Quantifying the infectiousness of post-kala-azar dermal leishmaniasis towards sandflies

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Running header: Xenodiagnosis in PKDL and VL patients

Abstract

Background: In the Indian subcontinent, visceral leishmaniasis (VL) incidence is on track to reach elimination goals by 2020 in nearly all endemic districts. Although not included in official targets, previous data suggest post-kala-azar dermal leishmaniasis (PKDL) patients can act as an infection reservoir.

Methods: We conducted xenodiagnosis on 47 PKDL patients and 15 VL patients using laboratory-reared *Phlebotomus argentipes*. In direct xenodiagnosis, flies were allowed to feed on the patient's skin for 15 minutes. For indirect xenodiagnosis, flies were fed through a membrane on the patient's blood. Five days later, blood-fed flies were dissected and examined by microscopy and/or PCR. A 3-mm skin snip biopsy (PKDL) or venous blood VL) was processed by quantitative PCR.

Results: Twenty-seven PKDL patients (57.4%) had positive results by direct and/or indirect xenodiagnosis. Direct was significantly more sensitive than indirect xenodiagnosis (55.3% vs 6.4%, p <0.0001). Those with positive xenodiagnosis had median skin parasite loads >1 $\log_{10}$ unit higher than those with negative results (2.88 vs 1.66, p<0.0001). In a multivariable model, parasite load, nodular lesions and positive skin microscopy were significantly associated with positive xenodiagnosis. Blood parasite load was the strongest predictor for VL. Compared to VL, nodular PKDL was more likely and macular PKDL less likely to result in positive xenodiagnosis, but neither difference reached statistical significance.

Conclusions: Nodular and macular PKDL, and VL, can be infectious to sand flies. Active PKDL case detection and prompt treatment should be instituted and maintained as an integral part of VL control and elimination programs.

Summary

We report xenodiagnosis results for 47 PKDL and 15 VL patients. Skin parasite load was strongly associated with positive xenodiagnosis. Compared to VL (66.7%), nodular PKDL was more likely (86%) and macular PKDL less likely (35%) to result in positive xenodiagnosis.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is characterized by prolonged fever, hepatosplenomegaly, wasting, and high mortality unless treated. Between 2005 and 2008, the estimated incidence in the Indian subcontinent ranged from 160,000 to 315,000 cases annually, figures amounting to 80% of the global burden of VL [1]. In this region, *Leishmania donovani* is transmitted by a single sand fly species, *Phlebotomus argentipes*, and VL patients provide the major infection reservoir during high incidence time periods [2].   In 2005, India, Bangladesh and Nepal announced a regional initiative aimed at the elimination of VL as a public health problem, defined as VL incidence < 1 case per 10,000 population per year at the subdistrict level [3].

In Bangladesh, rapid diagnostic tests were provided at health facilities starting in 2010, indoor residual spraying was introduced in 2012, and single dose AmBisome was implemented for VL treatment in 2014 [4]. However, VL incidence, which peaked in 2006, had already declined by 54% by 2009 and 80% by 2012, even before vector control began [4]. The cyclic incidence curve in Bangladesh is consistent with a century of observations in the subcontinent, where cycles of 5-10 years of high VL incidence followed by 10-20 years of low incidence have been documented since the early 20th century [2]. The fall in incidence after several peak years has been attributed to herd immunity, with resurgence occurring when the susceptible population has been replenished through births, in-migration and/or waning immunity in those previously infected [5, 6].

A dermatosis called post-kala-azar dermal leishmaniasis (PKDL) occurs in 5-15% of South Asian VL patients months to years after apparent cure [7]. PKDL is even more frequent in East Africa, particularly in Sudan [8]. Around 10% of PKDL patients report no history of VL

[5]. Characteristic lesions include hypo-pigmented macules, papules and nodules. Since PKDL patients are rarely systemically ill, most do not seek treatment and will be missed by passive surveillance [9]. Their public health importance stems from their potential role as reservoir hosts, capable of infecting the sand fly vector [10].

The only accepted proof of reservoir infectiousness is xenodiagnosis, which consists of feeding laboratory-reared sand flies on the putative reservoir host and demonstrating subsequent infection in the fly [11]. Indian VL patients were first shown to be infectious in 1924, findings confirmed in experiments over the subsequent decade [12-16] (Supplemental Table S1). In 1928, a patient with nodular PKDL lesions was shown to be infectious to *P. argentipes* [17]. By 1933, further experiments suggested that longer incubation and repeated feeding of flies, either on the same PKDL patient or uninfected laboratory animals, increased the yield; these same experiments showed that both nodular and macular PKDL patients could infect sand flies [18, 19]. These studies were followed by a hiatus of more than 50 years during which no human xenodiagnosis experiments were published.

In 1992, investigators in West Bengal described a community without previous VL that became an epidemic center in 1980 [10]. The authors postulated that the parasite was introduced by a single patient with nodular PKDL. The lack of previous VL cases implied lack of herd immunity, increasing the likelihood of rapid transmission. This history, together with positive xenodiagnosis results from 4 PKDL patients, including the community index case, provided data in support of the long-standing hypothesis that PKDL patients constitute an important inter-epidemic infection reservoir [6, 10].

Despite the evidence in the literature, no PKDL incidence target is included in South Asian VL elimination validation requirements, and recent papers have questioned the role of

PKDL as an infection reservoir [20]. With VL incidence at its lowest level in decades, elucidating the role of PKDL patients in transmission has become increasingly urgent. We report here the results of xenodiagnosis from 47 PKDL and 15 VL patients, the largest such study conducted to date. Our major aim is to provide quantitative data on the importance of PKDL patients as potential infection reservoirs in the context of regional VL elimination.

Materials and Methods

The protocol was approved by the Ethical Review Committee of ICDDR,B (#PR-14010). All patients provided written informed consent. Those with concurrent illness or a history of allergy to insect bites were excluded. Procedures were conducted at the Surya Kanta Kala-azar Research Centre (SKKRC), Mymensingh Medical College, under the supervision of two physicians. There were no adverse events. Following xenodiagnosis, patients were referred for treatment following national protocols.

Procedures. Lacking previous PKDL data, we based our sample size calculations on experience in *Leishmania* detection in cutaneous leishmaniasis (CL), and extrapolated to PKDL. Based on CL data, we assumed that 80% of probable PKDL cases would have parasites detectable by qPCR in skin specimens. We further assumed that at least 10% of confirmed PKDL cases would be infective to sand flies, with a precision of $\pm$5%. Based on these assumptions, we estimated that 44 confirmed PKDL cases would be needed for robust results. To allow a stratified analysis by lesion type, we decided to enroll a similar number of macular and nodular patients. The 3 patients enrolled in our previously published proof-of-concept paper [21] were not included in the present analyses. To provide a comparison group, we also included 15 VL patients.

PKDL patients 18 years or older were identified through active community searches, and were eligible if the diagnosis was confirmed by microscopy or PCR. Lesions were classified as macules, papules or nodules, and the affected area was quantified following published methods [22]. Following antisepsis, a snip biopsy was collected by elevating a 3-mm diameter cone of skin with a needle from an area with lesions and shaving it off with a scalpel. One-half was used for molecular assays; the other was used to prepare an impression smear, stained with Giemsa and examined by light microscopy.

VL patients were enrolled at the time of presentation at SKKRC. Blood was collected and separated into serum, buffy coat and red cells. VL patients fulfilled diagnostic criteria in the national guidelines (fever for at least 2 weeks, splenomegaly and positive results by rK39 rapid test (InBios, Seattle WA)). VL patients with a history of previous treatment were excluded.

Sand fly colony. A colony was established starting with wild blood-fed female *P. argentipes*. Twenty randomly chosen 1st and 2nd generation females were analysed by RT-PCR to rule out flavivirus and phlebovirus infection; all were negative. Sand flies used in xenodiagnosis belonged to these generations.

Xenodiagnosis. Direct xenodiagnosis was conducted as previously described [23]. The participant placed a hand into a small cage for 15 minutes. The cage contained 20 to 25 7-day-old female *P. argentipes* and 5 to 10 male flies. For patients without hand lesions, sand flies were placed in a 3-cm tube topped with gauze, which was held against a single lesion and the flies were allowed to feed for 15 minutes. Male and unfed female flies were removed with an electrical aspirator while blood-fed female flies were kept for 5 days at 27ºC and 85%-95% humidity, and fed on a 30% sucrose diet. Flies still living 5 days after the blood meal were anesthetized with CO_2 / chloroform, placed in a drop of sterile phosphate-buffered saline (PBS)

on a sterile microscope slide, and decapitated with sterile needles. The midgut was drawn out and placed in another drop of PBS, covered with a sterile cover slip and examined for promastigotes under microscope. For each patient, flies that died prior to 5 days and microscopy-negative flies were processed in separate pools by qPCR. For indirect xenodiagnosis, sand flies were allowed to feed on the patient's venous blood in a membrane feeder, following published methods [24]. Subsequent procedures were as outlined above.

qPCR: DNA was extracted from tissue, buffy coat and sand fly mid-gut specimens using Qiagen kits. Real time PCR was performed using Taqman primers and probes targeting the conserved region of *Leishmania* REPL repeats (L42486.1), following published methods [25]. To estimate parasite load, each run included a standard curve with DNA concentrations corresponding to 10,000 to 0.1 parasites and one reaction with molecular grade water as a negative control. Samples with cycle threshold (Ct) >40 were considered negative.

Analysis. The major outcome of interest was positive results by xenodiagnosis, defined as the detection of *L. donovani* promastigotes or DNA in at least one fly or pool of flies fed on that patient or their blood. Data were analyzed in SAS 9.0 and STATA 14.2. Univariate analyses utilized Fisher exact, Wilcoxon or Kruskal-Wallis tests as appropriate. Stepwise backwards elimination procedures were used to construct multivariable logistic regression models with p=0.05 for removal and addition. A receiver-operating-characteristic curve was constructed to identify the skin parasite load threshold with maximum sensitivity and specificity to differentiate PKDL patients positive and negative by xenodiagnosis (by maximizing Youden's index, the sum of the sensitivity and specificity). Bias-corrected 95% confidence intervals (CI) were computed for sensitivity and specificity, and area under the ROC curve (AUC) by bootstrapping with 10,000 replicates.

Results

From July 27 to November 29, 2017, 47 PKDL patients were enrolled. Of these, 22 (46.8%) were previously treated and 17 (36.2%) were female (Table 1). Two-thirds of PKDL patients came from the two highest-incidence subdistricts, Fulbaria and Trishal. All had been treated for VL prior to PKDL onset, 81% with pentavalent antimony. The median interval from VL treatment to PKDL onset was 3.9 years [interquartile range (IQR) 2.9, 5.8], and the median duration of lesions at the time of study was 3.5 years [IQR 1.7, 5.8]. Twenty-six (55.3%) patients had macular, papular or maculopapular lesions, while 21 (44.7%) had nodules or a combination of nodules and macules. All PKDL patients had positive results by qPCR in skin biopsies, with median parasite load of 275.5 parasites/μg tissue [IQR 41, 1232]; 32 (68.1%) also had positive results by microscopy. From August 6 to December 20, 2017, 15 VL patients were enrolled. VL patients were younger (p=0.05) and more likely to come from subdistricts other than Fulbaria and Trishal than PKDL patients (p=0.04 by 2-tailed Fisher exact test) (Table 2). Thirteen (86.7%) VL patients had positive results by qPCR in venous blood, with a median parasite load of 48 parasites/mL [IQR 8.5, 137.6].

Twenty-seven (57%) PKDL patients and 10 (66.7%) VL patients had positive results by xenodiagnosis. Supplementary Figures S1A-D show the median number of sand flies fed, surviving and examined by microscopy and qPCR, and composite xenodiagnosis results by patient. Of 47 PKDL patients, 26 had positive results by direct xenodiagnosis (17 by both microscopy and qPCR, 5 by microscopy only, and 4 by qPCR only). Three PKDL patients had positive results by indirect xenodiagnosis (1 by both microscopy and qPCR, and 2 by qPCR only); only one PKDL patient was positive by indirect but not direct xenodiagnosis. Among

PKDL patients, direct xenodiagnosis was much more likely than indirect xenodiagnosis to yield positive results (26 (55.3%) vs 3 (6.4%), p <0.0001). For VL patients, the difference in sensitivity was not significant (9 (60%) positive by direct vs 6 (40%) by indirect xenodiagnosis, p=0.47).

Among PKDL patients, factors associated with positive xenodiagnosis included having nodular lesions, younger age, positive microscopy and skin parasite load (Table 3). For patients with duration 3 to 12 months, 13 to 48 months and longer than 48 months, positive xenodiagnosis results were found in 50% (4/8), 59% (13/22) and 50% (10/20) (p>0.05 for all comparisons). Those with positive xenodiagnosis had median skin parasite loads >1 $\log_{10}$ unit higher than those with negative xenodiagnosis results (2.88 vs 1.66, p<0.0001) (Figure 1). Skin parasite load was significantly higher in nodular compared to maculopapular PKDL, and in those with positive microscopy (Supplemental Table S2). In the multivariable model, skin parasite load, nodular PKDL and positive microscopy all showed significant associations with positive xenodiagnosis (Table 4). Among VL patients, blood parasite load was the strongest predictor of positive xenodiagnosis; other factors with significant associations in univariable analyses included higher BMI and smaller liver size (Table 5). The small number of VLpatients precluded multivariable modeling. Compared to VL, nodular PKDL was more likely and macular PKDL less likely to result in positive xenodiagnosis (66.7% for VL, 85.7% for nodular [p=0.24] and 34.6% for macular PKDL [p=0.06]).

A receiver-operating-characteristic curve was constructed for skin parasite load as a classifier for positive xenodiagnosis in PKDL patients (Figure 2). The resulting threshold of 2.61 $\log_{10}$ parasites/µg genomic DNA showed sensitivity of 0.74 (95% CI 0.44-0.92) and

specificity of 0.90 (95% CI 0.64-1), and appears to be a better-than-random classifier since the lower bound of the 95% CI for the AUC is greater than 0.5 (AUC 95% CI 0.70–0.90).

Discussion

This study positions PKDL as one of the central challenges to VL elimination in the Indian subcontinent. Our data provide proof and quantification of the infectious potential of both nodular and macular PKDL patients, and strongly support the official policies in India and Bangladesh that all PKDL patients should be treated. However, without active case detection, most PKDL patients are never diagnosed and will continue to constitute a threat to sustained VL control [7, 9]. In our data, nodular PKDL and VL were both highly infectious, while macular PKDL patients were less so. However, PKDL patients go untreated for years. Hence their cumulative transmission potential may be higher than that of VL patients. Finally, our molecular results suggest a way forward for epidemiological studies, an endpoint for clinical trials, and a more effective public health approach to PKDL.

For some years succeeding peak VL incidence, herd immunity is high, especially within and close to households with previous VL cases [26]. Ninety percent of PKDL cases are themselves previous VL cases, so their households already have high immunity rates before onset of their skin lesions [5]. Onward transmission from PKDL cases will therefore become detectable only after sufficient time has passed for local susceptibility to rise, or when a PKDL patient migrates to a non-endemic community, as in the 1992 publication [10]. These factors may explain the failure to demonstrate transmission from PKDL patients in locations where herd immunity is likely still very high [20].

Our PCR cut-off was not a perfect predictor of xenodiagnosis results, and xenodiagnosis is not a perfect reflection of infectiousness in the field. Nevertheless, xenodiagnosis is still our best measure of infectiousness. Both measures have inherent biological variability. Recent animal models demonstrate that parasite distribution in skin is heterogeneous [27]; no human data exist to address the relative load in skin with and without lesions, or in different anatomical locations. Multiple skin biopsies from different locations would give a more nuanced picture of parasite load. Repeated blood meals were reported to increase xenodiagnosis yield in historical studies, and in an experimental animal-sand fly model system, a second blood meal caused a second cycle of parasite replication in flies and higher metacyclic promastigote production [18, 19, 28]. Serial xenodiagnoses, as in canine studies [29], would likely yield higher estimates of infectiousness. However, ethical considerations preclude repeated xenodiagnosis and multiple skin biopsies in humans.

Xenodiagnosis is not a practical assay for field studies. Less invasive skin specimens, if validated for accurate parasite load quantification, could open the door to population-based studies of transmission dynamics [30, 31]. Although there may be some individual-level misclassification, our data suggest that at the population level, qPCR results are likely to provide a useful reflection of transmission potential. Such studies would provide crucial inputs to model the interventions necessary to prevent VL resurgence when intensive elimination efforts are scaled back. For PKDL treatment trials, quantification of parasite load in skin could also provide a functional marker more relevant to disease control than, for example, repigmentation [7].

Xenodiagnosis has inherent challenges and potential biases. A sand fly colony is, by definition, a single population selected by captive breeding success, and different colonies of the same species show different feeding behavior and susceptibility to leishmanial infection [32].

Having more than one colony per region will contribute to more robust knowledge. Another limitation of our data was that flies were forced to feed in tubes from lesions; ethical considerations preclude allowing flies to feed on any accessible site as in canine xenodiagnosis [33]. Importantly, we did not demonstrate transmission from the infected fly to a subsequent mammalian host. Onward transmission has been clearly demonstrated in human studies in India [34] and more recently for other leishmanial species in experimental animals [35, 36]. Quantification of parasite loads and percentage of metacyclic promastigotes in individual flies infected via direct xenodiagnosis would be a first step towards assessing onward transmission potential and its relation to parasite load in infected humans [36].

In recent community-based studies in Bangladesh and India, 60 to 95% of PKDL cases were macular or maculopapular, while fewer than 10% were nodular [5, 9, 20]. Nevertheless 35% of macular PKDL patients also had positive xenodiagnosis results. Ignoring this form of the disease imperils the progress made in VL control in the Indian subcontinent. Our data, while generated in Bangladesh, also have implications for other regions of the world. No infectiousness data exist for East Africa, which now accounts for more than half the global VL disease burden [37]. In Sudan, PKDL is reported to occur in more than 50% of all VL patients, and unlike South Asia, nodular forms are frequent [8]. Because many PKDL cases resolve without treatment, long-standing policy in Sudan mandates withholding treatment for the first year unless the disease is severe [8]. Our data showed no difference in infectiousness based on PKDL duration and suggest that waiting 12 months for resolution provides a substantial parasite reservoir to sustain ongoing transmission.

In summary, our data indicate that all PKDL patients, regardless of lesion type or duration, should be treated promptly, and that active PKDL case detection should be instituted

and maintained to ensure comprehensive diagnosis and minimize the time that infectious patients go untreated. Great strides have been made in the control of VL in the Indian subcontinent. PKDL must be addressed in order to consolidate and sustain elimination, and perhaps eventually to permanently interrupt transmission.

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Figure Legends

Figure 1. Median $\log_{10}$ calculated parasites/µg genomic DNA in skin biopsies from post-kala-azar dermal leishmaniasis patients by composite xenodiagnosis results. Box indicates interquartile range, whiskers indicate minimum and maximum.

Figure 2. Receiver-operating-characteristic curve for skin parasite load as the predictor of positive results by xenodiagnosis in post-kala-azar dermal leishmaniasis patients. The indicated threshold of 2.61 $\log_{10}$ parasites/µg genomic DNA in skin biopsy shows sensitivity of 0.74 (95% CI 0.44-0.92) and specificity of 0.90 (95% CI 0.64-1). The threshold appears to be a better-than-random classifier because the lower bound of the 95% confidence interval for the area under the curve (AUC) is greater than 0.5 (AUC 95% CI 0.70–0.90). (All CIs are bias-corrected CIs computed by bootstrapping with 10000 replicates).

Supplemental files

Figure S1. Median numbers [interquartile range] of sand flies exposed and examined (top box), and composite xenodiagnosis results for VL and PKDL patients, Mymensingh, Bangladesh 2017. (A) direct xenodiagnosis of 47 post-kala-azar dermal leishmaniasis patients (PKDL) patients; (B) indirect xenodiagnosis of 47 PKDL patients; (C) direct xenodiagnosis of 15 visceral leishmaniasis (VL) patients; (D) indirect xenodiagnosis of 15 VL patients.

Table S1. Xenodiagnosis studies of visceral leishmaniasis and PKDL patients in the Indian subcontinent, 1924-2018.

Table S2. Skin parasite loads by xenodiagnosis results and type of PKDL lesions.

Table 1. Characteristics of post-kala-azar dermal leishmaniasis (PKDL) patients included in the xenodiagnosis study, Mymensingh, Bangladesh, 2017.

	PKDL patients N=47
Female N (%)	17 (36.2)
Age (years)	
Mean [SD]	35.3 [12.0]
Median [IQR]	33 [26, 45]
Residence	
Fulbaria	16 (34.0)
Trishal	15 (31.9)
Other upazilas of Mymensingh	12 (25.5)[1]
Outside Mymensingh District	4 (8.5)[2]
Antecedent visceral leishmaniasis (VL)	47 (100)
Initial KA treatment drug	
SSG monotherapy	38 (80.9)
AmBisome monotherapy	5 (10.6)[3]
Other	4 (8.5)[4]
Treated more than once for VL	6 (12.8)[5]
KA treatment to PKDL onset (years)	
Mean [SD]	5.2 [4.0]
Median [IQR]	3.9 [2.9, 5.8]
Duration of PKDL lesions (years)	
Mean [SD]	4.7 [4.0]
Median [IQR]	3.5 [1.7, 5.8]
PKDL Score	
Mean [SD]	146.4 [144.0]
Median [IQR]	97 [14, 255]
PKDL lesion types	
Macular, papular or maculopapular	26 (55.3)[6]
Nodular or nodules plus macules	21 (44.7)[7]
BMI (kg/m^2)	
Mean [SD]	20.8 [2.9]
Median [IQR]	20.6 [18.7, 22.9]
Skin biopsy positive by microscopy	32 (68.1)
Skin biopsy positive by qPCR	47 (100)
Parasite load (per µg genomic DNA)	
Mean [SD]	2164.7 [5636]
Median [IQR]	275.5 [41,1232]

[1]Bhaluka (6), Gaffargaon (1), Muktagachha (3), Mymensingh Sadar (2).

[2]Kaliakoir/Gazipur (2), Sreepur/Gazipur (1), Modhupur/Tangail (1).

[3]AmBisome single dose (2), multiple doses (2), unspecified (1).

[4]Miltefosine (2), AmBisome-paromomycin (1), miltefosine-paromomycin (1).

[5]Five treated twice, SSG then AmBisome (2); SSG followed by miltefosine (1); miltefosine followed by SSG (2); one patient treated 3 times, two courses of SSG followed by AmBisome.

[6]Macular only (24), papules only (1), maculopapular (1).

[7]Nodules only (1), mixed nodules and macules (20).

Table 2. Characteristics of visceral leishmaniasis (VL) patients included in the xenodiagnosis study, Mymensingh, Bangladesh, 2017.

	Visceral leishmaniasis patients N=15
Female N (%)	7 (46.7)
Age (years)	
Mean [SD]	28.7 [12.1]
Median [IQR]	24 [19, 35]
Residence	
Fulbaria	3 (20)
Trishal	2 (13.3)
Bhaluka	3 (20)
Other upazilas of Mymensingh[1]	2 (13.3)
Outside Mymensingh district[2]	5 (33.3)
BMI (kg/m^2)	
Mean [SD]	17.0 [2.4]
Median [IQR]	16.6 [15.8, 18.4]
Spleen size (cm below costal margin)	
Mean [SD]	8.7 [6.6]
Median [IQR]	6.0 [4, 12]
Liver size (cm below costal margin)	
Mean [SD]	4.0 [4.2]
Median [IQR]	3.0 [0, 8]
Blood positive by qPCR	13 (86.7)
Parasite load (per mL blood)	
Mean [SD]	159.8 [270.9]
Median [IQR]	48.0 [8.5, 137.6]

[1]Gaffargaon (1), Mymensingh Sadar (2)

[2]Ashuganj/Brahmanbaria (1), Gazipur Sadar/Gazipur (1), Gurudaspur/Natore (1), Dhonbari/Tangail (1), Gopalpur/Tangail (1)

Table 3. Factors associated with positive xenodiagnosis results among 47 PKDL patients, Mymensingh, Bangladesh, 2017.*

| PKDL patients | Xenodiagnosis results[1] | | |
	Negative N=20	Positive N=27	P value
Age			
Mean [SD]	40.0 [12.0]	31.9 [11.0]	
Median [IQR]	42.5 [28.5, 50]	30 [12, 40]	0.04
Female Sex n (%)	9 (45.0)	8 (30.0)	0.36
Previously treated PKDL	8 (40)	14 (51.9)	0.55
Duration of PKDL lesions (years)			
Mean [SD]	5.1 [4.5]	4.4 [3.7]	
Median [IQR]	3.6 [1.8, 8.9]	3.5 [1.5, 5.4]	0.84
PKDL Score			
Mean [SD]	153 [174]	142 [121]	
Median [IQR]	52 [11.5, 325]	111 [44, 230]	0.59
PKDL lesion types n (%)			
Macular, papular or maculopapular	17 (85.0)	9 (33.3)	0.0009
Nodular or nodules plus macules	3 (15.0)	18 (66.7)	
BMI (kg/m^2)			
Mean [SD]	21.2 [2.9]	20.5 [3.0]	
Median [IQR]	20.8 [18.6, 23.4]	20.5 [18.7, 22.0]	0.68
Skin biopsy positive by microscopy n (%)	10 (50.0)	22 (81.5)	0.03
Positive qPCR in skin n (%)	20 (100)	27 (100)	1.0
Parasite load (per µg genomic DNA)			
Mean [SD]	177.4 [304]	3636.8 [7130]	
Median [IQR]	46.1 [19, 212.6]	761.0 [205, 1958]	<0.0001
Log_{10} parasite load[2]			
Mean [SD]	1.76 [0.69]	2.92 [0.82]	
Median [IQR]	1.66 [1.26, 2.33]	2.88 [2.31, 3.29]	<0.0001

[1]Composite results by microscopy and/or PCR in sand flies from any of the xenodiagnosis experiments

[2]Parasite load per µg genomic DNA transformed as log_{10}(parasite load +1) to account for the possibility of zero parasite load

*We have reported both the median + IQR and mean + SD to provide some indication of the skew of the distribution

Table 4. Multivariable logistic regression model for factors associated with positive xenodiagnosis results in PKDL patients, based on stepwise backwards elimination with 0.05 significance level for removal and addition.

Factor	Odds ratio	95% confidence intervals	P value
Log_{10} parasite load in skin	7.25	1.78, 29.6	0.006
Nodular PKDL[1]	11.7	1.37, 100.7	0.03
Microscopy positive in skin	7.04	1.02, 48.7	0.05

[1]Compared to macular/maculopapular PKDL

Table 5. Factors associated with positive xenodiagnosis results among 15 visceral leishmaniasis patients, Mymensingh, Bangladesh, 2017.*

| Visceral leishmaniasis patients | Xenodiagnosis results[1] | | P value |
	Negative N=5	Positive N=10	
Age			
Mean [SD]	22 [2.9]	32.1 [13.7]	
Median [IQR]	23 [20, 24]	31.5 [19, 45]	0.33
Female Sex n (%)	3 (60.0)	4 (40.0)	0.61
BMI (kg/m^2)			
Mean [SD]	15.0 [1.8]	18.0 [2.0]	
Median [IQR]	15.2 [13.8, 15.8]	18.4 [16.4, 18.7]	0.03
Spleen size (cm below costal margin)			
Mean [SD]	12.0 [7.4]	7.1 [5.9]	
Median [IQR]	9.0 [7, 16]	5.5 [3, 12]	0.14
Liver size (cm below costal margin)			
Mean [SD]	7.0 [3.1]	2.5 [3.9]	
Median [IQR]	6.0 [6, 9]	0.75 [0, 3]	0.04
Blood positive by qPCR	3 (60.0)	10 (100)	0.10
Parasite load (per mL blood)			
Mean [SD]	13.5 [20.3]	232.9 [310.1]	
Median [IQR]	4.2 [0, 15.4]	93.3 [40.5, 321.6]	0.02
Log_{10} parasite load			
Mean [SD]	0.72 [0.75]	2.00 [0.64]	
Median [IQR]	0.72 [0, 1.22]	1.97 [1.61, 2.51]	0.01

[1]Composite results by microscopy and/or PCR in sand flies from any of the xenodiagnosis experiments

[2]Parasite load per mL blood transformed as log_{10}(parasite load +1) to account for zero parasite loads

*We have reported both the median+IQR and mean+SD to provide some indication of the skew of the distribution