Impact of the visceral leishmaniasis elimination initiative on *Leishmania donovani* transmission in Nepal: a 10-year repeat survey

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Summary

Background Nepal launched a visceral leishmaniasis (also known as kala-azar) elimination initiative in 2005. We primarily aimed to assess whether transmission of *Leishmania donovani* had decreased since the launch of the initiative. We also assessed the validity of the direct agglutination test (DAT) as a marker of infection, in view of future surveillance systems.

Methods We did a repeat survey in a population aged 2 years and older for whom baseline serological data were available from 2006. Data were from three districts in the eastern region of Nepal. The primary outcome of interest was prevalent infection with *L donovani* as measured with DAT (cutoff value ≥1:3200). We compared age group-specific and cluster-specific seroprevalences in 2016 with those in 2006, using χ^2 tests, with a specific focus on the comparison of seroprevalences in children born between 1996 and 2005, and those born between 2006 and 2015. To estimate the overall adjusted risk ratio for being seropositive in 2016 compared with 2006, we fitted a Poisson model controlling for age, sex, and cluster.

Findings Between Oct 17, 2016, and Dec 26, 2016, we assessed 6609 individuals. DAT prevalence in children younger than 10 years was 4·1% (95% CI 3·2–5·4) in 2006 versus 0·5% (0·1–1·7) in 2016 (p<0·0001). Seroprevalence was lower in 2016 than in 2006 in all age groups and in all repeated clusters. The overall adjusted risk ratio of being seropositive was 0·44 (95% CI 0·37–0·52) for 2016 compared with 2006, and 0·04 (0·01–0·16) in children younger than 10 years.

Interpretation Our findings show that transmission of *L donovani* in Nepal has decreased significantly between 2006 and 2016, coinciding with the elimination programme. DAT seems useful for monitoring of *L donovani* transmission.

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For the Nepali translation of the abstract see **Online** for appendix 1

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Introduction

Visceral leishmaniasis, or kala-azar, is a vector-borne disease, which is lethal in nearly all cases if untreated. An estimated 50 000–90 000 new cases occur annually worldwide, with more than 95% of cases in 2017 occurring in just ten countries, including Bangladesh, India, and Nepal—all three belonging to the Indian subcontinent.[1] In 2005, the governments of Bangladesh, India, and Nepal launched an initiative to eliminate visceral leishmaniasis as a public health problem from the region (the kala-azar elimination initiative), aiming to reduce the disease incidence to less than one visceral leishmaniasis case per 10 000 population at the sub-district or district level per year. The elimination strategy in the region is mainly based on improved case management, effective disease surveillance, and integrated vector control, with country-specific emphasis on different aspects of these activities.

Nepal was the first country to reach the targeted threshold in each of its endemic districts in 2013. However, this threshold was surpassed in one (supposedly non-endemic) district in 2017, and the disease is now observed in an increasing number of districts hitherto considered to be non-endemic. WHO has therefore not yet been able to validate the achievement of visceral leishmaniasis elimination as a public health problem in Nepal.

So far, the country-reported visceral leishmaniasis incidence is the only parameter used to assess the impact of the elimination initiative. These reported case numbers are highly susceptible to changes in the efforts made for case detection and surveillance. Moreover, efforts tend to fluctuate, especially in the period closer to elimination as international and national support dwindles in the face of competing health priorities. Also, the vast majority of infections do not result in overt clinical disease but will remain asymptomatic.[2] Monitoring of transmission through infection rather than disease incidence—as is already being done for several other infectious diseases

Research in context

Evidence before this study

We searched PubMed during the protocol writing process between Feb 1, 2016, and Aug 23, 2016, for any articles related to "leishmaniasis" and either "transmission", "epidemiology", "serology", or "surveillance". Additional background information was sought through references of the retrieved papers. Since the start of the visceral leishmaniasis elimination initiative on the Indian subcontinent in 2005, the caseload of the disease in the Indian subcontinent has reduced substantially, from 41 000 in 2005 to 6746 in 2016, according to WHO. However, as asymptomatic infection has been shown to outnumber incident visceral leishmaniasis by a factor of nine in India and Nepal, and risk factors for progression to disease are still largely unknown, this reduction in cases does not necessarily mean that transmission has also reduced.

Added value of this study

This study shows that the reported declining visceral leishmaniasis case load in Nepal is linked to declining seroprevalence rates. As the duration of seropositivity is unlikely to have changed over the past 10 years, we can safely assume that the incidence of *Leishmania donovani* infection, and hence the intensity of transmission, has lowered as well since the start of the elimination initiative in 2005. Given the rather robust features of the direct agglutination test as a marker for infection, especially at high titre cutoffs, serosurveillance of selected groups using the test could provide a powerful tool for tracking ongoing transmission. The eventual success of the elimination initiative will depend on such strengthened surveillance in the near future.

Implications of all the available evidence

The available evidence shows that transmission of *L donovani* infection has reduced over the past decade, coincidentally with the elimination initiative, which probably contributed to this decline. However, there is still an undetected pool of infected individuals present in villages that have not reported any visceral leishmaniasis cases for several years. Continued and adapted surveillance will be necessary.

such as malaria, tuberculosis, and schistosomiasis—could therefore be a powerful tool to detect changes in transmission earlier on, and corroborate (or not) any claims made about downward trends in disease incidence.[3–6] If there was a true decrease in transmission of *Leishmania donovani* in Nepal between 2006 and 2016, this decrease should be reflected in a substantial reduction or even absence of infection in those born after the start of the elimination initiative in 2005 (ie, in the children who were younger than 10 years old in 2016).

Correctly defining individuals who are infected, however, is not straightforward in the case of leishmaniasis. There are several tests available to identify *L donovani* infection, but none of these tests are suitable as a single comprehensive marker of infection.[2] To assess a difference over time, repeated measures of a single test can be assumed to be indicative. In the Indian subcontinent, the serological direct agglutination test (DAT) has been widely validated as a marker of disease, and has proven its use in several large-scale field surveys to document infection prevalence and incidence.

In this study, we compared the prevalence of *L donovani* infection as measured with the DAT in the baseline survey between Nov 10, 2006, and March 30, 2007,[7] with that in the revisit between Oct 17, 2016, and Dec 26, 2016, among communities exposed to heavy transmission at the start of the elimination initiative in 2005. We used the archived data from a serosurvey we did in 2006,[7] and replicated this serosurvey in 2016 in the same communities, 10 years after the launch of the kala-azar elimination initiative. The aim of the research in 2016 was to contribute to an evaluation of the elimination initiative and to guide future serosurveillance efforts for monitoring of transmission.

Methods

Study design and participants

In 2016, we did a repeat study using data from three districts of the visceral leishmaniasis endemic region of Terai in southeastern Nepal. All consenting individuals aged 2 years and older were included in the study. Between 2006 and 2009, the same research team did a cluster-randomised trial (Kalanet)[7] in ten purposefully selected highly endemic communities (clusters, corresponding to a subunit of a village development committee, the smallest administrative unit in Nepal, and identified with the codes C51 to C60). The communities had a mean visceral leishmaniasis incidence in 2003–05 of 0·8% or more per person-year. The Kalanet trial aimed to evaluate the effectiveness of long-lasting insecticidal bednets on the incidence of visceral leishmaniasis. The primary endpoint used in the trial was incidence of *L donovani* infection as measured by serology (DAT). Three cross-sectional surveys were done with 1-year intervals, but no difference was observed in visceral leishmaniasis incidence and seroconversion rates comparing clusters with and without long-lasting insecticidal bednets.[7] Further details regarding methods and outcomes of this study have been published previously.[6–9]

In 2016, 10 years after the baseline serosurvey was done for the 2006 Kalanet trial, we replicated the baseline survey in the same communities in eastern Nepal (Kalanet revisit survey) in the same season (October to December, 2016). However, we included only eight of the ten original trial clusters (C51–C53 and C56–C60), as for two semi-urban clusters within Dharan city (C54 and C55) we anticipated too much in-and-out migration over the past decade to make a meaningful interpretation

of any changes in seroprevalence at the cluster level. In addition to the eight repeated clusters, we added four new clusters with different levels of endemicity: two clusters without any visceral leishmaniasis cases reported in the past 20 years (C61 and C62); one cluster that reported visceral leishmaniasis cases up until 5 years ago (C63); and one cluster with ongoing reporting of visceral leishmaniasis cases (C64). All study clusters were located in three visceral leishmaniasis endemic districts in southeast Nepal: Sunsari, Saptari, and Morang (figure 1). Visceral leishmaniasis case data from the 3 years preceding the 2006 and 2016 study were collected for each of the clusters from the respective district public health offices; relative reductions in visceral leishmaniasis incidence ranged between 91% (C51) and 100% (C56 and C60). Exact numbers for visceral leishmaniasis incidence can be found in appendix 2 (p 1). We obtained written informed consent from all participants involved in this study. Participants aged between 12 and 16 years were asked for their assent in addition to the written informed consent of their parents or guardian. Ethics approval for this study was obtained from the institutional review board of the University of Antwerp, Belgium, and from the institutional review board at B P Koirala Institute of Health Sciences, Dharan, Nepal.

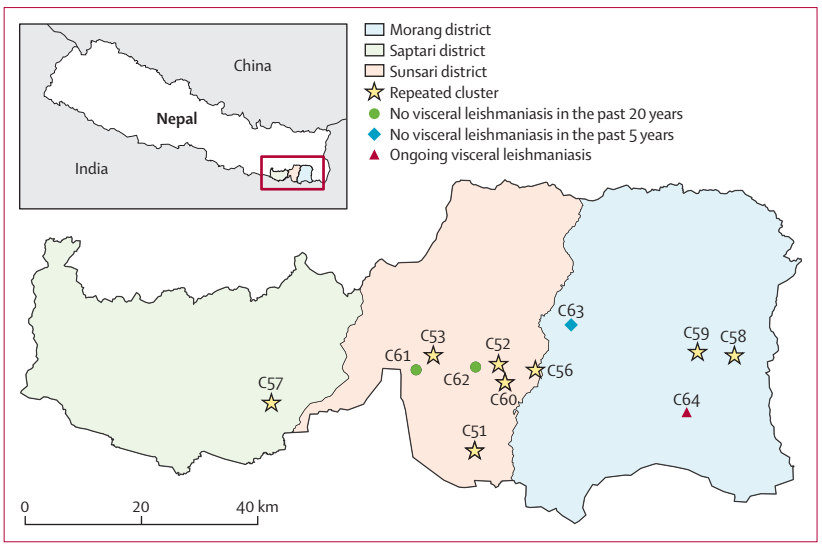

Figure 1: **Locations of study clusters**

Procedures

Trained fieldworkers did a door-to-door survey between Oct 17, 2016, and Dec 26, 2016. During a first visit, they sought informed consent from each head of the household and filled out a standard questionnaire on demographic details and visceral-leishmaniasis-related events (eg, previous visceral leishmaniasis or post-kala-azar dermal leishmaniasis history of any of the household members). Each individual was given a unique code; for individuals who had taken part in the Kalanet trial, the same code was reused in the Kalanet revisit survey. Data were recorded on an Android tablet using a custom-designed application developed in the open-source software application Open Data Kit. In a second visit, we invited the consenting households to a centrally located site within the cluster, after which all eligible household members (aged 2 years and older) were asked for their written consent (parents or guardians provided the written consent for those younger than 16 years; children aged 12 years and older were asked for their assent) to undergo venous blood sampling. The 2 mL collected blood was immediately transferred to pre-printed Whatman grade 3 filter paper, labelled with a unique barcode that was scanned using the same digital application containing the questionnaire data, thus linking all data from the same individual digitally. Dried filter papers were then placed in plastic bags containing silica gel and transported to the laboratory facilities of the B P Koirala Institute of Health Sciences, where we stored them at –20°C until analysis with DAT.

The primary outcome of interest was prevalent infection with *L donovani* as measured with DAT (cutoff value ≥1:3200). The laboratory analysis followed the same procedure and was done by the same team as for the Kalanet data in 2006.[7] In the laboratory of the B P Koirala Institute of Health Sciences, DAT was done on the filter paper blood sample using the freeze-dried antigen of fixed, trypsin-treated, and stained promastigotes of *L donovani* obtained from ITM, Antwerp, Belgium, as described by Jacquet and colleagues.[10] Results were expressed in serum dilution titres ranging between 1:400 or greater and 1:51200 or greater.

See **Online** for appendix 2

Statistical analysis

We defined prevalent infection by *L donovani* as the main outcome of interest, as measured by a DAT assay with a cutoff value to define seropositivity of 1:3200 or greater as suggested by Harith and colleagues.[11] We compared the age-specific seroprevalence from the baseline cross-sectional survey of the Kalanet trial to that of the Kalanet revisit survey for the eight repeated clusters (C51–C53 and C56–C60) taken together. Participants were grouped in age categories using 10-year bands, with a single category for those aged 50 years and older. We also compared cluster-specific seroprevalence from the baseline survey to that of the revisit survey, taking all age groups together. Proportions summarised seroprevalence data, and were compared by χ^2 tests and 95% CIs using Wilson's method. We then fitted a Poisson model to the data to estimate the adjusted risk ratio for seropositivity in 2016 compared with 2006, controlling for cluster, sex, and age group. As part of the secondary analysis, we repeated all the above procedures and tests for different DAT cutoff values ranging from 1:1600 or greater to 1:51200 or greater; appendix 2 (pp 1–6). Data analysis was done using RStudio version 3.6.1.

For the **Open Data Kit software** see https://opendatakit.org/

	2006 baseline clusters (n=5409)	2016 repeated clusters (n=4221)	2016 new clusters (n=2388)
Sex			
Male	2610 (48·3%)	1867 (44·2%)	1035 (43·3%)
Female	2799 (51·7%)	2354 (55·8%)	1353 (56·7%)
Median age, years	22 (10–37)	26 (14–44)	25 (13–40)
Age group, years			
2–9	1209 (22·4%)	414 (9·8%)	358 (15·0%)
10–19	1281 (23·7%)	1137 (26·9%)	636 (26·6%)
20–29	915 (16·9%)	760 (18·0%)	367 (15·4%)
30–39	770 (14·2%)	609 (14·4%)	346 (14·5%)
40–49	569 (10·5%)	574 (13·6%)	281 (11·8%)
≥50	665 (12·3%)	727 (17·2%)	400 (16·8%)
Cluster			
C51	1093 (20·2%)	961 (22·8%)	NA
C52	520 (9·6%)	367 (8·7%)	NA
C53	666 (12·3%)	357 (8·5%)	NA
C56	516 (9·5%)	482 (11·4%)	NA
C57	889 (16·4%)	739 (17·5%)	NA
C58	693 (12·8%)	500 (11·8%)	NA
C59	459 (8·5%)	381 (9·0%)	NA
C60	573 (10·6%)	434 (10·3%)	NA
C61	NA	NA	591 (24·7%)
C62	NA	NA	371 (15·5%)
C63	NA	NA	535 (22·4%)
C64	NA	NA	891 (37·3%)

Data are n (%) or median (IQR). NA=not applicable.

Table: **Baseline characteristics for the 2006 Kalanet study[7] and the 2016 revisit study**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 17, 2016, and Dec 26, 2016, 4221 participants were tested with the DAT in the eight repeated clusters, 2765 of whom had serological data from 2006 available; 2354 (55·8%) of 4221 were women, and the median age of participants was 26 years (IQR 14–44). In the 2006 baseline survey, 5409 participants were tested with the DAT in the eight repeated clusters. 2799 (51·7%) of 5409 participants were women, and the median age was 22 years (IQR 10–37). The age distribution was similar in 2006 and 2016, except for the category of children aged younger than 10 years, who were under-represented in the 2016 sample (414 [9·8%] of 4221 of the total sample in 2016 was aged younger than 10 years *vs* 1209 [22·4%] of 5409 in 2006). In the four new clusters that were added in the 2016 survey, another 2388 participants were included; 1353 [56·7%] of 2388 were women, 1035 (43·3%) of 2388 were men, and the median age of participants was 25 years (IQR 13–40). Baseline characteristics for the 2006 and 2016 surveys can be found in the table.

In individuals from all age groups and all repeated clusters combined, the overall seroprevalence decreased from 8·4% (455 of 5409) (95% CI 7·7–9·2) in 2006 to 4·7% (200 of 4221; 4·1–5·4) in 2016 (p<0·0001). Results for DAT cutoff values other than 1:3200 or greater are shown in appendix 2 (pp 1–2).

In the 2006 and 2016 data, the seroprevalence was higher in older age groups within a given survey (figure 2). Between 2006 and 2016, the seroprevalence changed from 4·1% (50 of 1209, 95% CI 3·2–5·4) in 2006 to 0·5% (2 of 414, 0·1–1·7) in 2016 in the youngest age group (p<0·0001), from 8·4% (107 of 1281, 7·0–10·0) to 1·7% (19 of 1137, 1·1–2·6) in those aged 10–19 years (p<0·0001), from 7·5% (69 of 915, 6·0–9·4) to 3·9% (30 of 760, 2·8–5·6) in those aged 20–29 years (p=0·0027), from 10·8% (83 of 770, 8·8–13·2) to 5·6% (34 of 609, 4·0–7·7) in those aged 30–39 years (p<0·0008), from 11·6% (66 of 569, 9·2–14·5) to 8·5% (49 of 574, 6·5–11·1) in those aged 40–49 years (p=0·11), and from 12·0% (80 of 665, 9·8–14·7) to 9·1% (66 of 727, 7·2–11·4) in those aged 50 years and older (p=0·088). Exact numbers on seroprevalence per age group for other DAT cutoff values can be found in appendix 2 (pp 1–2). A graphical comparison of the 2006 and 2016 data per age group for all DAT cutoff values is shown in appendix 2 (p 5).

Figure 3 graphically represents the seroprevalence per cluster, comparing the 2006 and 2016 data. Seroprevalence in 2016 was lower than in 2006 in all repeated clusters, although not significantly so for all. Between 2006 and 2016 the seroprevalence changed from 8·0% (87 of 1093) to 5·2% (50 of 961) in C51 (p=0·016), from 11·9% (62 of 520) to 4·9% (18 of 367) in C52 (p<0·0005), from 10·4% (69 of 666) to 5·9% (21 of 357) in C53 (p=0·022), from 13·4% (69 of 516) to 6·6% (32 of 482) in C56 (p<0·0006), from 9·3% (83 of 889) to 6·5% (48 of 739) in C57 (p=0·045), from 4·3% (30 of 693) to 2·2% (11 of 500) in C58 (p=0·067), from 5·9% (27 of 459) to 1·3% (5 of 381) in C59 (p=0·0011), and from 4·9% (28 of 573) to 3·5% (15 of 434) in C60 (p=0·34). Importantly, in the new clusters C61 and C62, in which no visceral leishmaniasis has been reported in the past 20 years, seroprevalence was similar to that of the other clusters investigated in 2016 at a DAT cutoff of 1:3200 or greater; however, no titres of 1:12 600 or greater were found in these clusters. Exact numbers on seroprevalence for the different DAT cutoff values can be found in appendix 2 (pp 3–4). A graphical comparison of the 2006 and 2016 data per cluster for all DAT cutoff values can be found in appendix 2 (p 6).

A Poisson model adjusting for sex, cluster, and age group yielded an overall risk ratio of 0·44 (95% CI 0·37–0·52) for *L donovani* infection for participants in 2016 compared with 2006. The age group-specific risk

ratio was 0·04 (95% CI 0·01–0·16) in those aged 2–9 years, 0·18 (0·11–0·29) in those aged 10–19 years, 0·43 (0·28–0·67) in those aged 20–29 years, 0·41 (0·27–0·61) in those aged 30–39 years, 0·74 (0·51–1·07) in those aged 40–49 years, and 0·83 (0·60–1·14) in those aged 50 years and older. Exact numbers on age group-specific risk ratios for the different DAT cutoff values are shown in appendix 2 (p 4).

Discussion

Our study shows that seroprevalence of *L donovani* infection was significantly lower in communities studied in 2016 than in 2006. Seroprevalence in 2016 was close to zero in children born since the start of the elimination initiative, and was lower in 2016 than in 2006 for all age groups and all repeated clusters, though not significantly for all. Multivariate analysis revealed that the adjusted risk of being seropositive was 56% lower in 2016 than in 2006 and even 96% lower in children younger than 10 years. Overall, the data point to a decrease in transmission over a 10-year period, coinciding with the start of the kala-azar elimination initiative.

In addition, our study shows that DAT could provide a useful tool for monitoring of *L donovani* transmission in the peri-elimination phase on the Indian subcontinent. DAT has a semi-quantitative read-out, allowing for several binary cutoff titres to be evaluated depending on the context. The inclusion of two new clusters without any reported visceral leishmaniasis in the past allowed us to establish the specificity of the different DAT cutoffs in a Nepalese setting. An unexpected finding was that we found DAT titres up to 1:6400 in clusters in which no visceral leishmaniasis cases have been reported in the past 20 years, and which were therefore assumed to be non-endemic (C61 and C62). Several possible explanations exist for this finding. Most probable is that these clusters, which are located within endemic districts, but considered non-endemic in the absence of reported visceral leishmaniasis cases, are falsely considered to be non-endemic, meaning that silent transmission might be present, while not leading to any clinical disease due to an epidemiological equilibrium as a result of herd immunity. Alternatively, inhabitants of these clusters might have travelled and could have been infected outside their own village. In addition, DAT is not 100% specific for leishmania infection; cross-reactivity with other pathogens has been described. Cross-reactivity can be pronounced in human African trypanosomiasis, but can also occur at low levels in malaria and autoimmune disorders.[11,12] For clinical diagnosis of visceral leishmaniasis, the standard cutoff is usually either 1:1600 or greater or 1:3200 or greater (serum dilution titres), depending on the context.[11,13–15] For the detection of asymptomatic infection on the Indian subcontinent, different cutoffs have been used in different studies.[16,17] Our results suggest that researchers, policy makers, and any others involved in epidemiological studies might consider using a higher, more specific DAT cutoff titre to accurately distinguish between low and zero transmission rates in an elimination setting, although this recommendation should be further evaluated.

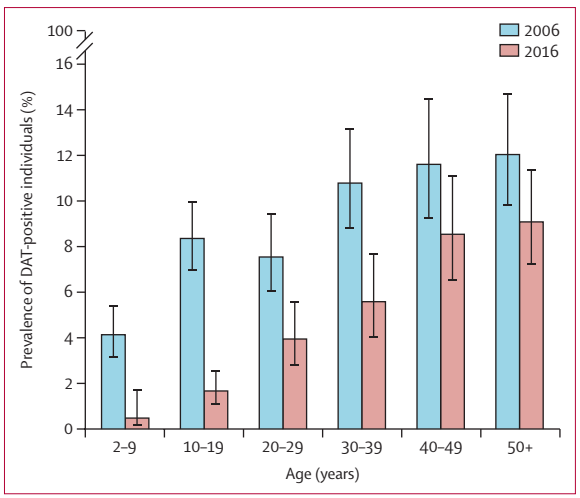

Figure 2: **Age-specific seroprevalence in 2006 and 2016**
DAT cutoff value of 1:3200 or greater. DAT=direct agglutination test. Error bars are 95% CIs. Only data from the eight repeated clusters are included in this figure.

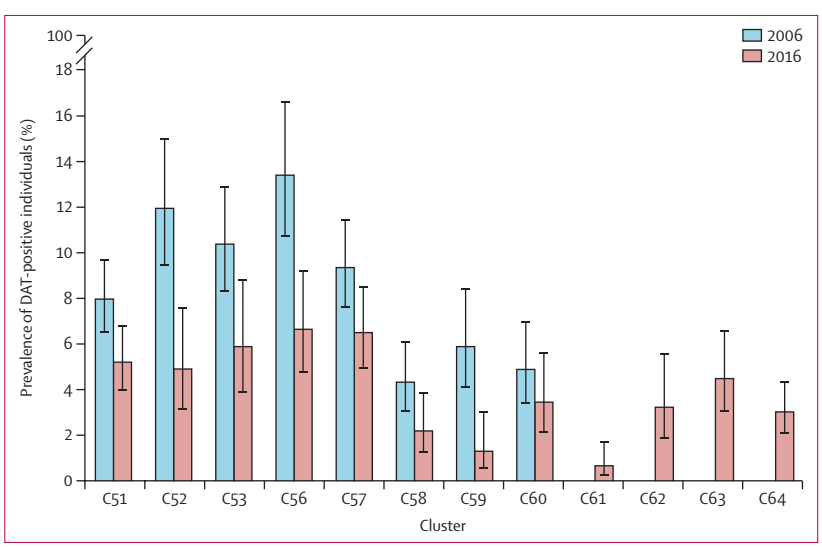

Figure 3: **Cluster-specific seroprevalence in 2006 and 2016**
DAT cutoff value 1:3200 or greater. No data on prevalence of DAT-positive individuals in 2006 were available for clusters C61 to C64. DAT=direct agglutination test. Error bars are 95% CIs.

Seroprevalence rates in the newly added clusters without visceral leishmaniasis cases in the past 5 years (C63) or 20 years (C61 and C62) were similar to those in clusters with continuous visceral leishmaniasis cases (C64 and repeated clusters). One explanation could be that currently, control activities such as active case finding and indoor residual spraying are being done reactively to new visceral leishmaniasis cases. In the absence of any visceral leishmaniasis cases in a cluster,

it is therefore possible that the intensity of the government's response and hence the effect of the elimination initiative in these particular clusters would be less substantial. This theory remains speculative though; without the 2006 baseline data for seroprevalence, we cannot draw any conclusions on the potential evolution of seroprevalence in these newly added clusters.

The main strength of this study was the availability of archived baseline serological data coinciding with the start of the elimination initiative, which allowed us to make a comparison of seroprevalence over a 10-year time period. Another strength was that utmost care was taken to make the 2016 survey procedures as much as possible a replicate of the 2006 ones, optimising the comparability between the two groups.

The main limitation of this study was the lower representation of children younger than 10 years in the sample taken in 2016, although the sample size in this age group remained sufficiently large to allow for robust conclusions based on the fact that the decrease in seroprevalence in this age group was still significant. While in 2006, children aged younger than 10 years constituted 22·4% of all participants, in 2016 their share was only 9·8%. Most probably, this discrepancy was because in 2016 venous sampling was used instead of a fingerprick to collect the blood, with a presumably lower acceptance rate for children. By directly transferring the venous blood to filter paper in the field, we were still able to perform the DAT analysis on whole blood, as had been the case for the samples collected through fingerprick in 2006, thereby optimising comparability between the two results.

In addition, it should be noted that most individuals who are seropositive will lose their antibodies over time and will become seronegative again, although little is known about the exact duration of this seropositive response. With such a large time gap between the two surveys, the 2016 seroprevalence will therefore be an underestimation of the true change in the cumulative incidence of infection compared between two timepoints. Without arguments for a change in the mean duration of infection, however, this underestimation can be assumed to be similar in 2006 and 2016 (prevalence of infection equals incidence of infection multiplied by mean duration of infection), and therefore both seroprevalences are comparable.

The current surveillance system in Nepal is facility-based monitoring of the trend in visceral leishmaniasis cases. This system is highly dependent on disease awareness among inhabitants and medical personnel, which is likely to further decline in the post-elimination phase. With an infection to disease ratio in Nepal of nine to one,[9] monitoring of infection would allow for more dynamic and accurate surveillance of transmission than monitoring of visceral leishmaniasis cases can ever achieve, not in the least because the long incubation period of this disease together with diagnostic and reporting delays will by definition lead to a delayed response if based on disease incidence.

Moreover, the current debate focuses on whether or not visceral leishmaniasis elimination as a public health problem should be followed by a more ambitious target such as elimination of transmission. Our study provides the first insight into the feasibility of this goal. At the same time, we should be careful when interpreting the declining seroprevalence as a direct result of the elimination initiative. All we are sure of is that the transmission rate has lowered over the past decade, coinciding with the elimination initiative in this region. The number of visceral leishmaniasis cases in India has shown to follow roughly 15-year cycles, making it difficult to assess whether the current decline is due to the success of the elimination initiative or to the natural periodicity of disease.[18] Most probably, it is a combination of both. Either way, a decrease in transmission will eventually result in fully susceptible populations again, making them prone to new outbreaks in the post-elimination phase. A solid surveillance system allowing for timely control measures will be paramount to prevent a resurgence of visceral leishmaniasis in the Indian subcontinent in the coming decade.

Contributors

MB and BO developed the conceptual framework. KC did the data analysis and drafted the manuscript. ELR and EH contributed to the data analysis, ELR to the creation of the figures. MB, SU, BO, ELR, AP, FC, EH, PK, and SR critically revised several versions of the manuscript. SU and BO organised the setup of the study; SU, BO, and KC coordinated the implementation of the field work. NRB and BK were responsible for the laboratory work involved in the study. AP, FC, and SR coordinated the original Kalanet trial. All authors approved the final version of the manuscript before submission.

Declaration of interests

We declare no competing interests.

Data sharing

Original data will not be shared, as the authors of this Article do not have transferable rights to all of the data. However, we will guide any requests to the owners of the respective databases.

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References

1 WHO Eastern Mediterranean Regional Office. Leishmaniasis. 2019. https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis (accessed July 15, 2019).

2 Hirve S, Boelaert M, Matlashewski G, et al. Transmission dynamics of visceral leishmaniasis in the indian subcontinent—a systematic literature review. *PLoS Negl Trop Dis* 2016; **10:** e0004896.

3 Al Abaidani I, Al-Abri S, Shaban M, et al. Decline in transmission of *Schistosomiasis mansoni* in Oman. *Infect Dis Poverty* 2016; **5:** 112.

4 Drakeley CJ, Corran PH, Coleman PG, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. *Proc Natl Acad Sci USA* 2005; **102:** 5108–13.

5 Miramontes R, Hill AN, Yelk Woodruff RS, et al. Tuberculosis infection in the United States: prevalence estimates from the National Health and Nutrition Examination Survey, 2011–2012. *PLoS One* 2015; **10:** e0140881.

6 Picado A, Das ML, Kumar V, et al. Effect of village-wide use of long-lasting insecticidal nets on visceral Leishmaniasis vectors in India and Nepal: a cluster randomized trial. *PLoS Negl Trop Dis* 2010; **4:** e587.

7 Picado A, Singh SP, Rijal S, et al. Longlasting insecticidal nets for prevention of *Leishmania donovani* infection in India and Nepal: paired cluster randomised trial. *BMJ* 2010; **341:** c6760.

8 Rijal S, Uranw S, Chappuis F, et al. Epidemiology of *Leishmania donovani* infection in high-transmission foci in Nepal. *Trop Med Int Health* 2010; **15** (suppl 2)**:** 21–28.

9 Ostyn B, Gidwani K, Khanal B, et al. Incidence of symptomatic and asymptomatic *Leishmania donovani* infections in high-endemic foci in India and Nepal: a prospective study. *PLoS Negl Trop Dis* 2011; **5:** e1284.

10 Jacquet D, Boelaert M, Seaman J, et al. Comparative evaluation of freeze-dried and liquid antigens in the direct agglutination test for serodiagnosis of visceral leishmaniasis (ITMA-DAT/VL). *Trop Med Int Health* 2006; **11:** 1777–84.

11 Harith AE, Kolk AH, Kager PA, et al. A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 1986; **80:** 583–86.

12 Harith AE, Kolk AH, Kager PA, et al. Evaluation of a newly developed direct agglutination test (DAT) for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis: comparison with IFAT and ELISA. *Trans R Soc Trop Med Hyg* 1987; **81:** 603–06.

13 Zijlstra EE, Ali MS, el-Hassan AM, et al. Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. *Trans R Soc Trop Med Hyg* 1991; **85:** 474–76.

14 Sundar S, Singh RK, Maurya R, et al. Serological diagnosis of Indian visceral leishmaniasis: direct agglutination test versus rK39 strip test. *Trans R Soc Trop Med Hyg* 2006; **100:** 533–37.

15 Mansour D, Abass EM, Mahamoud A, el Harith A. Qualitative and semi-quantitative comparison of an rK39 strip test and direct agglutination test for detection of anti-*Leishmania donovani* antibodies in the Sudan. *Iran J Immunol* 2009; **6:** 208–15.

16 Bimal S, Das VN, Sinha PK, et al. Usefulness of the direct agglutination test in the early detection of subclinical Leishmania donovani infection: a community-based study. *Ann Trop Med Parasitol* 2005; **99:** 743–49.

17 Schenkel K, Rijal S, Koirala S, et al. Visceral leishmaniasis in southeastern Nepal: a cross-sectional survey on *Leishmania donovani* infection and its risk factors. *Trop Med Int Health* 2006; **11:** 1792–99.

18 Rijal S, Sundar S, Mondal D, Das P, Alvar J, Boelaert M. Eliminating visceral leishmaniasis in South Asia: the road ahead. *BMJ* 2019; **364:** k5224.