Combination of Laboratory Research and Remote Sensing Applications toward Mitigation of Trypanosomiasis in Africa

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Abstract-

The need for a comprehensive bi-modal approach toward mitigation of trypanosomiasis in Africa, in both human and animal populations, is examined. Fundamental to such mitigation will be the continuance of basic research at the molecular level, which may hold promise of a better understanding of the organism, *Trypanosoma brucei,* and consequently thereby identify potential controlling mechanisms. Equally important to ultimate mitigation will be the intensification of monitoring both disease incidence and its vector, the Bantu-named tsetse fly (*Glossina morsitans*) habitat, with the assistance of remote sensing and Geographic Information Systems (GIS).

Trypanosomiasis, "African Sleeping Sickness" in human populations or "nagana" in animal populations, is sufficiently geographically dispersed throughout Africa as to require satellite monitoring in order to identify areas with vulnerable human and/or animal populations that are appropriate (De Wulff), ecologically indicated and cost-effective for sustainable intervention, including the application of trypanocides.

The current study describes basic research underway at the Seattle Biomedical Research Institute on *Trypanosoma brucei*, reviews existing monitoring programs utilizing remote sensing to assist in controlling the arthropod vector and makes the case for a coordinated *in vitro* and remote-sensing-assisted *in situ* bi-modal mitigation strategy.

The contributions to the remote sensing literature of the Trypanosomiasis and Land-Use in Africa (TALA) Research Group, of the Programme Against African Trypanosomiasis Information System (PAATIS) and of the Africa Real Time Emergency Management Information System (ARTEMIS) are reviewed. Further, the implications of combining course-resolution multi-temporal data with newly- available high-resolution remotely-sensed data are examined. The rationale for coordinating laboratory research and field-based interventions, informed by remote sensing, is articulated.

I. INTRODUCTION

African trypanosomiasis, more commonly known as African Sleeping Sickness, is caused by the protozoan parasite *Trypanosoma brucei*. Currently over 300,000 people are infected and 500,000 live in high-risk areas. The disease is transmitted to humans and other large mammals through the bite of an infected tsetse fly. The primary habitats of the tsetse are in the moist and forested areas of Sub-Saharan Africa. The initial bite of the tsetse fly usually results in a red sore or chancre, which is often painful. During the first stage of the disease, patients may experience swelling of the lymph nodes, headaches, fever, muscle and joint pain, as well as severe weakness as the parasite multiplies in the lymphatic and blood systems. During the second stage, the parasite invades the central nervous system. Victims may experience neurological disorders, slurred speech, behavioral changes, loss of motor control, extreme lethargy at daytime and insomnia at night. In the advanced stages, coma and death occur.

There are two sub-species, *T.brucei gambiense* and *T. brucei rhodesiense* that infect humans. The difference in pathology is mainly observed in the rate of disease progression. Eastern African sleeping sickness, caused by *T. brucei rhodesiense*, advances rapidly. Untreated cases can be fatal within weeks of initial infection. Western African Sleeping Sickness, caused by *T. brucei gambiense*, is usually fatal within several months to a few years after initial exposure. Current treatments for African sleeping sickness are expensive, highly toxic and largely inaccessible for the majority of those infected in endemic areas. Therefore, it is

imperative that more efficient and cost effective treatments and surveillance techniques be developed. Additionally, cattle can be infected by the by the parasite's *T. brucei brucei* strain. Infected cattle cause increased hardship because they can no longer be used for farm labor or nourishment.

Prevention may include avoidance of known vector habitats, as well as application of trypanocides, and /or the application of defoliants in areas thought to be primary habitat for the disease vector.

In recent years, two very distinct parallel research paths, one microscopic (i.e. gene expression disruption) and the other macroscopic (i.e. remote sensing of vector habitat), have provided some hope for tropical disease epidemiologists, medical practitioners and veterinarians contending with trypanosomiasis. Both of these approaches, each of which seeks ultimately to mitigate the disease prevalence, are described herein. Moreover, the case is made for coordination and collaboration among the seemingly disparate disciplines that are addressing disease prevention and treatment from divergent perspectives.

II. MOLECULAR LEVEL RESEARCH

The nucleolus, an organelle located within the nucleus of eukaryotic cells, is comprised mainly of a dense concentration of proteins and RNA and is also the site of ribosome biogenesis. The transcription of rDNA, processing of pre-rRNA, and the formation of pre-ribosomal particles all occur within this area (Melese and Xue 1995). Several factors are necessary for ribosome biogenesis to take place. Some required molecules, including proteins, are transported into the nucleus via interactions with a nuclear localization sequence and then retained within the nucleolus through interactions with other nucleolar proteins or nucleic acids.

Nopp44/46 is a 35 to 36 kDa phosphoprotein that has been found to localize in the nucleolus of *T. brucei*. The term 44/46 is representative of the fact that it migrates as a 44-46 kDa protein on SDS-PAGE (Das *et al.*, 1996). Four specific regions characterize Nopp44/46. The "unique region" (U), located at the amino terminus, is recognized because it shows moderate homology with other proteins contained within the nucleolus and it also contains sufficient information for retention of the protein within the nucleus (Das *et al.*, 1998). Nopp44/46 also includes a "junction region" (J), which contains phosphorylation sites and facilitates interaction with a protein kinase. The "acidic region" (A) enhances the interaction between Nopp44/46 and the protein kinase (Das *et al.*1998). Previous experiments suggest that the associated kinase may be protein kinase CK2. The "repeat region" (R), located at the carboxy-terminus, includes 22-26 arginine-glycine-glycine (RGG) repeats, which are common characteristics of nucleolar proteins (Das *et al.*, 1998). The "R" region is both necessary and sufficient for interactions with nucleic acids.

It is not yet known whether Nopp44/46 is essential to *T. brucei* or what its exact function is. The abundance of Nopp44/46 and its ability to bind both proteins and nucleic acids suggests that it may function as a scaffolding protein, assisting the binding of other nucleolar components. Nopp44/46 may also be involved in ribosome biogenesis, but a complete understanding of the function of the protein has not been drawn. In order to ascertain whether or not Nopp44/46 is essential to *T. brucei*, it is necessary to block functional Nopp44/46 from being expressed within the cell. Currently, there are two known methods of disrupting gene expression. One method, gene-knockout, is very difficult and can take up to six months to complete under optimal conditions. For this reason the newly developed method of RNA interference (RNAi) will be explored. If successful, a phenotypic analysis of *T. brucei* lacking Nopp44/46 will help determine both the role of the protein and whether or not it is essential to the life cycle of the parasite.

RNA Interference

It has been observed in several organisms that the presence of double-stranded RNA (dsRNA) can induce the degradation of mRNA transcripts containing the identical nucleic acid sequence as that of the dsRNA. Some believe that this process may have developed as a defense mechanism against retroviral attacks. It has been found that this phenomena, known as RNA interference (RNAi), or alternatively, post-transcriptional gene silencing (PTGS), can be artificially induced in *T. brucei.* A gene of interest can be cloned into a *T. brucei* expression plasmid in such a way that allows the mRNA transcript to form a hairpin loop. The presence of the dsRNA can initiate degradation of both the mRNA transcripts of the engineered gene and also the mRNA transcripts of the corresponding endogenous gene. This effectively creates a cell line that lacks the protein of interest and is much easier than performing gene knockout. Observations of the altered parasites can then be

made.

Materials and Methods

RNAi techniques were employed to study the function of Nopp44/46 and also to determine whether or not Nopp44/46 is an essential protein in *T. brucei.* Standard cloning methods were utilized to generate the RNAi construct. Polymerase chain reaction (PCR) was used to amplify the U and J regions of Nopp44/46 from the genomic DNA of *T. brucei*, strain Treu 667. The U and J regions were selected because the full-length sequence is not needed to induce degradation of Nopp 44/46 mRNA transcripts. Additionally, the A and R regions are slightly more difficult to clone. Two different amplification reactions were performed. The first created a fragment, Hind3UJXba, containing the U and J regions with a HindIII site on the 5' end and an XbaI site on the 3' end. The second reaction created a fragment, BamUJXba, containing the UJ regions with a BamHI site on the 5' end and an XbaI site on the 3' end. Since Taq polymerase was used in the amplification, an A overhang was created on both the 5' and 3' ends of each fragment. This facilitated cloning into a TA cloning vector. Each PCR reaction was run on a 0.8 % agarose gel. The amplified fragments were excised and purified with a Qiagen gel extraction kit. Each fragment was then ligated into the pGEM-T EZ vector. The ligations were transformed into XL1 blue *E. coli* and the transformants were screened for successful clones.

BamUJXba was cloned into the BamH1 and Xba1 sites of the pLew79 plasmid (Wirtz *et al*, 1999). Simultaneously, Hind3UJXba was ligated in to the pJM325 plasmid. PJM325 contains the intervening "stuffer" DNA (figure 2), which is necessary for the final mRNA transcript to form the hairpin loop.

A fragment containing Hind3UJXba as well as the stuffer DNA was then digested out of the pJM325 plasmid so that it could be ligated into the HindIII and XbaI sites of the pLewJU plasmid. This created the final construct known as pUJRNAi, which contains two copies of the U and J regions of Nopp44/46, the first in the sense direction (UJ) and the other in the anti-sense direction. (Figure 3) A fragment of stuffer DNA separates both.

The pUJRNAi construct was linearized with *Not1* and then electroporated into *Trypanosoma brucei brucei*, strain 29.13 cells. The linearized plasmid should incorporate into the rDNA regions of the host genome through recombination. Transfectants will be selected based on phleomycin resistance and cultured *in vitro*. When induction of the gene is induced through the addition of tetracycline, double-stranded mRNA transcripts of the UJ region of Nopp44/46 should be expressed. If all works well, then hopefully, both those mRNA transcripts, as well as, endogenous Nopp44/46 mRNA transcripts will be degraded and a Nopp44/46-free *T. brucei* cell line will be created.

The RNAi scheme has successfully been completed, and the constructed plasmid has recently been transfected into *T.brucei* cells. If RNA interference is attained, Nopp44/46 expression will be halted. The cells can then be observed for changes in their growth and nucleolar function compared to normal parasites.

III. REMOTE SENSING APPLICATIONS

An early major contributor to the application of remote sensing for monitoring insects in Africa was the United Nations Food & Agriculture Organization's (UN FAO) Africa Real-Time Emergency Management Information System (ARTEMIS). In addition to using Normalized Difference Vegetation Index (NDVI) derived from the Advanced Very High Resolution Radiometer (AVHRR) and the European Space Agency's Meteosat for deriving surrogate precipitation data (based upon cold cloud duration) for monitoring agricultural productivity (Snijders), ARTEMIS monitored the migration of the desert locust (*Schistocerca gregaria*) so as to provide early warning and to pinpoint areas at high risk, requiring pesticide application (Griguolo and Mazzanti). Time-series dekadal (10 day composite cloud-free) AVHRR Global Area Coverage (GAC) NDVI data for the ARTEMIS Project is available through FAO in Hammer Aitoff projection.

The methods developed for habitat identification in this instance facilitated the subsequent development of methods to monitor disease vector and host habitats, including the black fly (*Simulium damnosum*) in the case of onchocerciasis, the sand fly (*Phlebotomus paptasi, P. perniciosus, P. mascittii, P. argentipes*) in the case of leishmaniasis, the *Anopheles gambiae* mosquito in the case of malaria (*Plasmodium falciparum)*, two snail species (*Bulinus truncatus and Biomphalaria pfeifferi, B. smithi, B. alexandrina*) in the case of *Schistosoma mansoni, S. haematobium and S. interculatum* (Rochon, et al. 2002), and the tsetse fly (*Glossina morsitans and G. pallidipes*) in the case of trypanosomiasis.

Among the programs which have been in the vanguard of the campaign to monitor and eradicate trypanosomiasis is the Programme

Against African Trypanosomiasis (PAAT), described as an alliance of the FAO the World Health Organization (WHO), the International Association for Educational Assessment (IAEA) and the Organization of African Unity (OAU) Inter-African Bureau for Animal Resources (IBAR) "promoting integrated trypanosomiasis control through coordinated international action."

Other multi-lateral agencies involved in mitigation research and intervention with respect to trypanosomiasis include the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), which celebrated its 50th anniversary in 1999, by holding its 25th biennial meeting. The ISCTRC may hold the best hope for assembling scientists from a wide array of disciplines who share a common commitment to better understand and eradicate the disease; although, to date, the biological and chemical scientific community is far better represented therein than the remote sensing research community.

Other research groups more directly involved with remote sensing applications for tsetse mitigation include the Trypanosomiasis and Land Use in Africa (TALA) Research Group based at Oxford University (Hay, et al.); the Center for Health Applications of Aerospace Related Technologies (CHAART) at the NASA Ames Research Center; the British Overseas Development Agency's (ODA) Integrated Satellite and Field Data Analysis section; the Environment and Remote Sensing Institute (ERSI); the FAO's Sustainable Development Department; and the Center for Earth Science Information Network (CIESIN).

Although AVHRR continues to be used for monitoring *Glossina* habitat (Allsop), Landsat Thematic Mapper (TM) data has been utilized for this purpose in Kenya and for monitoring the *Cyclops* vector habitat for Dracunculiasis in Nigeria and Benin, Lyme disease in the USA, Malaria in Mexico and, in combination with SAR data, for Rift Valley Fever in Kenya. *Le Systeme Probatoire pour L'Observation de la Terre* (SPOT) sensors were used for monitoring *Culex* habitat for Rift Valley Fever in Senegal and *Anopheles albimanus* for Malaria in Belize. Beck, Lobitz and Wood, *inter alia,* speculate that other sensors with the capacity to measure soil moisture will become more important as tools for monitoring vector habitat (e.g. Almaz, ENVISAT, ADEOS II, Terra MODIS, Resurs, MSU and Priroda Mir MSU-SK and MOMS-2P). (Beck, et al.; Robinson, et al.; Rogers, et al.).

IV. COORDINATED INTERVENTION

Heretofore, the microscopic (molecular-based) research and the macroscopic (remote sensing) approaches to mitigating trypanosomiasis have proceeded in relatively total isolation from each other. This is an understandable phenomenon given the length of training required for mastery of each discipline, the highly specialized methodologies and vocabularies associated with each scientific genre and the absence of any bridging peer reviewed journal literature incorporating the findings of both approaches.

Having directly participated in research at the molecular level, while an intern at the Seattle Biomedical Research Institute, having participated as a public health research assistant on location in Madagascar, and having been involved in intensive review of the literature on remote sensing applications for monitoring tropical diseases, with specific reference to trypanosomiasis, I have identified specific complementarities and become convinced of the mutual benefit to be derived through communication between the research groups and, ideally, development of a coordinated strategy for trypanosomiasis mitigation and ultimate eradication.

Specifically, better awareness of the current laboratory research state-of-the-science on the part of the remote sensing community could potentially lead to more targeted approaches to monitoring vector habitat, a better understanding of organism vulnerability, thermal thresholds, and susceptibility to biogenic control mechanisms, as a supplement or alternative to trypanocides (Mangwiro, Torr & Cox). Moreover, the economic and ecological trade-offs between vector eradication and collateral damage to benign insect and bird species could become better understood (Grant; Wilson).

In like manner, greater awareness of the remote sensing literature on the part of laboratory-based researchers could potentially lead to improved methods for estimating vector population density, more botanical species-specific spectral signature libraries for vector habitat differentiation, particularly when utilizing high spatial resolution satellite data, and a better understanding of inter-seasonal variation in organism vulnerability and consequent improvements in the timing of control measure deployment and in the required dosages for best management practices and ecosystem sustainability.

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