LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF

IDRC-SUPPORTED RESEARCH

IDRC
CRDI
CIID

CANADA

The International Development Research Centre is a public corporation created by the Parliament of Canada in 1970 to support research designed to adapt science and technology to the needs of developing countries. The Centre's activity is concentrated in six sectors: agriculture, food and nutrition sciences; health sciences; information sciences; social sciences; earth and engineering sciences; and communications. IDRC is financed solely by the Parliament of Canada; its policies, however, are set by an international Board of Governors. The Centre's headquarters are in Ottawa, Canada. Regional offices are located in Africa, Asia, Latin America, and the Middle East.

Le Centre de recherches pour le développement international, société publique créée en 1970 par une loi du Parlement canadien, a pour mission d'appuyer des recherches visant à adapter la science et la technologie aux besoins des pays en développement; il concentre son activité dans six secteurs : agriculture, alimentation et nutrition; information; santé; sciences sociales; sciences de la terre et du génie et communications. Le CRDI est financé entièrement par le Parlement canadien, mais c'est un Conseil des gouverneurs international qui en détermine l'orientation et les politiques. Établi à Ottawa (Canada), il a des bureaux régionaux en Afrique, en Asie, en Amérique latine et au Moyen-Orient.

El Centro Internacional de Investigaciones para el Desarrollo es una corporación pública creada en 1970 por el Parlamento de Canadá con el objeto de apoyar la investigación destinada a adaptar la ciencia y la tecnología a las necesidades de los países en desarrollo. Su actividad se concentra en seis sectores: ciencias agrícolas, alimentos y nutrición; ciencias de la salud; ciencias de la información; ciencias sociales; ciencias de la tierra e ingeniería; y comunicaciones. El Centro es financiado exclusivamente por el Parlamento de Canadá; sin embargo, sus políticas son trazadas por un Consejo de Gobernadores de carácter internacional. La sede del Centro está en Ottawa, Canadá, y sus oficinas regionales en América Latina, Africa, Asia y el Medio Oriente.

This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

La présente série est réservée aux documents issus de colloques, aux rapports internes et aux documents techniques susceptibles d'être publiés plus tard dans une série de publications plus soignées. D'un tirage restreint, le rapport manuscrit est destiné à un public très spécialisé.

Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

Leishmaniasis control strategies

IDRC-MR322e
June 1992

Leishmaniasis control strategies: A critical evaluation of IDRC-supported research

Proceedings of a workshop held in Mérida, Mexico, November 25–29, 1991, sponsored by the International Development Research Centre, in collaboration with the Universidad Autónoma de Yucatán (UADY) and the Universidad Peruana Cayetano Heredia (UPCH)

Edited by
Pandu Wijeyaratne, Tracey Goodman
and Carlos Espinal

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
Ottawa • Cairo • Dakar • Montevideo • Nairobi • New Delhi • Singapore

ISBN 088936-653-5

TABLE OF CONTENTS

Clinical and Field Epidemiological Investigations of Kala-azar (Visceral Leishmaniasis) in West Pokot District of Kenya

M.J. Mutinga[1], C.M. Mutero[1], A. Ngindu[2], P.R. Kenya[3]
F. Amimo[1] and S.N. Nahashon[1]

Introduction

During the last few decades, research on kala-azar in Kenya has revealed that the disease is endemic in several arid and semi-arid areas of the country. In order to further understand the epidemiological factors whose interactions result in the creation and maintenance of such foci, a field study was initiated in West Pokot district of Kenya in October, 1985 and successfully completed in March 1988.

The main objectives of the study of were:

- To determine the prevalence of kala-azar in the human population in West Pokot district.
- To undertake ecological studies on the local sandfly species within an established kala-azar focus with particular reference to species composition, seasonal distribution, abundance in different habitats and to investigate their parasite infection rates.
- To investigate the vertebrate reservoirs of kala-azar from a range of local, wild and domestic animals.

Since malaria is also present in West Pokot, a prevalence survey of the disease was carried out parallel to that of visceral leishmaniasis.

Materials and Methods

A) Clinical Survey

Study population: The West Pokot district (35° 15' E, 10° 45' N) is situated in western Kenya along the border with Uganda (Fig. 1). Most of the land lies between 1000 m and 1500 m above sea level. A small area on the south eastern boundary covers part of the

[1] The International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya.

[2] Division of Vector Borne Diseases, Ministry of Health of Kenya.

[3] Kenya Medical Research Institute, (KEMRI).

malaria & leishmaniasis prevalence

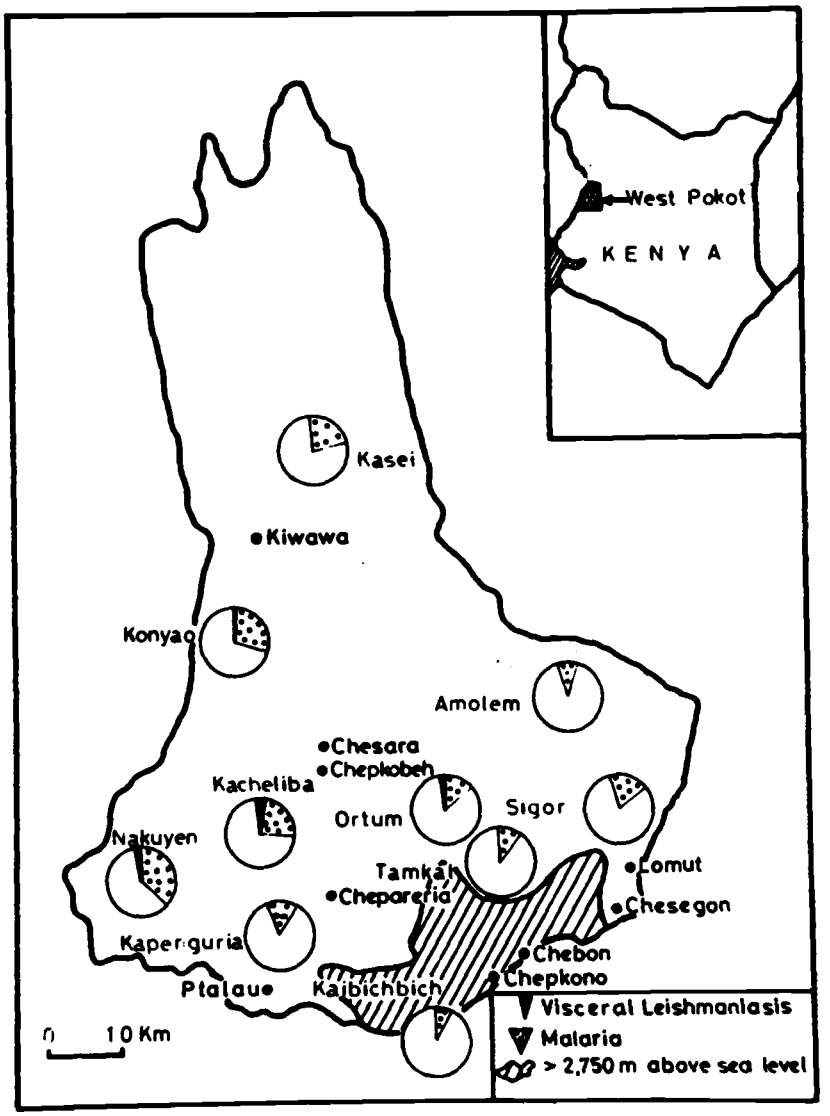

Figure 1: West Pokot District, showing location within Kenya and the proportion of people with Serologically Active Visceral Leishmaniasis and malaria parasites at different screening centres.

Cherangani hills which rise to more than 3000 m above sea level. The vegetation cover comprises acacia woodland and bushland in the lower-lying areas and forests at high altitudes. The mean annual rainfall ranges between 500 mm and 1300 mm. A large portion of the low-lying land is of marginal agricultural potential and the majority of the people are pastoralists. Cultivation of crops is also practised at around 1500m above sea level.

West Pokot has an estimated total population of 210,000 people distributed within 67 administrative sub-locations (Data obtained from the District office). According to the data from the District Office, proportions in the age groups 0-4, 5-14, 15-44, and >45 were 18.5%, 29.8%, 39.8% and 11.9% respectively. About 1% of the population, was used in the epidemiological survey. The sample was derived from nineteen sub-locations (clusters) selected at random. Individuals for screening were systematically selected from a queue at each screening centre. The number of people screened in various sublocations ranged from 50 to 200, in proportion to the relative population of each. In each sub-location, the number screened in each age-group was also proportional to the age group's representation in the whole population. Male and female persons were assumed to occur in equal proportions in the population. The number of each sex included in the screening sample was therefore approximately the same.

Diagnostic methods:

(a) Spleen examination: All persons being screened had their spleens physically checked by either of two techniques described by Bruce-Chwatt (1985). The spleen rate (percentage showing spleen enlargement) was calculated for each sub-location.

(b) Blood films: Thick and thin blood films were prepared for each person using methods described by Bruce-Chwatt (1985). The films were examined under oil immersion to identify malaria parasite species and determine malaria parasite rates.

(c) ELISA: The procedure for ELISA was as described by Ho et al. (1983). During the field survey, each person had one finger pricked with a sterile lancet and 70 µl of blood collected in a heparinized capillary tube. The blood was emptied within a circle of 1 cm in diameter drawn on Whatman No. 3 filter paper. Individual blood samples on filter paper were soaked in 0.5 ml distilled water at 40°C overnight and eluted to provide test sera. The sera were reacted in micro-ELISA plates against antigen prepared from Leishmania donovani previously isolated from a Kenyan kala-azar patient. A positive reaction corresponding to an active case of kala-azar was indicated by a vividly yelow colour with an absorbance value greater than 0.45 at 492 nm. Borderline cases representative of exposure but not the active disease had absorbances between 0.25 and 0.45 while negative cases were consistently below an absorbance of 0.25.

The proportion of reactions corresponding to active disease was expressed as a percentage to provide the kala-azar prevalence rate. Borderline reactions represented

the proportion of people expressing Leishmania antibodies but not having active disease. Active and borderline reactions combined provided the Leishmania antibody prevalence rate.

(d) Leishmanin test: Antigen for the Leishmanin test (Peters and Killick-Kendrick 1987) was prepared from a Kenyan L. donovani strain by Kenya Medical Research Institute (KEMRI) at a concentration of $2x10^6$ organisms per ml. The antigen was reconstituted in 0.5% sterile phenyl saline solution. For each person, one forearm was swabbed with 70% alcohol and injected intradermally with a 0.1 ml dose of the antigen. The other forearm was injected with a 0.1 ml control dose consisting of phenyl saline alone. The reaction on the fore arm was examined after 48 hours and the diameter of the swelling measured in millimetres.

(e) Statistical analysis: Chi-square with Yates correction (Bailey 1981) was used in 2x2 contigency tables to test the difference in disease prevalence between different sexes and age groups. Chi-square was also used in comparing infection rates obtained by various diagnostic methods.

B. Entomological Studies

Sandfly sampling and dissection for Leishmania parasites: On the basis of results from the clinical survey, Kacheliba was selected as an ideal locality for both entomological and reservoir studies. Sandfly sampling was carried out in three different sites around Kacheliba, namely, Sangakai, Kongelai, and Pole. In each site, sandflies were collected weekly from a variety of habitats including houses, termite hills, rodent burrows, tree holes, cow and goat enclosures. Sampling was done using a standard 1 m^2 sticky polythene trap which was set up at at the sites at 1800 hours and removed at 0600 hours the following day. Two such traps were placed in each habitat.

Flies removed form the various traps were washed in water with a little detergent added to it. A subsample was dissected and the gut area examined for Leishmania parasites. The undissected sample including the heads of those dissected were mounted on glass slides for identification into species. Positive guts were inoculated into NNN culture media and taken to Nairobi for further parasite characterization.

Isolation of Leishmania parasites form domestic and wild animals:

Spleen and liver tissue for parasite examination in domestic animals were regularly collected from the local butcher. Small pieces of the organs were homogenized in the field laboratory and inoculated in NNN culture media. Impression smears from the organs were also made on glass slides for the direct examination of Leishmania amastigotes. Media bottles and specimen slides were then transported to Nairobi for examination and further observation on parasite growth.

Results

A summary of results on the clinical survey is presented in Table 1. There was no correlation between the distribution of people reacting positively to the Leishmanin skin test and those having antibodies against Leishmania as demonstrated by ELISA. However, a highly significant positive correlation ($P>0.01$) was found between the distribution of enlarged spleens and the presence of either Leishmania antigens or malaria parasites.

According to Table 1 and Fig. 1, the proportion of people showing antibody levels that were indicative of active kala-azar ranged from zero to 5% in the various sub locations. The overall proportion of people showing either borderline positive reactions or active disease was much higher and ranged form zero to 20%. The highest incidence of active cases in a screening centre was 5% recorded in Kacheliba.

Presence of Leishmania antibodies was more common in low lying areas around Kacheliba than in the higher altitudes at Kaibichbich. The occurrence of active disease was even more limited to lower areas and was virtually absent beyond 2,000 metres above sea level. The distribution of kala-azar active cases and antibodies showed the incidence to be lowest in the age groups below 4 years and also above 45 years. It was highest in the age group 5-14 years.

The prevalence rate of malaria parasites was higher than that of Leishmania antibodies and also more widespread in the district (Fig. 1). A maximum parasite rate of 32.7% was recorded at Nakuyen, a few kilometres from Kacheliba (Table 1 and Fig. 1). Infection rates were highest in the younger age groups but steadily declined with age. Over 95% of malaria parasites belonged to Plasmodium falciparun, the rest being P. malariae and to a less extent P. ovale. Only one case of mixed infection of P. falciparun in association with P. malariae was recorded.

With regards to entomological investigations, a total of 11 sandfly species was recorded from all the three sampling sites. The most common species were Sergentomyia antenuatus, S. bedfordi and S. schwetzi. P. martini occurred in relatively lower numbers and was most common in termite hill and animal burrow habitats (Table 2). The termite hill habitat yielded the highest number of sandflies in Kongelai. Sangakai had the highest number of sandflies collected around houses while Pole had the lowest. Most of the sandflies in Pole were collected near animal burrows.

The monthly fluctuations in relative density of various species showed a major peak in numbers in January, followed by a steady decline until June when the lowest density was recorded. This population trend was most evident in the termite hill and house habitats in Sangakai. The peak sandfly season coincided with the dry period while the lowest numbers were collected a month after the rainfall peak in May.

Table 1. Summary of results from the clinical survey of West Pokot, 1986

Cluster	No. examined	Leishmanin test % positive	Enlarged spleen %	E L I S A (Visceral leishmaniasis) % active	% antibodies	Malaria % positive
Kacheliba	158	0.6	60.8	5.1	17.7	22.9
Nakuyen	101	7.9	22.8	3.0	7.9	32.7
Konyao	150	2.0	16.7	0.7	8.0	23.5
Kasei	50	2.0	2.0	0.0	2.0	15.6
Kapenguria	101	1.0	5.0	0.0	1.0	15.8
Kaibichbich *	50	0.0	0.0	0.0	0.0	4.0
Chepkono *	125	0.0	0.0	0.0	0.8	0.0
Ptalau	200	0.5	0.0	0.0	1.5	2.1
Chebon *	65	---	0.0	0.0	0.0	1.5
Chepareria	126	0.8	4.8	0.8	1.6	4.2
Ortum	142	3.5	5.6	1.4	19.0	11.3
Chepkobeh	87	6.9	26.4	3.4	20.7	6.9
Chesara	124	3.2	2.4	0.8	9.7	13.8
Lomut	103	4.9	17.5	1.0	6.8	13.6
Sigor	96	7.3	15.6	0.0	3.1	20.8
Tamkal	100	13.0	1.0	0.0	0.0	7.0
Chesegon	99	5.1	9.1	1.0	3.0	4.0
Amolem	125	12.0	12.8	0.0	0.0	9.1
Kiwawa	137	---	16.8	1.5	6.6	12.0
Total	2139	4.2	11.5	1.0	5.7	11.6

* Screening centre lying more than 2750 m above sea level.
--- Screening not done.

Table 2: Female P. martini collected from various habitats between January and December 1987

Locality		Housewall (Inside)	Housewall (outside)	Treehole	Termite Hill	Rodent burrow	Cow enclosure	Goat pen
Kongelai	No.	0	0	2	10	-	-	-
	%	0	0	16.6	83.4	-	-	-
Sangakai	No.	1	3	2	19	8	-	-
	%	3	9	6.1	57.6	24.2	-	-
Pole	No.	2	5	-	46	157	9	5
	%	0.9	2.2	-	20.5	70.1	4	2.2

- Sampling not done in the habitat

The relative density of flies collected inside and outside houses varied between the three sites during different months. However, generally more flies were collected inside houses than in traps placed next to the walls outside, as shown for all the three different sites in Figures 3-5.

Infection of phlebotomine sandflies with Leishmania parasites was observed in February and March, 1987. The overall total infection rate was 1.2%. The parasites were obtained from S. antennatus, S. bedfordi, Phlebotomus martini and S. schwetzi. Identification of an isolate from P. martini which grew in NNN medium is pending.

The processing of spleen and liver material from domestic goats was carried out throughout 1987 in order to investigate animal reservoirs. During the period, a total of nine goats, one sheep, two lizards and one rat were found infected with Leishmania parasites. The parasites from goat and sheep have been tentatively identified as L. donovani and L. major using isoenzymes.

Discussion

Several observations were made during the study which may be of major implications in leishmaniasis epidemiology in the East African region. Results on prevalence showed that within the West Pokot focus, the disease existed in several geographically defined micro-foci of varying severity. The disease was also found to be limited by altitude as was clearly indicated by an absence of anti-Leishmania antibodies in people living in high attitudes. Although familial clustering of leishmaniasis has been reported by other workers in the past (Mutinga et al. 1984) the present extensive study provides useful information on the geographical distribution of the disease. This knowledge will be particularly useful in the proper direction of disease control measures to those areas where they are most urgently needed.

In addition to prevalence of leishmaniases, this study yielded important data on the occurrence of malaria in West Pokot district. Malaria infection rates were generally higher than those of leishmaniasis. However, the ranges of these two diseases were found to overlap considerably. A significant positive correlation of the distribution of either disease with that of splenomegally was established, and the occurrence of enlarged spleens alone was therefore considered an inconclusive method for screening the diseases. Nevertheless, the results on the distribution of the two diseases underlined the importance of a multi-disease systems approach in future studies and during the implementation of disease control measures. This is in order to ensure that the resources available for research and improvement of the health of the affected populations are most effectively and economically utilized.

With regard to studies on animal reservoirs, the occurrence of Leishmania parasites in goats and sheep spleen and liver and in skin lesions was particularly

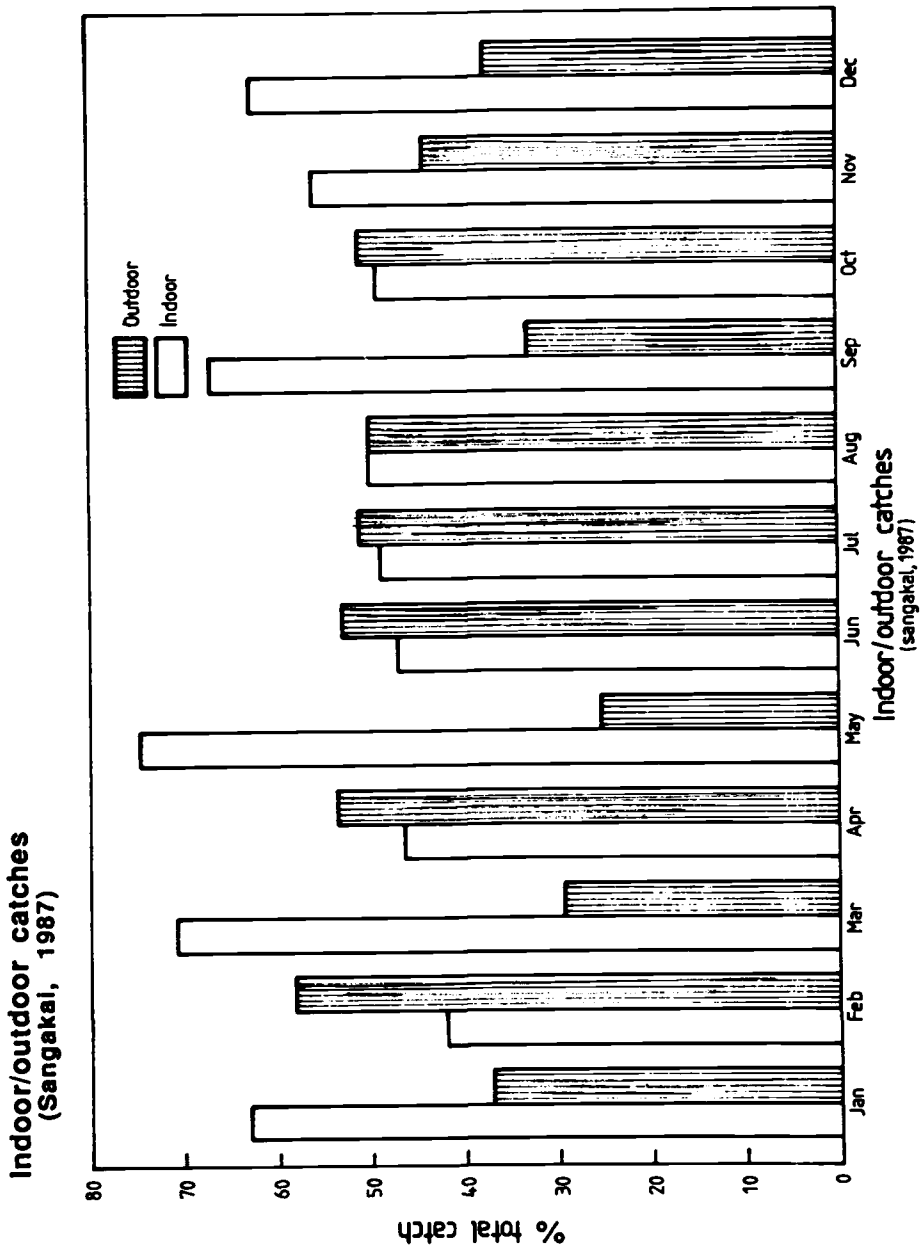

Figure 3: Proportion of sandflies collected outdoors and indoors in Sangakai.

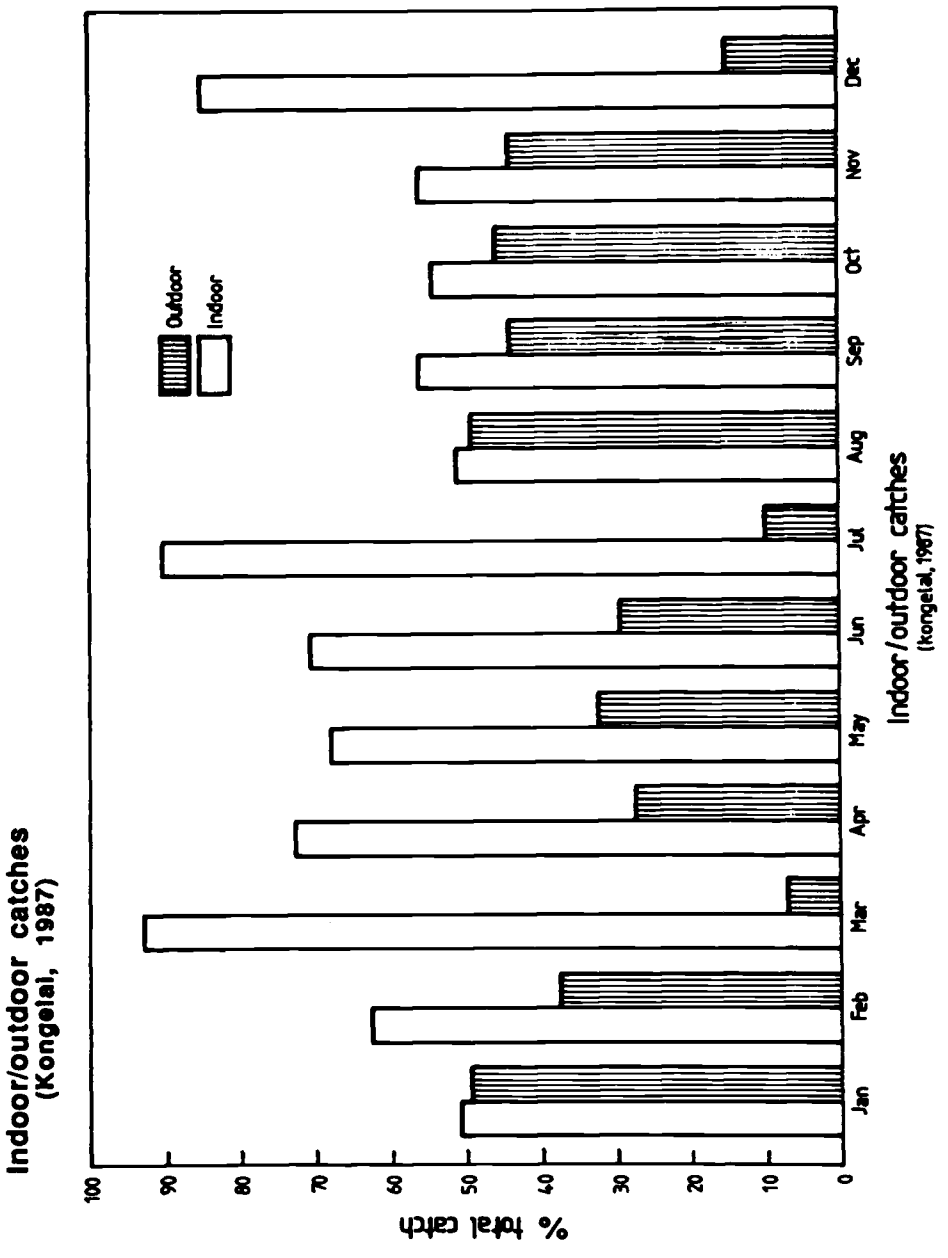

Figure 4: Proportion of sandflies collected outdoors and indoors in Kongelai.

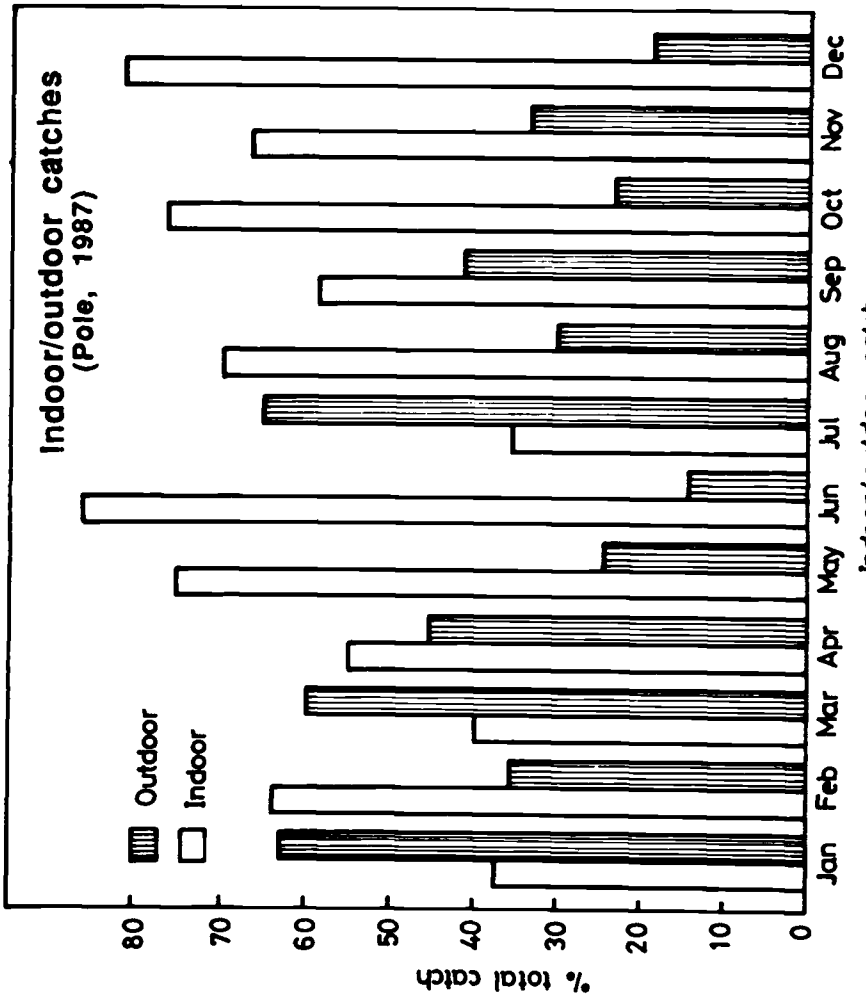

Figure 5: Proportion of sandflies collected outdoors and indoors in Pole.

significant. Workers in Africa and elsewhere have in the past shown very little interest in the possibility of domestic animals being reservoirs of leishmaniases. However, our finding of several infected goats and sheep from within such a small area might suggest that these animals could be reservoirs in the various leishmaniasis foci in the East African region. This observation is especially important in view of the close association that normally exists between people and their livestock in most kala-azar areas. Tentative charcaterization reveal that the goat and sheep leishmania are similar to those that affect man, i.e. L. donovani and L. major.

Results on phlebotomine sandfly studies revealed valuable information regarding their distribution in various habitats during different times of the year. Termite hills and rodent burrows were particularly the favoured concentration sites for the various species. Occurrence of flies in large numbers inside houses suggests probable larval breeding indoors; a phenomenon that has been observed in Marigat area of Kenya (Mutinga, et al. 1989). It is also worth noting that S. antennatus, in addition to being the most common species, also yielded several females with Leishmania parasites. Although Sergentomyia antennatus has not in the past been implicated in the transmission of kala-azar, its possible role requires further investigations both in the field and in the laboratory, in view of recent findings that S. garnhami and S. ingrami are probable vectors of mammalian Leishmania (Mutinga and Odhiambo 1982; Mutinga et al. 1986).

With the establishment of the resting and breeding sites of sandflies in major endemic foci of leishmaniases in Kenya, ICIPE is now well set to embark on a pilot control scheme of sandfly and mosquito populations. It will be possible to target integrated control measures inside houses, around termite hills and animal burrows appropriately as well as breeding places for mosquitoes. Inspired by the simplicity and low cost of the "ICIPE Sticky trap" (Mutinga 1981) for the control of sandflies, made of polythene sheets and smeared with castor oil, the Medical Vectors Research Programme has developed the "MBU Cloth"[4], a simple cloth made from cotton and hung on the wall and impregnated with permethrin insecticide at dosage of 0.5g per m^2. The cloth is hung along the wall of every dwelling of rural house; it is of appropriate size worked out to ensure elimination of female mosquitoes and sandflies before they are able to acquire infection. This technology has been tried by ICIPE on a small scale and has shown very promising results.

It now requires a larger scale trial with very well controlled scientific data gathering to provide information on the prevalence of vector and parasite populations before, during and after the intervention measures. The merits of this new control strategy are:

(a) The cloth is simple and is very easily accepted by the local population. For rural

[4] MBU is a swahili word meaning mosquito.

areas where beds are rarely used, a single wall cloth can protect an entire family sleeping in the same house. The effect of the cloth is apparent almost immediately therefore its usefulness is appreciated quickly by the people.

(b) It is made of locally available raw material, i.e. cotton, and hence it is affordable. Approximately 9 m^2 of the cheapest cotton cloth or material is required and lasts over 4 years.

(c) The permethrin insecticide used is safe and limited in application only to the cloth which hangs on the wall away from children and adults. The impregnated cloth controls a variety of vectors which frequent dwellings such as mosquitoes, fleas and a variety of houseflies.

(d) The insecticide is long-lasting (lasts for 6 months) and the cost of impregnating each house is approximately US$ 0.80.

This new innovation we feel, is an additional new tool to existing ones in an attempt to control leishmaniases and malaria vectors inside houses. For outdoor populations, we still have to continue to look for alternative control measures such as biological control agents, i.e. bacteria and other protozoa and fish.

For leishmaniasis, the question of animal reservoirs both wild and domestic remains still a very crucial issue. New findings strongly suggest the involvement of animals that have previously been least suspected, e.g. domestic goats and sheep . This area needs urgent attention, in our view, in order to know whether or not these very close associates of man contribute to the dissemination of leishmaniases or whether they are mere accidental hosts without threat to health.

REFERENCES

Bailey, N.T.J. 1981. Statistical methods in Biology (2nd Ed.). Hodder and Stoughton, London.

Bruce-Chwatt, L.J. 1985. Essential malariology (2nd Ed.). William Heinemann Medical Books, London.

Ho, M., Leeuwenburg, J., Mbugua, G., Wamachi, A. and Voller, A. 1983. An enzyme linked immunosorbent assay (ELISA) for field giagnosis of visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene, 32, 943.

Mutinga, M.J. 1981. An efficient trap for sandflies (Diptera, Phlebotomidae). Insect Insect Science and Applications, 9, 191-198.

Mutinga, M.J. and Odhiambo, T.R. 1982. Studies on infection rates of human-baited anthropophilic sandflies in Machakos District, Kenya. Insect Science and Applications, 4, 237-240.

Mutinga, M.J., Ngoka, J.M. and Odhiambo, T.R. 1984. Epidemiological investigations of visceral leishmaniasis in the West Pokot district, Kenya. Insect Science and Applications, 5, 521.

Mutinga, M.J., Kyai, F.M., Kamau, C.C. and Omogo, D.M. 1986. Epidemiology of leishmaniasis in Kenya - III. Host preference studies using various types of animal baits at animal burrows in Marigat, Baringo District. Insect Science and Applications, 9, 191-198.

Mutinga, M.J., Kamau, C.C., Kyai, F.M. and Omogo, D.M. 1989. Epidemiology of leishmaniases in Kenya. VI. Wider search for breeding habitats of Phlebotomine sandflies in three kala-azar endemic foci in Kenya. East African Medical Journal, 66, 173-182.

Peters, W. and Killick-Kendrick, R. 1987. The Leishmaniases in biology and medicine, Vol. 2. Clinical aspects and control. Academic Press, London.