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PRINCIPAL INVESTIGATOR: J. K. Omuse

CONTRACTING ORGANIZATION: Kenya Trypanosomiasis
Research Institute
P.O. Box 362
Kikuyu, Kenya

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J. K. Omuse

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P.O. Box 362
Kikuyu, Kenya

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Abstract

Careful collection of data from a mouse model, a Kenya Trypanosomiasis Research Institute (KETRI) vervet monkey model, and patients of the Alupe Human Sleeping Sickness Referral Hospital at Busia, Kenya, screening of Walter Reed Army Institute of Research (WRAIR) trypanocides with known therapeutic effects and potential for treatment of African Trypanosomal disease were undertaken. Clinical trials for efficacy of individual drug regimens for treatment of human trypanosomiasis with acute febrile attacks and chronic, central nervous system involvement in Kenya, and application of an ELISA antigen detection for early determination of efficacy in treatment of human sleeping sickness were assessed.

The vervet model, developed at KETRI is a useful live system. The trypanosomal disease clinical stages as seen in humans, including the progressive brain dysfunction leading to "sleeping sickness" with advancing cachexia and death were stepwise analyzed. The development and refinement of both chemotherapeutic and diagnostic skills were remarkably facilitated by using this nonhuman primate model. Five potential trypanocidal compounds from WRAIR were screened and evaluated. Three of the compounds proved to have efficacy in controlling or eliminating the disease. These merit further investigation and assessment, including controlling incidence and morbidity.

Advantage was taken to investigate the reported synergism between suramin and other therapeutic trypanocides. This combination therapy was recommended by the reviewers because of the availability of the vervet model. DFMO was used in conjunction with some trypanocides because of its recognized supportive effects.

An outbreak of African trypanosomal disease occurred in an area where African trypanosomiasis is known to exist along the Kenya-Uganda border during the year 1990. Ninety cases of the disease were reported, the majority of which were in the chronic, episodic fever with splenomegaly and swelliing of the lymph nodes clinical stage. Progressive brain dysfunction were observed in a few instances. Prompt action was taken to obtain a definite diagnosis and to treat the ill. The fly vector was controlled through insect spraying. The outbreak was brought under control by the above cited intervention. Active surveillance in the region, with fly control and treatment of the continuing sporadic human cases, has prevented a repeat occurrance.

Rift valley fever (RVF) studies have focused on the endemic cycle of the RVF virus in the vector (Aedes mosquito), vector competence, environmental conditions, and the potential for predicting outbreaks of the disease. Research entomological efforts have been able to establish models for predicting likely breeding sites for the vector mosquito and as such the likelihood of outbreaks of RVF disease. This endemic cycle and transmission of infection information make it possible to predict potential outbreaks of the disease as well as design specific means to prevent/control

RVF through surveillance and vector control. Studies have allowed development of models for predicting likely breeding sites for RVF vector mosquitoes and potential areas for outbreaks.

Entomological studies for Congo-crimean hemorrhagic fever (CCHF) have been directed toward collection of ticks from selective sites in Kenya and tested for tick-borne viruses at the United States Army Medical Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland. This Institution has screened more than 1100 ticks of the genera Amblyomma, Boophilus, and Rhipicephulus collected in Kenya. Additionally, 400 Hyalomma ticks have been collected from potential CCHF areas in kenya and screened for arboviruses. No CCHF viruses have been detected or isolated from these ticks. Greater than 2500 ticks of various genera await shipment to USAMRIID for virus detection and screening. Also, the tick collections have allowed the gathering of information on seasonal occurrance, geographical distribution, and host preference of ticks.

In endemic areas, the incidence of trypanosomal infection varies depending on such factors as the availability of mammalian reservoirs, the density of vector tsetse flies, and environmental factors, but as a rule, humans and their pathogenic trypanosomes cannot coexist without trypoanosomal disease. In man these flagellated protozoan parasites "homes" to the microvasculature of the brain and skeletal muscles, and the frequent paucity of organisms in the peripheral blood makes diagnosis and estimates of the total burden difficult. The difficulty in treating the disease is well documented in the literature and further highlighted in chemotherapeutic studies accomplished here. Future studies should continue to vigorously pursue improved means to accurately diagnose human trypanosomiasis. The antigen ELISA technique was successful in diagnosing well developed cases. Its reliability for detecting early as well as chronic inapparent cases should be further evaluated. Work must continue in the area of chemotherapy, particularly in testing various regimens of existing useful drugs and the search for combination and new single drug treatments to compliment the few useful ones need to be continued..

Rift Valley fever efforts have provided insights for strategic identification of vector mosquito breeding sites and as such allow strategies to be developed for controlling RVF disease outbreaks. Humans are susceptible to clinical and inapparent infections and as such may contribute to spread and outbreaks of the disease. Future studies should be directed to fill the vast gaps that remain in our present knowledge of cycles of the virus in mammals and the blood sucking arthropods.

Congo Crimean hemorrhagic fever studies have focused on isolation of the virus from various species of ticks without success. These studies are to be continued as well as future efforts should include serology tests for antibodies in humans and migratory birds (suspected reservoirs).

FOREWORD

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Diagnosis and Chemotherapy of Human Trypanosomiasis and Vector Ecology of Rift Valley Fever and Congo-Crimean Hemorrhagic Fever in Kenya

CONTENTS

I. HUMAN TRYPANOSOMIASIS IN KENYA

CHEMOTHERAPY AND DIAGNOSIS: This phase of research was accomplished and reported in the Midterm Report -- the background, introduction, conclusions, and references are repeated in this report. (See Midterm Report DAMD17-90-Z-0005).

Background

About 50 million people on the continent of Africa are currently at risk from the two forms of "sleeping sickness", caused by *Trypanosoma b. gambinese* in West and Central Africa and *Trypanosoma b. rhodesiense* in Eastern and Southern Africa. The gambinese disease is usually chronic, leading over months and years to invasion of the central nervous system by trypanosomes, resulting in a fatal encephalitic form if not treated. The rhodesiense disease, on the other hand, may be acute and rapidly fatal from pancarditis or more slowly from encephalitis. Treatment of trypanosomiasis is limited at present to the use of 3 or 4 drugs. The early disease may be treated with suramin or , in the case of T. gambinese infection, pentamidine. The late encephalitic form of the disease is treatable only with melarsoprol, an arsenical compound, which itself may result in encephalopathy in 10% of treated cases; 1-6% of these are fatal. Administration of these drugs requires careful patient monitoring and hospilalization. In addition, it has long been recognized that some strains of trypanosomes are refractory to treatment. Recently the use of DFMO has shown promising results in cases of T. gambiense infection in Sudan and other areas. The position with T. rhodesiense is less encouraging.

The objectives for the prypanosomiasis component of this project were firstly, to examine the therapeutic properties of compounds from th Division of Therapeutica, WRAIR, against Trypanosoma brucei brucei and Trypanosoma brucei rhodesiense infections in mice and vervet monkeys. Secondly, to explore the efficacy of combination therapy in encephalitic T. rhodesiense infected vervet monkeys.

Introduction

The Chemotherapy Division of KETRI has developed a protocol for testing novel compounds with trypansocidal activity for *T. b. brucei* and *T. b. rhodesiense* in murine and nonhuman primate models of human sleeping sickness. More recently this has been extended to include a trypanosome culture system. Together these systems, which include both the acute and the meningoencephalitic stages of trypanosomiasis, permit a full assessment of the potential of novel compounds or revised treatment regimens to be made prior to clinical trials.

The focus of attention for studies in the murine and nonhuman primate models of T. rhodesiense at KETRI has recently been directed towards a more detailed understanding of the behaviour of melarsoprol, of eflornithine (DFMO - Omidyl, Merrell Dow), and towards the evaluation of treatment regimens utilizing combinations of drugs already registered for use in humans, rather than towards experimental compounds. There continues to be an urgent need for new compounds for *T. rhodesiense* infection but there may be little hope of commercial manufacture or field use in the immediate future.

Previous studies in the vervet trypanosomiasis model indicated that combination therapy involving suramin and an experimental nitroimidazole from Merck Sharpe and Dohme, MK436, cured four of five encephalitic cases, thus giving the next best cure rate to melarsoprol. Although another nitroimidazole from Hoffman La Roche, Ro15-0216, did not give satisfactory results alone (2 cures in 14 treatment courses) in the early phase of the vervet mode., it has been shown to have a synergistic effect with other trypanocidal compounds, particularly DFMO, against *T brucei brucei* in culture and in mouse and sheep models of the acute disease.

Encouraging results (4 out of 5 non-encephalitic vervet cases cured) were achieved with suramin in combination with the arsenical Mel Cy or cymelarsen which is now being developed by Rhone Poulenc for *T. evansi* therapy in camels and horses.

It has also been demonstrated in encephalitic vervet cases, that dosages of melarsoprol lower than those recommended for routine use in human cases of both Gambian and Rhodesian sleeping sickness were effective and might therefore constitute a lowered risk from arsenical

toxicity. A double blind clinical trial in patients at Daloa was initated as a result of these findings. This has not been completed, however, because there is a strong suspicion amongst those running the trial that there is a greater than expected number of relapse in patients selected. Independent statistical analysis of the results of some 60 cases did not support this view but, because the confidence of the clinicians concerned has been undermined, completion of the trial was delayed.

The exact nature of the adverse reaction reported after treatment with melarsoprol in 1-15% of patients contnus to be ill defined. Studies have been pursued in mice at Glasgow and to a limited extent at KETRI to explore the aetiology of these reactions and to devise preventative therapeutic measures.

In encephalitic vervet monkeys infected with T. rhodesiense KETRI 2537, although far from ideal, melarsoprol continues to be the superior drug studied to date.

The limited number of reports of DFMO treatment of T. rhodesiense patients indicate variable responses to the drug while DFMO is successfully used in T. gambiese human patients refractor to melarsoprol. The reasons for this difference in response to the drug have yet to be explained, but the finding in mice that T. rhodesiense isolates exhibit a wide range of sensitivities to DFMO in important.

The results of extensive eflornithine (DMSO) treatment trials in T. rhodesiense KETRI 2537 infected encephalitic vervet cases indicated that the drug was not curative at any of the dosages or in any of the combinations tested. It was encouraging therefore to confirm the value of this drug used orally in Sykes monkeys (Cercopithecus mitis) and one vervet monkey, all wit encephalitic changes due to T. gambiense KETRI 2347 and 2569. In contrast to the T. rhdesiense KETRI 2537 cases, there was rapid and total remission of clinical signs and trypanosomes could not be detected.

Progress Report:

Experimental compounds with trypanocidal activity from Walter Reed Army Institute of Research were evaluated. Five compounds were sent by Dr. Willis Reid from a series that had been shown by scientists from the Division of Experimental Therapeutics to possess trypanocidal activity in T. rhodesiense infected mice. Since small quantities only were available, studies were initially confned to mice. However, we were informed that at this stage in development there is no likelihood of further supplies of these compounds being synthesized. Three of the compounds (3,4, & 5 --see table 2 of midterm report) were extremely effective in the mouse model and merit further investigational study in the vervet model.

II VECTOR ECOLOGY OF RIFT VALLEY FEVER IN KENYA

Background:

I In 1981 a collaborative research project was initiated between Kenyan, USAMRU-Kenya, and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) scientists to study Rift Valley Fever (RVF) in Kenya. Significant data has been accumulated on this important human and animal pathogen (1-10). A paper presented at the 7th Annual Medical Scientific Conference of the KEMRI/KETRI summarized the major results achieved, including the demonstration of a close link between the endemic cycle of RVF virus, *Aedes* mosquitoes, and specific types of mosquito breeding habitats known as dambos (11). However, almost all studies have been confined to a relatively small geographical area in Kenya. Virtually nothing is known about the role of ground pool *Aedes* mosquitoes and dambos in RVF maintenance and transmission in other parts of Kenya, even though RVF is known to be widespread (12) with the highest RVF antibody prevalence rate in humans in Kenya found in the northwest in Turkana District (13). Rift Valley Fever is an important human and livestock disease which cannot be controlled at this time. There is no licensed human vaccine available, and the livestock vaccine, producing up to 30-40% abortions, is not widely used.

Detailed studies have been conducted in the laboratory to investigate vector competence of many species. Some of the species tested are considered to be likely secondary vector species; however, because the important ground pool breeding *Aedes spp*. have not been colonized, only very limited vector competence (both horizontal and vertical) studies have been made of the primary maintenance vectors.

Using the National Oceanographic and Atmospheric Administration's (NOAA) satellite data, the potential for predicting outbreaks of RVF in Kenya has been demonstrated (14). A paper on this subject was presented at the organization of African Unity Symposium on viral diseases in Africa, held in Nairobi in May-June 1986 (15). The prediction of outbreaks, however, needs refinement and validation. In addition, other high resolution remote imagery technique,such as LANDSAT, SPOT, METEOSAT, or SAR radar imaging need to be evaluated in the detection of dry and flooded dambos.

Estimates of a recent (October-December 1987) serious RVF epidemic and epizootic in the Senegal River basin in Muritania and Senegal indicate that more than 20,000 people were infected (45% attack ratre in some areas), and that up to 90% of the livestock were infected. Human disease in this area had never previously been reported, even though antibodies had been detected. There is suggestive evidence that the epidemic was precipitated by the construction dams designed to maintain water level in the river at allow for the development of rice and other agricultural crops. The ecology of the area and the utilization of irrigation schemes is similar to the situation found in some areas in Kenya, in particular the Tana River Project. Cattle have been found to have RVF virus antibody in these regions in Kenya (12). The potential for occurrance of RVF epidemics caused by alterations in the natural ecology of such areas has not been assessed in Kenya. Studies on the geographic distribution of maintenance and secondary mosquito vectors and their potential breeding habitats could provide the information needed to assess the RVT threat both to the human and domestic animal population.

The unique relation between vectors, dambos, rainfall, and RVF makes the disease a likely candidate for control targeted against mosquito vectors and their habitats. Currently available safe insecticides or biological control methods might be efficient in controlling or eliminating RVF virus disease. No information is available concerning the efficacy of these insecticides in the dambos habitats.

Introduction:

Rift Valley Fever is an incapacitating and lethal disease of man and domestic animals in much of Africa. Daubney, et al. established that a virus was the causative agent of RVF during an epizootic in Kenya in 1930 (1). Since then, periodic outbreaks of RVF have occurred in Kenya as well as other African nations (2,3,4,5,6). Rift Valley fever virus causes acute clinical disease in man and most domesticated ungulates. It causes a flu-like illness in man which can have an immediate impact on soldier performance and reduce unit readiness during epidemics. An outbreak of RVF in Egypt during the years 1977-1978 demnstrated the potential for the virus to cause high morbidity/mortality in humans (7,8.9).

Mosquitoes are the primary vectors of RVF virus (4,8,10). The mosquito species *Aedes mcintoshi* has been implicated as the reservoir of RVF, and their is strong evidence for transovarial transmission of the virus by this mosquito (10,11). Sand flies and ticks have been experimentally infected with RVF virus and may serve as natural vectors of the virus (12,13).

Successful RVF vector abatement and RVF vaccination programs in Kenya will require identification of potential endemic areas of the virus. The use of data from remote sensing earth orbiting satellites and airborne imaging radar provide augmentative means to quickly and economically map dambo habitats and to predict their flooding (18,19,20,21).

Rift Valley fever control strategies have been directed at the control of *Ae mcintoshi.* the presumed reservoir for the virus. *Ae mcintoshi* is one of the first mosquito species to emerge from flooded dambos. It produces one generation, disapears and is replaced by other mosquito species (14,15). The majority of eggs in the soil hatch after initial flooding with very little hatching during subsequent flooding (22). Abatement efforts directed at *Ae. mcintoshi* may prevent or greatly reduce the severity of epizootics because of these characteristics in its biology. Studies have demonstrated that pretreatment of dambos with a sustained release formulation of methoprene prior to flooding effectively reduces or eliminates the emergence of adult *Ae. mcintoshi* populations (23, 24), but other chemical control strategies may not be effective in suppressing vector populations (25`). Controlled burning of oviposition habitat may also result in a significant reduction of *Ae. mcintoshi* emergence after flooding (26).

<u>Objectives:</u>

1. Identify critical ecological factors which influence the occurrence of RVF in Kenya

2. Determine the geographical distribution of floodwater *Aedes* species which are suspected reserviors and enzootic vectors of the RVF virus.

3. Evaluate cntrol strategies to suppress th emergence of RVF vectors and prevent the introduction of RVF virus into susceptible human and livestock populations.

Published Studies:

A complete listing of scientific publications with abstracts and summaries resulting form research during this grant period are included in the appendix.

Unpublished Investigations:

We undertook an initial study to determine the vector potential of *Ae. mcintoshi* and *Ae. circumluteolus* to transovarially transmit RVF virus to their progeny. A total of 97 adult female *Ae. mcintoshi* took blood meals from hamsters infected with RVF virus and produced 3500 adult progeny. Even though we established sound rearing and isolation techniques, we were unable to isolate RVF virus form any of the mosquito progeny. We believe this species is an inefficient vector of RVF virus. However, there were indications that the virus isolate we used had lost much of its virulence. It produced very low titers in our experimental hamster, and feeding mosquitoes may not have imbibed transmissible viral titers with their bloodmeal. Currently, we are analyzing the data for scientific understanding and possible publication. If we determine that insufficient virulent viral titers were used, we hope to repeat this experiment with remaining funds using a more potent RVF virus isolate.

We attempted to colonize *Ae. mcintoshi* in the laboratory. We reared mosquitoes from eggs in the laboratory and collected newly emerged mosquitoes from an artifically flooded dambo in the field. We placed newly emerged virgin male and female mosquitoes in holding cages measuring 61 cm x 61 cm x 122 cm tall and exposed them to a 12 hour light/dark cycle in the laboratory. We did not observe any mating in holding cages, or were we able to dissect any fertilized spermathecae from females. Our attempts to force-mate males and females in the laboratory were also unsuccessful.

We collected and tested sera for antibodies to RVF virus from 34 sheep, 40 goats, 6 cows and 5 camels in Turkana District near Lake Turkana in 1991. Two animals, one sheep and one goat, had circulating anti-RVF virus IgG antibody. We also recovered a small number of Aedes mosquito eggs from soil samples taken from the margin of Lake Turkana and from a seasonal roadside pool. These eggs conformed in size and appearance with eggs of *Aedes mcintoshi*. However, we were unsuccessful in our attempts to hatch these for positive identification. We used standard egg hatching methods and assume that the small number of eggs we collected were unexplainably nonviable.

We are currently attempting to identify the oviposiiton habitat for reservoir *Aedes* species involved in an outbreak of Rift Valley fever along the shores of Lake Naivasha in 1989. We have receovered Aedes mosquito eggs from the lake margin. We will rear these to adults to determine the species and assay from the RVF virus. The lake level is currently increasing, and we are closely monitoring it. If it should flood suspected oviposiiton habitat and produce mosquito larvae, we will collect Aedes larvae, identify them to species and assay them for virus. We will continue this investigation with remaining funds.

Summary of Key Achievements:

During this grant period, we have successfully demonstrated the value and effectiveness of remote satellite telemetry to rapidly and efficiently locate and map potential habitats of mosquito species that serve as reservoirs and amplification vectors of RVF virus (19,20,21). We have identified and evaluated effective control strategies for the Ae. mcintoshi and other vectors of RVF virus (23,24,26,27). Our studies have contributed to a better understanding of the biology and vector competence of *Aedes mcintoshi* (122 unpublished data). We also identified potential amplifying vectors of RVF virus, including one previously unreported mosquito, during an outbreak of the disease (2).

Recommendations for Future Research:

We believe that the opportunity exists for a better understanding of RVF epidemiology and epizoology in Kenya. The geographic distribution of RVF virus in Kenya has not been fully documented, particularly along the margins of Rift Valley lakes and streams. Should excessive rains in these areas cause lake levels to increase or rivers to flood, outbreaks of RVF virus may provide an opportunity to better understand RVF ecology and identify additional mosquito vectors.

Techniques using remote satellite telemetry to identify and map potential foci of RVF virus need refinement. To date, our research has been in dambo habitats at a single site. We need to expand this work to include various habitats and sites to determine the true value of this technology. The use of remote satellite telemetry to predict outbreaks of RVF virus also needs to by investigated more thoroughly.

We need a better understanding of the interactions between RVF virus and mosquito vectors, particularly the reservoir *Ae. mcintoshi.* Currently, there is little ifnormation available concerning the vector potential of *Ae. mcintoshi* or transovarial transmission rates of the virus by this species. This information is critical for the development of effective strategies to reduce or eliminate outbreaks of RVF. Laboratory colonization of *Ae. mcintoshi* would facilitate this research.

Finally, we feel that new pesticides vector control strategies need to be investigated as they become available.

III. TICK-BORNE VIRUSES IN KENYA

Introduction:

Ixodid ticks are thought to be the primary vectors of Crimean-Congo hemorrhagic fever (CCHF), Dugbe, Thogoto, Bhanja and Jos viruses [30]. Linthicum, et al. provided evidence that ticks may also be able to transmit Rift Valley fever virus [15].

CCHF virus has not been isolated from vertebrates in Kenya. However, limited surveys have demonstrated antibodies to the virus in humans and cattle [31, 32, 33, 34].

Ticks in the genus *Hyalomma* appear to be the primary vectors of CCHF virus. Six *Hyalomma* species have been collected in Kenya [35]. Three of these, *Hy. impeltatum, Hy. rufipes* and *Hy. truncatum*, serve as invertebrate hosts of CCHF virus in Senegal [36]. Ticks in this genus are generally restricted to arid and semi-arid habitats and do not survive in areas where there is high humidity and abundant rainfall [34, 36, 37]. Logan et al. demonstrated transtadial transmission of CCHF virus *Hy. truncatum* and horizontal transmission by feeding adults, but was unable to demonstrate transovarial transmission of the virus by this species [38].

Little is known about the distribution and epidemiology of other tick-borne viruses in Kenya. Steele and Nuttall compared the vector competency of *Amblyomma variagatum* and *Rhipicephalus appendiculatus* and found that *Am. variagatum* was a competent vector of Dugbe virus and that *Rh. appendiculatus* was not [39]. However, Linthicum et al. experimentally infected *Rh. appendiculatus* ticks with Dugbe virus, and these successfully transmitted the virus to susceptible hamsters [40].

Objectives:

1. Determine the status of CCHF in Kenya.

2. Determine the geographical and seasonal distribution of potential tick vectors of CCHF virus (*Hyalomma* spp.) in Kenya.

Research Summary:

We collected numerous *Hyalomma* spp. and other tick genera from throughout the Rift Valley region of Kenya. To date, personnel assigned to USAMRIID, Fort Detrick, Maryland, USA, have screened and failed to isolate any arbovirus, including CCHF virus, from more than 400 *Hyalomma* ticks. USAMRIID also screened more than 1100 ticks from the genera *Amblyomma*, *Boophilus* and *Rhipicephulus* and failed to isolate arbovirus. Recently more than 2700 ticks of the genera stated above have been shipment to Fort Detrick for arbovirus screening.

Continuing Efforts:

Planned was a study to conduct a mark release and recapture investigation with laboratory reared *Hyalomma rufipes* to determine the host seeking range of this species in its natural habitat. Early rotation of the investigator and termination of the contract precluded this study.

Recommendations for Future Research:

The status of CCHF and other tick-borne viruses in Kenya is not known. Tick collections should continue throughout Kenya, particularly in arid and semi-arid regions to get a better understanding of geographic and seasonal distribution of potential vectors of human arboviruses. Ticks should also be screened for these viruses. However, we do not currently have appropriate P4 containment laboratories to attempt virus isolations and must rely on laboratories at USAMRIID, Fort Detrick, Maryland to attempt virus isolations. There is little known about the habitat requirements of *Hyalomma* spp. in Kenya, and various aspects of the field biology of these ticks should be undertaken. We also need to investigate the potential of Remote Satellite Telemetry to identify and map *Hyalomma* tick habitats.

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APPENDIX

GRANT PUBLICATIONS

1989 - present

Davies, F.G., T.M. Logan, Y. Binepal and P. Jessen. 1992. Rift Valley fever activity in East Africa in 1989. The Veterinary Record 130: 247-248.

SUMMARY[1]: Outbreaks of Rift Valley fever that occurred in livestock in Kenya and Tanzania in 1989 are described. Cattle and sheep infected with RVF virus were recorded at three farms on the shores of Lake Naivasha in the Great Rift Valley, Kenya. At one farm approximately 50 % of the dairy cattle between 15-24 months old were infected. An outbreak of RVF was also recorded at two farms in Morogoro, Tanzania.

Linthicum, K.J., T.M. Logan, P.C. Thande, J.N. Wagateh, C.W. Kamau, C.L. Bailey, F.G. Davies and J.P. Kondig. 1989. Efficacy of a sustained-release methoprene formulation on potential vectors of Rift Valley fever virus in field studies in Kenya. J. Am. Mosq. Contr. Assoc. 5(4): 603-605.

SUMMARY: Dambos were artificially flooded and treated with Altosid *pellets applied at a rate of* 5.6 kg/ha. One dambo was treated the day after flooding, and one was treated 3 days after flooding. An untreated dambo was used as a control. There was 100% mortality of both *Aedes* and *Culex* pupae in treated dambos during week 2 of the study. This was significantly higher than in the control (untreated) dambo. There appeared to be some residual effect for *Culex* pupae for up to 3 weeks following treatment.

Linthicum, K.J., C.L. Bailey, C.J. Tucker, K.D. Mitchell, T.M. Logan, F.G. Davies, C.W. Kamau, P.C. Thande and J.N. Wagateh. 1990. Application of polar-orbiting, meteorological satellite data to detect flooding of Rift Valley fever virus vector mosquito habitats in Kenya. J. Med. Vet. Entomol. 4: 433-438.

ABSTRACT: Measurements of green leaf vegetation dynamics recorded by the advanced very high resolution radiometer instruments onboard National Oceanic and Atmospheric Administration satellites 7 and

9 were used to derive ground moisture and rainfall patterns in Kenya and monitor resultant flooding of mosquito larval habitats (dambos) likely to support Rift Valley fever virus vector mosquitoes (*Aedes* and *Culex* spp.). Satellite-derived data from mid-1981 to December 1988 have been analyzed with corresponding rainfall, flooding and vector population data as they relate to Rift Valley fever virus ecology. Single (7X& km) and multiple grid-cell image analysis (200X300 km) in small localized areas and large geographical regions, respectively, of vegetation data were used to quantify the potential for flooding of mosquito breeding habitats. The ability to detect accurately parameters, such as ground moisture, that determine flooding could provide local officials with sufficient warning to allow for implementation of specific mosquito control measures before a disease outbreak.

Linthicum, K.J., D. Angleberger, D. Ashby, G. Nelson, R. Whittle, J.N.Wagateh, P.C. Thande and C.W. Kamau. An automated technique to map mosquito-breeding habitats in Kenya. In preparation.

ABSTRACT: The flooding of mosquito-breeding habitats known as dambos has been associated with epizootics and epidemics of Rift Valley fever (RVF) virus in sub-Saharan Africa. Identification and mapping of these habitats are critical to the identification of foci of disease outbreaks and implementation of vaccination and vector control programs. An automated technique was tested for its abiility to map dambos with existing LANDSAT Thematic Mapper satellite data. Multivariate statistical techniques were used on a wet season (1987) and a dry season (1984) data set for a 135 square km test area (150,000 pixels) in central Kenya. Principal component analysis of all 14 available satellite bands found that most of the variation in the data was contained in the first 3-4 linear combinations of the original bands. Cluster analysis of the components was used to remove clouds and cloud shadows. Linear discriminant anlaysis was performed on the band data by using a training dambo data set previously identified and confirmed. Dambo habitats were accurately identified with a quantifiable degree of precision.

Logan, T.M., K.J. Linthicum, J.G. Ksiazek. 1992. Rift Valley fever antibody in human sera collected after an outbreak in domestic animals in Kenya. Transactions Roy. Soc. Trop. Med. Hyg.: Trans. Roy. Soc. Trop. Med. Hyg. 86: 202-203.

SUMMARY: Blood sera was collected from 30 herdsmen during an outbreak of Rift Valley fever in domestic livestock. Twelve (40%) of the herdsmen had detectable IgG antibodies specific for RVF virus. Five of the herdsmen with measurable IgG also had measurable IgM indicating recent exposure to RVF virus.

Logan, T.M., and K.J. Linthicum. 1992. Evaluation of a briquet formulation of *Bacillus thuringiensis* var *israelensis* (H-14) against *Aedes* spp. and *Culex* spp. larvae in dambos in Kenya. Biocontrol Science and Technology. In press.

ABSTRACT: *Bacillus thuringiensis* Berliner var. *israelensis* serotype H-14 (Bti) in briquet formulation (Bactimos) was tested in a field trial against ground-pool breeding mosquitoes in a dambo located in a Rift Valley fever virus-enzootic/epizootic area in central Kenya. Bactimos (10% Bti; 7000 AA International Toxic Units/mg) was tested for 30 days in 3 separate treatment areas at the rate of 1 briquet/9 m^2, later increased to 1 briquet/1.5 m^2 on day 13 postflood; 1 briquet/9 m^2, and 1 briquet/4.6 m^2. An estimate of the daily survival rate of larvae at different periods during the study revealed that mosquitoes in the area treated with 1 briquet/9 m^2 had a significantly lower (64%) survival rate than those in the control site (92%) against *Aedes* spp. and was not significantly different from the site treated with 1 briquet/4.6 m^2. Mosquitoes in the site treated with 1 briquet/1.5 m^2 on day 13 postflood had a much-reduced survival rate (25%) when compared to those in the control site (67%) during the 3rd *Culex* spp. generation (22-26 days postflood). This 5-fold increase above standard label dosage still failed to prevent *Culex* spp. emergence. There was no significant difference in survival rates between the control and any of the 3 treatment sites during the first or second generation (9-20 days postflood). Emerged adults collected as pupae from control and treatment sites indicated that *Aedes mcintoshi* Huang and *Culex antennatus* (Becker) were the most predominate *Aedes* and *Culex* species.

Logan, T.M., K.J. Linthicum, F.G. Davies, Y.S. Binepal and C.R. Roberts. 1991. Isolation of Rift Valley fever virus from mosquitoes (Diptera: Culicidae) collected during an outbreak in domestic animals in Kenya. J. Med. Entomol. 28(2): 293-295.

ABSTRACT: During an outbreak of Rift Valley fever (RVF) in livestock near Lake Naivasha, Rift Valley Province, Kenya, 61,347 mosquitoes (1287 pools) collected in CO^2-baited light traps yielded seven viral isolates. Five isolates of RVF virus were recovered from 18,831 *Culex zombaensis* Theobald and one from 4,439 *Mansonia africana* (Theobald). One of isolate of a Bunyamwera group virus was recovered from 1175 *Ades quasiunivittatus* (Theobald).

Logan, T.M., K.J. Linthicum, P.C. Thande, J.N. Wagateh, G. Nelson and C.R. Roberts. 1991. Egg hatching of *Aedes* mosquitoes during successive floodings in a Rift Valley fever endemic area in Kenya. J. Am. Mosq. Contr. Assoc. 7(1): 109-112.

ABSTRACT: Floodwater *Aedes* breeding habitats in central Kenya were sequentially flooded to determine the amount of egg hatch that occurred during each flooding. Approximately 90% of the total number of larvae sampled during four floodings emerged during the initial flooding. The number of *Aedes* eggs hatching during the second flood was lowest of all 4 floodings and no significant differences in the amount of egg hatching during floodings 3 and 4 were seen. Unhatched *Aedes* eggs were present in soil samples collected after the final flooding. The possible implications of these findings with regard to rift Valley fever virus control are discussed.

Logan, T.M., K.J. Linthicum, P.C. Thande, J.N. Wagateh and C.R. Roberts. 1992. Mosquito species collected from a marsh in western Kenya during the long rainy season. J. Am. Mosq. Contr. Assoc. 7(3): 395-399.

ABSTRACT: A total of 476,656 mosquitoes representing 10 genera and 43 species were collected from a marsh in the western Kenya highlands. *Culex pipiens* was the most common species, totalling 91.3% of the collection followed by *Cx. zombaensis* (2.1%), *Anopholes coustani* (1.1%), *An. squamosus* (0.8%), *Mansonia uniformis* (0.6%), *Ma. africana* (0.4%), *Coquilletidia aurites* ((0.4%) and *Uranotaenia mashonaensis 5*(0.3%).*Aedes quasiunivittatus* was the first floodwater species to

emerge from newly flooded areas and was the most abundant *Ae.* spp. collected representing 88% of all *Ae.* specimens. *Culex guiarti* and *Cx. zambaensis* colonized newly flooded areas soon after the areas became flooded.

Logan, T.M., K.J. Linthicum, J.N. Wagateh, P.C. Thande, C.W. Kamau and C.R. Roberts. 1990. Pretreatment of floodwater *Aedes* habitats (dambos) in Kenya with a sustained-release formulation of methoprene. J. Am. Mosq. Contr. Assoc. 6(4): 736-738.

ABSTRACT: Effectiveness of sustained-release Altosid pellets (4% AI methoprene) against floodwater mosquitoes in dambos treated a 5,3, and 1 week before and 1 day after flooding was determined. Only 2% of *Aedes* pupae (primarily *Aedes mcintoshi*) survived to adults in an area treated 5 weeks preflood, and no adult mosquitoes emerged from an area treated 1 day after flooding. In contrast, 12 and 16% of *Aedes* pupae successfully survived to the adult stage in areas pretreated 3 and 1 week, respectively, preflood. The effectiveness of the Altosid declined against *Culex* spp. (primarily *Cx. antennatus*) collected from dambos 15-31 days after flooding. The potential for using preflood treatment with methoprene to control *Aedes* vectors of Rift Valley fever virus in endemic areas is discussed.

Pope, K.O., E.J. Sheffner, K.J. Linthicum, C.L. Bailey, T.M. Logan, E.S. Kasischke, K. Birney, A.R. Njogu and C.R. Roberts. Identification of central Kenya Rift Valley fever virus vector habitats with Landsat TM and evaluation of their flooding status with airborne imaging radar. In preparation.

SUMMARY: This study demonstrated the potential for using LANDSAT satellite data to predict dambo habitats. It also demonstrated the potential for using aircraft mounted synthetic aperture radar (SAR) data to show dambo flooding.

Whittle, R.K., K.J. Linthicum, P.C. Thande, J.N. Wagati, C.M. Kamau, and C.R. Roberts. 1993. Effect of controlled burning on survival of floodwater *Aedes* eggs in Kenya. J. Am. Mosq. Contr. Assoc. In Press.

__ABSTRACT__: The effect of controlled burning on the survival of *Aedes* mosquito eggs was evaluated in 2 distinct dambo habitats. In a dambo dominated by grasses, egg survival was 3.3% after burning compared with 43.8% in a similar dambo that was not burned. In a dambo dominated by sedges, egg survival was 0.7% after burning compared with 28.5% in a similar dambo that was not burned. Mortality of mosquito eggs appeared to be caused by high temperatures associated with the fire and not elapsed time since egg survival did not decrease with time after burning. The potential for burning to control the mosquito vectors of Rift Valley fever virus is discussed.