INTRODUCTION

Since 1980, the central and eastern lowland region of Nepal has experienced a resurgence of visceral leishmaniasis (VL) in parallel with a large epidemic in the neighboring Indian states of Bihar, Uttar Pradesh, and West Bengal.\(^1\)\(^-\)\(^3\) Leishmania donovani is the parasite implicated in south Asia, and is transmitted by the bite of the infected female sand fly, Phlebotomus argentipes. In India and Nepal, VL has an anthroponotic transmission cycle, but the vector feeds on livestock and is attracted by their presence.\(^2\) Phlebotomus argentipes is most active from dusk to dawn, and spends the daylight hours in dark crevices and corners of houses or sheds where humid conditions favor its survival.\(^4\)\(^,\)\(^5\)

Visceral leishmaniasis was reported to be endemic in the Nepali lowlands in the 1950s,\(^1\) but no formal surveillance existed at that time. The intensive insecticide-spraying programs undertaken to eradicate malaria in the 1960s were apparently also effective in controlling VL.\(^6\) The first confirmed case of VL in Nepal was reported in 1980, after 3 years of epidemic VL in the adjacent Indian state of Bihar.\(^1\)\(^,\)\(^3\) Since the 1980s, because of limited funding and personnel, insecticide-spraying efforts were targeted only at villages where clusters of VL cases were reported.\(^6\) However, in spite of this spraying program, the incidence of disease has increased over the past decade.\(^1\)\(^,\)\(^8\)

The disease burden from VL in this region is large. In 1992, more than 80,000 cases were reported from northern India.\(^9\) In Nepal, between 1,300 and 1,800 VL cases were reported annually to the Ministry of Health from 1995–1997, corresponding to incidence rates of 2 to 8 cases per 10,000 population in VL-endemic districts.\(^3\) These passive facility-based surveillance data are widely considered to be a substantial underestimate of the true number of cases.\(^3\) The disease is chronic, and can be lethal if not treated. It is also expensive to treat, given the limited resources available for health care in these countries.\(^6\) In Nepal, the first line of therapy is intramuscular injection of sodium stibogluconate at 20 mg/kg/day for 30 days. If the response at 30 days is incomplete, 10 more days of therapy are given before making a diagnosis of clinical resistance.\(^1\) The second-line drug is intravenous injection of amphotericin B at 0.5 mg/kg/day for 14 days, which is a significantly more costly option. Recently, resistance to sodium stibogluconate (defined as lack of response after 28–30 days of 20 mg/kg/day) has been reported from India.\(^10\)\(^,\)\(^11\)

Given the burden of treating VL in Nepal, a strategy to prevent the disease would be extremely valuable. We therefore conducted a case-control study to identify household and individual risk factors that could be addressed using a practical intervention plan.

MATERIALS AND METHODS

We conducted the fieldwork from November 15 to December 15, 1999 in the VL-endemic districts of Dhanusha, Mahottari, and Siraha (Figure 1). We identified VL patients through several sources, including VL registries of the 4 government hospitals in the study area, and patient logs from 2 health-care practitioners who performed bone marrow aspirates. We specifically looked for VL patients with Leishmania-positive bone marrow smears. In the villages we visited, we also recruited patients with clinical syndromes that were consistent with VL, and those with a record of VL diagnosed parasitologically, or a convincing history of clinical VL that responded to documented anti-leishmanial therapy. We facilitated the diagnosis of sick patients by sending them to the nearest government hospital with a referral letter, and by paying their admission and diagnostic fees, including bone-marrow aspiration for parasitologic diagnosis. Standard drug treatment for VL was provided free at government hospitals by the Ministry of Health.

Control households were chosen at random from voting registries. All households in a Village Development Committee (VDC) are listed by household number and family name in the VDC registry. Village Development Committees are local political units that comprise 3 to 8 villages with a combined population of 3,000–12,000. The study districts of Mahottari, Dhanusha, and Siraha have 77, 103, and 112 VDCs respectively. We selected the number of the first control household using a random-number table. We then chose control households, using a sampling interval equal to the number of households in the VDC divided by the number of cases in the VDC plus 3. We chose one extra control
household per VDC (beyond the number of cases) to allow for later exclusions based on positive diagnostic tests. The other 2 extra control households provided alternates in case the chosen control households could not be reached for logistical reasons. Within control households, we chose a person to serve as the control in order to arrive at the same distribution by age group and sex as that of cases in the respective VDC. Controls were not individually matched to cases. To minimize the likelihood of including VL-infected persons among our controls, we excluded potential control households if there was a history of prolonged febrile illness consistent with VL, or if there had ever been a diagnosis of VL in any household member. In this case, a person who fit the age-group and sex requirements in the closest neighboring house was recruited as a control.

We interviewed participants using a structured questionnaire that focused on history of illness and treatment, housing conditions, socioeconomic indicators, animal husbandry practices, and sleeping habits. A capillary-puncture blood specimen, or in newly diagnosed cases, a venous blood specimen, was collected for rK39 dipstick testing (InSure Rapid Test for Detection of Visceral Leishmaniasis, InBios International Ltd., Seattle, WA) and for the direct agglutination test (DAT). The DATs were performed and interpreted according to published methods. In whole blood, the test was considered negative at a titer of < 1:2,000, borderline at 1:2,000, and positive at ≥ 1:4,000. In serum, the test was considered negative at a titer of < 1:3,200, borderline at 1:3,200, and positive at ≥ 1:6,400. All titers refer to the blood or serum dilution after the addition of antigen.

Definition of a VL case required a history of illness consistent with VL, response to anti-leishmanial drug therapy, and positive results on both the DAT and rK39 dipstick test. Illness was considered consistent with VL if the patient had a history of fever lasting 2 weeks or more, and either splenomegaly, abdominal swelling or pain, weight loss, or skin darkening. Response to therapy was defined as resolution of illness for cases fully treated before interview, and defervescence within 2 weeks of initiation of therapy for newly-diagnosed cases. We included cases diagnosed since April 14, 1999, corresponding to the beginning of the Nepali year 2056. Potential controls found to have a positive or borderline serology were excluded from analysis.

Analysis was performed using Epi-Info 6.04c (Centers for Disease Control and Prevention, Atlanta, GA) and SAS for Windows Version 6.12 (SAS Institute, Cary, NC). The significance of differences was tested using the Mantel-Haenszel chi-square test or a 2-tailed Fisher exact test for categorical variables, and the Wilcoxon 2-sample test for continuous variables. Multivariable models were constructed via a backwards stepwise elimination procedure using unconditional logistic regression.

The protocol was approved by the Centers for Disease Control and Prevention Human Subjects Institutional Review Board, the Nepal National Health Research Council Ethical Review Committee, and the Ministry of Health of His Majesty’s Government of Nepal. Informed written consent was obtained from all cases and controls.

RESULTS

We interviewed a total of 92 potential VL cases and 113 potential controls. We excluded 6 potential cases who had negative rK39 dipstick results, 2 of whom also had negative DAT results. Two more cases were excluded because diagnosis and treatment occurred before April 14, 1999. Eight potential controls had positive or borderline DAT. These exclusions left a total of 84 cases and 105 controls. Five sibling pairs and one mother-son pair of cases were interviewed. Both members of a pair were included in the analyses of clinical signs and symptoms and individual risk factors, but one of the pair (chosen at random) was excluded from analyses of household level risk factors.

Of the 84 cases, 54 had been diagnosed previously with VL, and had been treated or were under treatment at the time of interview. The other 30 were referred by the study team and newly diagnosed at the nearest government hospital. The cases and controls came from 28 VDCs in the 3 study districts. Our frequency matching ensured that the age and sex distribution was similar for VL cases and controls.
of 84 visceral leishmaniasis cases and 105 controls. Characteristics of these cases and controls are presented in Table 1. More than 80% of cases and controls were between 5 and 40 years of age. The median age was 25 years for cases and 26 years for controls; males slightly outnumbered females. Of 84 cases, 58 (69%) were reported to have Leishmania amastigotes visualized on bone-marrow or splenic-aspirate smear.

Patients reported classical symptoms of VL: all had fever, and most reported weight loss, abdominal swelling and pain, and the development of a black color in the skin (Table 2). The median duration of illness before treatment was 3 months, but ranged from 2 to 18 months, and tended to be longer for females than males (median 3.75 months for females versus 2.75 months for males; *P* = 0.15 by Wilcoxon 2-sample test). Seventy percent of males, but only 45% of females, were treated within 3 months of onset of illness (*P* = 0.04). Six of 7 patients ill for ≥ 8 months before treatment, including all 4 patients whose illness lasted more than 1 year, were female. Illness duration before treatment was equal for previously-diagnosed and newly-ascertained cases (*P* = 0.99).

Twenty-two newly-diagnosed cases were examined by the study-team physician (CB). One bone marrow-confirmed patient had massive ascites which precluded an adequate abdominal examination. Twenty (95%) of 21 others had palpable splenomegaly, with spleen size ranging from 2 cm to 12 cm below the left costal margin in the anterior axillary line. Of the 21 patients, nine had splenic tenderness and eight had spleens of a very firm consistency. Of the eight very firm spleens, five were also tender. Hepatic enlargement and lymphadenopathy were uncommon.

A number of factors were associated with altered risk of VL (Table 3). Sleeping on the second floor of a house, sleeping on a bed or cot (compared with sleeping on the floor or ground), and sleeping under a bed net were all associated with protection. Eighty-five percent of case houses, and 87% of control houses were constructed from mud and dung plastered over a stick framework. Ninety-four percent of case houses and 92% of control houses had thatched roofs (68% compared with 54% among control houses; OR = 1.8, 95% CI 0.9–3.4). Cracks in the walls of the house and dampness in the floor of the house were both associated with elevated risk.

In the multivariable model, 4 variables were significantly associated with altered risk of VL. Having 3 or more rooms in the house, ownership of a cow or buffalo, and use of a bed net during the warm months of the year were all strongly protective. In contrast, having a damp floor was a strong risk factor (Table 4). Excluding the “damp floor” variable from the model allowed us to include 77 cases and 104 controls in the model, but did not substantially alter the magnitude of the protective effect for the other 3 variables. Our data suggest that household resources play an important role in determining whether a household owned a bed net. Among controls, those with a bed net in the household owned significantly more land than those without a bed net (median 1.25 acres versus 0.08 acres among those with and without bed nets, respectively; *P* = 0.01).

**DISCUSSION**

This study is the first to systematically assess the epidemiology of visceral leishmaniasis in Nepal. Our findings highlight the continued transmission of VL in the lowlands of Nepal despite more than a decade of control efforts. As in another clinical study in Nepal, we found that most patients were ill for an average of 3 months before they were seen by health-care practitioners, and some were ill for more than a year. Nearly all of our patients manifest the classic clinical picture of VL, known in Nepal and India as kala-

**Table 1**

Characteristics of 84 visceral leishmaniasis cases and 105 controls. Controls were frequency-matched by age and sex to cases.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases no. (%)</th>
<th>Controls no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>5–4</td>
<td>21 (25)</td>
<td>26 (25)</td>
</tr>
<tr>
<td>15–40</td>
<td>50 (60)</td>
<td>61 (58)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>11 (13)</td>
<td>16 (15)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>25 (2–70)</td>
<td>26 (3–73)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44 (52)</td>
<td>53 (51)</td>
</tr>
<tr>
<td>Female</td>
<td>40 (48)</td>
<td>52 (50)</td>
</tr>
</tbody>
</table>

**Table 2**

Characteristics of illness in 84 visceral leishmaniasis patients included in the case-control study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases No./Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>84/84 (100)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>77/82* (94)</td>
</tr>
<tr>
<td>Darkening of skin</td>
<td>71/80* (88)</td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td>63/80* (79)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>57/81* (70)</td>
</tr>
<tr>
<td>Median duration of fever before treatment (range)</td>
<td>3 mo. (0.5–18 mo.)</td>
</tr>
</tbody>
</table>

* Data missing for some records.
† One patient had massive ascites which precluded estimation of hepatic and splenic size.
‡ Below the left costal margin in the anterior axillary line.
§ Anterior and posterior cervical, axillary, and epitrochlear nodes examined.

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azar (in Hindi, kala means “black” and azar “fever”). Blackening of the skin was a frequent and often spontaneously reported symptom. Splenomegaly, abdominal discomfort, and weight loss were also common among the patients in our study, but lymphadenopathy was not.

Our data contradicted the commonly held belief that transmission of VL in Nepal is facilitated by sleeping in the cattle shed or having animals sleeping in the house. In fact, ownership of large domestic animals such as cattle and water buffaloes are strongly protective even after adjustment for house size and landholdings. In an entomologic survey in Dhanusha district, the blood found in *P. argentipes* collected in animal sheds was predominantly bovine, while in human or mixed dwellings, the flies fed almost equally on humans and bovines (Lawyer P. unpublished data). The protective effect of owning a cow or water buffalo may reflect the role of the animal as a preferred blood source. Alternative explanations are that large animals indicate higher socioeconomic status, or that their ownership may be associated with better nutritional status among household members, and through this mechanism, may prevent the progression from subclinical infection to clinical VL.

Partway into the study, we noticed that case houses were more likely to have palpably-damp earthen floors. For the remainder of the study, we collected this information systematically, and confirmed that this was a strong risk factor, which makes sense in the context of a vector that requires fewer sand flies were caught under insecticide-treated than untreated bed nets. Insecticide-treated bed nets are therefore likely to have a stronger protective effect than that of untreated bed nets seen in our study.

Bed nets are already in wide use in this area, largely as protection against nuisance mosquitoes. More than 70% of our control group reported the regular use of bed nets. However, control households without bed nets owned significantly less land than those with bed nets, suggesting that lack of resources to buy nets may be an important constraint. In this setting, a campaign to increase bed-net usage combined with subsidized purchase for the poorest segment of the population could have a substantial impact on the incidence of VL.

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high humidity for survival. Improved house construction to increase ventilation and access to sunlight might address this risk factor. However, the poorest households are unlikely to be able to afford these improvements.

Our study had a number of limitations. We were unable to rule out other potential causes of chronic fever and splenomegaly, such as typhoid fever and malaria. For this reason, we included only cases that responded to specific anti-leishmanial therapy and were positive for 2 serologic tests. Although it is possible that a subset of our cases may have had other diseases, we are confident that most had VL.

Our data strongly suggest that an increased use of bed nets could substantially reduce the occurrence of visceral leishmaniasis in the Nepali lowlands. Further work is needed to confirm that bed nets decrease sand fly access to humans and to assess the effect of insecticide impregnation. A prospective intervention study would provide the most reliable test of the protective effect of bed nets against visceral leishmaniasis.

Acknowledgments: We are especially grateful to our field team, Tej Kumari Shrestha, Dorik Lal Kamait, Nand Kishor Shah, and Satyanarayan Pandit, without whom this study would not have been possible, and to Shishir Pant for essential laboratory work. The authors thank Thomas Navin, Barbara Herwaldt, Robert Gilman, Robert Wirtz, Phillip Lawyer, George Stroh, James Mendlein, and Matthew Brown for their scientific advice and support. In addition, we thank Cathy Thompson, Pandu Wijayeratne, Rosy Adhikari, Drubha Shrestha, Anjana Shrestha, and Shreedhar Pradhan for logistical support in Nepal.

Financial support: This study was supported by the United States Agency for International Develop (USAID) Nepal Infectious Diseases Program and by the Infectious Diseases component of the USAID-CDC Interagency Agreement.

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