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FOREWORD

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NA For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

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INTRODUCTION:

Kenya is endemic to several infectious diseases of military relevance and hence of interest to the US military. In the coast and Western lowlands hyperendemic malaria predominates. In contrast, the highlands, previously considered malaria-free, can experience epidemic or imported malaria. Leishmaniasis is focally distributed in the Baringo district, Machakos and the arid but vast northeastern regions of the country. Enteric pathogens cause significant morbidity and mortality in poor communities in urban and remote areas where opportunities for zoonotic diseases prevail.

This Cooperative agreement initially covered malaria immunology and vaccine development, microbiology and drug development and vector studies. In the later years of the contract, allowance was made to carry out initial work in Leishmania transmission, arboviral transmission, enteric pathogens and HIV. The malaria work was centered in Nyanza province around the city of Kisumu and its environs. It comprised malaria vaccine trial, malaria immunological studies, particularly, molecular mechanism for susceptibility to severe anemia and of pregnancy, and malaria vaccine trials. Microbiology and drug development included drug sensitivity testing for antimalarials and antibiotics, diagnosis of enteric and viral pathogens and vector surveillance for transmissible agents. Investigations were undertaken in the coast, urban slum communities and remote locations in Entosopia. The scope of work covered range from field studies to basic research on the diseases of interest. The methods, results and accomplishments are covered in the summary and research accomplishment sections.

SUMMARY OF RESEARCH ACCOMPLISHMENTS:

I. Immunology and Vaccine Development

A. Malaria Immunology

Western Kenya has one of the highest rates of malaria transmission in the world, variously reported between 40 and 300 infective bites per person per year. The burden of intense transmission falls on young children, who, if they survive, progressively develop immunity. In the vicinity of Kisumu, where our operations are centered, severe anemia is the most common cause of morbidity and death in children 6 - 24 months old suffering from *Plasmodium falciparum* malaria. In these children, the level of parasitemia cannot account for the RBC loss and hence, alternative mechanisms of destruction of uninfected RBCs must be active. In areas where the annual inoculation rate is less than 50, cerebral malaria in 3 - 5 year olds is the most important complication of infection. It is not known how *P. falciparum* induces two distinct, age related immune responses.

Attention was focussed on the study of RBC complement regulatory proteins (CR1, CD55 and CD59). These proteins control the complement activation cascade, remove immune complexes (IC) from circulation and protect RBCs from complement mediated damage. In addition, CR1 has been shown to be involved in rosette formation between infected and uninfected RBCs (Rowe et al. Nature. 338, 292-295, 1997), a phenomenon linked to the occurrence of cerebral malaria. We conducted a case control study involving children with severe *P. falciparum* anemia (hemoglobin ≤ 5 g/dL) admitted to the pediatric ward of the New Nyanza Provincial Hospital in Western Kenya. We used cytofluorometry to quantify in vitro erythrophagocytosis and to measure red cell surface

complement regulatory proteins (CRPs) CR1, CD55 and CD59. Red cells from patients with severe anemia were more susceptible to phagocytosis and also showed increased surface IgG and deficiencies in CR1 and CD55 compared to asymptomatic and symptomatic controls. Longitudinal case-control study of children with severe malarial anemia (hemoglobin ≤ 5 g/dL) were carried out to determine if the deficiencies identified are baseline or acquired, and, if acquired, their relationship to the development of anemia. It was shown that the deficiencies in erythrocyte CR1 and CD55 in children are acquired and develop progressively as IgG and C3b become deposited. This cumulative loss of CR1 and CD55 that occurs with repeated attacks of malaria may result in an inability of RBCs to regulate complement activation and RBCs are destroyed through complement-mediated lysis and/or phagocytosis.

Using cytofluorometry to quantify in vitro erythrophagocytosis and to measure the red cell surface complement regulatory proteins CR1, CD55 and CD59, it was shown that red cells from patients with severe anemia are more susceptible to phagocytosis and have increased surface IgG and are deficient in CR1 and CD55 compared to asymptomatic and symptomatic controls. The deficiencies in erythrocyte CR1 and CD55 in children are acquired progressively as IgG and C3b become deposited. This cumulative loss of CR1 and CD55 after repeated attacks of malaria may result in the inability of RBCs to regulate complement activation and RBCs to be destroyed by complement mediated lysis or phagocytosis².

B. Malaria Vaccine

USAMRU-Kenya has been involved in developing or testing candidate vaccines since 1986. In 1995 it tested a promising antigen construct from sporozoites (RTS, S) and it is now planning extensive tests of candidate vaccines based on merozoite surface proteins (MSP-1) or the apical merozoite antigen 1 (AMA-1). Under this CA, the safety of the experimental vaccine RTS, S in a malaria semi-immune population of western Kenya was determined. Field site development continues with the anticipation of testing other candidate vaccine antigens to include the WRAIR MSP-1.

II. Microbiology and Drug Development

A. Drug Testing

Although Plasmodium is adapted to wide areas of the world, today its most serious effects are felt in the Tropics. More than 300,000,000 people are estimated to be infected globally and Plasmodium falciparum caused mortality may be between 1,000,000 and 1,500,000 annually (WHO), mostly among children pregnant women in equatorial Africa. In the absence of a vaccine, anti-malarial drugs play an important role not only in treatment but in prevention. Therefore the consequences of drug resistance are especially profound. The Walter Reed Army Institute of Research, in Washington, DC, has long been in the forefront of malaria drug development. During the tenure of this

CA, two main missions have been undertaken - to determine the nature and extent of drug resistance and to test candidate drugs for the prevention of malaria

Resistance of *Plasmodium falciparum* to chloroquine, a cheap and well tolerated treatment and prophylaxis, emerged simultaneously in Southeast Asia and Central America during the early 1960s. Although chloroquine resistance took hold in Africa later, by the early 1990s it appears to have been widespread in East Africa. Resistance has also developed to nearly all promising, long lasting drugs introduced as replacements to chloroquine for individual malaria prevention. In a clinical study supported by this CA in 1998, Dr Bernhards Ogutu, found that 35% of a study group of children in Nairobi had some degree of resistance to Fansidar (pyrimethamine and sulfadoxine). Nearly all anti-malarials can be divided into two chemical classes, depending on where they interfere in the parasite pathway to DNA synthesis: folates (for example, chloroquine, primaquine), which inhibit the action of dihydrofolate reductase (DHFR), and anti-folates (e.g., pyrimethamine, sulfadoxine), which interfere with dihydropteroate synthetase (DHPS). Mutations in the genes coding for these two enzymes may develop under drug pressure and render the parasite resistant. The primary objective of the work was to map the extent of resistance in East Africa for a wide range of both classes of drugs and to provide this information to public health workers for the better prevention and treatment of malaria. To do this it is necessary to establish systematic surveillance based on a consistent method of in vitro testing. In June, 2001 attempts to establish the first automated in vitro drug testing facility for anti-malaria drugs in East Africa began. *P. falciparum* are cultured for two weeks and then exposed, in treated microtiter plates, to a battery of 16 pharmaceuticals. Resistant isolates are those that incorporate tritiated hypoxanthine, as compared to standard lines maintained at WRAIR. The data are stored and analyzed using a custom designed program. This system is now ready for surveillance of point mutations in DHFR (51, 59, 108, 164) and DHPS (437, 463, 581, 631) in both freshly collected and archived specimens, which will be correlated with the in vitro results and, when available, clinical outcomes. Of 53 specimens collected from a remote area of southern Kenya, a single DHPS mutation (437) was found in 92%, but a triple DHFR mutation (51, 59, 108) was found in 87%, indicating a high level of anti-folate resistance. Both resistance markers appear to be widely diffused in Kenya. The malaria drug resistance project now has the first in vitro malaria drug sensitivity laboratory in Equatorial Africa.

Resistance to antimalarial drugs is emerging faster than new drugs can be developed. Because western Kenya is subject to high malaria transmission rates - often higher than 100 inoculations per person per year - new prophylactic drugs can be evaluated in relatively small numbers of people. The highland areas, on the other hand, offer the opportunity to test therapeutic drugs in infected migrants under conditions that preclude re-infection. Tafenoquine, an 8-aminoquinoline analog of primaquine, that in preliminary testing has shown great promise as a safe, efficacious preventative. Tafenoquine, acts against the liver stages of the parasite, is better tolerated than primaquine, and is long-acting. Phase III trials were undertaken in western Kenya under this CA.

B. Diagnosis

In the Tropics, diarrhea is not only a significant cause of morbidity but the fourth leading cause of mortality. Worldwide, 4.6 million children die from severe diarrhea annually, or 12,600 deaths per day. The epidemiology of diarrheal disease is complex: viruses, bacteria or parasites may be responsible, while environmental and social factors modulate transmission. Studies on enteric pathogens were undertaken in 1998 to identify the causes of bacterial diarrhea, characterize the factors contributing to their virulence and dissemination, and to establish a surveillance system to detect epidemics and patterns of antibiotic resistance

Especially important in Kenya, because of their prevalence and severity, are enterotoxigenic *E. coli* (ETEC) and species of *Shigella*. Complicating the heavy disease burden has been the rapid development of antibiotic resistance. Treatment in East Africa is often based on syndromic diagnosis or self-medication, frequently resulting in inappropriate or insufficient drug administration. (In Bangladesh, susceptibility of *Shigella* to nalidixic acid dropped from 99.7% to 79.8% within four years.) Although the problem of resistance is widely recognized in Kenya, there has been no systematic surveillance for it. Nor is there much known about the epidemiology and resistance patterns of other enteric bacterial pathogens (e.g., non-ETEC pathotypes and *Campylobacter*).

Studies were initiated throughout Kenya. Each location represented a population with unique characteristics and exposure to bacterial pathogens that have yet to be fully described. For example, Machakos is a prosperous highland crop growing area; Entasopia, a southern center for rural Masai pastoralists; and Mathare, a vast Nairobi shantytown. Study locations are rotated throughout Kenya in an effort to obtain information from a wide variety of climates and communities. During the tenure of this CA, the first invasive, toxigenic *E. coli* from this region was isolated. The *E. coli* O6:HNM was positive by PCR for both shigatoxin-1 and VirG from Machakos was cytotoxic in Vero cell assay and invasive in the Sereny test. Additionally, the first isolation of *Shigella dysenteriae* type 12 from Kenya made during an outbreak of dysentery at Loyangalani. The isolation of *E. coli* O157:H7 Masai in Southern Kenya is of particular interest because the Masai have limited contact with visitors from outside Kenya. This pathogen has been documented only once before, in the coastal town of Malindi

C. Disease Surveillance

Machakos

Machakos District Hospital lies about 50KM east of Nairobi, on a high, semi-arid plateau devoted crops such as vegetables and coffee and animal husbandry. During March-September 1999, specimens were collected from 264 patients presenting with diarrhea. Case definition was the passage of three or more unformed stools in a 24-hour

or any one stool containing blood and / or mucous. Among the 264 patients meeting these criteria, 40 presented with bloody diarrhea.

After serotyping, *E. coli* isolates were examined by PCR for the presence of *shigatoxin 1* and *shigatoxin 2* genes; *Shigella dysenteriae* type 1,3 of which were *shigatoxin (stx)* gene positive. All 3 *stx* positive isolates produced this toxin in vero cells. Other serotypes of *Shigella* recovered during the study period included : *S. boydii* 3.8%, *S. flexneri* 5.7% and *S. sonnei* 6.4%. Pathogenic *E. coli* was isolated from 74(28%) of the patients. Among the *E. coli* isolates were 2 *E. coli* 0157:H- and 1 *E. coli* 06:HNM. These were *shigatoxin1(stx)* gene positive for *VirG* and were cytotoxic to Vero cells.

This table summarizes our findings on antibiotic susceptibility:

	TE	AM	CH	GE	ER	NA	SXT
Shigella	91.7	90.3	26.8	0	88.9	0	90.3
E.coli	58.6	88.6	20	1.4	98.6	10	74.3

Percent of each isolates resistant to given antibiotic. TE=Tetracycline, AM=Ampicillin, CH= Chloramphenicol, GE=Gentamycin, ER=Erythromycin, NA= Nalidixic Acid, SXT= Trimethprim-Su;famethoxazole

During this study, children 0-5 years old had the highest incidence of diarrhea caused by shigatoxin producing *E. coli* (STEC). Considering STEC alone, these isolates were responsible for 5.0% of bloody diarrhea cases and 2.7% of all *E. coli* isolated from the Machakos study area. All STEC isolates carried the *stx1* gene however, no isolate was positive for *stx2*. The strong association of *stx2* and the development hemolyticurmic syndrome (HUS) and the low incidence of this toxin during our study may be one explanation for why this syndrome is seldom reported from East Africa. The most interesting finding in this study, was the recovery of *E. coli* 06:HNM that carried both, *stx1* and *VirG*. We believe this is the first finding of a pathogenic *E. coli* capable of both toxin production and invasion.

Due to the low recovery of STEC during this study it is important to consider other possibilities for why HUS and this group of organisms is seldom reported. For instance, based on the low recovery of STEC, it is possible that STEC do not represent significant threat in this region. This study does not indicate further investigation of this organism's distribution is warranted in Kenya because it could be a more significant threat to populations with more risk factors for acquiring STEC infection.

Entasopia

Entasopia is a village about 100 km south of Nairobi, on the floor of Rift Valley. It is a gathering point for semi-nomadic Masai cattle herders, whom we anticipated would have higher than normal exposure to ruminants and lower than normal antibiotic use. The study ran from March to November 1999, and 165 patients were seen at the rural health center where the study took place. Diarrhea was defined as the passage of three or more unformed stools in a 24-hour period or any one stool containing blood and/or mucous. Specimens were placed in Cary-Blair transport media and transported to our laboratory in Nairobi. Once in Nairobi, each specimen was inoculated onto selective media and incubated for 24 hours at 37C. Identification of isolates was by standard biochemical

reactions. Isolates identified as *Shigella* sp. *Salmonella* sp. or *E.coli* were serotyped using commercial anti-sera. Each specimen was also examined on site for parasites. *E.coli* isolates were examined by multiplex PCR for the presence of shigatoxin1(stx1) and shigatoxin 2 (stx2), intimin(eae) invasiveness(VirG), and aggregative (Eagg) genes. These isolates were then examined for the ability to produce cytotoxic effects on Vero cells and for adherence to HEp-2 cells. *Shigella dysenteriae* isolates were also examined by PCR for the *shigatoxin* gene and positive specimens were tested under *in vitro* conditions using Vero cells. All bacterial isolates were tested by disc diffusion for antibiotic susceptibility.

The most common cause of acute bacterial diarrhea among the masai is due to *E.coli* and STEC (defined as the presence of either *stx1* or *stx2*) represented over 22% of the *E.coli* isolates. The close associations of the masai with ruminants along with certain cultural practices are possible explanations for this finding. *E.coli* 0157 was detected only twice during the study period indicating that non-0157STEC may play more significant role than *E.coli* 0157 in infection in this setting. The virulence properties of our isolates (Table1) show that the majority of isolates were *eae* negative, carry *stx2* and are capable of adherence to HEp-2 cells *in vitro*. Two isolates were *eae* negative and did not exhibit *in vitro* adherence and are believed to be non-pathogenic as these patients had a concurrent infection with *G. lamblia*. The remaining *eae* negative isolates all showed *in vitro* adherence and carried the *stx2* gene. Vero cells assays showed that three isolates were capable of producing toxin. Based on this information, we believe these were the only three isolates capable of causing human disease. The remaining five STEC isolates are most likely animal pathogens that were acquired through close contact of the patients with animals and their waste products.

Even though *E.coli* were the most significant cause of bacterial diarrhea during this study, parasitic causes of diarrhea were more common than any bacterial cause. *Giardia lamblia* was the most common parasitic cause of diarrhea, accounting for over 35% of all diarrhea cases. Other parasites observed during this period are *E.hystollica* (15.2%), *S.mansonii*(10.9), *Hookworm* (5.5%) and *T. trichuria*(4.2%).

The antibiotic data (Table2) indicates all isolates were resistant to three or more antibiotics, including at least one first line drug for Kenya. However no isolate showed resistance to nalidixic acid. This is in contrast to other developing nations, where nalidixic acid resistance is present. The resistance data indicates that overall, antibiotic resistance was lower among this population than others previously studied. Due to the economic conditions in Kenya, it is imperative that surveillance efforts on drug sensitivity continue, nalidixic acid is the last affordable drug to treat enteric infections in Kenya.

Table1.

	stx1	stx2	eae	Vero	HEp-2
027	+	-	-	-	-
06	-	+	-	-	++

086a:H10	+	-	-	+	+++
0157:HNM	+	+	+	-	+++
0164	-	+	-	-	-
0157:H7	-	+	+	+	++++
029	+	+	+	-	NT
028a	+	+	-	+	++

Table2.

	TET	AMP	CHL	GEN	ERY	NAL	NOR	SXT
Shigella	63	40.7	29.6	7.4	66.7	0	0	44.4
E.coli	80	62.9	31.4	11.4	91.4	0	0	68.6

Antibiotics resistance profiles of isolates. All *shigella sp.* and *E. coli* were resistant to 3 or more antibiotics including at least one of recommended first line antibiotics in Kenya.

Mathare 4B

Mathare 4B is a peri-urban area of Nairobi, with a population of approximately 200,000. The area is characterized with poor sanitary conditions and limited access to healthcare. Mathare 4B was selected following many cases of undiagnosed bloody diarrhea at a clinic. It is anticipated that predominant bacterial causes of diarrhea will be different from those observed in a rural setting and may show a different pattern of antimicrobial susceptibility. Of particular interest was the epidemiology of *campylobacter sp.* and non-0157STEC in this population.

III. Vector Studies

In FY01, the Entomology program at USAMRU-Kenya supported three primary research programs in Kenya. These programs covered malaria research in western Kenya and the city of Nairobi and arbovirus vector research along the coast of Kenya. Approximately 40 casual (full-time) employees were hired to assist in these programs and two vehicles were dedicated for support of the entomology project in western Kenya. An entomology laboratory was established at headquarters in Nairobi that supports specimen identification, storage (-70 C freezers), and PCR capabilities.

The project in western Kenya was designed to study the ecology of malaria vectors with the use of remote sensing and GIS to develop a new dry season malaria vector control strategy in Kenya. We used the combined vector, environmental, and map database to address questions about malaria transmission focality. We constructed maps of the site using the differential global positioning system (GPS). The maps include permanent water sources and areas where we find malaria vector eggs during the dry season. On-site mosquito collections provided data for Entomological Inoculation Rates

(EIRs) which indicated that the highest monthly EIR was 0.14, or an infective bite every three days in May. We also determined that *An. funestus* tend to seek blood meals earlier than infective *An. gambiae*. This finding will have significant impact on transmission, and on assessment of disease risk since early biting infective vectors are more likely to find unprotected hosts.

In Nairobi, we established a malaria vector surveillance program in the Kibera shantytown, a district of the city with >700,000 inhabitants. Urban malaria is having an increasing impact in sub-Saharan Africa and in Nairobi the ecology is further confounded by the fact that the city is at an altitude of approximately 1 mile. Two of the most important vectors, *An. gambiae* and *An. arabiensis*, were found throughout the year in Kibera although at extremely low population densities. It appears that during the drier seasons these vectors are breeding in polluted streams that border the edge of the shantytown. No infected vectors were collected however, our collections were associated with areas of suspected local transmission (based on travel histories of infected people).

Recent data from the Coast Province indicated ongoing dengue transmission. We established an arbovirus surveillance program that initially focused on vector surveillance in the localities of the identified infections. We conducted household vector surveillance throughout the year and collected eggs from ovicups to determine the presence/absence of the vector in the area. *Aedes aegypti* is the primary dengue vector but other potential vector species are present. *Ae. aegypti* is not usually found indoors although larvae are quite abundant especially during the rainy seasons. We collected temperature and humidity data for the study area. A human use protocol for a dengue serosurveillance study was submitted through KEMRI. Potential study sites were identified in Mombasa and Malindi. In addition to these studies, entomology provided support for CONUS based programs to include phase one of the Dengue Vector Control System device testing. This was a 15 week test program designed to evaluate the effectiveness of traps for collecting dengue vectors. We also provided support for several leishmaniasis programs/protocols.

A. Malaria Transmission

Many of the most important infectious diseases in Kenya are vector borne: malaria, yellow fever, typhus, Rift Valley fever, leishmaniasis, Congo-Crimean hemorrhagic fever, trypanosomiasis and dengue are examples. Because the vectors - mosquitoes, ticks, sand flies - are cold blooded, free living arthropods, climate and topography play important roles in transmission, and are factors that can be used in prediction of epidemics and in their control. The intensity of malaria transmission in many parts of equatorial Africa is the direct consequence of ecologies that promote the dissemination and longevity of the *Anopheles* mosquitoes that transmit it. The primary objective of the vector studies is better understanding the effect of environment on malaria transmission. Because of its high average elevation and diverse topographies, Kenya supports a wide variety of malaria situations, ranging from no transmission to holoendemicity. The basin of Lake Victoria has one of the highest transmission rates in the world. Around the provincial capital of Kisumu, malaria is directly or indirectly responsible for nearly one third of the deaths in children under 5 years old. The cost of nonfatal infections with *Plasmodium falciparum*, *P. malariae* and *P. ovale* has not been estimated but must be proportionately huge. As in most equatorial Africa, the principal vectors are *Anopheles gambiae*, *An. arabiensis*, and *An. funestus*. Western Kenya, which is heavily cultivated,

has two distinct wet seasons - April to August and November to January - when transmission is intense, and intervening dry periods when *Anopheles* are rare and incidence is low.

From September, 1999 to August, 2000 work was performed in the village of Kamonye, about 30 km west of Kisumu. The village encompasses 46 widely spaced family compounds housing 306 people. Houses are of renewable construction with mud plastered walls and grass or metal roofs. The village and surrounding area (about 80 hectares) was precisely mapped using GPS, topographic maps, and aerial photographs. All ground water was registered at monthly intervals, numbered, and sampled for larvae. Nine houses were selected for indoors, human landing collections during 1800-0600 twice each week for 12 months. *Anopheles* were then sorted by hour, checked for parity, and prepared for sporozoite ELISA detection PCR species identification. Mass blood surveys were done twice a year. Continuous records of indoor and outdoor temperature were made using microchips and local rainfall measured daily.

In a mass blood survey conducted in May, 1999, 72% of males and 60% of females were microscopically positive for *P falciparum*, with the highest prevalence in 2-9 year olds. The study period coincided with a drier than normal period: 302 *An gambiae* and 187 *An funestus* were caught; fewer than 10 *An arabiensis* were identified. The majority of host seeking *funestus* were caught before 2400, of *gambiae*, after 2400. Nearly 62% of all the *gambiae* were collected during the 3 wet months March-May, whereas there was no significant monthly variation in *funestus* numbers. The infectivity rate for *funestus* was nearly twice the 5% in *gambiae*, but because of its greater numbers, *gambiae* was responsible for a higher inoculation rate.

During the rains *gambiae* bred in a multitude of puddles, but during the dry intermissions when these disappeared the only suitable water was in seepages at bottom of widely spaced ravines. In this water *funestus* outnumbered *gambiae*.

A section of Kibera from where some crops were being cultivated was chosen. Three men made Systematic morning resting collections were made in a slum area where several cases of malaria had been found and that stood across a shallow river. During 2000, central Kenya experienced a severe drought and no *Anopheles* were found in collections during the first 11 months. But beginning in January 2001, as normal rain patterns resumed, small numbers of *Anopheles gambiae* and *An arabiensis*, as determined by PCR, began to be caught in the ratio of 12 *gambiae* to 1 *arabiensis*. Species determination were made by PCR. Thus far all have been negative for sporozoites by ELISA. Breeding sites have not yet been identified. Recently the project was expanded to analyze the records of parasitologically confirmed malaria in children reporting to the clinic. Active foci may be identified by concentrating collection efforts around clusters of cases.

B. Leishmania Transmission

Visceral leishmaniasis (VL) is a very serious disease often affecting the poorest of the poor. In the Old World, VL, or kala-azar, extends from the border of Bangladesh and India to the Middle East and southward through the Great Rift Valley into Kenya. Recently, the World Health Organization estimated that more than 500,000 new cases occur annually, most documented in India and Bangladesh. The disease is caused by a

microscopic parasite of blood tissue, *Leishmania donovani*, which is transmitted through the bites of sand flies. Endemic VL occurs predominately in Wajir, Baringo, Turkana, and West Pokot districts, in the Rift Valley Province, and Kitui, Meru, and Machakos districts, in Eastern province. Children are most severely affected. Through collaborations with researchers at WRAIR, KEMRI, CDC and commercial partners in Sydney, Australia (Cellabs Pty Ltd) and Camarillo, California (Medical Analysis Systems), a number of prototype assays were developed. First, an enzyme-linked immunosorbent assay (ELISA) was developed by our commercial partners in Australia and secondly, a prototype immunofluorescence assay (IFA). The same polyclonal antibodies used to assemble the IFA were used to develop a rapid immunochromatographic antigen-capture assay. Prospective studies are underway to evaluate these assays and other rapid tests that are commercially available from InBios, Inc. (Seattle, Washington). The goal of this program is to assist the WRAIR Diagnostics section validate any of these devices that prove to be accurate and practical for field use.

In April 2001 we a prospective study in the Baringo District was started with the primary goal of determining the seroprevalence of *Leishmania*-specific IgG. The antibody-capture ELISA and two InBios wicking assays were used. Thereafter, a complete census and GIS-mapping of two villages Parkarin and Loboï was undertaken. Over 500 subjects were enrolled to gather information on certain risk factors, signs and symptoms of VL. A small blood sample was obtained. Serum was extracted from all blood samples and serological testing done. The serological results was matched to the census and GIS coordinates, and spatial analysis utilized to establish focality of disease. During the next CA correlations between case clustering and certain risk factors (i.e., location of subjects to animal enclosures, sand fly habitats, latrines, etc.) will be sought.

C. Arboviral Transmission

Kenya is endemic for yellow fever, Rift Valley fever, West Nile fever and Congo-Crimean Hemorrhagic fever, among others. Many are zoonoses, infecting both animals and humans. Because of the involvement of other mammals and of arthropods in transmission, human epidemics often follow sudden changes in climate or environment. We know little about assessing risk or preventing most of these viruses in spite of mounting evidence that a number of other undescribed pathogenic viruses exist in Kenya. The USAMRU and, active Active surveillance was conducted in collaboration with the World Health Organization Reference Laboratory for Haemorrhagic Viruses at KEMRI to identify and characterize these viruses and the causative agents of typhus, the *Rickettsia*. Suspected yellow fever cases were reported at 22 hospitals and clinics around Kenya. Because other hemorrhagic viruses often present with symptoms of inhibited coagulation similar to those of classic yellow fever (and about 40% of yellow fever cases initially present without those symptoms), a network was designed to screen for a wide variety of other viruses. Case definition for sample collection was 2 of the following: temperature $>38^{\circ}\text{C}$, Jaundice, Hemorrhage, encephalitis, and renal dysfunction. Isolations were done from mosquito C6-36 and Vero cell cultures in which cytopathic effects (CPE) were noted. Viruses, as determined by presence of CPE or IFA, were inoculated into mice. Routine IFA using the NIH Arbovirus Pooled Grouping Serum for

alpha viruses, flaviviruses, and bunya viruses was employed. In October 2000, the Reference Centre diagnosed the first confirmed case of human Crimean-Congo Haemorrhagic Fever (CCHF) in Kenya. Using RT-PCR a diagnosis was made within 24 hours after receipt of the serum sample from one of the Kenya Yellow Fever surveillance sites. The tick-borne CCHF virus is notorious for secondary transmission in hospital settings but fortunately no secondary cases amongst the hospital staff or close family members were recorded. Follow-up studies suggested that exposure of the single fatal case to CCHF virus was most probably through the bite of an infected tick.

Ticks have the ability to transmit a wide range of viral pathogens, including CCHF virus mentioned above. The Reference Centre is currently investigating 16,250 ticks collected at two Nairobi slaughterhouses for viral pathogens. The ticks have been taxonomically identified to species level, pooled and inoculated in to newborn mice and cell culture systems for virus isolation. 39 viruses were isolated and are being characterized by indirect immunofluorescence, in conjunction with pooled immune grouping serum, and RT-PCR. Common East African tick-borne viruses including Dugbe virus (Bunyavirus) and Thogoto virus (Orthomyxovirus) have been characterized, but the majority of the virus isolates may require additional expertise to fully identify them. It is expected that some of the isolates may prove to be 'new' viruses

Dengue, a flavivirus transmitted by *Aedes* mosquitoes, is the most cosmopolitan and important arbovirus in the world. The virus occurs in four types (1, 2, 3, and 4). Infection with one type confers life long immunity to that type but not to the others. For reasons not yet fully understood, but apparently related to the type or sequence in which one is infected by different types, some people develop Dengue Hemorrhagic Fever Syndrome (DHF), which is often fatal. Although usually associated with Asia and the Americas, it has been recorded sporadically from Africa, including coastal Kenya, in 1980. Because cases of dengue have been found so infrequently in Kenya, epidemics have generally been thought to originate from introduced cases. In 2000 we began to test the hypothesis that dengue transmission was endemic to Kenya. In collaboration with the Oxford University / Wellcome Foundation research unit operating at Kilifi, about 50 Km north of Mombasa, three isolates were obtained from pediatric admissions with undiagnosed fever. All three isolations were of dengue, serotype 2. Further, about 8% of more than 160 other sera from ill children presenting to Kilifi District Hospital had elevated antibodies to dengue, including several neonates whose mothers were presumably recently infected. None of the positive cases manifested DHF. In 2001 a 12 month cohort study to measure both seroconversion in the human population and vector bionomics was started at a site near Malindi, 50 Km north of Kilifi.

Tick Typhus

Typhus is caused by *Rickettsia*, small, bacteria-like parasites that can invade the cells of humans and other mammals, but usually infect insects, mites and ticks, which pass them vertically in their eggs. Tick typhus has only recently been recognized as a serious endemic disease of humans in Africa. The species of parasite responsible, *Rickettsia africae*, had been recorded only once from Kenya, in a tourist diagnosed in UK. The disease caused by *R. africae* includes fever and rash but has never been recorded as fatal.

In 2000, DNA amplification and sequencing from a fatality at Kijabe hospital with symptoms consistent with typhus incriminated *R. africae*. This is the first known fatality attributed to *R. africae*. Dry season collection of ticks from vegetation and domestic and wild animals at Masai Mara refuge, where a group of school children from Kijabe had allegedly contracted a typhus like disease on a camping trip, yielded one *R. africae* positive pool of 7 ticks.

TRAINING:

Training was an important component of CA activities. Appendix 1 contains the list of individual trained with their corresponding degrees. The list comprises Kenyans, Americans and other Nationalities. Two workshops were co-sponsored – the First National Workshop on Antimicrobial Drug Susceptibility, Surveillance and Monitoring (FEB 1997) with the Center for Microbiological Research and the East African GCP Training Course, Nairobi (September 2001) with the U.S. Military HIV Research Program. Several Kenyans were sponsored to attend regional and International conferences, some to present their research results as listed under publications.

SUMMARY:

Under this Cooperative Agreement, work focussed on Malaria Immunology and vaccine development, microbiology and drug development and vector studies. The recombinant RTSS circumsporozoite vaccine was tested and showed to be efficacious but warranted further studies. Field site development continued in Western Kenya in preparation for future more effective vaccine candidates. In between vaccine trials basic immunological research was undertaken which led to novel hypotheses for the increased risk of primigravida mothers to malaria complications and the propensity of children in regions with hyperendemic malaria such as in Nyanza province to present with severe anemia. Phase 1 and 2 drug studies were undertaken leading to filing of an NDA for oral atovaquone/proguanil for the treatment of uncomplicated falciparum malaria and the identification of a promising long acting 8-amino quinoline for malaria prophylaxis. The effectiveness of antimalarial regimens routinely used in Kenya were evaluated in in vitro drug sensitivities studies. Results from these studies have led to re-evaluation of the national recommendations for first and second-line drug use in the country. Additional work was performed in the area Plasmodial drug resistance and the foundation set for surveillance studies to determine the distribution of drug resistance genes, in particular DFHR point mutations in the region. An automated in vitro drug sensitivity system was successfully established during the closing months of this contract. The prevalence of antibiotic resistance in enteric pathogens was also determined during the course of this CA. Surveys were undertaken in Machakos, Entosopia and Mathere 4B with surprising results that may impact the future antibiotic use in specific communities within the country. Surveillance for viral pathogens uncovered dengue type 2 in the coastal regions of the country, and the first reports of C-CHF and fatal *R. africae* in the country. The entomological dynamics supporting dry season malaria transmission and those underlying highland malaria epidemics was also investigated. Competent vectors for malaria were found in a slum area of Nairobi. However, proof of transmission must await

documentation of infected mosquitoes in the areas under surveillance. This CA has laid the foundation on which future work will be grounded. The field site in Western Kenya is now mature enough to support Phase 1, 2 and 3 clinical studies for antimalarial drugs and vaccines. The capacity to perform both antimalarial and antimicrobial drug sensitivities and characterize new enteric and febrile pathogens will support the effort to build a Global Emerging Infections System program. Another multi-year agreement is required to bring to fruition the efforts started in this contract.

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Shanks GD; Snow RW; Guyati H; Arness m; Bradshaw D; Maguire J and Biomndo K. Travel to malaria endemic areas as a factor in highland malaria in western Kenya.

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Sang, Willie., Davis, Jon., Smoak, Bonnie.

Verocytotoxin production and presence of VT genes in strains of *Escherichia coli* and *Shigella* isolates from feces of diarrhoeal patients at Machakos District Hospital in Kenya. The 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-producing *E. coli* Infections. Kyoto, Japan. October, 2000.

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Alam, U: Participated in the International WHO supported training course on molecular biology techniques in malaria, Kampala, Uganda, December 1999.

Alam, U; Adoyo, m; Mason, C: Were representatives at the 48th Annual Meeting of the American Society of Hygiene and Tropical Medicine held in Washington DC. November 1999.

Mbaisi A; Alam UH; Mwangi J; Cortese J; Smoak BL; Elson L and Mason CJ: Presented a poster at the 49th Annual Meeting of the American Society Of Hygiene and Tropical Medicine, Texas, USA 2000.

APPENDIX 1

Three-Year Contract Personnel*

	Stage	Payroll No.	Employee Name	Position
1	MR-10	80057	Fred Kiddy Onyango	Senior Lab Technologist
2	MR-10	80044	David Kiplangat Chumo	Senior Lab Technologist
3	MR-10	80068	Joseph Koros	Senior Lab Technologist
4	MR-9	80079	Joyce Macharia	Personnal Secretay I
5	MR-9	80113	Malachi Opollo	Laboratory Technologist I
6	MR-9	80183	Theresa a. Wesonga	Assistant Research Officer
7	MR-9	80182	Zakayo Kadenge	Accountant II
8	MR-9	80229	Margaret W. Muturi	Research Officer
9	MR-9	80234	Bindi A. Gadhi	Assistant Research Officer
10	MR-9	80231	Raphael Pundo Omondi	Computer Programmer I
11	MR-8	80080	Daniel Waema	Supplies Officer III
12	MR-8	80134	Lucy Lodenyi	computer Programmer II
13	MR-8	80071	Charles Asiago	Laboratory Technician I
14	MR-7	80066	Josphat Mwangi Kabui	Laboratory Technician II
15	MR-7	80164	Michael Ouma Opiyo	Laboratory Technologist III
16	MR-7	80233	Joseph Ouya Osoga	Laboratory Technologist III
17	MR-7	80232	Gordon M. Hongo	Laboratory Technologist III
18	MR-7	80039	John Kamanza	Laboratory Technician II
19	MR-7	80063	Christopher Oyaro (onger)	Laboratory Technician II
20	MR-7	80069	Michael Ouko	Laboratory Technician II
21	MR-6	80147	Charles Okundo Okelo	Laboratory Technician III
22	MR-6	80053	Ramadhan Mutalib	Laboratory Technician III
23	MR-6	80035	Agnes Nganga	Computer Operator II
24	MR-5	80049	Nerry Oluoch Ndiege	Junior Laboratory Tech.
25	MR-5	80166	Cathrine Wigwa	Computer operator III
26	MR-5	30029	James Gitonga	Computer Operator III
27	MR-5	80206	John G. Kamau	Driver Grade I
28	MR-5	80230	Valerie A. Oundo	Junior Laboratory Tech.
29	MR-5	80047	Dismas Achango	Junior Laboratory Tech.
30	MR-5	80074	Jecinta Wanjiru	senior Auxilliary Staff
31	MR-5	80042	Samwei Odour Wangowe	Junior Laboratory Tech.
32	MR-4	80045	Joram Osumo	senior Auxilliary Staff
33	MR-4	80034	Samwei K. Ligonzo	senior Auxilliary Staff
34	MR-4	80056	Alex Masinya	senior Auxilliary Staff
35	MR-4	80061	Philistus Oigo Ogilo	senior Auxilliary Staff
36	MR-4	80050	Consolata Onyango	senior Auxilliary Staff
37	MR-4	80167	David L. Madahana	Driver Grade II
38	MR-4	80181	George Nyawade	Driver Grade II
39	MR-4	80168	Abdi Ayub	Driver Grade II
40	MR-3	80054	Silas Ongonga Onguka	Auxilliary Staff I
41	MR-3		Edwin C. Mbwabi	Auxilliary Staff I
42	MR-2	80165	Raphael onyango	Auxilliary Staff II
43	MR-9		Melanie Atieno Onyango	Assistant Research Officer
44	MR-9		Joram Ogola Siagla	Assistant Research Officer
45	MR-2		Maurice Odongo Otieno	Driver Grade III

*about 100 shorter term employees not shown

APPENDIX 2

Personnel Trained at United States Army Medical Research Unit-Kenya

<u>Name</u>	<u>Nationality</u>	<u>Affiliation</u>	<u>Degree</u>	<u>Area</u>	<u>Completed</u>
Mebrahtu, Yemane	Kenya	Nairobi University	PhD, M.Sc	Leish	June-92
Njunge, Luna	Kenya	Kenyatta University	M.S.	Malaria	Jul-93
Ofulla, Ayub	Kenya	Kenyatta Univeristy	PhD, M.Sc	Malaria	Jun-94
Alwi, Shatri	Kenya	Kenyatta University	PhD.	Leish	May-95
Ngumbi, Phillip	Kenya	Nairobi University	PhD, M.Sc	Leish	Jun-95
Angile, Chris	Kenya	Nairobi University	Ph.D.	Leish	Sep-95
Iversen, Elsa	Denmark	University Copenhagen	M.D.	Enteric	Feb-96
Christensen, Melin	Netherlands	Leiden University	M.D.	Malaria	Jun-96
Andresen, Renee	Netherlands	Leiden University	M.D.	Malaria	Jun-96
Kariuki, Michael	Kenya	Nairobi University	M.Sc	Malaria	Jun-96
Malakoti, Mark	Navy	USUHS	MPH	Enteric	Jun-96
Taylor, Kathy	US	ILRI	PhD	African Tryp	Jul-96
Schmit, Margot	Netherlands	University Amsterdam	M.D.	Enteric	Jul-96
Tiemessen, M	Netherlands	University Amsterdam	M.D.	Enteric	Jul-96
Ohas, Eunita	Kenya	Kenyatta University	M.Sc.	Malaria	Aug-96
Masinde, Godfried	Kenya	Tulane University	Ph.D.	Malaria	Sep-96
Nyakeriga, Alice	Kenya	Kenyatta Univeristy	M.Sc.	Malaria	Sep-96
Van Doom, Olga	Netherlands	Univeristy Amsterdam	M.D.	Enteric	Oct-96
Van Eljk, Everline	Netherlands	University Amsterdam	M.D.	Enteric	Oct-96
Muturie, Margaret	Kenya	Kenyatta Univeristy	M.Sc	Malaria	Nov-96
Fried, Michal	Israel	NRC	PhD	Malaria	96-98
Kurtis, Jonathan	US	NRC	PhD	Malaria	96-98
Ngure, Peter	Kenya	Moi Univeristy	M.Sc.	Malaria	1997
Ngure, Veronica	Kenya	Moi Univeristy	M.Sc.	Malaria	1997
Ogola, Bilha	Kenya	Moi University	M.Sc.	Malaria	1997
Siangla, Joram	Kenya	Maseno Univeristy	M.Sc.	Malaria	1997
Obado, Michael	Kenya	Kenyatta Univeristy	M.Sc	Malaria	1997
Odhiambo, Rose	Kenya	Egerton University	Ph.D; M.Sc.	Malaria	1997
Honnas, Arne	Norway	University Norway	M.D.	Enteric/meningi	1997
Peta Petersen	Netherlands	St. Mary's Hospital	M.D.	Statistics	June-97
Dianne Olsen	Netherlands	St. Elizabeth Univ	M.D.	Statistics	June-97
Van Gee, Winfred	Netherlands	St. Elizabeth Univ	M.D.	Statistics	June-97
Caaca, Abrahams	Kenya	St. Mary's Hospital	M.D.	Statistics	June-97
Lars Petersen	Norway	St. Mary's Hospital	M.D.	Enteric	June-97
Ogutu, Ragama	Kenya	Kenyatta National Hosp.	MD	MS Paed.	Sep-98