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EPIDEMIOLOGY AND EPIZOOTIOLOGICAL INVESTIGATIONS
OF HEMORRHAGIC FEVER VIRUSES IN KENYA

FINAL REPORT

PETER M. TUKEI

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Virus Research Centre
Kenya Medical Research Institute
P.O. Box 20752
Nairobi, Kenya

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The following has been achieved. A virus containment facility was established in the Virus Research Centre (VRC) permitting the safe handling of specimens suspected to contain haemorrhagic fever viruses. Incidence and prevalence rates of disease and antibodies to Marburg and Ebola viruses have been determined in an area in Kenya suspected to be a focus for these diseases. A surveillance system for viral hemorrhagic fever viruses based on selected hospitals in Western Kenya has been instituted.					
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In depth studies of the circumstances surrounding suspected cases of AHVF were carried out with a view to identifying the source of infection.

Seroepidemiological studies of contacts of suspected cases have been made in order to determine the extent of spread of these viruses.

Indirect immunofluorescence has been utilized as a rapid method for antibody detection in the field laboratory as well as in the base laboratory. No method for antigen detection became available during the period of study.

Intensified field ecological studies within Kitum Cave and its environs have attempted to implicate vertebrates and/or invertebrates as reservoirs of Haemorrhagic fever viruses particularly Marburg virus. The final results of this particular investigation will be reported at a later date.

The grant has established a capability and capacity for VRC to investigate any future occurrence of epidemic or sporadic cases of viral haemorrhagic fevers.

Application for
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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council (DHHS, PHS, NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

Background Information on African Viral Haemorrhagic Fevers in Kenya

Research work done in Kenya has shown that three haemorrhagic fever viruses occur in the country. These are Rift Valley Fever Virus (RVF), Crimean - Congo Haemorrhagic Fever virus (CCHF) and Marburg virus. Serological evidence of the occurrence of Ebola Virus (strains Zaire and Sudan) has also been obtained. The specificity of these serological reactions have not been confirmed for certain. There has been no evidence of Lassa fever anywhere in East Africa. Dengue virus type 2 has been isolated in Coastal Kenya once but with no haemorrhagic manifestations.

Marburg virus was initially recognized in Marburg and Frankfurt, Germany and Belgrade in Yugoslavia in 1967. A very fatal nosocomial infection associated with tissues of imported Green Monkeys from Uganda affected tissue culture technicians and eventually hospital staff. Seven out of 31 infected scientists died. Field investigations in the trapping and holding areas in Uganda did not indicate the source of infection.

In 1975, however, a second episode of Marburg infection was described in South Africa in an Australian hitch-hiker and his girlfriend. The source of infection was most likely in Zimbabwe.

In 1980 in Kenya, an expatriate Frenchman working as an electrician in a sugar factory in Western Kenya died in a Nairobi hospital with severe haemorrhagic symptoms. He subsequently infected his attending physician from whom the diagnosis of Marburg virus infection was made. The physician recovered. Extensive investigations carried out by the staff of the Virus Research Center (VRC) indicated that the potential nosocomial outbreak was well contained. Out of 52 hospital contacts only two were infected. A survey carried out in Western Kenya revealed a seroprevalence of 1% out of 700 possible and susceptible individuals in the area.

One significant observation at this stage was that the index case had visited a cave (KITUM) in Western Kenya 9-12 days prior to falling ill.

In August 1987, a young Danish boy died in a Nairobi hospital with haemorrhagic symptoms. The VRC recovered Marburg virus from his serum in tissue culture. These findings were subsequently confirmed by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). Epidemiological information

available (see details later) indicated that the boy had visited the KITUM cave 9 days earlier with his parents.

Rift Valley fever virus was initially described by Daubney, Hudson and Garndham in 1931 in Kenya. Since then several other RVF virus isolations have been made in mainly animals and arthropods. There has never been any major human epidemic with RVF. Most of the human infections have been associated with laboratory accidents or contaminations. Rift Valley Fever in Kenya is therefore seen as a very significant veterinary problem. It causes abortions (90%) in sheep, cattle and goats. Epizootics of this nature have occurred sporadically in Kenya in a 10-year cyclic fashion associated with heavy flooding. Persistence of the virus in the inter-epizootic years is now thought to be in the eggs of certain flood-water species of Aedes mosquitoes.

In recent years, however, RVF virus seems to have moved northwards to Sudan and Egypt along the Nile delta. A large epizootic occurred in Sudan in 1974, and in 1977 a human epidemic was recorded in Egypt where an estimated 20,000 to 200,000 cases could have occurred with 600 deaths.

Crimean Haemorrhagic Fever is also caused by a virus of the Bunyaviridae family, genus Nairovirus and is related to Nairobi Sheep Disease virus. The virus is transmitted by ticks - Ixodid or Argasid which also act as reservoirs. The natural cycle of the virus is between ticks and rodents etc. Transmission to man is tangential through infected tick bite or contamination with infected ticks. In Kenya, however, this virus has been isolated only once from a sick cow and serological evidence in humans was detected on three other occasions. The illness was associated with haemorrhagic manifestations. The general sero prevalence is less than 1% even in pastoralists of Kenya.

Recent Serological Findings of Ebola Haemorrhagic Fever Virus in Kenya

In our progress report on Grant DAMD17-83-G-9535 (Dr. Peter M. Tukei and Dr. Bruce Johnson) covering the periods May 1984 - October 1985, we made the following observations:

1. 471 patients were detected by a passive surveillance system to have symptoms and signs compatible with viral haemorrhagic fevers i.e., high fever, headache, chest, abdominal, muscle, joint and back pain, diarrhea and vomiting with or without blood and sore throat.
2. The mean age of these patients was 21.4 years.
3. Ebola antibody seemed to have been the most common i.e., 96 (9.8%) of all suspected cases.
4. Both the Zaire and Sudan serotypes of Ebola virus reacted.
5. These Ebola seropositives were encountered in all the hospitals in Western Kenya that were participating although the highest rates were found in Nzoia and West Pokot areas.
6. The mortality rates, however, in Ebola seropositives and Ebola seronegative patients was not significantly different.
7. Increased Ebola virus activity was shown to coincide with the end of the long (June-July) rains and short rains (Dec-Jan). This is the first time seasonal activity has been demonstrated with filovirus activity.
8. There was a lack of virus isolation.
9. The specificity of the Ebola sero reactivity could not be confirmed in the absence of virus isolation.

Proposals for Active AHFV Surveillance in Western Kenya

Observations described in the previous reports although strongly indicating that filoviruses are active haemorrhagic fever viruses in Western Kenya, needed in-depth active surveillance studies to confirm the findings. It was unclear whether the Ebola seropositives are a result of the highly pathogenic Ebola virus or by as yet unidentified less pathogenic

but serologically related member of the filovirus group. Previous recorded outbreaks of Ebola virus in Zaire and Sudan, which were typically nosocomial, had mortality rates of 60-90%. In our observed cases in Western Kenya, the mortality rate was only 5%. This remarkable difference has been attributed by us to a much higher level of medical care in these hospitals in Western Kenya compared to the rural health units that were involved in Zaire and Sudan.

The reservoirs of these viruses in Western Kenya have as yet to be determined.

The proposals under Grant DAMD17-86-G-6016 had the objectives of:

1. Institute active surveillance in four hospitals in Western Kenya viz:
 - a. Ortum Mission Hospital (West Pokot District)
 - b. Misikhu Mission Hospital (Bungoma District)
 - c. St. Mary's Hospital, Mumias (Bungoma District)
 - d. St. Elizabeth Hospital, Mukumu (Kakamega District)The above hospitals were selected due to the stability of their medical staff and their willingness to cooperate with our research staff.
2. Institute active virus isolation attempts at the VRC.
3. Prepare contingency plans for further epidemiological and ecological investigations in the event of a virus being isolated.
4. Prepare virus isolates for further characterization of USAMRIID.

Activities Undertaken

1. Active Surveillance

Detailed discussions were held with the physicians in each of the four sentinel hospitals. Requirements for staff and materials were worked out. It was agreed that an experienced physician, preferably one who had worked in Western Kenya, be recruited to lead the field team and be on the spot to supervise the day to day activities. In July, Dr. Peter Petit, a Dutch

physician, who had worked in Western Kenya was recruited and took up residence in Kakamega.

A team of three public health nurses and one clinical officer were recruited, one for each hospital.

The hospitals were then equipped with long-term liquid nitrogen containers for storage of serum samples. Other laboratory and clinical supplies were made available.

In VRC, one senior laboratory technologist, Mr. David Ochieng, was hired and designated Chief of Central Operations. He supervised virus isolations, serological testing, storage of specimens and trans-shipment to USAMRIID. He coordinated purchases of supplies, liquid nitrogen and the distribution of these to the periphery. Twice a month he delivered liquid nitrogen to the peripheral hospitals and picked up sera and other specimens collected from the field.

In the field, Dr. Petit and his teams took detailed histories and clinical findings in patients suspected to have viral haemorrhagic fevers. Detailed epidemiological histories were also recorded including occupation, history of recent travel outside Western Kenya, family histories of similar illness etc. Ten ml of blood was collected from each suspect on the day of admission, another sample or two during the acute stage, and a final one on discharge from hospital. In families where there was a history of more than one case, the clinical officer or nurse made a brief follow up and obtained samples of blood from family members for serology and or virus isolation.

2. Virus Isolation Attempts in VRC

Haemorrhagic fever viruses are hazardous to culture and handle in conventional type I and two biohazard hoods. It was therefore necessary to construct an absolute virus containment facility within VRC. This consists of an anteroom, changing rooms, and airlock. The floors, windows, ceiling and walls were appropriately sealed so that the facility can be maintained at negative pressure to the exterior. Air is extracted and filtered through two absolute Hepa filters. Operators, who are the only persons permitted inside the facility, change their street clothing on entering and adorn special laboratory gowns, masks, headgear and gloves and boots. On exiting, they discard these into disinfectant, wash, and put on their street clothes. Specimens for virus isolation are manipulated inside three vickers plastic isolators which are also under negative pressure to the laboratory. Air from these isolators is discharged to the

exterior of the building through HEPA filters. Other support equipment within the unit include Revcos, deep freezers, refrigerators, incubators, water baths, microscopes, autoclaves etc.

Cell Cultures System

Four cell lines were regularly maintained: vero, SW-13, CV-1 and BHK21. A stock of these were stored in liquid nitrogen in one ml ampoules. Each cell line was grown up and passaged for no more than six times to prevent loss of sensitivity.

Acute serum from field material was maintained in liquid nitrogen in the field hospital, transported in LN to VRC and maintained in LN until used to inoculate cell lines in 25 ml plastic flasks. Serum was absorbed onto the cells for 1 hour at 37 degrees centigrade before adding maintenance medium. Cells were then observed daily for CPE. Any cells that showed a CPE were harvested and tested by indirect IF and the supernatant passaged. Where convalescent serum had been obtained this was also used in IF.

3. Epidemiological Follow-up Investigations

The field resident physician was able to visit each of the participating hospitals each week to review hospital records in order to detect any additional suspected cases. He also conferred with the hospital physicians and discussed possible diagnoses of the suspected cases. For those cases that diagnoses of AHF were highly suspicious, and particularly if another family member of a neighbor had a similar history, the resident physician and the public health nurse or clinical officer visited the family. More detailed histories of illness within the family were obtained, history of movements and activities were recorded and finally blood samples were taken from family members and the neighbors as well.

Twice every month, the principal investigator and/or Dr. Bruce Johnson from VRC in Nairobi visited the field to replenish supplies and to discuss progress and give a feedback on results obtained. Field specimens were then taken back to VRC in Nairobi.

The field physician also undertook basic field serological surveys in areas where seropositives were demonstrated. These included in Turkana, Ortum Valley and mountains and in Western Province, Nzoia Sugar Factory, Sangalo, Namirania area and Kakamega forest and Kisii.

RESULTS

Between June 1986 and July 1987 a total of 1,118 suspected viral haemorrhagic fever cases were observed in seven hospitals. The field physician included three more hospitals in his field rounds for only two months viz: Lodwar District Hospital, Kakuma Mission Hospital and Kilgoris Health Center, see Figure 1.

Antibody positives were detected in all the seven stations. Table 1 shows numbers positive at each station and for which virus. Over all positives 140/1,118 (12.5%) were positive to Ebola virus with a range of 10% - 16%. C-CHF and Marburg positives were found in 0.98% and 0.70%, respectively. No antibodies were detected against RVF and Lassa.

Ebola Virus Antibodies in Fever Patients

Ebola virus antibodies are more prevalent among patients in the more arid areas of Turkana compared to the higher rainfall areas of Western Province. In Kakuma and Lodwar the rate is 12/77 (15.6%) compared to 81/626 (13%) in Western Province (Mukumu, Mumias and Misikhu).

Table 2 shows the provisional clinical diagnosis of 140 patients who were found positive for Ebola antibody. Malaria diagnosis accounted for 21.5%, followed by fevers of unknown origin 12.1%, respiratory infection 12.1% and abortion premature delivery 10.7%.

The proportion of Ebola antibody positives with specific provisional diagnoses of the total number of patients admitted with that diagnosis reveals three provisional diagnoses which are not randomly distributed when tested for homogeneity. Respiratory infection with 23% having EBO antibody and abortion and premature delivery with 18% EBO positive. Both of these are much higher than expected ($P < 0.05$). The provisional diagnosis of dysentery had 6.25% EBO positives which is lower than expected ($P < 0.05$).

Typhoid fever has similar presentations to EBO virus infection but as a provisional diagnosis in this series only 10/140 (7%) had typhoid as a provisional diagnosis. The mortality among these EBO antibody positive was 6/140 (4.2%) which is no higher than in the general hospital population from all causes.

Table 3 shows that the mean days of admission for patients with EBO antibody positives was 9.2 days and the days of fever after admission was only 3 days whereas the illness prior to admission averaged 7 days. The mean white cell count of 5,900 cmm was well within the normal range.

Contacts of EBO Antibody Positives

Thirty-two EBO seropositives were traced back to where they lived during the presumed incubation period and prior to admission. A total of 147 contacts were bled and 28 of them (19.05%) were found positive for EBO antibody. A general survey in the same area but not linked to a fever case showed an EBO antibody prevalence of 87/809 (10.75%) which is much lower ($P < 0.05$) Table 4.

The range of prevalence rates in the various population surveys shows wide variations from 4% (3/75) to 39% (13/33). The more arid regions (Turkana and West Pokot Districts) have shown a significantly higher antibody prevalence rate in the general population than that of the higher rainfall areas of Western and Nyanza Provinces - i.e. 16.8% vs. 8.4%, respectively ($P < 0.05$). Comparing only Pokot to Western Province, the difference is even greater viz 21.3% vs. 8.4% ($P < 0.01$).

Marburg Virus Antibodies in Fever Patients

Only eight fever patients were positive for Marburg virus antibody (5M and 3F). The mean age of the females, 34.5 years, was higher than the mean age of the males, 17.3 years. All eight survived. Four of these patients were provisionally diagnosed as typhoid, one of whom had a positive widal test. One patient was admitted as a case of abortion, one as an enteritis and one had burns. Only three of the eight had haemorrhagic manifestations. The mean white cell count at 3,500 x cmm was depressed. The mean number of days prior to admission was seven similar to 7.2 for Ebola positives and the fever lasted 5 days after admission compared to 3 days for Ebola. Hospital stay averaged 17 days compared to 9 for Ebola positives suggesting that this group had a more severe illness. It is interesting to note that four of these patients all clustered into the same hospital (Ortum in West Pokot) in the same month - October 1986. Geographically however, these four cases did not seem to be connected.

Contact and Population Survey of Marburg Antibody Positives

No Marburg antibody positives were detected in any of the family contacts 0/80. In the general population surveys in Western Kenya, only 2/790 (0.25%) were positive (Table 4).

Congo-Cremean Haemorrhagic Fever Virus Antibodies in Fever Patients

A total of 12 adults were positive. The mean age for males was 36.3 years and for females 22.5 years. The provisional diagnoses ranged from malaria 5, abortion 2, typhoid 2 and one each for enteritis, pneumonia and epilepsy. Haemorrhagic manifestations were observed in 3. One of the confirmed malaria cases died. The mean WBC was 6,650 x cmm (3) which is within normal limits. Days of fever prior to admission were 5; days of fever after admission was 2.9 and the period of hospitalization averaged 8 days (Table 3).

CCHF, being a tickborne disease, would be expected to coincide with peaks of ixodid tick populations. Although the numbers observed here is small, the end of the rainy season July-August during which tick populations are high, accounted for 50% of the cases. Surprisingly antibody positive cases were detected more often in the arid areas, particularly Ortum in Turkana 7/12 (58%) of all cases of CCHF, Table 1.

Virus Isolation

No virus was recovered from 676 sera inoculated into cells. Failure to recover EBO virus which appears from the serological results to be prevalent in the area is difficult to explain (see discussion).

A Fatal Case of Marburg in Kenya 1987

1. Clinical Disease:

As mentioned in the background information, on August 13, 1987, a 15-year-old European male was admitted into Aga Khan Hospital, Mombassa with a 3-day history of fever, malaise and anorexia. He was treated as a case of malaria but failed to respond. He subsequently developed severe haemorrhagic diathesis, became septicaemic, and had bloody diarrhoea and vomiting. He was flown to Nairobi Hospital on August 18, 1987, where he continued to deteriorate. Laboratory tests revealed leucocytosis, thrombocytopenia and elevated coagulation times. On the 20th August he died of cardiac failure with severe

hypotension and disseminated intravascular coagulation (DIC). The attending physician had made a provisional diagnosis of viral haemorrhagic fever complicated by an overwhelming pseudomona euroginosa infection.

A postmortem examination indicated a severe haemorrhagic disease with massive petachial and purpuric haemorrhages in the skin, mucosal haemorrhages in GIT, GU and CVS. Histologically three pathological processes were noted: Diffuse coagulative necrosis resulting in the patient shock, microthrombi with surrounding tissue infection resulting in the clinical DIC and micro-abscesses containing pseudomona like filamentous rod shaped organisms. The final cuase of death was attributed to acute cardiac decompensation consequent on massive septicaemia.

2. Laboratory Diagnosis

An acute serum collected on the ninth day of illness (August 19th) was found positive by Indirect Immunofluorescence (IF) on a five way CREELM slide. The serum, however, appeared negative on the only remaining Marburg specific slide in VRC. Aliquots of the same serum was inoculated into vero tissue culture cells undiluted 1:100 dilution. Within 96 hours, cell darkening, rounding and floating cells were seen in the inoculated cultures and the controls were looking normal. The positive CPE vero cells were fixed in cold acetone and tested against the patient's serum and it was positive by IF. A portion of the positive vero culture fluids was pelleted and fixed in gluteraldehyde for E.M. which showed typical club and question mark shaped particles characteristic of filoviruses.

Serum and infected vero cell cultures were dispatched to USAMRIID for further investigation. It was then confirmed that acetone fixed vero cells reacted positively in IF with the patients acute serum, Marburg fever virus convalescent reference sera, and Marburg virus specific monoclonal antibodies. They reacted negatively with convalescent or monoclonal antibodies specific for the other known African haemorrhagic fever viruses (i.e. Ebola virus, Rift Valley fever virus, Congo-Crimean haemorrhagic fever virus, Lassa virus, Dengue virus, West Nile virus or Yellow Fever virus). Electron microscope examination of clarified cell culture fluid revealed Marburg virus-like filamentous and club shaped particles. A study of intact cells showed Marburg virus intracellular inclusion bodies, or pro-marburg virus particles. Serological testing of the acute serum further showed strong reactivity with only Marburg viral antigens.

Thus, the diagnosis of fatal Marburg virus haemorrhagic fever was confirmed to have occurred in Kenya for the second time; once in 1980 and the second in 1987.

3. Outbreak Containment

Sixty hospital staff and the patient's family members and other contacts were traced for evidence of secondary cases. All those tested serologically were negative for Marburg antibodies. One nurse who had tended the patient in Mombasa developed fever, vomiting and abdominal pain. She was admitted but her convalescent serum was negative for Marburg. The Nairobi hospital pathologist who did the P.M. felt feverish and developed a rash and a herpetic lesion on his upper lip three days after performing the P.M. His serum also tested negative for Marburg.

4. Epidemiological Background

The parents of this fatal case were European expatriates working in a technical school in Kisumu and living within Kisumu municipality. The index case arrived in Kenya from Europe on July 14, 1987 with his young sister. The significant family movements in Kenya were thus:

On Saturday, August 1, 1987, the family of four drove from Kisumu to Kitale for a weekend. The family camped in the compound of an elderly British couple living some 20 km north of Kitale town. This tent camp is used by many other tourists interested in ornithological expeditions. On Sunday, August 2, 1987, the family of four visited "KITUM CAVES" in Mount Elgon National Park. These caves are known to harbour many fruit eating and insectivorous bats, elephants, rock rabbits, water bucks, buffaloes and even leopards. Family members entered the cave dressed in the usual casual manner for a hot day, i.e., normal footwear, T-shirts for the children and no head gear or face masks.

The most significant activity inside the cave is that the index case was the most excited and probably penetrated deepest into the cave and also explored lots of stones with his bare hands because of his known interest in geology. Some of these stones are known to be covered with moss, needle sharp salt crystals and fungi. It was, however, never recalled whether there had been any specific pricks. No accidental falls or any other direct injuries were recalled. No special bites or unusual wetting of any of the party was recounted. The total time spent inside the cave was about one hour and thereafter the family drove back to Kisumu.

On August 8, 1987 the family drove from Kisumu to Mombasa for holidays via Nairobi. The family camped in the South Coast (Twiga Lodge) and were spending most of the time swimming in the sea.

On Monday, August 10, 1987, the index case started feeling unwell with headache, fever and loss of appetite. Despite treatment for malaria the condition worsened necessitating admission on August 13, 1987, into Aga Khan Hospital Mombasa. The patient was transferred into the intensive case unit on the night of Monday 17, 1987. He was then flown by AMREF to Nairobi hospital on August 18, 1987 where he died on August 20, 1987.

This same family had visited Lake Bogoria Hot Springs on Friday and Saturday, July 24th and 25th. The father had visited Kitum caves twice previously. On one of these occasions he had the worst food poisoning in the lodge.

5. Field Studies that were Proposed

Summary: After reviewing the travel history of the index case, "KITUM CAVE" in Mount Elgon National Park seemed to be the likely place where exposure could have occurred. First the 1980 case had been exposed to the same caves nine days prior to falling ill. Since this seemed to be the first time a localized area amenable to intense ecological investigations had been identified, plans were drawn up by VRC in conjunction with USAMRIID. Detailed ecological studies were planned to determine whether Marburg Virus is present in the cave environment. Additionally, hospital surveillance and seroepidemiological studies were planned to be conducted in the Mt. Elgon region to identify any additional Marburg Virus infections.

KITUM CAVE INVESTIGATIONS

1. Preliminary Preparations:

Authorizations had to be obtained from the following officers in order to conduct the proposed field investigations.

1. Office of the President
2. Ministry of Tourism and Wildlife
3. Ministry of Research, Science and Technology

Having obtained the necessary authorizations, the following group of scientists and staff were assembled to discuss activities, procedures and requirements.

1. Physicians (three)
2. Veterinarians (two)
3. Mammalogists (two)
4. Entomologists (three)
5. Primatologists (two)
6. Technologists (three)
7. Support staff (twelve)

The major scientific supplies were ordered through USAMRIID and were airfreighted to KEMRI in January. The USAMRIID team arrived in KEMRI early February. The teams moved to Mount Elgon National Park in the third week of February.

2. Research Activities

The following activities were carried out in the cave, cave environment, park environment and in the Kitale area generally. All scientists entering the cave wore positive pressure respirators and water-proof gowns, boots and gloves.

a. Sentinel Non-Human Primates:

Prescreened sero-negative baboons, sykes monkeys, vervet monkeys and guinea pigs were housed in open cages and strategically placed in different corners of Kitum Cave. Control monkeys and guinea pigs were similarly caged and maintained outside the park environment. These animals were checked twice daily for signs of illness for a period of three weeks.

At the end of three weeks, no animal showed any signs of sickness and they were all bled for evidence of sero-conversion and euthanized and postmortem specimens obtained.

b. Bat and Bird Studies:

Mist nests were used to trap bats and birds associated with Kitum Cave. All bats and birds captured were identified, combed for ectoparasites, bled and the following organs obtained:

lung, heart, kidney, liver, spleen and brain. Organs were immediately kept in liquid nitrogen for virus isolation. Serum obtained was also kept in liquid nitrogen for serology. Ectoparasites were identified and stored in liquid nitrogen for virus isolation.

c. Mammal and Rodent Trapping:

Mammal and rodent traps were baited with peanut butter, cassave, sweet potatoes, corn, meat etc. The traps were put out every evening inside the cave, outside the cave, and in the park environment. Another lot of traps was put out in the Park Lodge environment. Traps were checked every morning. All animals captured were identified, combed for ectoparasites, bled and in the case of rodents, the following organs were obtained: liver, spleen, lung, kidney, heart and brain. All organs and ectoparasites were stored in liquid nitrogen for attempted virus isolation. The two gnet cats that were captured were bled and released.

d. Entomological Studies:

Light traps with some using carbon dioxide were set every evening inside the cave, outside the cave and in the park environment. Every morning the traps were retrieved and blood sucking insects trapped were identified and put into liquid nitrogen for attempted virus isolation. Mosquitoes and sandflies dominated the catches.

Ticks were collected from outside the cave and in the park environment by hand picking or by the white sheet sweep method.

e. Domestic Animals:

Cows, goats, sheep and dogs were bled with the consent of the owners.

These were grouped into the following categories:

- (1) Animals associated with caves past or present
- (2) Farm animals
- (3) Animals associated with the park environment only

The sera obtained will be used for serology.

f. Human Survey:

(1) Hospital Records

Several health units associated with the park and the Kitale area were visited. Clinical officers were interviewed for past evidence of haemorrhagic fevers fatal or nonfatal. Records were also examined retrospectively for three years.

(2) Population Survey

Inhabitants of the park environment were bled for serology. They were classified as:

- associated with caves
- associated with park environment only
- associated with Kitale environment

3. Results

Specimens collected in the field were each divided into two aliquots. One set of specimens was airfreighted to USAMRIID and the second is being analyzed in VRC.

Table 1. Antibodies Against Five Haemorrhagic Fever Viruses in Fever Patients Admitted to Seven Hospitals in Western Kenya:
June 1986 - July 1987

	<u>EBO</u>	(%)	<u>MBG</u>	(%)	<u>CCHF</u>	(%)	<u>RVF</u>	(%)	<u>LAS</u>	(%)
HOSPITAL										
A KAKUMA	4/25	16	0/25	0	0/25	0	0/25	0	0/25	0
LODWAR	8/25	15.4	1/52	1.9	1/52	1.9	0/52	0	0/25	0
B ORTUM	22/190	11.6	4/190	2.1	7/190	3.7	0/190	0	0/190	0
C MISIKHU	30/235	12.8	1/235	0.4	1/235	0.4	0/235	0	0/235	0
MUMIAS	35/234	15	1/234	0.4	1/234	0.4	0/234	0	0/234	0
MUKUMU	16/157	10.2	1/157	0.6	0/157	0	0/157	0	0/157	0
D KILGORIS	25/225	11	0/225	0	2/225	0.5	0/225	0	0/225	0
Totals	140/1118	12.5	8/1118	0.7	11/1118	0.98	0/1118	0	0/1118	0

A. Turkana District

C. Western Province

B. West Pokot

D. Nyanza Province

Table 2. Provisional Diagnoses on Patients Found to be Ebola Virus Antibody Positive (N - 140)

DIAGNOSIS	NUMBER	% POSITIVE
Malaria	29	21.5
Fever of unknown origin	17	12.1
Respiratory infection/ bronchial pneumonia	17	12.1
Abortion/premature delivery	15	10.7
Typhoid syndrome	10	7.1
Septicaemia	6	4.3
Dysentery	5	3.6
Abscesses	3	2.1
Hepatitis	2	1.4
Kala Azar	2	1.4
Other conditions	34	24.3

Table 3. Percentage and Mean Values of Clinical Details
of Antibody Positive Haemorrhagic Fever Virus Suspects

Haemorrhage	25.7% (36/140)	37.5% (3/8)	30% (3/10)
Mean WBC level per mm cu.	6,650	3,500	5,900
Days of illness prior to admission	5.0	7.0	7.2
Days of fever after admission	2.9	5.0	3.0
Days of hospital admission	7.8	17.0	9.2
Mean age, female	27.2	34.5	36.3
Mean age, male	28.5	17.3	22.5
Mortality rates	4.3% (6/140)	0%	10% (1/10)

Table 4. Ebola and Marburg Virus Antibodies in Population Surveys in Western Kenya

AREA	EBO	MRG
Turkana	6/75	0/75
Pokot		
Mountain	10/80	0/80
Lowland	22/71	0/71
Western Province		
Nzoia	30/250	2/250
Sangalo	3/75	0/75
Namirama	8/133	0/133
Kakamega	5/89	0/89
Nyanza Province		
Kisii	3/36	0/36
Total	87/809	2/809

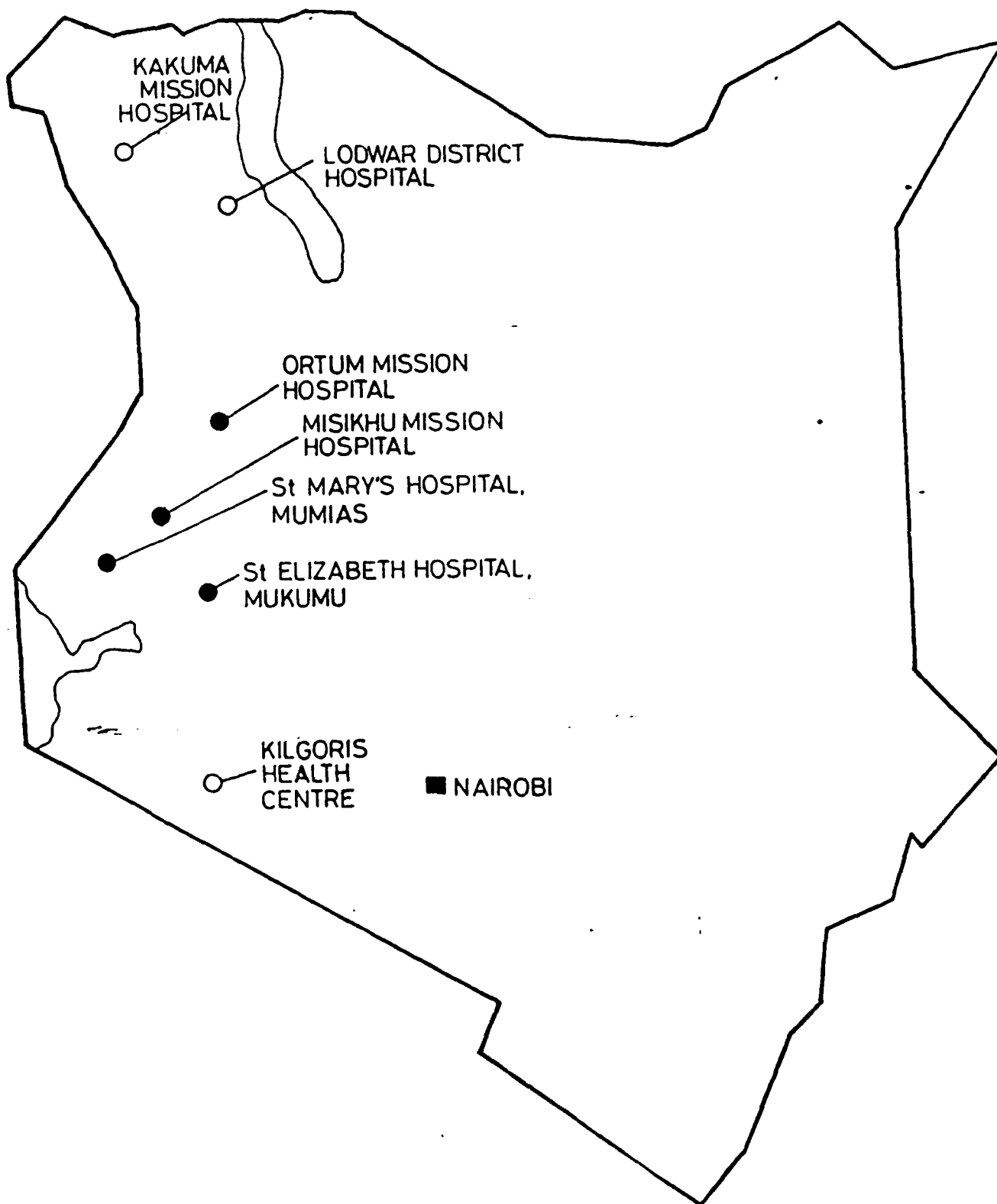


Figure 1. Location of hospitals within the study area. Open circles represent hospitals studied for short periods (up to 3 months).