

An outbreak of suspected cutaneous leishmaniasis in Ghana: lessons learnt and preparation for future outbreaks

Margaret A. Kweku^{1†}, Shirley Odoom², Naiki Pupilampu², Kwame Desewu², Godwin Kwakye Nuako³, Ben Gyan², Greg Raczniak⁴, Karl C. Kronmann⁴, Kwadwo Koram², Silvia Botero^{5,6}, Daniel Boakye² and Hannah Akuffo^{6*}

¹District Health Directorate, Ghana Health Service, Volta Region, Ghana; ²Noguchi Memorial Institute for Medical Research, Accra, Ghana; ³Department of Microbiology, University of Ghana Medical School, Korle-Bu Hospital, Accra, Ghana; ⁴US Naval Medical Research Unit No. 3, Ghana Detachment, Accra, Ghana; ⁵Department of Parasitology, Mycology and Environmental Biology, Swedish Institute for Infectious Disease Control, Stockholm, Sweden; ⁶Department of Microbiology, Cell and Tumor Biology, Karolinska Institutet, Stockholm, Sweden

Human cutaneous leishmaniasis (CL) has previously been reported in West Africa, but more recently, sporadic reports of CL have increased. *Leishmania major* has been identified from Mauritania, Senegal, Mali, and Burkina Faso. Three zymodemes (MON-26, MON-117, and MON-74, the most frequent) have been found. The geographic range of leishmaniasis is limited by the sand fly vector, its feeding preferences, and its capacity to support internal development of specific species of *Leishmania*. The risk of acquiring CL has been reported to increase considerably with human activity and epidemics of CL have been associated with deforestation, road construction, wars, or other activities where humans intrude the habitat of the vector. In the Ho Municipality in the Volta Region of Ghana, a localised outbreak of skin ulcers, possibly CL, was noted in 2003 without any such documented activity. This outbreak was consistent with CL as evidenced using various methods including parasite identification, albeit, in a small number of patients with ulcers.

This paper reports the outbreak in Ghana. The report does not address a single planned study but rather a compilation of data from a number of ad-hoc investigations in response to the outbreak plus observations and findings made by the authors. It acknowledges that a number of the observations need to be further clarified. What is the detailed epidemiology of the disease? What sparked the epidemic? Can it happen again? What was the causative agent of the disease, *L. major* or some other *Leishmania* spp.? What were the main vectors and animal reservoirs? What are the consequences for surveillance of the disease and the prevention of its reoccurrence when the communities see a self-healing disease and may not think it is important?

Keywords: *cutaneous leishmaniasis*; Ghana; Volta Region; West Africa; outbreak; follow-up

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Leishmania are intracellular protozoa that cause infections with diverse clinical manifestations ranging from chronic but often self-healing skin lesions, cutaneous leishmaniasis (CL), to erosive mucosal membrane destruction of the nasopharynx known as mucocutaneous leishmaniasis, and a life-threatening

systemic infection with hepato-splenomegaly in visceral leishmaniasis. *Leishmania* are characteristically transmitted by the bite of various species of sand flies. The disease is determined by complex interactions between the infecting species of *Leishmania* and the immunological status of the host. Many species of sand flies are potential vectors and some 100 species of animals could serve as reservoir hosts, such as various wild or domestic animals but also human beings.

[†]Present address: Onchocerciasis Chemotherapy Research Centre, Municipal hospital, Ghana Health Service, Hohoe, Ghana.

Effective prevention, control, and monitoring of leishmaniasis transmission require an identification of the involved specific sand fly vector, a detailed knowledge of the biology of the vector species, as well as their influence on the transmission of the *Leishmania*. In the Old World, including West Africa, the known vectors of leishmaniasis are species belonging to the genus *Phlebotomus*. Although *Sergentomyia* species are also common in these areas, they have not been reported as vectors of leishmaniasis.

Leishmaniasis is one of the major infectious diseases affecting the poorest regions and the poorest populations of the world. The CL forms are the most common ones. They produce skin ulcers on exposed skin, causing serious disability and permanent scarring after healing. These forms have been described in Northern and Eastern Africa but are said to be rare in West Africa and had not prior to 1999 been described in Ghana. In 1999, however, chronic ulcers were reported from one village that shares a border with the Republic of Togo and investigations were done to identify the aetiology of the disease. This is the point of departure for us describing the subsequent outbreaks in the present report.

Cutaneous leishmaniasis in West Africa

Sporadic cases of canine leishmaniasis were reported from Dakar, Senegal, as early as 1915 (1) and human leishmaniasis from Nigeria 1944 (2, 3). The CL is found in Senegal (4) and *L. major* has been identified in a number of non-primate mammals (5). Case reports of CL exist from The Gambia (6, 7).

The history of CL has been presented by Boakye et al. (8). In their review, the authors point out that CL is proposed to be endemic in parts of West Africa that 'may include northern Ghana.' Human disease requires an animal reservoir (typically mammalian) and a vector capable of transmitting disease to humans – sand flies of the genera *Phlebotomus* in the Old World. Neither the reservoir animals nor the vectors are well described in West Africa.

An ecological study of an epidemic of CL in Senegal between 1975 and 1978 allowed, for the first time, a description of a CL focus in West Africa (9–11). An outbreak of cases of CL was also reported in Ouagadougou, Burkina Faso in 1996; 1,845 cases were identified through retrospective and prospective studies in 1996 to 1998 (12). The *Leishmania* parasite was detected by positive smears in 28 of 52 (54%) patients tested in 1998. *L. major* was identified by Polymerase Chain Reaction (PCR) in one patient.

The reservoir hosts identified from Senegal and The Gambia are the rodents *Mastomys erythroleucus*, *Tatera gambiana*, *Arvicanthis niloticus*, and *Mastomys erythroleucus* plus a dog in The Gambia (13). In Nigeria,

Mastomys natalensis and *Tatera gambiana* were identified as probable reservoirs (14). No evidence of reservoir hosts has been published from Ghana.

Anthropophilic West African *Phlebotomus* sand flies identified from Ghana include *P. duboscqi* and *P. Rhodaini*. Sand fly species collected in 1997 and 2002 from Navrongo in the Upper East Region of Ghana showed the presence of 14 different species, 13 of which belonged to the genus *Sergentomyia*. The only *Phlebotomus* species was *P. duboscqi*, a known vector of CL in West Africa.

Aims of this report

The aim of this report is to collate the various reports of an outbreak of ulcerative skin disease in Ghana and to present additional data, to facilitate further studies and surveillance of what appears to be an intriguing form of a newly emerged CL in the country.

Description of the outbreak and results of investigations

Outbreak area

The suspected CL cases were noted for the first time in the Ho Municipality, Volta Region of Eastern Ghana, in 1999 (Ghana Ministry of Health, Annual Report 2004). This moist semi-deciduous forest ecosystem is not typically associated with leishmaniasis. Although there has not been any cases reported in Ghana before 1999, the West African CL belt cuts across the country's northern, arid, Sahel savannah regions that borders Burkina Faso.

The Ho Municipality, which is one of the 138 administrative municipalities/districts in Ghana, is located in the middle zone of the Volta Region (Fig. 1). It is bordered in the North by the Hohoe Municipality, in the East by the Ghana-Togo border, in the West by the Kpando district, and in the South by the North-Tongu and Akatsi Districts. It covers an area of about 2,564 square kilometres and has an estimated population of 249,000 (projection from the 2000 population census) with an annual growth rate of 1.8%. Ho town doubles as the capital of the Municipality and the Regional Capital (of the Volta Region). For the purpose of health care delivery, the Municipality is divided into six sub-districts: Tsito, Abutia, Adaklu, Kpetoe, Kpedze-Vane, and Ho-Shia.

The northern and eastern zones of the Municipality are mountainous and are covered with forest while the southern zone, on the other hand, is of mixed savannah and grassland with some marshy areas. The vegetation of the Municipality is mainly forest and forest savannah. There are two main seasons: wet and dry. The wet season encompasses the major rains from May to August and the minor from October to November. The rest of the



Fig. 1. Map of Ghana showing Ho District Outbreak area.

year is relatively dry. Malaria and onchocerciasis are the main diseases transmitted throughout the year. The main economic activities are farming and trading. There are also minor economic activities like pottery, woodcarving, kente weaving, and cattle rearing in the southern part of the Municipality.

Case detection

Patients reporting to health facilities

Ulcer biopsies were taken at the Volta Regional Hospital (VRH) from 15 patients from villages in two sub-districts (Ho-shia and Kpedze-Vane) with ulcers who agreed verbally to go to the hospital for a biopsy. Initially the patients had visited their respective health facilities in November 2002 with ulcers, which were not responding to antibiotic treatment. Apart from the ulcers, the patients did not have any other complaints. The biopsies were sent to the pathology laboratory at Korle-Bu Teaching Hospital in Accra. The presence of what the pathologist identified as L-Dbodies (intracellular kinetoplastic organisms also called Leishman-donovan bodies) was consistent with the diagnosis of CL that prompted further investigations. The background characteristics and microscopic result of the 15 patients is shown in Table 1.

Nine of the 15 patients were females. The median age was 33 years, ranging from 12–71 years. Their main occupations were farming ($n = 6$), trading ($n = 2$), and studying ($n = 5$). One was a teacher and another self-employed. The average length of stay in the community

was 22 years, ranging from 6 months to 71 years. The majority of the patients (12/15) had lived in the community for 12 years or more. The average duration of the ulcers before investigation was 6 weeks (range 3–12).

Survey at selected village schools

A survey was then made to three schools from where some of the cases examined at the health centres came to determine if other students had similar ulcers. This was done by the director of the municipal health services (Medical Officer) and the municipal disease control officer. Based on the clinical examination by the Medical Officer, 124 cases were found among the examined 675 students (Table 2).

Passive case search in sub-districts of the Ho Municipality

A list of patients with active lesions, who spontaneously sought help for skin ulcers at the health centres in July and August 2003, was compiled by trained health workers. The patients came from 42 different villages. A total of 790 cases were recorded out of which 471/790 (59.6%) were females and 319/790 (40.4%) males. It was observed that, some lesions were single, others multiple, although this information was not consistently recorded to assess the proportions. The disease was seen in all age groups and lesions were mostly located on the head, neck, and the limbs. The lesions healed spontaneously within 3–8 months.

Active case search in sub-districts of the Ho Municipality

An active search of cases was carried out in November 2003 during the first round of National Immunisation Days (NID) for poliomyelitis in the Kpando district, Ho, and Hohoe municipalities.

A team trained to administer poliomyelitis vaccine was also trained to search for lesions using laminated pictures of various forms of lesions. They visited every house in every community to carry out both activities. They asked if anybody had lesions similar to those in the pictures or 'Agbamekanu' (the local name meaning; gift from somebody who has returned from a journey) in 2002 (a year before) or in 2003 (the year of the case search).

As a result of this survey, a total of 8,876 possible cases that occurred between 2002 and 2003, were identified in three municipalities/districts in the Volta Region with 8,533 cases coming from Ho Municipality alone (Table 3). None of these areas had, prior to 1999, reported cases of such ulcers. In the Ho municipality 6,185 cases occurred in 2003 compared to 2,348 in 2002.

A breakdown of the results into sub-districts of the Ho municipality (Table 4) showed that out of a total of the 2,348 cases that occurred in year 2002 within the Ho municipality, Ho-Shia sub-district, where the disease

Table 1. Background characteristics of the patients who volunteered for ulcer biopsies and microscopy report

No	Code	Age	Sex	Occupation	Community	Length of stay in community (years)	Reported duration of lesion at time of diagnosis(weeks)	Location of lesion	LD bodies-seen
1	A.J.	65	F	Farming	Matse	13	3	Left arm	No
2	M.M.	42	F	Farming	Matse	42	3	Right thigh	Yes
3	D.J.	26	F	Hair dressing	Nyive	26	8	Left leg	Yes
4	A.A.	16	M	Student	Akoefe	16	3	Scalp	No
5	D.G.	40	F	Teaching	Taviefe	4	12	Left arm	No
6	K.D.	25	M	Farming	Klefe	23	12	Right arm	Yes
7	K.F.	33	F	Trading	Matse	33	4	Right arm	Yes
8	A.R.	45	M	Farming	Akoefe	45	8	Right arm	No
9	S.B.	12	F	Student	Akoefe	12	3	Right thigh	Yes
10	T.A.	40	F	Farming	Shia	6	6	Back of trunk	Yes
11	K.E.	17	M	Student	Nyive	17	8	Scalp	No
12	B.C.	16	M	Student	Akoefe	6 months	4	Scalp	Yes
13	T.E.	38	F	Trading	Taviefe	36	8	Right arm	Yes
14	B.M.	13	M	Student	Akoefe	13	4	Right calf	Yes
15	K.E.	71	F	Farming	Taviefe	71	8	Right leg	Yes

apparently started, recorded the highest numbers (1,536). This was followed by the adjoining sub-district Kpedze-Vane (770). The third highest sub-district was Tsito, which shares boundary with Kpedze-vane (42). It was also shown that more cases occurred within these three sub-districts in year 2003 compared to 2002 (2,725 vs 770; 2,854 vs 1,536; 556 vs 42, respectively).

Interviews of respondents from the three sub-districts where no cases occurred prior to 2003 (Kpetoe/Ziope, Adaklu, and Abutia) revealed that the cases in these three sub-districts had a link with the endemic communities (those where cases occurred prior to 2003). Some of the cases were residing in the endemic communities and had been transferred to new work stations. Others were diagnosed while visiting neighbouring districts from the endemic communities or had returned home from visits to endemic communities. This provides further evidence of an actual increase in disease incidence in 2003,

Table 2. Result of the follow-up to the schools from which initial cases were reported

Village	Number examined	Number of ulcer cases (%)
Akoefe kindergarten and primary	320	39 (12.2)
Akoefe JSS	175	60 (34.3)
Nyive JSS	180	25 (13.9)
Total	675	124 (18.4)

although an increased awareness of health care providers cannot be excluded.

Monitoring and understanding the outbreak

As of 2003, the disease had spread to all villages in the two biggest sub-districts (Kpedze/Vane and Ho/Shia) in the Ho Municipality as shown in the active case search and people who visited those communities were also affected. The disease affected all age groups. Based on these observations, the disease epidemiology appears to be more complicated than initially thought. However, no systematic case detection follow-up was done after this period. Cases continued to be detected in 2004, 2005, and 2006, but this was not based on a systematic data collection but a sample collection for parasite identification. In 2007 a small active case detection study was

Table 3. Active case search in three municipalities/districts in the Volta Region

Municipality/district	Number of cases before 2003	Number of cases in 2003	Total
Hohoe municipality	2	174	176
Kpando district	76	91	167
Ho municipality	2,348	6,185	8,533
Total	2,426	6,450	8,876

Table 4. A breakdown of the cumulative results of the active case search for leishmaniasis by November 2003 within the Ho municipality by sub-District

Sub-dis-trict	Number of communities with cases	Number of households with cases	Number of cases (before 2003)	Number of cases (in 2003)	Total number of cases by November 2003
Ho/Shia*	49	2,240	1,536	2,854	4,390
Kpedze/Vane*	31	1,493	770	2,725	3,495
Tsito*	17	340	42	556	598
Adaklu	8	18	0	18	18
Kpetoe/Ziope	6	16	0	17	17
Abutia	5	15	0	15	15
Total	116	4,107	2,348	6,185	8,533

*Referred to in the text as 'endemic communities'.

carried out in the Ho Municipality as part of a Master of Science thesis study.

Clinical presentation of lesions

Early lesions started as pustules then became nodules or deep ulcers with flat base and raised margins (Fig. 2). Patients presented with both single and multiple ulcerative lesions (Fig. 2). Some presented with healed lesions that had left unsightly scars (Fig. 3). All patients

photographed consented for their photographs to be taken and used for scientific purposes.

Parasite identification

Histology

Table 5 summarises the cases described for clinical and parasitological identification. Ulcer biopsies were taken from 15 patients aged 12–71 years who reported to the health centres in two sub-districts of the Ho Municipality of the Volta Region with chronic ulcers in 2002 (Table 1).

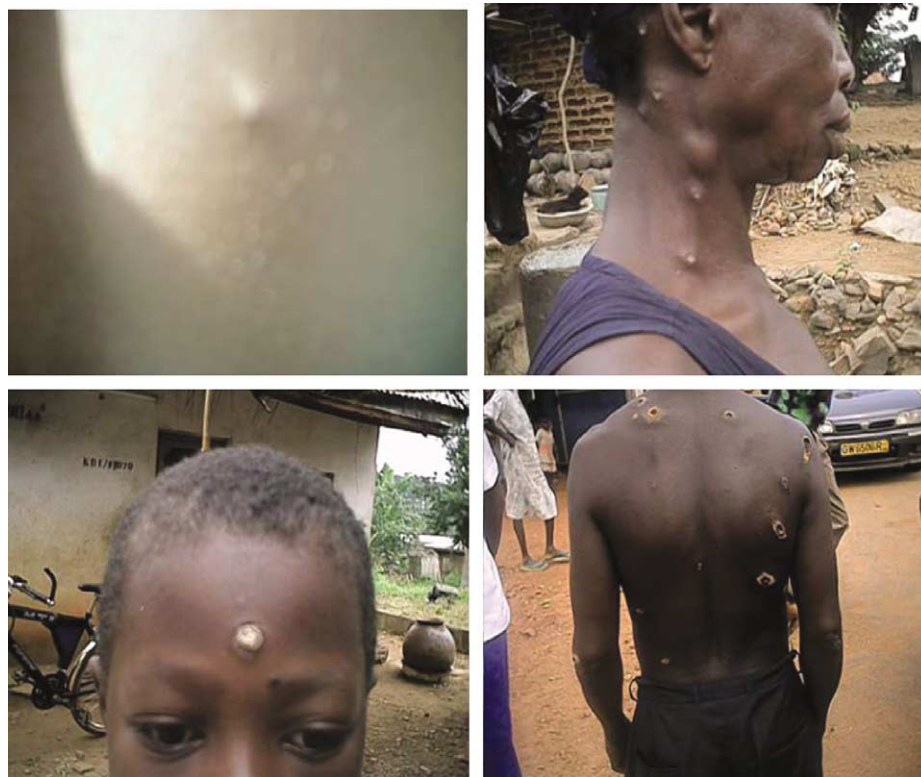


Fig. 2. Various stages of active lesions (Photographs from M. Kweku).



Fig. 3. Scars and ulcers that are healing (Photographs from H. Akuffo).

Microscopic examination of biopsies fixed in formalin and embedded in paraffin showed intracellular kinetoplastic organisms reminiscent of *Leishmania* parasites/amastigotes (so called LD bodies) in 10 out of the 15 biopsy specimens. The other five specimens showed a heavy infiltrate of histiocytes, plasma cells, lymphocytes, neutrophils, and giant cells in the ulcers, suggestive of CL. Due to lack of appropriate equipment no photographs were taken of the histological sections.

Smears and culture

To try to more clearly identify the nature of the kinetoplastic organisms found in the biopsies in 2003,

scrapings were taken from the edge of the ulcers from another four patients from the same area as the cases that underwent biopsies in 2002. At least two scraping samples were taken from each of the four patients and smears made on glass slides, dried, fixed, and stained with May Grünwald-Giemsa. The smears all showed kinetoplastic amastigotes albeit at very low numbers (Fig. 4).

Scrapings from the same four patients were also put onto NNN medium (standardised for use at the reference Parasitology Laboratory of the Swedish Institute for Infectious Disease Control) for *Leishmania* culture. None of the samples showed growth of parasites

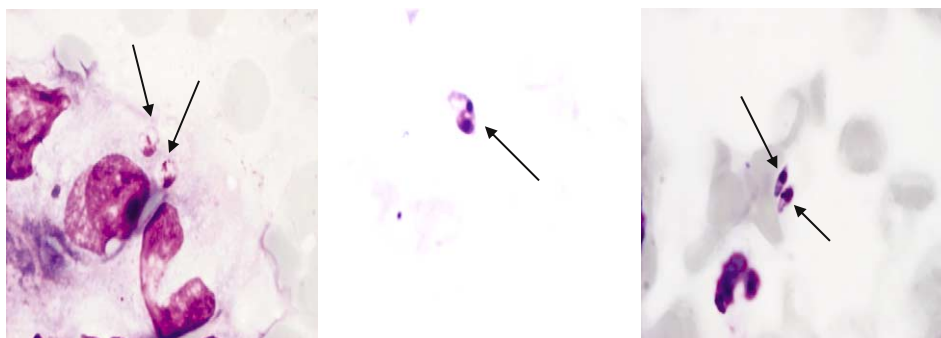


Fig. 4. Smears from lesions of patients with cutaneous lesions showing amastigotes (arrows).

Table 5. Summary of cases used for clinical and parasite identification

Year of sample taking	Year of analysis	Sample	Number	Analysis	Results
2002	2002	Biopsy of ulcer	Fifteen ulcer cases	Histology	10/15 LD bodies positive
	2004		Five LD bodies positive	PCR	No <i>Leishmania</i> DNA detected
2003	2003	Ulcer edge scrapings	Two samples from each of four ulcer cases	Smear	4/4 amastigote positive
				Culture	No parasite growth
2004 and 2005	2004 and 2005	Biopsy of ulcer		PCR	<i>L. major</i> identified
2006	2006	Biopsy of ulcer	Nine biopsies from five ulcer cases	PCR	Previously uncharacterised species of <i>Leishmania</i> identified
2007	2007	Biopsy of ulcer	One biopsy from ulcer case		

even after 8 weeks of culture despite the positive smear results.

Identification using Polymerase Chain Reaction

In 2004, skin biopsies obtained from five of the stored amastigote positive histological samples from 2002 were further examined using standard PCR used for identification of leishmaniasis in the reference laboratory in Sweden. Fifteen 10 µm thick sections per biopsy were cut and collected in the same tube and their DNA extracted as instructed by the protocol for fixed tissues found in the manual of the QIAamp DNA mini Kit (Qiagen Inc., Valencia, California). The extracted total DNA was freeze-dried and sent for PCR analysis at the Swiss Tropical Institute, Basel, Switzerland. This PCR assay is designed to target the gene for the spliced leader RNA (mini-exon) that is present as tandem repeats in the genus *Leishmania* and other kinetoplastida (15). The PCR-based analysis was performed twice on each sample but no *Leishmania*-specific DNA could be amplified from any of these five samples.

Skin tissue samples collected in 2004–2005 from Ho with cutaneous lesions were reported to be infected by *L. Major* (16). The exact PCR method used in this study is described in Fryauff et al. (16). During field investigations in January 2006 to identify the disease vector(s) and reservoir(s), nine biopsies were collected from five suspected CL cases in the village of Taviefe, 10 km north of Ho. Another sample was collected from the same village in February 2007. None of the samples taken in 2006 and 2007 showed *L. major* but rather a previously uncharacterised species of *Leishmania* spp., inferred by DNA sequence analysis of the small subunit ribosomal RNA gene and the internal transcribed spacer region (ITS1), was detected in tissue biopsies from these patients (17).

Entomological investigations

Studies done since 2004 in the outbreak area have concentrated on determining the possible vector species.

Sand flies have been collected using various trapping methods that include CDC light traps with or without baits (dry ice), malaise traps, and sticky paper traps. The sampling have mostly been done in three communities, Klefe (06°37'N–00°26'E), Taviefe (06°66'N–00°47'E), Hlefi (06°42'N–00°22'E) where cases had been reported. Sand fly species collected were identified morphologically, using the taxonomic keys of Abonnenc (18). In all, 20 species of sand flies were recorded from the outbreak area. Eighteen of these were *Sergentomyia* species with only two *Phlebotomus* species namely *P. duboscqi* and *P. rhodaini*. Although *P. duboscqi* has been reported from most West African countries as the vector of CL in the region, its importance in the Ho outbreak area is uncertain due to the low numbers collected in previous surveys. For example in a sample of 251 sand flies collected from October to December 2006, only one was *P. duboscqi* while none was identified from 1,918 sand flies collected from January to December 2007 on sticky traps. The 18 *Sergentomyia* species identified include species that have been reported to feed on man although these have not been found to be vectors of human leishmaniasis (8).

In 2008, collections were made from human dwellings in a bid to determine the human biting sand flies in the outbreak areas. A total of 295 blood-fed sand flies characterised yielded only *Sergentomyia* species; *S. similima* (38.2%), *S. ingrami* (34.5%), *S. africana africana* (26.9%), and *S. antennata* (0.4%) (Desewu, unpublished observation from 2008). The blood-meal analysis showed that with the exception of *S. antennata*, the others had mixed blood meals that included human blood. Other blood sources were from chicken (bird) and goat. Unfortunately the tests did not include rodent blood. Nevertheless, the presence of mixed-blood meals indicates the possibility of supporting the transmission of *Leishmania* parasites from an animal host to man but it is yet to be studied whether any of these species harbours the parasite in nature.

Discussion

The outbreak of skin ulcers in the Volta region of Ghana caused national anxiety when many patients were reporting to the hospital and health centres on seeing strange ulcers on their body.

Results of the active case search in the Ho Municipality shown in Table 3 seem to show a dramatic increase in the number of cases in 2003, though recall bias makes definitive conclusions impossible. Boakye et al (8) state that CL in West African countries seems to occur in epidemics characterised by an increase in frequency over a few years followed by a drastic reduction in incidence. A follow-up survey would help determine whether this pattern occurred in this outbreak, or whether there continues to be a significant number of unreported cases.

Nonetheless, the disease appears to have become endemic since cases were detected in 2004, 2005, and 2006 in this area. In 2007 a small active case detection study in the Ho Municipality, cases clinically compatible with leishmaniasis were identified (Godwin Kwakye Nuako unpublished data), all of whom were identified outside of the health system. It would appear that the affected communities have noted that the lesions tend to self-heal, thus making the likelihood of them choosing to visit the health facilities slim. Despite the fact that CL is a self-healing disease, there are many signs that this particular disease in Ghana is not identical to simple *L. major* infections previously identified in the region. The lesions in the outbreak area were often multiple, distributed all over the body including the face, the infecting organism seems difficult to identify by routine methods.

The cumulative findings using microscopic and molecular methods are consistent with the disease being CL. However, despite the identification of *L. major* by PCR in one study, other studies present initial intriguing results that implicate an as yet unidentified *Leishmania* species. The finding of few amastigotes in the small sample of skin scrapings, which did not grow in standard *Leishmania* culture medium, is unexplained as are the negative PCR results in LD positive biopsies. The sand fly vector implicated in the outbreak also remains unknown and interestingly, of the 20 species identified only two were *Phlebotomus* species with the bulk being *Sergentomyia*, a number of which may be man biting species.

The results suggest that this self-healing cutaneous ulcer disease is associated with *Leishmania*, but that the main causative agent is not the same as that described in other parts of West Africa. It is thus important to further study this disease more closely while it remains focal in nature, since this may give the opportunity to contain a possible future outbreak.

Such studies would need to involve active case detection in a broader area, surveillance within the health system and mapping, using improved parasite identifica-

tion methods, PCR identification of organisms from samples from more patients. Entomological surveillance should be continued and important studies on identification of the host in domestic animals, rodents, game, and wildlife including birds should also be intensified.

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The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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***Hannah Akuffo**

Department of Microbiology, Cell and Tumor Biology
Karolinska Institutet
P. O. Box 280
171 77 Stockholm, Sweden
Email: Hannah.Akuffo@sida.se