Natural History of a Visceral Leishmaniasis Outbreak in Highland Ethiopia

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Abstract. In May 2005, visceral leishmaniasis (VL) was recognized for the first time in Libo Kemken, Ethiopia, a highland region where only few cases had been reported before. We analyzed records of VL patients treated from May 25, 2005 to December 13, 2007 by the only VL treatment center in the area, maintained by Médecins Sans Frontières-Ethiopia, Operational Center Barcelona-Athens. The median age was 18 years; 77.6% were male. The overall case fatality rate was 4%, but adults 45 years or older were five times as likely to die as 5–29 year olds. Other factors associated with increased mortality included HIV infection, edema, severe malnutrition, pneumonia, tuberculosis, and vomiting. The VL epidemic expanded rapidly over a several-year period, culminating in an epidemic peak in the last third of 2005, spread over two districts, and transformed into a sustained endemic situation by 2007.

INTRODUCTION

In 2005, a new epidemic of visceral leishmaniasis (VL) was reported in the district of Libo Kemkem in northwestern Ethiopia.[1] The outbreak occurred in a highland region, where few cases of the disease had ever been reported before. The initiating event was hypothesized to have been the introduction of the parasite, probably on multiple occasions, by migrant agricultural laborers returning to their villages from seasonal work on the border with Sudan.[1–4] Health practitioners in the district had no previous experience with VL, and early cases were attributed to drug-resistant malaria, leading to a delay in recognition and high mortality early in the epidemic.[1] By the time the etiology was confirmed, available data suggested that some cases had symptom onset at least 2 years earlier, and the outbreak had already spread beyond the villages that were first affected. More than one species and several genotypes of *Leishmania* were identified in human and canine specimens, supporting the hypothesis that multiple introductions had occurred.[1] The Addis Zemen Health Center (AZHC), maintained by Médecins sans Frontières-Ethiopia, Operational Center Barcelona-Athens, was the only facility in the area with VL diagnostic capability and anti-leishmanial drugs, and their personnel identified the cause of the epidemic and treated almost all of the VL patients. Their records therefore provide a fairly complete picture of the course of the outbreak from the time it was first recognized. Community-based data collected in October 2005 suggested that a large proportion of patients who became ill in 2003 and 2004 may have died before their disease was recognized; however, once AZHC began offering treatment of VL, the mean duration of illness before diagnosis fell quickly, and ascertainment in villages near Addis Zemen was likely to be high.[1] Using data from AZHC, we therefore performed a series of analyses to describe the patient population and characterize the course of the epidemic from 2004 to 2007.

MATERIALS AND METHODS

Addis Zemen is the capital of Libo Kemkem wereda (district), in the Amhara Region of northwestern Ethiopia (average altitude 2,000 m above sea level). The town is located between Bahir Dar and Gondar on the major road connecting Addis Ababa to the Red Sea through Port Sudan. Most patients treated at AZHC lived in Libo and Fogera districts (estimated populations 198,374 and 226,595, respectively), located across from each other on the north and south banks of the Rib River.

The data in this analysis included all primary VL cases diagnosed and treated at AZHC between May 25, 2005 (when the etiology of the outbreak was first recognized as VL) and December 13, 2007. The WHO operational case definition for VL was used: a person showing clinical signs (prolonged irregular fever for > 2 weeks, splenomegaly, and/or weight loss) with serologic and/or parasitologic confirmation (when feasible).[5] Malaria was ruled out based on negative blood smears. Most patients had their diagnosis confirmed by the direct agglutination test (DAT). The DAT was performed using standard methods and antigen from ITG-Belgium; titers ≥ 1:3,200 were considered positive.[6] If the diagnosis could not be confirmed by the results of DAT on two separate occasions (because of discordant results or repeatedly borderline titers of 1:800 or 1:1,600), splenic aspirate was used to confirm the diagnosis. A small number of patients had their diagnosis confirmed using the InBios Kala-azar Detect rK39 rapid test (InBios International, Seattle, WA), which was not yet registered in the country at the time. These patients were diagnosed during and just after an earlier study that used these rapid tests; the study investigators provided the remainder to AZHC after the field work ended.[1]

We calculated dates of symptom onset retrospectively, based on dates of diagnosis and the duration of illness reported in the clinical record. Spleen size below the costal margin was measured in centimeters and categorized based on the distribution by age group; quartiles were defined for children < 2, 2.1–5,

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5.1–14, and 15 years of age or older. The top two quartiles were compared with the bottom two quartiles of spleen size as the referent. Hemoglobin testing was conducted for a proportion of patients. Until January 2007, a manual hemoglobinometer (Hemocue Hb 201+, HemoCue AB, Angelholm, Sweden) was used. Later in the course of the epidemic, the facility obtained a SYSMEX (KX-21 Automated Hematology Analyzer, Sysmex Corporation, Kobe, Japan) instrument that produced complete blood counts for the most severe patients. Adults were offered systematic HIV screening through a voluntary counseling and testing program beginning in December 2006. Parallel testing was performed using the Determine Rapid HIV1/2 Test (Inverness Medical, Waltham, MA) and Capillus HIV1/HIV2 Rapid Test (Trinity Biotech, Wicklow, Ireland). For specimens with discordant results, the Uni-gold Recombigen HIV (Trinity Biotech) was used as the third test. Written parental consent was obtained before testing children for suspected HIV infection.

Body mass index (BMI) was calculated (weight in kilograms divided by the square of the height in meters) for adults and children older than 5 years. Weight-for-height Z scores were calculated for children using software available on the World Health Organization website (http://www.who.int/childgrowth/software/en/index.html). This software uses newly released data from an international reference population.[7–9] Severe malnutrition was defined by the presence of bilateral lower extremity edema or the appropriate anthropometric criteria depending on age: weight-for-height Z scores < −3 for participants ≤ 5 years; BMI-for-age Z score < −3 for those between 5 and 19 years; and BMI < 16.0 kg/m² for those older than 19 years. Severe anemia was defined as hemoglobin < 7.0 g/dL.

Statistical analyses were performed in SAS 9.0. We modeled predictors of death first through univariate logistic regression analyses, followed by a backwards stepwise elimination procedure beginning with variables significant at 0.10. Collinear variables, such as edema and severe malnutrition, were not included in the same model. Cumulative annual incidence data were mapped at the kebele (subdistrict) level within Libo and Fogera, the two most affected districts.

RESULTS

A total of 2,543 leishmaniasis patients were treated at AZHC between May 25, 2005 and December 13, 2007. Of these, 2,364 (92%) were diagnosed with primary VL; 31 (1.2%) patients were recorded as having a relapse of previously treated VL, 39 (1.5%) as post–kala-azar dermal leishmaniasis, 106 (4.2%) as cutaneous leishmaniasis, and 3 (0.1%) as mucocutaneous leishmaniasis. The remainder of the analysis focused on the 2,364 patients with primary VL. A total of 2,174 patients were diagnosed by DAT, whereas 86 had the diagnosis confirmed by splenic aspirate and 104 by rK39 rapid test. The median age of VL patients was 18 years (range, 0.8–80 years); 1,834 (77.6%) were male. Female patients were significantly younger than male patients (median age, 11 versus 20 years; P < 0.0001). Of 578 patients younger than 10 years old, 235 (40.7%) were girls, compared with 21.0% (136/647) of patients 10–19 years old and 13.7% (156/1136) of patients 30 years or older (χ² for linear trend, 151.0; P < 0.0001). Of 2,306 patients with data regarding spleen size on admission, 2,168 (94%) had palpable splenomegaly; the median spleen size was 8.5 cm (range, 1–28 cm). Admission measurements of weight and height were available for 2,261 patients. A large propor-

tion of patients showed signs of severe malnutrition: 39% of children < 5 years had weight-for-height and 41% of children 5–19 years old had BMI-for-age below −3 Z scores, whereas 26% of adults had a BMI < 16 kg/m². Of 1,860 patients with data available, 365 (20%) had bilateral lower extremity edema on physical examination. The median hemoglobin on admission was 8.1 g/dL.

Ninety-seven percent (2,296/2,364) of VL patients were treated with sodium stibogluconate. In 2007, a limited supply of liposomal amphotericin was available but was used only for the most gravely ill patients; a total of 48 (2%) patients were treated with liposomal amphotericin, and 20 (0.8%) received a combination of sodium stibogluconate and liposomal amphotericin. Of 2,177 VL patients with outcome data, 87 (4%) were known to have died. Outcome data were missing for 187 patients, including 38 patients who defaulted and 13 who were transferred. The case-fatality rate showed a J-shaped curve: mortality was lowest in patients 5–29 years old, whereas adults 45 years or older were five times as likely to die as patients in the 5- to 29-year age group (Figure 1). In addition to age, other factors associated with increased risk of death included HIV infection, the presence of edema, severe malnutrition, pneumonia, tuberculosis, and vomiting (Table 1). Vomiting was more common in the small group of patients treated with liposomal amphotericin (23/48 [47.9%] compared with 74/2,295 [3.2%] patients treated with sodium stibogluconate; P < 0.0001 by two-tailed Fisher exact test). The severity of illness in this patient group lends support to the hypothesis that vomiting was a marker for more severe VL and not an outcome of antimonial therapy. Before December 2006, only 2% of patients had HIV testing compared with 82% of patients admitted in or after December 2006. Data were not systematically collected regarding jaundice or bleeding. In a multivariable logistic regression model, age group, edema, severe anemia, HIV status, and tuberculosis were identified as independent predictors of death (Table 2).

The duration of symptoms was recorded for 1,909 (80%) of 2,364 patients, allowing us to calculate the month of illness onset. Cases missing duration data did not differ from those with known duration in terms of age or sex distribution but were more likely to have been treated in 2005 than in 2006 or 2007 (P < 0.001 for 2005 compared with 2006–2007). The
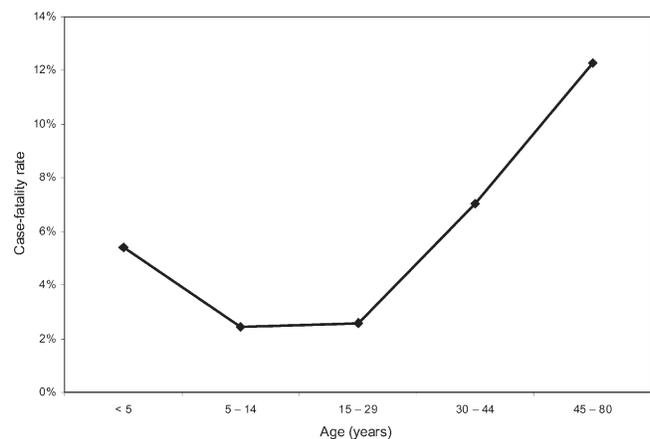

FIGURE 1. Case-fatality rate by age group among 2,177 primary VL patients treated from May 2005 to December 2007 at the Addis Zemen Health Center.

TABLE 1

Univariate analyses of factors associated with death among 2,177 primary VL patients* treated from May 2005 to December 2007 at the Addis Zemen Health Center, Libo District, Ethiopia

	N	Case fatality rate (%)	Odds ratio (95% CI)	P
Age group (years)				
< 5	239	5.4	2.2 (1.2–4.2)	0.02
5–14	655	2.4	Referent	
15–29	850	2.6		
30–44	327	7.0	2.9 (1.7–5.0)	< 0.0001
45–80	106	12.3	5.4 (2.8–10.5)	< 0.0001
Sex				
Male	1,689	3.9	Referent	
Female	487	4.3	1.1 (0.7–1.8)	0.69
Spleen size				
Below median for age	988	3.5	Referent	
50th to 75th percentile	531	4.0	1.0 (0.6–1.7)	0.94
Above 75th percentile	538	4.3	1.1 (0.7–1.9)	0.70
HIV status				
Positive	50	10.0	5.4 (1.5–19.4)	< 0.0001
Negative	248	2.0	Referent	
Unknown	1,879	4.1	2.1 (0.8–5.2)	0.12
Severe anemia				
Hemoglobin < 7.0	159	8.8	4.7 (1.86–11.9)	0.0011
Hemoglobin ≥ 7.0	348	2.0	Referent	
Unknown	1,670	4.0	2.0 (0.91–4.4)	0.084
Edema				
Yes	365	10.7	5.3 (3.3–8.6)	< 0.0001
No	1,495	2.2	Referent	
Severe malnutrition†				
Yes	922	6.1	2.6 (1.6–4.0)	< 0.0001
No	1,255	2.5	Referent	
Pneumonia				
Yes	230	7.0	2.1 (1.2–3.6)	0.02
No	1,937	3.5	Referent	
Diagnosis of TB				
Yes	22	16.7	5.7 (1.9–17.3)	0.002
No	2,145	3.9	Referent	
Vomiting				
Yes	95	9.5	2.8 (1.3–5.7)	0.0056
No	2,071	3.6		

* These analyses exclude 187 VL patients whose outcome data were missing or ambiguous.
† Severe malnutrition defined by presence of edema or weight-for-length/height Z scores < −3 for participants ≤ 5 years; body mass index (BMI) Z score < −3 for those between 5 and 19 years; BMI < 16.0 kg/m² for those older than 19 years.

AZHC treated a total of seven VL cases with calculated onset dates between March 2003 and June 2004. From June 2004 onward, VL incidence rose sharply, peaking in the latter half of 2005 and falling in 2006 (Figure 2). Beginning in mid-2006, a higher proportion of patients also came from more distant districts. These patients were older, more likely to be male, and

TABLE 2

Multivariable model of risk factors associated with death among 2,177 VL patients treated from May 2005 to December 2007 at the Addis Zemen Health Center, Libo District, Ethiopia

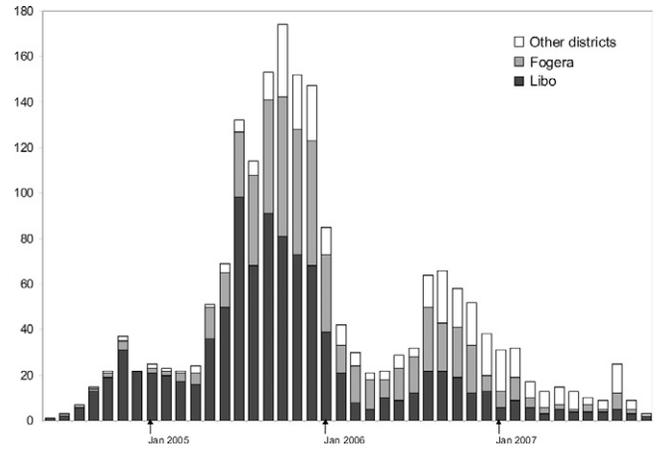| Risk factor	Odds ratio (95% CI)	P
Age (years)		
< 5	1.66 (0.74–3.75)	0.2226
5–29	1.0	—
30–44	3.32 (1.76–6.27)	0.0002
≥ 45	6.61 (3.15–13.9)	< 0.0001
Edema	4.93 (2.93–8.29)	< 0.0001
Hemoglobin < 7.0	4.43 (1.53–12.8)	0.006
HIV status		
Positive	5.96 (1.23–24.0)	0.027
Negative	1.0	—
Unknown	5.82 (1.73–19.6)	0.0044
Tuberculosis	5.18 (1.29–20.8)	0.0205

FIGURE 2. Epidemiologic curve showing cases by month of illness onset and location of residence, among 1902 primary VL patients with onset between June 2004 and December 2007.

had been ill longer at the time of admission compared with patients who came from Libo and Fogera, the two districts closest to AZHC (P < 0.001 for each comparison; Table 3). As noted in earlier analyses, the mean illness duration in patients from Libo and Fogera fell from 5.8 months in May to August 2005 to 3.0 months thereafter.

Within Libo and Fogera districts, VL cases became more geographically dispersed from 2004 to 2006 (Figure 3A–C). Sixty-seven percent of cases with onset in 2004 came from Bura kebele, where the epidemic began (Figure 3A). However, only 15.7% of 2005 cases and 3.9% of 2006 cases came from Bura kebele. In the latter 2 years of the outbreak, more patients came from other kebeles of Libo (43.1% of 2005 and 31.7% of 2006 cases) and from Fogera district (30.6% of 2005 and 39.3% of 2006 cases) than from Bura kebele. By 2006, the cumulative annual incidence had diminished throughout the districts (Figures 2 and 3C).

DISCUSSION

Data from VL cases treated at AZHC tell the story of an epidemic that expanded rapidly over a several-year period, culminating in an epidemic peak in the last third of 2005, spread over two districts, and transformed into a low incidence endemic situation by 2007. During the first 3 months after recognition of the epidemic, the mean duration of illness fell sharply, reflecting the identification and treatment of patients whose diagnosis had been delayed during the period that the etiology was unknown. The response to the epidemic by AZHC staff was instrumental in maintaining the case fatality at or near 4%, a low rate under field conditions. A substantial proportion of patients undoubtedly died at home before recognition of the epidemic,[1] and some deaths that occurred after discharge likely went unrecorded. Case follow-up in this area is limited by distance, poverty, lack of public transport, and resulting constraints on the ability to travel to facilities. The fact that VL patients from more distant districts were significantly older and more likely to be male compared with those from Libo and Fogera suggests that patients who are younger and/or female may be less likely to be able to travel to AZHC, reflecting more marked barriers to health care access for these groups. Deaths from untreated VL may be occurring

TABLE 3

Characteristics of 2,364 primary VL patients treated from May 2005 to December 2007 at Addis Zemen Health Center, Libo District, Ethiopia, by district of residence

	Libo	Fogera	Other districts	All patients
Sex				
Male	933 (73.9%)	536 (75.5%)	365 (94.1%)*	1834 (77.7%)
Female	330 (26.1%)	174 (24.5%)	23 (5.9%)	527 (22.3%)
Age (years)				
< 5	173 (13.7%)	74 (10.4%)	6 (1.6%)	253 (10.7%)
5–14	431 (34.1%)	226 (31.8%)	33 (8.5%)	690 (29.2%)
15–29	411 (32.5%)	264 (37.2%)	251 (64.7%)	926 (39.2%)
30–44	183 (14.5%)	107 (15.1%)	82 (21.1%)	372 (15.7%)
45–80	67 (5.3%)	39 (5.5%)	16 (4.1%)	122 (5.2%)
Mean age (years)	18.1	19.5	24.7*	19.6
Months of illness before treatment	3.35	3.12	4.27*	3.44

* Distribution by sex, age, and duration of illness differs significantly for patients from other districts compared with patients from Libo and Fogera; $P < 0.001$ for each comparison.

in these districts without being recognized. Low awareness of the disease both by health practitioners and local population may also decrease recognition of relapses, especially among HIV-VL co-infected patients who often have atypical presentations. Because of these factors, the measured case-fatality rates are likely to be underestimates.

In common with data reviews from other VL treatment facilities in the Horn of Africa, we found that young children and older patients were much more likely to die than older children or young adults.[10] Other predictors of mortality, such as severe anemia, severe malnutrition, edema, HIV status, and the presence of pneumonia or tuberculosis, were not unexpected.[10–12] Severe malnutrition, anemia, and immunosuppression from HIV all intensify VL morbidity. Bacterial pneumonia and tuberculosis are typical secondary infections during VL and are often the immediate cause of death. Jaundice has also

been recognized as an indicator of poor prognosis.[12] We lacked systematic data on jaundice, but vomiting may have been associated with hepatic dysfunction. Edema also may have been a composite measure, reflecting both severe malnutrition and, in some cases, hepatic dysfunction. These data support the continued use of a severity score to triage patients in need of more intensive therapy.[10]

Although the large number of patients included in this analysis lends robustness to our findings, the inevitable limitations of clinical data constrained our ability to precisely define risk factors and their impact. One major limitation is the fact that data on HIV status were only available starting in December 2006; the impact of this factor is almost certainly underestimated in our analysis. Furthermore, we cannot evaluate the previously documented association between treatment with antimonials and higher mortality in HIV co-infected patients,[13]
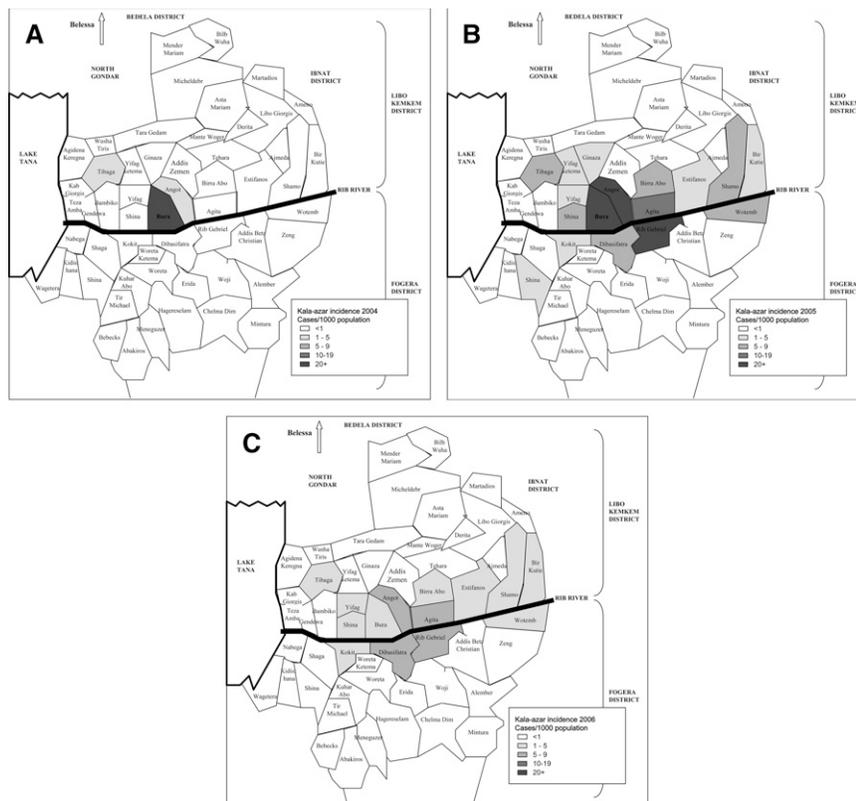

FIGURE 3. Cumulative annual incidence of VL by *kebele* (subdistrict) in Libo and Fogera districts in (**A**) 2004, (**B**) 2005, and (**C**) 2006.

because our HIV data are incomplete and sodium stibogluconate was the only anti-leishmanial drug available for most of the study period. Because of the extremely limited supply, liposomal amphotericin was reserved for the most gravely ill patients. Other risk factors, such as hemoglobin level, were more likely to be measured in the patients with more severe illness; nevertheless, the comparison within the subgroup with measured hemoglobin supports the view that severe anemia is associated with increased mortality risk.

Most importantly, these data offer the unique opportunity to document the early stages of an epidemic followed by the transformation into a situation of sustained endemic transmission. We know that local VL transmission occurred in this highland region in the 1970s, when two apparently autochthonous VL cases were described in the neighboring district of Belessa.[2] More than 25 VL cases were treated at Gondar Hospital in the 1970s, all in migrant workers returning from the Sudan border areas.[3] The authors estimated in 1978 that 50,000–100,000 highland men spent an agricultural season in the Sudan border area each year[3]; the current number of seasonal migrants is likely to be even higher. *Phlebotomus orientalis*, the known lowland vector, was trapped in Belessa in 1972 and in Libo in 2006.[2,14] Although none of the trapped sand flies were shown to be infected with *Leishmania*, the highland vector is assumed to be capable of sustaining leishmanial transmission.[2,14]

Between the 1970s and 2003, *Leishmania* introductions must have occurred, but in 2004, a threshold seems to have been passed, leading to a period of intense transmission in Bura kebele. In the second half of 2004 and in 2005, the incidence of VL in Bura was > 2% per year. In the 2005 survey of the most affected village in Bura, 14% of the population was reported to have had VL since 2004, and 50% of those tested were leishmanin skin test positive.[1] In 2005, during the peak of the epidemic, kebeles adjacent to Bura showed equally high incidence. However, by 2006, the incidence of new cases of VL had fallen sharply in Bura and its neighbors; like the high positive skin test prevalence, the decline probably reflected saturation of the susceptible population. Nevertheless, low-level transmission has continued into 2007 and shows no signs of disappearing. This region must now be added to the list of sustained endemic foci of visceral leishmaniasis in Ethiopia.

Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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