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Community-based Detection of Post Kala-azar Dermal Leishmaniasis (PKDL) and its Risk-Factors in an Endemic Region of Bihar, India

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Abstract

There have been relatively few large-scale studies of spread of Post Kala-azar Dermal Leishmaniasis (PKDL) and factors that affects its prevalence. Consequently, little is known about the dynamics of PKDL, or the confounding factors that may give rise to its spread. A large-scale survey-based study of PKDL prevalence was conducted in the endemic region of Araria in Bihar, India. The results of the study indicate a sample prevalence of as high as 7.9 cases per 10,000 individuals. Socio-economic and demographical factors were recorded for each study participant, and the influence of these factors on PKDL and non-PKDL incidence in the sample populations was analysed. Our results suggest that factors of caste, cattle shed proximity, and gender all contribute to the characterization of the PKDL-afflicted population. The mean household size for PKDL households was found to be 4.9, almost doubled the number observed for non-PKDL households. Individuals in the age-group 10-19 years old, Hindus, or those belonging to Schedule Caste are more likely to get PKDL than others in the population. Consideration of these factors can provide a clear starting point for further in-depth examination of their contribution to PKDL patterns.

Keywords: Epidemiology; Bihar; Post Kala-azar Dermal Leishmaniasis; Prevalence; Community survey; Socio-demographic factors

Introduction

Visceral leishmaniasis (VL) is a neglected potentially fatal disease transmitted by sand flies. Most of the estimated 200,000 to 400,000 annual new VL cases reported worldwide are located in India, with its state of Bihar accounting for 90% of cases [1]. Government of India, with aid from World Health Organization in 2005 initiated programs to eliminate visceral leishmaniasis. The initial elimination target was 2010, but this has now been put forward to 2015. However, with current reporting levels of VL cases (which are under estimated) it seems unlikely that 2015 goals will be achieved as objective of the VL elimination initiative is to reduce VL incidence below 1 per 10,000 per year at block level (i.e. just smaller administrative unit) within the district, but current incidence is around 20 per 10,000 per year at block level in many endemic regions. Surveillance of Post Kala-azar Dermal Leishmaniasis (PKDL) is critical to elimination of VL, as PKDL cases may serve as reservoirs for the VL disease transmission during the inter-epidemic period. Hence, one of the objectives for reducing the annual incidence of VL is to reduce cases of PKDL thus to interrupt VL transmission [2]. However, there are currently no accurate information/data on specific characteristics and burden of PKDL.

PKDL is generally regarded as a secondary complication of VL infection [3]. In Bihar, India, where PKDL and VL are endemic, both are caused exclusively by the leishmania donovani (LD) parasite [4]. Nearly 10-20% of patients cured of visceral leishmaniasis in India (where it is known as Kala-azar, or KA), and about 50% of patients cured of VL in the Sudan go on to develop PKDL as a result. In India, this can occur anywhere between 2 to 3 years after recovery from VL, whereas this number is between 0 to 6 months for Sudan [5-7]. Clinical presentation of PKDL usually begins with the appearance of macules on the face, followed by the development of papules and nodules in the skin, and plaques spread across the body's surface. PKDL is not fatal even without treatment, and given the apparent absence of animal reservoirs in India, patients with PKDL are a primary source of L. donovani-and thus they act as reservoirs for continued VL incidence for the region [8,9]. Treatment for PKDL requires an average of 6 months to a year for full recovery, though many cases take much longer because of the low treatment adherence rate among poor patients. Symptoms of PKDL are also often confused with several dermatological conditions, such as leprosy. With the exception of cosmetic considerations, PKDL does not cause any physical limitation, and thus, patients do not tend to seek medical care for it [10]. The risk of developing PKDL seems to be associated with incomplete sodium Sodium Stibo-Gluconate (SSG) treatment [11,12]. But PKDL has also been reported in patients treated with other anti-leishmanial drugs (e.g. miltefosine, amphotericin-B and paromomycin) and in individuals with no history of VL [13-15]. Considering the high numbers of asymptomatic VL cases thought to exist, it is plausible that PKDL cases without prior VL infection originate from asymptomatic VL infection, though the nature of this potential relationship remains unknown [16]. Given its close relationship to VL and the uncertainty surrounding prevalence and risk factors, more knowledge of PKDL's mechanism of action is required before undertaking any preventative endeavor or health policy recommendation. When considering the under reporting percentages of VL thought to exist in Bihar, India, and high incidence in this resource limited region [17,18], if PKDL does indeed arise out of complications from VL while also serving as a primary leishmania donovani reservoir, its study is vital to VL control and incidence reduction. Despite the potential for significant impact on the effectiveness of VL control measures, the number of studies estimating the burden of PKDL is limited. A study conducted by Singh

et al. in 2012 documented the overall prevalence of PKDL cases as ranging from 4.4 to 7.8 per 10,000 [19,20].

In 2005, Health Ministers of India, Bangladesh, and Nepal signed a Memorandum of Understanding for the elimination of VL from the Indian subcontinent by 2015. Active VL and PKDL case detection and their proper management is two important strategies of the elimination program. Until now, no definite method has been identified for active PKDL case detection but one proposed plan includes a house-to-house search for cases by public-health workers. The present household survey study and analysis reports these metric and other findings. We use cross-sectional data from Araria district of Indian state of Bihar to study the burden of PKDL and associated risk factors. This analysis revealed significant socio-economic and demographic factors associated with the differentiation of a sample PKDL population. These factors and the study from which they originate are presented in an effort to provide a clear means of inquiry and direction for future PKDL and VL research and prevention.

Methodology

Study area, population and study approach

The present study was a cross-sectional study, conducted in the areas of Forbesganj and Raniganj Block PHC's under Araria district of Bihar, India. Araria was selected as study district because of its consistently high endemicity and treatment of VL with Sodium Stibo Gluconate (SSG) in the district. Increased risk of PKDL is associated with treatment of VL with SSG.The district is in eastern part of the state and bordering with Nepal. It stretches in 9 Block/PHC areas (block; smaller administrative unit within the district, PHC; smaller health system unit to deliver health services at peripheral level). The study was conducted in randomly selected five health sub-centre (smallest health system unit) areas, based on the sample size requirement. Finally, a list of villages was prepared for each selected sub-centre and villages were selected randomly as per the sample size requirement. Households (HH) within the village were the smallest sampling unit under the study. Household survey approach was attempted to capture potential suspected case of PKDL. Trained field research investigators performed a census in Forbesganj and Raniganj. During the census, field research investigators assessed household number and the number of people in each household (household: consists of one or more people who live in the same dwelling and also share at meals or living accommodation and is the basic unit of analysis in many social, microeconomic and government models; Number of people: Individual sharing meals, dependant on the same dwelling and related by blood or law, they constitute a family i.e. One household). They also asked and recorded whether any member within the family suffered from Kala-azar in the past and if any of them currently had skin rash. The study was conducted between the financial years 2010-2011.

Study design

The study was design as community based cross-sectional study.

Sampling design

Multi-stage sampling technique was adopted for the selection study samples. Health sub-centre and villages were selected randomly; however within selected villages blanket approach was adopted as per the sample size requirement.

Sample-size

Assuming a PKDL prevalence of 0.17%, (95% CI 0.15–0.20), the required sample-size needed to search for PKDL cases was 55,225. A total population of 60,000 in the study area was sufficient for our study.

Trained field investigators

After consulting the local community leaders and obtaining their consent, the field investigators were trained by the RMRI resource personnel on clinical manifestations of PKDL, how did a PKDL case looks like, and how to look for the suspected PKDL case. One-day training was imparted to the workers, and pictures of skin lesions from PKDL patients were utilized. Additionally, the trained field investigators firstly performed a census includking socio-demographic information of household, using a specially designed pre-tested semi structured questionnaire for family members. A detailed history of infection, complications, treatments and response was obtained. In addition to that, photographic documentation of skin rashes of individual was done for each case. Medical records and histories were verified where possible. Prior to start of the survey it was decided to first complete the census and after completing census of the study area, survey workers along with study clinician visited each suspected household captured during census, for searching suspected PKDL cases and, if any individual suspected for PKDL was examined on the spot by the clinician. Study physician assessed patients with a history of VL and skin rash. Household members who had a history of VL but were not present during the home-visits were invited through the fellow villagers to visit the study clinician for assessment.

Evaluation and procedures

The study physician examined the suspected PKDL cases and requested an rK39 strip test (Kala-azar Detect TM Rapid Test, InBios International, Seattle, WA, USA) as needed. A trained field investigator performed the rK39 strip test as per the manufacturer's instructions. If the results were positive, the patients were considered probable PKDL cases (see definition below) and were requested to undergo slit skin scraping and blood collection. A qualified pathologist from the Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Agamkuan, in Patna performed slit skin scraping and collected skin specimens for staining, culture, and PCR test in the study clinic. The study physician collected up to 5 ml of venous blood in EDTA tubes and gently shook. After collection, the blood and skin specimens were transported to the Laboratory of the RMRIMS maintaining cold chain. PCR tests were performed in the Microbiology Laboratory of RMRIS on the following day, and the study physician was informed about PCR results immediately after testing.

Treatment and follow-up

We referred all cases positive for the LD parasite or positive for LD DNA by PCR to the nearest primary health centre/district hospital/ RMRIMS for treatment as per the national guideline for Kala-azar elimination. After referral, we followed up patients to find out whether they went to the hospital for admission, and if admitted, whether they completed full treatment courses. Further, we followed all the probable PKDL patients after one year from the date of their referral by household-visit and collected information on the status of their skin rash. Patients who were rK39 test-negative were referred to the respective higher health facility available in the district Hospital for further medical consultation.

Microscopy for LD bodies and PCR

A qualified and experienced pathologist of RMRI, Patna examined the Giemsa stained slit skin slides for amastigotes and also examined the wet film of culture for promastigotes. PCR LD antibody detection test was performed using described techniques [3,21,22].

Case definition

A suspected PKDL patient was a person from a Kala-azar-endemic area with a past history of Kala-azar and a skin lesion. A probable PKDL case was a patient with suspected PKDL who also had a positive rK39 test result. A confirmed PKDL case included those probably PKDL cases who also had the LD parasite identified by slit skin examination or culture, or had a PCR test positive for LD DNA.

Age-groups

The study population was divided into five age-groups: 0-14 years, 15-29 years, 30-44 years, 45-59 years and 60 & above years.

Duration of onset of PKDL

Duration was defined in months between the past treatment history of VL and the onset of skin lesion as reported by the patient.

Grading of PKDL

Skin lesions were graded into three major categories as described as grade I included scattered maculopapular or nodular rash in the face with or without some involvement in the upper chest or arm; grade II included maculopapular or nodular rash mostly of the face and extending to the chest, back, upper arms, and legs; and grade III included maculopapular or nodular rash covering most parts of the body, including hands and feet.

Progression of skin lesions

Based on case history given by the patients, the study physician categorized progression of skin lesions into rapid (conspicuous/obvious progression every month from onset), gradual (clear, steady progression from onset but at less detectable pace), and slow/stagnant (appearance, with little or no progression throughout early months).

Treatment management

All the confirmed PKDL cases were referred to concerned PHC if treatment is available otherwise RMRI/Higher health facility where treatment of PKDL was available. Delivery of PKDL Treatment was as per the national guideline for Kala-azar elimination in the country. A close monitoring was conducted by the study physicians with a team of research worker and with mobile connectivity to find out whether the referred PKDL individual went to the hospital for admission or not. If not, again attempt was made with the help of survey workers to visit such household and try to find out the reason/reasons of not attending the facility. Once again, such individual was invited to visit study clinician for further motivation to start treatment immediately. If admitted, whether they were completed full courses of treatment or not was recorded. If not completed the full course of treatment, they were again advised and motivated to get complete treatment of the disease.

Cure of PKDL

Cure was defined as complete disappearance of skin lesion(s) after treatment, as reported by the patient and assessed by the study physician.

Data analysis

Analysis of collected data was conducted in the open-source statistical computing package R. For each survey factor, it is assumed they follow a multivariate hyper geometric distribution with a probability mass function denoted by:

$$pmf = \frac{\prod_{i=1}^{l} \binom{K_i}{k_i}}{\binom{N}{n}}$$

Where l is the total number of distinct levels or categories within a factor, ki is the expected count of individuals found in the Kith level, and N is the total size of the sample population.

Mutually exclusive sample populations for individuals with PKDL and for those without were assumed to be separate and independent distributions, with the factors and associated levels,comprising the only commonalities between the two. For example, in Table 1, one of the factors is gender, and its levels are male and female.

Factor	Relative Frequency-Cases, Control	Odds Ratio	95% Confidence Interval	p-value
Subcenter				p = 0: 001
Halhaliya	20.0%/6.9%	3.37	1.42-7.13	00:01
Parwaha	4.4%/13.5%	0.3	0.03-1.14	00:05
Rampur	24.4%/35.8%	0.58	0.27-1.17	
Pachira	40.0%/17.2%	3.22	1.67-6.06	0: 001
Dumaria	4.4%/13.5%	0.3	0.03-1.14	00:05
Dwelling Type				p = 0: 10
Kutcha	66.7 %/54.6%	1.67	0.87-3.33	
Brick	8.9%/8.1%	1.11	0.29-3.07	
Pucca	4.4%/7.1%	0.61	0.07-2.35	
Thatched	20.0%/30.3%	0.58	0.24-1.22	
Gender				p = 0: 11
Male	42.2 %/53.3%	0.64	0.34-1.20	
Female	57.8%/46.7%	1.56	0.83-2.98	
Cattle Shed Type				p = 0: 109
No Cattle Shed	48.9 %/47.7%	1.05	0.57-1.97	
Inside	48.9%/40.9%	1.38	0.74-2.60	
Outside	2.2%/11.4%	0.18	0.004-1.04	
Caste				p = 0: 001

General	20.0 %/34.7%	0.47	0.20-1.00	00:05
OBC	20.0%/32.6%	0.52	0.22-1.09	00:01
SC	57.8%/27.6%	3.59	1.91-6.87	0: 001
ST	2.2%/5.1%	0.43	0.01-2.50	
Religion				p = 0: 01
Muslim	20.0 %/46.5%	0.29	0.12-0.61	0: 001
Hindu	80.0%/52.5%	3.61	1.71-8.53	0: 001
Sikh	0.0%/0.5%	0	0.00-16.59	
Christian	0.0%/0.5%	0	0.00-19.24	
Age				p = 0: 093
< 10 years old	13.0 %/28.2%	0.38	0.13-0.91	00:05
10 <20 years old	34.8%/23.6%	1.73	0.88-3.27	00:01
20 <30 years old	19.6%/16.3%	1.25	0.53-2.63	
30 <40 years old	17.4%/12.8%	1.43	0.58-3.11	
40 <50 years old	8.7%/9.2%	0.94	0.25-2.60	
50 <60 years old	4.3%/4.9%	0.88	0.10-3.37	
60 <70 years old	0.0%/3.5%	0	0.00-2.31	
70 <80 years old	0.0%/1.1%	0	0.00-7.47	
80 <90 years old	2.2%/0.3%	7.8	0.19-46.32	
90+ years old	0.0%/0.1%	0	0.00-131.16	
Household Size				p = 0: 001
1 individual	20.5 %/55.9%	0.2	0.09-0.43	0: 001
2 individuals	0.0%/6.6%	0	0.00-1.26	00:02
3 individuals	6.8%/8.6%	0.78	0.15-2.46	
4 individuals	6.8%/9.4%	0.72	0.14-2.26	
5 individuals	20.5%/7.6%	3.2	1.35-6.82	0: 002
6 individuals	22.7%/5.2%	5.43	2.38-11.31	0: 001
7 individuals	11.4%/3.1%	4.08	1.25-10.44	00:04
8 individuals	6.8%/1.7%	4.23	0.83-13.37	00:05
9+ individuals	2.3%/2.0%	2.74	0.07-16.32	

Table 1: Factors recorded in household study.

These two sample populations were tested with Pearson's Chi-square and Fisher's exact test in order to identify survey factors able to significantly differentiate the un-infected population from the PKDL population. Where the number of individuals in a level was low (< 6), Fisher's exact test was used. Statistical tests for factor levels were conducted by testing a single factor level against the summation of all other levels for a given factor.

Odds ratios giving the relative risk of contracting PKDL for an individual found in a particular level were estimated by Fisher's exact test. The binary response variable was the occurrence of PKDL in the sample population. Independent categorical variables consisted of the factors described in Table 2. A univariate and multivariate logistic regression using the generalized linear model methodology was also conducted, the results of which are displayed in Tables 3 and 4 respectively.

Levels	Estimate	Std. Error	Z value	CI-2.5%	-97.50 %	p-value
Age Class						
< 10 years old –	Ref	Ref	Ref	Ref	Ref	Ref
< 20	-1.219	0.475	-2.567	0.116	0.75	0.0103**
< 30	-0.951	0.527	-1.804	0.137	1.085	0.071
< 40	-0.786	0.578	-1.361	0.147	1.413	0.173
< 50	-0.716	0.646	-1.109	0.138	1.732	0.267
< 60	-0.647	0.817	-0.792	0.106	2.597	0.429
< 70	13.673	655.133	0.021	0	Inf	0.983
< 80	13.673	1162.804	0.012	0	Inf	0.991
< 90	-2.806	1.083	-2.591	0.007	0.505	0.0096**
< 100	13.673	4742.128	0.003	0	Inf	0.998
Cattle Shed						
No Cattle Shed-Ref	Ref	Ref	Ref	Ref	Ref	Ref
Cattle Shed inside	-0.155	0.302	-0.514	0.474	1.547	0.607
Cattle shed outside	1.657	1.023	1.62	0.707	38.899	0.105
Dwelling Type						
Kutcha – Ref	Ref	Ref	Ref	Ref	Ref	Ref
Brick	0.103	0.533	0.193	0.39	3.148	0.847
Pucca	0.664	0.731	0.909	0.464	8.132	0.363
Thatched	0.616	0.38	1.62	0.879	3.9	0.105
Caste						
General – Ref	Ref	Ref	Ref	Ref	Ref	Ref
OBC	-0.062	0.472	-0.131	0.373	2.369	0.896
SC	-1.29	0.387	-3.335	0.129	0.587	0.0009***
ST	0.274	1.054	0.26	0.167	10.379	0.795
Religion						
Hindu – Ref	Ref	Ref	Ref	Ref	Ref	Ref
Muslim	1.264	0.373	3.391	1.705	7.351	0.0007***
Sikh	12.842	627.187	0.02	0	Inf	0.984
Christian	12.842	674.767	0.019	0	Inf	0.985

Gender						
Male – Ref	Ref	Ref	Ref	Ref	Ref	Ref
Female	-0.444	0.302	-1.471	0.355	1.159	0.141
Subcenter						
Halhaliya	Ref	Ref	Ref	Ref	Ref	Ref
Parwaha	2.174	0.782	2.782	1.9	40.678	0.005***
Rampur	1.444	0.45	3.21	1.754	10.23	0.0013***
Pachira	0.216	0.409	0.528	0.557	2.765	0.597
Dumaria	1.935	0.558	3.468	2.32	20.676	0.0005***

Table 2: Univariate level analysis (**stands for significant and*** means highly significant).

Ethical Considerations

The study was conducted by the Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, one of the institutes under administrative control of Indian Council of Medical Research (ICMR), Department of Health Research, Ministry of Health and Family Welfare, Govt. of India and approval of Institutional Ethics Committee and Scientific Advisory Committee were obtained. Written informed consent was obtained from individuals before performing diagnostic tests.

It was also obtained from study households (HH) or their guardian for those aged less than 18 before en-rolling them in the study and conducting household interviews. Care was taken that all the proceedings (questions and replies) during household surveys will be kept confidential. The respondents were assured of the confidentiality of the information revealed by the respondents by non-dissemination of their name during presentation of the data.

Results

The census performed by the Field Research Assistants revealed a total population of 57,099 people from 11,653 households: 53% male and 47% were female. Significantly a much higher proportion (42%) were aged between 0-14 years and the median family size within each household was 5 (IQR 3-6).

Prevalence and incidence

For the study sample population of roughly 60,000 individuals, the prevalence of PKDL in the sample was found to be as high as 7.9 cases per 10,000 individuals. Using survey questionnaire on symptom history and laboratory tests, yearly sample incidence of PKDL from 2001 to 2011 was computed and is given in (Figure 1).

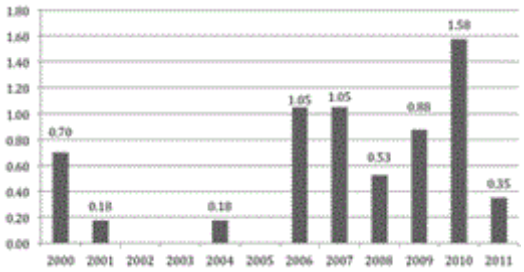

Figure 1: Yearly sample PKDL incidence per 10,000 individuals using survey questions on symptom history and laboratory tests.

Factors

Note: For all figures, the PKDL population is shown as dark, while the non-PKDL population is lighter in shade.

Geographical location

The physical distribution of PKDL incidence appears quite different than that of the non-PKDL population (Figures 2a and 2b).

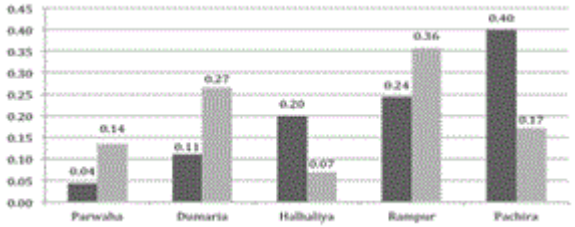

Figure 2a: Normalized PKDL, non-PKDL sample populations by sub-centre.

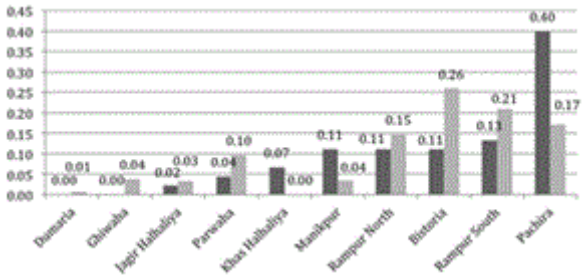

Figure 2b: Normalized PKDL, non-PKDL sample populations by village.

Prevalence was significantly higher or lower than expected values for specific subcenters and villages compared to the null hypothesis that PKDL is evenly diffuse throughout the study population. This finding is strongly significant both at the subcenter and village level, with p<0: 01 for Fisher's exact test of distributional independence for either level. Results of ANOVA on both the univariate and multivariate

logistic regression indicate that subcenter as a factor is of great importance in determining model significance. Despite this significance, this factor was excluded from the final multivariate regression analysis because the chosen study subcenters were selected for their prior high PKDL incidence, which would bias any significant multivariate model by its inclusion.

Household size

The mean household size for PKDL households was found to be 4.9; mean household size for non-PKDL households was nearly half that value. Thus, there is a marked difference in the distribution of household size for PKDL and non-PKDL populations, as shown in (Figure 3a and Figure 3b).

This is strongly supported by results of Fisher's exact test for distributional independence at $p<0.01$.

Despite the significance of household size in differentiating the PKDL population from the non-PKDL population, this factor was not significant in univariate analysis. This can partially be attributed to the fact that the PKDL population's maximum household size was 13 with empty intermediate levels, while for the non-PKDL population it was 22 with fewer empty levels.

The increased degrees of freedom for the test model lessened its significance. This can partially be attributed to the fact that the PKDL population's maximum household size was 13 with empty intermediate levels, while for the non-PKDL population it was 22 with fewer empty levels. Hence, the increased degrees of freedom for the test model lessened its significance.

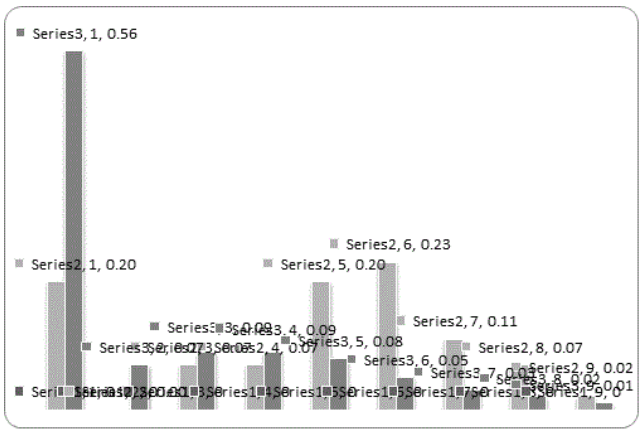

Figure 3a: Normalized PKDL, non-PKDL sample populations by household size.

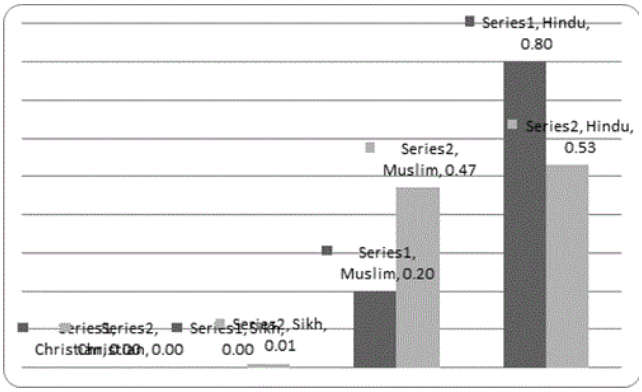

Figure 3b: Normalized PKDL, non-PKDL sample populations by religion.

The study was conducted by the Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, one of the institutes under administrative control of Indian Council of Medical Research (ICMR), Department of Health Research, Ministry of Health and Family Welfare, Govt. of India and approval of Institutional Ethics Committee and Scientific Advisory Committee were obtained as shown in Table 3.

Written informed consent was obtained from individuals before performing diagnostic tests. It was also obtained from study households (HH) or their guardian for those aged less than 18 before en-rolling them in the study and conducting household interviews.

Care was taken that all the proceedings (questions and replies) during household surveys will be kept confidential.

Factor	Residual Deviance	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	p-value>chi
Age Class	14.56	-3.973	0.027	0.04	0.035	0.044	0.111	0.104
Gender	2.196	-3.841	0.035	0.035	0.036	0.044	0.044	0.138
Caste	18.035	-3.993	0.03	0.031	0.035	0.057	0.057	0.0004***
Cattle Shed	5.646	-4.19	0.04	0.04	0.036	0.043	0.043	0.059
House Type	3.46	-3.901	0.032	0.044	0.036	0.044	0.044	0.326
Religion	15.077	-3.997	0.026	0.049	0.035	0.049	0.049	0.002 **
Subcenter	27.975	-4.064	0.026	0.033	0.034	0.033	0.068	0.00001***
Household Size	12.794	-4.007	0.032	0.041	0.035	0.046	0.053	0.849

Table 3: Univariate regression against null model** stands for significant and *** means highly significant.

The respondents were assured of the confidentiality of the information revealed by the respondents by non-dissemination of their name during presentation of the data. The study results yielded markedly different distributions of categorical data for the respective sample populations. Although further exploration of these sociological and demographical trends is needed before PKDL incidence can be directly tied thereto, these trends nonetheless provide possible paths of exploration into PKDL patterns previously little understood or altogether unknown. Similar socio-economic factors have already proven significant to VL prevalence in Bihar and thus it is reasonable to suggest further research towards establishing those factors common to both VL and PKDL.

Under the assumption that PKDL-afflicted individuals act as parasite reservoirs for continued VL incidence, future investigation of these factors and their contributions to PKDL prevalence is made even more urgent further exploration of these sociological and demographical trends is needed before PKDL incidence can be directly tied there, these trends nonetheless provide possible paths of exploration into PKDL patterns previously little understood or altogether unknown.

Similar socio-economic factors have already proven significant to VL prevalence in Bihar and thus it is reasonable to suggest further research towards establishing those factors common to both VL and PKDL. However, it is significant to the best t model of the multivariate regression.

Religion

As shown in (Figure 3b), there appears to be a significant difference between the PKDL and non-PKDL sample populations for religious affiliation. For the non-PKDL population, there is an insignificant difference between the frequency of Muslim individuals and those identifying as Hindu.

The PKDL population, however, is pre-dominated by a high number of Hindu individuals. This is strongly supported by the results of Fisher's exact test for distributional independence for p value equal to 0.01. In addition, religion was a significant factor in univariate regression. It was not, however, significant to the best t model of the multivariateregression.

Dwelling type

The factor of dwelling type was found to be statistically insignificant, both in differentiation of the PKDL population, and in univariate and multivariate regression.

Though it seems plausible that a dwelling built with less permanent and more porous materials (e.g. Kutcha and Pucca dwellings) might yield higher PKDL incidence, this was not the case, even for rescaled data.

With the idea that impermanent versus permanent dwellings might yield some statistical difference between populations, Kutcha and Pucca dwelling types were grouped together (both reasonably impermanent dwellings), and in the same manner brick and thatched dwellings (more permanent structures). This new grouping, however, did not yield statistical significance.

Caste

Controlling for caste, it is clear that there are marked differences in the distribution of the PKDL population as compared with the non-PKDL population as shown in (Figure 4a). Frequency for the General, OBC, and SC classes appears relatively even in the non-PKDL population, while the PKDL population shows a large spike in frequency for those who identify as a member of the 'SC' class. This trend is strongly significant ($p<0.01$) for Fisher's exact test of distributional independence. The factor of caste was highly significant to model formulation in both univariate aznd more importantly, multivariate regression.

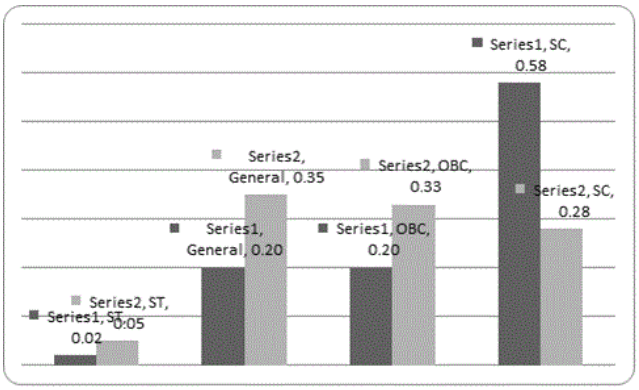

Figure 4a: Normalized PKDL, non-PKDL sample populations by caste.

Controlling for caste, it is clear that there are marked differences in the distribution of the PKDL population as compared with the non-PKDL population. Frequency for the General, OBC, and SC classes appears relatively even in the non-PKDL population, while the PKDL population shows a large spike in frequency for those who identify as a member of the 'SC' class. This trend is strongly significant ($p<0.01$) for Fisher's exact test of distributional independence. The factor of caste was highly significant to model formulation in both univariate and more importantly, multivariate regression.

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This trend is strongly significant ($p<0.01$) for Fisher's exact test of distributional independence. The factor of caste was highly significant to model formulation in both univariate and more importantly, multivariate regression as shown in (Figure 4b).

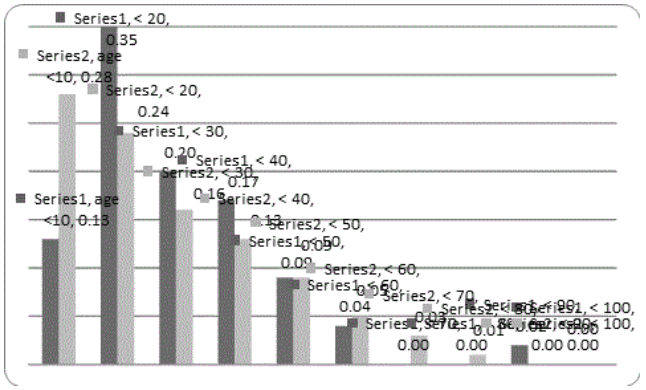

Figure 4b: Normalized distributions of PKDL, non-PKDL sample populations by age class (mutually exclusive).

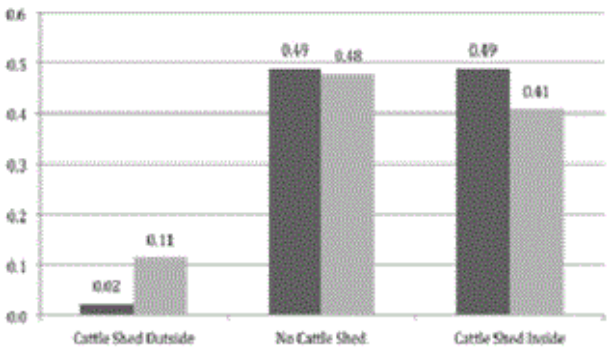

Figure 5a: Normalized distributions of PKDL, non-PKDL sample populations by cattle shed proximity.

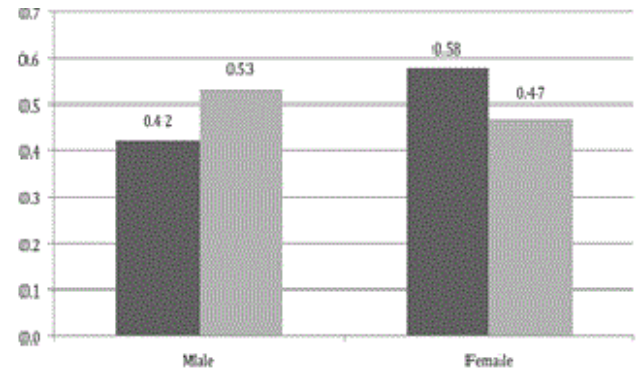

Figure 5b: Normalized distributions of PKDL, no n-PKDL sample populations by gender.

Cattle Shed Proximity

The cattle shed factor was not significant for the exact count data obtained in the study. If rescaled modestly (as frequency out of 100 instead of the PKDL sample population total), however, a significant result was obtained at ($p<0.05$), though the meaning of this result is ambiguous.

Model	AIC
Caste + Cattle Shed + Gender	721.95
Caste + Cattle Shed	722.07
Religion + Caste + Cattle Shed + Gender	722.74
Caste + Cattle Shed + Gender + Cattle Shed and Gender Interaction	722.76
Caste + Gender	722.87

Table 4: Multivariate analysis.

Less ambiguous, however, was this factor's contribution to model importance for both univariate and multivariate regression, in particular. Single predictor regression yielded weak significance to model importance ($p=0.059 > chi$), but multivariate regression yielded an increased response, a finding suggestive of interactions with other covariates.

Though not the best t multivariate model, it is of note that a model including an inter-action term between cattle shed proximity and gender came in as the 4th best t model as shown in Figure 5a and 5b.

Thus by its appearance in the majority of the multivariate best- t models, it is clear that the cattle shed factor is highly important to the formulation of best- t models, as shown in Table 4.

Gender

Despite an odds ratio of 1.56 for women contracting PKDL, gender did not appear to play a statistical role in differentiating the PKDL population from the non-infected general public. However, the factor of gender was found to be highly significant to the best- fitting multivariate model, despite having less significance in the univariate regression.

Further analysis of gender as a factor might investigate incidence among mothers rather than women as a whole, though this demographic was not recorded in the present study. There has been limited information on the PKDL prevalence and its key features. In this study, we collected and analyzed a cross-sectional survey to identify prevalence and primary aspects of PKDL in hyper endemic regions for VL as shown in Table 5.

	Mean	Mode
PKDL Cases	4.9 individuals	6
Non-PKDL	2.6 individuals	1

Table 5: Parameters of household size distributions for PKDL and non-PKDL populations.

Though this demographic was not recorded in the present study. There has been limited information on the PKDL prevalence and its key features. In this study, we collected and analysed a cross-sectional survey to identify prevalence and primary aspects of PKDL in hyper endemic regions for VL.

Additionally, the significance of number of children on PKDL incidence for mothers could be investigated, as well the nature of the potential interaction term between gender and cattle-shed proximity in the 4th best t model as shown in Table 6.

	Mean	Std Dev
PKDL Cases	22.82 years	15.83
Non-PKDL	22.53 years	17.45

Table 6: Parameters of age distributions for PKDL and non-PKDL populations.

Dwelling type

The factor of dwelling type was found to be statistically insignificant, both in differentiation of the PKDL population, and in univariate and multivariate regression. Though it seems plausible that a dwelling built with less permanent and more porous materials (e.g. Kutcha and Pucca dwellings) might yield higher PKDL incidence, this was not the case, even for rescaled data. With the idea that impermanent versus permanent dwellings might yield some statistical difference between populations, Kutcha and Pucca dwelling types were grouped together (both reasonably impermanent dwellings), and in the same manner brick and thatched dwellings (more permanent structures). This new grouping, however, did not yield statistical significance.

Discussion

Post kala-azar dermal leishmaniasis (PKDL) is a neglected complication of visceral leishmaniasis (VL) and is thought to be a reservoir for continued transmission of VL. Hence, understanding PKDL risk factors are critical to the elimination programs of VL. However, there has been limited information on the PKDL prevalence and its key features. In this study, we collected and analyzed a cross-sectional survey to identify prevalence and primary aspects of PKDL in hyper endemic regions for VL.

For the present study, the estimated PKDL prevalence ranged from 2.0 to 7.9 per 10,000 individuals. These findings are identical to those estimated in two other similar studies for India and Bangladesh [12,22]. For the statistically significant factors presented above, and even for those factors without proven significance, there is much insight to be gained from interpreting their potential role in PKDL prevalence.

In analyzing the distribution of age in PKDL, a potential explanation for the high incidence found in the second age class (ages 10-19) could lie in familial work roles. As a course of growing older and being allotted a larger share of responsibility in the family household, second age class children could be assigned increased duties relating to family livestock care, where, because of preferential feeding on livestock, a higher density of sand flies might be present. Until they are 10, younger children might be more likely to be kept inside by their mothers. This would explain the lower-than-expected incidence for the first age class. These results are in contrasts with findings from Nepal and Bangladesh, where young VL patients were not found to be at higher risk of developing PKDL than older children [11,22].

If the Nepal and Bangladesh studies also found that young kids were "not at higher risk" of developing PKDL compared to older children, this study is in agreement with those results, rather than contrasting them.

Explanations for the factor of religion on PKDL prevalence need not have any ideological basis, but rather grounding in the traditions and customs of each religion. For the two most common religions, Islam and Hinduism, differences in the traditional attire worn by respective followers might explain the differences found in PKDL prevalence for each. For example, the traditional attire of a Muslim follower generally leaves a smaller area of skin exposed, as compared to the attire worn by Hindu followers. Accordingly, it is possible that a biting female sandy encounters less of a barrier in landing on a human host and taking a blood meal for those with more exposed skin. This theory could explain the significantly higher prevalence for Hindu followers, although tests of the ability of sand flies to penetrate clothing of varying thickness would yield more definitive results.

As to the factor of geographical location, it is difficult to draw any conclusions there from, despite its significance in univariate regression. This again is due to the study's mandate of selecting geographical sub centers with known high rates of PKDL incidence. There is significant difference in the composition of castes from the PKDL population compared to its non-PKDL counterpart. Clearly, the caste with the highest prevalence of PKDL is the SC class. In India, this caste has traditionally been of lower socioeconomic standing, and it is this disadvantage which could predispose those SC individuals to higher rates of PKDL incidence, owing to the decreased access to health care and generally poorer states of health which can be associated with those who are economically less fortunate. Caste as a factor was found to be highly significant in multivariate regression.

There has been a debate as to whether the proximity of livestock influences the spread of VL or PKDL. The primary vector of VL and PKDL in Bihar and India, P. argentipes, preferentially feeds on livestock [4]. Logically, one may assume that increased presence of livestock would increase the presence of sand flies, and thus PKDL incidence would in-crease. It is also logical to assume, however, that because sand flies are feeding on livestock preferentially, they feed on human beings comparatively less. Many diverse assumptions could be formulated as to the reason for this factor's statistical ambiguity and weak significance in univariate regression, but it is clear that it is highly significant to multivariate model formulation.

A number of socio-economic and demographic factors have been explored and presented as important to the differentiation of PKDL populations from non-PKDL populations. The study results yielded markedly different distributions of categorical data for the respective sample populations. Although further exploration of these sociological and demographical trends is needed before PKDL incidence can be directly tied thereto, these trends nonetheless provide possible paths of exploration into PKDL patterns previously little understood or altogether unknown. Similar socio-economic factors have already proven significant to VL prevalence in Bihar [23], and thus it is reasonable to suggest further research towards establishing those factors common to both VL and PKDL. Under the assumption that PKDL-afflicted individuals act as parasite reservoirs for continued VL

incidence, future investigation of these factors and their contributions to PKDL prevalence is made even more urgent.

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Conflict of interest

The authors declare that they have no conflict of interest.

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