J. & Boelaert, M. A meta-analysis of the diagnostic performance of the direct agglutination test and rK39 dipstick for visceral leishmaniasis. *Br. Med. J.* **333**, 723 (2006).
22. Sundar, S. & Rai, M. Laboratory diagnosis of visceral leishmaniasis. *Clin. Diagnostic Lab. Immunol.* **9**, 951–958 (2002).

SUPPLEMENTARY MATERIAL

Is linked to the online version of this paper at: <http://dx.doi.org/10.1038/nature16042>

ACKNOWLEDGEMENTS

The authors thank D. Mondal, O. Courtenay, C. Bern, L. Chapman, A. Ghani and S. Laney for informative discussions. G.F.M. and E.R.A. gratefully acknowledge funding from the Bill and Melinda Gates Foundation through the Diagnostics Modelling Consortium based at Imperial College. G.F.M., E.R.A. and T.D.H. gratefully acknowledge funding of the NTD Modelling Consortium by the Bill and Melinda Gates Foundation in partnership with the Task Force for Global Health. The views, opinions, assumptions or any other information set out in this article are solely those of the authors. P.L.O. is a staff member of the WHO; the authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy, or views of the WHO.

COMPETING FINANCIAL INTERESTS

E.R.A. is a consultant for FIND in the evaluation of the diagnostic tool LAMP for leishmaniasis. Financial support for this publication has been provided by the Bill & Melinda Gates Foundation.

ADDITIONAL INFORMATION



This work is licensed under the Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0>