Am. J. Trop. Med. Hyg., 95(1), 2016, pp. 104–108
doi:10.4269/ajtmh.15-0738

Visceral Leishmaniasis in Benishangul-Gumuz Regional State, Western Ethiopia: Reemerging or Emerging?

Adugna Abera,[1] Geremew Tasew,[1,3] Teshome Tsegaw,[1] Asfaw Kejella,[2] Abate Mulugeta,[4] Dagimlidet Worku,[5] Abraham Aseffa,[1] and Endalamaw Gadisa[1]*

[1]*Armauer Hansen Research Institute, Addis Ababa, Ethiopia;* [2]*Benishangul-Gumuz Regional Health Bureau, Assosa, Ethiopia;* [3]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia;* [4]*Disease Prevention and Control Programs, World Health Organization, Ethiopia Country Office, Addis Ababa, Ethiopia;* [5]*KalaCORE Consortium, Ethiopia Country Office, Addis Ababa, Ethiopia*

Abstract. Kala-azar is a growing public health problem in Ethiopia. Benishangul-Gumuz regional state was previously not known to be endemic for the disease. In response to a case report from the region, we conducted a rapid assessment survey. A pretested questionnaire was used to capture sociodemographic and clinical histories pertinent to kala-azar. Study participants with complaints of fever and headache for 2 weeks or more were tested for kala-azar and malaria. All participants were screened with the leishmanin skin test and the direct agglutination test for exposure to *Leishmania*, defined as a positive result with either or both tests. Of 275 participants, 20 were exposed giving an overall leishmaniasis seroprevalence rate of 7.3%. Among the 20 positive individuals, 19 were farmers and nine of them reported no travel history outside their district. It appears that kala-azar is emerging in Dangur and Guba districts of Benishangul-Gumuz regional state, probably in connection with human encroachment into one or several previously out-of-reach zoonotic foci. We recommend integrated epidemiological surveys for confirmation and early containment of disease transmission in the area.

INTRODUCTION

East Africa, the second largest visceral leishmaniasis (VL or kala-azar) focus, contributes 15% of the estimated annual global burden of 0.2–0.4 million cases.[1] The major contributors to the kala-azar burden in the region are Sudan, South Sudan, and Ethiopia.[1] In Ethiopia, over 3.2 million people live at risk of infection with up to 4,500 new cases of kala-azar per year.[2,3] The known endemic foci are the Segen-Woito valleys, lower Omo river plains, Lake Abaya area in the southwest, and Metema-Humera area in the northwest.[4]

The east African region has experienced several outbreaks of kala-azar in recent years. Outbreaks were recorded in Somali refugee camps in Kenya in 2000[5]; in northern Jonglei and eastern Upper Nile states of South Sudan[6] in 2002; in Bakool, Huddur, and Tijelow regions of Somalia in 2002–2005[7]; and in Libo Kemkem and Fogera districts of northwest Ethiopia in 2005–2007. More recently, Tahtay Adiabo District in northern Ethiopia has turned out to be highly endemic for VL.[8–10] Further cases of kala-azar are being reported from Oromia (Borena zone) and Somali (Imey zone) regional states to the Federal Ministry of Health of Ethiopia from places previously not known to be endemic for the disease.[3] Large-scale movement of temporary laborers to endemic localities, malnutrition, and human immunodeficiency virus coinfection have been identified as the main risk factors for these resurges of kala-azar in Ethiopia.[11–14]

The spread of kala-azar outside the historically known endemic foci is a growing public health concern in Ethiopia. Recently, we came across a kala-azar case on treatment at the Addis Zemen Health Center in northwest Ethiopia. The case was a migrant laborer from Wanberma District of west Gojjam zone, Amhara regional state, who had gone to work at a commercial farm in the adjoining Benishangul-Gumuz regional state. Parts of the Benishangul-Gumuz regional state fall under high and very high risk zones according to the kala-azar environmental factor–based risk map.[2] However, to our knowledge, there has so far been no report of kala-azar from the region although it is currently home to new small- and large-scale development programs that penetrate into formerly uninhabited or sparsely inhabited areas that attracted influxes of immigrants and shift population bases, all factors which could potentially lead to the establishment of new foci. We conducted a rapid assessment to verify if there is autochthonous transmission in the area of Benishangul-Gumuz where the kala-azar case came from so that appropriate measures could be taken for early intervention.

MATERIALS AND METHODS

Study design and period. A community-based cross-sectional survey was conducted from April 20 to May 10, 2015, in Dangur and Guba, the two suspected districts in Benishangul-Gumuz regional state, western Ethiopia.

Study area. The survey followed the travel history of the kala-azar case treated at Addis Zemen Health Center. This kala-azar case was initially working in Bruhoy/Demelash Farm in Kota village of Dangur District. Later, he traveled to the Almehal kebele of the Guba District. The two districts are bordered by Quara, Jawi Sirba Abay, and Wonbera (in Ethiopia) and the Jebel Geri and Dindir National park areas (in Sudan). The districts are located between 34°55.4′ to 36°26.7′ E longitude and 10°54.8′ to 12°00′ N latitude (Figure 1).

The average altitude is 824.6 and 717.3 for Kota and Almehal areas, respectively. The area had an average temperature of 26°C and an annual rainfall of 1,323 mL over the last 20 years (data from the Ethiopian Meteorology Agency). The major soil type found in Almehal kebele is vertisols, whereas alisols and nitosols are the dominant soil types in Kota kebele (based on the United Nations Food and Agriculture Organization Soil data, 2000).

Data collection methods and analysis. Local community elders were consulted on how to effectively access the community before the survey was initiated. A house-to-house

*Address correspondence to Endalamaw Gadisa, Armauer Hansen Research Institute, Jimma Road, P.O. Box 1005, Addis Ababa, Ethiopia 1005. E-mail: endalamawgadisa@yahoo.com

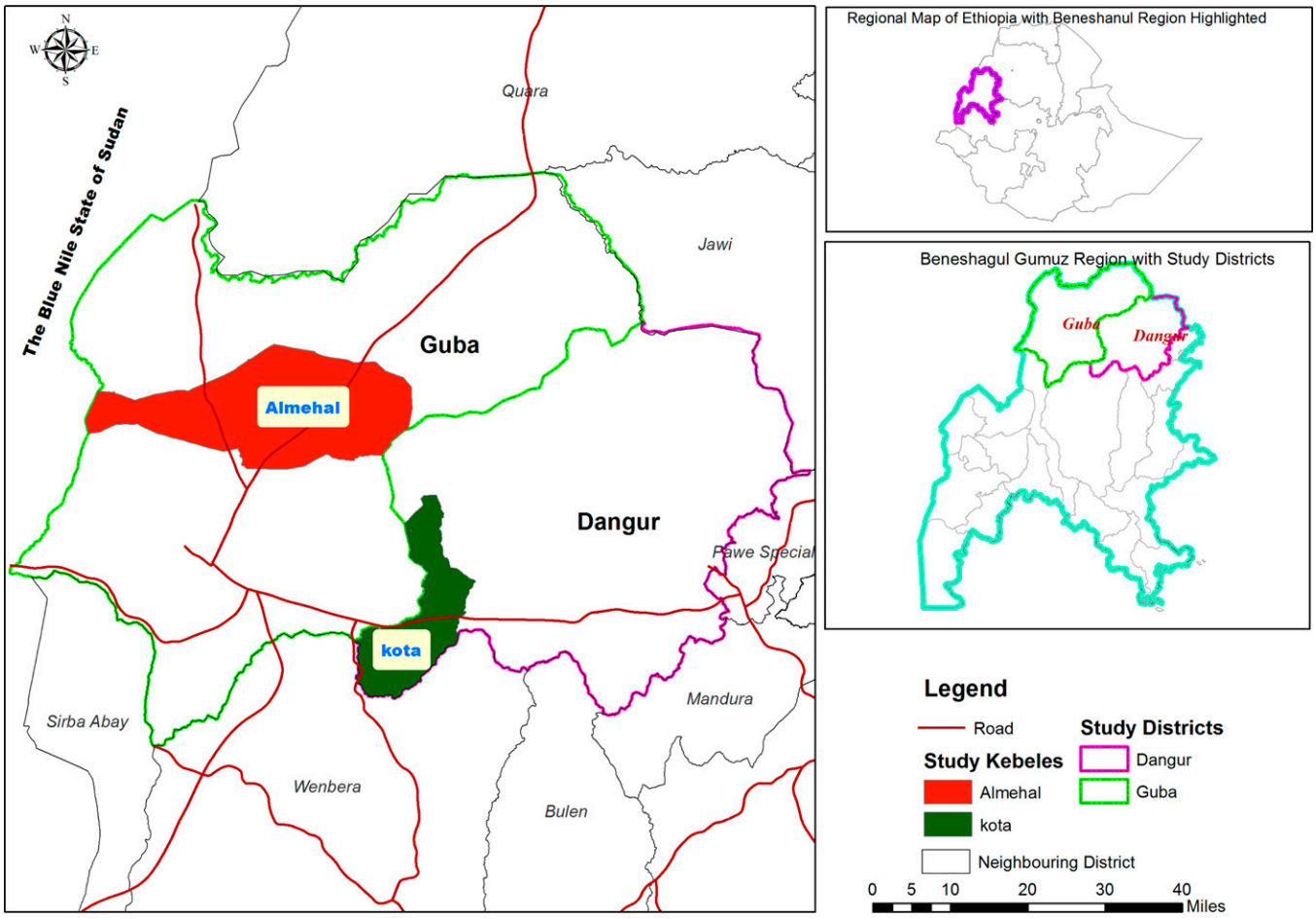

FIGURE 1. Map of study kebeles, Almehal and Kota, in perspective of Guba and Dangur districts, Benishangul-Gumuz regional state, April–May 2015.

survey was conducted starting from the center of each Gott. Only those individuals who had lived in the area for at least 6 months before the study were considered. All volunteers and/or their guardians (for children between 11 and 18 years of age) were adequately informed about the study and signed a consent form. Heads of the households were interviewed about kala-azar history in the household. A pretested structured questionnaire was used to record participants' travel history and other sociodemographic characteristics. Up to 40 volunteers were enrolled per Gott. For camp residents in large-scale farms, all consenting temporary and permanent employees (laborers), who were found in the camp during our visit, were enrolled.

Individuals suspected for clinical kala-azar based on signs and symptoms were examined by the study physician at the nearby health post where privacy could be provided. General clinical and physical examinations were done with particular reference to fever, splenomegaly and/or hepatomegaly, and enlargement of lymph nodes. Suspected febrile cases were screened with rapid tests: DiaMed-IT LEISH rK39 (DiaMed AG, Cressier sur Morat, Switzerland) and CareStart™ (ACCESS BIO, Inc., Somerset, NJ) for kala-azar and malaria, respectively. The test results were interpreted according to the manufacturer's instructions.

The leishmanin skin test (LST; Pasteur Institute of Iran, Tehran, Iran) was administered to all consenting participants by injecting 0.1 mL leishmanin solution intradermally on the volar surface of the left forearm using a 1.0-mL sterile syringe and disposable needle. The reaction was read after 48–72 hours. Induration was measured with the ballpoint technique, and a cutoff of ≥ 5 mm average diameter was considered positive.

Direct agglutination test (DAT) assay: dried blood spot (DBS) samples were collected aseptically from all consenting study participants using sterile disposable blood lancets on 3MM Whatman paper (Whatman Internationl Ltd., Springfield Mill, United Kingdom) and tested with DAT (Institute of Tropical medicine, Antwerp, Belgium) as per the manufacturer's instructions. In brief, a 5-mm disk from each of the DBS samples was punched out into each well of the second row of a V-shaped microtiter plate. Then 125 μL physiological saline (0.9% NaCl) containing 0.78% β-mercaptoethanol was added and incubated overnight at 4°C. A 2-fold series of serum dilution was prepared from 1:200 to 1:204,800. Positive and negative controls were included in the first and every fifth plate thereafter. Results were read after 18 hours of incubation. A cutoff titer of 1:6,400 was considered positive and titers between 1:1,800 and 1:3,200 were reported as borderline.

Data were double entered in an access database, cleaned, and verified. The χ^2, McNemar's, and Fisher's exact tests were used to assess the relationship between seroprevalence values and socioepidemiological factors. Statistical analysis was

performed with STATA version 11 (StataCorp LP, College Station, TX), and a P value ≤ 0.05 was considered statistically significant.

Ethical consideration. This work was approved by the Armauer Hansen Research Institute/All Africa Leprosy Rehabilitation and Training Center Ethics Review Committee. A support letter for the study was obtained from the Benishangul-Gumuz Regional Health Bureau. All participants were informed about the purpose of the study before obtaining their own or their guardian's consent. Assent was obtained for children above 12 years of age, assuming that they would not have given samples otherwise. The community elders were informed that further investigations would follow if this study indicated possible transmission of the disease in their area. Malaria rapid diagnostic test (RDT)–positive cases were linked to the nearby treatment facilities. Study participants with *Leishmania* infection were given appropriate advice, and those symptomatic were assisted to reach the Gondar University Hospital Kala-azar Center for further evaluation and treatment.

RESULTS

A total of 289 participants (139 male and 150 female) were enrolled in the survey. Among these, 170 were from Kota kebele of Dangur District and 119 were from the Almehal kebele of Guba District (Table 1). The mean age (± standard deviation) of the participants was 32 ± 16.1. On clinical examination, 25 participants had complaints of fever and headache for 2 weeks or more and 14 of them were febrile (axillary temperature ≥ 37.5°C) during examination. None of those examined had hepato- or splenomegaly. Of the 14 febrile participants, six tested positive with malaria RDT and one was positive for *Leishmania* infection (rK39 positive and LST negative) and malaria RDT negative. This "probable" kala-azar case had a borderline DAT titer. He complained of fever, headache, and chest pain, and upon examination had wasting and lymphadenopathy but no organomegaly. He was taken to the Gondar University Hospital, where he again tested positive with a similar brand of rK39 but his bone marrow and spleen aspirates were negative for Leishman-Donovan bodies. He was later diagnosed as a case of smear-positive tuberculosis (TB) and was put on anti-TB medication.

Of 275 study participants for whom results were available, 20 were positive by LST and/or DAT/rK39. The overall leishmaniasis seroprevalence rate was thus 7.3% (Table 1). There was no trend of increase in both LST and DAT positivity as age increased (Table 1). There were 12 LST-positive cases of which five were DAT negative and three borderline. Among the 18 DAT positive cases, 14 were LST negative, one was lost for reading, and three were positive for both DAT and LST. Test positivity for *Leishmania* infection (LST and/or DAT) appeared to correlate with occupation but not with travel history. Among the 20 cases positive for *Leishmania* infection, nine reported no travel history outside their village or immediate neighborhood.

DISCUSSION

The survey revealed a leishmaniasis seroprevalence rate of 9.3% using LST and/or DAT in a region previously unknown as a focus although located within a high-risk area for kala-azar through prior geographical information–based risk mapping.[2] The lack of correlation of positivity rate with age, as is commonly observed in established endemic foci[15,16] (Table 1), suggests that there is no cumulative response to infection from historical exposures, and thus infection in the area is likely to be of recent origin. A similar association of *Leishmania* infection with farming practices, as indicated in our survey, has previously been reported by Fuller and others[17] in the Humera focus. It is interesting to note that there was large-scale expansion of agriculture in Humera then, as it is taking place in our study area now. Our data may thus indicate emergence of new foci following intrusion of people into existing zoonotic cycles as large-scale farming is introduced in high-risk areas.

Established vectors of kala-azar in Ethiopia are to date *Phlebotomus orientalis*, *Phlebotomus martini*, and *Phlebotomus*

TABLE 1

Sociodemographic characteristics and prevalence of *Leishmania* infection among the study participants (LST and/or DAT positive), May–June 2015, Dangur and Guba districts of Benishangul-Gumuz regional state

		LST		DAT		
	N (%)	Positive	*P* value	Positive	Borderline	*P* value
District						
Dangur	170 (58.8)	2	0.001	8	1	0.04
Guba	119 (41.2)	10		10	5	
Sex						
Male	139 (48.1)	8	0.253	15	5	0.001
Female	150 (51.9)	4		3	1	
Occupation						
Farmer (own land)	186 (64.4)	10	0.393	7	5	0.029
Temporary farm laborer	52 (18.0)	1		8	1	
Other	51 (17.6)	1		3	0	
Age group (years)						
< 21	29 (10.03)	0	0.434	1	1	0.168
21–31	108 (37.4)	3		6	1	
31–41	42 (14.5)	3		1	3	
> 41	110 (36.06)	6		10	1	
Travel history						
Yes	88 (30.5)	3	0.760	8	2	0.386
No	201 (69.6)	9		10	4	

DAT = direct agglutination test; LST = leishmanin skin test.

celiae,[18,19] and transmission is considered zoonotic. Data on the contribution of human intrusion into a zoonotic cycle for its reemergence at a site not known for autochthonous human kala-azar are very scanty. Ayele and Ali (1984) reported a possible origin of human infections from intrusion into a zoonotic cycle. According to them, the cases they came across during their survey were soldiers from the highland naive to kala-azar deployed to patrol uninhabited areas in north and northwestern parts of Ethiopia. On the other hand, there are data on zoonotic kala-azar in neighboring Sudan, bordering our study area.[20] A high infection rate of *P. orientalis* with *L. donovani* has been reported from uninhabited areas of Dinder National Park.[20] Subsequent studies identified infected mongoose[21] and humans in neighboring villages.[20] Moreover, a recent study done in Dinder National Park encountered *Phlebotomus rodhaini* infected with *L. donovani*. This finding suggests that *P. rodhaini* might be the vector responsible in maintaining a zoonotic cycle in this area uninhabited by humans.[22]

The spread of kala-azar is a significant public health concern in Ethiopia and the east African countries in general. In the last few decades, outbreaks of kala-azar have claimed the lives of thousands in the region outside the previously known endemic foci.[11,23] The increase in kala-azar burden in the lowland areas of north and northwestern Ethiopia since the 1990s has been associated with the arrival of large numbers of seasonal laborers and settlers from the neighboring highland areas.[24,25] It is likely that a similar situation is currently developing in the Benishangul-Gumuz regional state, again probably due to human encroachments into existing zoonotic cycles because of land clearance for agriculture and/or development of other natural resources in areas that in the past used to be outside of human contact or had very minimal human contact until recently. The economy of the region is growing fast attracting increased movement of seasonal laborers and settlers. Since the study area falls in the high-risk category for kala-azar according to the environmental factor–based risk model,[2] consolidation of an endemic focus with further adaptation from a zoonotic animal reservoir to human transmission is a strong possibility. Therefore, we recommend an integrated epidemiological study in the area to validate the findings and more importantly determine responsible vectors and potential reservoirs so that a baseline is established to monitor the trend as control measures are put in place before outbreaks and fatalities occur.

Limitations of the study. This report is a rapid cross-sectional population-based survey conducted in targeted communities and/or farms following the travel history of a human kala-azar case treated at a health center. Thus, the infection prevalence may not be representative of the whole population in the region. Moreover, the study did not include a survey of potential vectors or reservoirs involved in the suspected transmission to humans. Nevertheless, the findings strongly suggest possible acquisition of kala-azar infection in a previously nonendemic but potentially high-risk area for transmission.

Received October 10, 2015. Accepted for publication February 19, 2016.

Published online May 2, 2016.

Acknowledgments: We would like to thank the Benishangul-Gumuz Health Bureau and community elders of the study kebeles who facilitated the field work. We acknowledge the EETB-RTP program (grant no. D43 TW009127) of Emory University for the support.

Financial support: This work was supported by WHO-Ethiopia and AHRI core budget (supported by Norad and SIDA).

Authors' addresses: Adugna Abera, Teshome Tsegaw, Abraham Aseffa, and Endalamaw Gadisa, Armauer Hansen Research Institute, Addis Ababa, Ethiopia, E-mails: adugnabe@yahoo.com, teshomegis@gmail.com, aseffaa@gmail.com, and endalamawgadisa@yahoo.com. Geremew Tasew, Ethiopian Public Health Institute, Addis Ababa, Ethiopia, E-mail: getas73@yahoo.com. Asfaw Kejella, Benishangul-Gumuz Regional Health Bureau, Assosa, Ethiopia, E-mail: kejellaasfaw@yahoo.com. Abate Mulugeta, World Health Organization, Ethiopia Country Office, Addis Ababa, Ethiopia, E-mail: abatem@who.int. Dagimlidet Worku, KalaCORE Consortium, Ethiopia Country Office, Addis Ababa, Ethiopia, E-mail: dagimlidet@gmail.com.

REFERENCES

1. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team, 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One 7:* e35671.
2. Tsegaw T, Gadisa E, Seid A, Abera A, Teshome A, Mulugeta A, Herrero M, Argaw D, Jorge A, Aseffa A, 2013. Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach. *Geospat Health 7:* 299–308.
3. Federal Ministry of Health, 2013. *Guidelines for Diagnosis, Treatment and Prevention of Leishmaniasis in Ethiopia*, 2nd edition. Addis Ababa, Ethiopia: Ethiopian Federal Ministry of Health.
4. Gadisa E, Tsegaw T, Abera A, Elnaiem DE, den Boer M, Aseffa A, Jorge A, 2015. Eco-epidemiology of visceral leishmaniasis in Ethiopia. *Parasit Vectors 8:* 381.
5. Boussery G, Boelaert M, van Peteghem J, Ejikon P, Henckaerts K, 2001. Visceral leishmaniasis (kala-azar) outbreak in Somali refugees and Kenyan shepherds, Kenya. *Emerg Infect Dis 7:* 603–604.
6. Abubakar A, Ruiz-Postigo JA, Pita J, Lado M, Ben-Ismail R, Argaw D, Alvar J, 2014. Visceral leishmaniasis outbreak in South Sudan 2009–2012: epidemiological assessment and impact of a multisectoral response. *PLoS Negl Trop Dis 8:* e2720.
7. Raguenaud ME, Jansson A, Vanlerberghe V, Deborggraeve S, Dujardin JC, Orfanos G, Reid T, Boelaert M, 2007. Epidemiology and clinical features of patients with visceral leishmaniasis treated by an MSF clinic in Bakool region, Somalia, 2004–2006. *PLoS Negl Trop Dis 1:* e85.
8. Kirstein OD, Faiman R, Gebreselassie A, Hailu A, Gebre-Michael T, Warburg A, 2013. Attraction of Ethiopian phlebotomine sand flies (Diptera: Psychodidae) to light and sugar-yeast mixtures (CO_2). *Parasit Vectors 6:* 341.
9. Abbasi I, Aramin S, Hailu A, Shiferaw W, Kassahun A, Belay S, Jaffe C, Warburg A, 2013. Evaluation of PCR procedures for detecting and quantifying *Leishmania donovani* DNA in large numbers of dried human blood samples from a visceral leishmaniasis focus in northern Ethiopia. *BMC Infect Dis 13:* 153.
10. Gebresilassie A, Yared S, Aklilu E, Kirstein OD, Moncaz A, Tekie H, Balkew M, Warburg A, Hailu A, Gebre-Michael T, 2015. Host choice of *Phlebotomus orientalis* (Diptera: Psychodidae) in animal baited experiments: a field study in Tahtay Adiyabo district, northern Ethiopia. *Parasit Vectors 8:* 190.
11. Alvar J, Bashaye S, Argaw D, Cruz I, Aparicio P, Kassa A, Orfanos G, Parreno F, Babaniyi O, Gudeta N, Canavate C, Bern C, 2007. Kala-azar outbreak in Libo Kemkem, Ethiopia: epidemiologic and parasitologic assessment. *Am J Trop Med Hyg 77:* 275–282.
12. Herrero M, Orfanos G, Argaw D, Mulugeta A, Aparicio P, Parreno F, Bernal O, Rubens D, Pedraza J, Lima MA, Flevaud L, Palma PP, Bashaye S, Alvar J, Bern C, 2009. Natural history of a visceral leishmaniasis outbreak in highland Ethiopia. *Am J Trop Med Hyg 81:* 373–377.

13. Lyons S, Veeken H, Long J, 2003. Visceral leishmaniasis and HIV in Tigray, Ethiopia. *Trop Med Int Health 8:* 733–739.
14. Shanks L, Ritmeijer K, Piriou E, Siddiqui MR, Kliescikova J, Pearce N, Ariti C, Muluneh L, Masiga J, Abebe A, 2015. Accounting for false positive HIV tests: is visceral leishmaniasis responsible? *PLoS One 10:* e0132422.
15. Ali A, Ashford RW, 1993. Visceral leishmaniasis in Ethiopia. I. Cross-sectional leishmanin skin test in an endemic locality. *Ann Trop Med Parasitol 87:* 157–161.
16. Hailu A, Gramiccia M, Kager PA, 2009. Visceral leishmaniasis in Aba-Roba, south-western Ethiopia: prevalence and incidence of active and subclinical infections. *Ann Trop Med Parasitol 103:* 659–670.
17. Fuller GK, Lemma A, Haile T, Atwood CL, 1976. Kala-azar in Ethiopia I: Leishmanin skin test in Setit Humera, a kala-azar endemic area in northwestern Ethiopia. *Ann Trop Med Parasitol 70:* 147–163.
18. Gebre-Michael T, Balkew M, Berhe N, Hailu A, Mekonnen Y, 2010. Further studies on the phlebotomine sandflies of the kala-azar endemic lowlands of Humera-Metema (north-west Ethiopia) with observations on their natural blood meal sources. *Parasit Vectors 3:* 6.
19. Gebre-Michael T, Lane RP, 1996. The roles of *Phlebotomus martini* and *P. celiae* (Diptera: Phlebotominae) as vectors of visceral leishmaniasis in the Aba Roba focus, southern Ethiopia. *Med Vet Entomol 10:* 53–62.
20. Elnaiem DA, Hassan HK, Ward RD, 1997. Phlebotomine sandflies in a focus of visceral leishmaniasis in a border area of eastern Sudan. *Ann Trop Med Parasitol 91:* 307–318.
21. Elnaiem DA, Hassan MM, Maingon R, Nureldin GH, Mekawi AM, Miles M, Ward RD, 2001. The Egyptian mongoose, *Herpestes ichneumon*, is a possible reservoir host of visceral leishmaniasis in eastern Sudan. *Parasitology 122:* 531–536.
22. Elnaiem DE, Hassan HK, Osman OF, Maingon RD, Killick-Kendrick R, Ward RD, 2011. A possible role for *Phlebotomus* (*Anaphlebotomus*) *rodhaini* (Parrot, 1930) in transmission of *Leishmania donovani. Parasit Vectors 4:* 238.
23. Marlet MV, Sang DK, Ritmeijer K, Muga RO, Onsongo J, Davidson RN, 2003. Emergence or re-emergence of visceral leishmaniasis in areas of Somalia, north-eastern Kenya, and south-eastern Ethiopia in 2000–01. *Trans R Soc Trop Med Hyg 97:* 515–518.
24. Anema A, Ritmeijer K, 2005. Treating HIV/AIDS and leishmaniasis coinfection in Ethiopia. *CMAJ 172:* 1434–1435.
25. Hailu A, Gebre-Michael T, Berhe N, Balkew M, 2006. Leishmaniasis. Berhane Y, Mariam DH, Kloos H, eds. *Epidemiology and Ecology of Health and Disease in Ethiopia*. Addis Ababa, Ethiopia: Shama Books.