Sero-Prevalence and Risk Factors associated with Visceral Leishmaniasis

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ABSTRACT

Background and Objectives: The epidemiological studies, clinical presentation and pathogenesis of visceral leishmaniasis (VL) are reviewed along with the current control strategies and research challenges to launch a regional VL elimination programme. VL causes considerable morbidity and mortality both in terms of geographical spread and incidence. Combinations of different risk factors are responsible for leishmaniasis. However, a disease control strategy is still unpractical because the reservoir host, the ecology and behaviour of the vector have not been fully clarified yet. Therefore, this sero-epidemiological study was designed to analyze socio- economic status, Socio-behavioural and environmental factors associated with the prevalence of visceral leishmaniasis. **Materials and Methods:** The descriptive cross sectional study based on non-experimental design among 300 study subjects were carried out at Bindhi Village of Dhanusha district in the central development region of Nepal. Data were collected using close type of questionnaire; direct personal interview, observation and Statistical analysis were performed using the DBASE IV and EPI-INFO version 6 STATA. The direct agglutination test was performed for screening the population. **Results:** The prevalence and sero- prevalence of the disease was found to be 1% at the initial screening and 13.4 % respectively. The ratio of clinical disease to sero-positive was 1:13.3 in the initial examination. Based on the past history, the majority of the cases were among the age group 5-35, years in male, farmers, students, housewives, labour and others. More of the cases were among the illiterate, having primary education and less in higher educated person. **Conclusion:** Poverty, thatched roofed houses and not using mosquito nets were found to be risk factors. The sensitivity and specificity of the DAT was satisfactory for field application. Early diagnosis and treatment are essential for both individual patients and for the community.

Key words: Direct Agglutination Test, Sero-prevalence, Socio-Economic factor**,** Visceral Leishmaniasis

Introduction

Urbanisation, climate change and variability, trade and human developments , pollution , land use, water storage and irrigation are aspects of global change that are likely to influence the vector-borne disease incident rate and the distribution of diseases transmitted by vectors [1]. Visceral Leishmaniasis is a vector-borne disease caused by obligate intra-macrophage protozoa and transmitted by phlebotomus argentipes [2]. It is often very crucial within an endemic large area of the tropics, subtropics and the Mediterranean basin leading to 'hotspots' of disease transmission [3,4] which is characterized by both diversity and complexity [2]. A total of about 21 *Leishmania* species have been identified to be pathogenic to human. The four main clinical syndromes are cutaneous leishmaniasis (CL); muco-cutaneous leishmaniasis (MCL; also known as espundia); visceral leishmaniasis (VL; also known as kala-azar); and post-kala-azar dermal leishmaniasis (PKDL). VL is caused by two leishmanial species, *L. donovani* or *L. infantum*, depending on the geographical area. *L. infantum* infects mostly children and immunosuppressed individuals whereas *L. donovani* infects all age groups [5].

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Nowadays, especially CL and VL forms have undoubtedly a wider geographical distribution than before. The increase in leishmaniasis incidence is mainly attributed to several risk factors such as environmental conditions, human behavior, socioeconomic status, immunogenic profile, and genetic factors pose a major risk to human populations [6,7]. Important environmental risk factors including living in houses with cracked mud or thatched plastered house walls, damp earthen floors, sleeping on floor or outside, and vegetation near house can facilitate sand fly survival and enhance vector abundance via providing diurnal resting places, breeding sites, and humidity [8].

Prolonged fever, hepatosplenomegaly, lymphadenopathy, emaciation, skin becomes dry, rough , darkly pigmented, jaundice, epistaxis, bleeding gums, substantial weight loss, progressive anemia are the clinical sign and symptoms of leishmaniasis and even death may occur due to amoebic or bacillary dysentery, pneumonia, pulmonary tuberculosis, cancrum oris, and other septic infections due to immunosuppressive effect [9]. Diagnosis of VL is complex because common diseases such as malaria, typhoid, and tuberculosis have clinical features similar to VL [10]. Moreover, some of VL cases have been misdiagnosed as autoimmune hepatitis, acute lymphoblastic leukemia, malignant lymphoma, and acute myeloid leukaemia [11-14].

The diagnosis of visceral leishmaniasis is complicated both at laboratory and field level. The specifications for VL diagnostic tests show inconsistency among the different endemic regions which are based upon the detection of antibodies or antigens in serum, plasma or urine samples. They are highly sensitive, non-invasive and more suitable [15]. The new methods such as Fast Agglutination Screening Test (FAST) and immunochromatographic strip test (based on rK39 antigen) have also been developed for use in field conditions [16]. The Direct Agglutination Test (DAT) detects agglutinating antibodies against surface antigens of Leishmania is widely used [17-21] and found to be 91-100 % sensitive and 72- 100 % specific [22,23]. For long time DAT remained first line diagnostic tool in resource poor countries. The method uses whole, stained promastigotes either as a suspension or in a freeze dried form. The freeze-dried form is heat stable and facilitates the use of DAT in the field [24]. Recently, detection of parasite DNA in tissues,[25] serum [26] and urine [27] in peripheral blood [28] via polymerase chain reaction (PCR) is used for early and more specific diagnosis.

Visceral leishmaniasis is a major global public health problem touching some 65 countries, with an estimated annual incidence of 500 thousand new cases, 90% of which occur in India, Nepal, Sudan, Bangladesh, and Brazil [29]. Case fatality is sky-scraping, and an estimated 59 thousand persons depart their life due to this disease every year [30]. Estimated disease burden has been found to be 23,57,000 Disability-Adjusted Life Years (DALYs) lost due to leishmaniasis and has been termed to be "significant" among communicable diseases [2].

Kala-azar is mostly confined to the plain areas of Nepal. It does not occur in altitude over 2000 ft. However, presently cases have been reported from the hilly areas of the country. Knowledge on transmission dynamics including incidence, distribution of infection and disease in Nepal remains far less than desired. Health and disease are distributed unequally in populations. These inequalities are often associated with the racial, social, cultural, behaviors and health beliefs in the different endemic areas of the country. The aim of the current study was thus to identify sociobehavioural risk factors, demographic factors associated with visceral leishmaniasis incidence in the urban area, prevalence rate of VL. This study also highlights the Direct Agglutination Test (DAT) to identify Leishmania foci and the ratio between VL and sub clinical cases in this region.This research may be an important tool for the policy makers at national level and health managers in the district level to formulate appropriate planning of Kala-azar control programme.

Materials and Methods

Study Design and Area

The descriptive cross sectional study based on non-experimental design was carried out in the central development region of Nepal. Bindhi Village development committee (VDC) of Dhanusha district was chosen for the study where the VDC consists of 3 villages i.e Bindhi, Bela, Lado and is divided into 9 wards.

Study Population and Sampling technique

The highest numbers of Kala-azar cases were reported from Bindhi VDC of Dhanusha district. VDC was composed of 736 households consisting 4409 populations. Altogether 300 study subjects from the ward number 5, 6, 7, 8 and 9 took participation in the

study and was followed non-probability judgement sampling technique.

Sample Collection and Processing

Finger prick blood samples were collected on printed circles of 12.7 mm diameter in triplicate over Whatman number 3 filter paper (Whatman Ltd, Maid Stone, England) strips each with three circles. One circle was used for screening DAT, in positive case second circle was used for repeat testing using serial dilution up to 1: 6400 and the third one was preserved as stand by.The filter paper strips after collection of blood were left to dry at room temperature (+24^{o} C), was bundled together with a rubber band and placed in sealed plastic bags containing silica gel dehydrant and were transferred to -20^{o} C until testing. All the samples were tested within a period of 10 weeks. The blood soaked filter paper circles (one from each Individual) was cut down with scissors and placed in glass test tubes containing 2ml of saline citrate, and was transferred to +40^{o} C for elution. For the period ranging from 6 to 18 hours, depending upon the time of collection before elution (recently collected samples taking lesser time for complete elution). Such eluted blood sample provided a dilution of 1:60.

The direct agglutination test was performed in commercially available V-shaped micro titre plates (Greener, West Germany) with a twofold dilution of blood samples from 1: 100 to 1: 6400. The diluents used were saline citrate containing 0.2% gelatin and 0.78% mercaptoethanol. Blood samples were diluted over the microlitre plates over horizontal row of wells, each well having 50 micro liter of the diluted sample. However, the first well of each horizontal row was charged with 50 micro liter diluents only, to serve as control. Following addition of 50 micro liter of antigen to each well including the control wells, the plates were incubated at room temperature over night and examined for the development of agglutination [31]. For each sample, the last well showing definite agglutination and bigger than the bottom of the control well (first well of each raw) was considered as the end point.

Criteria for VL case diagnosis

A serological technique was employed to determine the antibody titre against VL in samples of the blood drawn in individuals from the study population. The serological tests were correlated with clinical examination. This study was designed to determine the epidemiological features of VL by measuring antibody prevalence clinical examination confirmation in gender, age, occupation, education and types of houses.

Table 1:Anti-leishmanial agglutinational antibodies titre value

Parameters	Antibody Titre value
Positive	> 1:800
Negative	< 1: 800
VL cases	1: 3200.
Sub-clinical kalazar	1: 800 to 1: 3200
Apparent infection	1: 800 to 1: 1600
VL non-case	< 1: 800

Data Collection and Processing

The primary data were collected through a close type of questionnaires, direct personal interview and observation. The data which was obtained from primary and secondary sources was edited reviewing the completeness, consistency and accuracy of the data; it was coded and entered into computer using data bases DBASE IV and EPI-INFO version 6. Percentage in parentheses was used to see the relationship of distribution of the disease with regard to age, sex, social ethnic group, education, economic level, occupational, behavioural risk factors and environmental sanitation.

Ethical Consideration

The study was ethically approved by Research Ethical Committee Review Board of Institute of Medicine, TUTH Maharajgunj, Kathmandu, Nepal. An informed verbal consent was received from each study subjects. For young children who were not capable to respond for questions that explore exposure status and knowledge level, parents or guardians were provided consent and responded to the questionnaire. Strict confidentiality was also maintained through coding of questionnaire anonymously.

Reliability and Validity

Ten percent of the total samples were taken for the Pre-testing from Mujailiya VDC of Dhanusha district. Interview was taken individually. Research guide and donor agency had supervised during the collection of data. Frequent supervision and cross check of the filled form had helped to maximize the reliability and validity of the study.

Results

Age group and gender distribution

A total number of 300 subjects were enrolled for the study. Out of total, 59.7 % were more males than 40.3 % were female. There are differences between male and female population in all the age groups (Table 2).

Table 2: Age group and gender distribution

Age Groups (yrs)	Male (%)	Female (%)	Total
<5	14(4.7)	7(2.3)	21 (7)
5-10	71(23.7)	30(10)	101(33.7)
11-15	32 (10.7)	9(3)	41(13.7)
16-20	13(4.3)	6(2)	19(6.3)
21-25	7(2.3)	8(2.7)	15(5)
26-30	12(4)	18(6)	30(10)
31-35	14(4.7)	17(5.7)	31(10.3)
36-40	5(1.7)	10(3.3)	15(5)
41-45	2 (0.7)	4(1.3)	6(2)
46-50	2(0.7)	1(0.3)	3(1)
51-55	5(1.7)	8 (2.7)	13(4.3)
56-60	1(0.3)	1(0.3)	2(0.7)
61-90	1(0.3)	2(0.7)	3(1)
Total	179 (59.7)	121(40.3)	300 00)

Pattern of occupation and educational attainment

Among total study population, 20 % were working as farmers and 2.7 % were service holder, 30 % were students and 14.7 % were housewives of the total population. There was one high school within the community and the majorities of the population were uneducated (64.3 %) and 23.3 % has only completed primary education. About 6.7 % had secondary education, 2 % had higher education. The results are shown in Table 3.

Table 3: Occupation and educational attainment

Variables	Male (%)	Female (%)	Total
Occupation			
Farmer	31(10.3)	29(9.7)	60 (20)
Service holder	8(2.7)	0(0)	8(2.7)
Labour	20(6.7)	13(4.3)	33(11)
Business	2(0.7)	0(0)	2(0.7)
Students	78(26)	12(4)	90(30)
Housewives	1(0.3)	43(14.3)	44(14.7)
others	39(13)	24(8)	63(21)
Attainment of Education			
Illiterate	87(29)	106(35.3)	193(64.3)
Literate	11(3.7)	0(0)	11(3.7)
Primary	57(19)	13(4.3)	70(23.3)
Secondary	18(6)	2(0.7)	20(6.7)
Higher	6(2)	0(0)	6(2)
Total	179(59.7)	121(40.3)	300(100)

Occupational groups with relation to educational status

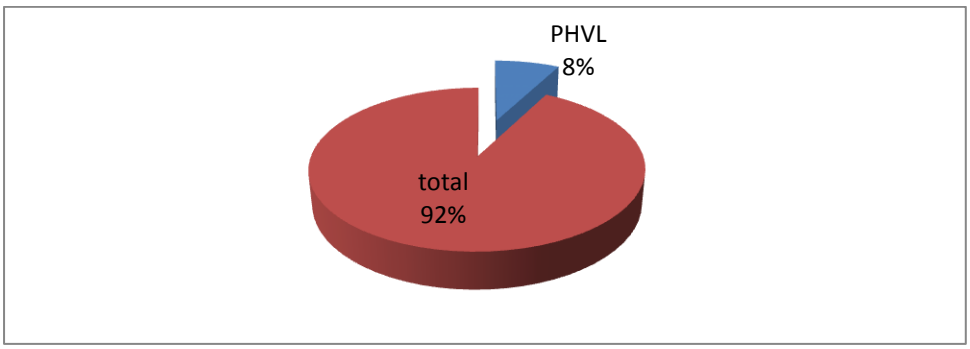
The literacy rate varies among different occupation groups. A majority of the housewives (13.7%) and farmers (17.3 %) were illiterate. Of the total study population 64.3 % are illiterate and 3.7 % are literate. Among this illiterate population a majority are from low caste. The results are shown in table 4.

Table 4: Association of Occupational groups to educational status

Occupation	Attainment of Education					Total
	Illiterate (%)	Literate (%)	Primary (%)	Secondary (%)	Higher (%)	
Farmer	52 (17.3)	3 (1)	1 (0.3)	3 (1)	1 (0.3)	60 (20)
Service holder	4(1.3)	1(0.3)	0 (0)	2(0.7)	1 (0.3)	8 (2.7)
Labor	31(10.3)	0(0)	2(0.7)	0(0)	0(0)	33 (11)
Business	1(0.3)	0(0)	0(0)	1(0.3)	0(0)	2(0.7)
Student	0(0)	6(2)	65(21.7)	13(4.3)	4(1.3)	88(29.3)
Housewives	41(13.7)	0(0)	2(0.7)	1(0.3)	0 (0)	44(14.7)
Others	64(21.3)	1(0.3)	0(0)	0(0)	0(0)	65(21.7)
Total	**193(64.3)**	**11(3.7)**	**70(23.3)**	**20(6.7)**	**6(2)**	**300 00)**

Past history of visceral leishmaniasis (PHVL)

To understand the endemicity of the disease, history of kalaazar during the past five years was enumerated. Assessment of people's experience related to VL infection was based on their recollection of the past infection of kala-azar, checking their medical records, consulting their local medical practitioner. Information regarding past kalaazar infections among different occupations, educational groups, economic status were collected from the Bindhi village. The respondents were questioned as to whether they had a history of kalaazar infection. Of the total 300, 25 (8%) of the individuals recalled a past history of kalaazar within the period of five years (figure 1). The proportional morbidity was calculated to interpret the Data in terms of risk. This used total population as the denominator. Although there was no authentic record of their diagonosis methods which were used to reach to such a diagnosis. This information was utilized to get a rough idea about past incidence. However, in view of long persistence of anti-leishmanial antibodies these samples were subjected to DAT.



PHVL=Individuals with past history of VL

Figure 1: Individuals recalled a past history of kala azar within the period of five years

Age and Gender wise distribution of past history of VL

The higher numbers of cases were found among the age groups 5 to 15 and 16 to 35. The overall past history of VL was 8.3 % .Over the period of five years both genders were infected with kala azar. The past history prevalence among male was 2.7 % and in female was 5.7 %. The results are shown in table 5.

Table 5: Age and Gender specific past history of VL

Age groups (yrs)	Past history of VL (N=300)	
	Positive (%)	Negative (%)
1-4	0(0)	21(7)
5-15	9(3)	133(44.3)
16-35	8(2.7)	87(29)
36-60	8(2.7)	31(10.3)
61-90	0(0)	3(1)
Gender		
Male	8(2.7)	171(57)
Female	17(5.7)	104(34.7)
Total	25(8)	275(92)

Occupational and Educational Specific past history of VL

The information was obtained directly from questionnaires regarding occupation. The majority of the cases were among farmers, students, others and housewives. All educational groups were found infected with kalaazar. A higher number of cases were reported among the literate (Table 6).

Table 6: Occupational and educational distribution of past history of VL

Variables	Past History of kalazar	
	Yes (%)	No (%)
Occupation		
Farmer	6(2)	54(18)
Service holder	1(0.3)	7(2.3)
Labor	3(1)	30(10)
Business	1(0.3)	1(0.3)
Students	5(1.7)	83(27.6)
Housewives	4(1.3)	40(13.3)
Others	5(1.7)	60(20)
Education		
Illiterate	17(5.7)	176(58.7)
Literate	1(0.7)	10(3.3)
Primary	4(1.3)	66(22)
Secondary	2(0.7)	18(6)
Higher	1(0.3)	5(1.7)
Total	25(8.3)	275(91.7)

Sero epidemiological Study

In this study, 300 populations had serological and clinical examination. All the blood samples were subjected to DAT screening using three dilutions. Of the total 40 (13.3 %) were reactive on screening at 1:400. All these individuals were examined clinically for the presence of fever, hepatospleenomegaly, lymphadenopathy, skin lesions and also asked about the past history of anti-leishmanial therapy to exclude the residual circulating antibodies. Of the total 20 (6.7%) were DAT positive at dilution of 1: 6400. Three individuals in this group showed serological as well as clinically positive and were considered active VL cases. The three cases were all male. Positive sero reactive cases were higher among males than females. A higher number of seropositive cases were detected in age groups below 35 years. However, other age groups were also seropositive. The prevalence of clinical disease was found to be 1% (3 /300) during the initial examination. The ratio of clinical disease to sero positive was 1:13.3 (3/40) (fig.2). Those sero-reactive at lower dilution were considered to be sub-clinical or asymptomatic cases. The results are shown in table 7.

Table 7: Gender and age wise Specific Prevalence of VL

Sex	Total Population Examined	Serological and Clinical Examination				
		Non-reactive	Reactive at Dil. 1: 400	Reactive at Dil. 1: 800	Reactive at dil. 1:6400	VL confirmed cases
Male	179	156(52)	23(7.7)	19(6.3)	11(3.7)	3(1)
Female	121	104(34.7)	17(5.7)	16(5.3)	9(3)	0(0)
Age group (yrs)						
1-4	21	21(7)	0(0)	0(0)	0(0)	
5-15	142	125(41.7)	17(5.7)	14(4.7)	9(3)	3(1)
16-35	95	80(26.7)	15(5)	13(4.3)	5(1.7)	
36-60	39	32(10.7)	7(2.3)	7(2.3)	6(2)	
61-90	3	2(0.7)	1(0.3)	1(0.3)	0(0)	
Total	300	260 (86.7)	40 (13.3)	35 (11.7)	20(6.7)	3(1)

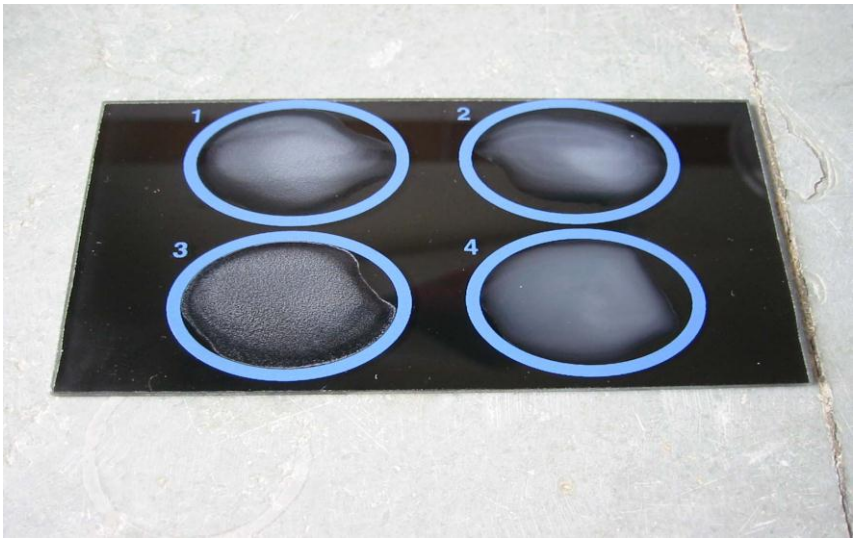


Figure 2: Sample (D3) shows sero-positive.

Occupational Specific prevalence of VL

The higher percentages of sero positivity were found among farmers, housewives, students and others respectively. The results are shown in table 8.

Table 8: Occupational Specific prevalence of VL

Occupation	Total Population Examined	Serological and Clinical Examination				
		Non-reactive	Reactive at Dil. 1: 400	Reactive at Dil. 1: 800	Reactive at dil. 1:6400	VL confirmed cases
Farmer	60	50(16.7)	10(3.3)	9(3)	5(1.7)	0
Service holder	8	7(2.3)	1(0.3)	1(0.3)	1(0.3)	0
Labor	33	30(10)	3(1)	2(0.7)	2(0.7)	0
Business	2	2(0.7)	0(0)	0(0)	0(0)	0
Students	88	80(26.7)	8(2.7)	7(2.3)	3(1)	2(0.7)
Housewives	44	34(11.3)	10(3.3)	10(3.3)	4(1.3)	0
Other	65	57(19)	8(2.7)	6(2)	5(1.7)	1(0.3)
Total	300	260(86.7)	40(13.3)	35(11.7)	20(6.7)	3(1)

Educational Specific prevalence of VL

In the villages most of the population were illiterate (64.3%) and only some had got primary school education (23.3 %), very few had finished secondary school and college. The population of anti-leishmanial antibody was higher in the illiterate group. However, anti-leishmanials antibody was detected among all other education groups. The results are shown in table 9

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Table 9: Educational Specific prevalence of VL

Education	Total Population Examined	Serological and Clinical Examination results				
		Non-reactive	Reactive at Dil. 1: 400	Reactive at Dil. 1: 800	Reactive at dil. 1:6400	VL confirmed cases
Illiterate	193	164 (54.7)	29(9.7)	25(8.3)	15(5)	1(0.3)
Literate	11	9(3)	2(0.7)	2(0.7)	1(0.3)	0(0)
Primary	70	63(21)	6(2)	6(2)	3(1)	2(0.7)
Secondary	20	18(6)	2(0.7)	2(0.7)	1(0.3)	0(0)
Higher	6	6(2)	0(0)	0(0)	0(0)	0(0)
Total	300	260(86.7)	35(11.7)	35(11.7)	20(6.7)	3(1)

Relationship of DAT reactivity during initial survey with VL past history

Of the total examined, 8% (25/300) had a past history of VL. On further analysis it was observed that 13 persons (4.3%) showed serologically reactive at different dilution and 12 person (4%) showed serologically non-reactive (table 10)**.**

Table 10: Past History of VL in relation to serological tests

Past History	Total Population Examined	Serological and Clinical Examination			
		Non-reactive	Reactive at Dil. 1: 400	Reactive at Dil. 1: 800	Reactive at dil. 1:6400
Yes	25	12(4)	13(4.3)	13(4.3)	12(4)
No	275	248(82.7)	27(9)	22(7.3)	8(2.7)
Total	300	260(86.7)	40(13.3)	35(1.7)	20(6.7)

Socio-behavioural risk factors

Respondents were asked about the risk factors for the disease transmission. Positive risk factors were listed as " not using a mosquito net" , "sleeping on the ground floor" and sleeping outside and inside during warm or hot season. Of the total 169 (56.3 %) used mosquito net and 131 (43.7 %) did not use mosquito net. Eleven (3.7%) people who used mosquito net had past history of kala-azar and 24 (8%) serologically reactive at different dilution whereas people who did not use mosquito net had 14 (4.7 %) with past history of kala-azar and 16 (5.3 %) serologically reactive at different dilutions. In the same way, 250 (83.3 %) people slept outside and 50 (16.7 %) of the people slept inside the house during warm and hot season. People who slept outside the house had 21 (7%) past history of kala azar and 37 (12.3%) had serologically reactive at different dilution.

Socio-economic factors

In present study, Most of the respondents had own houses. Few of them were not having their own house. Of the total 198 (66%) had land and 102(34 %) people were landless. Hundred sixty four (54.7 %), 64 (21.3 %), 17 (5.7 %), 18(6 %) and 37 (12.3 %) had production sufficient for their family up to 3, 6, 9, and 12 months respectively. Out of people who had production sufficient up to 3 months to 9 months, 20 (6.6 %) had past history of positive kala azar and 32 (10.6 %) serologically reactive at different dilutions but people who had production sufficient up to 12 months to > 12 months were 5 (1.6%) with past history of positive kala-azar and 8 (2.7 %) serologically reactive at different dilutions.

Environmental factors

In most areas, the types of houses are good indicator of the economic status of the population. In this study

area 10.7 % had pucca (cemented) houses, 32 % brick wall with thatched, 28.7 % mud wall with thatched roof and 50 % thatched roof and wall. Twenty (6.7 %) out of 25 (8.3 %) people living in such houses had past history of kala azar positive and 24 (8%) out of 40 (13.3 %) had serologically positive at different dilutions.

Discussion

Leishmaniasis is still prevalent in many tropical and sub-tropical countries and can only survive in areas where sand flies, reservoir hosts (dogs, foxes and rodents) and infected human population are high. In relation to the urbanization of visceral leishmaniasis, studies have suggested that the form of occupation of space, in particular the environmental, social, and economic transformations associated with migratory movements and population growth and increased population density in large cities are involved in the genesis of the phenomenon [32-34]. Diagnostic tests however are based on antigens of a single Leishmania donovani subspecies that might limit the diagnosis of the disease in some regions [35].

The study comprises 300 population from Bindhi village participated in the study. Of which 59.7% were male and 40.3 % were female. There was numerical differences between male and female population in all age groups. Similar findings were also obtained from the study conducted by Sah et al [2].

This study reveals a majority of the population of Bindhi area worked as farmers, students, labours and house wives. A few people were in government services. Majorities of the population was uneducated and had finished only primary education. A few had secondary and college level education. The literacy rate varied between different occupation groups. The majority of the farmers, housewives, labour and other were illiterate population. Among illiterate population, majority were from low caste ethnic groups. NK Sah et al highlighted that in the endemic countries, Kala azar affects the poorest among the poor. The very poor had little knowledge about the disease and hence they were unlikely to seek early treatment, and most of those who started treatment cannot afford to complete it. The occurrence of the disease drags them further into the downward spiral of poverty from which they were unable to recover. Kala-azar worsens the poverty amongst people. It contributes to poor development of the area and stresses the overstretched health system [2].

Assessment of people's experienced related to VL infection was based on their recollection of their past infection with kala-azar checking their medical records and consulting their local medical practitioners. Information regarding past kala-azar among different occupations, educational groups, economic status, behaviour of people and environmental sanitation of people was collected from the study areas. Of the total three hundred population, 25 (8%) of individuals recalled a past history of kala-azar within a period of five years. The proportional morbidity was calculated to interpret the data in terms of risk. This was based on total population as denominator, based on the past history the majority of cases were among age group 5-35 years, farmers, students, housewives, labours and others. More of the cases were reported among the illiterate and primary education and less in higher educated person.In this study, 300 populations have serological and clinical examination. The DAT was employed to screen the population for the presence of anti-leishmanial antibody against VL and attempts were made to correlate the finding with clinical observations in gender, age, occupation, education, type of houses, socio-economic and behaviours of population. All the blood samples were subjected to DAT Screening Test using three dilutions (1: 400, 1: 800 and 1: 6400). Of the total, 13.4 % (40/300) were reactive in different dilution of sera and 20 (6.7 %) were reactive at 1: 6400. Among these sero positive three people were clinically positive for the diseases and considered to have active leishmaniasis and were referred to Janakpur Zonal Hospital for anti-leishmanial therapy. The prevalence of disease was found to be 1 % at the initial screening.

This study reveals sero-prevalence of the disease was found to be 13.4 % (40/300). The ratio of clinical disease seropositive was 1: 13.3 in comparison to initial examination. These sero reactive at lower dilutions were considered to be sub clinical or asymptomatic cases. A similar study was carried out in Kenya, where 15% of 321 people examined were sero positive [36] as were 7.5 % of more than 2500 children tested in Brazil [37]. Sero prevalence of VL have been reported at 13 % in the western upper Nile region to Sudan [38]. In Nepal, an accurate prevalence of this disease was difficult to determine, since not all the cases had been reported to the Hospital. The higher percentage of seropositivity was found among farmers, students, housewives, labors and the other. The proportion of anti-leishmanial antibody was higher in illiterate groups. The higher severity of disease was responded by various socio-economic classed individuals. The highest socio economic status reported in Kenya and Uganda was protective to VL infection [36]. Similar findings were also obtained in the study

conducted by Belo et al [39] . The possible explanation for this could be that low income can affect over all status of household and individuals in many aspects. Low income can be associated with poor housing conditions, poor environmental hygienic conditions, poor nutritional status and increased risk of infections. Different types of houses were assessed in relation to the severity of the disease, e.g not using mosquito nets, sleeping in cattle shed, storage of firewood and bricks around the house. Though 50% of the houses were with thatched roof and wall and such houses are suitable for the sand fly vector. Presence of dog was an important predictor for VL in the study carried out by Kindie et al [40]. People living in above mentioned circumstances had severe risk of kala azar. The socioeconomic characteristics identified as risk factors of VL in this study which could help in strengthening existing control strategies.

Conclusion

The present study concluded that the prevalence of confirmed disease was found to be 1 % at the initial screening whereas sero-prevalence of disease was found to be 13.4 %. The ratio of clinical disease to sero-positive was 1:13.3 in the initial examination. Low socio-economic condition, wall with thatched roofs, illiteracy and not using mosquito nets were risk factor for kalaazar. For diagonosis DAT testing, clinical examination and history of endemicity were found to be appropriate, low in cost, simple, specific and sensitive. Ongoing case surveillance and treatment, entomological study, health education, community based income generating programmes and mosquito small mesh net purchasing projects should be the most appropriate ways to prevent and control the disease. Moreover, this study suggests that the intricate role of sandfly and other domestic animals in the transmission cycle should be further investigated and well recognized.

Limitations

Limitations included the poor compliance of the referred subjects for follow-ups were not done when the serological titre was more than 1: 800. Serologically, positive cases were not followed for clinical examination due to time constraints and also the sample size was small which was not sufficient to represent the larger number of the population .These aspects can further be studied for better understanding of the effects and control of VL patients in countries like Nepal.

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