Kala-Azar Outbreak in Libo Kemkem, Ethiopia: Epidemiologic and Parasitologic Assessment

Jorge Alvar,* Seife Bashaye, Daniel Argaw, Israel Cruz, Pilar Aparicio, Askal Kassa, Giannos Orfanos, Fernando Parreño, Olusegan Babaniyi, Nigussu Gudeta, Carmen Cañavate, and Caryn Bern

Department for the Control of Neglected Tropical Diseases (CDS/NTD/IDM), Leishmaniasis Control Program, World Health Organization, Geneva, Switzerland; Malaria and Other Vector Borne Diseases, Prevention and Control Program, Ministry of Health, Ethiopia; Disease Prevention and Control Programmes, World Health Organization, Ethiopia; WHO Collaborating Center for Leishmaniasis, National Center of Microbiology, Instituto de Salud Carlos III, Madrid, Spain; National Center of Tropical Medicine, Instituto de Salud Carlos III, Madrid, Spain; Health Center, Addis Zemen, Ethiopia; Greek Medical Council, Athens, Greece; Area de Salud 7, Madrid, Spain; World Health Organization, Ethiopia, Regional Health Bureau, Bahar-Dar, Amhara State, Ethiopia; Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. In May 2005, visceral leishmaniasis (VL) was recognized for the first time in Libo Kemkem, Ethiopia. In October 2005, a rapid assessment was conducted using data from 492 patients with VL treated in the district health center and a household survey of 584 residents of four villages. One subdistrict accounted for 71% of early cases, but the incidence and number of affected subdistricts increased progressively throughout 2004–2005. In household-based data, we identified 9 treated VL cases, 12 current untreated cases, and 19 deaths attributable to VL (cumulative incidence, 7%). Thirty percent of participants were leishmanin skin test positive (men, 34%; women, 26%; $P = 0.06$). VL was more common in men than women (9.7% versus 4.5%, $P < 0.05$), possibly reflecting male outdoor sleeping habits. Molecular typing in splenic aspirates showed *L. infantum* (six) and *L. donovani* (one). Local transmission resulted from multiple introductions, is now well established, and will be difficult to eradicate.

INTRODUCTION

In the Horn of Africa, visceral leishmaniasis (VL, kala-azar) has a focal distribution in two distinct ecologic settings: the semi-arid regions in the north where *Phlebotomus orientalis* breeds in cracks in the black cotton clay soil and the savanna and forest areas in the south where the vectors *P. martini* and *P. celiae* are found in association with *Macrotermes* termite mounds.[1,2] In Ethiopia, long-recognized VL-endemic foci are situated in Metema and Humera along the border with Sudan in the northwest, reflecting the first ecologic pattern, and in the regions of Lake Abaya, Omo River, and the Aba Roba plains in the south, following the second, with an estimated country-wide incidence of 2,000 cases/yr (Figure 1).[3,4] In the northwest Ethiopian foci close to the Sudan border, 20–30% of patients of VL are also infected with HIV, the highest co-infection rate in the world.[5] Anthroponotic transmission seems to predominate in the Horn of Africa, but leishmanial infection has been detected in wild animals and domestic dogs in Sudan.[6,7] In Ethiopia, the balance between anthroponotic and zoonotic VL transmission is unknown. In western Ethiopia, sporadic VL cases are often attributed to the in-migration of patients infected in eastern Sudan and Sudanese border localities.[8] While epidemics of leishmaniasis have not previously been reported in Ethiopia, East Africa, as a whole has seen an increasing burden of VL over the last two decades.[9,10]

Before 2005, kala-azar cases had never been recognized in Libo Kemkem wereda (district). In 2004, the Amhara Regional Health Bureau reported a 5-fold increase in crude mor-

tality rates in Libo, attributed initially to an outbreak of drug-resistant malaria; coartem was provided without effect. In May 2005, after ~200 reported deaths, Médecins sans Frontières-Greece (MSF-G) identified the outbreak as kala-azar (MSF-Greece, unpublished data). From May to June 2005, MSF-G diagnosed 201 new VL cases with seven additional deaths and reported that the outbreak had spread to eight kebeles (subdistricts) with an estimated 50,000 inhabitants at risk. In October 2005, a rapid outbreak assessment was conducted by local and national health authorities with technical support from the World Health Organization to define the extent of the outbreak and describe basic epidemiologic features.

MATERIALS AND METHODS

Location. Libo Kemkem wereda is located in the Amhara Region of northwestern Ethiopia at an altitude of 2,000 m above sea level. The district is made up of 30 kebeles with an estimated population of 196,813 in 2004 (Figure 2). Addis Zemen (the district capital, population 19,755) is located between Bahir Dar and Gondar on the major road connecting Addis Ababa to the Red Sea, crossing known foci of intense VL transmission in Metema, Ethiopia, and Gedaref, Sudan (dotted line in Figure 1). The district has one health center and 10 health posts.

Data sources and case definitions. The outbreak study was conducted from October 8–22, 2005. Two main data sources were used: 1) records of patients with VL diagnosed and treated in the MSF-G/Addis Zemen Health Center (AZHC), the only facility in the district with VL diagnostic capability and antileishmanial drugs, and 2) a household survey carried out in four villages.

Kala-azar patients at AZHC were diagnosed predominantly based on clinical assessment and a positive direct agglutination test (DAT). We calculated dates of symptom on-
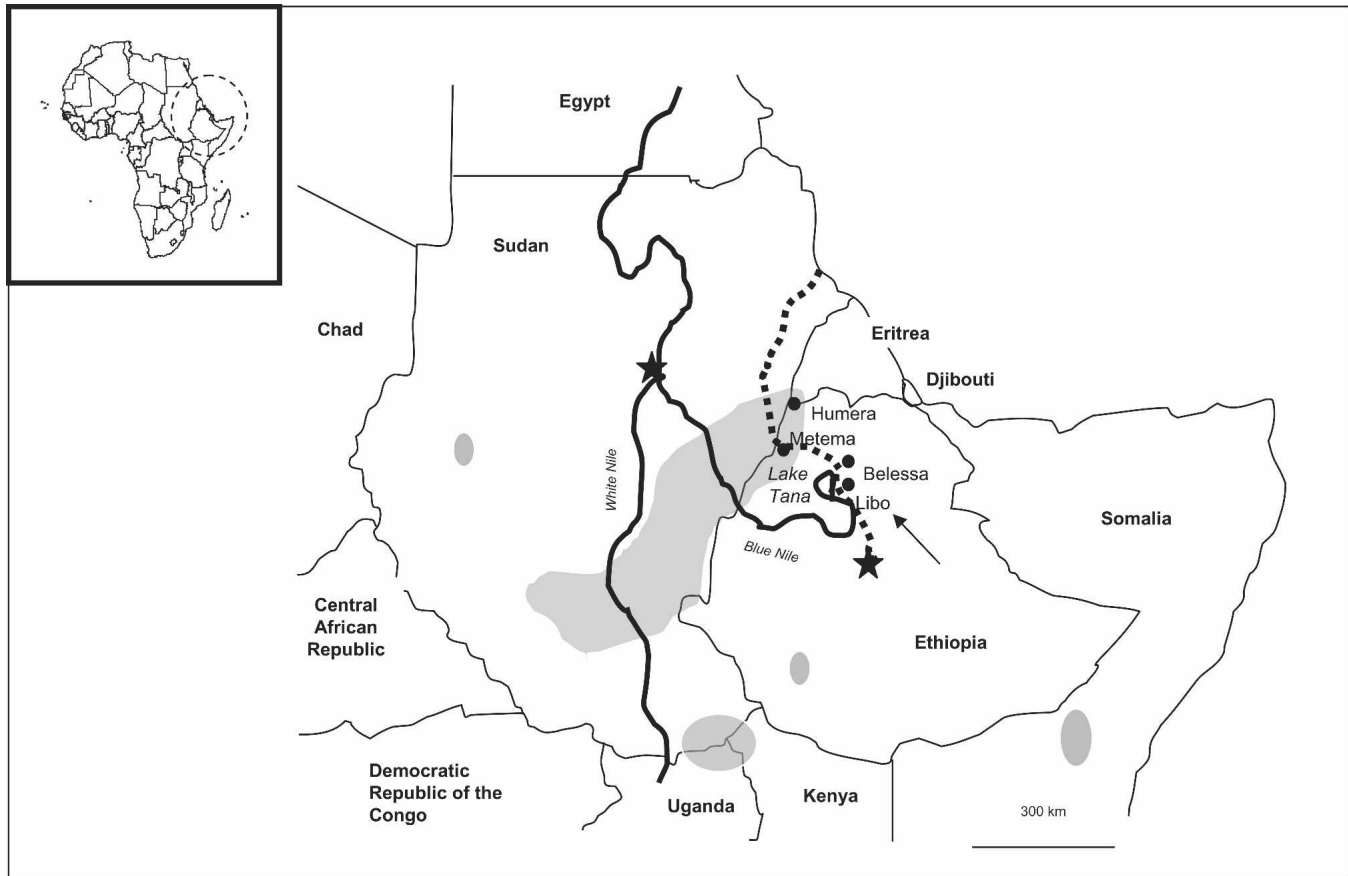
FIGURE 1.   The major foci of kala-azar in the Horn of Africa, indicating the location of Libo Kemkem district.

set retrospectively, based on dates of diagnosis and reported months of illness in the clinical record. The DAT was performed using standard methods and antigen from ITG-Belgium; the recommended screening cut-off was used, considering titers ≥ 1:3,200 to be positive.[11] During the field study, patients suspected of having kala-azar were tested using two recombinant K39 antigen-based immunochromatographic strip tests, the KalazarDetect (batch FC1098; InBios, Seattle, WA) and IT-Leish test (Diamed AG, Cressier sur Morat, Switzerland), in addition to the DAT. They were also tested using a rapid test for *Plasmodium falciparum* malaria (Paracheck Pf; Orchid Biomedical Systems, Goa, India).[12,13] All rapid tests were performed following the manufacturers' instructions.

MSF-G patient record data were delinked from personal identifiers at the time of data entry. Written informed consent was obtained from patients with VL who underwent splenic aspirate for parasitologic diagnosis.

Household survey. No population estimates were available for entities smaller than kebeles; representative sampling was therefore not possible. To validate the facility-based data and confirm the epidemic nature of the disease in Libo Kemkem, we therefore performed a rapid epidemiologic assessment that included three villages reported to be highly affected and one village close to the highly affected villages but with only a few reported cases. The three highly affected villages were chosen from Bura kebele (population 6,321), the apparent center of the epidemic; all had multiple recent

cases of kala-azar reported in AZHC data. The less affected village was selected from Shina (population 4,480), a few kilometers away from Bura, to ascertain the presence or absence of a background of existing endemic VL transmission. The team attempted to enumerate and include all households in each survey village, but because of logistic constraints and the uncertainty of village limits, some houses were not included.

The survey was conducted by Ethiopian nurses specifically trained in the use of the survey questionnaires and application and reading of the leishmanin skin test (LST). Oral informed consent was obtained from each person included in the household survey. Structured questionnaires were used to enumerate all members residing in the household for ≥ 6 months in the last 2 years. For each individual, age and sex were recorded, and the nurses used an abbreviated medical history to ascertain probable and definite kala-azar cases with symptom onset since New Year's Day 1996 by the Ethiopian calendar, equivalent to September 11, 2003. Patients with kala-azar were characterized as confirmed past cases (diagnosed at AZHC and treated with 28 days of intramuscular sodium stibogluconate at a dose of 20 mg/kg/day; SSG; Albert David Ltd., Kolksta, India); confirmed current cases (> 1 month of fever and malaria ruled out by rapid test, plus at least one of the following: weight loss, abdominal pain or swelling, and positive DAT serology during the field survey), or probable kala-azar deaths (death after an illness with > 1 month of fever plus weight loss, abdominal pain or swelling, and no
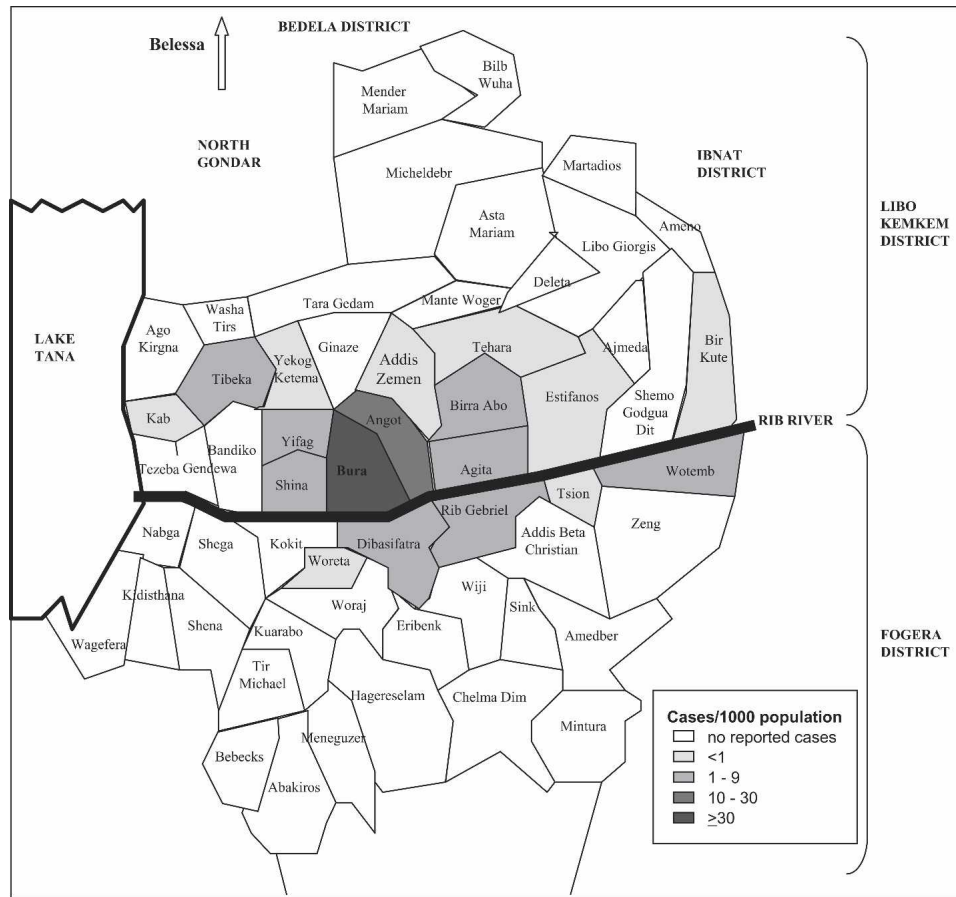
FIGURE 2. Map of Libo Kemkem and Fogera weredas (districts) indicating the kebeles (subdistricts) affected by the visceral leishmaniasis outbreak.

other known cause of death). Because kala-azar is close to 100% lethal without treatment, we sought to identify people who might have died of kala-azar, especially before the availability of diagnostic and treatment capability in AZHC. Family members were asked to describe all deaths that occurred during the study period. Interviewers probed for the characteristics outlined above, other descriptors that would indicate probable cause of death, and what the family had been informed was the cause of death. Only deaths meeting the above case definition, and without another reported or probable cause, were included as probable kala-azar deaths. Leishmanin skin testing was conducted for all consenting participants older than 1 year of age, using *L. major* antigen (leishmanin batch 122; Pasteur Institute, Tehran, Iran).

Parasitologic analysis. Splenic aspirates were obtained from seven patients clinically suspected to have kala-azar. These samples were examined microscopically and inoculated in NNN medium. To identify the *Leishmania* species in the samples collected, three different approaches were used. Two techniques identified all Old World *Leishmania* species and *L. donovani* to the complex level: 1) sequence analysis of the LnPCR product (SSUrRNA region)[14] and 2) *Hae*III restriction fragment length polymorphism (RFLP) analysis of the ITS-1 PCR product (ITS-1 region).[15] To differentiate between *L. infantum* and *L. donovani,* a third technique, *Hae*III

RFLP analysis of the T2B4 PCR product (repetitive nuclear DNA sequence), was used.[16]

In addition, venous blood specimens were collected from 40 asymptomatic dogs living in the three highly affected survey villages in Bura. Molecular characterization of the *Leishmania* species infecting dogs was done with the same methodology used for human specimens.

RESULTS

Facility-based data. Records were available for 492 kala-azar patients diagnosed at AZHC between May 10 and October 18, 2005. Of these, 344 (74%) were men and 121 (26%) women (data missing for 27). The mean age was 17.4 years (median, 16 years; range, 0.7–60 years); female patients were younger than men (mean, 14.5 versus 18.6 years for men; *P* < 0.001). Of 490 patients with outcome data, 21 (4%) were known to have died. Patients came from 47 kebeles in 3 weredas (Libo Kemkem, Fogera, and Mecha), but 87% of patients came from just 10 kebeles and 42% from Bura kebele (Table 1; Figure 2). Dates of symptom onset could be calculated for 357 patients: the earliest onset date was April 2003 (Figure 3). Of the 110 cases with symptom onset between April 2003 and December 2004, 78 (71%) occurred in Bura kebele. The outbreak began to expand beyond Bura by late 2004: among 247

Geographic distribution, age, and duration of illness among kala-azar patients diagnosed at Addis Zemen Health Center from May to October 2005

By location of residence		N (%)	Month of diagnosis					
			May	June	July	August	September	October
Wereda	Kebele		n	n	n	n	n	n
Libo	Agita	25 (5)	0	2	4	7	8	4
Libo	Angot	66 (13)	11	2	6	12	28	7
Libo	Birra Abo	12 (3)	2	2	4	1	2	1
Libo	Bura	205 (42)	114	24	20	15	10	22
Libo	Shina	14 (3)	1	3	2	3	3	2
Libo	Tibaga	20 (4)	2	3	5	2	5	3
Libo	Yifag	18 (4)	1	7	3	6	0	1
Fogera	Dibasifatra	25 (5)	3	3	4	6	4	5
Fogera	Rib Gebriel	30 (6)	0	4	2	3	12	9
Fogera	Wotemb	11 (2)	0	0	0	3	4	4
Other locations		66 (13)	6	4	12	17	14	13
All locations		492 (100)	140	54	62	75	90	71
Mean months of illness		N = 357*	6.1	5.6	5.2	4.2	2.9	2.8
Median age in years among								
Patients from Bura kebele		N = 205	15.5	11.5	16.0	19.0	8.5	4.5
Patients from all other kebeles		N = 266†	18.0	17.0	20.0	17.0	11.5	15.0

* Illness duration before diagnosis; data missing for 135 patients.
† Data missing for 21 patients.

patients with onset in January 2005 or later, 170 (69%) came from outside Bura kebele. The patients with kala-azar diagnosed during the first massive screening effort in May 2005 had been ill for a mean of 6 months, but the mean duration fell to < 3 months by September 2005 (Table 1). Only three cases of post-kala-azar dermal leishmaniasis (PKDL) were diagnosed from April to October 2005.

Community survey. The survey included 592 members of 125 households in the four villages. Among the 584 individuals for whom kala-azar status could be determined, 40 (6.8%) met a case definition for confirmed or probable kala-azar (9 cases diagnosed and treated at AZHC, 12 current untreated cases, and 19 deaths after an illness consistent with kala-azar). The epidemiologic curve based on community data (Figure 4) was consistent with that for facility-based cases (Figure 3). The cumulative incidence was higher among men (9.7%) than women (4.5%; $P < 0.05$). The median age of kala-azar patients was 15.5 years compared with 17 years for the population as a whole ($P = 0.53$). A total of 27 deaths were reported in the study population; deaths attributed to kala-azar occurred in significantly older individuals (median age, 16 versus 3 years; $P < 0.05$), who were more likely to be men (68% versus 25%, $P < 0.05$). Seventy-nine percent of deaths attributed to kala-azar occurred before VL diagnostic testing be-
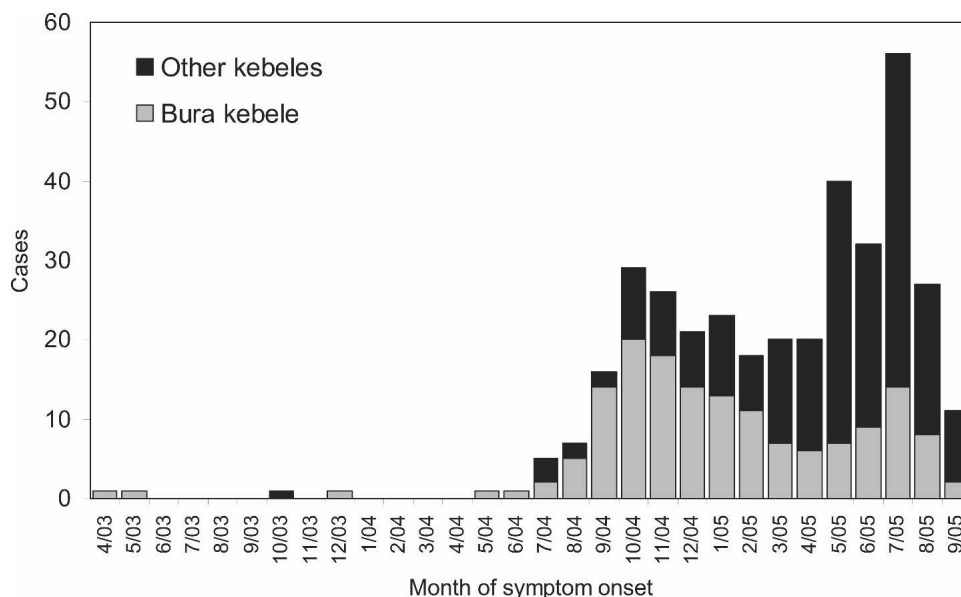

FIGURE 3. Epidemiologic curve for 357 patients with kala-azar diagnosed from May to October 2005 by MSF-Greece at Addis Zemen Health Center. Date of symptom onset missing for 135 patients.
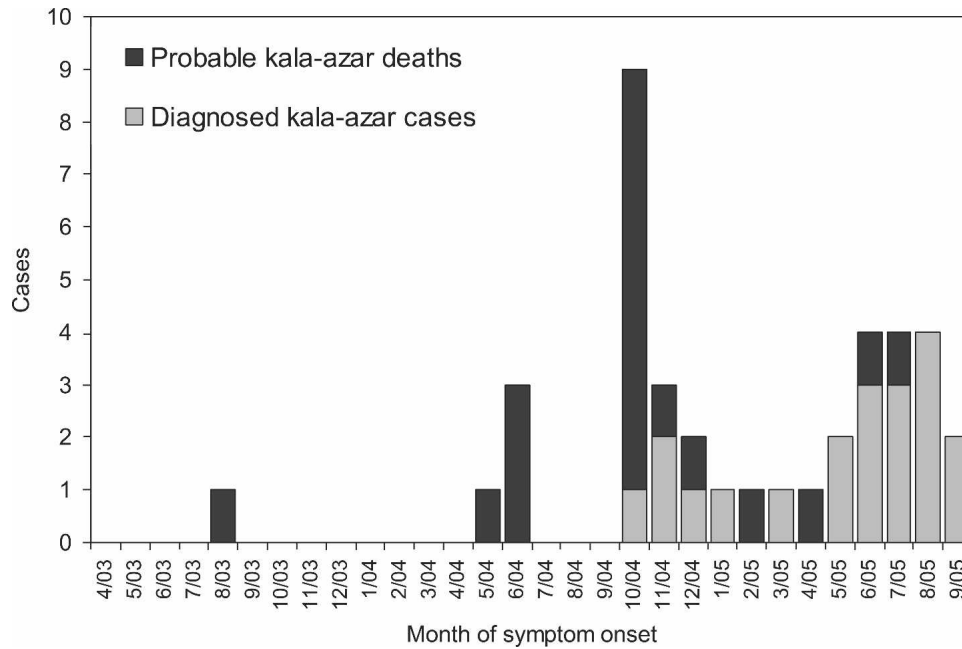
FIGURE 4. Kala-azar cases and probable kala-azar deaths by month of symptom onset; household survey data are from four villages in Libo, October 2005. Month of symptom onset could not be determined for one kala-azar case.

came available at AZHC (Figure 4). Among 39 patients with definite or probable kala-azar, 16 (76%) of 21 with onset in 2003 or 2004 died compared with 3 (16%) of 19 with onset in 2005 ($P < 0.0001$).

Leishmanin skin tests (LSTs) were applied and read 48 hours later for 459 participants. The prevalence of positive LST was higher among men (34%) than women (26%; $P = 0.06$) and rose significantly with age among men (χ^2 for trend 28.5, $P < 0.00001$) but not women (χ^2 for trend 2.9, $P = 0.09$; Table 2). There were consistent differences in the cumulative incidence of kala-azar and the prevalence of positive LST by village (Table 3); the ratio of positive LST to clinical kala-azar ranged from 2.5:1 to 4:1. A total of 14 men were reported to have spent time in Metema, Humera, or other areas bordering Sudan, but no significant association was found with risk of kala-azar ($P = 0.63$). Men who reported travel to these areas were more likely to be LST positive (8/11; 73%) than other men ≥18 years of age (43/89; 48%), but this difference did not reach statistical significance ($P = 0.13$).

Parasitology. *Leishmania* DNA was detected in all seven splenic aspirates by all three PCR techniques used.

In addition, NNN culture was positive in six and microscopy in three of seven specimens. Sequence of LnPCR products and RFLP analysis of the ITS-1 PCR products confirmed that all belonged to the *L. donovani* complex, whereas RFLP analysis of the T2B4 PCR product indicated that six isolates were *L. infantum* and one was *L. donovani*.

LnPCR amplified *Leishmania* DNA in the blood of 2 of the 40 dogs studied. Both samples belonged to the *L. donovani* complex, but further analysis of the ITS-1 and T2B4 regions was precluded by the scarcity of *Leishmania* DNA.

DISCUSSION

This rapid epidemiologic assessment confirmed a major new outbreak of VL in Libo Kemkem wereda, a highland district of Ethiopia where the disease had never before been reported. The epidemiologic picture, with a few cases over a 1-year period followed by an explosive increase, is consistent with the rapid emergence of the disease in a population with little pre-existing immunity. The data indicate that the outbreak began in Bura kebele as one or more foci of limited transmission in early 2003, followed by a marked increase in cases and deaths in the last several months of 2004, when it

TABLE 2
Prevalence of positive leishmanin skin test by sex and age, household survey in Libo Kekmen wereda, Ethiopia, October 2005

| Age group (years) | Leishmanin skin test positive [*N* (%)]/total tested | | |
	Men [*n*/N (%)]	Women [*n*/N (%)]	Both sexes [*n*/N (%)]
< 5	2/34 (6)	2/20 (10)	4/54 (7)
5–9	8/45 (18)	6/34 (18)	14/79 (18)
10–14	13/45 (29)	10/33 (30)	23/78 (30)
15–19	14/27 (52)	10/32 (31)	24/59 (41)
20–39	26/57 (46)	13/54 (24)	39/111 (35)
≥ 40	17/31 (55)	15/47 (32)	32/78 (41)
Total	80/239 (34)	56/220 (26)	136/459 (30)

TABLE 3
Cumulative kala-azar incidence from September 11, 2003 through October 22, 2005, and prevalence of positive leishmanin skin test by village, household survey in Libo Kekmen wereda, Ethiopia

Kebele	Village	Cumulative kala-azar incidence [*n*/N (%)]	Positive leishmanin skin test [*n*/N (%)]
Bura	1	18/132 (14)	50/100 (50)
Bura	2	12/133 (9)	48/107 (45)
Bura	3	8/163 (5)	32/122 (26)
Shina	4	2/156 (1)	6/130 (5)
Total		40/584 (7)	136/459 (30)

was first brought to the attention of health authorities. Our community data suggest that the reported cases may represent less than one half the true total, with many patients dying before treatment was available. An even greater increase in cases occurred in 2005, and the outbreak showed no sign of abating. As of July 2006, a total of 1,841 patients with kala-azar had been treated by MSF-G in Addis Zemen (MSF-Greece, unpublished data, 2006).

The mean illness duration before diagnosis fell from 6 months in May 2005 to 3 months in October 2005, reflecting increased awareness in affected communities and improved diagnostic availability at the AZHC. Based on the community data, case-fatality rates seem to have fallen sharply as well, confirming that the current clinical approach is functioning well. With adequate treatment, kala-azar case-fatality rates are < 5%, and patients generally respond quickly to antimonials in the Horn of Africa.[9] However, this outbreak has placed the local health care system under substantial strain. Moreover, our community data, in which we ascertained more currently ill, undiagnosed VL cases than treated cases, suggest that the patients reaching AZHC represent only a fraction of the total who would be identified by active case finding. Support and commitment are urgently needed for ongoing diagnostic and treatment services and to increase clinical capacity to allow active case detection in communities.

Kala-azar has been known to occur in migrant workers returning from the cotton, sesame and sorghum fields of Humera and Metema on the Sudan border since the 1970s.[4,17,18] Migrant workers sleep in rough shelters or the open air under acacia trees, placing them at high risk for exposure to bites of the leishmanial vector *P. orientalis*.[17,18] Leishmanin surveys in the border area suggest intense transmission in the migrant population.[17] The only previous report of kala-azar in the highlands east of Lake Tana described six cases in Belessa wereda, another highland area around 130 km north from Libo, with very similar ecology. In Belessa, *P. orientalis* was implicated as the vector, and at least two patients were children who had never traveled outside of the district.[8] No further transmission in the area was reported.

In both the Belessa cluster[8] and the Libo outbreak, investigators hypothesized that agricultural migrant workers returning from the Sudanese border area introduced VL into highland communities. Among adult men surveyed in Libo in 2005, 4% had worked in Metema and 8% in Humera in the previous 3 years. Those who worked in the border zone were more likely to be skin test positive, suggesting that they had leishmanial exposure there, but they had no higher incidence of kala-azar than their neighbors who had not traveled. The outbreak study thus could not prove the hypothesis that leishmaniasis was introduced into Libo by returning seasonal workers but supported the possibility. Nevertheless, in contrast to the isolated report from Belessa, VL transmission is now well established in Libo Kemkem and spreading into neighboring districts; the area seems destined to become a VL-endemic focus. Spurred by recurrent famine and droughts, the Ethiopian government established a program to resettle thousands of people from drought-affected areas that have no leishmanial transmission to the more fertile but VL-endemic areas bordering Sudan. The Libo outbreak raises the possibility that both resettled people and highland communi-

FIGURE 5. Bed adjacent to shelter in Bura. Sleeping outside may be a risk factor for infection because of higher exposure to sand fly bites.

ties to which they return will be at high risk for lethal epidemics of VL.

The limitations of this rapid assessment leave several unanswered questions. The small number of leishmanial isolates from humans and dogs does not allow us to draw a definitive conclusion regarding the relative contributions of zoonotic and anthroponotic transmission. Moreover, the responsible sand fly species has not yet been proven. In the facility-based data, some records lacked data on specific variables such as illness duration or sex. In the community-based data, we ascertained probable deaths from kala-azar based on the report of family members, and some of the deaths may have had some other unidentified cause. Nevertheless, the data from the two sources taken together present a consistent picture of an explosive focal outbreak with high mortality followed by sustained transmission. Both disease and infection rates were significantly higher in men than women. This finding may reflect the male tendency to sleep outdoors to guard their cattle (Figure 5) and suggests that transmission may occur predominantly outside the house, hypotheses that should be tested in a more detailed study of behavioral and other risk factors.

Despite the relatively small number of isolates, both *L. donovani* and *L. infantum* were identified in Libo Kemkem, as previously reported in Kenya,[19] Sudan,[20] and Ethiopia (A. Hailu, personal communication, 2005). The detection of two *Leishmania* species and multiple genotypes (data not shown) indicates that multiple parasite introductions must have occurred into the outbreak area. *L. infantum* was more frequently identified than *L. donovani,* consistent with the low reported PKDL prevalence and raising the potential role of dogs as an epidemiologically important reservoir host. Indeed, the presence of leishmanial infection in the dog population was confirmed by the amplification of *Leishmania* DNA from two dogs. Molecular analysis of the amplified products indicated infection by the *L. donovani* complex, but species-level identification was impossible because of the scarcity of DNA in the samples. Although suggestive, these preliminary results are not sufficient to clarify the role of the dog in this outbreak. Further study may clarify the epidemiologic significance of our findings and help resolve the ongoing controversy surrounding the taxonomy of the *L. donovani* complex in the region.[21]

However, the most important unanswered question is why transmission in Libo Kemkem and neighboring districts reached epidemic levels in 2004 and has continued to increase since then, rather than burning out, as seems to have happened in the past.[8] In the Horn of Africa, VL epidemics frequently occur in the wake of war, population displacements, famine, and drought.[10,22] However, the population of Libo was stable through 2005, and no dramatic changes in agricultural production or cattle populations were reported. Based on the limited available data, we hypothesize that VL transmission in Libo may have been sustained focally and at low levels for many years, occasionally coming to light in studies such as the 1973 study.[8]

In summary, the VL outbreak in Libo Kemkem and neighboring weredas seems to be fueled by a well-established transmission cycle caused by two different *Leishmania* visceralizing species. The geographic spread and rapid, sustained progression over the last 2 years suggest that VL transmission will not be easy to eradicate. The most urgent need is to strengthen the capacity of local health facilities to ensure availability of early, accurate diagnostic testing followed by appropriate antileishmanial treatment. Until the detailed transmission cycle, sand fly species and risk factors are fully described, the use of insecticide-treated bed nets may help to stem the growth of this epidemic. Further studies in humans and dogs, as well as entomologic studies during the appropriate season, will be essential to develop a comprehensive long-term control strategy.

Authors' addresses: Jorge Alvar, Department for the Control of Neglected Tropical Diseases, World Health Organization, 20 Avenue Appia, CH-1211Geneva 27, Switzerland. Seife Bashaye, Malaria and Other Vector Borne Diseases, Prevention and Control Program, Ministry of Health, Addis Ababa, Ethiopia. Daniel Argaw and Olusegan Babaniyi, Disease Prevention and Control Programmes, World Health Organization. P.O. Box 3069, Menelik Avenue, UNECA Compound, Addis Ababa, Ethiopia. Israel Cruz, WHO Collaborating Center for Leishmaniasis, Servicio de Parasitología, National Center of Microbiology, Instituto de Salud Carlos III, 28220-Majadahonda, Madrid, Spain. Pilar Aparicio, Centro Nacional de Medicina Tropical, Instituto de Salud Carlos III, c/ Sinesio Delgado s/n, 28029 Madrid, Spain. Askal Kassa, Health Center, Addis Zemen, Ethiopia. Giannos Orfanos, Greek Medical Council, Athens, Greece. Fernando Parreño, Centro de Salud Area 7, Cea Bermúdez núm. 10, 28015-Madrid, Spain. Nigussu Gudeta, Regional Health Bureau, Bahar-Dar, Amhara State, Ethiopia. Carmen Cañavate, WHO Collaborating Center for Leishmaniasis, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid, Spain. Caryn Bern, Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341.

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