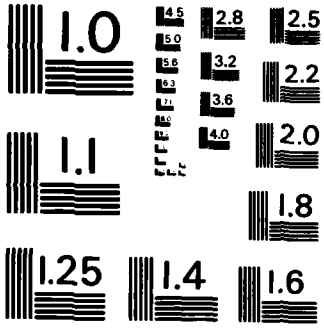
AD-A158 545 STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS(U) 1/1
ARMY MEDICAL RESEARCH UNIT-KENYA APO NEW YORK 09675
I E MURIITHI SEP 82 DAMD17-82-G-9498

UNCLASSIFIED F/G 6/5 NL



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

AD_____

STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS

Annual/Final
1 Oct 81-30 Sep 82

I.E. Muriithi

U.S. Army Medical Research Unit, Kenya
Box 401, APO New York 09675

September 1982

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-82-G-9498

Government of Kenya
Kabete, Kenya

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A158545
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE *(and Subtitle)* STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS	5. TYPE OF REPORT & PERIOD COVERED Annual/Final 1 Oct 81-30 Sep 82
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) I.E. Muriithi	8. CONTRACT OR GRANT NUMBER(s) DAMD17-82-G-9498
9. PERFORMING ORGANIZATION NAME AND ADDRESS Government of Kenya* Kabete, Kenya	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A871.AH.088
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701-5012	12. REPORT DATE September 1982
	13. NUMBER OF PAGES 14
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)* Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. RIBUTION STATEMENT *(of this Report)*
App. oved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES
*P.I. Address:
U.S. Army Medical Research Unit--
Kenya
Box 401, APO New York 09675

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*
Kenya
Trypanosomiasis
Vaccine
Africa
Cattle
Goat
Immunity

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
During this period the investigators continued to monitor the antigenic stability of parasites from western Kenya. Studies were conducted to assess the possible role of mechanical transmission in spread of T. rhodesiense. In a cow-cow study it appeared that this mode of transmission is unlikely to play a major role and would not therefore negate a metacyclic vaccine. It is not possible to totally rule out this form of transmission in cow-man or man-man epizootics but the (see reverse)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Block 20 (continued):

negative data would not support a human volunteer study. In conjunction with local health authorities extensive patient follow up studies have been initiated to determine the extent of treatment failure/relapse and reinfection after a course of therapy recommended by WHO. An experimental model utilizing the goat is being evaluated since it appears that uniform central nervous system disease can be produced in a short period of time. This CNS disease is uniformly fatal if not successfully treated.

Introduction

Vaccine development remains a high priority in African trypanosomiasis research efforts. The Lambwe Valley, Kenya study area countinues to yield a stable serodeme of T.b. rhodesiense. The 1980-81 zoonotic in humans and domestic animals was brought under control in Feb-Apr 1981 largely as a result of an aerial application of insecticide. Monitoring of the tsetse population and epidemiologic surveys of human and cattle populations were initiated immediately after the spray campaigns and continue to the present. These studies will furnish new data on the effect of environmental pressures on tsetse and trypanosome populations.

Visceral leishmaniasis in East Africa, although subjected to much study, remains poorly understood. There is a paucity of data concerning host-parasite-drug interactions. Adequate second and third line drugs do not exist and the subject of parasite resistance vs patient non-responsiveness to therapy remains a subject of debate. Vector-reservoir relationships also are poorly understood. Objectives include better documentation of the action of available drugs both in vivo and in vitro, better definition of "resistance", biochemical typing of both parasites and vectors, and expansion of vector-reservoir field studies.

African Trypanosomiasis

Approximately 11,300 individuals from 1,300 households were either interviewed or accounted for by the head of the household. Figure 1 shows the survey areas and the location of cases. During the survey blood films were prepared for microscopic examination. Individuals with histories or clinical signs of trypanosomiasis were contacted later for more extensive laboratory workups. These examinations included the collection of blood for rodent subinoculation, complement fixation studies and serum protein electrophoresis. Analysis of these specimens is underway.

A limited survey of Kisegi (Fig 1) was performed in the vicinty of the home of a patient that had no contact with the Lambwe Valley. Two hundred fifty-two (252) humans were examined and 157 cattle. No further human cases were identified but 15 of 157 (10%) cattle were found to have Trypanosoma brucei type parasite by rodent subinoculation. G. pallidipes was not found in the area but G. fuscipes were collected in moderate numbers. G. fuscipes feeds predominately on cattle but will bite man. Studies are underway to characterize the parasites isolated from cattle and to compare these to those collected from humans and cattle in the Lambwe Valley.

New patients began to present in Oct 1981 but remained few in number until July 1982. From July to October 1982 approximately 20 cases presented to Homa Bay District Hospital. A corresponding increase in isolates of T.b. brucei from cattle and a return of tsetse flies in numbers equal to or greater than found in the Lambwe Valley during the 1980-81 outbreak strongly suggest the onset of a new transmission cycle which may become epidemic in scope.

The Kenya Trypanosomiasis Research Institute is in the process of establishing a central treatment facility for the care of sleeping sickness cases. This decision has been prompted by the sudden increase in patient load placed on the Homa Bay District Hospital, the need to have a more specialized facility and staff to adequatly evaluate and treat these patients and the need for a research-oriented patient care facility if clinical drug trials were to be performed. The Walter Reed Army Institute of Research has been requested to provide a clinician and to participate in this program.

Mechanical transmission studies utilizing a bovine-tsetse-bovine system in the laboratory indicate that this mode of transmission is probably not a significant factor in field transmission.

Interrupted feedings of teneral, laboratory-reared Glossina morsitans morsitans were used to study mechanical transmission of Trypanosoma brucei rhodesiense. Intervals between exposure of individual flies on parasitemic rats and refeedings on clean rats were varied from 5 min to 24 hr. Direct transmissions were demonstrated at each interval up to 160 min post-exposure. Proboscis dissections showed that active trypanosomes were present up to 320 min post exposure. No mechanical transmissions from bovine-to bovine occurred in 39 attempts, when groups of 20-120 flies exposed on parasitemic bovines were transferred immediately to uninfected cattle, but 2/40 individual flies exposed on parasitemic bovines mechanically transmitted trypanosomes to clean rats. Proboscis dissections done immediately after flies were exposed to a bovine with a parasitemia of 4.8×10^4 trypanosomes per mm^3 of blood showed that 11/20 (55%) had active trypanosomes in the food canal. The mean number of trypanosomes per proboscis (±1SD) was 29.4 (±20.5). Of 20 flies exposed on a bovine with a low parasitemia, however, only 1 trypanosome was seen in proboscis dissections. The parasitemia of the infected donor was an important factor in mechanical transmission. It appeared that an individual mechanically infected fly might not transmit a human-infective dose during refeeding. Previous work demonstrating transmission by probing and the more frequent feedings of infected flies more likely explain high transmission rates with a low percentage of infected flies.

Leishmaniasis

A comparison of three dosage regimens of sodium stibogluconate (Pentostam(R)) in the treatment of visceral leishmaniasis in Kenya was undertaken. Previously untreated patients were randomized to receive 31 doses of sodium stibogluconate, 10 mg Sb/kg per dose, administered once daily for 31 days (group A), every 12 hours for 15 days (group B) or every 8 hours for 10 days (group C). Of the 29 patients who completed treatment, 26 appeared cured 3 to 12 months later. Two patients in group B who initially responded to treatment relapsed 6 weeks after discharge but appear to have been cured by further treatment with sodium stibogluconate at 20 mg Sb/kg/day for 60 days. A third patient in group B failed to respond to initial treatment. None of the treatment regimens was toxic. Parasites disappeared from splenic aspirates more quickly and hemoglobin levels rose more rapidly in patients receiving sodium stibogluconate every 8 hours. Treatment of visceral leishmaniasis in Kenya with sodium stibogluconate at a dose of 10 mg Sb/kg every 8 hours for 10 days appears to be a safe and effective alternative to conventional treatment.

Quantitation of amastigotes of Leishmania donovani in smears of splenic aspirate from patients with visceral leishmaniasis. During a 19 month period, more than 500 splenic aspirations were performed in 79 patients with suspected or proven visceral leishmaniasis. The two complications which occurred (intra-abdominal bleeding and penetration of the intestine in one patient each) both resolved with conservative management. Parasite density in splenic aspirate smears was graded on a logarithmic scale from 0 (no parasites in 1,000 microscopic fields) to 6+ (> 100 parasites per microscopic field). Among 39 newly diagnosed and 17 relapsed or drug resistant patients with visceral leishmaniasis, the average initial parasite grade was 4.29 ± 0.97 (mean ± s.d.) and 4.15 ± 1.37, respectively. The grading system was useful in measuring the speed of response to treatment and in distinguishing slow responders from non-responders. This was especially valuable for managing patients with drug-resistant visceral leishmaniasis. The system also provided a means of comparing the efficacy of different tratment regimens and for calculating the optimum duration of treatment.

A comparison of microscopy and culture in the detection of Leishmania donovani from splenic aspirates. Three culture media were compared with Giemsa stained smears for the detection of Leishmania in splenic aspirates from Kenyan patients with visceral leishmaniasis. Ninety-nine splenic aspirates obtained from 26 patients at various times before, during and after treatment were cultured in Schneider's Drosophila medium and RPMI medium 1640, both supplemented with 20% fetal bovine serum, and McConnell's modification of Senekje's medium overlayed with 0.9% saline. From 13 splenic aspirates obtained before treatment, amastigotes were identified microscopically in all and promastigotes were cultured

in 12. During and after treatment. Schneider's medium was the most sensitive method for detecting parasites, followed by microscopic examination of stained smears which was more sensitive than either of the other two media tested.

Experimental East African cutaneous leishmaniasis. Eleven strains of cutaneous leishmania (8 East African, 2 Old World and 1 New World) were inoculated into BALB/c mice and lesion development and progression of infection were studied. BALB/c mice were susceptible at varying degrees to 8 of the 11 strains tested. In general, infections with Leishmania aethiopica were variable and inapparent. Parasites could be cultured from the noses of infected mice, however no swelling or lesions appeared. One strain of L. aethiopica produced lesions in 2 of 5 mice inoculated at 60 days post-inoculation. L. aethiopica infections did not visceralize in BALB/c mice. Inoculation with L. tropica minor also resulted in parasites in the nose without visible lesions. L. mexicana lesion development was slow and progressive with visible nose swelling beginning at 40 days PI. Visceralization did not occur with either L. tropica minor or L. mexicana in this strain of BALB/c mice. Inoculations with L. major produced fulminating, fatal infections in BALB/c mice. Visceralization and metastasis of lesions occurred in all animals. Albino WRAIR mice and golden hamsters were also susceptible to L. major.

A survey to examine small mammals for leishmanial parasites was initiated in the Perkerra Settlement Scheme, Baringo District, Rift Valley Province, Kenya. A total of 789 animals of 10 different species were trapped and examined. Leishmanial parasites were isolated from the spleens of 9 animals of 5 different species: 7 from Tatera robusta, 2 from Taterillus emini, 5 from Arvicanthis niloticus, 1 from Aethomys kaiseri and 2 from Mastomys natalensis. The isolations of Leishmania from Taterillus and Aethomys are the first recorded from these rodents in Africa.

Transmission of Leishmania donovani by experimentally infected phlebotomine sandflies. Evidence that the sandfly Phlebotomus martini Parrot is a vector of Leishmania donovani in Kenya includes its anthropophilic biting habits, its presence in areas where kala-azar is endemic or epidemic and the isolation, from this species, of leishmania parasites which are infective to man and indistinguishable, based on enzyme typing, from human-derived strains of L. donovani Additional confirmation of the vector status of this sandfly is provided in the present study. P. martini females, from a recently established laboratory colony, were experimentally infected with L. donovani and subsequently transmitted the parasite to hamsters while taking a blood meal.

Two problems arise in doing transmission work with P. martini. Hamsters, infected with L. donovani are not, in our experience, always infective to sandflies that feed on them; and female sandflies, which produce eggs following a single blood meal, usually die in the act of oviposition and are therefore not available to take a second 'transmission' blood meal. To circumvent these problems, fly infections in the present study were achieved by membrane feeding 2 day old females on cultures of L. donovani promastigotes (RPMI 1640 plus 20% FBS; 10^5 parasites/ml). All P. martini fed this way develop heavy midgut infections but do not produce eggs. Such flies continue to display normal biting behavior and can therefore be used in attempts to infect hamsters.

To assess the vector competence of P. martini with this system, 20 females were infected with L. donovani via membrane feeding, held for 7 days, then allowed to engorge on uninfected hamsters (5 females/hamster). The hamsters were held for 45 days at which time cultures (RPMI 1640 plus 20% FBS) were made to detect the presence or absence of parasites. Three out of 4 hamsters bitten by P. martini were culture positive for L. donovani. Larger scale experiments, using different concentrations of parasites for fly infection are currently underway.

Phlebotomus (Phlebotomus) duboscqi from Kenya: a new record. A group of light trap-captured sandflies collected in Baringo District, Rift Valley Province; Kenya (0° 30'N. Lat., 36°E long); included a single male which has been identified as Phlebotomus (Phlebotomus) duboscqi Neveu-Lemaire, 1906. This is a new record for Kenya and represents the first time any member of this medically important subgenus has been taken in the country.

In conjunction with this finding it is interesting to note that P. duboscqi is thought to be a vector of cutaneous leishmaniasis (Leishmania major) in a wide area of North Africa. While no cases of human disease due to L. major have been reported in Kenya, Leishmania from rodents, captured at Baringo, are biochemically and serologically identical to parasites isolated from human cases of cutaneous leishmaniasis in Senegal suggesting a reservoir of L. major in Baringo. The presence, here in Kenya, of an L. major-like parasite together with a putative vector of cutaneous leishmaniasis is an interesting situation that merits further investigation.

RECOMMENDATIONS

African trypanosomiasis

It is recommended that the Lambwe Valley study be continued with emphasis on case followup and evaluation of the demographic data. The typing studies using VAT, isoenzymes and neutralization techniques should continue and be coupled with attempts to identify immunologically important antigens. In the light of the reported relapse data, increased efforts should be made to reevaluate existing drugs and added emphasis should be placed on new drug development. The use of serological testing should be expanded.

Leishmaniasis

Drug efficacy and pharmacokinetic studies should continue on currently available compounds until such time as new compounds or new formulations are available for field trials. Vector-reservoir field studies should be expanded. Controlled biochemical typing, morphologic taxonomy and transmission studies should be implemented as colony raised sandfly become available.

Publications

1. Beach, R.F., Young, D.G. and Mutinga M.J.: Phlebotomus (Phlebotomus) duboscqi from Kenya: a new record. Trans. Roy. Soc. Trop. Med. Hyg. 76(5), 707.

2. Preston, J.M., Kovatch, R.M. and Wellde, B.T.: Trypanosoma congolense: Thrombocyte Survival in Infected Steers. Exp. Parasit. 54, 129-133.

3. Roberts, L.W., Boyce, W.L. and Lyerly, W.H.: Cordylobia anthropophagia (Diptera: Calliphoridae) Myiasis in an Infant and Dog and a Technique for Larval Rearing. J. Med. Ent. 19(3), 350-351.

Manuscripts In Press

1. Beach, R., Young. D.G. and Mutinga, M.J.: New Phlebotomine Sandfly Colonies: Rearing Phlebotomus martini, Sergentomyia schwetzi and Sergentomyia africanus (Diptera: Psychodidae). J. Med. Ent.

2. Chulay, J.D., Bhatt, S.M., Muigai, R., Ho, M., Gachihi, G., Were, J.B.O., Chunge, C. and Bryceson, A.D.M.: A Comparison of Three Dosage Regimens of Sodium Stibogluconate (Pentostam$^{(R)}$) in the Treatment of Visceral Leishmaniasis in Kenya. J. Inf. Dis.

3. Chulay, J.D. and Bryceson, A.D.M.: Quantitation of Leishmania donovani Amastigotes in Splenic Aspirate Smears. Am. J. Trop. Med. Hyg.

4. Chulay, J.D., Haynes, J.D. and Diggs, C.L.: Plasmodium falciparum: Assessment of in vitro Growth by [^{3}H] Hypoxanthine Incorporation. Exp. Parasit.

5. Lightner, L.K., Chulay, J.D. and Bryceson, A.D.M.: A Comparison of Microscopy and Culture in the Detection of Leishmania donovani from Splenic Aspirates. Am. J. Trop. Med. Hyg.

6. Wellde, B.T., Chumo, D.A., Adoyo, M., Kovatch, R.M., Mwongela, G.N. and Opiyo, E.A.: Haemorrhagic Syndrome in Cattle Associated with Trypanosoma vivax Infection. Trop. An. Hlth. Prod.

Manuscripts Submitted

1. Chulay, J.D., Spencer, H.C., Warshow, M.M., Mson, L., Saio, M.A., Musoke, S.S., Masembe, J.B., Rollins, A.J. and Churchill, F.C. II: Fatal Chloroquine Resistant Plasmodium falciparum Malaria from Tanzania.

2. Lightner, L.K.: In Vitro Cultivation of Kenya Leishmania

3. Lightner, L.K. and Reardon, M.J.: Dipetalonena dracunculoides in Dogs and Spotted Hyena (Crocuta crocuta) in the Turkana District of Kenya.

4. Lightner, L.K., Reardon, M.J. and Giture, J.I.: Isolation of Leishmanial Parasites from a Dog in the Turkana District of Kenya.

5. Roberts L.W., Wellde, B.T., Reardon, M.J. and Onyango, F.K.: Mechanical Transmission of Trypanosoma brucei rhodesiense by Glossina morsitans (Diptera: Glossinidae).

6. Spencer, H.C., Kipingor, T., Agere, R., Koech, D.K. and Chulay J.D.: Plasmodium falciparum in Kisumu, Kenya: differences in sensitivity to amodiaquine and chloroquine.

7. Spencer, H.C., Masaba, S.C., Chulay, J.D. and Nguyen-Dinh, P.: Field Evaluation in Kenya of the 48-Hour In Vitro Test for Plasmodium falciparum Sensitivity to Chloroquine.

8. Watkins, W.M., Sixsmith, D.G. and Chulay, J.D.: The Activity of Proguanil and Its Metabolites, Cycloguanil and p-Chlorophenyl-biguanide, Against Plasmodium falciparum In Vitro.

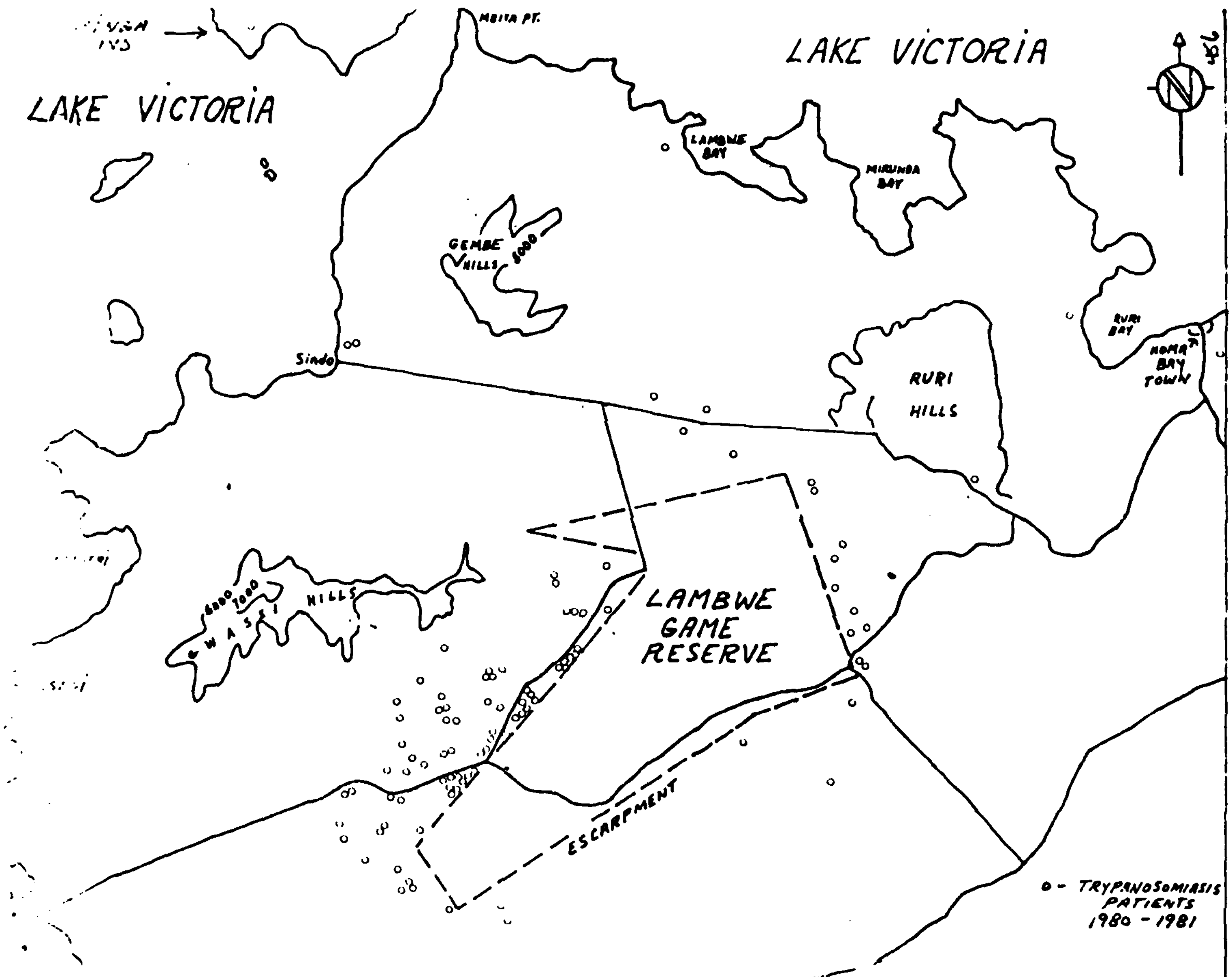

LAKE VICTORIA
LAKE VICTORIA
MBITA PT.
LAMBWE BAY
MIRUNDA BAY
GEMBE HILLS 8000
RURI BAY
HOMA BAY TOWN
Sindo
RURI HILLS
GWASSI HILLS
8000
7000
LAMBWE GAME RESERVE
ESCARPMENT
o - TRYPANOSOMIASIS PATIENTS 1980 - 1981
N

Fig 1

DISTRIBUTION LIST

Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
ATTN: SGRD-UWZ-C
Washington, DC 20307-5100

Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick, Frederick, Maryland 21701-5012

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

END

FILMED

10-85

DTIC