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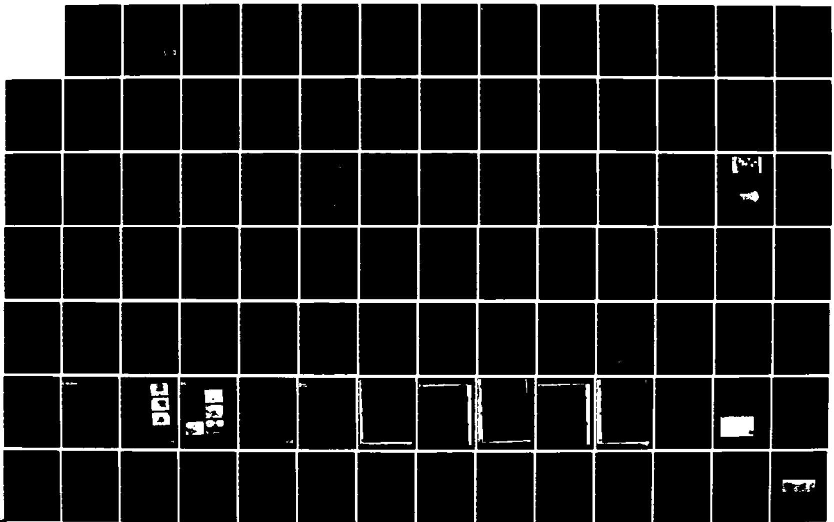

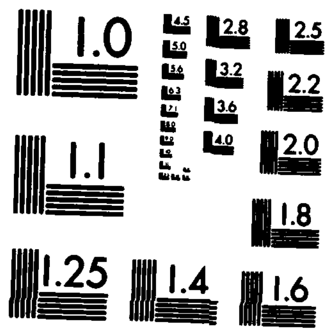

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STUDIES ON AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS

Final Report, Volume 2

Dr. I.E. Muriithi

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July 1984

(For period 1 October 1982-14 November 1983)

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Eight hundred seven individuals have been identified as having had sleeping sickness between 1960 and 1984. Of these,medical records and present health status have been evaluated for 560 individuals. This information is unique in that no other study has produced a documented overview of the disease patterns, efficacy of varied treatment regimens and long term survival rates. Findings from this study continue to be used in preparation of research protocols for the Alupe Treatment Center. The Center received first patients in December 1983.

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Block 20 (continued)--

By agreement with the Government of Kenya all individuals with sleeping sickness are being treated according to accepted procedures. Upon the approval of research protocols qualifying patients will be enrolled. Non-consenting or otherwise inappropriate cases will be treated as medically indicated by the staff.

Lambwe Valley Trypanosome Studies--
Studies within our laboratory and in conjunction with the Kenya Trypanosomiasis Research Institute utilizing isoenzyme analysis and serum neutralization techniques continue to show a high degree of correlation. New cases are being found outside of the valley but parasites appear to be of Lambwe Valley origin indicating spread from the endemic focus rather than new strains imported from other areas.

This volume consists of 16 papers on work done under the grant. Volume 1 is a summary of the work.

PROGRESS IN TRYPANOSOMIASIS RESEARCH

Epidemiology Survey and Followup Patient Studies

Eight hundred seven individuals have been identified as having had sleeping sickness between 1960 and 1984. Of these medical records and present health status have been evaluated for 560 individuals. This information is unique in that no other study has produced a documented overview of the disease patterns, efficacy of varied treatment regimens and long term survival rates. Findings from this study continue to be used in preparation of research protocols for the Alupe Treatment Center.

Alupe Treatment Center

This facility received the first patients in December 1983. By agreement with the Government of Kenya all individuals with sleeping sickness are being treated according to accepted procedures. Upon the approval of research protocols qualifying patients will be enrolled. Non-consenting or otherwise inappropriate cases will be treated as medically indicated by the staff. This facility offers the best professional and technical care available in western Kenya.

Lambwe Valley Trypanosome Studies

Studies within our laboratory and in conjunction with the Kenya Trypanosomiasis Research Institute utilizing isoenzyme analysis and serum neutralization techniques continue to show a high degree of correlation. New cases are being found outside of the valley but the parasites appear to be of Lambwe Valley origin indicating spread from the endemic focus rather than new strains imported from other areas.

Progress in Leishmaniasis Research

This report consists of recently published articles and submitted papers describing the progress of Leishmaniasis research. The papers are listed below.

J. Med. Entomol. Vol. 21, no. 2: 243 30 March 1984

MECHANICAL TRANSMISSION OF *LEISHMANIA MAJOR* BY *GLOSSINA MORSITANS MORSITANS* (DIPTERA: GLOSSINIDAE)[1]

Abstract. Laboratory-reared *Glossina morsitans morsitans* mechanically transmitted 2 strains of *Leishmania major* following interrupted feedings on infected hamsters and mice. Since *L. major* foci in Africa occur within the ranges of 1 or more species of tsetse, *Glossina* spp. could transmit *Leishmania* mechanically in nature.

Although phlebotomine sand flies are considered to be the principal vectors of both Old and New World *Leishmania* spp., mechanical transmission of these parasites may also be a factor in the epidemiology of leishmaniasis (Garnham, 1965, Am. Zool. 5: 141; Lewis, 1966, Trans. R. Soc. Trop. Med. Hyg. 60: 419). Transmission of cutaneous leishmaniasis by contaminated mouthparts of both biting and nonbiting insects has been demonstrated (Thomson & Lamborn, 1934, Br. Med. J. 2: 506–09; Berberian, 1938, Proc. Soc. Exp. Biol. Med. 38: 254–56). Under experimental conditions, Berberian (1966, Trans. R. Soc. Trop. Med. Hyg. 60: 277–78) was able to transmit *Leishmania tropica* mechanically from man to man with the stable fly, *Stomoxys calcitrans* (L.). Lainson & Southgate (1965, Trans. R. Soc. Trop. Med. 59: 716), also using *S. calcitrans*, achieved mechanical transmission of *Leishmania mexicana* from hamster to hamster. In the present study, mechanical transmission of *Leishmania major* (=*Leishmania tropica major*) was demonstrated with the tsetse *Glossina morsitans morsitans* Westwood.

Two strains of *L. major* were used: a biochemically characterized strain from Israel isolated from a human (NLB 070; LRC-L137) (Brazil, 1978, Ann. Trop. Med. Parasitol. 72: 289–91) and a strain (NLB 056) isolated from the spleen of a naturally infected gerbil, *Taterillus emini*, collected in Baringo District, Kenya. The NLB 056 strain is considered to be *L. major*, based on preliminary biochemical characterization by means of cellulose acetate electrophoresis, using the technique of Kreutzer & Christen (1980, Am. J. Trop. Med. Hyg. 29: 199–208).

In the 1st experiment, a golden hamster was infected with *L. major* (NLB 070; LRC-L-137) by subcutaneous injection on the bridge of the nose. An ulcerated nose lesion developed on the animal approximately 25 days postinoculation, and individual male tsetse were exposed to the lesion using the interrupted feeding method (Roberts, 1981, Am. J. Trop. Med. Hyg. 30: 948–51). Flies were allowed to probe for 30 s on the lesion of the infected donor and were then transferred immediately to the nose of an uninfected, anesthetized hamster and allowed to feed to repletion. Time from exposure to refeeding was ≤2 min. Ten flies and 10 uninfected hamsters were exposed in this manner. At 30 days postexposure, 1 of the 10 recipient hamsters developed a papule on its nose. A needle aspirate culture of this papule (as described by Hendricks & Wright, 1979, Am. J. Trop. Med. Hyg. 28: 962–64) was positive for leishmanial parasites. By 55 days postexposure, the papule had developed into an ulcerated lesion. This hamster was then used as a donor animal and the experiment described above was repeated. Six male flies were exposed, and 4 of these subsequently fed on 4 uninfected hamsters. One of the 4 hamsters developed a culture-positive nose lesion at 67 days postexposure.

The 2nd experiment was similar to the 1st, except that BALB/c mice were used instead of hamsters, and the donor animals were infected with the strain of *L. major* (NLB 057) isolated from the naturally infected gerbil. Sixteen male flies and 16 uninfected mice were exposed in this experiment. Three of the 16 mice developed culture-positive papules and lesions between 32 and 48 days postexposure. *Leishmania major* normally visceralizes in BALB/c mice (Leclerc, Modabber, Deriaud & Cheddid, 1981, Trans. R. Soc. Trop. Med. Hyg. 75: 851–54), and spleen cultures from the 3 mice infected mechanically by tsetse were also positive for leishmania.

Results from our study with *G. morsitans* concur with those of earlier workers with stable flies, and it appears that both stable flies and tsetse are capable of mechanically transmitting cutaneous leishmaniasis. Lainson & Southgate (1965, loc. cit.) suggested that with some forms of leishmaniasis, e.g., diffuse cutaneous leishmaniasis or post kala-azar dermal leishmaniasis where multiple nodules and lesions are present, mechanical transmission may occur naturally. *Leishmania major* occurs in foci scattered throughout the Ethiopian zoogeographical region of Africa (Chance, Schnur, Thomas & Peters, 1978, Ann. Trop. Med. Parasitol. 72: 533–42), many of which are within the ranges of 1 or more species of tsetse. Under favorable conditions, *Glossina* spp. could transmit leishmania mechanically in nature.

We thank Dr R.F. Beach for characterization of the *L. major* strain, NLB 056, and Dr M.J. Reardon for review of the manuscript. In conducting the research described in this note, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Academy of Science—National Research Council.—**Lawrence Lightner and Lyman W. Roberts**, U.S. Army Medical Research Unit (WRAIR) Kenya, Box 401, APO New York 09675, USA.

[1] This work was supported by Research Grant No. DAMD17-82-G-9498 from the U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, Maryland 21701, USA, and was published with the approval of Dr I.E. Muriithi, Director of Livestock Production, Ministry of Livestock Development, Kenya.

Comparison of Three Culture Media for Isolating *Leishmania donovani* from Splenic Aspirates in Kenyan Visceral Leishmaniasis

SUMMARY

Three culture media were compared for their sensitivity in isolating *Leishmania donovani* from splenic aspirates from patients with visceral leishmaniasis. A total of 151 spelnic aspirates wer obtained from 18 patients before, during and after chemotherapy. Aspirates were cultured in Schneider's Drosophila medium supplemented with 20% fetal bovine serum (SCH), a rabbit blood-agar diphasic medium (NNN) overlayed with normal saline (NS), and NNN overlayed with SCH. Gimesa stained aspirate smears were microscopically examined. Of the 77 aspirates that were positive by any method, 88% were positive on smears, 57% were positive in NNN/SCH, 29% were positive in NNN/NS, and 25% were positive in SCH. Microscopy plus culture gave complementary results. We suggest that for optimal diagnostic and evaluation of response to treatment of visceral leishmaniasis in Kenya, splenic aspirates should be examined by microscopy and cultured in NNN/SCH.

INTRODUCTION

Isolation of promastigotes in culture is the most specific diagnostic test for leishmnaiasis. A previous study in Kenya found that culture was more sensitive than microscopy for detecting parasites in splenic aspirates from patients with visceral leishmaniasis, and that Schneider's medium was more sensitive than either RPMI 1640 or NNN medium with saline overlay (1). Schneider's medium is more sensitive than NNN with Locke's solution overlay in the diagnosis of human cutaneous leishmaniasis (2).

However, experimental studies have shown that NNN medium overlayed with Locke's solution is more senisitve than either Schneider's, RPMI 1640, or Iscoves medium in the isolation of a Kenyan strain of *Leishmania donovani* from infected hamster spleen (3). In addition, NNN overlayed with Schneider's medium appears to be more sensitive than either medium alone for isolating *Leishmania* spp from rodents in Kenya (Githure and Lightner, unpublished observations).

The aim of the present study was to compare the sensitivity of Schneider's medium and NNN overlayed with either normal saline or Schneider's medium for isolating parasites from splenic aspirates in patients with visceral leishmaniasis.

MATERIALS AND METHODS

Schneider's Drosophila medium (4) was prepared using chemicals obtained from Sigma Chemical Co.,St. Louis, MO, USA. (When prepared in our laboratory, the amount of tyrosine and cystine in the medium did not dissolve completely into solution). This medium was supplemented with 20% (v/v) fetal bovine serum (FBS, Kenya Meat Commission) which was heat-inactivated at 56°C for 30 minutes. One ml of medium was dispensed into 16 x 110 mm plastic tissue culture tubes (Ambitubes; Lux Scientific Corp., Newbury Park, CA, USA). NNN medium was prepared using DIFCO blood-agar base and 15% defibrinated haemolysed rabbit blood as previously described (2), except that 1.5 ml of rabbit blood-agar base was slanted in the Ambitubes, and 1 ml of either 0.9% saline, or Schneider's medium with 20% FBS, was added as the overlay. All cultures were modified by the addition of 500 I.U. pencillin and 500 μg streptomycin per ml.

Splenic aspirates were performed as previously described (5) using a 5 ml syringe and 21 gauge needle. A portion of the splenic aspirate material was squirted into a plastic vial containing 0.5 ml sterile 0.9% saline, mixed by shaking, and equal liquots added to the three culture media. From the remaining splenic aspirate material, smears were prepared and quantitated as previously described (5), using a logarithmic scale from 1+ (1-10 parasites per 1000 microscopic fields) to 6+ (> 100 parasites per field). Cultures were incubated at 25°C and examined for promastigotes daily for up to 14 days before being considered negative.

RESULTS

A total of 151 splenic aspirates were obtained from 18 patients over a period of 6 months. Nine patients had single aspirates, six had 2-4 aspirates and three had 5-9 aspirates. Among the 151 aspirates, 48 were culture positive and 68 were smear positive (Table 1). NNN overlayed with Schneider's medium was more sensitive than either of the other two culture media. Microscopy and culture gave complementary results: 31 of the 77 positive aspirates were detected only by smear, and nine of the positive aspirates were detected only by culture. NNN overlayed with Schneider's medium yielded the only positive result in six aspirates, while the other two media each yielded the only positive result in one aspirate. Cultures became positive most quickly in NNN medium overlayed with Schneider's medium (Fig. 1).

Most of the smear-positive, culture-negative aspirates had very few amastigotes (mean parasite grade 2+). Twenty-six of these 31 were obtained during the course of treatment. Of the remaining five that were obtained before treatment, all cultures from two aspirates had bacterial contamination, which could have inhibited parasite growth, and the other three aspirates had low parasite densities (all 3+).

DISCUSSION

In this study, combining NNN medium and Schneider's medium into a single diphasic medium gave a medium which was more sensitive than either medium alone for isolating parasites from splenic aspirates in patients with visceral leishmaniasis. It is unlikely that this or any other single medium will be ideal for culturing all species and strains of Leishmania, but NNN overlayed with Schneider's medium appears to be the best medium currently available for isolating L. donovani in Kenya.

In contrast to studies in hamsters (6) and a previous study from our laboratory (1), microscopy was more sensitive than culture in detecting parasites in splenic aspirates. Additionally, Schneider's medium was more sensitive than NNN in our previous study (1), but these two media had approximately equal sensitivity in the present study. The only changes in methodology between the two single were the source of Schneider's medium (obtained in liquid form from GIBCO in the previous study vs prepared from powdered chemicals in this study), the method of diluting the aspirated material to inoculate cultures (1 ml saline in glass bottles vs 0.5 ml saline in plastic bottles), the container in which NNN medium was dispensed (glass bottles vs plastic tubes), and the FBS used (mycoplasma-screened FBS from Flow Laboratories vs unscreened FBS from a local slaughter house). Tyrosine and cystine did not completely dissolve into solution when Schneider's medium was prepared from powdered chemicals, but neither of these is generally considered an essential amino acid, since tyrosine can be formed from phenylalanine and cystine can be formed from methionine (7). It is possible, however, that this or other differnces between commercially prepared and laboratory prepared Schneider's medium contributed to the discrepant results. Different lots of FBS are known to vary in their ability to support the growth of Leishmania (8), and this is another possible contributing factor. In addition, almost all of the discrepancies between culture and smear occurred when the parasite density was low. After a small portion of the aspirate was used to inoculate the cultures, the remaining, larger portion was used to prepare slides. Finally, since most of the culture-negative, slide-positive aspirates were obtained during the course of chemotherapy, it is possible that the amastigotes seen in smears were not viable and thus could not transform into promastigotes and multiply in culture.

NNN medium was prepared in Ambitubes, which have a flat surface at one end which allowed the fluid phase to be examined by inverted microscopy without opening the culture tube. This reduced the risk of bacterial and fungal contamination, and may have increased sensitivity, since virtually the entire fluid phase was examined when checking for the presence of promastigotes.

This study confirms our impression that combining NNN and Schneider's medium provides a superior medium for isolating L. donovani. For optimal diagnosis and evluation of response to treatment, we suggest that this culture medium should be used in addition to microscopy for examining splenic aspirates from patients with visceral leishmaniasis in Kenya.

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TABLE 1

Sensitivity of microscopy and culture for detecting *Leishmania donovani* in splenic aspirates*

Test	Sensitivity+	Predictive value of negative test#
Smears	68/77 (88%)	74/83 (87%)
NNN/SCH-20% FBS	44/77 (57%)	74/107 (69%)
NNN/saline	22/77 (29%)	74/129 (57%)
SCH-20% FBS	19/77 (25%)	74/124 (56%)

* Based on 151 splenic aspirates from 18 patients

+ Sensitivity = no. of positive in test/total no. of positives

Predictive value = no. of true negatives/total no. of negatives in test.

Legend to Figure 1

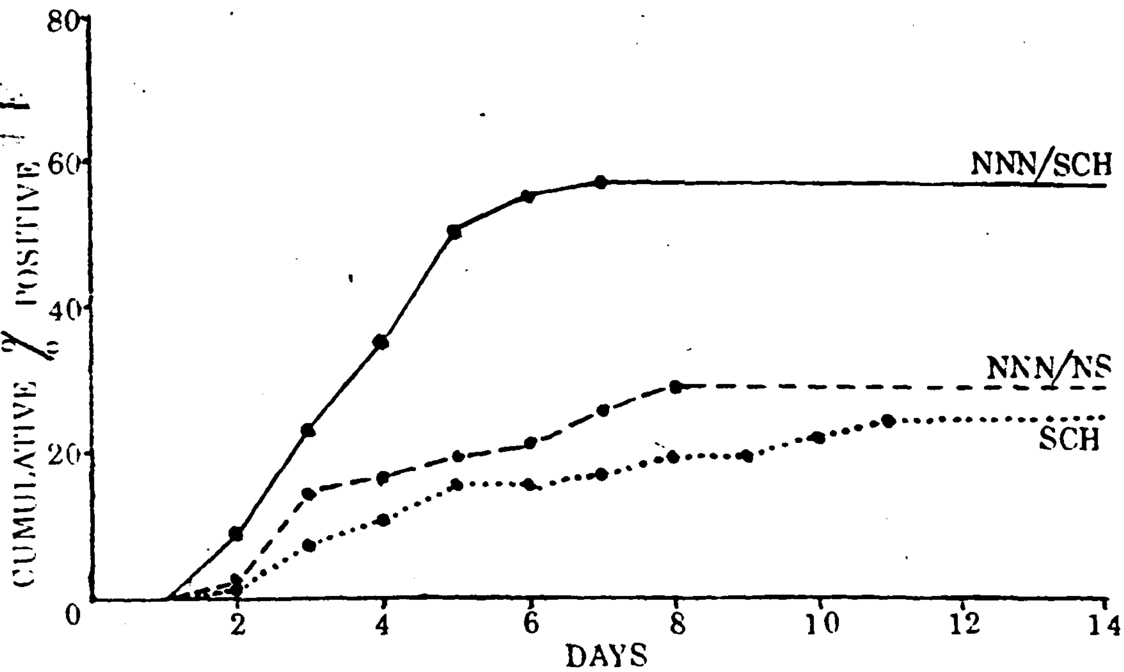
Detection of promastigotes in cultures of splenic aspirates from patients with visceral leishmaniasis.

NNN = rabbit blood-agar medium.

SCH = Schneider's medium with 20% fetal bovine serum.

NS = Normal saline.

Fig. 1



80
60
40
20
0
CUMULATIVE % POSITIVE
NNN/SCH
NNN/NS
SCH
2
4
6
8
10
12
14
DAYS

Leishmania donovani Parasitemia in Kenyan Visceral Leishmaniasis

SUMMARY

Twenty Kenyan patients with visceral leishmaniasis were evaluated for the presence of *Leishmania donovani* in peripheral blood. Smears, cultures, or hamster inoculations detected parasitemia in 11, 10 and six patients, respectively, and at least one method detected parasitemia in 15 patients (75%). The likelihood of detecting parasitemia correlated with the density of parasites in splenic aspirate smears. We conclude that parasitemia with *L. donovani* occurs frequently in Kenyan patients with visceral leishmaniasis.

Introduction

Parasitemia with Leishmania donovani is common in visceral leishmaniasis. In India, amastigotes are seen in peripheral blood smears in 67 to 79% of patients (KNOWLES & DAS GUPTA, 1924; SHORTT et al, 1927), and cultures of peripheral blood are positive in 96 to 97% (BRAHMACHARI and MAITY, 1925; DAS GUPTA, 1930). In China, at least one pre-treatment blood culture was positive in 31 of 34 patients, and at least one pre-treatment blood smear was positive in all nine patients studied (YOUNG & VAN SANT, 1923). In Sudan, however, peripheral blood smears are positive in only 35% of patients; adding the technique of hamster inoculation increased the detection of parasitemia to 65% (ROHRS, 1964). In Kenya, parasites have not been found in smears of peripheral blood, but cultures of whole blood on NNN medium were positive in 7 of 21 patients (MANSON-BAHR, 1959).

We have re-evaluated the question of parasitemia in Kenyan visceral leishmanisis, using a variety of techniques (cultures, smears, hamster inoculation, and feeding sandflies) with various blood fractions to detect parasites in peripheral blood. We report here our results in 20 patients, 15 of whom had parasitemia detected by at least one method.

Materials and Methods

Blood was obtained from consenting patients with untreated visceral leishmaniasis. The diagnosis was confirmed in all patients by finding amastigotes in splenic aspirate smears. Parasites in these smears were quantitated as previously described (CHULAY & BRYCESON, 1983), using a logarithmic scale from 1+ (1-10 parasites per 1000 microscopic fields) to 6 + (100 parasites per field). Ten milliliters venous blood was placed in a sterile heparinized tube (Beckton-Dickinson #6480, Scientific Products, Columbia, MD, USA) and fractionated as outlined in Figure 1. Two and one-half milliliters blood was mixed with 12 ml 0.9% saline and centrifuged at 250 x G for 15 min. (This and all subsequent centrifugations were done at room temperature.) The platelet-rich plasma was removed, centrifuged at 1000 x G for 5 min, and the pellet resuspended in 0.1 ml supernatant fluid. From the sediment remaining after removing the platelet-rich plasma, approximately 0.1 ml of the buffy coat was transferred with a Pasteur pipet to another tube, and approximately 0.1 ml of the red cell pellet from the bottom of the tube was similarly transferred to a third tube. These three fractions are essentially those described by YOUNG & VAN SANT (1923). From the original blood sample, 3 ml was mixed with 6 ml 0.9% saline and 1.8 ml 4.5% dextran (MW 200-275,000, Sigma Chemical Co., St. Louis, MO, USA) and allowed to sediment 45 min at room temperature. The leukocyte-rich plasma was removed, 6 ml was layered over 3 ml of a mixture of 63.5 g ficoll 400 (Pharmacia, Uppsala, Sweden) and 100 G sodium diatrizoate (Winthrop Laboratories, Surbiton-upon-Thames, UK) per liter (sp. gr.= 1.075 - 1.084) and centrifuged at 1000 x G for 20 min. The mononuclear leukocytes at the interface and the pellet of polymorphonuclear leukocytes were collected and washed with 0.9% saline. The mononuclear leukocytes, polymorphonuclear leukocytes and 1 ml leukocyte-rich plasma were centrifuged at 1000 x G for 5 min and resuspended in 0.1 ml supernatant fluid. From the six fractions and the original whole blood, 20 1 aliquots were added to tubes containing either 1 ml Schneider's Drosophila medium (HENDRICKS & WRIGHT, 1979) (prepared using chemicals obtained from Sigma) or NNN medium overlayed with Schneider's medium (GITHURE & CHULAY, 1984). Cultures were incubated at 25^{o}C and observed daily for 3 weeks.

To the remainder of each suspension (excluding whole blood), 0.25 ml 0.9% saline and 0.1 ml newborn calf serum (GIBCO, Grand Island, NY, USA) was added, and 0.1 ml aliquots were used to prepare smears with a cytocentrifuge (Cytospin, Shandon Southern Instruments, Sewickley, PA, USA). Thin films were prepared from the original whole blood in the standard fashion (with a feathered edge) and also according to the method of SHORTT, et al. (1927) (with the edge of the second slide lifted just before the blood was ex- hausted). Thick blood films were prepared by spreading 5 1 evenly through- out a 2.5 x 10 mm rectangle, and also according to the method of KNOWLES & DAS GUPTA (1924), using four drops at the corners of a 0.5 x 0.5 inch square near the center of a slide. Thick films were dehaemoglobinized with 0.4% tartaric acid in 2% glacial acetic acid (KNOWLES & DAS GUPTA, 1924). All slides were stained with Giemsa and examined at 1000 X magnification for at least 5 min. If all 10 slides from any patient were negative, a second set of 10 slides was examined.

Hamsters were inoculated intracardially with 0.5 ml whole blood or leukocyte-rich plasma. Three months later they were sacrificed and impression smears and cultures (in the above two media) obtained from liver and spleen.

Results

Parasites were detected in peripheral blood from 15 of the 20 patients (Table I). Cultures from at least one of the seven fractions (Figure 1) were positive from 10 patients. Positive cultures were obtained from the buffy coat in nine and from the mononuclear cell fraction in eight. Cultures from the red cell pellet and leukocyte-rich plasma were each positive from seven samples. Cultures from the platelet-rich plasma, the polymorphonuclear leukocyte fraction, and whole blood were each positive from six samples. Twenty-nine of 140 cultures in Schneider's medium and 44 of 140 cultures in NNN/ Schneider's were positive.

At least one of the initial 10 smears was positive from 11 patients. Smears from the mononuclear cell fraction, leukocyte-rich plasma, Shortt's thin films, buffy coat and the polymorphonuclear leukocyte fraction were positive from 5, 4, 3, 3 and 1 patient, respectively. Parasites were always found within monocytes (Figure 2), even in the PMN fraction. A total of 24 cells were found infected, with between one and seven (mean = 2.6) parasites per cell. Parasites were not found in thick films, normal (feathered edge) thin films, or slides of platelet-rich plasma or the red cell pellet. In no instance were parasites found in the second set of slides when all in the first set were negative.

Blood from six patients caused visceral infection in hamsters. Six hamsters inoculated with whole blood and five inoculated with leukocyte-rich plasma became infected.

Parasite density in pre-treatement splenic aspirate smears correlated with the detection of parasites in peripheral blood (Table I). From the seven patients with a 5+ splenic smear, 37/98 (38%) cultures, 9/70 (13%) blood smears, and 7/14 (50%) inoculated hamsters were positive. From the seven patients with a 4+ splenic smear, 31/98 (32%) cultures, 4/70 (6%) blood smears, and 2/14 (14%) inoculated hamsters were positive. From the two patients with a 3+ splenic smear, 5/28 (18%) cultures, 2/20 (10%) blood smears, and 2/4 (50%) inoculated hamsters were positive. From the four patients with splenic smears of grade 2+ or 1+, only 1/40 (2%) blood smears were positive, and all cultures and hamster inoculations were negative.

Discussion

Our results indicate that *L. donovani* parasitemia is common in patients with visceral leishmaniasis in Kenya. The use of several methods of detection enabled us to document parasitemia in 75% of patients.

NNN medium overlayed with Schneider's medium was more sensitive than Schneider's medium alone for detecting *L. donovani* in cultures of peripheral blood. This medium is also more sensitive than either NNN or Schneider's medium alone for culturing *L. donovani* from splenic aspirates (GITHURE & CHULAY, 1984).

The level of parasitemia, as estimated by the percentage of specimens in which parasites were detected, correlated with the density of parasites in splenic aspirate smears. However, even when splenic parasites were abundant, parasitemia levels were low; most slides had no infected cells detected microscopically, and more than one infected cell was found on only one slide. A more sensitive and rapid method of examining peripheral blood might make detection of parasitemia useful diagnostic aid. Biotin-labeled DNA probes (LEARY et al, 1983) may provide these advantages and should be evaluated for detecting *L. donovani* parasitemia. At the present time, however, splenic aspiration remains the most sensitive method for confirming a diagnosis of visceral leishmanisis.

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Table I. Detection of <u>Leishmania donovani</u> parasitemia in Kenyan visceral leishmaniasis.

		Cultures*						
Pt. No.	Spleen grade**	whole blood	buffy coat	red cell pellet	platelet -rich plasma	leukocyte -rich plasma	mononuclear leukocytes	polymorpho-nuclear leukocytes
1	5+		+(N)	+(N)	+(N)	+(N)	+(N)	+(N)
2	5+							
3	5+							
4	4+							+(N)
5	4+		+(S)		+(S)			
6	5+	+(N)	+(N)	+(B)	+(B)	+(N)	+(N)	+(B)
7	5+	+(N)	+(B)	+(N)	+(S)	+(N)	+(S)	+(N)
8	4+							
9	1+							
10	3+							
11	5+							
12	1+							
13	1+							
14	5+	+(N)	+(B)	+(B)	+(B)	+(B)	+(B)	+(B)
15	4+	+(B)	+(B)	+(B)		+(B)	+(S)	+(N)
16	4+	+(N)	+(B)	+(B)				
17	4+							
18	2+							
19	4+	+(B)	+(B)	+(B)	+(B)	+(N)	+(B)	+(B)
20	3+		+(N)			+(B)		+(B)

Table I. (Continued)

Smears					Hamster Inoculation	
thin film	buffy coat	mononuclear leukocytes	polymorpho-nuclear leukocytes	leukocyte -rich plasma	whole blood	leukocyte -rich plasma
		+			+	+
			+		+	+
		+				
+	+					
+	+	+				
					+	+
+		+			+	+
	+				NA***	NA
				+	+	NA
				+		
				+		
		+		+	+	+

Footnotes to Table I.

* +(N) = positive only in NNN medium overlayed with Schneider's medium, +(S) = positive only in Schneider's medium, +(B) = positive in both media. Blanks indicate all cultures were negative.

** Density of parasites in splenic aspirate on a logarithmic scale from 1+ (1 - 10 parasites per 1000 microscopic fields) to 6+ (> 100 parasites per field).

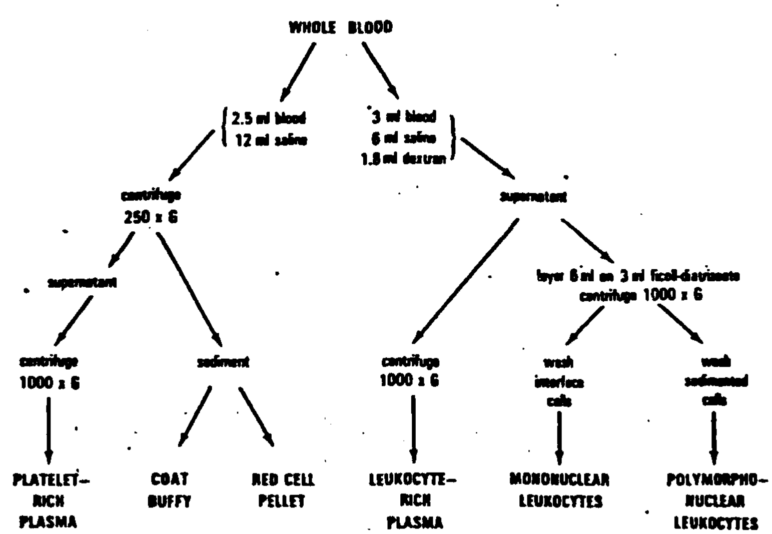
*** Not available (hamster died 1-2 days after inoculation).

Legends to Figures

Fig. 1 Outline of the method used to fractionate blood. The fractions which were cultured are in bold type.

Fig. 2. _Leishmania donovani_ amastigotes in peripheral blood monocytes from patients with visceral leishmaniasis in Kenya. (A & B) From a thin blood film. (C,D & E) From a cytocentrifuge preparation of mononuclear leukocytes purified by dextran sedimentation and ficoll-diatrizoate gradient centrifugation. (F) From a cytocentrifuge preparation of the buffy coat.

FIGURE 1



WHOLE BLOOD
2.5 ml blood
12 ml saline
3 ml blood
6 ml saline
1.8 ml dextran
centrifuge
250 x G
supernatant
supernatant
layer 6 ml on 3 ml ficoll-diatrizoate
centrifuge 1000 x G
centrifuge
1000 x G
sediment
centrifuge
1000 x G
wash
interface
cells
wash
sedimented
cells
PLATELET-
RICH
PLASMA
COAT
BUFFY
RED CELL
PELLET
LEUKOCYTE-
RICH
PLASMA
MONONUCLEAR
LEUKOCYTES
POLYMORPHO-
NUCLEAR
LEUKOCYTES

FIG 2

New Phlebotomine Sandfly Colonies II: Laboratory Colonization of *Phlebotomus duboscqi* (Diptera:Psychodidae)*

Abstract. *Phlebotomus duboscqi*, a vector of *Leishmania major* in Kenya is being maintained in a closed laboratory colony, currently in its 5th generation. The rearing techniques developed for three previously colonized species of phlebotomine sandflies are also suitable for maintaining this new species. *P. duboscqi* is comparatively easy to rear because 75% of the females survive oviposition, take additional blood meals and lay 2nd and in some cases 3rd egg batches thereby substantially increasing the productivity of each generation. Adult female longevity averages 25 days. Because this species takes mutiple blood meals and survive oviposition it has been possible to experimentally infect females with *L. major* and achieve sandfly-rodent-sandfly transmission of the parasite.

The techniques that we used to colonize *Phlebotomus martini*, *Sergentomyia schwetzi* and *S. africana* (Beach, Young & Mutinga, 1984, J. Med. Entomol. 20: 579-584) are also being employed to raise *Phlebotomus* (*Phlebotomus*) *duboscqi* Neveu-Lemaire, 1906. This is the only colony of *P. duboscqi*, a suspected vector of *Leishmania major* in a wide area of North Africa (Lariviere, Quenum & Abonnenc, 1961, Bull. Soc. Med. Afrique Noire Langue francaise *6*: 431-435; Lewis, 1974, Colloques Internationaux du Centre de la Researche Scientifiques, Paris, pp 133-138), that we are aware of.

Begun in June 1983 with eggs from 12 females captured in Baringo District, Rift Valley Province, Kenya (0° 30'N lat., 36°E long.), the colony is currently in its 5th laboratory-bred generation. *P. duboscqi* is relatively easy to rear because females of this species do not die while ovipositing eggs, a serious problem in our previous colonization efforts

Beach et al., 1984, loc cit.). Instead 75% of the P. duboscqi females in our colony survive oviposition, take additional blood meals and complete a 2nd and in some cases a 3rd gonotrophic cycle. Thus, instead of doubling in size each generation, the situation in our P. martini colony, P. duboscqi achieves a 5 to 10 fold increase per generation. Like P. papatasii, a close taxonomic relative, P. duboscqi females take up to 4 blood meals during each gonotrophic cycle. This repetitive biting behavior, in additional to an average female longevity of 25 days (range: 19-30; n=30 females) makes this species ideal for experimental transmission of Leishmania major (Beach, Kiilu & Leeuwenburg, 1985, Am. J. Trop. Med. Hyg. (in press)).

The presence of P. duboscqi in Kenya was reported in 1982 when one of us, DGY, identified a male from a light trap collection made in Baringo District, (Beach, Young & Mutinga, 1982, Trans. R. Soc. Trop. Med. Hyg. 76: 707). The discovery was significant in that it revealed the presence of a candidate vector of L. major, a parasite known to occur in the Baringo area (Chance, Schnur, Thomas & Peters, 1978, Ann. Trop. Med. Hyg. Parasitol. 72: 533-542; Githure, Beach & Lightner, 1984. Trans. R. Soc. Trop. Med. Hyg. (in press)). During the past year we have collected 278 additional P. duboscqi females in Baringo, one of which, prior to capture, bit and infected the senior author with L. major (Beach, Kiilu, Hendricks, Oster & Leeuwenburg, 1984, Trans. R. Soc. Trop. Med. Hyg. (in press)), an event demonstrating that P. duboscqi is a vector of L. major in Kenya.

Our P. martini and S. schwetzi colonies, now over 2 years old, are in thier 14th and 17th laboratory generations respectively. Our S. africana colony was terminated after 8 generations.

*This work was supported in part by contract number DAMD17-83-G-9517 from the U.S. Army Medical Research and Development Command.

For submission to Trans. R. Soc. Trop. Med. & Hyg. - Communications

Rapid identification of *Leishmania* isolates in Kenya

In Kenya, we encounter cases of human cutaneous leishmaniasis caused by *Leishmania donovani* (post kala-azar dermal leishmaniasis), *L. major* and *L. aethiopica* (OSTER, personal communication; BEACH et al., 1984; CHULAY et al., 1983). To identify the species of *Leishmania* involved in each infection we biochemically characterize parasites isolated from patients with suspected cutaneous disease by cellulose acetate electrophoresis (CAE) (KREUTZER & CHRISTENSEN, 1978). Identification is based on the similarity between zymogram banding patterns for an 'unknown' isolate and 3 already characterized marker strains, *L. major*, *L. donovani* and *L. aethiopica*. When the banding pattern of an unknown visually matches that of a marker, a tentative identification can be made. The probability of making a false identification is reduced by repeating the electrophoresis step several times and staining for different enzymes.

Initially we mass cultured each unknown isolate in order to produce a sufficient volume and concentration of enzyme extract to carry out CAE analysis for the following enzymes: lactate dehydrogenase (LDH), malic enzyme (ME). 6-phosphogluconate dehydrogenase (6PGDH), glucose-6-phosphate dehydrogenase (G6PDH), aspartate amino transferase (ASAT), hexokinase (HK), phosphoglucomutase (PGM) and phosphate glucose isomerase (PGI) (BEACH, *et al.*, 1984). The results of this work indicated that 1 enyzme, PGI, could consistently be used to identify each isolate to species without relying on the additional discretionary power provided by banding patterns on the zymograms stained for the other 7 enzymes (figure 1).

Isolates come to our laboratory directly from clinical wards where material aspirated from patient skin lesions (HENDRICKS & WRIGHT, 1979) is inoculated into a culture tube containing NNN media overlaid with 2 ml of Schneider's Drosophila media (GIBCO) supplemented with 20% heat inactivated foetal bovine serum (SCH 20FBS) plus gentamicin (25 μg/ml) and 5-fluorocytosine (500 μg/ml). Upon arrival in our laboratory the cultures are incubated at 25°C and monitored for 2 weeks to confirm the presence or absence of *Leishmania* organisms in the original aspirate. If promastigotes are present in the culture, 1 ml of the overlay is subcultured in a larger volume of SCH 20FBS, grown to a concentration of 10^5-10^6 promastigotes/ml and cryopreserved for future reference. The second ml of overlay is now immediately used to produce an extract for CAE identification based on PGI banding patterns, without further propagation of the parasite. This can be done because only a small number of parasites are needed to produce an extract of high PGI activity. We find that the 10^5 parasites contained in 1 ml of overlay produce enough extract for 10 separate CAE runs when staining for PGI. However, this concentration of extract is not adequate when assaying most of the 7 other enzymes. Extraction of promastigotes as well as electrophoresis and staining, the basic steps in CAE, can all be done in less than 1 hr.

The fact that a PGI zymogram discriminates between *L. donovani*, *L. major* and *L. aethiopica* and can be easily produced with only a small number of promastigotes suggests that it may be used as a rapid identification technique which could be coupled to our routine procedure for confirmation of *Leishmania* organisms in patient aspirates.

To support our observation that PGI can separate these three species of *Leishmania* consistently, we have used this enzyme alone to identify *Leishmania* isolates from 10 cases of human cutaneous leishmaniasis. Of these 2 were identified as L. donovani (post kala-azar dermal leishmaniasis), 7 as L. aethiopica and 1 as L. major. When the same isolates were mass cultured, extracted and characterized using ME, the identifications remained unchanged. We chose ME for verification because this enzyme cleanly separates L. major from L. donovani and L. aethiopica (figure 2). There is some overlapping of L. major PGI bands with those of L. donovani and L. aethiopica (figure 1). Therefore, the most obvious source of error in relying on PGI alone, mistaking L. major for L. aethiopica or L. donovani was eliminated. L. donovani bands on PGI zymograms do not overlap with L. aethiopica (figure 1).

Success in discriminating between the species of Leishmania based only on PGI relies on the use of freshly prepared buffers and extracts which, if stored, as is the case with our marker strains, are held in liquid nitrogen. Realizing the inherent danger in relying on only 1 enzyme to identify Leishmania species (KREUTZER et al., 1983) we feel that these preliminary results need confirmation in other laboratories and are arranging for such 'repeatability' studies to be carried out.

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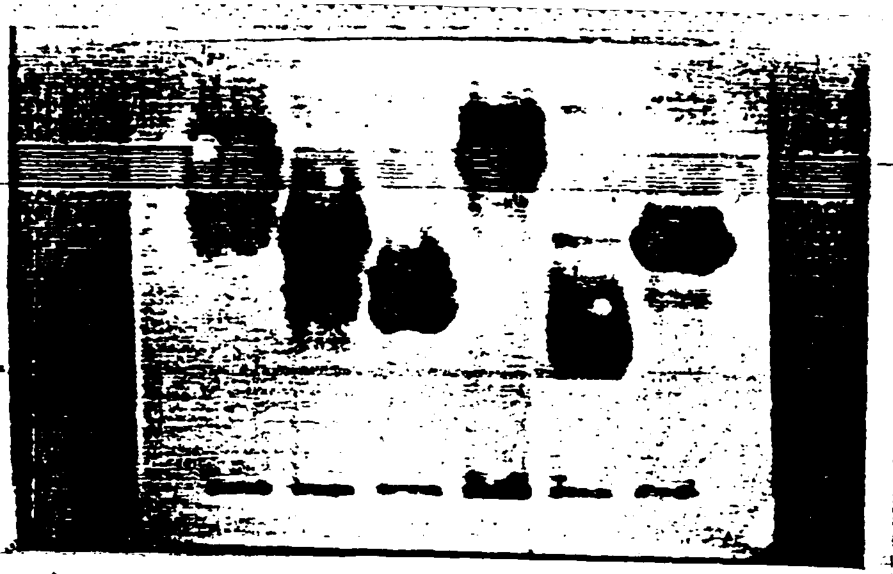

Figure 1. A CAE zymogram stained for PGI. 1-*L. donovani* marker 2-*L. major* marker, 3-*L. aethiopica* marker, 4-*Crithidia* sp (non-human marker, 5-*L. adleri* (nonhuman) marker, 6-unknown isolate from cutaneous lesion identified as *L. major*

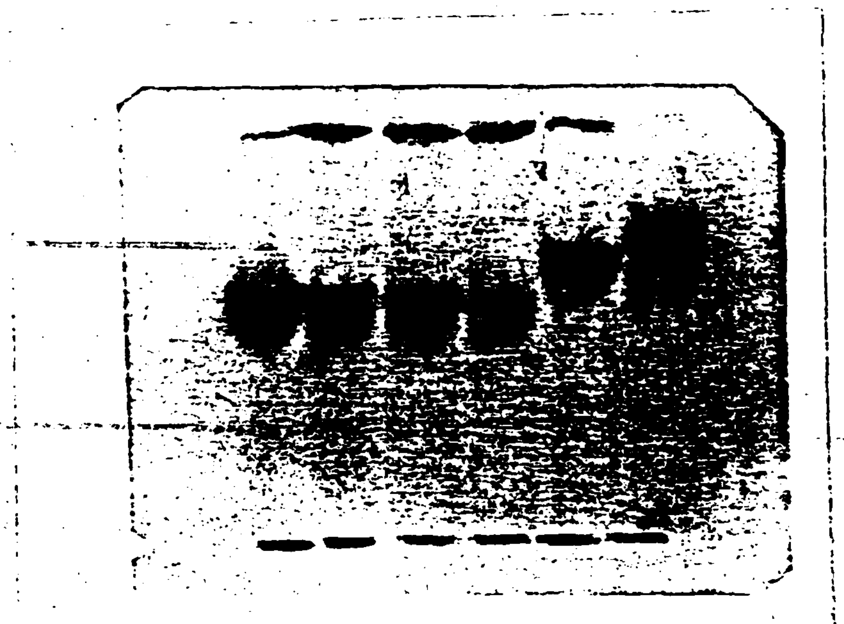

Figure 2. A CAE zymogram stained for ME. 1-3 unknown isolates from cutaneous lesions identified as *L. major*, 4-*L. major* marker, 5-*L. donovani* marker, 6-*L. aethiopica* marker.

Modification of Sandfly Biting Behavior by *Leishmania* Leads to Increased Parasite Transmission

ABSTRACT

To attempt rodent-sandfly-rodent transmission of *Leishmania major*, laboratory-reared *Phlebotomus duboscqi* were fed on *L. major*-infected mice and then refed on uninfected mice 21 days later. Flies which refed either probed 1-2 times and took a full blood meal in less than 10 min or probed 3 or more times and took little or no blood during a period of 15 min or more. When dissected, 7 of 8 flies which experienced difficulty in obtaining a blood meal had flagellates in their cibaria, an observation supporting the hypothesis that parasites in this part of the alimentary canal modify normal blood feeding behavior. None of the infected females which robed 1-2 times had similar anterior station infections. Infected sanflies transmitted *L. major* to uninfeeted mice and one female, transferred from one mouse to the next while repeatedly attempting to take blood, infected 5 mice.

During a year-long survey in Baringo District, Kenya, we collected 9182 female sandflies. Only 2 of the 278 *P. duboscqi* captured during this collection were infected with *L. major*. Parasite interference with normal blood feeding and the associated increase in number of infective bites/female may explain how a relatively small population of *P. duboscqi*, only a few of which are infected with *L. major*, can nonetheless maintain a parasite reservoir in local rodents.

Beach *et al.*[1] reported transmission of *Leishmania major* by the bite of a naturally-infected *Phlebotomus duboscqi* sandfly, captured in Baringo District, Kenya. During this incident the infected fly probed r peatedly, transmitting parasites at 11 different biting sites, but was unable to take blood. The number of infective bites delivered by this female was greater than that predicted based on the 1-2 bites delivered by uninfected females in the course of obtaining a blood meal.[2] Laboratory studies[3-8] have also described how *Leishmania* infections interfered with sandfly blood feeding and, in some cases, elicited a greater-than-expected number of bites by females attempting to take blood. It has been suggested that such changes in sandfly biting behavior are of adaptive value to the parasite because they promote transmission.

The transmission experiments discussed in this paper were carried out using laboratory-reared *P. duboscqi* infected with *L. major*. We characterized the blood feeding behavior of infected females and then dissected them to determine if flagellates were present in the cibarium, an experiment predicated on the hypothesis that such infections interfer with cibarial blood meal-sensing receptors, thereby changing normal blood feeding behavior.[8,9]

The need for a mechanism to increase the number of infective bites delivered by a female sandfly tallied with the relatively low *L. major* infection rate and population density of *P. duboscqi* measured in an area with a sizeable *L. major* rodent reservoir.

MATERIALS AND METHODS

Sandflies

P. duboscqi females were taken from the first and second generations of a laboratory colony begun in June 1983. The flies were reared according to previously described procedures.[10] Because they took mutiple blood meals, females were fed 3-4 times on *L. major*-infected BALB/c mice and then refed on uninfected mice 21 days after first taking blood. Unless otherwise stated 4 or 5 randomly selected females were used in each transmission attempt. Infected, as well as uninfected, females were held in individual cages at 95% rh, 27^{o}C and had access to a small slice of apple which was changed daily. All blood feeding was done in the early morning when ambient temperatures were lowest and light intensity diminished.

After attempting transmission, infected flies were dissected in saline to determine presence and location of parasites in the alimentary canal. The head was severed from the body and the anterior midgut removed and examined for promastigotes. Finally the pharynx, cibarium and mouth parts were dissected, intact, from the head and examined for parasites.

Probing behavior was quantified by recording the number of probes, that is the number of times the proboscis was raised and lowered with subsequent insertion of the feeding stylets. We also recorded the time between the first and last attempt to take blood or until successful completion of the blood meal. The volume of blood imbibed was determined by comparison of the visible blood meal taken by infected sandflies with that taken by paired uninfected controls.

Leishmanial strains

Nairobi *Leishmania* Bank isolate 144, a strain of *L. major* isolated from a wild caught *P. duboscqi* and characterized by cellulose acetate electrophoresis,[1] was used in this study. The spleen from a mouse, already infected with 144, was homogenized in 1 ml of saline and 0.1 ml of the resulting amastigote suspension inoculated subcutaneously into the noses of 5 mice. Approximately 45 days later, sandflies were allowed to feed around the lesions which developed at the site of inoculation.

Mice

The BALB/c mice used in transmission attempts were sacrificed 60 days after being bitten on the nose by infected *P. duboscqi*. To verify infection, tissue from the nose and spleen of each animal were cultured in Schneider's Drosophila Medium (GIBCO) supplemented with 20% heat inactivated fetal bovine serum,[11,12] 250 µg/ml gentamicin and 500 µg/ml 5-fluorocytosine (SCH-20FBS). The cultures were incubated at 25°C and monitered for promastigotes over a 14 day period.

Field work

Field studies were carried out near the town of Marigat, Baringo District, Rift Valley Province, Kenya (0°30' N lat., 36° E long.). Sandflies were collected daily during the period February 1983 - January 1984 inclusive using CDC light traps and hand aspirators. All flies were dissected, examined for parasites and mounted for taxonomic identification.

Flagellates from infected flies were inoculated into SCH-20FBS, sent to our laboratory in Nairobi and characterized using the technique of cellulose acetate electrophoresis.[13]

RESULTS

The effect of L. major on P. duboscqi biting behavior

Twenty-four P. duboscqi were used in a transmission experiment 21 days after first feeding on an infected mouse. The biting behavior, amount of blood imbibed and duration of the biting period were compared with the presence of parasites in cibarium of each sandfly (Table 1). When dissected, 7 of 8 flies which probed 3 or more times and took little or no blood over a period of 15-20 min had parasites in their cibaria. Of the remaining 16 females which probed 1-2 times and took a full blood meal within 10 min, 10 had midgut infections which had not spread to the anterior station, here defined as the part of the alimentary canal located in the head of the insect and including the pharynx, cibarium and remainder of the buccal cavity, and 6 were uninfected.

Transmission of L. major

When sacrificed, 2 of the 5 mice bitten by potentially infective P. duboscqi in the preceeding experiment were culture positive for Leishmania

Three L. major-infected P. duboscqi, observed to probe repeatedly without engoring, were subsequently used in a multiple transmission experiment 25 days after their first infective blood meal. Each fly was exposed to a different series of 5 BALB/c mice. When a fly had probed 1-2 times on the first mouse in the series it was transfered to the next until all five mice had been bitten or until the fly showed no further interest in attempting to take blood. Upon dissection, all three flies were found to have heavy midgut infections extending forward into the anterior station. Fly I (Table 2) transmitted L. major to all 5 mice it fed upon; Fly II infected 3 of 5 mice and the last fly (III) bit and infected only 1 mouse. Control females, I-c, II-c, III-c, uninfected but otherwise identical in age and prior blood feeding experience to experimental flies, probed only on the first mouse, took a full blood meal and thereafter showed no inclination to feed on mice 2-5 in each series.

L. major infections in natural populations of P. duboscqi

Sandlfies were collected by light trap and aspiration over a 12 month period in Baringo District, Kenya. These collections yielded a total of 9182 female sandflies of which 278 (0.3%) were P. duboscqi. Two of the P. duboscqi females (0.7%) were infected with L. major (Table 3).

DISCUSSION

Experimental and natural Leishmania infections interfere with sandfly blood feeding. Increased biting, a corollary of this interference, is important because it amplifies the transmission potential of an infective sandfly and therefore the contribution made by these insects to the spread of leishmaniasis. Parasite interference with cibarial sensilla controlling engorgement is thought to be a cause of obstructed blood feeding behavior in Leishmania-infected sandflies.[8,9] The presence of parasites in the cibaria of 7 of the 8 sandflies in our study, which displayed excessive biting and had difficulty taking blood, provides only circumstantial support ror this hypothesis. However, because L. major-infected P. duboscqi fail to engorge and probe repeatedly this model may be of value in conducting a more rigorous investigation of this idea.

An alternative explanation of how Leishmania interfer with sandfly blood feeding involves mechanical blockage of thepharynx or esophagus by large numbers of parasites, some of which are flushed forward as the flow of blood, unable to pass the parasite block, moves back through the mouth parts into the wound.[14] According to this hypothesis, 'blocked' sandflies would also have difficulty engorging on blood, however 10 of our experimental flies that took full blood meals after probing once or twice were found to have heavy infections in their anterior midguts. Thus, in our work,large numbers of parasites in the anterior midgut fail to interfere with normal blood feeding. Whether flies with heavy infections in their anterior midguts transmit parasites when they feed is not known, but the biting behavior of such insects is not the same as that observed in flies with parasites in the cibarium as well as the anterior midgut.

The tsetse fly, Glossina m. morstians, infected with Trypanosoma (T.) brucei probes more frequently than uninfected controls,[15] a behavioral change attributed to parasite interference with mechanoreceptors monitering blood flow rate in the proboscis. However, this explanation has been recently questioned.[16] A similar increase in probing behavior occurs in G. m. morsitans, infected with T. (N.) congolense.[17] In addition, changes in blood feeding behavior are known to occur in mosquitoes infected with malaria and arboviruses[18,19] and in plague-infected fleas.[20] The strategy of eliciting increased probing or other transmission-favoring events in hematophagus insects appears to have been invoked during the evolution of several vector-pathogen relationships.

The natural infection rate in P. duboscqi from Baringo District, 0.7%, is low when compared with promastigote infection rates for other species of sandflies,[21] which range from 0.2% to 10.5%. Based on our collection methods, 6 of the 11 species of sandflies captured in Baringo were more abundant than P. duboscqi, suggesting that the population size of this species is relatively small. The prevalence of L. major in the Baringo rodent population, where 18 of 789 animals (2.2%), representing 15 species in 6 genera were infected with L. major,[22] may be due to the occurrence of a greater-than-expected number of infective bites by P. duboscqi with its potential for increasing the amount of transmission attributable to a small population of vector sandflies.

Despite our attempts at standardization, infectivity, localization of L. major in the sandfly alimentary canal and biting behavior of our experimental flies varies when we attempt transmission making our results inconsistent. However, we do experience some success employing colonized P. duboscqi in experimental transmission studies. Two possible explanations for this are suggested. Other colonized sandflies suffer high mortality rates during oviposition.[8] Due to this oviposition-associated death, most females infected for transmission work die while laying eggs, prior to seeking additional blood and transmitting parasites. However, 75% of our P. duboscqi females survive oviposition and thereafter readily take as many as 4 additional blood meals. This allows us to avoid the somewhat artificial treatment of denying females an oviposition site in order to keep them alive until attempting transmission, or of infecting flies by membrane feeding them on suspensions of promastigotes in media that does not stimulate oogenesis, two methods from other studies[8,23] for circumventing oviposition. In addition, the prolonged survival of adult P. duboscqi, which averaged 25 days in this study, favors transmission by affording more time for L. major infections to develop. Finally, the use of NLB 144, only recently isolated from a sandfly and passaged only once in mice, reduces the possibility of loss of infectivity to the insect as a result of continual passage in laboratory culture or animals, a problem known to occur with certain rodent malarias.[24]

This study demonstrates that Leishmania interfere with normal blood feeding eliciting a greater-than-expected number of infective bites/sandfly. It is suggested that such changes favor the spread of Leishmania in Kenya where populations of vector sandflies have relatively low densities and infection rates.

TABLE 1

P. duboscqi infected with L. major: Biting behavior, blood meal volume and duration of biting period compared with presence of parasites in sandfly's cibarium

Biting behavior, blood meal volume and biting period duration	Parasite in cibarium Yes	no
flies probing 1-2x; taking full blood meal in 10 min	0/16	16/16[1]
		10/10[2]
flies probing ≥ 3x; taking small or no blood meal in 15-20 min	7/8	1/8[3]

[1]when dissected 10 females with midgut infections only; 6 females with no apparent infection

[2]uninfected controls

[3]heavy midgut infection, no parasites observed in anterior station

TABLE 2

Mutiple transmission of *L. major* by individual *P. duboscqi* females

	mice				
Sandfly[1]	1	2	3	4	5
I	+[2]	+	+	+	+
I-c[3]	-	0	0	0	0
II	+	+	+	-	-
II-c	-	0	0	0	0
III	+	0	0	0	0
III-c	-	0	0	0	0

[1]for each experiment a single infected female (I, II, III) allowed to probe on each of 5 BALB/c mice, numbered 1-5 in the table, in succession.

[2]presence(+)/absence(-) of promastigotes in mouse spleen cultures made 60 days after transmission attempted. 0 = no attempt by sandfly to bite.

[3]uninfected control: a female of the same age and with similar blood feeding history as the preceeding infected female.

TABLE 3

Female sandflies caught in Baringo District, Kenya during the period February 1983-January 1984 inclusive: total and number infected.

species	aspiration total	aspiration positive	light trap total	light trap positive
	collection method			
P. duboscqi	52	2[2]	226	0
P. martini	35	0	302	0
P. rodhaini	1	0	1	0
S.[3] *antennatus*	1182	9	232	1
S. bedfordi	3613	10	849	1
S. schwetzi	368	7	756	11
S. africanus	220	1	72	0
S. clydei	114	2	689	5
S. squamipleuris	7	0	435	3
S. adleri	7	0	16	0
S. grangeri	1	0	1	0
TOTAL	5603	31	3579	21

[1] positive for flagellates upon dissection

[2] flagellates identified as *L. major* by cellulose acetate electrophoresis

[3] *Sergentomyia*

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Transactions Paper 83327—

The isolation of *Leishmanaia major* from rodents in Baringo District, Kenya*

An examination of small rodents for leishmanial parasites was initiated in Marigat, Baringo District, Rift Valley Province, Kenya, an area where kala-azar is endemic (MCKINNON, 1962). Trapped rodents were examined for cutaneous lesions and then necropsied. Samples of the livers and spleens were individually cultured in NNN medium overlayed with Schneider's Drosophila medium supplemented with 20% foetal bovine serum. 789 rodents of 10 different species were examined over an eight-month period. Leishmanial parasites were isolated in culture from the spleens and livers of 17 (2·2%) rodents namely, seven of 168 (4·2%) *Tatera robusta*, five of 466 (1·1%) *Arvicanthis niloticus*, two of 17 (11·8%) *Taterillus emini*, two of 51 *Mastomys natalensis* and one of two *Aethomys kaiseri*. Impression smears of the livers and spleens from these animals were all negative and none of the 17 showed any signs of cutaneous lesions. No leishmanial parasites were isolated from 29 *Acomys* sp., 22 *Rattus rattus*, 15 *Elephantulus rufescens*, 15 *Crocidura* sp. and four *Graphiurus* sp. Isolations of *Leishmania* from *Taterillus*, *Arvicanthis*, *Mastomys* and *Aethomys* are the first recorded from these rodents in Kenya.

Experimental studies with all these isolates have shown a course of infection in mice and hamsters similar to a previously characterized strain of *Leishmania major* from Israel (J. Githure, unpublished observations). Representative isolated from each rodent species have been biochemically characterized using cellulose acetate electrophoresis (KREUTZER & CHRISTENSEN, 1980) and found to be identical with a previously characterized strain of *L. major* (R. Beach, unpublished data). Isolates of *Leishmania* from *Xerus rutilus* (see HEISCH, 1957) and *Tatera robusta* (see HEISCH *et al.*, 1959) from Baringo have also been characterized as *L. major* (see CHANCE *et al.*, 1978). In addition, the vector of West African cutaneous leishmaniasis (*L. major*), *Phlebotomus duboscqi*, is also present in this area (BEACH *et al.*, 1982). Cutaneous leishmaniasis in Baringo probably occurs as a rodent-vector-rodent cycle with no recorded human cases to date, although inapparent benign cases may occur.

Isolation of *L. major* from rodents in Baringo does not rule out the possibility that rodents may also be hosts for *L. donovani* as demonstrated in Sudan (CHANCE *et al.*, 1978). The epidemiology of leishmaniasis in this district needs re-evaluation as a result of these recent discoveries. A search for cases of human cutaneous leishmaniasis and biochemical characterization of all the recent and cryopreserved isolates from visceral leishmaniasis patients in this area is currently in progress.

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THOR'S PROOF
PLEASE RETURN

Leishmania major in Kenya (East Africa): Transmission to a Human by Bite of a Naturally Infected *Phlebotomus duboscqi* Sandfly

SUMMARY

We isolated Leishmania from a *Phlebotomus duboscqi* female captured in Baringo District, Kenya, and from papular lesions that developed at sites where this sandfly had fed on a human. When characterized by cellulose acetate electrophoresis (8 enzymes examined) these isolates proved to be identical to known *Leishmania major* strains from a human and a rodent (*Arvicanthis* sp.) and different from *Leishmania donovani* and *Leishmania adleri* which also occur in Baringo. This is the first case of human cutaneous leishmaniasis caused by *L. major* reported from Kenya.

INTRODUCTION

The sandfly *Phlebotomus duboscqi*, a vector of *Leishmania major* in Senegal (DEDET *et al.*, 1978), breeds in Baringo District Kenya (BEACH *et al.*, 1982), where it presumably transmits *L. major* among various species of rodents, known to be infected with this parasite (CHANCE *et al.*, 1978). Given the opportunity, Kenyan *P. duboscqi* readily bite man (BEACH, unpublished observation) yet there have been no reported cases of human cutaneous leishmaniasis associated with the *L. major* zoonosis in Kenya. This paper presents evidence that *P. duboscqi* does transmit *L. major* in Baringo District and in the process of doing so can infect humans.

The cellulose acetate electrophoresis (CAE) technique for taxonomic identification of *Leishmania* spp. (KREUTZER & CHRISTENSEN, 1980) is a means of identifying *Leishmania* isolates from areas such as Baringo where *L. donovani* and *L. adleri*, as well as *L. major*, occur (MCKINNON & FENDALL, 1955; JAHN & DIESFELD, 1983; BEACH, unpublished observations). Due to the existence of two or more species of Leishmania in one location it is necessary to characterize isolates from humans, animal reservoirs and sandflies before drawing conclusions about transmission of each parasite.

METHODS

Sandfly collection: The infected sandfly was one of 12 *P. duboscqi* females aspirated from an animal burrow on 10 June 1983 in Baringo District, Rift Valley Provine, Kenya (0° 30' N. lat., 36°E. lon.).

Isolation of Leishmania: The sandfly was shaken in 2% detergent saline then transferred to a drop of sterile saline for removal of the midgut. The isolated midgut was moved to a second drop of sterile saline, teased apart and the contents drawn up in a syringe for inoculation into NNN media overlaid with Schneiders Drosophila medium supplemented with 20% (v/v) heat inactivated fetal bovine serum 250 µg/ml gentamicin and 500 µg/ml 5-fluorocytosine (KIMBER *et al*., 1981). Material derived from needle aspiration of the human lesions (HENDRICKS & WRIGHT, 1979) was inoculated into similar cultures. All cultures were examined daily with an inverted microscope.

Electrophoresis: All CAE-associated techniques used in this study have been previously described (KREUTZER & CHRISTENSEN, 1980). At least 4 assays of each enzyme substrate system were made for all the Leishmania isolates examined.

Leishmanial strains: Four reference strains, representing the species of Leishmania known to occur in Baringo District, were used to characterize our unidentified isolates. These are listed in Table 1. The *P. duboscqi*- and human-derived isolates were compared with these 4 reference strains by concurrently electrophoresing extracts of each then staining such preparations for one of 8 different enzymes listed in Table 2. The zymograms produced by this procedure had visually distinct banding patterns for each species in the reference group (Fig. 1). The relationship of an unidentified isolate to one or more of the reference strains was determined by comparison of its banding patterns with those of the reference strains. If the banding patterns for a reference strain and an unidetnfied isolate proved to be similar for all enzymes tested the two were considered to belong to the same species group.

In the present study we were not concerned with L. aethiopica, a human Leishmania restricted to certain highland areas of Kenya (MUTINGA, 1975A; MUTINGA & MONGOLA, 1974), but with those species of Leishmania which occur on the floor of the Rift Valley, where climatic conditons favor neither the vector, P. pedifer, (MUTINGA, 1971) nor the reservoir, rock and tree hyraxes, (MUTINGA, 1975B) of L. aethiopica.

RESULTS

On 10 June 1983 the senor author was bitten 11 times on the hand by a single sandfly later identified as P. duboscqi. The fly was subsequently dissected and found to be infected with promastigotes which were inoculated into culture and propagated for CAE identification. These parasites were assigned Nairobi Leishmania Bank (NLB) number 144.

At each of the sites where the sandfly probed the patient developed pruritic, erythematous papules, 1 to 2 mm in diameter, within several hours of the feeding. These early lesions were typical of those that develop whenever sandflies bite, however unlike normal sandfly bites which fade and disappear in a few days time, these lesions persisted, remaining as small asymptomatic erythematous papules which gradually enlarged over the next month, reaching diameters of 4 to 12 mm, and becoming indurated. Aspirates from 3 of these lesions, taken in August 1983, also yielded promastigotes. These isolates were designated NLB 173, 174 and 175. The patient was begun on heat treatment at this time, soaking his hand in a hot water bath (41°C) for 2 hours daily (NEVA, 1982). At the time this report was written the papules had developed central ulcerations (Fig. 2).

Skin testing was carried out using an L. donovani test antigen. When read at 48 hours the induration at the site of antigen inoculation was 11 mm, a positive reading based on previous skin test results in Baringo District (LEEUWENBURG et al., 1983).

The enzyme banding patterns for NLB 173 and NLB 144 are diagrammed with those of the reference strains NLB 061 (L. donovani), NLB 070 (L. major human), NLB 095 (L. major -rodent) and NLB 005 (L. adleri) as they appeared in the actual zymograms in Fig. 3. For 7 to 8 enzymes tested, the banding patterns for NLB 173 and NLB 144 were the same as the patterns for NLB 070 and NLB 095, the L. major reference strains. The banding patterns characteristic of this group (NLB 173, NLB 144, NLB 070 and NLB 095) were visually different from both NLB 061 and NLB 005. Based on these results NLB 173 and NLB 144 were designated as L. major.

The only instance of polymorphism detected in the L. major isolates (NLB 070, NLB 095, NLB 144 and NLB 173) was in the 6PGDH profile where the NLB 070 band was slightly more anodal than the NLB 095, NLB 144 and NLB 173 bands (Fig. 3). Thus NLB 095, NLB 144 and NLB 173 were identical to each other in all enzyme profiles and identical to NLB 070 in 7 of 8 profiles (88% similar). There were visible differences between NLB 005, NLB 061 and the L. major isolates in each enzyme profile (100% different).

DISCUSSION

L. major has been isolated from rodents in Senegal, Kenya and Ethiopia, from the sandfly P. duboscqi in Senegal, and from humans with cutaneous leishmaniasis in Senegal, Sudan and Ethiopia (CHANCE et al., 1978; DEDET *et al.*, 1978). In this study *Leishmania* isolates from a *P. dubosci* sandfly trapped in Kenya and from cutaneous lesions which developed at sites where this sandfly had bitten a human are also identified as *L. major*.

There are a number of possible explanations for the absence of other confirmed reports of human *L. major* infections in Kenya. Previous cases may have been misdiagnosed. MUTINGA and NGOKA (1970) suggested that reports of cutaneous and mucocutaneous leishmaniasis which occurred in kala-azar areas of Kenya were actually aberrant visceral infections similar to these observed in the Sudan (HOOGSTRAAL & HEYNEMAN, 1969). Baringo District is part of an endic focus of visceral leishmanisis in Kenya (MCKINNON & FENDALL, 1955) and similar conclusions about cutaneous disease which occurs there may have led to a diagnosis of visceral leishmaniasis when, infact, the causative agent was *L. major*. Cases of cutaneous leishmaniasis in Kenya may also have occurred but not been reported. *L. major* lesions normally heal without treatment within 3 to 12 months (MANSON-BAHR & APTED, 1982; FARAH & MALAK, 1971; NADIM & FAGHIH, 1968) and therefore may be ignored by infected individuals. A third explanation for the lack of reported cases involves the large rodent population, living in close proximity with humans, in Baringo. This population may serve as a nearly exclusive blood meal source for *P. duboscqi* due to prolonged contact with the sandfly, which favors rodent burrows as a resting site (DEDET *et al.*, 1979; BEACH, unpublished observations). A reluctance to take blood outside the burrow would restrict the parasites to the rodent population in all but a few cases where some unusual activity resulted in inadvertent contact between infected sandfly and man, such as in the present report. If this assumption is correct then programs which could potentially reduce the rodent population in Baringo should be undertaken with caution. A comparison of the biology of *L. major* in Senegal, where human cases occur, with that in Baringo District may also provide insights into what conditions give rise to transmission of L. major to humans.

MOLYNEUX (1977) cites several examples of transmission of Leishmania by infected sandflies which have difficulty engoring. Various physiological explanations for this altered behavior are reviewed by KILLICK-KENDRICK (1979), who also noted its adaptive advantage for the parasite. In the present case as single P. duboscqi female probed 11 times without taking blood apparently transmitting L. major during each probing episode. Cases of cutaneous leishmaniasis involving mutiple lesions or the occurrence of two or more cases in one household may be due to such repeated probing behavior by one sandfly rather than an attack by several such insects. This efficient transmission strategy is further enhanced by the fact that all P. duboscqi females captured by us take two or more blood meals during each gonotropic cycle, a behavior that allows for transmission during the first ovarian cycle following emergence. The vector of L. donvoani in Kenya, P. martini, takes only one blood meal during each ovarian cycle (BEACH et al., 1983) and therefore can transmit only during the 2nd and subsequent cycles. F_1 adult female P. duboscqi, reared from eggs laid by wild captured females, agressively bite humans as well as hamsters suggesting that this sandfly is an opportunist when seeking blood (BEACH, unpublished data).

Our CAE results support the conclusions of KREUTZER et al. (1983) who concluded that at the species level Leishmania have a high level of isozyme similarity. Given these authors precautions regarding the use of multiple identification systems rather than one or two enzymes only, CAE appears to be a quick and relatively easy method of characterizing Leishmania isolates from areas where multiple Leishmania species exist.

Fig. 1. Photograph of banding patterns in a malic enzyme (ME) zymogram. 1 NLB 095 (L. major -rodent). 2-NLB 173 (human isolates), 3-NLB 144 (sandfly isolate), 4-NLB 070 (L. major -human), 5-NLB 061 (L. donovani), 6-NLB 005 (L. adleri). Bands below the number are located at the origin. The anode is at the top of the figure.

Fig 2. L. major skin lesions (approximately 4.0 -10.0 mm dia.) 60 days post infection by bite of a naturally infected P. duboscqi.

Fig. 3. Diagramatic representation of electrophoretic patterns of 8 enzymes obtained from 6 species of Leishmania. Dotted bands represent lighter zymogram bands. The anode is at the top of each drawing. 1-NLB 095 (L. major -rodent), 2 NLB 173 (human isolate), 3-NLB 144 (sandfly isolate), 4-NLB 070 (L. major -human), 5-NLB 061 (L. donovani), 6-NLB 005 (L. adleri). The horizontal axis in each figure marks the origin.

FIG1

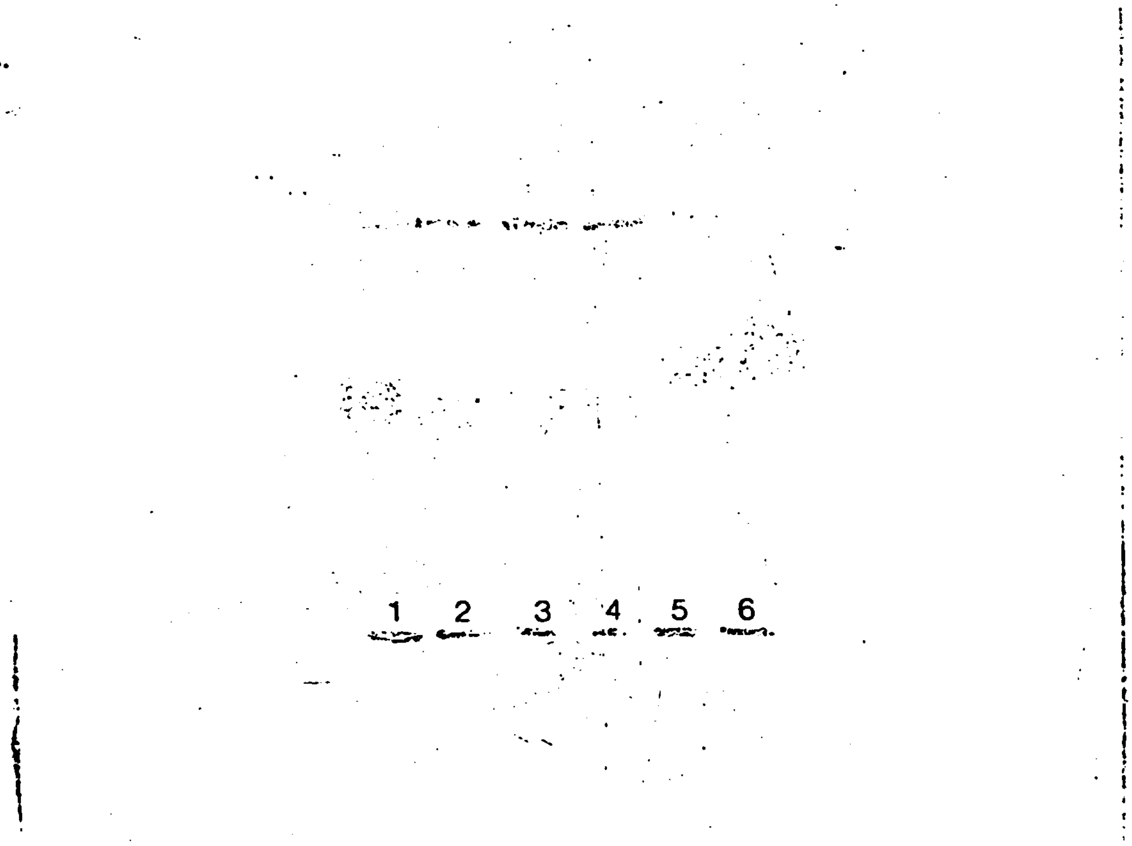
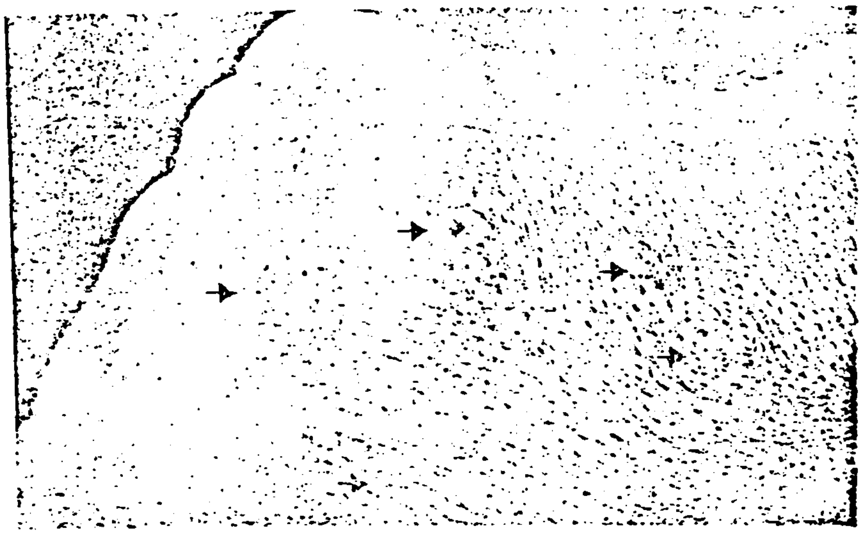
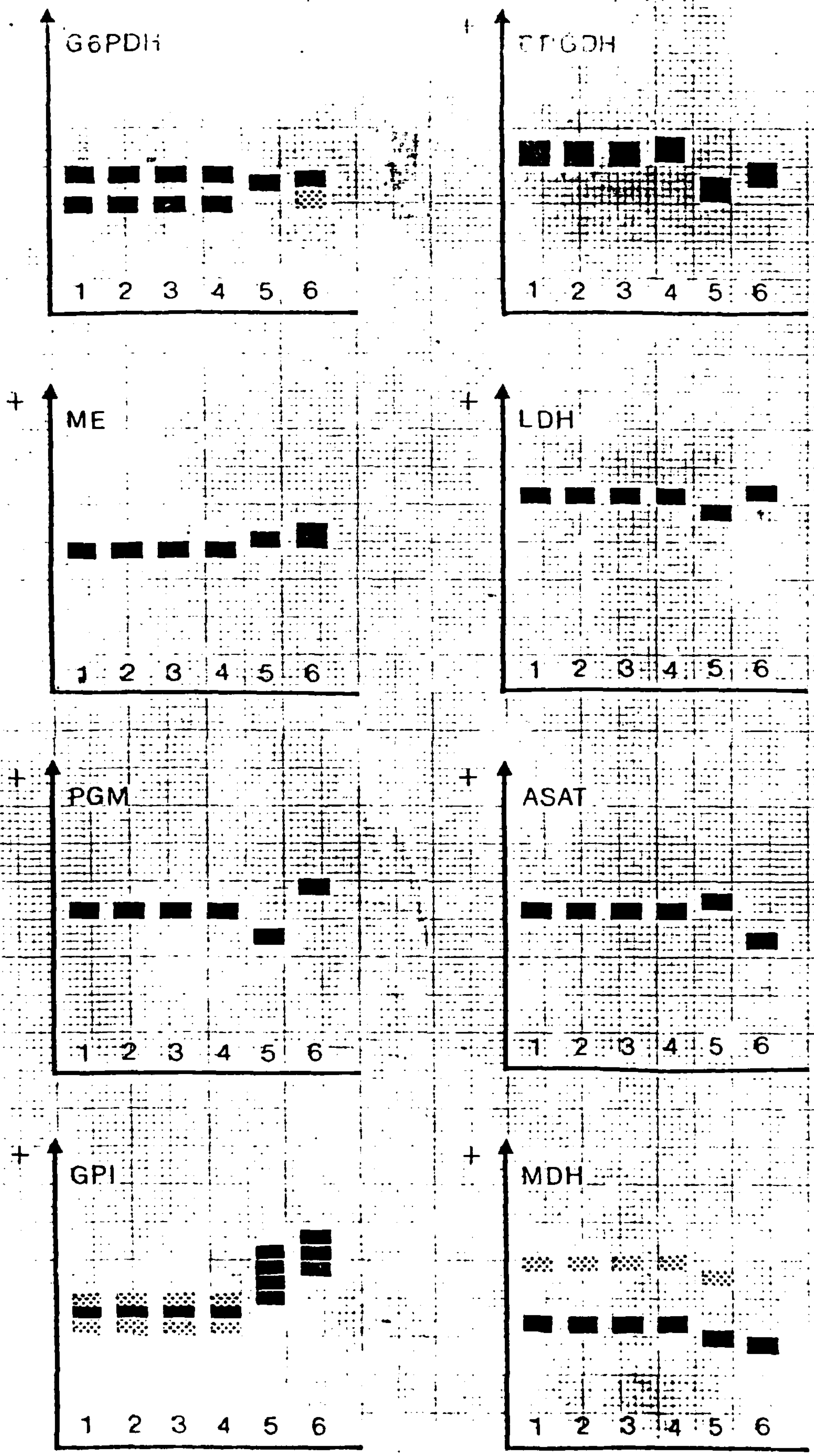


FIG 2 black/white





G6PDH
1 2 3 4 5 6
+
1 2 3 4 5 6
+
ME
1 2 3 4 5 6
+
LDH
1 2 3 4 5 6
+
PGM
1 2 3 4 5 6
+
ASAT
1 2 3 4 5 6
+
GPI
1 2 3 4 5 6
+
MDH
1 2 3 4 5 6

TABLE 1. *LEISHMANIA* REFERENCE STRAINS

NLB NUMBER[1]	ORIGIN	SPECIES	OTHER DESIGNATORS	HOST	REFERENCE
061	Ethiopia	*L. donovani*	LV9 LRC-L133	human	CHANCE *et al.*, 1978
070	Israel	*L. major*	LV561 LRC-L137 Jericho II	human	SCHNUR & ZUCKERMAN, 1977
095	Kenya Baringo District	*L. major*	none	rodent	BEACH, unpublished data
005	Kenya	*L. adleri*	LV34	sand fly	KREUTZER & CHRISTENSEN, 1980

[1] Nairobi Leishmania Bank

C3

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High-dose sodium stibogluconate treatment of cutaneous leishmaniasis in Kenya

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Summary

Cutaneous leishmaniasis caused by *Leishmania aethiopica* usually responds poorly to conventional doses of pentavalent antimonial drugs. We treated three patients with cutaneous leishmaniasis acquired in Kenya, presumed or documented to be caused by *L. aethiopica*, with intravenous sodium stibogluconate, 18 to 20 mg Sb/kg body-weight twice daily for 30 days. All patients had a good response to treatment, with disappearance of parasites from skin smears and cultures after 14 to 27 days, clinical healing of the lesions, and no recurrence during a three to 18-month follow-up. Side effects of treatment were minor. We conclude that this high dose sodium stibogluconate regimen is safe and effective for treating cutaneous leishmaniasis caused by *L. aethiopica* in Kenya.

Introduction

Leishmania aethiopica is a parasite of hyraxes transmitted by the high-altitude sandflies *Phlebotomus longipes* and *P. pedifer* in Ethiopia and Kenya (ASHFORD *et al.*, 1973; MUTINGA, 1975a, 1975b; CHANCE *et al.*, 1978). Zoonotic human infection with this parasite generally causes simple cutaneous leishmaniasis (LEMMA *et al.*, 1969), although diffuse cutaneous leishmaniasis (DCL) develops in a small proportion of infected persons (BRYCESON, 1969). DCL is characterized by multiple nodular skin lesions, absence of delayed hypersensitivity to leishmanial antigens, a chronic course without spontaneous healing, and a generally poor response to treatment (BRYCESON, 1970). Simple cutaneous leishmaniasis caused by *L. aethiopica* generally heals spontaneously within one to five years, but lesions are usually on the face and are often disfiguring (LEMMA *et al.*, 1969). These lesions are generally unresponsive to antimonial drugs (Bryceson & Chulay, unpublished observations) or relapse after initial clinical response (KUNG'U *et al.*, 1972; P. H. Rees, personal communication). Local heat therapy has been tried in Kenya (MUTINGA & MNGOLA, 1974), but this treatment is also associated with relapse (T. K. Siongok, personal communication).

Sodium stibogluconate, the antimonial drug generally used to treat both visceral and cutaneous leishmaniasis in East Africa, has usually been administered at a maximum dose of 10 mg Sb per kg body-weight per day. Higher doses have not been used because of fears of toxicity. However, a careful literature review suggested that the toxicity of pentavalent, as opposed to trivalent, antimonial drugs has been exaggerated (BRYCESON, 1983). We have recently used sodium stibogluconate at doses of 20 to 30 mg Sb/kg/day to treat patients with visceral leishmaniasis and observed minimal toxicity (CHULAY *et al.*, 1983). We have also used higher doses (60 mg Sb/kg/day) to treat relapsed visceral leishmaniasis patients unresponsive to lower doses of sodium stibogluconate, although this dose was often toxic (Bryceson *et al.*, in preparation). We therefore decided to treat patients with Kenyan cutaneous leishmaniasis with sodium stibogluconate at a dose of 20 mg Sb/kg twice daily (40 mg Sb/kg/day). We report here our experience with three such patients.

Methods

Slit skin smears from patients with skin lesions suggestive of cutaneous leishmaniasis were performed as follows: the lesion was wiped with 70% ethanol and allowed to dry. The margin was squeezed between thumb and forefinger until bloodless, incised with a sterile number 23 blade, and the cut edges scraped with the blade. Blood and tissue fluid on the blade were rinsed into Schneider's *Drosophila* medium (HENDRICKS & WRIGHT, 1979) and the saline overlay of a diphasic rabbit blood agar medium (MCCONNELL, 1963). Additional material was used to prepare thin films which were stained and quantitated as described for *L. donovani* (CHULAY & BRYCESON, 1983). Skin biopsies were obtained from two patients. Antileishmanial antibodies were measured by complement fixation (CF) (HOCKMEYER *et al.*, 1983) and ELISA (HO *et al.*, 1983b) using *L. donovani* antigen. Delayed cutaneous hypersensitivity and *in vitro* lymphocyte blastogenesis were measured using *L. donovani* antigens as previously described (HO *et al.*, 1983a). Isoenzyme analysis was performed by the method of KREUTZER & CHRISTENSEN (1980).

Sodium stibogluconate (Pentostam®, Wellcome) was administered by slow intravenous injection (2 to 3 ml per minute) of the undiluted drug (100 mg Sb/ml) through a fine (23 to 26 gauge) needle. During treatment, slit skin smears were repeated, and drug toxicity was sought by measuring haemoglobin, leucocyte and platelet counts, serum alanine aminotransferase, aspartate aminotransferase, bilirubin, albumin, total protein, blood urea nitrogen, and urinalysis weekly, and electrocardiograms (ECGs) biweekly.

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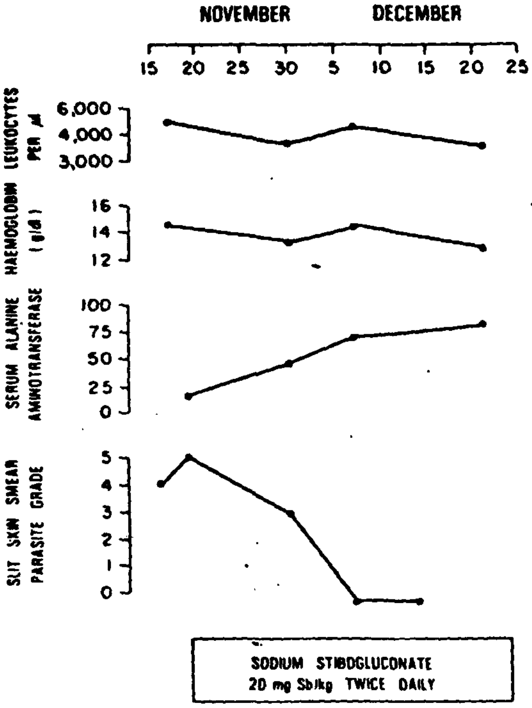

Fig. 1. Response of patient 1 to sodium stibogluconate treatment. Alanine aminotransferase values are in Sigma-Frankel units/ml. Parasite grading scheme: 6+ = >100 parasites (p)/oil immersion field, 5+ = 10–1000 p/field, 4+ = 1–10 p/field, 3+ = 1–10 p/10 fields, 2+ = 1–10 p/100 fields, 1+ = 1–10 p/1000 fields, and 0 = no p/1000 fields (see CHULAY & BRYCESON, 1983).

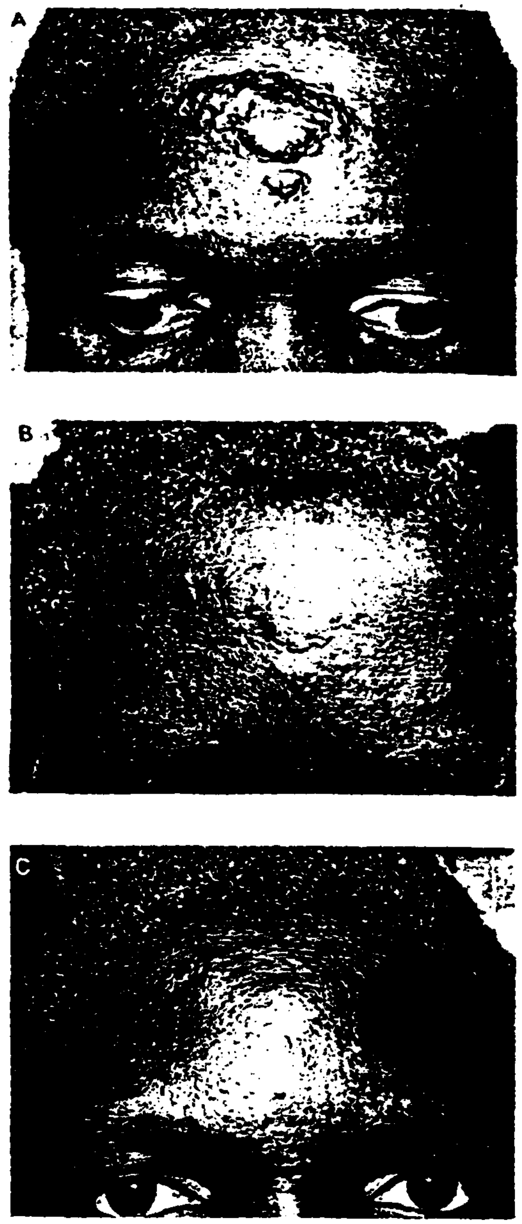

Fig. 2. Lesion on forehead of patient 2. (A) Before treatment. (B) Immediately after treatment. (C) Three months after treatment.

Results

Case 1. A 21-year-old Luhya man from Chebukwabi (1650 m elevation on the slopes of Mt. Elgon) was admitted in November 1981 with a lesion on the end of his nose. It began five months earlier as a papule which increased to become a 3·5 × 2 cm indurated plaque. Skin biopsy showed diffuse infiltration with lymphocytes and foamy macrophages, rare giant cells, and many amastigotes in most macrophages. Slit skin smear and culture were positive. Leishmania skin test was positive (8 mm induration). Leishmanial CF test and ELISA were negative. He was treated with sodium stibogluconate, 20 mg Sb/kg (12·4 ml) twice daily for 30 days. Slit skin smears and cultures became negative by treatment day 14 (Fig. 1), and the lesion improved, becoming noticeably flatter by day 9. Side effects of treatment were thrombosis of superficial veins at the site of drug injections, elevation of liver enzymes (Fig. 1), and decreased QRS and T wave amplitude in frontal and precordial ECG leads with T wave inversion in right precordial leads. Further healing was noted at follow-up examination two months after finishing treatment. The affected skin was completely normal when examined 6 and 18 months after treatment.

Case 2. A 23-year-old Luhya man from Kakilongo (2100 m elevation on the slopes of Mt. Elgon) was admitted in June 1982 with a lesion on his forehead. It began nine months earlier as a pruritic papule which gradually enlarged to become a 5 × 3 cm multinodular lesion with a 1 × 0·7 cm satellite lesion below (Fig. 2A). Slit skin smear and culture were positive. Skin biopsy showed many well-formed granulomata surrounded by cuffs of lymphocytes, occasional giant cells, and few amastigotes.

Leishmanin skin test caused no induration, but his lymphocytes responded to leishmanial antigens *in*

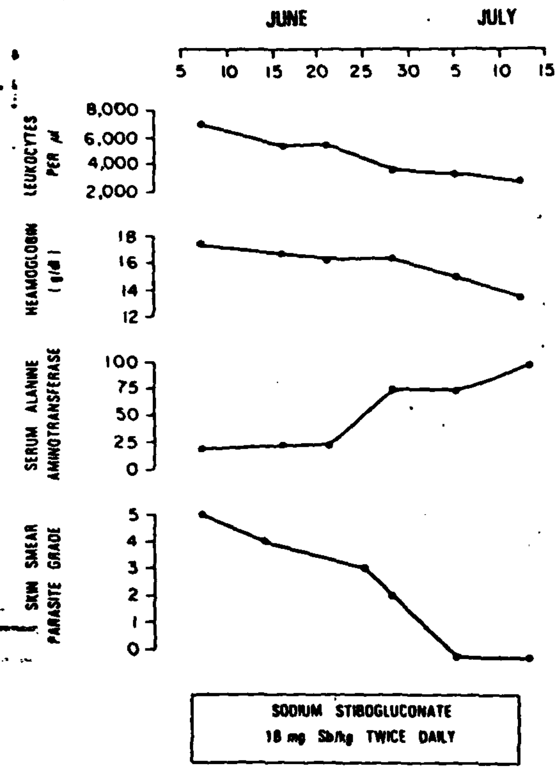

Fig. 3. Response of patient 2 to sodium stibogluconate treatment. Scales as in Figure 1.

Fig. 4. Lesion on nose of patient 3. (A) Before treatment. (B) Immediately after treatment. (C) Two months after treatment. (D) Four months after treatment.

vitro (stimulation index = 2·3). Leishmanial CF test and ELISA were negative. He was treated with intravenous sodium stibogluconate, 20 mg Sb/kg (12·4 ml) twice daily. After receiving six doses he complained of anorexia. The dose was reduced to 18 mg Sb/kg (11 ml) twice daily for the remaining 54 doses, and his appetite returned. Slit skin smears and cultures became negative by treatment day 27 (Fig. 3), and the lesion became flatter and smoother (Fig. 2B). Side effects attributed to treatment included occasional pain proximal to the intravenous injection site, multiple thrombosed superficial veins, elevation of liver enzymes and a fall in haemoglobin and leucocyte counts (Fig. 3), and decreased amplitude of QRS and T waves in frontal and precordial ECG leads. A repeat leishmanin skin test at the end of treatment was positive (7 mm induration). When seen in follow-up in October 1982 healing was complete (Fig. 2C).

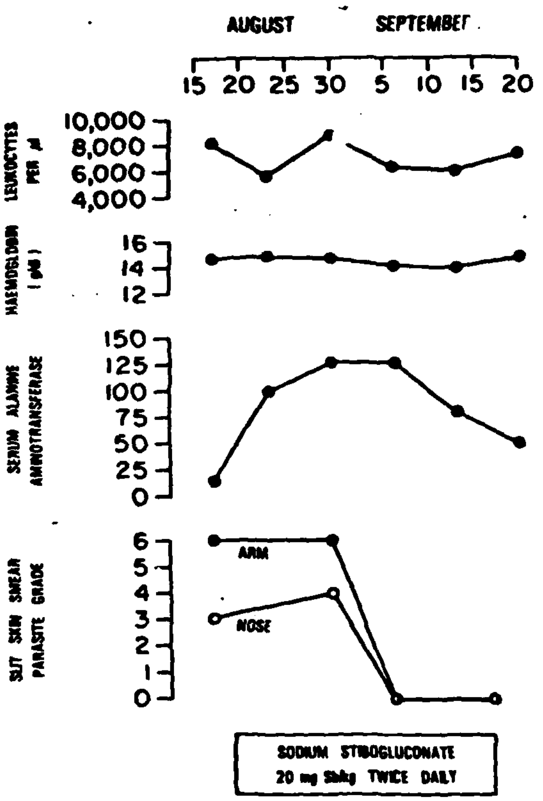

Fig. 5. Response of patient 3 to sodium stibogluconate treatment. Scales as in Figure 1.

Case 3. An 18-year-old Kikuyu man was admitted in August 1982 with lesions on his nose, arm, and neck. They began seven months earlier as pruritic papules which gradually enlarged with marked crusting. He lived near Wanjohi, on the eastern edge of the Rift Valley, at 2375 m elevation. 200 m from his house was a cliff face with caves where he had seen hyrax. He had never visited Mt. Elgon. The largest lesion was a 5 × 2·5 cm elevated, crusted ulcer on the bridge of his nose (Fig. 4A). He also had a 4 × 2·5 cm crusted lesion with a nodular edge and multiple satellite papules on his left upper arm, and a similar 3 × 2·5 cm lesion with satellite papules behind the left ear. Slit skin smears and cultures from the nose and arm lesions were positive. The isoenzyme pattern of cultured parasites was identical with a marker *L. aethiopica* strain. A leishmanin skin test was positive (12 mm induration), but his lymphocytes did not respond to leishmanial antigens *in vitro* (stimulation index = 1·0). Leishmanial CF titre was 1:5 but ELISA was negative. He was treated with intravenous sodium stibogluconate, 20 mg Sb/kg (10·6 ml) twice daily for 30 days. Slit skin smears and cultures became negative by treatment day 19 (Fig. 5), and the lesions improved slowly during treatment (Fig. 4B). Side effects of treatment were thrombosis of superficial veins at injection sites, elevation of liver enzymes (Fig. 5), and T wave inversion in frontal and left precordial ECG leads. Continued healing of lesions was noted at follow-up examination two and four months after finishing treatment (Figs. 4C & D).

Discussion

All three patients had chronic indurated facial lesions which were cosmetically disfiguring. In view of the long time required for spontaneous healing, and the marked scarring which often results, treatment was indicated. Previous experience suggested that usual doses of sodium stibogluconate would not be effective, and that higher doses could be given safely.

Parasitological response to treatment was rapid in all three patients; smears and cultures were negative within 14 to 27 days. Clinical response was slower. The nodular and indurated lesions were all improved at the completion of treatment, but complete healing took many months. The total time from initial onset to complete healing (11, 13 and 15 months) was shorter than the usual duration of lesions in Kenya (MUTINGA & MNGOLA, 1974).

Drug toxicity was not a major problem. The dosage had to be reduced slightly for one patient because of anorexia. The slight decrease in haemoglobin and leucocyte counts in one patient were of no clinical importance. Mild transiently increased liver enzymes and ECG changes occurred in all patients. Local venous thrombosis at the site of intravenous injections was common but did not prevent intravenous administration of all doses. It is possible that diluting the drug in 50 ml dextrose or saline before injections, as done by OSTER *et al.* (1983), might reduce the local venous irritation, but facilities for sterile preparation of such dilutions were not readily available.

Although promastigotes grew in cultures from all three patients, only one proliferated sufficiently in subculture for isoenzyme analysis. The clinical and epidemiological features of the other two cases indicates they were in all probability also caused by *L. aethiopica*.

Failure of leishmaniasis to respond to pentavalent antimonials is often dose-dependent. Our success in treating some visceral leishmaniasis patients with higher doses of sodium stibogluconate after relapse or unresponsiveness to lower doses prompted the evaluation of higher doses to treat cutaneous leishmaniasis. The results presented here indicate that cutaneous leishmaniasis in Kenya can be treated safely and effectively with sodium stibogluconate at a dose of 18 to 20 mg Sb/kg twice daily for 30 days. We suggest that similar therapy should be evaluated for treating diffuse cutaneous leishmaniasis caused by *L. aethiopica*.

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Addendum: Patient 2 returned for further follow-up evaluation in July 1983, at which time he had recurrent leishmaniasis. It was unclear whether this was a relapse or reinfection. In November 1982 he killed an insect feeding on his forehead and two weeks later a papule developed at this site and gradually increased in size. By July 1983 he had multiple nodular lesions, many of which had coalesced to form a 3 × 4 cm indurated mass involving the lower two thirds of the site of his previous infection and extending inferiorly. There were also multiple 1 mm satellite papules. The upper third of the previous lesion consisted of slightly elevated scar tissue. Slit skin smears contained many amastigotes (grade 5+). Leishmanin skin test was positive (7 mm induration). He is currently receiving local heat treatment, and after six weeks there has been a gradual reduction in the density of parasites in slit skin smears and gradual clinical improvement.

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Paper No. 58/83

Localization of *Leishmania donovani* in Experimentally Infected Sandflies: An Indicator of Vectoral Competence

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Introduction

Only three of the more than forty sandfly species occurring in Kenya are thought to transmit *Leishmania donovani* (Abonnenc 1972). These are *Phlebotomus martini, Phlebotomus celiae* and *Phlebotomus vansomerenae.* The vector competence of these species reflects their anthropophilic biting behaviour and their ability to sustain *L. donovani* in the fore-gut of the alimentary system, from where the promastigote forms of the parasite are probably transmitted during blood feeding (Adler and Theodore 1957). The present study compares the fate of *L. donovani* in *P. martini* and *Sergentomyia schwetzi*, a non-vector sandfly. At regular intervals, following infection, both species were dissected to determine if and when promastigotes moved from the mid-gut to the head of the insect (Figure 1). Prior to dissection flies were also allowed to feed on hamsters, thereby correlating parasite localization in the alimentary system with transmission of *L. donovani*. The results suggest that anterior migration is a prerequisite for transmission of *L. donovani* and that the physiological conditions which promote such transmission-favouring movement do not occur in the non-vector species.

Materials and methods

Our experiments used a *Leishmania* isolate obtained from an 8-year-old kala azar patient from Rift Valley Province, Kenya. The parasite was passaged once in hamsters, re-isolated, mass cultured in Schneider's *Drosophila* medium supplemented with foetal bovine serum (Hendricks *et al.* 1978; Childs *et al.* 1978), a culture system used throughout this study, and cryopreserved. Aliquots of the cryopreserved stock were grown up as needed for use in this experiment.

Preliminary biochemical characterization of this isolate by cellulose acetate electrophoresis (Kreutzer and Christensen 1980) indicates that it is *L. donovani*. The enzyme profile of this isolate, derived from zymograms for 5 enzymes (glucosephosphate isomerase, glucose-6-phosphate dehydrogenase, malic enzyme, phosphoglucomutase and aspartate-amino transferase) is the same as that of an already characterized *L. donovani* and different from that of known *L. major* and *L. adleri* isolates, two other *Leishmania* which occur in the Rift Valley.

This study utilized 5th and 6th generation, laboratory-bred (Beach *et al.* 1983) *P. martini* females. Our colony was established with flies captured in Rift Valley Province, Kenya. *Sergentomyia schwetzi*, a sandfly also found in Kenya's kala azar areas, is likewise maintained in our laboratory. There is no evidence that *S. schwetzi* is involved in the transmission of visceral leishmaniasis and it was used in this

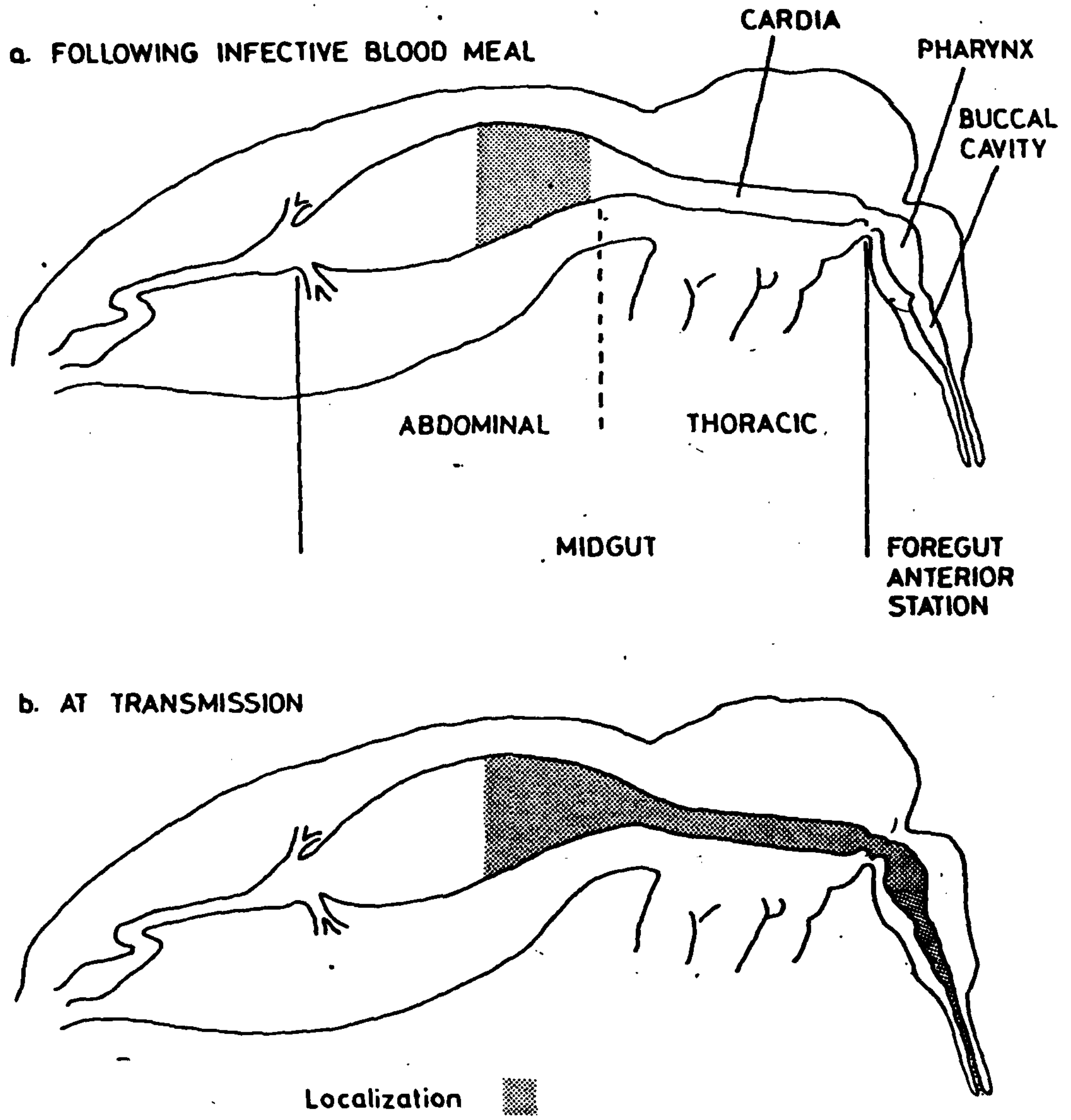

Figure 1. Leishmania in sandflies

study as a non-vector comparison species. Our colony originated from material collected in Rift Valley Province, Kenya.

Two problems associated with experimental transmission of *Leishmania donovani* from fly to hamster determined the methodology used in this study. First, we have been unable to infect sandflies by feeding them on the skin of *L. donovani*-infected hamsters. However, an alternative means of delivering parasites to the fly's mid-gut, membrane feeding, is acceptable, in varying degrees, to our sandflies. A second problem involves oviposition death, a term referring to the fact that under laboratory conditions almost all female sandflies die either during or shortly after egg laying. This, of course, precludes the taking of a 2nd 'transmission' blood meal unless multiple blood meals are taken during each gonotrophic cycle, which is not the case for either of the sandflies used in this study. Feeding flies on promastigotes in culture media circumvents oviposition-associated problems in transmission studies, since all females fed in this manner, at least initially, develop heavy midgut infections, do not mature eggs, and continue to display normal biting behaviour in the presence of a suitable host, thus becoming available for a potential transmission blood meal.

Results

Our results were obtained using the experimental design outlined in Figure 2, which was repeated several times, using sandflies which

Figure 2. Design

Day 0	3-day-old flies infected* Held at 26°C, 95% RH, sugar source — apple
Day 4 or Day 8	*Fly-hamster transmission***
Day 64 or Day 68	Hamster sacrificed, spleen cultured

*Membrane feeding on 10^6 promastigotes/ml
**5–20 flies feeding/hamster

emerged on the same day and were therefore of similar age. In all, a total of 223 *P. martini* and 248 *S. schwetzi* were fed by the method described.

Flies were dissected (Minter and Wijers 1962) at intervals during the first 8 days of the experiment to determine if they sustained their infections and also to see if the parasites moved from the mid-gut, where they were initially deposited, to anterior station sites in the head of the sandfly. Table 1 quantifies the results of these dissections. Note that while the infection rate in *P. martini*, the vector species, remained at 100% on days 4 and 8, it decreased to less than 50% in the non-vector, *S. schwetzi*, during this period. Also, 60% of the *P. martini* dissected on day 8 had fore-gut infections, whereas none of the *S. schwetzi* examined at this time had parasites in the anterior station.

The results of the transmission experiments are presented in Table 2 along with the data on per-cent foregut infections from Table 1 for comparison. On day 4, *P. martini* had no fore-gut infections and none of the flies were able to infect hamsters at this time. By day 8, 60% of the *P. martini* had fore-gut infections and 4 out of 10 hamsters bitten by such flies were found to be positive for *L. donovani*. No fore-gut infections were observed in *S. schwetzi* and no transmission of *L. donovani* to hamsters was detected.

Discussion

The gradual disappearance of parasites from the alimentary system of *S. schwetzi* and the

Table 1. L. donovani in colonized sandflies: Infection rate and localization

Day	Flies dissected	% Positive	% Mid-gut infections		% Fore-gut infections	
			Abdominal	Thoracic	Pharynx	Buccal cavity
P. martini						
0	10	100	100	0	0	0
4	94	100	100	100	0	0
8	90	100	20	100	60	21
S. schwetzi						
0	10	100	100	0	0	0
4	93	40	30	20	0	0
8	96	30	30	10	0	0

Table 2. L. donovani in colonized sandflies: experimental transmission

Day 4		Day 8	
% Fore-gut infections	% Hamsters* infected	%Fore-gut infections	% Hamsters* infected
P. martini			
0	0	60	40
S. schwetzi			
0	0	0	0

*N=10

apparent inhospitability of the fore-gut for *L. donovani* suggest that conditions in the alimentary system of this sandfly discourage development and transmission of *L. donovani*. If so, the adverse factor(s) involved in the elimination of the parasite are absent in *P. martini*, whose gut condition may favour anterior development as well. A similar pattern of resistance to infection with *L. donovani* was recorded in the Sudan when a known vector was compared with a non-vector sandfly species (Heyneman 1963). Do such observations imply that the alimentary systems of most Kenyan sandflies are unsuitable for parasite development? The results of *Leishmania*-infection studies using other Old World sandflies, summarized by Killick-Kendrick (1979), support this generalization: that the *Leishmania* parasites of the Old World develop well only in the species of sandfly by which they are transmitted in nature. In this regard, parasite-localization studies, carried out in *P. celiae* and *P. vansomerenae*, might help to strengthen the assumption that they too serve as vectors of kala azar in Kenya (Wijers and Minter 1962).

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MOSQUITO SPECIES SUCCESSION IN A DAMBO IN AN EAST AFRICAN FOREST[1, 2]

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ABSTRACT. The mosquito larval and pupal fauna of a dambo in a primary forest in Nairobi Area, Kenya was monitored during the short rainy season. The density of the immature stages of 6 species was recorded daily for a 3 month period. *Aedes cumminsii mediopunctatus, Ae. lineatopennis* and *Ae. sudanensis* were the first 3 species collected following flooding. *Culex quasiguiarti, Anopheles coustani* and *Cx. theileri* were collected beginning 15, 17 and 33 days respectively following flooding. Each of the 3 *Aedes* spp. disappeared after one generation. No immature mosquitoes were recovered after day 48.

INTRODUCTION

The mosquito fauna associated with the temporary and infrequent flooding of dambos (vleis) (Ackermann 1936) is being studied in Kenya in areas where Rift Valley fever (RVF) has occurred. Between outbreaks, RVF virus is believed to be maintained (Shope et al. 1982) in forests or secondarily derived grasslands of comparatively high rainfall and humidity. These zones generally lie between the 15°C and 20°C isotherm (ecological zone II, Pratt et al. 1966). During this investigation we studied the mosquito fauna found in a dambo in a forest of the type commonly occurring in ecological zone II.

According to Mackel (1974), dambos are shallow streamless depressions at the headwaters of drainage systems in eastern and southern Africa. They are seasonally waterlogged and grass covered to varying extents, and without a true woodland vegetation. The surrounding woodland or scrub woodland usually stops abruptly at the dambo margin. The existence and form of a dambo is dependent upon relative relief of the surrounding terrain, seasonality of climate, vegetation, soil types and water. They are varied in shape and extend from a few meters to over several kilometers in length and up to several hundred meters in width. Despite their different shapes, dambos show a similar zonation from the margin toward the centers. A typical dambo cross profile is illustrated in Fig. 1. The washbelt slopes downward from the woodland and is characterized by the presence of surface pebbles. The central part is marked by a network of seepage lines forming the seepage zone. Flooding of the seepage zone, producing surface water, may only occur after extended periods of continuous heavy rainfall. Between the washbelt and the seepage zone there is a transitional zone marked by the decreasing size of the surface pebbles and increasing moisture content. The washbelt is composed of discontinuous grassland, and toward the center the vegetation becomes

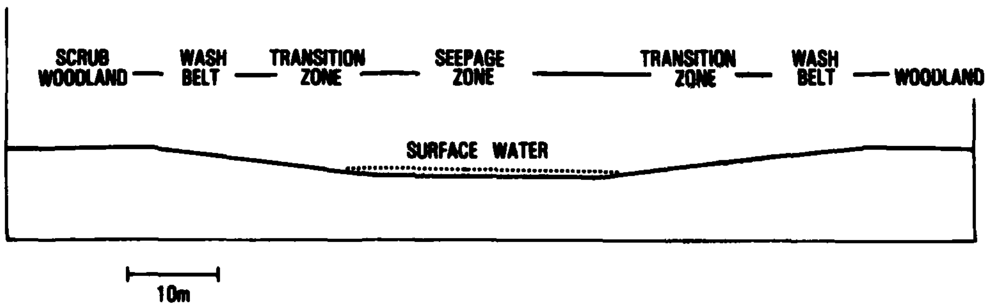

Fig. 1. Cross profile of long axis of the Karura Forest dambo.

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denser with an increasing number of erect rhizomatous herbs. The seepage zone contains both a moderately dense herb and tufted grass cover. Seasonal changes affect the vegetation of the dambos, and by the end of the rainy season the area of bare ground is reduced by the expansion of the vegetation.

Dambos are ideal habitats for mosquito larvae as they exist in geographical areas marked by distinct wet and dry seasons and are subject to seasonal flooding. Our 3 month study describes the daily relative density of immature stages of six species of mosquitoes in a dambo in a primary forest in Nairobi Area, Kenya during the short rainy season (October 1 to December 31, 1982). Emphasis was placed on studying species succession of developmental stages and relative population levels.

MATERIALS AND METHODS

The dambo monitored in this study (Fig. 2) is located at approximately 1° 14′ 30″ S. latitude and 36° 50′ E. longitude in the central part of the Karura Forest, Nairobi Area, Kenya. The Karura Forest is one of several small relict forest stands distributed along the foothills of the eastern edge of the Kikuyu Escarpment, between Nairobi and Thika, at an elevation of 1700 m. The dambo, situated in a forest clearing, is roughly elliptical in shape, measuring approximately 50 × 70 m. The dambo exhibits the typical zonal differentiation common to most dambos (Fig. 1). Prior to the rainy season (October 1, 1982), the washbelt varied from 15–20 m wide with approximately 50% of its area remaining bare of vegetation; the transitional zone varied from 5–10 wide and was approximately 10–20% bare ground. After the rainy season (December 31, 1982), bare ground represented only approximately 35% of the washbelt and 5% of the transitional zone. The seepage zone, about 20 m in diameter, was 5% bare prior to the rains and 1% after the rains. When the seepage zone became flooded, approximately 75% of the surface area of the standing water had extensive emergent vegetation. The emergent vegetation in the central area was primarily the rhizomatous herbs *Typha domingensis* (Persoon) and *Typha latifolia* (Linnaeus) while in the marginal areas along the transitional zone the short tufted grass *Eragrostis exasperata* (Peter) predominated. The entire surface of the dambo remained in direct sunlight for approximately 10 hours each day and the temperature of the water, as measured at 1100 hr each morning varied between 18°–30°C.

Fig. 2. The Karura Forest dambo, Nairobi Area, Kenya on November 10, 1982.

The rains in the Nairobi Area generally fall during 2 distinct periods, known locally as the long and short rains. The long rains usually occur from March to July and the short rains usually from October to December. The total rainfall is usually greater during the long rains; however, the short rains monitored in this study were greater in terms of total mm of rainfall than many previous long rains, including those of 1982. Daily rainfall data was recorded from October 1, 1982 to December 31, 1982 at the Karura Forest Station located 0.8 km SE of the study site. The degree of flooding (water level) in the dambo was recorded daily by measuring the water depth in mm at the lowest point in the seepage zone (the first area to flood). The flooding of dambo formations as described here is a relatively infrequent occurrence and may not occur over periods of several years, based upon local observations. The dambo did not flood during the previous long rain season.

Immature stages of mosquitoes were sampled with a pint (0.47 liter) dipper daily from October 19, 1982 (the first day of flooding) to December 31, 1982. Each day 100 dip samples were collected and transported to the laboratory. Samples were collected along 4 lines transecting the dambo by dipping equally in each of 3 predetermined zones to reduce the possible effects of non-random horizontal distribution of specimens. The zones (A,B,C) as shown in Fig. 3 were roughly defined as 3 concentric ellipses and were primarily distinguished by the type and amount of emergent vegetation predominating. Zone A contained abundant emergent *Typha domingensis* and *T. latifolia*, zone B contained no emergent vegetation and zone C contained abundant emergent *Eragrostis exasperata*. In the laboratory the larvae were separated by stage, counted and, when possible, reared to adults. The pupae were also counted and reared to adults. Subsamples of associated larval and pupal skins, whole larvae and adult male genitalia were mounted in Euparal® (G.B.I. Laboratories, England) and examined with a compound microscope to identify species using reference keys (Edwards 1941, Gillies and de Meillon 1968, Hopkins 1952, Mattingly 1971, Tyson 1970). Subsamples of associated reared males and females were mounted on paper points and examined with a dissecting microscope to confirm species identification.

RESULTS

The daily rainfall at and water level in the Karura dambo are illustrated in Fig. 4. During the 29 rainy days that occurred during the study period, 652.4 mm of rain fell on the Karura dambo. The first water in the dambo was observed on October 19 when the level was 55 mm. Between October 24 and December 3, the level gradually increased and the average depth during this interval was 410 mm. From December 4 to 7 the level increased to a maximum of 690 mm and then declined from December 19 to 31 to 150 mm. The water was cloudy and brown from the first to the 46th day following flooding (day 46), but from day 47 to the end of the study period the water color changed to a red to reddish-brown.

Between October 20 (day 1) and December 5 (day 47), 20,926 larvae and pupae were collected, separated according to developmental stage and identified. Figure 5 shows the daily total of immatures collected for each species during the study period. The mosquito species identified included: *Aedes (Aedimorphus) cumminsii mediopunctatus* (Theobald) (36%), *Ae. (Mucidus) sudanensis* (Theobald) (4%), *Ae. (Neomelaniconion) lineatopennis* (Ludlow) (22%), *Culex (Culex) quasiguiarti* (Theobald) (18%), *Anopheles (Anopheles) coustani* (Laveran) (3%) and *Cx. (Cux.) theileri* (Theobald) (17%). *Aedes cumminsii mediopunctatus* was first collected on

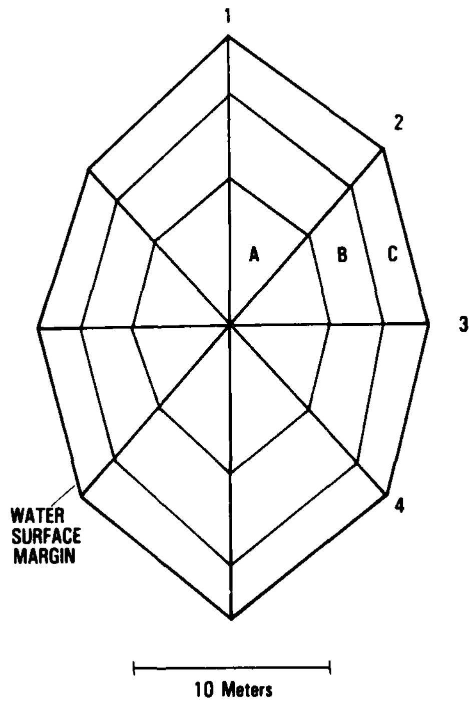

Fig. 3. Diagramatic overhead view of Karura dambo standing water demonstrating transecting sampling lines (1–4) and concentric collection zones (A,B,C).

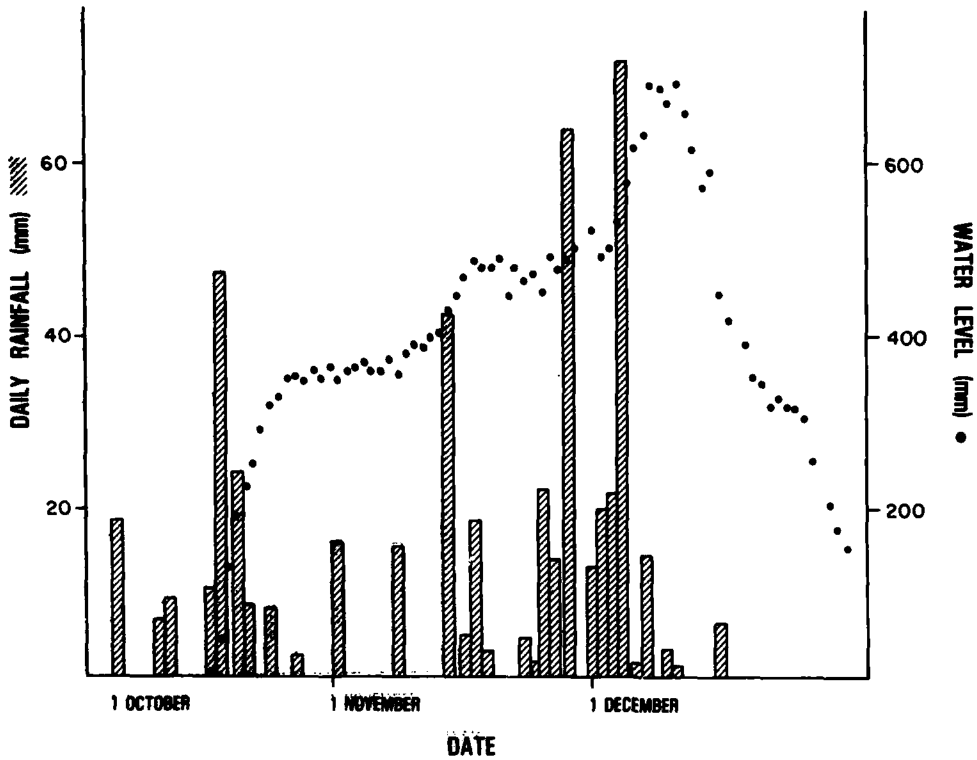

Fig. 4. Daily rainfall in mm (histogram) and daily flooding level in mm (dots) for Karura dambo.

day one and last collected on day 15 with greatest numbers occurring on days 4–9. *Aedes sudanensis* was collected on day 2 and persisted at a low level to day 29 with its largest collection (day 3) only 12% of that of *Ae. cumminsii mediopunctatus*. *Aedes lineatopennis* was first collected on day 9 and last collected on day 21 with peak populations on days 12 and 13. After numbers of the 3 species of *Aedes* dropped to zero they were not collected again during the study period. On day 15 the first *Cx. quasiguiarti* larvae were collected and its density remained fairly constant to day 30 whereafter numbers peaked on days 38, 42 and 46. *Anopheles coustani* larvae were collected on days 17 and 22, and starting again on day 30, its numbers remaining low to day 47. *Culex theileri* was not collected until day 33 but numbers increased rapidly to a maximum on day 47. On day 48 and for the next 26 days of the study no mosquitoes were collected from the dambo. Populations of *Cx. quasiguiarti, Cx. theileri* and *An. coustani* abruptly dropped to zero. Figure 6 illustrates, for each of the 6 species, the mean number of individuals of each developmental stage collected per dip on each collection day following flooding of the dambo. Table 1 lists the number of specimens of each stage collected and the percentage of the total number collected for each species.

DISCUSSION

The rainfall during the study period nearly equalled the previous 10 year mean yearly rainfall for the study area. The distribution and amount of rainfall prevented the dambo water level from dropping significantly during the study. The heavy rains of late November and early December may have caused excessive deposition of red laterite soil into the dambo and the resultant mosquito die off on day 48. The specimens of *Ae. cumminsii mediopunctatus, Ae. lineatopennis* and *Ae. sudanensis* collected were the result of a single, progressive and generalized flooding of eggs in the soil of the dambo. There is no indication that water recession and reflooding significantly contributed to population levels in any of the *Aedes* spp. Maximum numbers of *Aedes* first stage larvae were reached 1–3 days after hatching and declined rapidly thereafter. There was no recurrence of *Aedes* immatures following their brief population in-

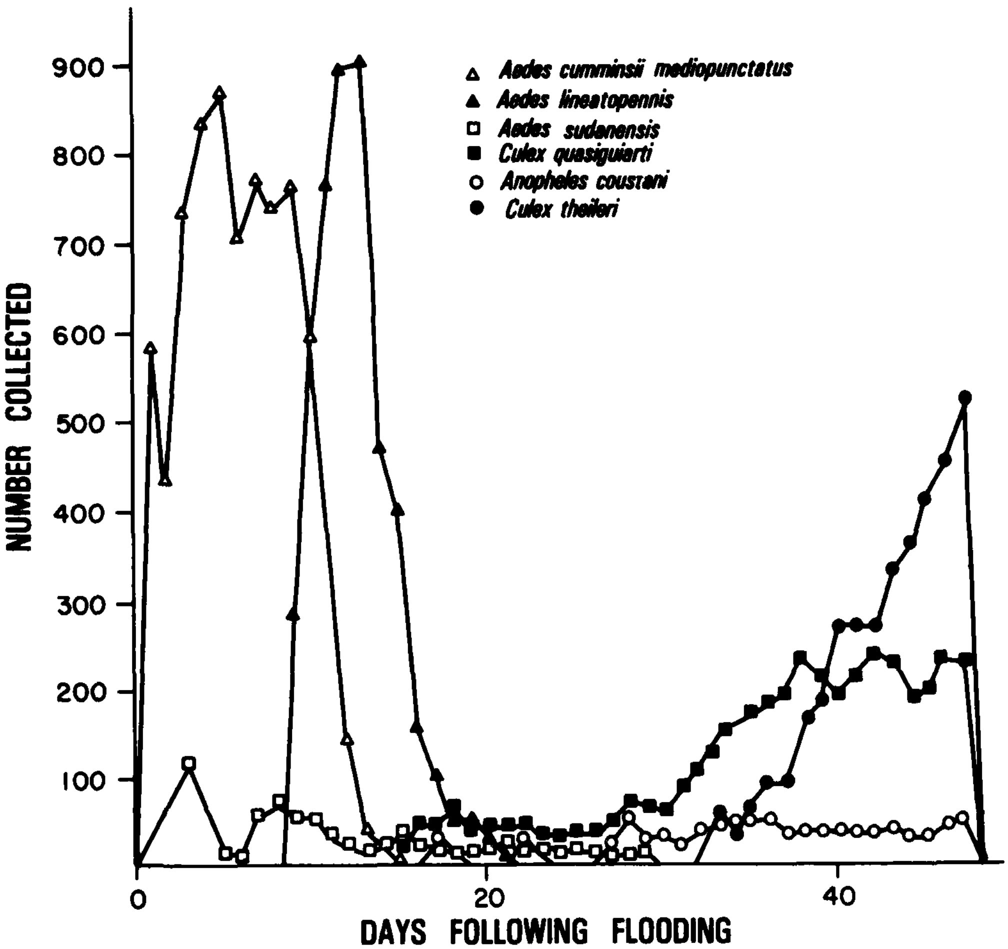

Fig. 5. Daily total of specimens for each species collected from Karura dambo.

crease and decline; each of these species disappeared after one generation. The occurrence of *Ae. lineatopennis* 8 days following *Ae. cumminsii mediopunctatus* may indicate that either the eggs of the 2 species required different hatching stimuli or that they were located in different areas of the dambo and were flooded at different times as the water level rose. Figure 4 shows that the water level had risen to a plateau coinciding with the appearance of *Ae. lineatopennis* on day 9. This suggests that the eggs of *Aedes lineatopennis* were located away

Table 1. The number of specimens of each stage collected and the percentage of the total number collected for each species.

	Larval stage					
Species	1st	2nd	3rd	4th	Pupae	Total
Ae. cumminsii	2489(33%)	2308(30%)	1560(21%)	817(11%)	407 (5%)	7581
Ae. sudanensis	316(43%)	148(20%)	107(14%)	96(13%)	72(10%)	739
Ae. lineatopennis	2003(43%)	1070(23%)	696(15%)	551(12%)	341 (7%)	4661
Cx. quasiguiarti	1613(43%)	1046(28%)	643(17%)	286 (8%)	124 (4%)	3712
An. coustani	186(25%)		525(72%)		42 (3%)	753
Cx. theileri	1730(49%)	723(21%)	575(16%)	379(11%)	91 (3%)	3498

from the center of the dambo in higher areas which flooded later. *Aedes sudanensis* required approximately twice the developmental period of either *Ae. cumminsii mediopunctatus* or *Ae. lineatopennis.* This is indicative of a long developmental cycle common to some predacious species. *Aedes sudanensis* was observed to be predacious in the field and it had to be provided with mosquito larvae in the laboratory as a source of nutriment. The development from first stage larva to pupa, as interpreted from Fig. 6, required 5–8 days for *Ae. lineatopennis* and 18–19 days for *Ae. sudanensis.* The occurrence of *Cx. quasiguiarti, An. coustani* and *Cx. theileri* 15, 17 and 33 days, respectively, following flooding and the general increase in population levels of all stages to day 47 indicate that these 3 species had successfully developed to adults and were producing multiple generations. Presumably, the absence of all mosquito immatures from day 48 followed some abrupt change in the aquatic environment of the dambo. Other aquatic ar-

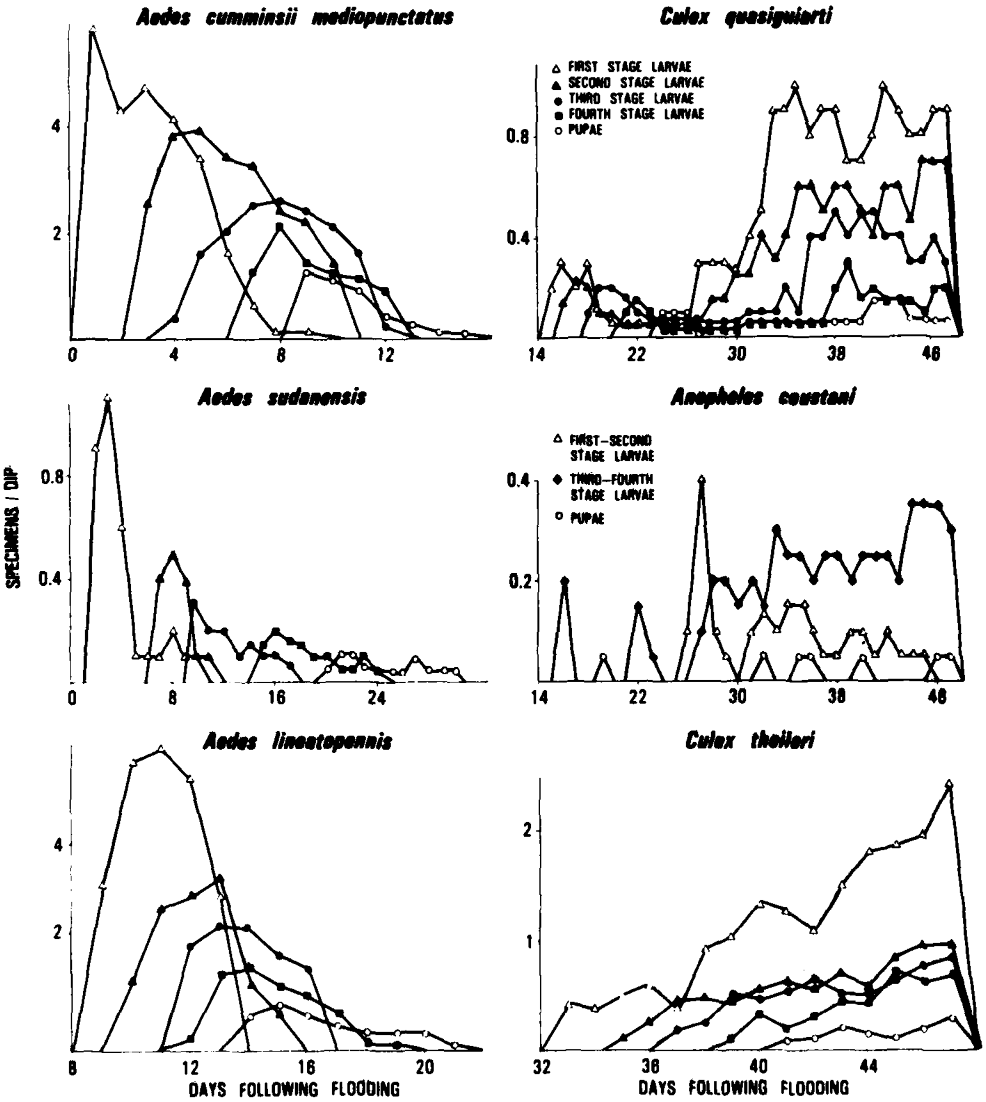

Fig. 6. Mean number of specimens collected per dip in Karura dambo for the developmental stages of *Aedes cumminsii mediopunctatus, Aedes sudanensis, Aedes lineatopennis, Culex quasiguiarti, Anopheles coustani* and *Culex theileri.*

thropods were also absent in collections after day 47. The appearance of first stage larvae of aedine species soon after flooding, compared with a delay of 15–33 days until the appearance of the *Culex* and *Anopheles* larvae and the occurrence of only one generation of aedine mosquitoes, are important observations with respect to determining field sampling strategies for mosquitoes during RVF investigations.

Rift Valley fever virus has been previously isolated from 3 of the 6 species encountered in the dambo. *Aedes lineatopennis* and *An. coustani* have been incriminated by McIntosh (1972) in Zimbabwe, and by Davies and Highton (1980) in Kenya. Gear et al. (1955) and McIntosh (1972) incriminated *Cx. theileri* in South Africa. McIntosh et al. (1980) have incriminated both *Cx. theileri* and *Ae. lineatopennis* in South Africa. The developmental cycles, the influence of environmental factors upon them, and particularly the conditions necessary to produce high population densities require further study to evaluate the role of these species in the transmission of RVF. Davies (1975) states that "the most interesting problem relating to RVF in Kenya and elsewhere has been to define the interepizootic maintenance cycle." This remains a fascinating problem in the natural history of RVF.

ACKNOWLEDGMENTS

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Mosquito Species Encountered in a Flooded Grassland Dambo in Kenya

ABSTRACT

The larvae and pupae of mosquitoes found in a flooded dambo on a bushed grassland in Central Province, Kenya were monitored during the short rainy season. The densities of the immature stages of 8 species were recorded daily for a one month period. *Aedes cumminsii mediopunctatus*, *Ae. lineatopennis* and *Ae. sudanensis* were collected for 8, 9 and 18 days respectively and each disappeared after one generation. *Aedes lineatopennis* and the *Culex* spp. specimens were collected in much greater densities than in a forest dambo studied previously.

INTRODUCTION

Davies (1975) states that Rift Valley fever (RVF) virus is believed to be maintained in Kenya in forests or secondarily derived and natural grasslands of comparatively high rainfall and humidity (ecological zones II, III, Pratt et al. 1966). In these areas the mosquito fauna associated with temporary pools in dambos (Ackermann 1936) is being studied. Observations concerning the immature mosquito fauna of a dambo in a forest of the type commonly occurring in ecological zone II have been reported previously (Linthicum et al. 1983). This study follows the succession of mosquito species found in a grassland dambo in ecological zone III in Kenya.

MATERIALS AND METHODS

The dambo monitored in this study (Fig. 1) is located along the Kamiti River 7 km SE of Ruiru, Thika District, Central Province, Kenya (1°12'S, 37°E) at an elevation of 1500 m. This area is principally comprised of bushed grasslands. The dambo is sinuous in shape, measuring approximately 75 x 200 m. It exhibited the zonal differentiation described by Mackel (1974) and Linthicum et al. (1983) with the exception that the washbelt area on one side was much reduced by the presence of the river. The seepage zone was extensive and extended 20-30 m on either side of the longitudinal axis of the dambo. The emergent vegetation in the central area was primarily the sedge <u>Cyperus immensus</u> B. Clarke. The grass <u>Digitaria abyssinica</u> (A. Richard) Stapf predominated in the remainder of the dambo. The entire surface of the dambo remained in direct sunlight for approximately 12 hrs each day and tbe water temperature at 1430 hr varied between 23-34°C.

Daily rainfall data were recorded during the short rains from October 1 to December 31, 1982 at a station 2 km NW of the study site. Rainfall data for previous years were obtained from the Kenya Meteological Department. The degree of flooding (water level) in the dambo was recorded daily by measuring the water depth in mm at the lowest point in the seepage zone (the first area to flood). The flooding of dambos occurs infrequently and usually coincides with periods of unusually heavy rainfall.

Immature mosquitoes were sampled with a pint (0.47 liters) dipper daily from November 27 (the first day of flooding) to December 31, 1982. Twenty-five samples were taken by dipping at equal intervals along each of 2 lines transecting the dambo. The ends of the transecting lines always coincided with the margins of the standing water. All samples were transported to the laboratory where the larvae were separated by stage, counted and, when possible, reared to adults. To simplify sorting procedures, the larvae were separated into 2 groups consisting of (first and second) and (third and fourth) stages. The pupae were counted and reared to adults. Species identification was determined following the methods described by Linticum et al. (1983). Each day the total number and stage of each <u>Aedes</u> collected was determined. The

Culex larvae and pupae were grouped together each day and not sorted to species. Random samples, consisting of 10 specimens, of the Culex larvae were taken daily and reared to adult to determine relative abundance of the individual species.

RESULTS

The daily water level in the Kamiti dambo and daily rainfall for the areas are illustrated in Fig. 2. During the 29 rainy days that occurred during the study period an unusually high precipitation of 436.5 mm fell on the Kamiti dambo. The previous 20 year mean annual rainfall for the area was 488.2 mm. The first standing water in the dambo was observed on November 27 when the level was 160 mm. Between November 27 and December 6 the water level rose to a depth of 605 mm. The water level gradually declined thereafter until December 16 when the level dropped rapidly. On December 31 there was no standing water in the dambo. The water appeared cloudy and brown during and immediately following periods of rainfall. At other times the water was only slightly to moderately turbid.

A total of 29,570 larvae and pupae were collected and separated according to developmental stage between November 28, the first day after flooding (day one), and December 27 (day 29). The mosquito species identified were: *Aedes* (*Aedimorphus*) *cumminsii mediopunctatus* (Theobald), *Ae.* (*Mucidus*) *sudanensis* (Theobald), *Ae.* (*Neomelaniconion*) *lineatopennis* (Ludlow), *Culex* (*Culex*) *antennatus* (Becker), *Cx.* (*Cux*) *pipiens* Linnaeus, *Cx.* (*Cux.*) *theileri* Theobald, *Cx.* (*Cux.*) *zombaensis* Theobald and *Cx.* (*Lutzia*) *tigripes* De Grandpre and De Charmoy. Table 1 lists the total numbers of mosquitoes collected. The 3 *Aedes* spp. represented 19.1% of the total collection and 87% of these specimens were *Ae. lineatopennis*. *Culex pipiens* was found in all samples and it represented 54.6% of the individuals in the sample collections. *Culex* *zombaensis* was found in all but 4 samples and it represented 28.6% of the total collection. *Culex antennatus*, *theileri* and *tigripes* represented 12.2% 2.9% and 1.7% respectively of the sample collections. Figure 3 illustrates the daily total of immatures collected for each of the 3 *Aedes* spp. and for the *Culex* spp. *Aedes cumminsii mediopunctatus* was first collected on day one and last collected on day 8, with greatest numbers occurring on days 2 and 3. Small numbers of *Ae. sudanensis* were collected on days 1-18. *Aedes lineatopennis* was first collected on day 5 and last collected on day 13, with largest numbers collected on days 6-8. After the *Aedes* larvae disappeared they were not collected again during the study period. The first *Culex* specimens were collected on day 7 and numbers increased to a maximum on days 11 and 12. After this time their numbers declined but still remained numerous until day 22. The last *Culex* specimens were collected on December 27 (day 30). Figure 4 illustrates for each of the 3 *Aedes* spp. and the *Culex* spp. the mean number of individuals of each developmental stage collected per dip on each collection day following the presence of standing water. The time from first hatch to first pupae was 6 days for *Ae. cumminsii mediopunctatus* and *lineatopennis*, and 9 days for *sudanensis*.

DISCUSSION

Rift Valley fever virus has been isolated from 6 of the 8 species of mosquitoes collected in this grassland dambo. *Aedes lineatopennis* has been found infected in Kenya (Davies and Highton 1980), South Africa (McIntosh et al. 1980, McIntosh and Jupp 1981) and Zimbabwe (McIntosh 1972). *Culex pipiens* was found infected during a 1977 epizootic in Egypt (Meegan 1979), Hoogstraal et al. 1979) and *Culex theileri* has been found infected in South Africa (Gear et al. 1955, McIntosh 1972, McIntosh et al. 1980, McIntosh and Jupp 1981). McIntosh et al. (1983) made 7 isolations of RVF from

Cx. zombaensis during an outbreak in the coastal region of Natal Province, South Africa in 1981. Virus has recently been isolated in Kenya from Ae. cumminsii mediopunctatus and Cx. antennatus (Linthicum, Davies, Kairo and Bailey 1983, unpublished data). The study of the breeding habits of these and other species of mosquitoes may be important to understand better the ecology of RVF.

The rainfall during the study period almost equalled the yearly mean total for the area and there was a sufficiently high water table to produce standing water in the dambo. This may be of importance as RVF epizootics are known to coincide with periods of heavy rainfall which flood low lying areas (Schulz 1951, Davies 1975, McIntosh and Jupp 1981). RVF did not occur although the short period of flooding of this grassland dambo produced large numbers of potential vectors.

Aedes cumminsii mediopunctatus, sudanensis and linetopennis larvae were collected after the dambo was flooded. The eggs of Ae. cumminsii mediopunctatus and sudanensis must have been flooded immediately as larvae were found on day one and maximium numbers were collected on day 2. Figure 2 shows that by day 4 the water depth had more than doubled since the first day of flooding. This increase coincided with the first collections of Ae. lineatopennis on day 5 and suggests that its eggs were located in areas not flooded until the water level reached 380 mm at the lowest point in the seepage zone. Each of the Aedes spp. disappeared after a single generation. The period from first hatch to first pupae for Ae. cumminsii mediopunctatus and sudanensis was 2 and 9 days respectively less than that reported for the same species in a forest dambo (Linthicum et al. 1983). The same period for Ae. lineatopennis required 1 more day than that reported in the forest dambo. In comparison with the forest dambo the grassland dambo was more exposed to direct sunlight, the water temperatures was higher and was located at a 200 m lower elevation. The number of specimens of Ae. cumminsii mediopunctatus and sudanensis collected per dip was less than one-half the number collected per dip in a flooded forest dambo (Linthicum et al. 1983). However, the number of specimens of Ae. lineatopennis collected per dip was more than twice the number collected per dip in the forest dambo. The number of Culex spp. collected per dip was almost ten fold the number collected per dip in the forest dambo. Although Culex (Cux.) quasiguiarti Theobald and Cx. theileri were found in the forest dambo in approximately equal numbers, Cx. quasiguiarti was absent from the grassland dambo and Cx. theileri represented only 2.9% of the Culex collected. These species were replaced by Cx. pipiens and zombaensis which together represented 83.2% of the sample Culex collections. The decline in numbers of Culex spp. collected in the grassland dambo correspond to a lowering of the water level (Fig. 2) starting at day 17 and a dramatic drop after day 25.

The Aedes spp. described in this study were found in very large numbers only in association with the flooded dambo situations. Immature have been collected in a variety of other habitats but in comparatively small numbers (Hopkins 1952, Linthicum et al. unpublished data). Light trap and dipping collections conducted in the same area in Kenya between 1981 and 1983 show that small numbers of the 3 Aedes spp were collected during each rainy season when temporary ground pools (other than the dambo formations) are flooded, and that large numbers were collected only when the dambos flooded (Linthicum et al. unpublished data). These collections also indicate that the 5 Culex spp. are collected in small numbers year-round with much higher numbers collected during the rainy seasons, particularly when the dambos flooded. The only ecological change that increases the surface area of the standing water in this area and produces the conditions necessary for large numbers of mosquitoes to be produced is the flooding of the dambos.

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Table 1. The number of specimens collected by species and life stage at the Kamiti dambo, Ruiru, Kenya from November 28 to December 27, 1982 by dipping.

Species	Larval Stage 1st-2nd	Larval Stage 3rd-4th	Pupae	Total
Ae. cumminsii	405	161	42	608 (2.1%)
Ae. sudanensis	100	32	16	148 (0.5%)
Ae. lineatopennis	2811	1579	507	4897 (16.5%)
Cx. spp.	14607	7226	2087	23917 (80.9%)

Fig. 1. The Kamiti dambo, Ruiru, Kenya on November 15, 1982.

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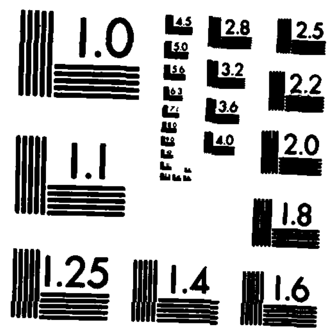

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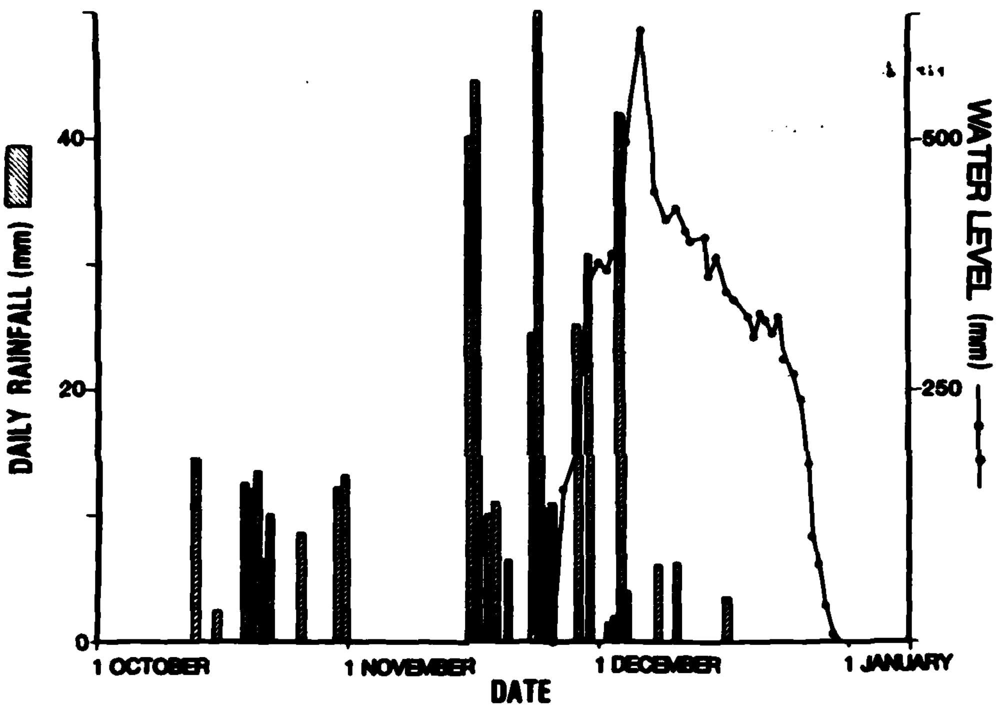

Fig. 2. Daily rainfall in mm (histogram) and water level in mm (solid line) at the Kamiti dambo, Ruiru, Kenya from October 1, 1982 to January 1, 1983.

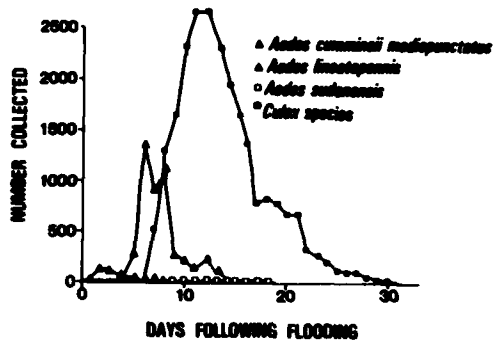

Fig. 3. Daily total of specimens collected by dipping from the Kamiti dambo, Ruiru, Kenya from November 28 to December 27, 1982.

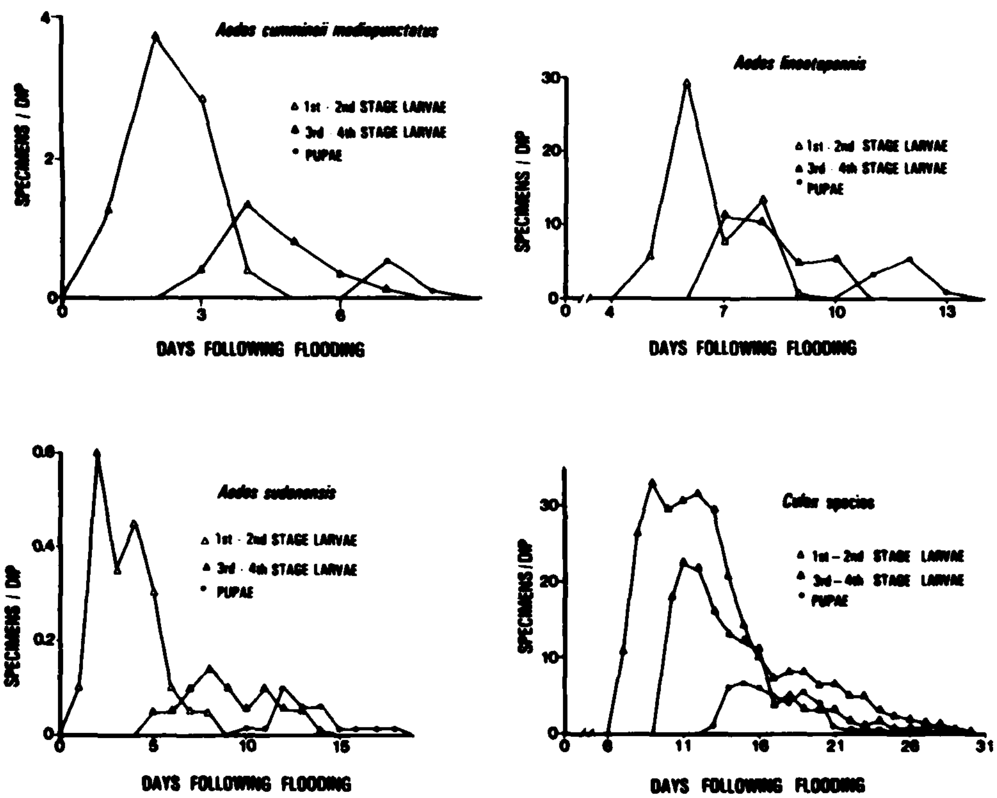

Fig. 4. Mean number of specimens collected per dip in the Kamiti dambo, Ruiru, Kenya from November 28 to December 27, 1982 for the developmental stages of *Aedes cumminsii mediopunctatus*, *sudanensis*, *lineatopennis* and *Culex* species.

Fig. 1. The Kamiti dambo, Ruiru, Kenya on November 15, 1982.

Fig. 2. Daily rainfall in mm (histogram) and water level in mm (solid line) at the Kamiti dambo, Ruiru, Kenya from October 1, 1982 to January 1, 1983.

Fig. 3. Daily total of specimens collected by dipping from the Kamiti dambo, Ruiru, Kenya from November 28 to December 27, 1982.

Fig. 4. Mean number of specimens collected per dip in the Kamiti dambo, Ruiru, Kenya from November 28 to December 27, 1982 for the developmental stages of *Aedes* *cumminsii* *mediopunctatus*, *sudanensis*, *lineatopennis* and *Culex* species.

Blood Feeding Activity of Mosquitoes at a Flooded Dambo in Kenya

ABSTRACT

The biting activity of mosquitoes encountered after flooding of a grassland dambo in Kenya was examined using human and calf bait. A total of 2,319 female mosquitoes, representing 9 species, were collected during a 96 hr period at human bait and a 48 hr period at calf bait. *Aedes lineatopennis* was the most commonly captured species. It represented 85% of the specimens collected at human bait and 96% of the specimens collected at calf bait. Diel biting activity was established for *Ae. lineatopennis*, *Ae. cumminsii mediopunctatus* and *Ae. dentatus.*

The biting habits of mosquitoes associated with dambos (Ackermann 1931) in Kenya may be of importance to understand their role in the ecology of Rift Valley Fever (RVF) and other viruses. There have been numerous studies of the biting habits of mosquitoes in East Africa (Haddow 1942, 1945a, 1945b, 1954, 1956a, 1956b, 1960, 1964; Haddow et al. 1947, 1951; Lumsden 1952; Haddow and Ssenkubuge 1963); but none specifically dealt with the dambo associated species. A more detailed knowledge of the biology of mosquitoes found in such habitats is important, for several of these have been incriminated as vectors of RVF (Linthicum et al. 1983).

RVF is primarily a cattle and sheep disease with man becoming infected when in close association with these animals (W.H.O. 1982). In areas in Kenya where the disease occurs herdsman graze their cattle in and around dambo formations. The purpose of this study was to determine (1), the mosquito species feeding on man and cattle, and (2), the time(s) of the diel in which blood feeding activity occurred in a dambo in an area known to have RVF outbreaks.

MATERIALS AND METHODS

Study area

This study was conducted on a ranch at the northeastern margin of the Athi plains, 7 km SE of Ruiru, Thika District, Central Province, Kenya (1°12'S; 37°E) at an elevation of 1500 m. The dambo was situated in an area of bushed grasslands along the Kamiti River (ecological zone III, Pratt et al. 1966). It showed the typical zonal differentiation (Mackel 1974, Linthicum et al. 1983) with predominantly sedge (*Cyperus immensus* C.B. Clarke) in the seepage zone with the grass *Digitaria abyssinica* (A. Richard) Stapf in the remainder of the dambo. On 26-27 April, 1983 overflow from the Kamiti River flooded the study site. A generation of mosquitoes emerged from the flooded dambo that contained standing water remained until 9 May 1983. This provided the opportunity to study the biting habits of mosquitoes associated with a flooded dambo.

Collection procedure

The study consisted of a continuous 96 hr collection by 2 men (1000 hrs 10 May 83 - 1000 hrs 14 May 83) and a continuous 48 hr collection at a calf (1100 hrs 12 May 83 - 1100 hrs 14 May 83). The 2 men were positioned together in the dambo so that they could capture mosquitoes landing on their partner as well as on themselves. The men worked 4 hr shifts before being relieved by 2 other men. They collected females as they came to feed by placing a tube over them as they started to probe. The time (East African Standard Time) and date of capture of each specimen was noted on the tube. A yearling calf, which weighed approximately 100 kg, was tethered on a 5 m rope to a stake and positioned 50 m from the 2 human collecters. The calf was given water and allowed to graze in the dambo. Another man continually collected specimens as they landed and started to probe on the calf. Specimens were placed in a tube and marked by time and date of capture. A flashlight was used for illumination by all collectors during dark periods. The tubes were returned to the laboratory where specimens were identified using a steroscopic dissecting microscope.

Data analysis

The number of specimens of the 3 most common species captured during each hourly period (30 minutes before and after the hour) were totalled for each sampling date. These sums were then converted to "Williams' mean" (Williams 1937). Williams' modification of the geometric mean was chosen as a statistic as it accomodates zero values and measures central tendency (Haddow 1960). It is denoted by the symbol M_w and defined as $\log (M_w + 1) = \frac{\log (n+1)}{N}$ where $n_1, n_2 \ldots$ represent the actual values of a series of N observations. M_w, the mean capture rate, for each hour is obtained by subtracting one from the antilogarithm of the quantity found by first adding one to each of the original observations, then summing the logarithms of these numbers, and finally dividing by the total number of observations (N days). In this study N = 4 for the human collections and N = 2 for the calf collections. Relative blood feeding rate for the 3 most common species collected was determined by calculating from M_w values the percent of total blood feeding occurring during any one hr period.

Climatic observations

Meterological data was recorded at the study site on an hourly basis. This information included air temperature, relative humidity and wind velocity. Observations concerning cloud cover and precipitation were also made during the study period. These observations were augmented by 24 hr weather and winds forecast provided by the Jomo Kenyatta International Airport Meterological Office. The times of local sunrise and sunset were provided by the Kenya Meterological Department, Nairobi, Kenya.

RESULTS

Climatic observations

Air temperatures ranged from a maximum of 33°C to a minimum of 13°C during the 4 day period in which observations were made. Relative humidity varied from a maximum of 95% during the night to a minimum of 30% during the day. There were complete cloud cover during each of the 4 nights, with ceilings ranging from 150 to 1070 meters above ground level. During the day cloud coverings ranged from thin-partial to complete with 300-1200 meters above ground level ceilings. Surface winds were calm and did not exceed 10 k.p.h. at any time. Local sunrise and sunset were at 0628 and 1830 hrs Eastern African Standard Time, respectively.

Mosquito collections

A total of 2,319 female mosquitoes were captured and identified to species. Table 1 lists the species collected and their numbers. Nine species, represented by 1446 specimens, were collected on human bait over a 96 hr period. Seven species represented by 873 specimens, were collected on calf bait over a 48 hr period. *Aedes* (*Neomelaniconion*) *lineatopennis* (Ludlow) was by far the most common species at both human and calf bait. Species other than *Ae. lineatopennis* represented only 15% and 4% of the total taken at human and calf bait, respectively.

Feeding activity

The biting of *Ae. lineatopennis* is shown in Table 2 and Fig. 1. The biting cycle is distinctly bimodal at human bait with 30% of the biting activity occurring in a 2 hr period just before and after sunset and over

30% occurring in the 4 hr period extending from 1 hr before to 3 hrs after sunrise. Biting activity was somewhat higher at night than during the day. This is reflected by the M_w values which ranged from 2.98-8.77 during darknes (2000-0500 hrs) while only ranging from 1.06-4.58 during midday (1000-1600 hrs). The biting activity at the calf was also bimodal with peaks occurring 2 hrs before sunset, and just before and at sunrise. The range of M_w values for darkness (2000-0500 hrs), 3.24-22.66, compared to those for midday (1000-1600 hrs), 0-14.49, indicate less biting activity in the middle of the day compared with the middle of the night.

The biting activity of <u>Ae</u>. (<u>Aedimorphus</u>) <u>cumminsii</u> <u>mediopunctatus</u> (Theobald) is shown in Table 2 and Fig. 2. Only 3 of the 63 specimens collected were captured at the calf. The biting activity at human bait is strongly unimodal, 50% of the specimens were captured in the 2 hr interval immediately before and after sunset (1800-1900 hrs). Diurnal biting (0700-1700 hrs) represents only 6% of the specimens captured.

<u>Aedes</u> (<u>Aedimorphus</u>) <u>dentatus</u> (Theobald) biting activity is shown in Table 2 and Fig. 3. There is a single pronounced peak at human bait, representing 57% of the total activity, during the interval from 1 hr before sunset to 2 hrs after sunset (1800-2000 hrs). Biting activity continued at a low level during most toher times of the day. Activity peaks are ill-defined at calf bait, but almost 70% of the captures were made between 1600 and 2200 hrs. Only one specimen was found on the calf between 0600 and 1500 hrs.

The biting activity of the less abundant species collected are shown in Table 3. At human bait <u>Ae</u>. (<u>Mucidus</u>) <u>sudanensis</u> (Theobald) exhibited a crepuscular and nocturnal biting preference. Only one specimen was taken at the calf. <u>Aedes</u> (<u>Neomelaniconion</u>) <u>unidentatus</u> (McIntosh), <u>Anopheles</u> (<u>Anopheles</u>) <u>coustani</u> (Laveran) and <u>An</u>. (<u>Cellia</u>) <u>gambiae</u> (Giles) were not collected at the calf. Aedes (Neo.) circumluteolus (Theobald) and Coquillettidia (Coquillettidia) aurea (Edwards) were collected both at human and calf bait. Coquillettidia aurea was captured at night. A single Culex (Culex) pipiens (Linnaeus) was collected at the calf.

DISCUSSION

Epizootics of Rift Valley Fever occur in bushed grasslands in Kenya after periods of heavy rainfall have caused flooding in low lying areas (Davies 1975). At these times certain mosquitoes have been found infected with the virus and may be vectors (McIntosh 1972, Davies and Highton 1980).

Aedes lineatopennis was the most common species of mosquito feeding on humans and cattle in our study area. This observation is of particular significance as an isolation of RVF virus has been made from a pool of 12 Ae. lineatopennis females collected very near our study site during an epizootic in cattle (Davies and Highton 1980). Although 85% of the specimens collected on the human bait were Ae. lineatopennis, it represented 96% of the specimens captured at the calf bait. Herds of 300-400 cattle grazed through the dambo area each day during this study period, and they were accompanied by one or two herdsmen. These cattle were held in night pens some distance away (1 km) from the nearest dambo and the herdsmen returned to their homes 2-3 km away. The large numbers of bovine hosts available to the dambo populations of Ae. lineatopennis during the day explain why blood meal analysis has shown that these feed almost exclusively on cattle (Linthicum 1983, unpublished data). The vast majority of Ae. lineatopennis captured in Western Kenya had fed on bovines (Chandler Boreham, Highton and Hill 1975, 1976; Chandler, Highton and Boreham 1976). A study of the dispersion of Aedes lineatopennis from individual dambos is needed to better understand the potential for the dissemination of RVF. Haddow (1942, 1960) reports that *Ae. lineatopennis* was encountered commonly during the day in the open and occasionally in huts in Kisumu, Kenya at an elevation of 3700 feet. Surtees (1970) collected 14 specimens of *Ae. lineatopennis* at human bait between 1800-2300 hrs. Our data show some low level activity at man in the daytime.

Since this species represented 4.5% of the species captured at human bait and only 0.3% at the calf. Cattle seem to offer little attraction to *Ae. cumminsii*. The crepuscular peak and night biting activity we observed was similar to that noted by Haddow (1945b, 1960) during studies in forests and banana plantations in Bwamba County, Uganda. *Aedes dentatus*, the second most frequently captured species, represented only 8% and 3% of the total captures at human and calf bait, respectively. Peak host seeking activity occurs at sunset with intermittent biting activity at other times. Cattle evidently are not a preferred host for *Ae. sudanensis* which preferentially feeds on humans and is most active at night and sunset. We do not know whether the sparse collections of the remaining 6 less abundant species is a result of low population levels at our study site or a low level of feeding activity at human and calf bait. *Aedes circumluteolus* is a common species in Western Uganda where it is found in the day at human bait collections (Haddow et al. 1947, Haddow 1960). On the Kano plain in Kenya *Ae. circumluteolus* populations were abundant after rains and represented 4.5% of the total catch at human bait (Chandler, Highton and Hill 1976). In Nigeria Mattingly (1949) encountered this species commonly in day and night collection at human bait following the rainy season. McIntosh (1971) in South Africa found *Ae. circumluteolus* feeding on man during the night and only rarely during the day. *Aedes circumluteolus* has been incriminated as a vector of RVF in both Uganda (Weinbren et al. 1957) and South Africa (Kokernot et al.

1957). Aedes unidentatus has been reported to feed voraciously on man and sheep in South Africa, with peak biting activity half-an-hour after sunset (McIntosh 1971). Anopheles gambiae, in timed night captures inside houses in one village on the Kano Plain, Kenya, represented 65% of the total catch (Surtees 1970), while outside in the same area it represented only 1% of the specimens captured at human bait (Chandler, Highton and Hill 1976). Blood meal identification studies conducted by Chandler, Boreham, Highton and Hill (1975) on An. gambiae collected on the Kano plain indicated a high proportion of human blood feeds (81%). Anopheles coustani represented less than 1% of the total catch at human bait in 2 villages in Kenya (Surtees 1970). Davies and Highton (1980) have collected An. coustani infected with RVF in Kenya. The feeding habits of Cq. aurea are little known, as it has only been collected in very small numbers (Lumsden 1951, Haddow 1954). Culex pipiens quinquefasciatus represented 5% of the specimens captured at human bait (Surtees 1970) in a village but less than 1% outside on the Kano plain (Chandler, Highton and Hill 1976).

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Table 1. The biting frequency of mosquitoes at human and calf bait.

Species	Human *		Calf **	
	No.	%	No.	%
Ae. lineatopennis	1235	85.0	835	96.0
Ae. cumminsii	60	4.5	3	0.3
Ae. dentatus	116	8.0	28	3.0
Ae. sudanensis	18	1.5	1	0.1
Ae. circumluteolus	5	0.3	1	0.1
Ae. unidentatus	2	0.1	-	-
An. gambiae	2	0.1	-	-
An. coustani	6	0.4	-	-
Cq. aurea	2	0.1	5	0.4
Cx. pipiens	-	0.1	1	0.1
Totals	1446		873	

* Collections are from 2 men over a period of 96 hrs (10 May-14 May 1983).

** Collections are from one calf over a period of 48 hrs (12 May-14 May 1983).

Table 2. Numbers of <u>Aedes lineatopennis</u>, <u>Ae. cumminsii</u> and <u>Ae. dentatus</u> captured at human and calf bait during 96 and 48 hrs of continuous collection

	Ae. lineatopennis				*Ae. cumminsii*				*Ae. dentatus*			
	Human		Calf		Human		Calf		Human		Calf	
Hour	No.	Mw	No.	Mw	No.	Mw	No.	Mw	No.	Mw	No.	Mw
0100	22	3.24	9	3.24	3	0.57	0	0	3	0.41	3	1.00
0200	24	3.95	26	10.49	5	1.21	0	0	0	0	2	0.73
0300	20	2.98	22	3.80	1	0.19	0	0	1	0.91	0	0
0400	23	3.09	33	11.25	0	0	0	0	2	0.41	1	0.41
0500	27	4.74	28	13.97	0	0	0	0	0	0	1	0.41
0600	77	7.85	53	22.96	0	0	0	0	1	0.19	0	0
0700	144	13.95	114	48.02	0	0	0	0	4	0.50	1	0.41
0800	128	15.03	25	12.42	0	0	0	0	0	0	0	0
0900	109	12.33	4	1.83	0	0	0	0	1	0.19	0	0
1000	44	4.58	28	11.69	0	0	0	0	0	0	0	0
1100	68	3.78	24	11.00	0	0	0	0	0	0	0	0
1200	9	1.30	25	12.42	0	0	0	0	2	0.32	0	0
1300	10	1.83	3	1.45	0	0	0	0	3	0.41	0	0
1400	9	1.63	0	0	4	0.50	0	0	7	0.93	0	0
1500	7	1.06	16	4.66	0	0	0	0	6	0.97	0	0
1600	15	2.31	36	14.49	1	0.19	0	0	2	0.32	4	1.83
1700	83	3.20	88	35.73	0	0	1	0.41	2	0.32	2	0.73
1800	180	36.55	70	34.87	15	3.24	0	0	31	2.63	2	0.73
1900	74	15.28	49	12.71	14	2.72	0	0	23	4.01	2	0.73
2000	40	8.77	51	13.00	8	1.51	2	0.73	13	2.08	3	1.00
2100	23	4.48	64	22.66	4	0.5	0	0	10	0.82	3	1.00
2200	16	2.98	28	14.00	0	0	0	0	1	0.19	3	1.45
2300	38	4.28	11	5.32	4	0.68	0	0	1	0.19	0	0
2400	45	7.97	28	9.20	1	0.19	0	0	3	0.41	1	0.41
Totals	1235	-	835	-	60	-	3	-	116	-	28	-

Table 3. The biting activity of the less abundant species collected at human and calf bait.

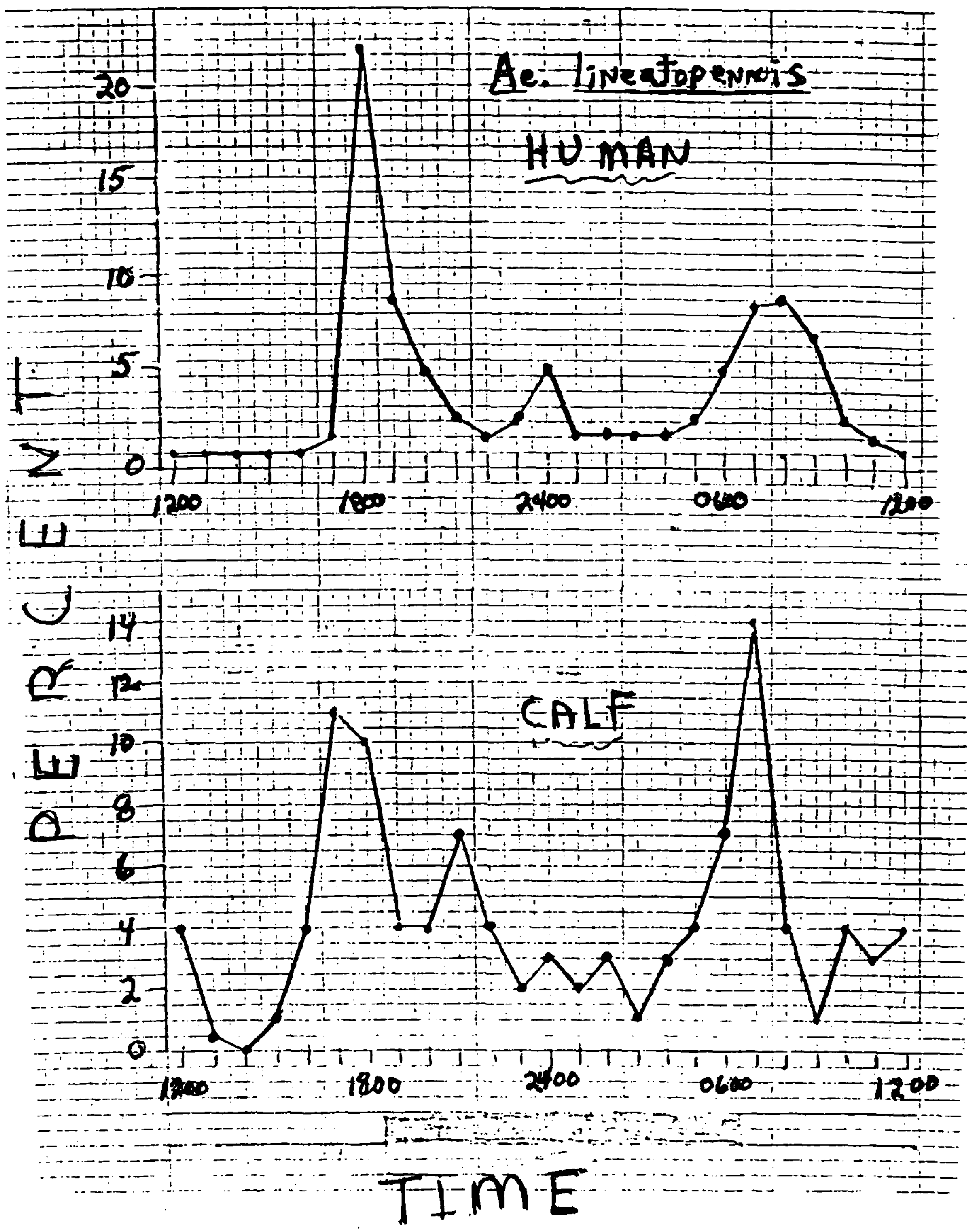
	Ae. sudanensis		*Ae. circumluteolus*		*Ae. unidentatus*		*Ae. gambiae*		*An. coustani*		*Cq. aurea*		*Cx. pipiens*	
Hour	Human	calf	Human	calf	Human	calf	Human	calf	Human	calf	Human	calf	Human	calf
0100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0200	2	0	1	1	0	0	0	0	0	0	1	1	0	0
0300	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0400	1	1	0	0	0	0	0	0	0	0	0	1	0	0
0500	0	0	0	0	0	0	0	0	0	0	0	2	0	0
0600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0700	1	0	0	0	0	0	1	0	0	0	0	0	0	0
0800	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0900	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1300	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1400	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1800	2	0	0	0	0	0	1	0	0	0	0	0	0	0
1900	1	0	2	0	1	0	0	0	2	0	0	0	0	0
2000	5	0	1	2	0	0	0	0	3	0	0	1	0	1
2100	0	0	0	1	0	0	0	0	0	0	1	0	0	0
2200	2	0	0	0	0	0	0	0	0	0	0	0	0	0
2300	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2400	4	0	0	0	1	0	0	0	1	0	0	0	0	0
Total	8	1	5	5	2	0	2	0	6	0	2	5	0	1

Fig. 1. Numbers of _Aedes_ _lineatopennis_ captured from human and calf bait. Collections were conducted continuously for 96 and 48 hours. Calculated as Williams' mean and expressed as a percent of the total.

Fig. 2. The biting cycle of _Aedes_ _cumminsii_ _mediopunctatus_ at human and calf bait. Calculated as Williams' mean and expressed as a percent of the total.

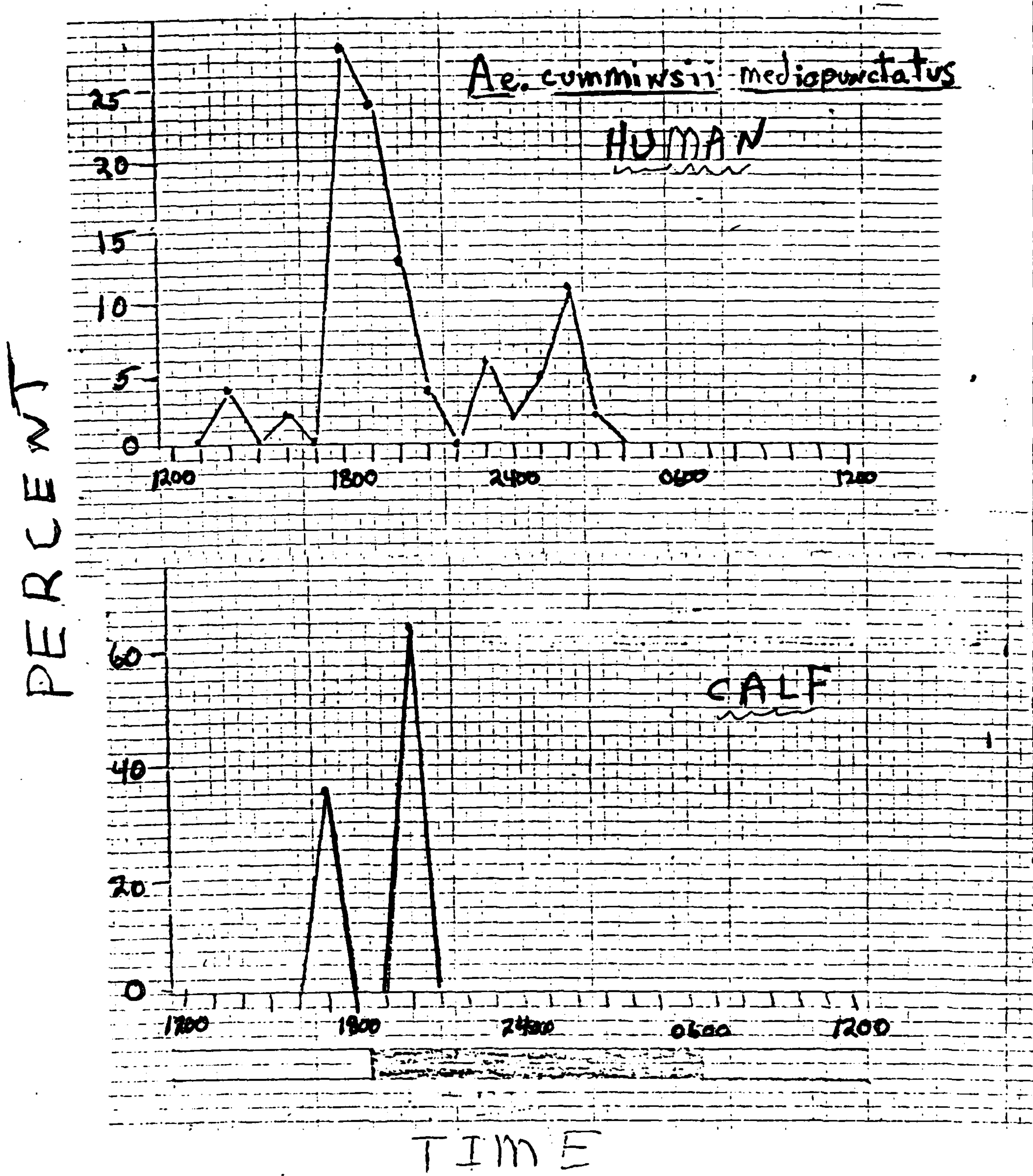
Fig. 3. The biting cycle of _Aedes_ _dentatus_ at human and calf bait. Calculated as Williams' mean and expressed as a percent of the total.

Fig. 1



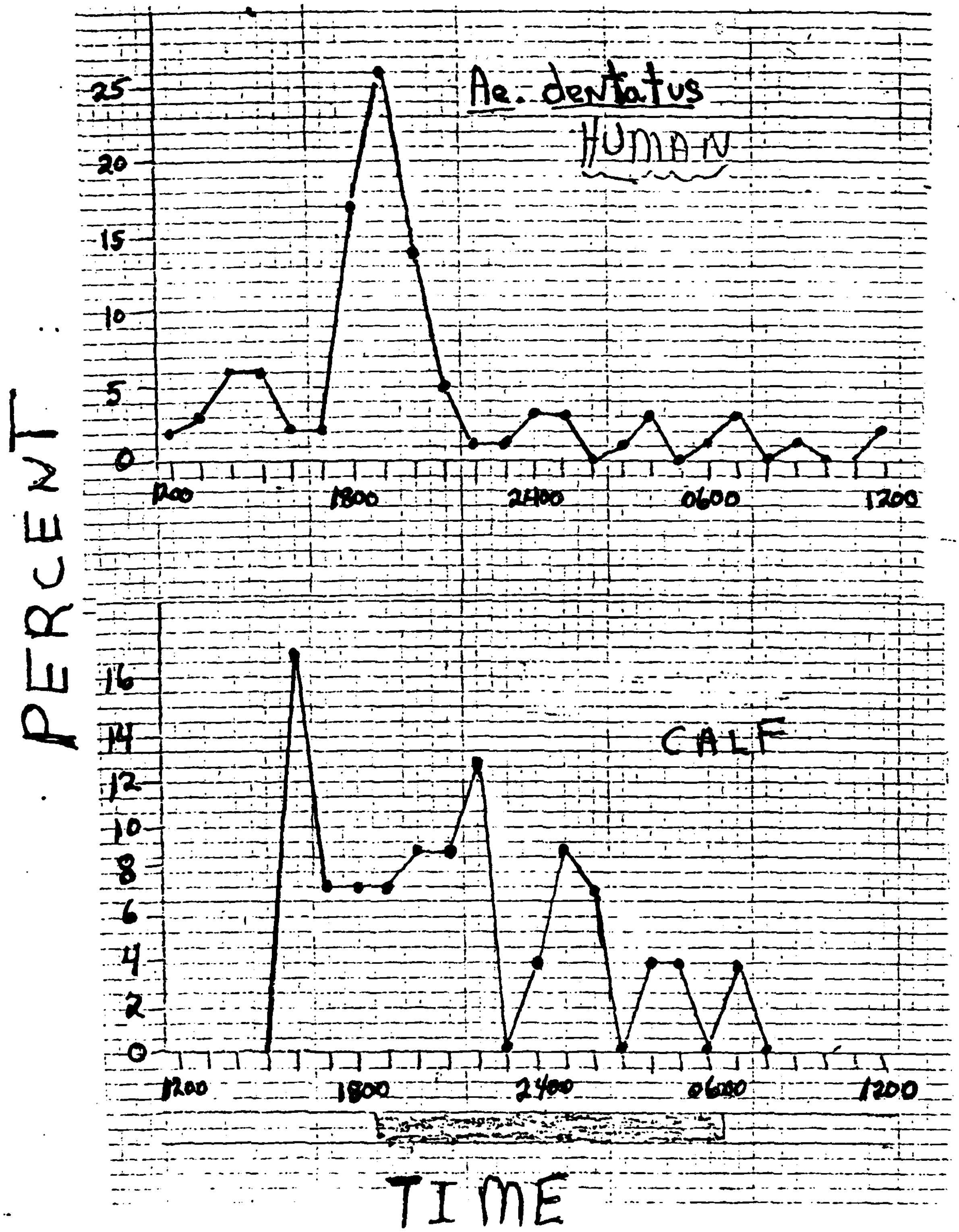
Ae. lineatopennis
HUMAN
CALF
PERCENT
TIME
20
15
10
5
0
1200
1800
2400
0600
1200
14
12
10
8
6
4
2
0
1200
1800
2400
0600
1200

Fig. 2



Ae. cumminsii mediopunctatus
HUMAN
CALF
PERCENT
25
20
15
10
5
0
1200
1800
2400
0600
1200
60
40
20
0
1200
1800
2400
0600
1200
TIME

Fig. 3


Ae. dentatus
HUMAN
25
20
15
10
5
0
1200
1800
2400
0600
1200
CALF
16
14
12
10
8
6
4
2
0
1200
1800
2400
0600
1200
PERCENT
TIME

Aerial Collection of *Culicoides schultzei* (Diptera: Ceratopogonidae) in Kenya

The feeding patterns and abundance of species of the *Culicoides schultzei* group strongly suggest that they are probable vectors of bluetongue and ephemeral fever viruses in Kenya, (Davies and Walker 1974, Walker and Boreham 1976). The spread of bluetongue and ephemeral fever viruses, within and outside endemic areas in East Africa, is thought to be caused by the movement of infected *Culicoides* which becomes airborne during the passage of the Intertropical Convergence Zone (ITCZ) (Sellers 1980). However, there has been no direct evidence of *Culicoides* involvement in the ITCZ air movements. The ITCZ is an equatorial belt of low barometric pressure where, at low levels, air flowing from the northern and southern hemispheres converge. Within the ITCZ air rises, expands and cools, producing frequent weather activity (Lamb 1972). The zone moves in East Africa from approximately 20°N in July to 20°S in January and is comprised of a zonal (East-West) branch continuous with a meridional (North-South) branch. This paper describes an attempt to make aerial insect collections within this area in Kenya.

Collections were attempted by making 3 flights (Jan 7,9,12 1984) in a Cessna 150 over a 200 km² section of bushed grassland (1°S, 37°E; 1500 m) in ecological zone III (Pratt et al. 1966). Each flight was made in the late afternoon and consisted of 15 minutes of collection time at 1800, 1950 and 2100 m above mean sea level. Fine mesh collection nets were placed over the cabin ports of the 2 wing ventilators whenover the collection area and were replaced at each change in altitude. The intake surface areas of the ventilators totalled about 26 cm². All screens and filters along each of the 30 cm long vent tubes were removed prior to the flight. Flights were concentrated in areas of upward air movement to maximize the possibility of obtaining specimens. Updrafts were found by observing the flight of 3 species of soaring birds: the Black Kite (Milvus migrans), the Augur Buzzard (Buteo rufofuscus) and the Maribou Stork (Leoptoptilos crumeniferus). The updrafts encountered on all flights were in the 70-240 m/min range, as determined by noting the change in rate of climb indication as the updraft was entered. The plane was trimmed for a slow flight configuration with 10° extended flaps and an indicated air speed of approximately 85 kph (45 knots). The true airspeed was approximately 100 kph at the pressure altitudes and temperatures of the flights. A continuous standard rate turn (360°/2 min) was initiated upon encountering updrafts to remain within their bounds. All collection nets were returned to the laboratory after the flights for examination. The approximate alignment of the ITCZ between Jan 7 and 12 1984, in relation to the collection area, is illustrated in Fig. 1.

Twelve specimens of the Culicoides schultzei group (sensu Khamala and Kettle 1971), were collected on Jan 9 at 1950 m. The collection was comprised of 9 males and 3 females. A precise identification was not possible due to the damaged condition of the specimens. No specimens were collected on Jan 7 or 12. The total volume of air sampled during the collection flights was approximately 580 m^3.. The mean density of insects sampled was one insect in 48.3 m^3. Rainey (1973) reported a density of one insect (predominantly Thysanoptera) in 230 m^3 in aircraft collections traversing the ITCZ at 30 m and a density of one insect (predominantly Cercopidae and Cicadellidae) in 30 m^3 in collections on a tower at 15 m during the passage of the ITCZ in Sudan. The number of male specimens in our collection suggests that the collection flight may have flown through a mating swarm. The collection of a vector of bluetongue and ephemeral fever virus at 450 m above ground level lends support to the theory that airborne wind carriage of insects is a means of disease dissementation. Further studies are needed to demonstrate the role of the ITCZ in stimulating vectors to become airborne and how environemental conditions aasociated with the ITCZ can maintain the infectivity of the vector.

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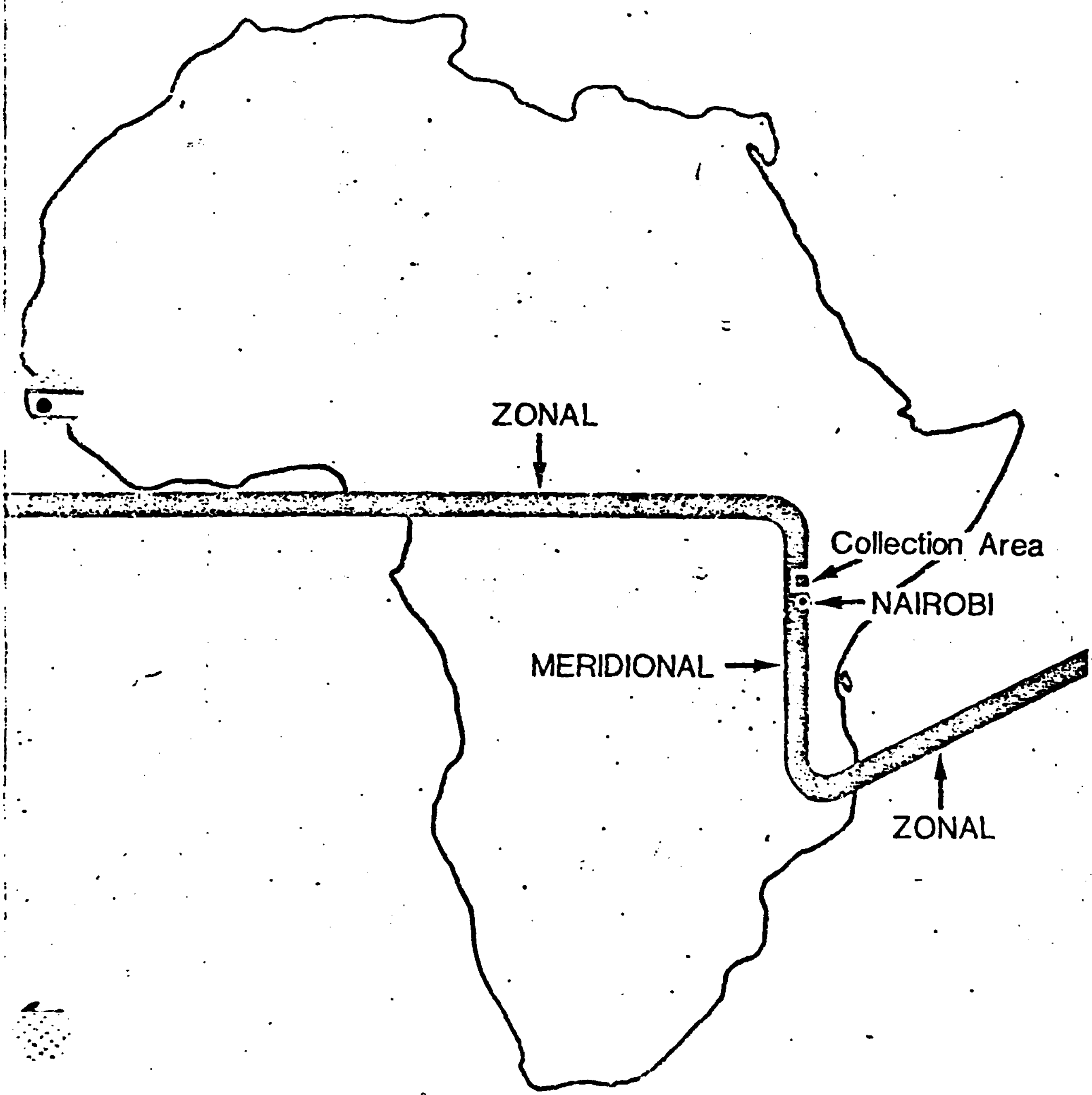
Fig 1. The approximate alignment of the ITCZ between Jan 7 and 12 1984 in the relation to collection area. The position of the ITCZ was interpreted from surface and 850 milibar synoptic weather charts, and from visible and infrared image data collected by the satellite Meteosat 2.

Figure 1



The Sudan dioch (*Quelea quelea aethiopica*) and Rift Valley fever

Dr. R.A. Alexander made two important observations on the epidemology of Rift Valley fever (RVF) at a meeting of the Inter African Advisory Committee on Epizootic Diseases, in Dakar in 1957. (Proc. IV Meeting I.A.C.E.D, 1957). First, he reported that the seasonal migrations of the Sudan dioch (*Quelea quelea*) were responsible for the introduction of RVF into different parts of the Rift Valley in Africa. The species "was susceptible and infected birds were bitten by mosquitoes during the viraemic state". Secondly, he stated that the reservoir for RVF between epizootics was in mosquito eggs found in the dried mud of lakes. When this mud was moistened and the eggs hatched, the adults produced were "infective for a period of 17 days". This remarkable report described transovarial transmission of an arbovirus by mosquitoes some 16 tears before this phenomenon was conclusively demonstrated by Watts et al. (1973) and Pantuwatana et al. (1974). His latter observation has recently been substantiated at this laboratory.

An experiment was carried out to determine whether the Sudan dioch was susceptible to RVF, as indicated by Alexander. Previous work with birds and RVF has shown that viraemia does not occur in canaries, parakeets, pigeons nor domestic fowl (Findlay 1931; Davies, unpublished data). No neutralising antibody was found in bird sera collected in RVF enzootic areas in Uganda (Davies and Addy 1979). Some 45 Quelea were trapped in mist nets at a roost in the Rift Valley in Kenya (1°S; 36°E). These were kept in 1 m^3 cages with adequate water and food. Nine birds were bled for serum and none contained nuetralising antibody in a microserum neutralisation test against 80 $TCID_{50}$ of RVF virus.

A strain of RVF isolated from *Aedes lineatopennis* in 1978, (Davies and

Highton 1980) was used to inoculate the birds. This had been passaged 3 times in infant mouse brain and once in Vero cells. Thirty-six brids were inoculated with $10^{5.5}$ $TCID_{50}$ in 0.' ml volumes into the subcutaneous tissue and muscle of the pectoral area. These birds were killed at 12 h intervals thereafter for five days. Blood and liver samples were taken from each bird for virus isolation attempts. Two golden Syrian hamsters (*Mesocricetus auratus*), were inoculated intraperitoneally with 10^{-1} dilutions of blood and two with 10% suspensions of liver homogenate in a transport medium. No isolations of RVF virus were made from any sample. No antibody to RVF was detected in 3 birds which had received RVF virus, although there was a slight reduction of the cytopathic effects at 1/10 dilutions of their sera, compared with uninoculated control bires sera. A control RVF sheep immune serum had a titre of 1/1280.

Thus Dr. Alexander's observation on the possible mode of RVF movement into different parts of Africa cannot be supported by the results of this experiment. It is possible that *Q. quelea aethiopica* used in this experiment differs from the southern race *Q. quelea lathamii* probably studied by him, or that a five day period of sampling for viraemia was inadequate. Both are unlikely to be valid criticisms on the basis of our knowledge of the suspectibility of bird species to RVF.

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An Hypothesis to Explain the Natural History of Rift Valley Fever in Africa

SUMMARY

Any hypothesis is presented which suggests that transovarial transmission of Rift Valley fever virus occurs in some Aedes mosquitoes. This explains the natural history of the virus disease in Africa and particularly the mechanism by which the epizootics occur in many different foci simultaneously after long interepizootic periods in which there is little or no evidence of virus activity. Virus has been isolated from laboratory reared adult Aedes lineatopennis collected as larvae in an epizootic area in Kenya.

INTRODUCTION

Rift Valley fever (RVF) has been recognized in East Africa for some 50 years[1] and in South Africa for 30 years.[2] Epizootics of the disease in domestic ruminants persist for some 1-3 years and are then often separated by 5-15 year intervals.[3-5] In most parts of Kenya and South Africa there is little or no evidence of any virus activity during the interepizootic periods other than some few seroconversions in cattle in higher rainfall parts of the country or an occasional clinical case in humans or in domestic animals.[5,6,7,8]

The maintenance cycle for RVF between epizootics in East and South Africa is not understood.[5,7-10] There is evidence available to show that wild birds, rodents, primates and ruminants are unlikely to be involved in some cryptic virus maintenance cycle in the bushed savannah and grasslands where the epizootic disease is encountered.[5,9-18] More relevant has been accorded ot this problem by the extension of RVF into Egypt in 1977, beyond its usual sub-Saharan range.[19-24] The disease, which is a highly pathogenic zoonosis, now has greater significance as its potential for establishing itself in new ecological habitats has become recognised.[18,24,25]

HYPOTHESIS

An hypothesis has been made that RVF virus is maintained during the interepizootic periods by transovarial transmission in mosquito species of the genus *Aedes* that breed in temporary ground pools. Transovarial transmission in *Aedes* species has been demonstrated to be an overwintering mechanism for LaCrosse[26-29] and trivittatus viruses[30,31] and is considered likely for Keystone[32] and snowshoe hare[33] viruses. Vertical transmission in *Aedes* spp. may also play a part in the maintenance of Koutango,[34] California encephalitis,[35-37] Jamestown Canyon,[38] Japanese encephalitis,[39] yellow fever,[40,41] San Angelo[42] and dengue[43] viruses.

The hypothesis can explain the patterns of RVF virus activity encountered in Africa. An annual emergence of infected *Aedes* mosquitoes may occur in the high rainfall tropical forest belt which traverses much of the continent. Serological studies of human and ruminant populations in riverine and forest edge settlements in such areas suggest that this could be the case.[5,6,9,44-46] Some emergence of RVF infected *Aedes* mosquitoes may be expected to occur at 2-4 year intervals coincident with excessive rainfall which succeeds in flooding their breeding sites in the moderately high rainfall bushed and wooded savannah and grasslands that are contiguous with the tropical forest belt. Such areas comprise a large portion of the continent south of the Sahara. They are characterized by a high plateau terrain east and south of the tropical forest. Epizootics of RVF generally occur at 5-15 years intervals throughout the lower rainfall areas of East and South Africa,[3-5] where cycles of drought are followed by periods of excessive rainfall. The excessive rainfall raises the level of the water table sufficiently to allow widespread flooding of dambo depressions.[47,48] Huge hatches of the ground pool breeding species of *Aedes* mosquitoes then follow,[49,50] these mosquitoes feed preferentially upon the highly susceptible ruminant hosts[51] and should they become infected with RVF by transovarially transmitted virus, secondary transmission cycles can then become established. Many other species of Culicine and Anopheline mosquitoes rapidly build up population numbers in this temporary habitat,[49,50] and it is these conditions that generate epizootics of RVF. A similar sequence may follow in the very low rainfall savannah following exceptional rains. Here the occurrence of RVF epizootics is much rarer, at 15-30 year intervals, but there appear to be foci of RVF infection in these zones.[17,52,53]

EVIDENCE IN SUPPORT OF THE HYPOTHESIS

RVF virus has been isolated from Aedes (Neomelaniconion) lineatopennis during epizootics in Zimbabwe[4,54] and in Kenya.[55] This mosquito occurs in very large numbers following exceptionally heavy rainfall[49,50] as happens in the early stage of RVF epizootics. The species is a floodwater breeding mosquito that oviposits in depressions that are common in the bushed and wooded savannah and grasslands where RVF epizootics occur.[49,50] These depressions may be identical with or related to the geological formations known as "dambos" which have been described in the RVF epizootic areas of Zambia and Zimbabwe.[47,48] They are subject to flooding during the periods of excessive rainfall, which are associated with RVF epizootics in Kenya. Figure 1 illustrates the monthly rainfall in Kenya for the period from 1950-1982 and shows the close relationship between RVF epizootics and periods of excessive rainfall. Other Aedes mosquitoes may be associated with such habitats in different parts of Africa. RVF virus has been isolated from Aedes (Aedimorphus) dentatus in Zimbabwe,[4] Ae. (Aed.) durbanensis in Kenya,[56] Ae. (Aed.) tarsalis group in Uganda,[57] Ae. (Neo.) circumluteolus in South Africa[58-60] and Uganda,[57] Ae. (Neo.) palpalis in Central African Republic,[46] Ae. (Ochlerotatus) caballus in South Africa,[61,62] Ae. (Och.) juppi in South Africa,[53] Ae. (Stegomyia) africanus in Uganda[60] and Ae. (Stg.) dendrophilus (as deboeri demeilloni), in Uganda.[57,63]

Exceptionally heavy short rains in Kenya during October, November and December 1982 totalled some 431 mm. This produced flooding in grasslands on a ranch where RVF had been know to occur (1° 12 S, 37°E at 1510 m). One flooded depression was up to 2 m deep, ovoid in shape and measured 50 x 100 m with many of the characteristics of a dambo. It showed a typical zonal differentiation with predominantly sedge (Cyperus immensus C B Clarke) in the seepage zone with the grass Digitaria abyssinica (A Richard) Stapf in the remainder of the dambo.[47,48] The presence of standing water and a hatch of Ae. lineatopennis eggs in the dambo allowed sampling before any possible exposure to RVF viremic hosts could occur. Third and fourth stage larvae were collected immediately after flooding by dipping at the base of the emergent vegetation. They were placed in plastic pans and allowed to emerge in holding cages at the laboratory. As adults emerged they were removed from the cages and killed by chilling at - 70 C. Pools of approximately 100 individuals were made according to sex and were triturated for virus isolation. This was attempted by the inoculation of a pair of golden Syrian hamsters (Mesocricetus auratus) by the intraperitoneal route.

RVF virus was isolated and reisolated from a pool of 100 adult female Ae. lineatopennis. The epidemiological and ecological association of this species with RVF epizootics supports the hypothesis that the virus may be maintained during interepizootic periods by this mechanism.

FURTHER STUDIES

Transovarially infected Ae. lineatopennis must be shown to be capable of transmitting virus to susceptible hosts. The rate or rates of transovarial infections with RVF virus must be examined in individual Ae. lineatopennis mosquitoes and their F_1 progeny in demes thoroughout epizootic areas, and in populations of widely separated geographic origin. The population biology of Aedes mosquitoes should be examined in the flooded dambo like situations with which epizootic RVF is associated in Kenya and elsewhere. The results of these studies will allow some judgement to be made on the necessity or otherwise for a vertebrate host to be involved in the RVF maintenance system. The importance of other haematophagous arthropods (Culicidae and non-Culicidae) in the transmission and maintenance of RVF virus must also be determined.

CONCLUSIONS

The transovarian maintenance of RVF virus can explain that aspect of the epidemiology which is least understood. The apparent disappearance of the disease from epizootic areas and its sudden reappearance can be related to the ecological conditions which alter the population densities of Aedes mosquitoes. The dambo formations which are subject to periodic flooding are features of many parts of East Africa, Zambia, Zimbabwe and South Africa where RVF epizootics occur. Such flooded depressions appear to play a significant role in the population dynamics of Aedes mosquitoes and may prove to be the key ecological factor in RVF epidemiology.

ACKNOWLEDGEMENTS

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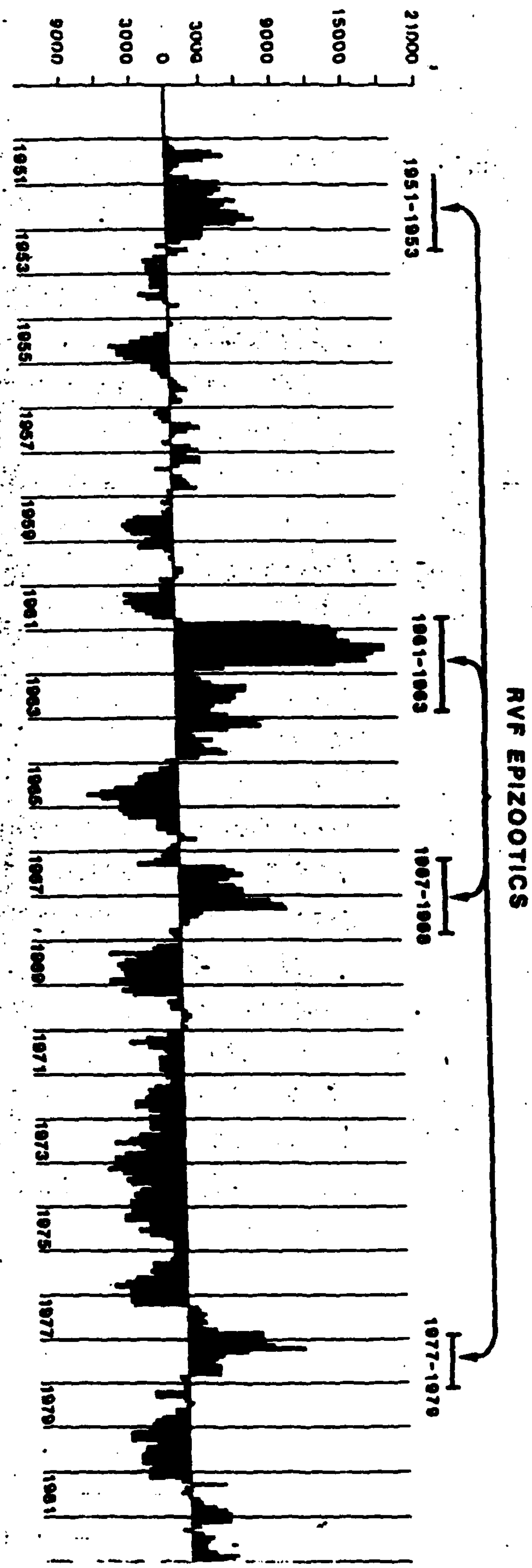
Fig 1. The relationship between RVF epizootics and rainfall in Kenya for the years 1950 - 1982. The graph depicts a composite of mean rainy days and mean rainfall for each month at 3 sites in Kenya where RVF epizootics occur. The zero line represents the 33 year mean rainfall for each month. Anything plotted above the zero line represents a period of excess rainfall.

Composite rain days x rainfall surplus
(12 month rollup)



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