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Quantifying the Infectiousness of Post-Kala-Azar Dermal Leishmaniasis Toward Sand Flies

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Background. On 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 polymerase chain reaction (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%, $P < .0001$). Those with positive xenodiagnosis had median skin parasite loads >1 $\log_{10}$ unit higher than those with negative results (2.88 vs 1.66, $P < .0001$). 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.

Keywords. xenodiagnosis; post-kala-azar dermal leishmaniasis; visceral leishmaniasis; transmission.

Visceral leishmaniasis (VL), or kala-azar, shows prolonged fever, hepatosplenomegaly, wasting, and high mortality if not treated. Between 2005 and 2008, the estimated incidence on the Indian subcontinent ranged from 160 000 to 315 000 cases annually, representing 80% of the global burden [1]. There, *Leishmania donovani* is transmitted by a single sand fly species, *Phlebotomus argentipes*, and VL patients are the main infection reservoir during high incidence periods [2]. In 2005, India, Bangladesh, and Nepal announced a regional initiative aimed at eliminating VL as a public health problem, defined as VL incidence <1 case per 10 000 population annually at the subdistrict level [3].

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

Post-kala-azar dermal leishmaniasis (PKDL) dermatosis occurs in 5%–15% of South Asian VL patients months to years after cure [7]. PKDL is even more frequent in East Africa, particularly in Sudan [8]. Lesions include hypopigmented 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, infecting sand flies [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

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to be infectious in 1924, and findings were confirmed 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, additional experiments suggested that longer incubation and repeated feeding of flies on the same PKDL patient increased the yield; furthermore, both nodular and macular PKDL patients could infect sand flies [18, 19]. These studies were followed by more than 50 years without human xenodiagnosis publications.

In 1992, investigators in West Bengal described a community without previous VL which became an epidemic center in 1980 [10]. They postulated that the parasite was introduced by a single nodular PKDL patient in a naive population, leading to rapid transmission. This history, together with positive xenodiagnosis results from 4 PKDL patients, including the community index case, supported the long-standing hypothesis that PKDL patients constitute an important interepidemic reservoir [6, 10].

Despite the above evidence, no PKDL target is included in South Asian VL elimination validation requirements; moreover, a recent article questioned the role of PKDL as an infection reservoir [20]. With VL incidence at its lowest level in decades, elucidation of the role of PKDL patients in transmission is urgent. Here, we report 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.

METHODS

The protocol was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (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, Bangladesh, under the supervision of 2 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 cutaneous leishmaniasis extrapolated to PKDL. Therefore, we assumed that 80% of probable PKDL cases would have parasites detectable by quantitative polymerase chain reaction (qPCR) in skin specimens and that at least 10% of confirmed PKDL cases would be infectous to sand flies (precision of ±5%). Considering these assumptions, we estimated that 44 confirmed PKDL cases would be needed for robust results. To allow for a stratified analysis by lesion type, we enrolled a similar number of macular and nodular patients. The 3 patients described in our previous proof-of-concept study were not included here [21]. Fifteen VL patients served as a comparison group.

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

VL patients were enrolled at the time of presentation at SKKRC if they fulfilled diagnostic criteria in the national guidelines (>2 weeks fever, splenomegaly, and positive results by rK39 RDT [InBios, Seattle WA]). VL patients with a history of previous treatment were excluded. Collected blood was separated into serum, buffy coat, and red cells.

Sand Fly Colony

A colony was established starting with wild blood-fed female *P. argentipes*. Twenty randomly chosen first- and second-generation females were analyzed using real-time (RT) PCR to rule out flavivirus and phlebovirus infection; all were negative.

Xenodiagnosis

Direct xenodiagnosis was conducted as previously described [23]. The participant placed a hand into a cage for 15 minutes; the cage contained 20 to 25 seven-day-old female *P. argentipes* and 5 to 10 male flies. For patients without hand lesions, sand flies were placed in a 3-cm diameter tube topped with gauze, and held against a single lesion for flies to feed for 15 minutes. Male and unfed female flies were removed with an aspirator while blood-fed female flies were kept for 5 days at 27°C, 85%–95% humidity, and fed on a 30% sucrose diet. Flies still living 5 days after the blood meal were anesthetized with carbon dioxide/chloroform, placed in a drop of sterile phosphate-buffered saline (PBS) on a microscope slide, and decapitated with needles. The midgut was drawn out and placed in another PBS drop, covered with a cover slip, and examined for promastigotes under a microscope. For each patient, flies that died prior to 5 days and microscopy-negative flies were processed in separate pools using qPCR. For indirect xenodiagnosis, sand flies were allowed to feed on patients' venous blood in a membrane feeder, following published methods [24], with subsequent procedures as described above.

qPCR

DNA was extracted from tissue, buffy coat, and sand fly mid-gut specimens using Qiagen kits. RT-PCR was performed following published methods using Taqman primers and probes targeting the conserved region of *Leishmania* REPL repeats (L42486.1) [25].

To estimate parasite load, each run included a standard curve with DNA concentrations corresponding to 10 000 to 0.1 parasites and 1 reaction with molecular-grade water as a negative control. Samples with cycle threshold >40 were considered negative.

Analyses

The major outcome was positive results by xenodiagnosis, defined as the detection of *L. donovani* promastigotes or DNA in at least 1 fly or pool of flies fed on that patient or their blood. Data were analyzed using SAS 9.0 and Stata 14.2. Univariate analyses utilized nonparametric tests as appropriate. Stepwise backward elimination procedures were used to construct multivariable logistic regression models with $P = .05$ for removal and addition. A receiver-operating-characteristic (ROC) 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, ie the sum of the sensitivity and specificity). Bias-corrected 95% confidence intervals (CIs) were computed for sensitivity and specificity and area under the ROC curve (AUC) by bootstrapping with 10 000 replicates.

RESULTS

From July 2017 to November 2017, 47 PKDL patients were enrolled; 22 (46.8%) were previously treated and 17 (36.2%) were female (Table 1). Two-thirds came from the 2 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 were positive by skin biopsy qPCR, with median parasite load of 275.5 parasites/μg tissue (IQR, 41, 1232); 32 (68.1%) were also positive by microscopy. From August 2017 to December 2017, 15 VL patients were enrolled. VL patients were younger ($P = .05$) and more likely to come from subdistricts other than Fulbaria and Trishal compared with PKDL patients ($P = .04$ by 2-tailed Fisher exact test; Table 2). Thirteen (86.7%) VL patients had positive results by qPCR in venous blood, with 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 S1*A–D* show the median number of sand flies fed, surviving, and examined using microscopy and qPCR, as well as 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, 2 by qPCR only); only 1 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%], $P < .0001$). For VL patients, the difference in sensitivity was not significant (9 [60%] positive by direct vs 6 [40%] by indirect xenodiagnosis; $P = .47$).

Table 1. Characteristics of Post-Kala-Azar Dermal Leishmaniasis Patients Included in the Xenodiagnosis Study: Mymensingh, Bangladesh, 2017

Characteristic	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)[a]
Outside Mymensingh District	4 (8.5)[b]
Antecedent VL	47 (100)
Initial VL treatment drug	
SSG monotherapy	38 (80.9)
AmBisome monotherapy	5 (10.6)[c]
Other	4 (8.5)[d]
Treated more than once for VL	6 (12.8)[e]
VL 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)[f]
Nodular or nodules plus macules	21 (44.7)[g]
Body mass index (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 quantitative polymerase chain reaction	47 (100)
Parasite load (per microgram genomic DNA)	
Mean [SD]	2164.7 [5636]
Median [IQR]	275.5 [41, 1232]

Abbreviations: IQR, interquartile range; PKDL, post-kala-azar dermal leishmaniasis; SD, standard deviation; SSG, sodium stibogluconate; VL, visceral leishmaniasis.

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

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

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

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

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

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

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

Table 2. Characteristics of Visceral Leishmaniasis Patients Included in the Xenodiagnosis Study: Mymensingh, Bangladesh, 2017

Characteristic	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[a]	2 (13.3)
Outside Mymensingh District[b]	5 (33.3)
Body mass index (kg/m^2)	
Mean [SD]	17.0 [2.4]
Median [IQR]	16.6 [15.8, 18.4]
Spleen size (centimeters below costal margin)	
Mean [SD]	8.7 [6.6]
Median [IQR]	6.0 [4, 12]
Liver size (centimeters below costal margin)	
Mean [SD]	4.0 [4.2]
Median [IQR]	3.0 [0, 8]
Blood positive by quantitative polymerase chain reaction	13 (86.7)
Parasite load (per milliliter blood)	
Mean [SD]	159.8 [270.9]
Median [IQR]	48.0 [8.5, 137.6]

Abbreviations: IQR, interquartile range; SD, standard deviation.

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

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

Among PKDL patients, factors associated with positive xenodiagnosis included 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) ($P > .05$ for all comparisons). Positive xenodiagnosis patients had median skin parasite loads >1 $\log_{10}$ unit higher than negative xenodiagnosis patients (2.88 vs 1.66, $P < .0001$) (Figure 1). Skin parasite load was significantly higher in nodular compared with maculopapular PKDL patients and in positive microscopy cases (Supplementary Table S2). In the multivariable model, skin parasite load, nodular PKDL, and positive microscopy showed significant associations with positive xenodiagnosis (Table 4). Among VL patients, blood parasite load was the strongest predictor of positive xenodiagnosis; Table 5 shows other factors with significant associations in univariable analyses. The small number of VL patients precluded multivariable modeling. Compared with VL, nodular PKDL was more likely and macular PKDL was less likely to result in positive xenodiagnosis (66.7% for VL, 85.7% for nodular [$P = .24$] and 34.6% for macular PKDL [$P = .06$]).

Figure 2 shows an ROC curve for skin parasite load as a classifier for positive xenodiagnosis in PKDL patients. 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 >0.5 (AUC 95% CI, 0.70–0.90).

DISCUSSION

This study positions PKDL as one of the central challenges to VL elimination on the Indian subcontinent. Our data provide proof and quantification of the infectious potential of nodular and macular PKDL patients, strongly supporting India's and Bangladesh's official policies that indicate treatment for all PKDL patients. 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 following 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 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 nonendemic community, as noted by Addy and Nandy [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 cutoff was not a perfect predictor of xenodiagnosis results, nor is xenodiagnosis a perfect reflection of infectiousness. Nevertheless, xenodiagnosis is still the best measure of infectiousness. Both measures have inherent biological variability. Recent animal models demonstrate that skin parasite distribution is heterogeneous [27]; no human data exist to address the relative load in skin with and without lesions or in different locations. Multiple skin biopsies from different locations would give a more nuanced picture of parasite load. In historical studies, repeated blood meals increased xenodiagnosis yield; in an experimental animal–sand fly model, 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

Table 3. Factors Associated With Positive Xenodiagnosis Results Among 47 Post-Kala-Azar Dermal Leishmaniasis Patients: Mymensingh, Bangladesh, 2017

Factor	Xenodiagnosis Results[a]		*P* Value
	Negative	Positive	
PKDL patients	N = 20	N = 27	
Age			
Mean [SD]	40.0 [12.0]	31.9 [11.0]	…[b]
Median [IQR]	42.5 [28.5, 50]	30 [12, 40]	.04
Female sex n (%)	9 (45.0)	8 (30.0)	.36
Previously treated PKDL	8 (40)	14 (51.9)	.55
Duration of PKDL lesions (years)			
Mean [SD]	5.1 [4.5]	4.4 [3.7]	…[b]
Median [IQR]	3.6 [1.8, 8.9]	3.5 [1.5, 5.4]	.84
PKDL score			
Mean [SD]	153 [174]	142 [121]	…[b]
Median [IQR]	52 [11.5, 325]	111 [44, 230]	.59
PKDL lesion type n (%)			
Macular, papular, or maculopapular	17 (85.0)	9 (33.3)	.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]	…[b]
Median [IQR]	20.8 [18.6, 23.4]	20.5 [18.7, 22.0]	.68
Skin biopsy positive by microscopy n (%)	10 (50.0)	22 (81.5)	.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]	…[b]
Median [IQR]	46.1 [19, 212.6]	761.0 [205, 1958]	<.0001
Log_{10} parasite load[c]			
Mean [SD]	1.76 [0.69]	2.92 [0.82]	…[b]
Median [IQR]	1.66 [1.26, 2.33]	2.88 [2.31, 3.29]	<.0001

We report both the median + IQR and mean + SD to provide some indication of the skew of the distribution.

Abbreviations: IQR, interquartile range; PKDL, post-kala-azar dermal leishmaniasis; SD, standard deviation.

[a]Composite results by microscopy and/or polymerase chain reaction in sand flies from any of the xenodiagnosis experiments.

[b]Mean + SD shown to provide indication of skew of the distribution; *P* value based on Wilcoxon rank sum test

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

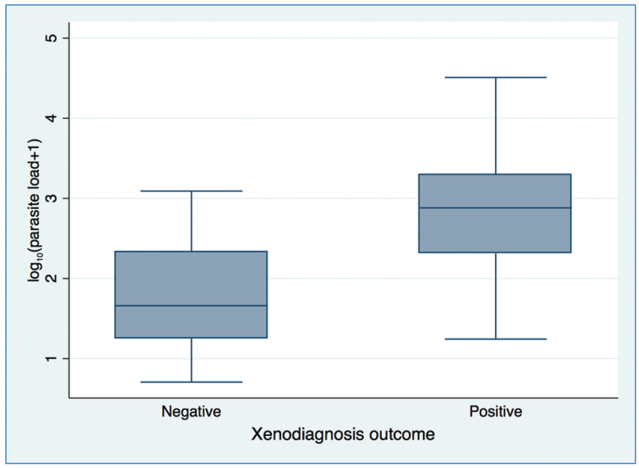

Figure 1. Median log_{10} calculated parasites per microgram genomic DNA in skin biopsies from post-kala-azar dermal leishmaniasis patients by composite xenodiagnosis results. Box indicates interquartile range; whiskers indicate minimum and maximum.

considerations preclude repeated xenodiagnosis and multiple skin biopsies in humans.

Xenodiagnosis is not practical 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 for the prevention of VL resurgence when intensive elimination efforts are scaled back. For PKDL treatment trials, skin parasite load quantification could also provide a functional marker that is more relevant to disease control than, for example, repigmentation [7].

Xenodiagnosis has inherent challenges and biases. A sand fly colony is, by definition, a single population selected by captive breeding success, and different colonies of the same species show

Table 4. Multivariable Logistic Regression Model for Factors Associated With Positive Xenodiagnosis Results in Post-Kala-Azar Dermal Leishmaniasis Patients

Factor	Odds Ratio	95% Confidence Interval	*P* Value
Log_{10} parasite load in skin	7.25	1.78, 29.6	.006
Nodular post-kala-azar dermal leishmaniasis[a]	11.7	1.37, 100.7	.03
Microscopy positive in skin	7.04	1.02, 48.7	.05

Based on stepwise backward elimination with 0.05 significance level for removal and addition.

[a]Compared to macular/maculopapular post-kala-azar dermal leishmaniasis.

different susceptibility to infection [32]. Multiple colonies per region will provide more robust knowledge. Another limitation is 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 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 toward assessing onward transmission potential and its relation to parasite load in infected humans [36].

In recent community-based studies in Bangladesh and India, 60%–95% of PKDL cases were macular or maculopapular, while fewer than 10% were nodular [5, 9, 20]. Nevertheless, 35% of macular cases also had positive xenodiagnosis results. Ignoring this form of the disease imperils the progress made in VL control on the Indian subcontinent. Our data, while generated in Bangladesh, have implications for other regions. No infectiousness data exist for East Africa, which now accounts for more than half the global VL disease burden [37]. In Sudan, PKDL occurs in more than 50% of all VL patients, and unlike South Asia, nodular forms are frequent [8]. Because many PKDL cases resolve spontaneously within 1 year, long-standing policy in Sudan mandates withholding treatment for the first year unless the disease is severe [8]. Our data show no difference in infectiousness based on PKDL duration, suggesting that waiting 12 months for resolution provides a substantial parasite reservoir to sustain ongoing transmission.

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 on the Indian subcontinent. PKDL must be addressed in order to sustain elimination and perhaps eventually to permanently interrupt transmission.

Table 5. Factors Associated With Positive Xenodiagnosis Results Among 15 Visceral Leishmaniasis Patients: Mymensingh, Bangladesh, 2017

	Xenodiagnosis Results[a]		
Factor	Negative	Positive	*P* Value
Visceral leishmaniasis patients	N = 5	N = 10	
Age			
Mean [SD]	22 [2.9]	32.1 [13.7]	…[b]
Median [IQR]	23 [20, 24]	31.5 [19, 45]	.33
Female Sex n (%)	3 (60.0)	4 (40.0)	.61
BMI (kg/m^2)			
Mean [SD]	15.0 [1.8]	18.0 [2.0]	…[b]
Median [IQR]	15.2 [13.8, 15.8]	18.4 [16.4, 18.7]	.03
Spleen size (cm below costal margin)			
Mean [SD]	12.0 [7.4]	7.1 [5.9]	…[b]
Median [IQR]	9.0 [7, 16]	5.5 [3, 12]	.14
Liver size (cm below costal margin)			
Mean [SD]	7.0 [3.1]	2.5 [3.9]	…[b]
Median [IQR]	6.0 [6, 9]	0.75 [0, 3]	.04
Blood positive by qPCR	3 (60.0)	10 (100)	.10
Parasite load (per mL blood)			
Mean [SD]	13.5 [20.3]	232.9 [310.1]	…[b]
Median [IQR]	4.2 [0, 15.4]	93.3 [40.5, 321.6]	.02
Log_{10} parasite load[C]			
Mean [SD]	0.72 [0.75]	2.00 [0.64]	…[b]
Median [IQR]	0.72 [0, 1.22]	1.97 [1.61, 2.51]	.01

We report both the median + IQR and mean + SD to provide some indication of the skew of the distribution.

Abbreviations: IQR, interquartile range; SD, standard deviation.

[a]Composite results by microscopy and/or polymerase chain reaction in sand flies from any of the xenodiagnosis experiments.

[b]bMean + SD shown to provide indication of skew of the distribution; *p* value based on Wilcoxon rank sum test.

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

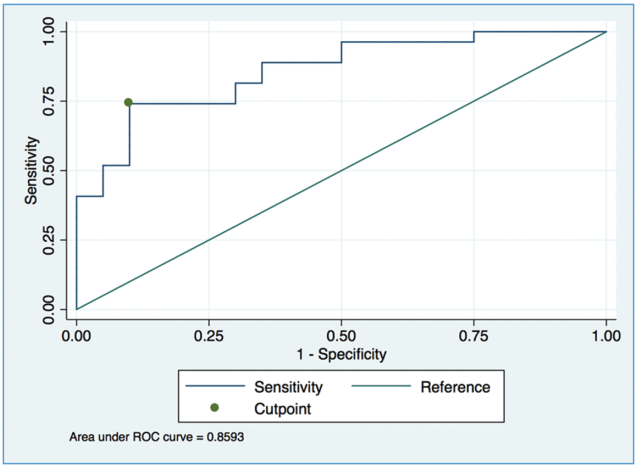

Figure 2. Receiver-operating-characteristic curve for skin parasite load as the predictor of positive results by xenodiagnosis in post-kala-azar dermal leishmaniasis patients. The indicated threshold of 2.61 $\log_{10}$ parasites per microgram genomic DNA in skin biopsy shows sensitivity of 0.74 (95% confidence interval [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% CI for the area under the curve (AUC) is >0.5 (AUC 95% CI, 0.70–0.90). (All CIs are bias-corrected CIs computed by bootstrapping with 10 000 replicates.) Abbreviation: ROC, receiver-operating-characteristic.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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