From Sentinel Surveillance for Sleeping Sickness Treatment Failure to the Development of a Pharmacovigilance Approach

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Summary

In the late 1990’s a disturbing trend of geographically distinct melarsoprol treatment failures accompanied a dramatic resurgence of Human African Trypanosomiasis. These studies present results from a sentinel surveillance network (HATSENTINEL) that was established at nine sites in five countries in response to this crisis, recommendations on the need for continued trypanosome specimen collection and a call for pharmacovigilance.

Seven sites are located in Democratic Republic of the Congo (DRC), Angola, or Sudan, where *T. b. gambiense* is endemic and two sites are located in Uganda and Tanzania, areas endemic for *T. b. rhodesiense*. The facilities included health centers and hospitals operated by ministries of health and nongovernmental organizations. The HATSENTINEL network addressed the lack of standard diagnostic and treatment protocols used for HAT in African facilities by using a standardized form to collect data about the specific diagnostic methods and treatment regimens in use at the sentinel facilities.

The melarsoprol failure rates detected by HATSENTINEL in northern Angola (98%) and in East Kasai Province of DRC (61%) are substantially higher than previously reported. There was no evidence of geographic spread of melarsoprol-refractory infection. The drug remained effective at a site in DRC during the 6 years of surveillance, despite its proximity to the northern Angola site.

The failure rates did prompt the MOHs to switch protocols to an alternative drug, eflornithine. The four centers that used eflornithine as first line treatment for Stage II patients had failure rates ranging from 2.3% to 3.9%; this led to overall reductions in the number of cases.

The study attempted to isolate and investigate parasite strains resistant to melarsoprol but the limited results did not find this to be the case. The investigation for resistant strains should continue.

The results from the HATSENTINEL network has reinforced the need for continued sentinel surveillance. This has also revealed the need for improved pharmacovigilance. There will be many barriers to establishing a reliable pharmacovigilance network, yet these challenges do not mean that pharmacovigilance is not possible in Africa or should be set to a lower standard. In the case of human African trypanosomiasis we argue that the most rigorous approaches advocated by WHO should be embraced.
Zusammenfassung

Die Rückfallraten veranlassten die Gesundheitsministerien, die Behandlung auf das alternative Medikament Eflornithin umzustellen. In den vier Zentren, welche Eflornithin für die Behandlung der Zweitstadiumspatienten zuerst verwendeten, wurden Rückfallrate von 2.3% bis 3.9% beobachtet, was zur Reduktion der gesamten Fallzahl beitrug.
Im Rahmen der Studie wurde versucht, Parasiten zu isolieren, um deren Melarsoprol - Resistenz zu analysieren, was aber in der begrenzten Anzahl der isolierten Stämme nicht der Fall war. Die Untersuchungen über resistente Parasitenstämme werden fortgesetzt werden.
Die Resultate des HATSENTINEL Netzwerks unterstrichen die Notwendigkeit einer gezielten und anhaltenden Überwachung der Medikamentenresistenz (Surveillance) und hat auch den Bedarf für eine verbesserte generelle Arzneimittelüberwachung aufgezeigt (Pharmacovigilance). Um dies zu erreichen werden noch viele Hürden überwunden werden müssen, aber die bestehenden Schwierigkeiten bedeuten nicht, dass Pharmakovigilanz in Afrika nicht oder nur bedingt möglich ist. Im Falle der menschlichen afrikanischen Schlafkrankheit (Trypanosomiasis) sind wir der Meinung, dass der rigoroseste Ansatz, welcher von der Weltgesundheitsorganisation WHO empfohlen wird, angewendet werden sollte.
Chapter 1

General information on HAT and current epidemiology

Literature review and background

Human African Trypanosomiasis (HAT) is a parasitic disease transmitted through the bite of the tsetse fly (Glossina ssp.) and is found in 36 countries in sub-Saharan Africa (Fevre, Picozzi et al. 2006; Maudlin 2006). It was a major public health problem for the first half of the 20th century. Intensive control programs reduced the disease to fewer than five thousand cases annually by the 1960’s, the majority of which were T.b. rhodesiense. However in the past two decades there was a resurgence of disease. This reemergence was due predominantly to cases of T.b. gambiense in central Africa (Van Nieuwenhove, Betu-Ku-Mesu et al. 2001). There has also been an increase of cases of T.b. rhodesiense in Tanzania and into new areas of Uganda but these make up less than 5% of the total(Simarro, Franco et al. 2006). Despite a historical presence, there has been no increase of cases noted in West Africa. The increase in cases was most evident in Angola, DR Congo and Sudan (Moore, Richer et al. 1999; Lutumba, Robays et al. 2005), countries that have all been suffering from conflict and war. In 1995 WHO issued a statement declaring that over 25,000 new cases were being reported
per year but that was due to inadequate surveillance the annual number of newly acquired infections was estimated to be between 300,000 and 500,000. This was a dramatic increase from the levels reported in the 1960’s and was a reminder that when ignored this disease could reach epidemic levels. The disease had been controlled in the past and had been considered to no longer be a public health problem. The levels reported in 1995 therefore were shocking to many and exposed many issues that had led to the resurgence. It became evident that there was inadequate surveillance (Cattand, Jannin et al. 2001), that there was a lack of funding for case detection, and that the elevated cost of the drugs for treatment often lead to a lack of availability. There has been an improvement since 1995 due to more resources being made available for control of the disease and the donations of drugs to WHO in 2001 (Jannin and Cattand 2004; Jannin 2005). In the period from 1997 to 2004 the number of people screened has increased from 1,345,000 to 3,300,000 while the number of new infections reported has dropped from 37,000 to 17,000 (WHO 2006).

This reduction has led WHO to declare that the elimination of *T.b. gambiense* is feasible. A goal has been set for elimination by 2015. The African Union has an even greater target of eradicating the African continent of both *T.b. gambiense* and *T.b. rhodesiense* (Kabay 2004).
Control Methods

There are two main approaches for control, the reduction of the disease reservoir and vector control. *T.b. rhodesiense* is a zoonosis and therefore control efforts focus on the vector. Epidemiologically, man is the most important reservoir for HAT disease from *T.b. gambiense* (Leak 1999). The reduction of the reservoir has been the cornerstone of control and has been proven effective when used alone or combined with vector control. The disease is spread by the bite of a tsetse fly that has previously taken a blood meal from an infected host (Leak 1999). Active case detection (ACD) in the known foci allows for the identification of *T.b. gambiense* infected individuals that are serving as reservoirs (Chappuis, Loutan et al. 2005). The primary method for ACD is the use of mobile teams of examiners who move from community to community in search of infected individuals (Robays, Bilengue et al. 2004). The mobile teams have increased their ability to do mass screening of populations with an antibody assay, the card agglutination trypanosomiasis test (CATT)(Chappuis, Loutan et al. 2005) (Inojosa, Augusto et al. 2006). This is not a test that can provide definitive diagnosis but allows for the identification of suspected cases. Individuals identified by the CATT will have additional testing to determine if they are infected with
the disease, and if so, at what stage (Lejon and Buscher 2001). The primary method for determining disease stage is through an examination of the CSF (Lejon and Buscher 2001).

There are also effective methods for the control of the vector with the use of fly traps that can be maintained by the local populations. Vector control can help reduce disease but without treatment of sick people disease, elimination is not possible (Cattand 2006) as humans are the primary reservoir for T.b. gambiense (Burri and Brun 2003).

The key factors to address if a disease can potentially be controlled or eliminated are the biologic and technical feasibility, the costs and benefits, and the societal and political considerations (Cattand 2006). The current arguments for T.b. gambiense HAT elimination include: the current improved political will (WHO and AU statements); new cost estimates that show the costs associated with control are lower than previously assumed and the benefits are higher; better access to patients in geographic regions previously cut-off due to conflict; and greater availability of treatment drugs through donation programs (Robays, Lefevre et al. 2007; Robays, Raguenaud et al. 2008). Access to effective chemotherapy is important even if elimination is not realistic, as it is a key factor for any HAT control program (Simarro, Jannin et al. 2008).
If *T.b. gambiense* elimination is implemented, a monitoring and evaluation plan is needed to continuously track the success or failure of control and elimination activities (Bouchet, Legros et al. 1998; Cattand, Jannin et al. 2001). It will be helpful in setting objectives rationally and in choosing appropriate indicators of progress. M&E tools are a critical component of ongoing elimination campaigns for other tropical disease, e.g. onchocerciasis and lymphatic filariasis (WHO 2005).

**Treatment**

Effective treatment is a key element of disease control for *T.b. gambiense*, as noted above. The treatment of infected individuals is vital as HAT is fatal when left untreated. Humans are the primary reservoir for *T.b. gambiense* associated HAT and therefore effectively identifying and treating those infected will reduce the levels of disease (Leak 1999; Jannin and Cattand 2004). This has been noted in foci where the disease has been eliminated such as Bioko Island (Simarro, Franco et al. 2006).

There are two stages of the disease. Stage I disease is limited to the haemo-lymphatic system with the trypanosomes circulating in the blood and lymph fluid (Lejon and Buscher 2001). Stage II disease is characterized by invasion of the central nervous system by the parasite (Fevre, Picozzi et al. 2006). Untreated HAT is fatal, and a substantial proportion of infected persons
remain undiagnosed and untreated (Odiit, Coleman et al. 2005). Of those who ultimately receive treatment, the majority have central nervous system involvement (stage II disease) at the time of diagnosis (Checchi, Filipe et al. 2008).

Patients presenting with relapse after treatment for either stage I or stage II disease tend to have central nervous system involvement.

There is a real lack of medication options with only four drugs currently licensed for treatment (Croft, Barrett et al. 2005). Pentamidine and suramin are the drugs used for treating Stage I (Croft, Barrett et al. 2005); pentamidine is the preferred drug to treat *T. b. gambiense* while suramin is used for *T. b. rhodesiense* (Pepin and Milord 1994). Pentamidine is less effective for *T. b. rhodesiense* and this is why suramin is used (Fevre, Picozzi et al. 2006). Since both pentamidine and suramin are unable to cross the blood-brain barrier, they are not effective for Stage II. Eflornithine and melarsoprol are licensed for Stage II disease though eflornithine is not effective against *T. b. rhodesiense*. This is due to the parasite not being susceptible to the compound because of the high ornithine decarboxylase turnover (Burri and Brun 2003). A nifurtimox-eflornithine combination therapy for Stage II *T. b. gambiense* is a promising option (Priotto, Kasparian et al. 2007), but the lack of treatment alternatives should be of dire concern (Moore 2005).
Melarsoprol was introduced in 1949 (Friedheim 1974) and has been the primary drug used for stage II patients for the last fifty years. It is an arsenic derivative and is trypanocidal. The current treatment regimen for *T. b. gambiense* takes ten days of in-patient care (Schmid, Nkunku et al. 2004; Schmid, Richer et al. 2005), an improvement over the month long hospital stay previously required. Due to the toxicity of the drug there are adverse effects, the most severe being an encephalopathic reaction (Blum, Nkunku et al. 2001); skin reactions are also observed. Despite these concerns, melarsoprol remained the first line drug of choice instead of eflornithine for most national control programs for several reasons. The primary factors against eflornithine were the frequent IV administration, the cost of infusion sets (now donated), concerns about sterile preparation of infusions and the risk of possible catheter site infections (Burri and Brun 2003).

A mounting area of concern is the fact melarsoprol is showing evidence of reduced efficacy in certain foci (Brun, Schumacher et al. 2001; Ollivier and Legros 2001). Historically, melarsoprol efficacy (91-95%) had shown little change in 50 years of use. However, elevated rates of melarsoprol treatment failure were observed in foci with Angola, Sudan, and Uganda in the late 1990s (Legros, Evans et al. 1999; Moore, Richer et al. 1999; Brun, Schumacher et al. 2001). The simultaneous appearance of treatment failure in
geographically distinct and widely separated areas was worrisome, particularly because treatment failure in most HAT-endemic areas were not being closely monitored and methodology for calculating failure rates was not standardized. Protocols between countries and centers were not standard and patient follow-up was often incomplete making the data difficult to interpret. The cause of the observed treatment failure is unclear. Potential causes include factors related to drug, factors related to host, and factors related to parasite (Burri and Keiser 2001).

Several factors related to drug have been examined (Brun, Schumacher et al. 2001). Less work has been done on factors related to host; however, no difference has been noted in drug pharmacokinetics between new patients and treatment failures (Burri and Keiser 2001). Limited data suggest HIV coinfection may play a role (Moore, AM personal communication) but is not likely to be the primary factor. Parasite drug resistance is suspected, but few isolates from patients have undergone drug susceptibility testing to date because of the technical difficulties in isolation, cryopreservation and study of the parasite (Likeufack, Brun et al. 2006).

Melarsoprol resistance has been induced in laboratory isolates and is linked to the loss of an aminopurine transporter, P2, that mediates the uptake of the drug and of its metabolite, melarsen oxide (Carter and Fairlamb 1993;
Barrett, Zhang et al. 1995; Carter, Berger et al. 1995). This laboratory resistance, along with the presence of melarsen oxide, has raised questions of whether the in vitro methods used previously are adequate for detecting melarsoprol resistance in field isolates.

Alternative therapies to melarsoprol are being used. Eflornithine monotherapy has been the main alternative as it is less toxic than melarsoprol yet more difficult to administer. There are also some adverse events that can be of concern including anemia and leucopenia (Milord, Pepin et al. 1992). Eflornithine may be even more vulnerable to the development of resistance than the other drugs, which is why combination treatment options are evolving and it will be necessary to monitor new regimens that might become widespread such as eflornithine-nifurtimox combination therapy (Priotto, Fogg et al. 2006) (Bisser, N'Siesi et al. 2007) or any new compounds that become available.

HATSENTINEL

HATSENTINEL was created in order to establish sentinel surveillance for treatment failure and drug resistance in the affected countries.

HATSENTINEL is currently WHO + CDC sponsored with STI, MOHs and NGO working partners. It currently includes nine sites in five countries.
There were four sites in DR Congo, two sites in Angola and one site in Sudan for *T.b. gambiense*. The sites in Uganda and Tanzania were for *T.b. rhodesiense*.

Unlike most sentinel surveillance systems, which collect data for cases of treatment failure only, HATSEN TinEL collected reports for all stage II patients. This approach provided denominator data and the ability to calculate actual rates of treatment failure. It also allowed denominator flexibility in the choice of case definitions for data analysis.

Data collection began in July 2002. The collection was achieved with a standardized report form for all Stage II patients. Geographic, demographic, diagnostic and treatment information was collected. The report form ascertained if a patient was previously treated and what the current treatment regimen was, allowing for the pinpointing of problem areas. In addition to the patient report form, there was also a facility form that was used to collect information about each site, including the medications used, the treatment schedules, diagnostic methods and tests used, other medications used prior to treatment, number of beds, and number of patients seen each year at the time of site enrollment. The facility questionnaire allowed for differentiation between centers, something important for the surveillance since staging and diagnostic techniques and capabilities varied between sites. This allowed for
the magnitude and geographic distribution of treatment failure to be better
defined. It is also provided a mechanism for obtaining and performing drug
susceptibility testing of isolates.
Chapter 2

Rationale of the project:
Monitoring temporal and geographic trends in treatment efficacy are needed not only for the selection of optimal first line therapy in specific areas endemic for HAT but also to provide timely data on refractory infection especially in view of the limited arsenal of chemotherapy.

Specific Objectives:
Collect and analyze data for HAT treatment failure risk factors to determine magnitude and geographic distribution of the problem. In sites where treatment failure is documented, collect specimens for drug susceptibility testing to determine if drug resistance is the cause of treatment failure.

Study design
Data were collected on all stage II patients seeking treatment at the selected sentinel surveillance sites. The patients were followed longitudinally. The collection was achieved with a standardized report form for all Stage II patients. A facility form collected information about each treatment site and their methods of diagnosis and treatment. There was also a mechanism for
obtaining and performing drug susceptibility testing of isolates. Cryopreservation of specimens was hampered by the low parasitemias in *T. b. gambiense* infections and the fragility of the parasite. Reto Brun’s group at STI developed a new preservation medium (Maina, Oberle et al. 2007) that was used.

**Study population**

The study registered and followed all stage II HAT patients from the sentinel sites. The population was comprised of the local communities, groups that ranged from rural farmers and pastoralists to diamond miners. Men and women were enrolled as well as children. HATSENTINEL collects case reports for all patients admitted to the participating facilities for treatment of infection with central nervous system involvement (stage II) and whose HAT diagnosis was made with a positive parasitological test (i.e., trypanosomes were observed in blood, lymph node aspirate, or cerebrospinal fluid (CSF)). Patients were included in the analysis if they fulfilled the following case definition for stage II infection: parasites observed in CSF, and/or CSF white blood cell count (WBC) >5/µl. After completing a treatment course, patients
are instructed to return for follow-up examinations of CSF every 6 months for 2 years.

**Human subjects issues**

This project was classified as surveillance (non-research) by the CDC IRB and, as such, is exempt from IRB review. Patients were followed prospectively by hospitals, but all data reported to the surveillance system and the specimens provided are without personal identifiers. Linkage data are not available to the surveillance system.

**Data analysis methods**

Analysis of the sentinel surveillance data was done using the EpiInfo and SAS computer programs. There was univariate and multivariate statistical analysis along with more advanced procedures such as logistic regressions and Kaplan-Meier survival analysis. Trends in treatment failure rates were examined by stratifying data by quarter of admission as well as using methods of survival analysis including the ‘survivor function’ $s(t)$ representing proportions of patients successfully treated after any given day $t$ since treatment. Treatment failure at any given day $t$ is presented by the
inverse function 1 minus s(t). The statistical methods selected were made in consultation with CDC statisticians.

**Case definitions:**

**Confirmed case:** a case with direct demonstration of the parasite, compatible or not with clinical description.

**Stage 1 sleeping sickness:** parasite seen in blood and/or lymph nodes, with CSF containing no detectable trypanosomes and a leukocyte count \( \leq 5/\mu l \).

**Stage 2 sleeping sickness:** CSF containing trypanosomes and /or leukocyte count \( > 5/\mu l \).

**Treatment failure, confirmed:** any confirmed trypanosomiasis patient with trypanosomes observed microscopically in an examination of CSF, blood or lymph gland fluid during a two-year follow-up period after receiving a complete treatment regimen appropriate for the initially diagnosed disease stage.
**Treatment failure, suspected**: a confirmed trypanosomiasis patient without direct demonstration of the parasite that is compatible with the clinical description and/or with a positive serology, during a two-year follow-up period after receiving a complete treatment regimen appropriate for the initially diagnosed disease stage.

*Suspicion is based on the local risk of contracting the disease and local disease historical background.*

Surveillance in the past was challenging since determining accurate treatment failure rates are difficult because sites have been using different case definitions and denominators. Analysis was further complicated because there is not an effective test to show that a patient has been cured. Using a standard approach through HATSENTINEL provided a more accurate picture of treatment failure issues.
Specimen collection methods

The HATSENTINEL cryopreservation protocol followed the methods developed by Dr. Reto Brun’s lab at STI (Maina, Oberle et al. 2007).

Samples of 2-3ml of venous blood was collected, concentrated in a centrifuge and re-suspended in Triladyl. The samples were then stored in a liquid nitrogen dry shipper until they were ready to be injected in rodents for amplification.

Specimen testing for drug susceptibility

In vitro drug susceptibility

The collected specimens were to be tested using the cryopreserved parasites from the infected HAT patients. The methodology called for isolate propagation in susceptible rodents (Mastomys or SCID mice) and testing for drug susceptibility in with the following assay: incorporation of \(^{3}\text{H}\)-hypoxanthine in culture at serial drug dilutions for measuring the IC\(_{50}\). Due to technical problems, in vitro testing was not successful.
In vivo drug susceptibility

Balb/c mice were used for the testing. The techniques developed in Reto Brun’s lab were followed (Maina, Maina et al. 2007). The mice were immunosuppressed with 300 mg/kg bwt cyclophosphamide (Endoxan®) 48 hours days prior to infection and on days 14 and 28 post infection. These specimens were used to infect mice intraperitoneally. The mice were then separated into three groups; one group of mice served as controls, another group received a melarsoprol dose for 4 days at 1 mg/kg, and a last group received a melarsoprol dose for 4 days at 4 mg/kg. Parasitemia was determined by examining tail blood on day 3, 7, 10, 14, 17, 21, 24, 28 and 31.

Pharmacovigilance

Pharmacovigilance (PV) and pharmacoepidemiology (PE) is based in the monitoring of adverse events for drugs that have been approved and are being marketed (ISPE 2007). The World Health Organization has advocated for greater monitoring of medicinal products (WHO 2002) worldwide and in the dissertation pharmacovigilance will be defined as, “the science and activities relating to the detections, assessment, understanding and prevention of adverse effects or any other possible drug-related problems.”
Pharmacovigilance is a subset of pharmacoepidemiology, a field that applies “epidemiologic methods to pharmacological issues” (ISPE 2007).

The dissertation will present the need for a pharmacovigilance approach to HAT treatment.
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Chapter 3

Sentinel surveillance for human African Trypanosomiasis treatment failure
Sentinel surveillance for human African Trypanosomiasis treatment failure

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Abstract

A limited number of drugs are available for treatment of human African trypanosomiasis. Reports of reduced effectiveness of melarsoprol, which had long been a mainstay of therapy, began to surface a decade ago from multiple sites in central Africa. A sentinel surveillance network, HATSENTINEL, was created in 2002 to monitor temporal and geographical trends in the efficacy of chemotherapy. We analyzed longitudinal surveillance data collected from 2002 to 2008 in facilities treating Trypanosoma brucei gambiense infection. Although melarsoprol remains effective in some sites, markedly elevated treatment failure rates are present in areas of Democratic Republic of the Congo (61%) and northern Angola (98%). The cause of melarsoprol-refractory infection has not been identified. Eflornithine, the alternative drug, remains effective. Continued monitoring of treatment effectiveness for human African trypanosomiasis is needed.
INTRODUCTION

Human African trypanosomiasis (HAT), also known as sleeping sickness, is caused by *Trypanosoma brucei*, a protozoan parasite that is transmitted by the tsetse fly (*Glossina* spp.) It is a neglected disease that continues to pose a health threat to rural Africans. A decade ago, central Africa faced a major resurgence of the West African form of HAT, caused by *T. b. gambiense* (Burri and Brun 2003). At the same time, the East African subspecies, *T. b. rhodesiense* was spreading to areas not previously endemic (Fevre, Picozzi et al. 2005). Improved HAT control in recent years has reduced the incidence by ~70%, however, the 12,000-15,000 cases reported annually to the World Health Organization are thought to underestimate the true incidence of the infection (Simarro, Jannin et al. 2008).

Effective drugs for HAT treatment are essential because the untreated infection is invariably fatal. Furthermore, successful control of *T. b. gambiense*, which accounts for >90% of HAT cases, depends on chemotherapy, because humans constitute the principal infection reservoir. However, treatment of HAT currently relies on a very limited arsenal of drugs. Of the 4 drugs registered for HAT, only 2 are useful for the majority of patients, who have central nervous system involvement (stage II HAT) at
the time of diagnosis. Melarsoprol has been the mainstay of therapy for stage II HAT since it was introduced in 1949. It is the only effective drug for treatment of stage II *T. b. rhodesiense*. Eflornithine is an alternative to melarsoprol for stage II *T. b. gambiense*, either given as monotherapy or, recently, given in combination with nifurtimox (Priotto, Kasparian et al. 2007), a drug indicated for Chagas disease.

Melarsoprol efficacy (91-95%) showed little change in the first 50 years of use. However, during the recent HAT resurgence, elevated rates of melarsoprol treatment failure were reported from *T. b. gambiense*-endemic areas of Uganda (Legros, Evans et al. 1999), Angola (Brun, Schumacher et al. 2001), and Sudan (Moore, Richer et al. 1999). The simultaneous appearance of treatment failure in geographically distinct and widely separated areas was of concern. The scope of the problem was unknown, because treatment failure in most HAT-endemic areas was not closely monitored and methodology for calculating failure rates was not standardized. A sentinel surveillance network (HATSENTINEL) was created in 2002 to gather data for monitoring geographical and temporal trends in treatment efficacy and to ascertain risk factors for treatment failure. We present an analysis of 6 years of longitudinal HATSENTINEL data for areas endemic for *T. b. gambiense*. 
METHODS

Surveillance sites

The HATSENTINEL network collects data from 9 HAT treatment facilities (Figure 1) in 5 countries. The countries and specific sites were selected because of their high burden of disease and/or proximity to areas where treatment failure had been reported. Seven sites are located in Democratic Republic of the Congo (DRC), Angola, or Sudan, where *T. b. gambiense* is endemic: Mbuji Mayi (DRC 1), Katanda (DRC 2), Kionzo (DRC 3), Kimpese (DRC 4), Caxito (Angola 1), Mbanza Congo (Angola 2) and Kiri (Sudan 1). Two sites are located in areas endemic for *T. b. rhodesiense* (Serere, Uganda and Kailua, Tanzania). The facilities included health centers and hospitals operated by ministries of health, nongovernmental organizations, and a Catholic mission. The level of care provided, compared to Western standards, would be considered basic. Participating facilities varied in size (number of beds 9-100) and patient volume (35-650 HAT inpatients per year). Data collection began in 2002 at 4 sites. Five additional sites were added during 2003-2004.

Surveillance data

Two types of data are reported through the HATSENTINEL network. To address the lack of standard diagnostic and treatment protocols used for HAT
in African facilities, data about the specific diagnostic methods and treatment regimens in use at the sentinel facilities are collected on a standardized form. A second report form is used to collect individual, anonymous patient data. These data include demographic information (age, sex, village of residence), HAT diagnostic information, treatment history, and current therapy. This project conformed to the policies of the CDC Institutional Review Board.

**Inclusion criteria and case definitions**

HATSENTINEL collects case reports for all patients admitted to the participating facilities for treatment of infection with central nervous system involvement (stage II) and whose HAT diagnosis was made with a positive parasitological test (i.e., trypanosomes were observed in blood, lymph node aspirate, or cerebrospinal fluid (CSF)). Patients were included in the analysis if they fulfilled the following case definition for stage II infection: parasites observed in CSF, and/or CSF white blood cell count (WBC) >5/μl. After completing a treatment course, according to the guidelines of the National HAT control programs, patients were instructed to return every six months over a two year period for follow-up examinations of their CSF. We used the following case definition for treatment failure: any patient with parasite-
confirmed HAT who presents with trypanosomes in CSF and/or a CSF WBC>50 cells/μl within a 2-year period following receipt of a complete treatment regimen appropriate for the initially diagnosed disease stage.

Case reports for 5110 patients treated and followed from July, 2002 through September, 2008 were examined. These reports were from the 6 sites endemic for *T. b. gambiense* in which >50 patients were treated during the surveillance period. Data from a pilot study conducted at the site Angola 2 were not included in the total 5510 but were used in the survival analysis. Reports for 206 patients were received from the 2 sites treating *T. b. rhodesiense*. These patients are not included in this analysis, but our data show no evidence of elevated treatment failure rates in *T. b. rhodesiense*-endemic areas.

**Statistical Analysis**

Data were entered into an EpiInfo database (version 6) and were analyzed using SAS (version 9.1). Univariate and multivariate analyses along with logistic regressions and Kaplan-Meier analysis for the probability of failure-free survival were performed. Trends in treatment failure rates were examined by stratifying data by quarter of admission as well as using
methods of survival analysis including the ‘survivor function’ s(t) representing proportions of patients successfully treated after any given day t since treatment. Treatment failure at any given day t is presented by the inverse function 1 minus s(t).

RESULTS

Site characteristics

The sites all use serologic testing with the card agglutination test for trypanosomes (CATT) to screen patients but methods for performing parasitological exams varied. Detection of peripheral trypanosomes was through microscopy of blood or lymph node aspirate at all centers; some centers also used blood concentration techniques. One center used Quantitative Buffy Coat (QBC) routinely (Sudan 1), one used capillary tube centrifugation (CTC) routinely (Angola 2) and three used anion exchange columns (mAECT) occasionally (DRC 1, DRC 2, DRC 3). Three centers staged by examining for trypanosomes in the CSF after double centrifugation (Angola 1, DRC 1, Sudan 1) while the other centers lacked centrifuges. Patients were treated for malaria if they are infected and most are presumptively treated for intestinal helminthes prior to receiving treatment for HAT. When surveillance began, melarsoprol monotherapy was the
treatment of choice for stage II HAT in 4 centers, although 3 centers subsequently changed to eflornithine monotherapy. Two centers (Angola 1, Sudan 1) used eflornithine as their primary treatment throughout the surveillance period.

**Patient Characteristics**

A total of 5110 patients met the criteria for stage II HAT during the surveillance period. While all ages were affected, most patients were adults with a median age of 31 and an inter-quartile range from 23-40. Male patients were the majority 3052 (60%), although the gender distribution varied with treatment center (Table 1). A marked male predominance (67%) was found at DRC 1, where the facility catchment area included a large number of alluvial diamond miners.

Overall, the proportion of patients receiving retreatment was high (36%). Most of these patients were admitted at the largest facilities (DRC 1 and DRC 2) where melarsoprol was first line therapy.
Treatment Failure

More than 98% of the treatment failure detected by the HATSENTINEL network was after the use of melarsoprol (Figure 2). Although the drug remained effective at 1 site (DRC 3), failure rates at the other 3 facilities using it were elevated (Table 2), compared to the rates observed historically (5-8%), regardless of whether we used for analysis the standard treatment failure case definition or a stricter case definition requiring trypanosomes to be found in CSF (Table 2).

The mean interval between initial therapy and readmission for retreatment was 389 days. However, this interval was considerably shorter at one site (Angola 2). This site was atypical in both the rapidity of relapse and the high proportion of patients failing treatment. In all sites, a small proportion of patients were diagnosed with treatment failure more than 720 days after initial therapy. These were classified as new infections.

Although facilities with elevated treatment failure rates ultimately adopted alternative first line therapy for stage II infections, we were able to monitor melarsoprol failure rates in 2 sites (DRC 1 and DRC 2) for 22 consecutive quarters. We detected no rising or declining trends in treatment failure rates. Eflornithine was used to treat 2026 patients in 5 facilities (Sudan 1, DRC 1, DRC 2, Angola 1, Angola 2). Failure rates for eflornithine were less than
5%, consistent with the reported efficacy of the drug (Pepin, Khonde et al. 2000; Balasegaram, Young et al. 2009).

Patient Characteristics Associated with treatment failure.

Patient age, sex, and CSF WBC, trypanosomes in the CSF, and HAT relapse at the time of admission were examined as potential risk factors for treatment failure. Having a CSF WBC > 100 was associated with treatment failure for melarsoprol with a relative risk of 1.82 (1.29-2.51 95% CI). No significant association was found for other variables.

DISCUSSION

Elevated rates of melarsoprol failure were first noted a decade ago and were reported from HAT treatment programs at sites within Uganda (Legros, Fournier et al. 1999), Sudan (Moore, Richer et al. 1999), Angola (Stanghellini and Josenando 2001), and, more recently, from northern DRC (Robays, Nyamowala et al. 2008). However, interpretation of these data and comparison of sites has been complicated by the use of different case definitions, variable completeness of follow-up, and the use of different denominators in calculating failure rates. Our standardized data collection
and the use of Kaplan-Meier survival analysis confirm that melarsoprol-refractory *T. b. gambiense* infection is present in several foci in central Africa.

The melarsoprol failure rates documented by HATSENTINEL in northern Angola (98%) and in East Kasai Province of DRC (61%) are substantially higher than previously reported. Because parasites cannot always be demonstrated in the CSF of relapsing patients, the diagnosis of treatment failure often is based on clinical criteria and CSF WBC count. However, melarsoprol failure rates unquestionably are elevated at these sites. When a strict case definition requiring observation of CSF trypanosomes is used for analysis, the probability of failure within the 2 year follow-up period is 23-36%, which is significantly higher than the 5-8% rates seen in previous decades. Melarsoprol failure at our sentinel sites was associated with elevated pre-treatment CSF levels of WBC, as observed by others (Legros, Evans et al. 1999; Schmid, Nkunku et al. 2004; Pepin and Mpiia 2005; Lejon, Roger et al. 2008), but it was not associated with age, sex, or relapse status at the time of hospital admission.

The elevated melarsoprol failure rates were independent of the specific treatment regimen administered, at least in the DRC sites, where both the traditional lengthy empirical schedule and the 10-day protocol(Schmid,
Nkunku et al. 2004) were used. No significant increasing or decreasing trends in melarsoprol failure rates were observed during nearly 2 years of longitudinal data collection in East Kasai Province. We also found no evidence of geographic spread of melarsoprol-refractory infection. The drug remained effective at site DRC 3 during 6 years of surveillance, despite its proximity to the Angola 2 site, which had high rates of melarsoprol failure when surveillance began in 2002. A similar observation was made at site Sudan 1. Melarsoprol, which was used until 2003, remained effective (Balasegaram, Young et al. 2009) even though treatment failure had been reported from sites in the same province and nearby in Uganda for several years.

The HATSENTINEL data have limitations. Definitive methods for HAT cure assessment are lacking, and patients are regarded as cured after a 2-year disease-free interval after treatment. This arbitrary cut-off may cause misclassification of patients. Relapse patients who are readmitted for treatment after intervals longer than 2 years are classified as re-infected, resulting in lowered treatment failure rates. Conversely, cured patients who are re-infected during the 2 year follow-up period may be misclassified as relapses. However, because the HAT annual incidence is \( \leq 1\% \) at these sites, misclassification of re-infected patients is unlikely to alter treatment failure
rates significantly. Another limitation arises from the level of patient compliance with follow-up, which varies with facility. HATSENTINEL generally underestimates failure rates, because the network does not record patients who die from undiagnosed relapse away from the treatment center. However, one facility, DRC 1, occasionally provides retreatment to patients who were initially treated elsewhere, resulting in an apparent failure rate that may be somewhat higher than the true rate at that site.

The underlying cause of melarsoprol-refractory infection has yet to be identified. The likelihood that relapsing patients received sub-therapeutic doses of drug is low, because melarsoprol is given only as inpatient therapy by specialized health facilities experienced in HAT management. Incremental dosing, which has been associated with increased treatment failure (Pepin and Mpiia 2005) was not used in the HATSENTINEL sites. Host-related factors, e.g. altered pharmacokinetics or immune status, may be playing a role. However, to date, no pharmacokinetic differences between new and relapse patients have been observed (Burri and Keiser 2001). The HIV status of patients at our sentinel sites is unknown, because testing is not routinely performed. Although it is possible that reduced melarsoprol efficacy in HIV-infected patients is contributing to the high treatment failure
rates, it is probably not the sole cause. In a Sudanese HAT focus where melarsoprol-refractory infection was present and patient HIV status was determined, failure rates were elevated for HIV-negative as well as for HIV-positive patients (Moore, author’s unpublished data). HAT is not an opportunistic infection in HIV-infected persons (Noireau, Brun-Vezinet et al. 1987; Meda, Doua et al. 1995), and the low HIV prevalence in rural areas endemic for *T. b. gambiense* cannot account for the high treatment failure rates observed.

The role of resistant trypanosomes in human treatment failures is unclear. The existence of resistance to veterinary drugs is well established in trypanosome species pathogenic to animals. Drug pressure on *T. b. gambiense* may have existed during the 1990’s, because nearly half of the melarsoprol administered in the past 6 decades was given during the recent HAT resurgence. Melarsoprol resistance has been induced in laboratory strains of *T. brucei* and has been linked to the loss of an aminopurine transporter that mediates drug uptake (Carter and Fairlamb 1993). The transporter is encoded by the TbAT1 gene (Maser, Sutterlin et al. 1999; Matovu, Stewart et al. 2003), but, although there are TbAT1 alleles circulating in the field that might be involved in resistance (Matovu, Geiser et
al. 2001), an association of these alleles with melarsoprol relapse has not been established. Drug susceptibility testing of isolates from patients infected with *T. b. gambiense* has been limited by the difficulty of parasite isolation and propagation. However, specimens from newly diagnosed HAT patients in sites of melarsoprol refractoriness in Uganda (Matovu, Enyaru et al. 2001) and Sudan (Maina, Maina et al. 2007) are susceptible to melarsoprol when tested either in vitro or in immunosuppressed mice. We have studied a small number of isolates from relapsing patients at HATSENTINEL site DRC 1 and these are sensitive to melarsoprol when tested in vivo (B Dahl, author’s unpublished results). These findings suggest either that melarsoprol-sensitive parasites have a selective advantage during the process of cryopreservation and multiple sub-passages in rodents prior to susceptibility testing or that factors other than resistant trypanosomes are responsible for treatment failure.

The magnitude of melarsoprol treatment failure was not recognized by the country HAT programs or participating facilities until formal surveillance was implemented with HATSENTINEL. Continuing surveillance for efficacy and safety of HAT drugs is advisable for multiple reasons. Although melarsoprol efficacy remains high at the HATSENTINEL sites where *T. b.*
*rhodesiense* is endemic, melarsoprol-resistant parasites have been found in a limited number of patients (Kibona, Matemba et al. 2006). There is no alternative treatment for stage II infection with this subspecies. The use of eflornithine monotherapy as first line treatment for stage II *T. b. gambiense* has expanded considerably. While our data show that eflornithine remains fully effective at present, there is some concern about its widespread use as monotherapy. Eflornithine may be particularly vulnerable to the development of resistance because of its short half-life, cytostatic mode of action, and uncertain efficacy in patients co-infected with HIV (Pepin, Ethier et al. 1992). Furthermore, it requires 4 daily intravenous infusions and a level of nursing care that is difficult to achieve in some facilities, so monitoring for adverse events as well as for efficacy is needed. Eflornithine is currently the only alternative to melarsoprol for treatment of *T. b. gambiense* infection. However new combination regimens are being explored (Bisser, N'Siesi et al. 2007; Checchi, Piola et al. 2007). Nifurtimox given in combination with a reduced dose of eflornithine appears very promising (Priotto, Kasparian et al. 2007), based on the results of recently completed clinical trials, and it may be in field use in the near future. Two product development groups, the Drugs for Neglected Disease Initiative and the Consortium for Parasitic Drug Development, have new HAT drugs in
their pipelines. Because of the limited number of treatment centers and the challenges of conducting trials in remote facilities, HAT clinical trials generally do not enroll large numbers of patients. Continued surveillance and monitoring of newly introduced therapy will be needed.

**Competing interests**
The author(s) declare that they have no competing interests.

**Acknowledgements:**
We would first like to thank the staff and patients at the participating sites as without their cooperation this project would not have been possible. Additionally we would like to thank the Atlanta Research and Education Foundation (AREF); Christian Burri, Reto Brun, Cecile Schmid and Jorge Seixas from the STI; Jackie Roberts, Jodi Vanden Eng, Shannon McClintock and Karen Hawkins-Reed from the CDC; Jacqui Mukoyogo and Ya Ching Lin from MSF; Simon Van Nieuwheove, Jean Jannin and Pere Simarro from WHO; Pierre Cattand, Stafford Kibona, Dawson Mbulamberi, Philippe Buscher, Veerle Lejon, Mike Barret, Annette MacLeod, Jeremie Ilunga, Jean Claude Dinanga, Jean Albert Kabulu, Eme Ntumba, Phelimant Kalala, Medard Ilunga, Stomy Karhemere, Pati Pyana, and Mr. Matondo.

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Table 1. Demographic and clinical characteristics of HATSENTINEL patients with stage II *T. b. gambiense* infection, 2002-2008 (n=5110).

<table>
<thead>
<tr>
<th></th>
<th>DRC 1</th>
<th>DRC 2</th>
<th>DRC 3</th>
<th>Angola 1</th>
<th>Angola 2</th>
<th>Sudan 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>2754</td>
<td>1421</td>
<td>177</td>
<td>73</td>
<td>141</td>
<td>542</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5 (%)</td>
<td>8 (0.3)</td>
<td>70 (4.9)</td>
<td>11 (6.2)</td>
<td>1 (1.4)</td>
<td>3 (2.1)</td>
<td>24 (4.5)</td>
</tr>
<tr>
<td>6-15 (%)</td>
<td>55 (2)</td>
<td>137 (9.6)</td>
<td>28 (16)</td>
<td>9 (12.3)</td>
<td>25 (17.7)</td>
<td>145 (26.8)</td>
</tr>
<tr>
<td>16-55 (%)</td>
<td>2561 (93)</td>
<td>1125 (79)</td>
<td>129 (72.9)</td>
<td>58 (79.5)</td>
<td>105 (74.5)</td>
<td>352 (64.9)</td>
</tr>
<tr>
<td>56- (%)</td>
<td>130 (4.7)</td>
<td>89 (6.3)</td>
<td>9 (5)</td>
<td>5 (6.8)</td>
<td>8 (5.7)</td>
<td>21 (3.9)</td>
</tr>
<tr>
<td>Male patients (%)</td>
<td>1837 (67)</td>
<td>689 (49)</td>
<td>87 (49)</td>
<td>41 (57)</td>
<td>87 (62)</td>
<td>311 (58)</td>
</tr>
<tr>
<td>Admission for new HAT diagnosis</td>
<td>1512</td>
<td>867</td>
<td>167</td>
<td>70</td>
<td>126</td>
<td>521</td>
</tr>
<tr>
<td>-Mean CSF WBC (cell/μl)</td>
<td>331</td>
<td>138</td>
<td>272</td>
<td>247</td>
<td>237</td>
<td>231</td>
</tr>
<tr>
<td>-Trypanosomes in CSF (%)</td>
<td>1080 (71)</td>
<td>460 (53)</td>
<td>101 (61)</td>
<td>70 (100)</td>
<td>76 (60)</td>
<td>230 (44)</td>
</tr>
<tr>
<td>Admission for HAT retreatment</td>
<td>1242</td>
<td>554</td>
<td>10</td>
<td>3</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>-Mean CSF WBC (cell/μl)</td>
<td>238</td>
<td>137</td>
<td>213</td>
<td>127</td>
<td>149</td>
<td>337</td>
</tr>
<tr>
<td>-Trypanosomes in CSF (%)</td>
<td>642 (52)</td>
<td>200 (36)</td>
<td>14 (82)</td>
<td>2 (67)</td>
<td>11 (73)</td>
<td>14 (67)</td>
</tr>
</tbody>
</table>
Table 2. Treatment failure within 730 days in patients with stage II T. b. gambiense infection, 2002-2008 (n=5110).

<table>
<thead>
<tr>
<th>Site</th>
<th>Melarsoprol</th>
<th></th>
<th>Eflornithine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total treated</td>
<td>Total treated</td>
<td>Failed treatment, standard case definition (%)</td>
<td>Total treated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Failed treatment, trypanosomes in CSF (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2754</td>
<td>2022</td>
<td>1113 (55)</td>
<td>732</td>
</tr>
<tr>
<td></td>
<td>1421</td>
<td>866</td>
<td>541 (62)</td>
<td>555</td>
</tr>
<tr>
<td></td>
<td>177</td>
<td>177</td>
<td>10 (6)</td>
<td>0</td>
</tr>
<tr>
<td>Angola 1</td>
<td>73</td>
<td>5</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>Angola 2</td>
<td>143</td>
<td>14</td>
<td>14 (100)</td>
<td>129</td>
</tr>
<tr>
<td>Sudan 1</td>
<td>542</td>
<td>0</td>
<td>0</td>
<td>542</td>
</tr>
<tr>
<td>Total</td>
<td>5110</td>
<td>3084</td>
<td>1790 (58)</td>
<td>2026</td>
</tr>
</tbody>
</table>
Figure 1. Surveillance for Human African Trypanosomiasis Treatment Failure. Sentinel Sites, 2002-2008. (1=DRC 1; 2=DRC 2; 3=DRC 3; 4=DRC 4; 5=Angola 2; 6=Angola 1; 7=Sudan 1; 8=Uganda; 9=Tanzania)
Figure 2. Proportion of patients failing treatment (by center) for stage II T. b. gambiense infection during the two year follow-up period. Shown as inverse Kaplan-Meir curves (1-s(t)). *(includes data from pilot study at Angola 2)
References


Chapter 4

Surveillance for human African trypanosomiasis treatment failure: effectiveness of eflornithine monotherapy for Trypanosoma brucei gambiense at four sentinel sites
Surveillance for human African trypanosomiasis treatment failure: effectiveness of eflornithine monotherapy for *Trypanosoma brucei gambiense* at four sentinel sites

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Introduction:

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is still a public health problem in several countries in sub-Saharan Africa, with close to 12,000 new infections reported in 2006 (Simarro, Jannin et al. 2008). The West African form of the disease, which is caused by *Trypanosoma brucei gambiense*, accounts for more than 95% of cases. Although the incidence is lower than other infectious diseases, such as malaria or tuberculosis, the impact of HAT on the health sector and on endemic communities is considerable. Untreated HAT is fatal, and a substantial proportion of infected persons remain undiagnosed and untreated (Odiit, Coleman et al. 2005). Of those who ultimately receive treatment, the majority have central nervous system involvement (stage II disease) at the time of diagnosis. These patients require inpatient therapy, which is often intensive and lengthy.

Only a limited number of drugs are available for treatment of HAT (Croft, Barrett et al. 2005; Moore 2005). Reduced effectiveness of melarsoprol, a mainstay of HAT chemotherapy for more than 50 years, is being reported from an increasing number of sites endemic for *T. b. gambiense* (Legros, Evans et al. 1999; Brun, Schumacher et al. 2001; Robays, Nyamowala et al.)
2008). Eflornithine is the only alternative to melarsoprol that is registered currently for *T. b. gambiense* infection, and its use has expanded considerably in recent years.

A network of sentinel health facilities (HATSENTINEL) was established in 2002 to collect standardized surveillance data about geographic and temporal trends in HAT treatment efficacy. Initial data from HATSENTINEL showed a consistently high probability of melarsoprol treatment failure in East Kasai Province in the Democratic Republic of the Congo (DRC) (B Dahl, author’s unpublished data). The high level of melarsoprol-refractory infections led the DRC sleeping sickness program to change first line therapy for stage II infection to eflornithine in 2006. We present HATSENTINEL data collected from 2003 to 2008 on eflornithine effectiveness from 4 sentinel hospitals, including 2 sites where eflornithine was introduced as first line therapy in 2003 and the 2 sites in DRC where it has been used since 2006. We also examine the impact of the change in first line therapy on the health care facilities and staff.
Methods

Surveillance sites

The HATSENTINEL network collects data from 9 HAT treatment facilities in 5 countries. Four of these sites used eflornithine for stage II *T. b. gambiense* infection: Mbanza Congo, Angola (since 2003), Kiri, Sudan (since 2003), Mbuji Mayi, DRC (since 2006), and Katanda, DRC (since 2006). The sites in Angola and DRC are government-run hospitals. The site in Sudan was run by Médecins Sans Frontières, Switzerland during the surveillance period. The facilities varied in size (9-100 beds) and patient volume (35-650). The hospital at Mbuji Mayi differed somewhat from the others in that it admitted patients from the local community, but it functioned also as a reference center and handled admissions of patients who had unsuccessfully been treated for HAT elsewhere.

Data Collection

*Facility data.* To address the lack of standard diagnostic and treatment protocols for HAT in African facilities, data about the specific diagnostic methods and treatment regimens in use at the sentinel facilities were reported on a standardized form. In the 2 facilities in DRC (Mbuji Mayi and Katanda)
where first line therapy was changed during the surveillance period, we collected information about the impact of the change through semi-structured interviews with hospital staff and patients.

The staff were asked about the impact the change of therapy had on their workload and stress, changes in the frequency and types of adverse reactions, issues related to the addition of an overnight shift, and which drug they preferred administering.

In similar interviews patients were asked about reasons for selecting the treatment facility, how they were responding to treatment, and if it was a repeat treatment, how it compared with the previous.

*Patient data.* A standardized form was used to report individual, anonymous patient data that included demographic information (age, sex, village of residence), HAT diagnostic information, treatment history, and current therapy. This project conformed to the policies of the CDC Institutional Review Board.
Inclusion criteria and case definitions

HATSENTINEL collects case reports for all patients admitted to the participating facilities for treatment of stage II infection and whose HAT diagnosis was made with a positive parasitological test (i.e., trypanosomes were observed in blood, lymph node aspirate, or cerebrospinal fluid (CSF)). We used the following case definition for stage II infection: parasites observed in CSF, and/or CSF white blood cell count (WBC) >5/ul. After completing therapy, patients are followed with examinations of CSF every 6 months for 2 years. We defined treatment failure as: any patient with parasite-confirmed HAT who presents with trypanosomes in CSF and/or a CSF WBC>50 cells/µl within a 2-year period following receipt of a complete treatment regimen appropriate for the initially diagnosed disease stage. Our analysis includes case reports for all patients meeting the criteria for stage II infection and who were treated with eflornithine at the participating facilities between January 2003 and September, 2008.

Statistical Analysis

Data were entered into an EpiInfo database (version 6) and were analyzed using SAS (version 9.1). Univariate and multivariate analyses along with
logistic regressions and Kaplan-Meier analysis for the probability of failure-free survival were performed. Trends in treatment failure rates were examined by stratifying data by semester of admission as well as using methods of survival analysis including the ‘survivor function’ $s(t)$ representing proportions of patients successfully treated after any given day $t$ since treatment. Treatment failure at any given day $t$ is presented by the inverse function $1 - s(t)$.

**RESULTS**

**Eflornithine effectiveness**

The four centers that used eflornithine varied in patient volume (Table 1), but patient demographic characteristics were similar, with the exception of the facility in Mbuji Mayi, which had a higher proportion of male patients. For patients who failed eflornithine, the mean interval between initial therapy and admission for retreatment was 408 days (30-723). The cumulative incidence of treatment failure within 730 days ranged from 2.3 % to 3.9% (Table 1). This incidence did not differ significantly between treatment-naïve patients and patients who had failed previous therapy with melarsoprol. No increasing or decreasing trends in the probability of failure-free survival were noted when data were analyzed by semester.
Patient age, sex, WBC count in CSF, and the presence of CSF trypanosomes were examined as potential risk factors for failing eflornithine therapy. We found no statistically significant association of these variables with eflornithine treatment failure. However, at all four centers, trypanosomes were found in the CSF at initial diagnosis more frequently in patients who failed treatment with eflornithine (16 of 24) than those who had failed with melarsoprol (204 of 452) with an odds ratio of 2.43 (95% CI, 1.02-5.8; p-value=0.039).

**Eflornithine as first line therapy: impact on health facilities**

The two facilities in DRC that changed from melarsoprol to eflornithine for initial stage II HAT therapy experienced a substantial reduction in the proportion of patients seeking retreatment (Figure 1). This resulted in a marked decrease of HAT admissions. At the Katanda facility, the introduction of eflornithine reduced the number of admissions from an annual mean of 268 in 2003-2006 to 220 in the period 12-24 months after making the change. A similar decrease in patient volume was seen at the Mbuji Mayi hospital, from 499 patients annually to 301.
Interviews with hospital staff found near unanimous support for the switch to eflornithine (11 of 12 staff interviewed). Initial reluctance due to the increased frequency of dosing changed to an overwhelming (92%) endorsement because of the reduction of adverse events, near elimination of deaths, and more manageable workload. The sole issue of contention was the near universal dislike of working the overnight shift.

Discussion

Our longitudinal surveillance data for HAT treatment failure at these 4 sentinel sites show that eflornithine remains an effective therapy for stage II disease, even in areas where melarsoprol-refractory infection is present. The cumulative incidence of 2.3%-3.9% eflornithine failure at 2 years post-therapy is actually even lower than previously reported for this drug (Milord, Pepin et al. 1992; Balasegaram, Young et al. 2009). No difference in failure rate is found between new patients and those who receive eflornithine for relapse after melarsoprol treatment.

Previous studies have identified high initial CSF WBC counts (Chappuis, Udayraj et al. 2005; Checchi and Barrett 2008) and, in some reports (Checchi and Barrett 2008), the presence of CSF trypanosomes as risk factors for eventual treatment failure. These were not associated with failure in our analysis, probably because of the limited number of patients who relapsed
and the lack of statistical power. Additional studies are needed to interpret our finding that CSF trypanosomes are present more frequently in patients who ultimately relapse after eflornithine therapy than those relapsing after melarsoprol.

These data may underestimate eflornithine failures, because our surveillance fails to record patients who may be treated at other facilities or who die during the 2 year follow-up period. However, assuming that patients with adequate follow-up are similar to those who are lost, the adjusted failure rates would still be consistent with the known efficacy of eflornithine (Milord, Pepin et al. 1992; Balasegaram, Young et al. 2009). The facility at Mbuji Mayi serves as a referral center and admits patients who have failed therapy elsewhere, so failure rates at that site may be overestimated. Cured patients who are reinfected during the follow-up period are classified as relapses, but these are not likely to alter failure rates significantly because the HAT incidence in the catchment areas of the sentinel sites is <1%.

HATSENTINEL surveillance data found markedly elevated melarsoprol treatment failure rates (55%--62%) at the Mbuji Mayi and Katanda sites in 2003-2005 (B Dahl, authors unpublished data). The change to eflornithine as
initial therapy yielded multiple benefits. Not only did patients experience sharply lower rates of treatment failure but also a lower case fatality rate. The mortality rate for patients treated with eflornithine was 2% at these facilities compared with 7% for patients who received melarsoprol. HAT patients were well aware of the superior outcomes with eflornithine and preferred it to melarsoprol.

The change in therapy also provided benefits to the health system. HAT is prevalent in poor countries where health care spending is minimal and access to care limited. Health centers and hospitals are often understaffed and those who are present are overworked. For example, the Katanda center functioned with only one nurse for several years. In these settings, patients returning for treatment a second or third time can burden the staff and overwhelm the system. Centers may be forced to have patients share a bed or delay treatment. After introduction of eflornithine as initial treatment at the DRC sites, patient volume became more manageable. In 2005, the hospital in Mbuji Mayi admitted 612 stage II HAT patients for treatment; in the last year of our surveillance (September 2007-September 2008) that figure had been cut to 301. This reduction is primarily attributable to a decrease in the number of patients seeking retreatment. The Katanda site also had reductions
in patient volume from 390 in 2005 to 220 in 2007-2008. Interviews with hospital staff showed an evolution of how eflornithine was perceived. While hospital staff in Mbuji Mayi were familiar with the medication before the switch and realized it was an excellent option for melarsoprol-refractory cases, the health care workers also noted that the 4 daily intravenous infusions needed for eflornithine administration were labor intensive and required close monitoring for catheter infections. This led to an initial reluctance to administer it to every stage II patient, especially because of the large patient volume. This view began to change as the staff became more familiar with administering the drug to more patients. Providing care with eflornithine was viewed as less stressful, because melarsoprol-induced the encephalopathic reactions were avoided. Although eflornithine is donated free of charge, its use of eflornithine generated additional costs to the sleeping sickness programs, because overnight staff must be present to administer it. However, these costs were balanced by the lower overall patient volume.

It has been recognized for some time that eflornithine is effective and safer than melarsoprol in treating stage II HAT (Chappuis, Udayraj et al. 2005; Checchi and Barrett 2008; Balasegaram, Young et al. 2009) but the
logistically difficult schedule of administration was viewed as a barrier to its widespread use. Our data show that acceptability of eflornithine is high among hospital staff and patients. We anticipate that its use will continue to increase. Eflornithine is trypanostatic, rather than trypanocidal. Because of the difficult schedule of administration, especially in understaffed facilities, doses may be missed. This may lead to subtherapeutic drug levels, given eflornithine’s short half-life. The question of potential development of drug resistance has been raised. Combination of a reduced dose of eflornithine with oral nifurtimox appears to be very promising (Priotto, Kasparian et al. 2007) and is expected to be in field use in the near future. This may offer some protection against the development of drug resistance, however, the dosing schedule has peaks and troughs in eflornithine levels. Continued surveillance for treatment failure of eflornithine monotherapy and combination therapy will be needed.
Table 1. Characteristics and Clinical Outcomes of Patients treated for stage II HAT with eflornithine, 2003-2008 (n= 1969)

<table>
<thead>
<tr>
<th>Site</th>
<th>Mbuji Mayi</th>
<th>Katanda</th>
<th>Kiri</th>
<th>M'Banza Congo</th>
</tr>
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<tbody>
<tr>
<td>Total treated</td>
<td>732</td>
<td>555</td>
<td>553</td>
<td>129</td>
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<tr>
<td>Mean age (range)</td>
<td>34 (3-80)</td>
<td>30 (1-88)</td>
<td>29 (1-65)</td>
<td>29 (1-73)</td>
</tr>
<tr>
<td>No. female (%)</td>
<td>244 (33)</td>
<td>286 (52)</td>
<td>228 (41)</td>
<td>56 (39)</td>
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<td>Mean CSF WBC (range)</td>
<td>291 (5-5520)</td>
<td>136 (6-2890)</td>
<td>223 (5-7311)</td>
<td>234 (5-1565)</td>
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<tr>
<td>CSF trypanosomes (%)</td>
<td>454 (62)</td>
<td>222 (40)</td>
<td>249 (45)</td>
<td>79 (61)</td>
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<tr>
<td>Treatment history</td>
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<td>Treatment naïve (%)</td>
<td>482 (66)</td>
<td>387 (70)</td>
<td>520 (94)</td>
<td>110 (85)</td>
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<tr>
<td>Treatment failure (%)</td>
<td>15 (2.1)</td>
<td>12 (2.2)</td>
<td>21 (3.8)</td>
<td>4 (3.9)</td>
</tr>
<tr>
<td>Deaths (%)</td>
<td>15 (2.1)</td>
<td>12 (2.2)</td>
<td>2 (0.4)</td>
<td>7 (5.4)</td>
</tr>
<tr>
<td>Previously treated (%)</td>
<td>250 (34)</td>
<td>168 (30)</td>
<td>33 (5.9)</td>
<td>19 (14.7)</td>
</tr>
<tr>
<td>Treatment failure (%)</td>
<td>2 (0.27)</td>
<td>1 (0.18)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Deaths (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

*Admitted for retreatment within 730 days of initial therapy
Figure 1. Proportion of sleeping sickness patients readmitted for treatment at two sites in the East Kasai Province of the Democratic Republic of Congo, by year.
References:
Chapter 5

Trypanosoma brucei gambiense samples from sleeping sickness patients in the Democratic Republic of The Congo: isolation, propagation and melarsoprol susceptibility
*Trypanosoma brucei gambiense* samples from sleeping sickness patients in the Democratic Republic of The Congo: isolation, propagation and melarsoprol susceptibility

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Introduction

Human African Trypanosomiasis (HAT) is a parasitic disease transmitted through the bite of the tsetse fly (*Glossina* ssp.) found in 36 countries in sub-Saharan Africa (Fevre, Picozzi et al. 2006; Maudlin 2006). There are two subspecies or strains that infect man. *T. b. rhodesiense* is a zoonosis and control efforts focus on the vector. *T. b. gambiense* is the other subspecies, responsible for 90-95% of infections in humans. The most important reservoir for *T. b. gambiense* is man (Leak 1999), and effective treatment is vital for control efforts, because identifying and treating those infected will reduce the prevalence of disease. There are only four drugs available for the treatment of HAT, pentamididine and suramin for stage I disease and eflornithine and melarsoprol for stage II(Croft, Barrett et al. 2005). Melarsoprol has been the primary drug used since 1949, with an efficacy of 91-95%. In the late 1990’s elevated melarsoprol failure levels for *T. b. gambiense* were observed in Angola, Uganda and Sudan (Legros, Evans et al. 1999; Moore, Richer et al. 1999; Burri and Keiser 2001). This led to questions about resistant parasites(Maser, Luscher et al. 2003), but few isolates from patients have undergone drug susceptibility testing because of the technical difficulties in isolation and cryopreservation (Likeufack, Brun et al. 2006).
Recently there have been new developments in cryopreservation that have the potential to improve the feasibility of collecting field samples (Maina, Kunz et al. 2006; Maina, Oberle et al. 2007). To date, testing has been performed for a limited number of specimens collected from HAT foci in which 30% to 50% of patients relapse after treatment with melarsoprol. These were melarsoprol sensitive when tested in vitro (Brun, Schumacher et al. 2001; Maina, Maina et al. 2007).

Melarsoprol resistance has been induced in laboratory isolates and is linked to the loss of an aminopurine transporter, P2, that mediates the uptake of the drug and of its metabolite, melarsen oxide (Carter and Fairlamb 1993; Barrett, Zhang et al. 1995; Carter, Berger et al. 1995). This laboratory resistance, along with the presence of melarsen oxide, has raised questions of whether the in vitro methods used previously are adequate for detecting melarsoprol resistance in field isolates.

Three sites participating in the HATSENTINEL network documented levels of melarsoprol-refractory HAT infection that exceeded 50%. To investigate the cause of treatment failure, the new cryopreservation method was used to isolate parasites from patients at one of these sites, the Dipumba Hospital in the town of Mbuji Mayi in the East Kasai province of the Democratic Republic of the Congo. These specimens were collected through
HATSENTINEL and another study (Pyana, unpublished data). *T. b.*
*gamblense* from 15 patients at this site were melarsoprol-sensitive when
tested *in vitro* (Pyana, unpublished results). This report describes parasite
isolation and melarsoprol susceptibility testing *in vivo* of specimens from
Dipumba Hospital.

**Methods**

*Specimen source and collection*

Procedures developed by Reto Brun’s lab (Brun, Schumacher et al. 2001;
Maina, Kunz et al. 2006) were followed. Technicians collected 2ml of blood
from patients at Dipumba Hospital who had been diagnosed with stage II
disease; for a patient to be included, trypanosomes had to be identified
through microscopy. Blood specimens were centrifuged for 10 minutes at
1000g (11k rpm with model of centrifuge we used). The pellets were
resuspended in 1ml of Triladyl® (1:1).

*Cryopreservation and propagation*

The samples were divided into 4 aliquots (0.5ml), labeled, and placed in a
liquid nitrogen dry shipper for storage. The patient’s admission data was
recorded in a notebook with identifying information such as name or hospital
ID number removed to assure anonymity (table 1).
HATSENTINEL specimens were collected during 2 time periods. Twenty specimens were obtained in the first two weeks of September 2004 and 11 specimens were obtained in August 2008. These were stored in a dry shipper and transported to the Swiss Tropical Institute in Basel. The cryopreserved parasites from the infected HAT patients were administered to susceptible rodents (SCID and BALB/c mice) for propagation. We examined an additional 4 specimens that were obtained from patients at Dipumba Hospital who had failed melarsoprol that were previously collected (Pyana, unpublished) in 2006 and stored at the Swiss Tropical Institute.

In vitro drug susceptibility

Four specimens, which were collected in 2006, had been previously been tested for drug susceptibility in vitro using an incorporation of $^3$H-hypoxanthine in culture at serial drug dilutions for measuring the IC$_{50}$ (Pyana, unpublished). The trypanosomes were harvested from the infected rodents through a cardiac puncture and separated according to the methods used by Maina et al (Maina, Maina et al. 2007).
In vivo drug susceptibility

Balb/c mice were used for the testing. The techniques developed in Reto Brun’s lab were followed (Maina, Maina et al. 2007). The mice were immunosuppressed with 300 mg/kg bwt cyclophosphamide (Endoxan®) 48 hours prior to infection and on days 14 and 28 post infection. Four isolates were selected from samples collected during a previous study at the same location [Pyana, unpublished data]. These specimens were used to infect 11 mice intraperitoneally. The mice were then separated into three groups; 3 mice served as controls, 4 received a melarsoprol dose for 4 days at 1 mg/kg, and 4 received a melarsoprol dose for 4 days at 4 mg/kg. Parasitemia was determined by examining tail blood on day 3, 7, 10, 14, 17, 21, 24, 28 and 31.

Results

The protocol at STI for monitoring for parasitemia in the rodents calls for examining of blood every other day for 60 days. However, we were unable to propagate parasite from any of the 20 specimens collected in 2004. No rodents became parasitemic. It is unclear what caused the failure but theories include problems with the freshness of the egg yolks procured on-site, cold-
chain issues related to the Triladyl®, and concerns about the rapidity of freezing.

The 2008 samples were inoculated in SCID mice and parasitemia was followed, however the results were the same as with the stabilates from 2004 with no samples being viable for drug susceptibility testing. This lack of success compares poorly to the results previously seen (Maina, Oberle et al. 2007) where approximately 45% of stabilates propagated viable trypanosomes.

Melarsoprol drug susceptibility in vivo was examined for 4 stabilates collected in 2005. These were all sensitive to the drug (table 2). Six out of seven mice were free of parasites after 7 days and all 7 were cured by day 31.

Discussion

The samples that were tested in vivo and were susceptible to melarsoprol are consistent with results seen elsewhere (Brun, Schumacher et al. 2001; Matovu, Enyaru et al. 2001; Likeufack, Brun et al. 2006). This supports the argument that there is not resistance to melarsoprol in T.b. gambiense and that other factors must be responsible for refractory cases. The samples came from patients who had previously failed melarsoprol treatment; the isolates
had additionally been tested in vitro where they were also sensitive to melarsoprol [Pyana, unpublished data].

Resistance has been observed with *T.b. rhodesiense* (Kagira and Maina 2007) and *T.b. brucei* (Fairlamb, Carter et al. 1992) so the idea of demonstrating resistance with *T.b. gambiense* was an interesting prospect. The lack of success propagating trypanosomes from an area with elevated rates of treatment failure was disappointing. The cryopreservation methods have worked in rural South Sudan (Maina, Oberle et al. 2007), in an environment that is arguably more technically challenging than the site in DRC. One of the first questions concerned the quality and age of the Triladyl® and if it had potentially been outside of the cold-chain long enough to render it unusable. However, the STI had identical stock remaining on-site in Basel and this was tested by cryopreserving an existing strain; the trypanosomes remained viable after freezing. Therefore, it is likely that our Triladyl® quality was adequate.

A second hypothesis is that there was a problem with the freezing and that the sudden drop into the vapor phase of a nitrogen dry-shipper could have damaged the trypanosomes so that they were not viable upon arrival in Basel. It will be difficult to ascertain if this is true, however we followed the exact protocol that was used successfully in Sudan (Maina, Oberle et al. 2007). This raises the possibility that the strains of *T.b. gambiense* in central DRC
are different than those in Sudan and are less stable for cryopreservation though this is doubtful.

We found that parasites from patients with melarsoprol-refractory infection are sensitive to melarsoprol when tested \textit{in vivo}. These results are consistent with previous \textit{in vitro} testing of these and other stablilates collected in sites with high melarsoprol failure rates (Brun, Schumacher et al. 2001; Maina, Maina et al. 2007; Maina, Oberle et al. 2007)).

It should be noted that the \textit{in vitro} and \textit{in vivo} drug sensitivity testing was conducted using parasites that had undergone multiple sub-passages in rodents. It is not possible to perform direct testing with patient specimens, because the level of parasitemia in \textit{T. b. gambiense} infection is low, the parasites do not grow well \textit{in vitro}, and they have low virulence in rodents. Therefore, we cannot rule out the possibility that clonal selection is occurring, with a selective advantage for melarsoprol-sensitive parasites. It will be difficult to address this issue until molecular markers for resistance are available or resistance testing can simplified (Stewart, Krishna et al. 2005; Njiru, Mikosza et al. 2008). Laboratory-induced melarsoprol resistance is associated with the P2 transporter (Carter and Fairlamb 1993; Barrett, Zhang et al. 1995; Carter, Berger et al. 1995). We did not try to
determine if mutant alleles of the *TbAT1* gene, which codes for the transporter (Maser et al. 1999; Matovu et al. 2001), were present in our specimens, however, an association of these alleles with clinical treatment failure has not been found when examined previously (Maina, Maina et al. 2007).

It is possible that factors other than reduced melarsoprol susceptibility are responsible for the high rates of clinical treatment failure. Host-related factors need further investigation. Patient HIV status is not usually determined in the hospitals where sleeping sickness is treated, and therefore data about patient immune competence is very limited at present. Parasite-related factors other than drug sensitivity also should be examined. This might involve studying strain differences in the affinity of the parasite for certain tissues or sites that may have minimal exposure to melarsoprol. The WHO is in the process of creating a specimen bank that will include samples collected in the East Kasai foci. It is hoped that those samples will be available for study and that a definitive answer to the cause of melarsoprol treatment failure will be found.
Conflicts of interest

The authors declare they have no competing interests.

Acknowledgments

We would like to thank the staff and patients at the Dipumba Hospital in Mbuji Mayi, DRC as without their cooperation this project would not have been possible.

Additionally we would like to thank Dr. Vet. Pati Pyana of the Institut National de Recherche (INRB), Kinshasa, DRC.
Table 1 Characteristics of patients who provided specimens for *T. b. gambiense* drug susceptibility testing, Mbuji Mayi, DRC

<table>
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<th>sample #</th>
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<th>age</th>
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Table 2 *In vivo* melarsoprol drug sensitivities of *T. b. gambiense* isolates from Mbuji Mayi, DRC

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Chapter 6

Meeting the Challenge of Pharmacovigilance and Pharmacoepidemiology for Human African Trypanosomiasis
Meeting the Challenge of Pharmacovigilance and Pharmacoepidemiology for Human African Trypanosomiasis

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Introduction

Human African Trypanosomiasis is a parasitic disease spread by the bite of the tsetse fly (*Glossina* ssp.) and was a major public health problem for the first half of the 20th century (Burri and Brun 2003). Intensive control programs reduced the disease to fewer than five thousand cases annually by the 1960’s, the majority of which were *T. b. rhodesiense*. However priorities shifted and cases began to increase; by the early 1990s the prevalence was estimated to be between 300,000 and 500,000 cases (Cattand, Jannin et al. 2001; Fevre, Picozzi et al. 2006). The reemergence was predominantly a geographical spread of central African cases of *T. b. gambiense*. The increase in cases was most evident in Angola, DR Congo and Sudan, countries that have all been suffering from conflict and war (Moore, Richer et al. 1999; Van Nieuwenhove, Betu-Ku-Mesu et al. 2001; Abel, Kiala et al. 2004). A concerted effort to control the disease has led to a decrease from the peak numbers seen in the late 1990’s. Currently there are under 12,000 cases registered annually and prevalence is estimated to be between 50,000 and 70,000 (WHO 2006; Simarro, Jannin et al. 2008). The majority of these cases are treated at remote facilities that offer only basic levels of care.
Treatment of HAT is necessary since the disease is 100% fatal left untreated and yet there are currently only four drugs licensed for treatment (Croft, Barrett et al. 2005). Pentamidine and Suramin are used to treat Stage I disease, the period before the parasite has crossed the blood-brain barrier. The other two drugs available, eflornithine and melarsoprol (Balasegaram, Young et al. 2009), are for Stage II disease where the parasites have entered the central nervous system; eflornithine, however, is only effective against *T. b. gambiense*. Eflornithine’s use for treatment has recently increased dramatically and is being advocated (Chappuis, Udayraj et al. 2005). Melarsoprol has been the primary drug for second stage disease for the past 50 years. In certain foci, elevated levels of treatment failure began to be recognized from 1999-2001 in distinct foci (Legros, Evans et al. 1999; Legros, Fournier et al. 1999; Brun, Schumacher et al. 2001; Burri and Keiser 2001). Additional sentinel surveillance revealed high rates of melarsoprol failure in several more foci [HATSENTINEL]. Therapeutic alternatives such as nifurtimox-eflornithine combination therapy are being examined (Checchi, Piola et al. 2007; Priotto, Kasparian et al. 2007). These changes should be followed with appropriate care.
Challenges for implementing pharmacoepidemiology/pharmacovigilance for HAT

Pharmacovigilance (PV) and pharmacoepidemiology (PE) has its basis in the monitoring of adverse events for drugs that have been approved and are being marketed (ISPE 2007). The World Health Organization has advocated for greater monitoring of medicinal products (WHO 2002) and this paper will be working with their definition of pharmacovigilance, “the science and activities relating to the detections, assessment, understanding and prevention of adverse effects or any other possible drug-related problems.” Pharmacovigilance falls under the broader topic of pharmacoepidemiology, a field that applies “epidemiologic methods to pharmacological issues” (ISPE 2007).

In the US, the Food and Drug Administration offers guidance and recommendations for how pharmacovigilance should be approached (US Health and Human Services 2005). Companies are required to monitor for drug safety as part of the post-licensing procedure. This includes the reporting of adverse events, a process often dependant on spontaneous reporting of the events by health care providers. The driving force behind the FDA guidance documents is to identify potential risk to those being treated.
Because of the basic care provided, barriers to communication and generally weaker disease surveillance systems, pharmacovigilance in Africa lags behind Europe and the US. It will therefore be necessary to adapt the methods of strong pharmacovigilance to a field applicable standard to improve overall surveillance. A Pubmed search found 1423 articles related to pharmacovigilance but only 29 articles for pharmacovigilance and Africa; of those 10 are related to malaria and 5 are overviews of suspected adverse events related to veterinary drugs in South Africa. There were no articles on Human African trypanosomiasis and pharmacovigilance. Despite the fact that there is not much of a history of pharmacovigilance in Africa, there are lessons that can be learned. The experiences of the Mectizan Donation Program detailing serious adverse events related to the use of ivermectin for the treatment of onchocerciasis (Twum-Danso 2003) can be a guide and offer hope.

Although there are barriers to implementing pharmacovigilance in Africa, certain aspects of HAT distribution and drug use may be advantageous such as the fact that the medication is donated to the World Health Organization, the number of cases are relatively low, the drugs are distributed through country programs to a limited number of facilities and there are a limited number of countries involved. One challenge to pharmacovigilance in
developing countries has been the lack of infrastructure and commitment of the pharmaceutical industry. Having the WHO and Ministries of Health take the lead investigative role will help alleviate this problem.

**Approaches for collecting HAT**

**pharmacoepidemiology/pharmacovigilance data**

**What data to collect**

To start, it is necessary to determine what information will be collected. Will the system focus on stage I, stage II or both? Due to the nonspecific and intermittent symptoms associated with stage I, the majority of patients have stage II at the time of diagnosis (Odiit, Coleman et al. 2005). When this fact is combined with the current lack of resources for the active population screening necessary to find stage I patients in large numbers, we propose that the data collected be for drugs used to treat stage II disease. The system should be flexible enough to incorporate new regimens as they are introduced.

The next question is one not unique to HAT; will the pharmacovigilance system look for adverse events (AEs), adverse drug reactions (ADRs) or a combination? In this case we will use the WHO definitions for both
terms (WHO 2002). An AE is described as “any untoward medical occurrence that may appear during treatment with a pharmaceutical product but which does not necessarily have a causal relationship with the treatment.” An ADR is “a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function.”

There are several types of ADRs already associated with HAT treatment including encephalopathic syndromes (Blum, Nkunku et al. 2001), skin reactions, anemia and leucopenia (Milord, Pepin et al. 1992). We recommend that all ADRs be recorded and reported, especially because new regimens and drug combinations are being introduced. In addition to ADRs, drug utilization should be examined. Stage II treatment is administered as inpatient care. Several of the medications are given parenterally so injection site infections are a concern and should also be reported.

As noted previously, in certain foci melarsoprol has become less effective prompting a switch to eflornithine (Moore 2005). Due to this phenomenon, treatment failures should also be monitored by the pharmacovigilance/pharmacoepidemiologic mechanism. Determining an accurate denominator for treatment failures is vital and therefore all stage II patients need to be monitored, not solely those failing treatment.
The system should not be overwhelmed by adverse events and yet there is a need to address reporting of unexpected as well as expected AEs. An expert committee could meet at the ISCTRC conference or at a WHO summit to determine the AE list and the severity level at which events would trigger a response.

**Reporting methods and channels**

A method of reporting will need to be established. The goals of CDC’s Morbidity and Mortality Weekly Report (MMWR) Surveillance Guidelines (1988) to give credible, accurate, timely and useful scientific information can also be applied here; the system should also be simple and flexible.

The two main reporting approaches are passive surveillance such as spontaneous reporting and more active pharmacoepidemiologic methods (US Health and Human Services 2005; Talisuna, Staedke et al. 2006; ISPE 2007). Both methods can serve as useful tools in assessing drug safety. Spontaneous reporting can be useful in finding rare adverse events that were not seen during clinical trials (Ineke Neutel and Walop 2000). Spontaneous reporting is dependent on the people detecting the events and therefore there is a potential for under-reporting and differences between sites which could lead
to varying levels of sensitivity. There are several major challenges to reporting in order to avoid biased or incorrect reporting. To address these issues will require training at the national level, onsite tutoring at the local level and data checks/quality control at all levels.

The biological and geographical challenges associated with the control of sleeping sickness point to a pharmacovigilance approach best addressed through the use of both passive and active reporting methods.

Active surveillance would require that programs seek out cases and AEs. This could be implemented through intensive reviews of hospital records at sentinel sites. The active surveillance is an area the sensitivity of the system would be tested and ideally validated. A pharmacovigilance system, by collecting demographic data, is more than counting AEs but is also a method to suggest which persons or groups may be at a higher risk for developing an AE. Using active surveillance can provide data to allow for pharmacoepidemiologic studies.

Two potential approaches for using pharmacoepidemiology in the field would be the implementation of case control and cohort studies. Case control
studies are frequently used when adverse events are first detected in order to determine potential exposures that could have led to the adverse event. This can be appropriate and is a relatively inexpensive and quick approach once the events have been detected. Cohort studies follow patients prospectively in order to see if an event will take place. This can be expensive and the sample size potentially would have to be large in order to detect rare adverse events.

The fact that all medication is channeled through WHO allows for an accurate denominator in regards to the number of treatments provided; in theory this could also be a way to get a rough count how many patients were treated but in reality this would not be optimal since it would not account for expired medication or unused treatments. To address this, treatment data from the centers would still be critical. The problem is the speed in which these data are reported back to the MOHs from the treatment centers and how quickly the MOHs transmit the information to WHO. Sentinel sites would allow for detection at a more rapid pace. The sites would report the information simultaneously to WHO and the MOH. A critique of using sentinel sites is that there is potential for selection bias but in situations where drugs are exclusively administered in a health care setting, they can be effective tools for pharmacovigilance (WHO 2002).
Sentinel sites would be the ideal location to set up cohort studies discussed previously. The sites would allow for in-depth pharmacoepidemologic study and would be active in surveillance. Selection of sites should be weighted so that the countries with the greatest burden (DRC, Angola and Sudan) are represented. Currently those three countries account for more than 87% of global cases (Simarro, Jannin et al. 2008).

An example of what a PV/PE system for HAT would look like
The following approach could help in the goal of establishing pharmacovigilance for HAT. A simple and secure website managed by WHO could be used to enter the adverse events. These data would be collected at the Ministry of Health level through a National Pharmacovigilance Center (WHO 2002) and then uploaded for compilation, analysis and dissemination. If a country feels it can not create a dedicated Center, the section of the MOH charged with HAT control could still take on the role of “collecting and analyzing case reports of ADRs” (WHO 2002). The proposed routes for communication are illustrated in Figure 1. It is recommended that data flows from the health centers to the Ministries to WHO and back but that there should be mechanisms for WHO to contact the health centers
directly for quality control. This would be necessary for the sentinel surveillance sites.

Figure 1
Communication channels for reporting HAT pharmacovigilance

The necessity for inpatient care assures that self-medication is not an issue for HAT as well as a reduced risk for under dosing. There is no commercial
market for the drugs unlike the situation for malaria (Talisuna, Staedke et al. 2006). These are some positive points that will make HAT pharmacovigilance more feasible though still with plenty of challenges.

To address these challenges additional measures will need to be tackled. Tools that can be of help to the WHO and the MOHs are to use maps of old foci to identify areas potentially at risk for cases (Chappuis, Loutan et al. 2005). Reporting of pharmacoe vents via cell phones is another rapid and inexpensive tool that should be used. In countries with few cases, adapting methods from the Guinea Worm Elimination Program approach of identifying every case and follow them through successful treatment would be a preferred approach (Ruiz-Tiben and Hopkins 2006). Once a reporting system and standard is established there will need to be capacity building to ensure that the methods are applied correctly (WHO 2002; US Health and Human Services 2005).

Spontaneous reporting will still be encouraged, necessary and one of the primary methods for detecting ADRs. These events should be reported to the MOH. If the mechanism for sentinel surveillance has been established, sites not included in the sentinel network would be encouraged to report to the MOH Pharmacovigilance Center. If the network had not been established,
those observing the ADRs would report to their district HAT contact person who would then report to the MOH. This is possible since all medication is delivered through a central WHO/MOH mechanism. In centers run by NGOs they would be advised/required to report to the WHO/MOH contact as part of their responsibility to receive medication.

A key to any successful reporting system will be its ease of use. Reporting that is too complicated or redundant will lead to errors. National Pharmacovigilance Centers, as advocated by WHO (WHO 2002), can help address these problems by having a level of expertise and capacity building potential. Having a central location to report to would also help avoid confusion and the loss of data. The central location also makes sense since the medication all flows through the national programs; to run a parallel or decentralized reporting system would just add unnecessary levels of paperwork and bureaucracy. In the Democratic Republic of The Congo, there is currently a project in tandem with the Institute of Tropical Medicine in Antwerp that is attempting to improve pharmacovigilance for HAT treatment [personal communication]. The overall goals to improve surveillance are to be lauded however the methods to create a decentralized system do not seem to be in line with the goals established by the WHO. In
order to have a methodology, standards and results that can be compared between countries we advocate following WHO’s approach.

Summary

There are many challenges to establishing a reliable pharmacovigilance network. These are not issues limited to the African setting are common in resource limited situations often resulting in the lack of infrastructure and difficult work environment. These challenges do not mean that pharmacovigilance is not possible in Africa or should be set to a lower standard. In the case of human African trypanosomiasis we argue that the most rigorous approaches advocated by WHO and the FDA should be embraced (WHO 2002; US Health and Human Services 2005). WHO and the international community should be realistic about the challenges and understand that building new mechanisms will take time and new approaches.

The increased use of eflornithine and possibly eflornithine/nifurtimox combination therapy will offer alternatives to melarsoprol for the treatment of *T. b. gambiense* associated HAT. These therapies have the potential to be safer with fewer adverse events but much is still unknown. Additionally there
are new drugs in the pipeline and the system has to be flexible to accommodate them. Improving pharmacovigilance at same time these medications are being introduced or given expanded use is an opportunity that should not be missed.

Conflicts of interest

The authors declare they have no competing interests.

Acknowledgments

We would like to thank Pere Simarro from WHO. We would additionally like to thank our friends and colleagues at the treatment centers and Ministries of Health in the countries suffering from Human African Trypanosomiasis. Finally, we would like to thank our colleagues in Basel and Atlanta.
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Chapter 7

Summary and discussion

The epidemiology of Human African Trypanosomiasis (HAT) can be used as a guide to highlight the dangers of complacency and the neglect given to tropical diseases. The disease which had been a major public health problem at the start of the 20th century had been successfully controlled and was no longer a priority for the international health community or the leaders of the newly independent countries where the disease is endemic (Burri and Brun 2003; Simarro, Jannin et al. 2008). The neglect of the disease allowed it to fester in remote areas of Africa (Legros, Fournier et al. 1999; Stanghellini and Josenando 2001; Van Nieuwenhove, Betu-Ku-Musu et al. 2001), building a reservoir that allowed for a dramatic resurgence of the disease at the end of the 20th century that approached the levels seen at the start of the century. There was a recognition by WHO, the endemic countries and others in the international health field that a concerted effort must be made to control the disease once again (WHO 1998; Cattand, Jannin et al. 2001).
Surveillance for HAT treatment failure

One key issue that allowed for the resurgence to gain traction was the overall weakness of the surveillance systems (WHO 1998). Countries did not communicate with WHO or their neighbors, there were different definitions of the disease, civil conflicts isolated regions and slowed the distribution of medicine (Berrang Ford 2007), and poor infrastructure combined with a lack of resources did not allow for the detection and communication necessary to have surveillance that was effective and efficient (Shaw 1989; CDC 1999). It was in this context that another disturbing issue was discovered, elevated melarsoprol treatment failure (Pepin and Mpiia 2005). HAT is a neglected disease that has a number of challenges to face for successful control and one of the greatest barriers is the lack of treatment options (Molyneux 2004). There are currently only four drugs licensed for the treatment of HAT, with melarsoprol having been the most important for the treatment of the more severe meningo-encephalitic stage of the disease (stage II) (Croft, Barrett et al. 2005). The drug, though toxic, had worked successfully since its introduction in 1949 (Friedheim 1974). This began to change in the late 1990’s when patients treated for T. b. gambiense in several areas of Angola, Sudan and Uganda (Moore, Richer et al. 1999; Brun, Schumacher et al. 2001; Matovu, Enyaru et al. 2001) began to fail melarsoprol at levels much higher
than the historical rate of 5-8%. As the disease is fatal left untreated, the loss of the most important chemotherapy could have devastating consequences (Burri and Keiser 2001; Fevre, Picozzi et al. 2006).

The overall poor surveillance system combined with a new phenomenon of treatment failures led to the creation of a sentinel surveillance based study to examine the issue and to get a better understanding of the geographic and epidemiologic scope of the problem. This study was called HATSENTINEL. To successfully implement this study a standard approach to surveillance had to be taken in order to have data that was comparable between countries. A standardized questionnaire, case definitions and study protocol allowed for this and was used in this study.

The HATSENTINEL study began in July 2002 and eventually comprised 9 sentinel sites in 5 countries collecting data on all stage II patients (see the dissertation chapters 1 and 2 for a more detailed description). Seven of the sites were in areas endemic for \textit{T.b. gambiense} and two were in regions with \textit{T.b. rhodesiense}. We did not find elevated treatment failure issues at the \textit{T.b. rhodesiense} sites and the remainder of the discussion will focus on the results from the sites monitoring \textit{T.b. gambiense}.

The study detected a focus in the East Kasai province of the Democratic Republic of The Congo (DRC) that was previously unknown to have
melarsoprol treatment failure problems. The sentinel sites in Katanda, DRC and Mbuji Mayi, DRC had melarsoprol failure rates of 62% and 55% respectively and were unexpected results. Treatment failures at the site in northern Angola in M’banza Congo had been previously detected but not at the levels we found, with 98% of patients not responding to melarsoprol. The elevated failure levels detected by HATSENTINEL when contrasted to the historical rates of 5-8% led the Ministries of Health in the DRC and Angola to switch their first line therapy for stage II disease to eflornithine. The longitudinal and flexible design of the HATSENTINEL study allowed for us to accommodate such changes in therapy and continue analyzing results from the data collected.

Two of the sentinel sites for the study (Sudan and Caxito, Angola) were using eflornithine to treat second stage disease from the debut of HATSENTINEL. By including data from the three sites that switched to eflornithine, we were able to demonstrate and replicate the positive results that has previously described. Melarsoprol treatment failure rates that had ranged from 55-98% fell to 2-4% for patients treated with eflornithine. This led to decreases in the number of patients seeking retreatment, reducing the overall caseload by as much as 40%.
The results from the eflornithine treatment data are encouraging and will be of interest to national programs, possibly accelerating the calls for a full-scale switch for first line usage (Chappuis, Udayraj et al. 2005; Balasegaram, Young et al. 2009). This could be the right approach but should be taken with caution and the understanding that surveillance will need to be continued and actually strengthened. Melarsoprol will continue to be a necessary drug as it is the only treatment option for *T.b. rhodesiense* patients and will be used for *T.b. gambiense* patients who do not respond to eflornithine.

**Lessons learned from implementing HATSENTINEL**

Both care and record-keeping are basic in the facilities where HAT is treated, and formal surveillance for treatment failure had not previously been attempted. However, once implemented, the system worked well. Acceptability of the surveillance system by the facilities was high and data quality remained high throughout the 6 year surveillance period. The country programs were able to use the data for decision-making and changing policy. This demonstrates how HATSENTINEL met the criteria (MMWR 1988) for a successful surveillance system by being simple and flexible while able to give credible, accurate, timely and useful scientific information.
An additional observation from the HATSENTINEL data could have implications for future clinical trials (WHO 2007). The current recommendations are to follow patients for 24 months after treatment to assure that they are disease free. Data from the two largest sites in the HATSENTINEL network showed that the majority of patients who fail treatment do so in the first 18 months. By extending the data collection to the full 24 months we detected an additional 6.5% of treatment failures for melarsoprol. This raises the question of what advantages or disadvantages would arise if the follow-up period was reduced to 18 months.

**Pharmacovigilance**

Future efforts to control HAT should incorporate pharmacovigilance. Compared to other infections, clinical trials for HAT enroll a limited number of patients. This is due the logistical difficulties of conducting the clinical trials in settings with shrinking patient populations. New regimens or drugs are likely to be introduced with less data than for other diseases and therefore, surveillance will be important to assess drug safety and efficacy. Expanded use of eflornithine for the treatment of second stage disease is underway and being advocated (Chappuis 2007). A combination therapy of
nifurtimox-eflornithine (Priotto, Kasparian et al. 2007) looks like an additional promising treatment option.

There is considerably less experience with eflornithine (Burri and Brun 2003) than with melarsoprol and this can raise questions about its long-term efficacy. To address these questions surveillance must continue and the adoption of a pharmacovigilance approach should be considered (WHO 2002; WHO 2004).

The situation for HAT therapy is somewhat unique in that all medication is funneled through WHO to the Ministries of Health. This allows for a level of control of the drug supply and the potential for accurate data collection. The countries do suffer from a lack of infrastructure that is needed for strong surveillance however this should not lead to avoiding the creation of a pharmacovigilance system (Talisuna, Staedke et al. 2006). The limited number of cases and the control of the medication can be used as an opportunity to create such a network and to tailor it to the needs of the Human African Trypanosomiasis community. Through the collection of data according to international standards, programs will be better able to address potential future challenges to the chemotherapy of the disease. This data will also be necessary if new drugs are to be developed and if clinical trials are to be implemented (WHO 2007).
Unanswered research questions and recommendations

More research is needed on several fronts including the cause of treatment failure and the future direction of pharmacovigilance.

The cause of melarsoprol failures is still unknown. This should be examined further.

Technical issues hampered the propagation of trypanosome specimens collected at the sentinel sites. Improving methods of isolation and propagation in an effort to minimize clonal selection should be a priority. Improving tools for molecular epidemiology with the ability to compare strains and correlate those strains with clinical results would allow for foci specific treatment and control measures.

In vivo melarsoprol sensitivity testing on a limited number of specimens did not show resistance to the drug.

More studies need to be done in order to determine risk factors for treatment failure, with a special emphasis on host immune status.

Continued surveillance and the implementation and concrete development of a pharmacovigilance system should be a priority. There should be an expansion of the number of facilities in any future surveillance network.

Calls from the WHO and the heads of African states for the elimination of
the disease make the need for the data collected with a strong surveillance system even more timely.

There should be the development of a second generation of treatment failure tools that allowed for surveillance of stage I drugs and the flexibility to collect data on new drugs and treatment regimens.

There should also be an emphasis on surveillance capacity building in the countries suffering from HAT. This would allow for faster and more effective data sharing.

The expanded use of eflornithine and nifurtimox-eflornithine combination therapy offer hope for the reduction of this disease. At the same time, this should be approached cautiously and viewed in the historical context so that progress made towards the reduction of cases is sustainable. Through continued surveillance, timely data will be available for making public health decisions.
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resistance to other drugs and trypanothione metabolism."


infections following melarsoprol therapy."


## Appendix: HATSENTINEL TREATMENT CENTER FORM

- **facility name:** ______________________________________________
- **facility location:** ____________________________
- **country**  
  **province**  
  **county**  
  **town/village**
- **geographical coordinates:**  
  N/S: ___° ___’ ___’’  
  E/W: ___° ___’ ___’’  
  (o GIS  o map)
- **catchment area:** ____ sq km  ____ population  
  (o estimated  o census data; year____)
- **facility type:**  
  o hospital  
  o health center  
  o private  
  o other  
- **administration:**  
  o government  
  o NGO  
  o joint  
  o other
- **patient capacity:**  
  ___ no. beds  
  ___ no. mats  
  ___ no. other
- **other**

### DISEASE PREVALENCE IN CENTER CATCHMENT AREA

- **sleeping sickness** ___%  
  (o estimated  o data-based  o prevalence unknown)
- **malaria (peak season)** ___%  
  (o estimated  o data-based  o prevalence unknown)
- **malnutrition** ___%  
  (o estimated  o data-based  o prevalence unknown)
- **HIV** ___%  
  (o estimated  o data-based  o prevalence unknown)
- **other** ___%  
  (o estimated  o data-based  o prevalence unknown)

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<td></td>
</tr>
<tr>
<td>number stage II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>case detection: number active</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>number passive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mortality: % stage I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% stage II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TRYpanosomiASIS DIAGNOSIS

<table>
<thead>
<tr>
<th>method</th>
<th>frequency of use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>routine</td>
</tr>
<tr>
<td>serology CATT</td>
<td>o</td>
</tr>
<tr>
<td>CATT with serum dilutions</td>
<td>o</td>
</tr>
<tr>
<td>IFAT</td>
<td>o</td>
</tr>
<tr>
<td>other (______________________)</td>
<td>o</td>
</tr>
<tr>
<td>trypanosomes, microscopy, lymph node</td>
<td>o</td>
</tr>
<tr>
<td>peripheral, microscopy, blood(wet/stained)</td>
<td>o</td>
</tr>
<tr>
<td>microscopy, buffy coat</td>
<td>o</td>
</tr>
<tr>
<td>QBC</td>
<td>o</td>
</tr>
<tr>
<td>anion exchange column</td>
<td>o</td>
</tr>
<tr>
<td>other (______________________)</td>
<td>o</td>
</tr>
</tbody>
</table>

### DRUG THERAPY USED IN THIS CENTER

<table>
<thead>
<tr>
<th>STAGE I</th>
<th>as first line</th>
<th>as alternative</th>
<th>not used</th>
<th>dose</th>
<th>duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>pentamidine</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(mg/kg/d) x ___ days</td>
</tr>
<tr>
<td>suramin</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(grams/dose) x ___ doses</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STAGE II</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pentamidine, pretreatment</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(mg/kg/d) x ___ days</td>
</tr>
<tr>
<td>suramin, pretreatment</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(grams/dose) x ___ doses</td>
</tr>
<tr>
<td>melarsoprol, with rest periods</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>see schedule below</td>
</tr>
<tr>
<td>melarsoprol, no rest periods</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>see schedule below</td>
</tr>
<tr>
<td>nifurtimox monotherapy</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(mg/kg/d) x ___ days</td>
</tr>
<tr>
<td>DFMO monotherapy</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(mg/kg/d) x ___ days</td>
</tr>
<tr>
<td>combination therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>melarsoprol/DFMO</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>see schedule below</td>
</tr>
<tr>
<td>melarsoprol/nifurtimox</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>see schedule below</td>
</tr>
<tr>
<td>DFMO/nifurtimox</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>see schedule below</td>
</tr>
<tr>
<td>other (______________________)</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>___</td>
<td>(mg/kg/d) x ___ days</td>
</tr>
</tbody>
</table>

### MELARSOPROL ADMINISTRATION AT THIS CENTER

#### DOSING (mg/kg)

<table>
<thead>
<tr>
<th>Injection</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>series 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>series 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>23</td>
<td>24</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>series 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>30</td>
<td>31</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>series 4</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td>37</td>
<td>38</td>
<td>39</td>
<td>40</td>
</tr>
</tbody>
</table>
**COMBINATION THERAPY FOR STAGE II TRYPANOSOMIASIS**

**MELARSOPROL/NIFURTIMOX**

<table>
<thead>
<tr>
<th>Days</th>
<th>Melarsoprol Dose</th>
<th>Nifurtimox Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Constant dose</td>
<td>Constant dose</td>
</tr>
<tr>
<td>2</td>
<td>Increasing dose</td>
<td>Increasing dose</td>
</tr>
<tr>
<td>3</td>
<td>Other (specify)</td>
<td>Other (specify)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>Melarsoprol Dose</th>
<th>Nifurtimox Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Constant dose</td>
<td>Constant dose</td>
</tr>
<tr>
<td>9</td>
<td>Increasing dose</td>
<td>Increasing dose</td>
</tr>
<tr>
<td>10</td>
<td>Other (specify)</td>
<td>Other (specify)</td>
</tr>
</tbody>
</table>

Mark days of administration of melarsoprol with **X** and days of nifurtimox with **O** in chart at left.

**MELARSOPROL/DFMO**

<table>
<thead>
<tr>
<th>Days</th>
<th>Melarsoprol Dose</th>
<th>DFMO Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Constant dose</td>
<td>Constant dose</td>
</tr>
<tr>
<td>2</td>
<td>Increasing dose</td>
<td>Increasing dose</td>
</tr>
<tr>
<td>3</td>
<td>Other (specify)</td>
<td>Other (specify)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>Melarsoprol Dose</th>
<th>DFMO Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Constant dose</td>
<td>Constant dose</td>
</tr>
<tr>
<td>9</td>
<td>Increasing dose</td>
<td>Increasing dose</td>
</tr>
<tr>
<td>10</td>
<td>Other (specify)</td>
<td>Other (specify)</td>
</tr>
</tbody>
</table>

Mark days of administration of melarsoprol with **X** and days of DFMO with **O** in chart at left.

**DFMO + NIFURTIMOX**

<table>
<thead>
<tr>
<th>DFMO Dose</th>
<th>Nifurtimox Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg/d</td>
<td>mg/kg/d x days</td>
</tr>
</tbody>
</table>

**CRITERIA USED TO DEFINE TREATMENT FAILURE OR RELAPSE**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Used</th>
<th>Not Used</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosomes identified in CSF</td>
<td><strong>X</strong></td>
<td><strong>O</strong></td>
<td><strong>☐</strong></td>
</tr>
<tr>
<td>CSF WBC &gt; 50 (or &gt; ____ specify alternative WBC cut-off value)</td>
<td><strong>X</strong></td>
<td><strong>O</strong></td>
<td><strong>☐</strong></td>
</tr>
<tr>
<td>CSF WBC &gt; 5 (or &gt; ____ specify alternative cut-off) AND an increase from previous exam of more than ____% (specify)</td>
<td><strong>X</strong></td>
<td><strong>O</strong></td>
<td><strong>☐</strong></td>
</tr>
<tr>
<td>Does not meet criteria above, but clinical symptoms strongly suggest relapse in the absence of other diagnosis</td>
<td><strong>X</strong></td>
<td><strong>O</strong></td>
<td><strong>☐</strong></td>
</tr>
<tr>
<td>Other (specify: )</td>
<td><strong>X</strong></td>
<td><strong>O</strong></td>
<td><strong>☐</strong></td>
</tr>
</tbody>
</table>
## THERAPY OF TRYPANOSOMIASIS AFTER RELAPSE

<table>
<thead>
<tr>
<th>therapy</th>
<th>relapse 1</th>
<th>relapse 2</th>
<th>relapse&gt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>first line alternative</td>
<td>first line alternative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>melarsoprol, with rest periods</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>melarsoprol, no rest periods</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>nifurtimox monotherapy</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>DFMO monotherapy</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>combination therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>melarsoprol/DFMO</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>melarsoprol/nifurtimox</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>DFMO/nifurtimox</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>other (________________)</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
</tbody>
</table>

## OTHER THERAPY PROVIDED ROUTINELY FOR TRYPANOSOMIASIS PATIENTS

<table>
<thead>
<tr>
<th>therapy</th>
<th>frequency of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>prednisolone pretreatment</td>
<td>by exam</td>
</tr>
<tr>
<td>(dose: ___________ mg/kg/d or o mg/d)</td>
<td></td>
</tr>
<tr>
<td>prednisolone during melarsoprol therapy</td>
<td>o</td>
</tr>
<tr>
<td>(dose: ___________ mg/kg/d or o mg/d)</td>
<td></td>
</tr>
<tr>
<td>multivitamin pretreatment</td>
<td>o</td>
</tr>
<tr>
<td>antimalarial pretreatment</td>
<td>o</td>
</tr>
<tr>
<td>antiparasitic pretreatment for intestinal helminths</td>
<td>o</td>
</tr>
<tr>
<td>antibacterial agent, pretreatment</td>
<td>o</td>
</tr>
<tr>
<td>antibacterial agent during melarsoprol therapy</td>
<td>o</td>
</tr>
</tbody>
</table>

## FOLLOW-UP OF PATIENTS TREATED AT THIS CENTER

LP performed immediately post treatment: o yes o no
mean duration between treatment and 1st follow-up exam: ___ months(estimated calculated)
mean number of follow-up exams per patient in a 2-year post treatment period: ___ exams (estimated calculated)
proportion of treated patients with at least one follow-up exam: ____%
date of form completion: ____/____/____ (dd/mm/yy)
form completed by: name________________________position________________________
# HATSENTINEL
## TREATMENT CENTER UPDATE FORM

<table>
<thead>
<tr>
<th>Facility Name</th>
<th>__________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility Location:</td>
<td>__________________________</td>
</tr>
<tr>
<td>Country</td>
<td>Province</td>
</tr>
<tr>
<td>Time Period Covered by this Report:</td>
<td>from <em><strong>/</strong></em>/___ to <em><strong>/</strong></em>/___ (dd/mm/yy)</td>
</tr>
</tbody>
</table>

### Patients Treated in Center

<table>
<thead>
<tr>
<th>Description</th>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients Treated, Sleeping Sickness Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Stage I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Stage II, Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Stage II Not Previously Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Stage II Previously Treated for Stage I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Stage II Previously Treated for Stage II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Case Detection

<table>
<thead>
<tr>
<th></th>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Passive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th>Stage</th>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Stage I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Stage II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Number of Follow-up Exams Performed

<table>
<thead>
<tr>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
</table>

### Recent Changes in Diagnosis or Treatment Practices in This Facility

<table>
<thead>
<tr>
<th>Decision</th>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(If yes, note changes on center information form)

### Date of Completion of This Form

<table>
<thead>
<tr>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
</table>

### Form Completed By

<table>
<thead>
<tr>
<th>Year to Date</th>
<th>This Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Position</td>
</tr>
</tbody>
</table>

### Comments

____________________________________________________________________________________

___________________________________________________________________________________

___________________________________________________________________________________

___________________________________________________________________________________

___________________________________________________________________________________

___________________________________________________________________________________

___________________________________________________________________________________
Appendix : HATSENTINEL CASE REPORT FORM

REPORTING FACILITY

facility name: __________________________________________
facility code: __________________________
facility location: __________________________
country________________________________________
town/village____________________________________

form completed by: __________________________
position______________________________________

PATIENT DATA

patient identification number: __________
age: ______(years)
signed consent for medical record release: □ male □ female □ unknown

residence location: ____________  ____________  ______________
country province town/village

SPECIMEN COLLECTION

specimen(s) collected/stored for this patient? □ yes □ no □ unknown

facility name: __________________________
specimen handled by: __________________________

facility code: __________________________
position____________________________________

informed consent obtained: □ yes □ no

patient ID number: __________________________

DIAGNOSIS AND STAGING

initial diagnosis of sleeping sickness (date: __/__/___ (dd/mm/yy))
parasitologic confirmation: □ confirmed ( □ lymph node fluid □ blood □ CSF □ unknown)
(check all that apply) □ not confirmed

Was the patient previously treated for sleeping sickness?
□ yes □ no □ unknown
(If yes, complete the following)

| CSF examination for CURRENT ADMISSION | CSF examination at the time of initial diagnosis | CSF examination (most recent exam prior to current admission) |
### Trypanosomes:
- yes □
- no □
- unknown □

### WBC:
**cell count**
- Cells __________
- unknown □

**IgM**
- titer > 1: ______ □
- titer > 1: ______ □
- titer > 1: ______ □
- not done □
- unknown □

---

### Previous Therapy

*Complete this section if patient has been treated for stage I or stage II sleeping sickness in the past.*

**Skip to Current Therapy if this report covers the patient’s first treatment.**

**previous therapy for stage I:**
- yes (treatment drug: □ pentamidine □ suramin □ unknown)
- no □

**number of previous treatments for stage II:**
- 0 □
- 1 □
- 2 □
- 3 □
- > 4 □
- unknown □

---

### Date

<table>
<thead>
<tr>
<th>(dd/mm/yy)</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>/</strong>/___</td>
<td><strong>/</strong>/___</td>
<td><strong>/</strong>/___</td>
<td></td>
</tr>
</tbody>
</table>

**Treatment Type**

(check all that apply)
- mel (with rest periods) □
- mel (no rest periods) □
- nifurtimox alone □
- DFMO alone □
- DFMO + nifurtimox □
- melarsoprol + DFMO □
- melarsoprol + nifurtimox □
- other/unknown □

**Therapy duration**
**CURRENT THERAPY**

*for patients previously treated for stage II trypanosomiasis* (check all that apply)

decision to re-treat based on: ☐ CSF tryps ☐ CSF WBC ☐ CSF IgM ☐ clinical exam

☐ other (__________________________)

*for all patients with stage II trypanosomiasis* treatment start date __/__/__

trypanosomiasis treatment: therapy duration: other drug administered:

☐ melarsoprol, with rest periods ☐ full course ☐ prednisolone

☐ melarsoprol, no rest periods ☐ incomplete course ☐ antimalarial agent

☐ DFMO monotherapy ☐ unknown ☐ antibacterial agent

☐ DFMO + nifurtimox ☐ other

☐ melarsoprol + DFMO ☐ unknown

☐ melarsoprol + nifurtimox ☐ other (__________________________)

clinical status at admission: ☐ symptomatic ☐ asymptomatic
clinical status at discharge: ☐ clinical improvement ☐ no improvement ☐ death

☐ unknown

CSF examination post treatment: ☐ not done

☐ done—results: trypanosomes: ☐ yes ☐ no

WBC _______ cells

Comment: __________________________________________

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________
Curriculum vitae
Benjamin A. Dahl, M.P.H.
183 Forest Glen Way • Avondale Estates, GA 30002 USA • (404) 292-8585 • bid5@cdc.gov

Qualifications
Summary
Over fourteen years’ experience in public health programs providing epidemiological surveillance, research, project evaluation, database development, data analysis and the supervision of budgets and staff. This has included experience working for and collaborating with the CDC, WHO and international organizations.

EDUCATION
PhD, Epidemiology, September 2006- April 2009 • UNIVERSITY OF BASEL • Basel, Switzerland
*Dissertation title: “From Sentinel Surveillance for Sleeping Sickness Treatment Failure to the Development of a Pharmacovigilance Approach”

Master of Public Health, International Health, May 2001 • Rollins School of Public Health, Emory University • Atlanta, GA
*Emory Global Health Organization, President 2000-2001. I led a student organization dedicated to promoting international public health. My roles included supervising the budget, selecting topics to advocate for and representing the organization at university events.
*Thesis topic- Lymphedema treatment in Leogane, Haiti: An effective, sustainable and replicable model program for lymphatic filariasis morbidity control
*Rollins School representative, Emory University Senate, 2000-2001. In my role as representative for the Rollins School, I attended monthly meetings to ensure that the needs of public health students were heard in regards to University policy decisions. I reviewed new policy and changes to policy as well as making recommendations to the University President and Board.
Bachelor of Arts, History, May 1994 • Macalester College
• St. Paul, MN
*Attended the School for International Training in Chile-Spring, 1993 (a study abroad program)
*Honors Preceptor, Fall, 1992
*Editor, Photographer of MacWeekly (Student Newspaper)

EXPERIENCE

July 2002- present

Epidemiologist
DIVISION OF PARASITIC DISEASES, CENTERS FOR DISEASE CONTROL AND PREVENTION • Atlanta, GA (employed by AREF)
*Worked for more than six years with the WHO Collaborating Center for African trypanosomiasis at CDC to establish and manage a sentinel surveillance and monitoring and evaluation system for African trypanosomiasis treatment failure and drug resistance
*Implemented and supervised data collection in two sites in Angola, four sites in Democratic Republic of Congo, one site in southern Sudan, one site in Tanzania and one site in Uganda in collaboration with WHO and the Ministries of Health (MOH) of the participating countries.
*Provided epidemiologic training and technical guidance in sentinel surveillance for more than six years to Ministry of Health staff in five African countries.
*Created data collection forms and electronic databases for a multi-country sentinel surveillance program to track treatment failure. The forms were used by Ministry of Health and local health center staff.
*Responsible for overall project management, including site selection, site visits to participating hospitals, specimen collection and data analysis
*Established and maintained a regular system of project evaluation and progress monitoring via site visits, trip reports, annual reports, data analysis and collaboration with WHO and the MOHs.
*Collaborated with and reported to the World Health Organization and Ministries of Health in five countries
*Used SAS and EpiInfo for analysis of data collected by the surveillance system

I had the opportunity to serve as a **W.H.O. Advisor/International Technical Consultant** in 2/08

*Led one of four teams performing an external evaluation of the Malian Guinea Worm Eradication Program

*Created a country specific, eight section questionnaire to assess the quality of the surveillance and elimination program. This allowed for data to be collected by the team I led as an external expert.

*Interviewed and evaluated activities of more than 200 persons, including health care workers and village members, in the Gao region of Mali using the questionnaire

*Wrote a 100 page country report based on data collected and analyzed. The results were presented to the Ministry of Health and WHO representative

**Epidemiologist**

**DIVISION OF PARASITIC DISEASES, CENTERS FOR DISEASE CONTROL AND PREVENTION • Atlanta, GA (employed by AREF)**

*Evaluated the effectiveness of the self-treatment program for the lymphedema elimination campaign in Leogane, Haiti using both quantitative and qualitative methods

*Hired and trained seven staff members for year-long, double-blind CDC/Proctor & Gamble $40,000 study in Leogane, Haiti

*Coordinated the logistics, created a database and presented a proposal to local IRB for a CDC lymphatic filariasis study

*Collaborated with CDC epidemiologists, statisticians and Haitian medical and field staff

*Analyzed and managed data from research conducted in Leogane, Haiti

*Translated surveys from Creole to English

*Provided programmatic and technical support to supervisor of lymphatic filariasis program

September 2000-July 2002
January-March 2002

**Public Health Technical Advisor and Consultant**

Global 2000, **THE CARTER CENTER • KARA, TOGO**

*Collaborated with Togo’s Ministry of Health’s Guinea Worm Eradication Program (GWEP) to assess the sensitivity of the surveillance system in the 14 prefectures of Northern Togo. Provided recommendations to local and national GWEP to correct flaws in the eradication efforts and strengthen capacity where the surveillance system was not active.

*Supervised and evaluated field supervisor performance in active case detection and management, chemical pond treatment and health education activities.

*Organized and facilitated training session of 30 local, regional and national guinea worm supervisors.

Summer 2000

**Public Health Researcher**

LYMPHEDEMA CLINIC, **HOPITAL STE. CROIX • Leogane, Haiti**

*Researched, wrote proposal, and received funding for thesis project in Leogane, Haiti

*Conducted survey on home treatment methods of 200 lymphedema patients in CDC funded clinic

*Analyzed existing databases and created new data sets using EpiInfo

*Used GIS for documenting and analyzing geo-spatial patterns of patient referral to lymphedema clinic

September 1999-May 2000

**Research Assistant**

Women And Children’s Center, Emory University • Atlanta, GA

*Prepared a 37-page report on the Vaccine for Children (VFC) Program

*Analyzed VFC questionnaire survey data from 57 states and territories

*Performed a literature review on vaccine distribution systems

1997-1999

**Program Officer**

East Central European Scholarship Program (ECESP), Georgetown University • Washington, DC

*Researched issues related to Eastern Europe including Health Care, Banking and Education reform

*Prepared and edited reports for USAID in compliance with
USAID reporting requirements
*Arranged travel logistics for over 250 visiting scholars
*Managed and maintained ECESP database for all programs and for alumni

1995-1996

**Agriculture/Agroforestry Extensionist**

UNITED STATES PEACE CORPS ● Bangassou, Central African Republic (CAR)

*Supervised the construction of the community health center of Ngounpalo, CAR and the thirteen surrounding communities to be accessible to over 1450 people, while organizing logistics and managing $4500 in grant money
*Developed an agribusiness project to raise funds for a community pharmacy
*Collaborated jointly with Ministry of Agriculture and European Union agribusiness program to train community leaders
*Promoted environmental awareness with flora/fauna preservation campaign
*Worked with youth to create a basketball league, English Club and safe sex education program that reached over 75 league members.

1988-1994

**Crew Member, First Mate**

NICOLE RENEE CORPORATION ● Gloucester, MA 1988-1994

*Worked on a long-term seasonal basis as a crew member on a recreational fishing vessel ● GLOUCESTER, MA

**SKILLS**

Languages

*French-Fluent
*Spanish-Basic
*Haitian Creole-Working knowledge
*Sango (National language of CAR) - Working knowledge

Computer

*Have worked with a number of programs on PC and Macintosh platforms including MS Word, MS PowerPoint, MS Excel, MS Access, Endnote, WordPerfect, Quattro Pro, EpiInfo, SAS, Arc View
*Have worked in, traveled to and/or lived in Angola, Benin, Brazil, Cameroon, Canada, Central African Republic, Chile, Democratic Republic of Congo, Ethiopia, France, Ghana, Haiti, Ireland, Italy, Mali, Panama, Peru, Poland, South Africa, Sudan, Switzerland, Tanzania, Togo, Turkey, Uganda, United Kingdom

International experience

| AWARDS AND MEMBERSHIPS | *2008 Award of Excellence-Public Health Epidemiology and Laboratory Research-Group- Acanthamoeba keraatitis Outbreak Research Team- CDC/NCZVED  
*2002 Secretary’s Award for Distinguished Service (for work related to the Anthrax attacks) –U.S. Secretary of Health and Human Services  
*Who’s Who of American Colleges and University Students Award - Rollins School of Public Health, 2001  
*Trans-cultural Scholarship Award Recipient -Rollins School of Public Health, 2000  
*Member, International Students for Health and Human Rights (ISAHHR)  
*Member, American Public Health Association (APHA)  
*Member, American Society of Tropical Medicine and Hygiene (ASTMH)  
*Dean’s List – Macalester College, 2002-2003 |

Presentations and Publications | Dahl BA  
*Patterns of Melarsoprol Treatment Failure*  
55th ASTMH Annual Meeting, Atlanta, Georgia, 2006

Dahl BA, Burri C, Bilenge CMM, Kande V, Josenando T, Mbulamberi D, Chappuis F, Moore AC  
*Sentinel Surveillance for HAT Treatment Failure*  
28th ISCTRC Meeting, Addis Ababa, Ethiopia, 2005
Dahl BA Moore AC Burri C Bilenge CMM Josenando T Chappuis F Jannin J
HATSEN TinEL: Surveillance for Human African Trypanosomiasis Treatment Failure

Evaluation of Antibacterial Soap for Treatment of Filarial Lymphedema, Leogane, Haiti

Benjamin A. Dahl, Antoine Michelus, Joyanna Wendt, David G. Addiss
Sustainability of Lymphedema Treatment Among Patients in Leogane, Haiti
50th ASTMH Annual Meeting, Atlanta, Georgia, 2001
David G Addiss, Jacky Louis-Charles, Joyanna Wendt, Benjamin A. Dahl, and Marie-Denise Milord
Lymphedema Treatment in a Filariasis-Endemic Area, Haiti
129th APHA Conference, Atlanta, Georgia, 2001

Workshops
Third International Course on Trypanosomoses
Lisbon, Portugal, May 12-30, 2003

Tools for Change: Problem Solving for Community Health
Community-Based Participatory Research workshop, Southeast Community Research Center, November 10, 2001

Geographic Information Systems (GIS) in International Health
Appalachian State University, July 24-29, 2001