NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH

Research Programmes

2012 – 2014

ACKNOWLEDGEMENTS

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Special mention must be made of NMIMR's present director, Professor Kwadwo Koram who provided direction and leadership for putting this report together. The Director, besides leading the process, was deeply involved in formatting and editing the report.

Appreciation also goes to all heads of department who collated and submitted contributions from their various departments, as well as wrote the department's profile.

Thanks also go to all persons, especially the senior members who contributed in one way or the other to make this report see the light of day.

A debt of gratitude is owned to Hudson Odoi for his excellent photographic skills that produced photographs to enrich the report.

Thanks to all the hardworking staff of the institute whose efforts have made it possible to produce this report, ***Ayekoo!***

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MESSAGE FROM THE DIRECTOR

Japan-Ghana Medical Cooperation was established nearly 50 years ago between University of Ghana Medical School (UGMS) and the Fukushima University Medical College soon after the inception of UGMS. This led to the establishment of the Noguchi Memorial Institute for Medical Research more than three decades ago to undertake research mainly on infectious diseases in the spirit of Dr Hideyo Noguchi, after whom the Institute is named.

The early studies centred on child survival issues and saw researchers establishing field stations in the Gomoa area to study the effects of simple interventions such as environmental sanitation, childhood immunisations and health promotion on child mortality. These studies showed clearly the marked reduction of child mortality that could be attained by simple application of the tenets of primary health care as the child mortality rate in the Gomoa area was reduced from approximately 150/1000 to 47/1000 after 4 years of activities. To maintain these gains, a clinic was established and donated to the community after the project. Other studies then covered nutrition, diarrhoea, and food hygiene among others.

In the second decade of the Institute's life, research activities were intensified with researchers competing for and winning research grants from international funding agencies. Studies in the area of Malaria, HIV, Neglected Tropical Infections, Mycobacterial infections etc. became important areas in response to the international drive and concentration on HIV, malaria and tuberculosis. Lately, the Institute has begun studies in response to the changing patterns of diseases by establishing programmes on chronic kidney diseases, biological basis of certain cancers in the Ghanaian population, exploration of the interfaces between infection and chronic non communicable diseases and a big push in the area of medicinal plant research. Following the production of the Institute's Readers as part of the 65th anniversary celebration of the University, a decision was taken to document the research activities being undertaken in the Institute as a follow up to the annual report last published in 2011.

It is hoped that this volume of research activities, covering the period 2012 – 2014 will be a guide to studies being undertaken at the Institute that will be revised, at least every other year, to inform stakeholders on how the Institute is fulfilling its mandate and living up to its vision and that of the founding fathers. The Institute is particularly grateful to Professors Michael D. Wilson and Prof. Dorothy Yeboah-Manu, and Dr. Collins Ahorlu who worked tirelessly to edit the various departmental reports.

We are also grateful to all our benefactors and sponsors for their generous support over the years. We are especially grateful to JICA, GNPC, WHO Country Office, who supported our activities during the Ebola outbreak. I wish you an enjoyable reading.

Kwadwo A. Koram

DEPARTMENT OF ANIMAL EXPERIMENTATION

Prof. Phyllis Addo, HOD

Animal Experimentation contributes to the discharge of NMIMR's mandate to research into human diseases of public health importance, by using animals as stand-ins or surrogates for humans. Through the Department's comparative medicine research, it contributes to the elucidation of various human disease conditions with respect to modes of transmission, pathogenesis, diagnosis, prevention and treatment.

The Department of Animal Experimentation produces and supplies various laboratory animals for research, testing, diagnosis and teaching, for users within and outside Ghana. The animals include: mice, rats, hamsters, guinea pigs, rabbits, poultry, grasscutters, sheep/goats, and non-human primates.

The Department trains technicians from other institutions in laboratory animal science and technology. The Department also uses the animals it produces to undertake exclusive departmental projects or collaborative projects with departments and institutions within and outside Ghana. In the year under review, the following routine and research activities were undertaken by the Department:

1. Production, Characterization and Maintenance of Laboratory Animals for Research, Testing, Diagnosis and Teaching (Routine)
2. Maintenance and Characterization of Environmentally-Controlled Animal Facilities (Routine)
3. Development of Animal Models for Infectious and Non-Infectious Human Diseases
4. Preclinical Studies on the Safety and Efficacy of Natural Products for *Salmonella typhi*, *Vibrio cholera Mycobacterium ulcerans*; *Trypanosoma brucei* infections; Type II Diabetes, Epilepsy, and uterine leiomyoma (Fibroid).
5. Buruli ulcer Research: Transmission and Treatment

Table 1: Animals Produced and Maintained in the NMIMR Animal Facility in 2013

Animal Type	Strain Name	Genetic Status	Microbiological Status	Usage in 2013
1. Mouse	BKS.Cg/ BomTacm$^{+/+}$Lepr^{db}	Inbred	SPF	Type II Diabetes studies
	Hsd:ICR (CD-1)	Outbred	SPF	Efficacy and Toxicity tests, Susceptibility test, Epilepsy
	BALB/cO1aHsd	Inbred	SPF	Efficacy and Toxicity tests, Susceptibility test
	C3H/HeNHsd	Inbred	SPF	Efficacy and Toxicity tests, Susceptibility test
	C57BL/6Jo1aHsd	Inbred	SPF	Efficacy and Toxicity tests, Susceptibility test
2. Rat	Hsd:SD	Outbred	SPF	Efficacy and Toxicity tests, Susceptibility test & Teaching
	F344/NHsd	Inbred	SPF	Efficacy and Toxicity tests, Susceptibility test & Teaching
	NTac:SHR	Outbred	SPF	Hypertension studies
	EKER	Outbred	SPF	Fibroid studies
3. Hamster	Hsd:Han.Aura	Outbred	SPF	General purpose use
4. Guinea pig	Undefined	Undefined	Clean Conventional	Influenza studies, Buruli ulcer, Feeding of mosquitoes & Teaching
5. Rabbit	Undefined	Un-defined	Clean Conventional	Blood, Feeding of mosquitoes Toxicity test & Teaching
6. Grasscutter	Undefined	Un-defined	Clean Conventional	Buruli ulcer
7. Sheep	Undefined	Un-defined	Conventional	Blood/Plasma/Serum/Antiserum
8. Goat	Undefined	Un-defined	Conventional	Blood/Plasma/Serum/Antiserum
9. Poultry	Undefined	Un-defined	Conventional	Influenza studies

SPF - Specific Pathogen Free

DEPARTMENT OF BACTERIOLOGY

Prof. Dorothy Yeboah-Manu

The Department of Bacteriology works to improve the health of populations internationally and nationally through excellence in research, teaching, training and diagnostic services to improve patient care. In addition to working on enteric pathogens and sexually transmitted diseases, the department's current main focus is on the two most important mycobacterial diseases of public health importance to Ghana, namely Buruli ulcer (BU) and tuberculosis (TB).

The department collaborates with other research partners on the global fight against TB and BU by conducting studies aimed at improving understanding of disease epidemiology, genetic diversity within the causative agent, host-pathogen

interactions and laboratory diagnosis. In order to make our research findings beneficial for disease control, we work closely with endemic communities and these field activities are carried out in close collaboration with the respective national control programmes and district health management teams of the Ghana Health Service. Furthermore, senior members in the department teach at various departments within the Faculty of Science and the College of Health Sciences. They also supervise Masters and PhD students from these departments as well as host international students.

In addition to research and teaching, we conduct free reference laboratory services to national control programmes such as BU and TB. Within BU, the current main method for confirmation PCR, cannot be done at the facilities of the Ghana Health Service as it lacks the expertise and infrastructure to conduct such assays. This is very important to prevent unnecessary surgical excision and/or anti-mycobacterial drug treatment of patients with non-BU lesions and for epidemiological surveillance. Thus our department has been supporting the national programme by confirming cases and this is crucial as instances of false diagnosis based on clinical judgment is reported. Samples are received across the country and our department has worked on more than 2,000 samples within the past five years. In addition we are working to develop new diagnostic tools such as LAMP and also improve the sensitivity of microscopy by concentrating on using immuno-magnetic beads.

The department is supporting TB control and patient care by testing sputum from smear positive relapse and treatment failure cases for drug susceptibility to guide retreatment regimen. Laboratory staff from the department also assist in development of manuals, training and quality assurance activity to improve the quality of TB diagnosis within the country.

A research assistant working in the microbiology lab

DEPARTMENT OF CLINICAL PATHOLOGY

Dr. Regina Appiah-Opong

The Clinical Pathology Department conducts research that contributes to intervention strategies and safeguarding of public health in Ghana. The Department carries out research into plant medicine development for non-communicable and communicable diseases including cancers, diabetes mellitus, malaria, HIV/AIDS, as well as drug interactions and regulation, bio-monitoring and prevention of poisoning from environmental toxicants (e.g. mycotoxins specifically aflatoxins and toxic heavy metals). The Department also provides laboratory services in support of public health programmes. This includes oversight of a Clinical laboratory that supports various projects, health facilities and individual patient's requests, and the laboratory for screening of new born babies for sickle cells disease. The department also provides support for the H3 Africa Kidney Disease Research Network.

Key Research Areas

- Drug development
- Drug-drug/ herb-drug/ food-drug interactions
- Heavy metal studies
- Mycotoxin studies

Research Activities

Research projects carried out in 2014 include:

- Screening Ghanaian traditional medicinal plants for bioactive anti-cancer agents
- Studies on anti-viral and anti-parasitic compounds from selected Ghanaian medicinal plants: Toxicity studies on medicinal plants
- Onshore environmental assessment of the Jubilee oil field in the Western Region of Ghana
- Determination of aflatoxin levels in Bokina, a beverage in Ghana

DEPARTMENT OF ELECTRON MICROSCOPY AND HISTOPATHOLOGY

Dr. Michael Fokuo Ofori

The Electron Microscopy & Histopathology (EMH) Department houses the only Transmission Electron Microscope in Ghana. The main research focus of the department is in the area of Enteric Diarrhoea with special emphasis on Rotaviruses. Through its diarrhoea surveillance studies, the department helped to firmly establish rotavirus as a major cause of diarrhoea in children less than five years of age. The EMH department is a key member of the African Rotavirus Surveillance Network, a network set-up to initiate and facilitate the determination of the burden of rotavirus diarrhoea disease across Africa and to provide evidence based data for the introduction of rotavirus vaccines in Africa and the rest of the developing world.

The Department currently is a WHO supported Reference Centre for the identification of rotavirus using Electron Microscopy, Enzyme Immunoassay and Molecular techniques. The Department is also involved in studies measuring the burden and characterization of Noroviruses, Caliciviruses, Astroviruses and other enteric viruses. The Department also conducts studies in Buruli ulcer and some plant viruses and also provides technical support to various African scientists in the area of rotavirus research and diagnosis.

Research Assistants at the bench in the EM&H Department

The EMH Department hosted the 6th and 7th Africa Regional Workshop on Rotavirus Surveillance, Diagnosis and Characterization with participants from Nigeria, Côte d'Ivoire, Senegal, Guinea Bissau, Gambia, Benin, Liberia, Niger, Togo, Sierra Leone and Ghana.

The Department received a number of Institutions including the Department of Microbiology and the Department of Medical Laboratory Technology of Kwame Nkrumah University of Science and Technology as well as the Department of Microbiology and Biotechnology of the University of Cape Coast.

Research Activities

The Department undertook some specific projects. These included the following:

- Estimating the economic burden of gastroenteritis in Ghanaian children (in collaboration with PATH, USA).
- Investigation into the prevalence of *Shigella* and/or ETEC infections in five communities in Ghana to measure the natural immune response
- Assessment of faecal exposure pathways in low income settings in Accra (in collaboration with Rollins School of Public Health, Emory University, USA)
- Africa Regional Workshop on Rotavirus Surveillance, Diagnosis and Characterization

Research Collaborations

- Plant toxicology studies with the Centre for Scientific Research into Plant Medicine, Mampong.
- WHO and PATH
- Emory University, USA
- Centres for Disease Control (CDC), Atlanta, USA
- Ghana Health Service, Ministry of Health, Ghana
- University of Allied Health Sciences, Ho, Ghana
- Department of Paediatrics, University of Ghana Medical School
- Department of Child Health Komfo Anokye Teaching Hospital, Kumasi, Ghana
- London School of Hygiene and Tropical Medicine, UK

DEPARTMENT OF EPIDEMIOLOGY

Dr. Collins S. K. Ahorlu

In line with the vision and mission of the Institute, the Epidemiology Department continued to work on varied areas such as basic and applied epidemiological research on malaria and other diseases (both communicable and non-communicable) of public health importance in Ghana. The Department houses the budding Social Science Unit of the Institute, the Health Support Centre for HIV/AIDS. The Department is in charge of the Institute's Clinical Trials Unit and has oversight responsibility for the Computer and Information Technology sections as well as the Data Management unit. The Department provides technical assistance to other researchers of the Institute in the areas of research design, implementation, data management and analysis.

Staff of the Department includes a mixed array of medical epidemiologists, health social scientists, clinicians, pharmacists, nurses, statisticians, information technologists and laboratory technicians as well as clerical workers.

During the period under review the Department's research activities covered the following areas.

- Malaria Vaccines: Clinical Research and Trial Sites preparations in Endemic Areas.
- Monitoring the Therapeutic Efficacy of Artersunate Combination Therapies (ACTs) in the Treatment of Uncomplicated Malaria in Ghanaian Children.
- Reaching the Poor in Ghana's National Health Insurance (SHINE Ghana) project.
- Rapid Mortality Monitoring Births and Deaths Registration plus (RMM-BDR+) project to evaluate progress towards MDG 4 .
- Enhancing and sustaining health insurance participation in Ghana through improved client-oriented quality of care.
- "Health Inc. Research":-Exploring social exclusion as a determinant of social health protection in West Africa and India .
- Accelerating progress towards attainment of MDG 4 and 5 in Ghana through basic health systems function strengthening.
- Sexual and Reproductive Health Resilience of Adolescents in Ghana and Tanzania.
- Stop Buruli Project: Early case detection, reporting and treatment interventions in the Ga East and South Municipalities.
- Characterization of Molecular Markers of Antimalarial Drug Resistance.
- Prevalence of *Plasmodium falciparum* parasitaemia and anaemia in children under five years of age at baseline and following annual vs. biannual indoor residual spraying (IRS) in Bunkpurugu-Yunyoo district, northern Ghana.
- Assessment of malaria transmission in a potential site for clinical trials in Ghana.
- National malaria prevalence surveillance in all 10 regions of Ghana.

Training activities

Faculty members of the department were involved in teaching and supervision of research students at masters and/or doctoral levels from the School of Public Health, School of Nursing and Department of Sociology, University of Ghana as well as from other local and foreign universities.

Staff of the Department also provided training through workshops and in-service training to various technical and professional groups, as well as internship programmes. They support the Ghana health service, notably the malaria and Buruli ulcer control programmes and served on a number of committees of the Ghana health service.

DEPARTMENT OF IMMUNOLOGY

Prof. Ben A. Gyan

The overarching focus of the Department has been the exploitation of the discipline of immunology for improved diagnosis, treatment, control and prevention of the major infectious diseases prevalent in Ghana. Whilst this is consistent with the overall strategy of the Institute, the on-going reorganization of the University with additional mandate for the Institute places a demand on the department to strategically reposition in a field that is fast evolving. This is also to ensure that the department and its activities receive the expected visibility in a university poised to focus on research.

We have therefore initiated efforts to meet demands. Key amongst these that were fulfilled during the period under review were:

- expansion of the portfolio of research activities to cover disease areas endemic in Ghana
- promoting multidisciplinary and collaborative initiatives that enhance research activities within the department, e.g. initiating multi-investigator grant proposal in thematic disease areas;
- developing research support platforms for departmental members (e.g., refurbished culture facility to ensure sterility and general working environment; provision of office space for students and other staff).

Staff

There are two tenured Associate Professors, one Senior Research fellow, two Research fellows and a postdoctoral fellow with diverse expertise in the department. In addition, there are currently research assistants and technical staff who contribute to the research and training mission of the department.

Teaching and Training

Based on their knowledge and expertise, our departmental faculty directly engages in the teaching and training of undergraduate and graduate students as well as post-doctoral fellows in immunology, biochemistry, parasitology and microbiology.

Key Research Areas

- Standardization of Immunological Assays in Preparation for Vaccine Trials
- Pregnancy Associated Malaria
- Immunological Correlates of Protection against Clinical Malaria in a Cohort of Young Children
- Pathogenesis of Severe Malaria in Children
- Developing Immuno-Epidemiological Tools for the Assessment of Malaria Transmission Intensity
- Evaluation of Immunogenicity of Vaccines
- Phenotypic Characterization of Host-Pathogen Interaction in Tuberculosis
- Understanding Natural Immune Responses to Buruli Ulcer Infection
- Natural Immune Responses to Shigella and Enterotoxigenic Escherichia Coli Infections.
- Immunological characterization of Cutaneous Leishmaniasis

DEPARTMENT OF NUTRITION

Dr. Gloria Folson

The Nutrition Department has extensive experience in the broad area of applied nutrition research. The goal of the department is to conduct research in the field of nutrition towards the establishment of a solid evidence base on which national policies and decisions can be made to solve nutritional problems in Ghana. The research agenda of the Department also focuses on issues of global importance such as the nutrition transition and its attendant problems which include the surge in non-communicable diseases, nutrition and HIV and hidden hunger. In pursuance of these goals, the Nutrition Department's main research areas are maternal, infant and young child nutrition, food consumption and food security studies, micronutrients and nutrition intervention studies.

The Nutrition Department currently houses the International Iodine Deficiency Laboratory which it runs in collaboration with the Department of Nutrition and Food Science, University of Ghana. Our laboratories also provide services such as chemical analyses of foods to the academic community and the public. Worthy of mention is accreditation for Vitamin A analyses.

Visitors to the Department

Dr Eva Monterossa of DSM Sight and Life, and Mr Emmanuel Paa Nii Quaye, Country Manager, Global Alliance for Improved Nutrition (GAIN) visited the Department in November and December 2013, respectively.

Training and Teaching

Staff of the Department taught courses in Nutrition to students of the Schools of Allied Health Sciences and Public Health of the College of Health Sciences, and supervised MPhil and PhD students in other institutions in Ghana and abroad.

Extension services

The Department has represented the Institute on a number of national committees such as the

Scaling Up Nutrition (SUN) Movement in Ghana

Cross Sectoral Planning Committee (SCPG) for Nutrition

Review of WHO Recommendations for Micronutrient Supplementation

DEPARTMENT OF PARASITOLOGY

Dr. Irene Ayi

The Department of Parasitology conducts basic and applied research on communicable diseases; malaria, neglected tropical diseases including trypanosomiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, leishmaniasis, soil-transmitted helminthiasis and on non-communicable diseases such as Buruli ulcer as well as zoonotic diseases of public health importance notably, toxoplasmosis and babesiosis. It has a major research programme on non-communicable diseases specifically asthma and allergies because of the linkages with helminths infections. Almost all the studies have field components which are conducted at sites located all over Ghana.

The in-house expertise includes:

- **Entomology:** vector biology and systematics, vector transmission studies, field monitoring of disease control interventions, insecticide susceptibility testing, and efficacy testing of insecticide-treated nets and textiles.
- **Malacology:** study of schistosomiasis host snails in disease transmission, prevention and control, field monitoring of snail distribution and molluscicide efficacy testing

- **Parasitology:** parasite biology and systematics, life cycle studies in disease transmission, prevention and control applications, accurate diagnoses, development and evaluation of diagnostic tools for early detection of infections.
  * Parasite biochemistry and immunology
  * Applied molecular biology and genomics

The Department also provides technical training on field and laboratory research, consultancies and specialized technical services to international and local industries, and agencies including Ministries, Governmental and non-governmental organizations. For effective and efficient execution of the Department's core and supportive roles, The Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine is supporting the Department to build capacity for ISO 15189 accreditation which was initiated in 2012.

AFFILIATED CENTERS AND FACILITIES:

1. Lymphatic Filariasis Support Centre For Africa (LFSCA),
2. West African Centre For International Parasite Control (WACIPAC)
3. NMIMR-Vestergaard Research Facility (NVRF)

Lymphatic Filariasis Support Centre for Africa

The LFSCA was established in 2006 to complement the activities of existing Lymphatic Filariasis Support Centres to provide technical and operational support as well as develop capacity to support LF elimination programmes in Africa. It serves as a platform for support and exchange of knowledge among the various national programmes of Global Alliance to Eliminate Lymphatic Filariasis (GAELF) in Africa. The main activities of the centre include training, research and general advocacy on LF in line with the approach adopted by the Global Programme to Eliminate Lymphatic Filariasis (GPELF). The major stakeholders are the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) and GlaxoSmithKline (GSK), Task Force for Global Health, Centre for Neglected Tropical Diseases (CNTD), Liverpool and the Noguchi Memorial Institute for Medical Research (NMIMR),

THE WEST AFRICAN CENTRE FOR INTERNATIONAL PARASITE CONTROL (WACIPAC)

The WACIPAC was established to build capacity to strengthen malaria and neglected tropical diseases control programmes in West Africa. WACIPAC was supported by the Government and People of Japan through JICA from 1st January 2004 till 31st December 2008. Thereafter, WACIPAC partnered with the East and Southern African Centre for International Parasite Control (ESACIPAC), KEMRI, Kenya and the Partnership for Child Development (PCD), Imperial College, UK to continue its capacity strengthening functions and to add on Nutrition programmes. Four (4) International Short Courses on School Health and Nutrition (SHN) held in Nairobi (24rd to 31st October 2011) and, Kilifi (19th to 28th June 2012), Kenya, Vientiane, Vientiane Province, Lao PDR (10th to 20th February 2013) and Elmina, Ghana (10 – 20th June 2013). Among the dignitaries

who visited WACIPAC were Prof Don Bundy of the World Bank, Dr Lesley Drake, Director of PCD, Dr Sammy Njenga, Director of ESACIPAC and Professor Sir Roy Anderson, Director of the Neglected Tropical Diseases Centre in London.

A group picture of facilitators at the Asian training course on School Health and Nutrition

A picture of the speakers at the opening ceremony of the short course at Elmina. Insert: A section of participants

The Department is spearheading the establishment of a Centre for Neglected Tropical Diseases for the West African sub-region, using the platforms of its two affiliate centres (LFSCA and WACIPAC).

NMIMR-Vestergaard Research Laboratories

The NMIMR-Vestergaard Research Laboratory

The NMIMR-Vestergaard Research Laboratories (NVRL) was established as a partnership between NMIMR and Vestergaard-Frandsen on 30th November 2011. The facility has a fully functional *An. gambiae* insectary, bioassay and molecular laboratories. The insectary holds colonies of both susceptible and resistant strains of *An. gambiae s.s.*, which allows for high volume of bio-efficacy testing for product development, field trials, and molecular investigations into insecticide resistance mechanisms in *An. gambiae* species. The insectary also supports the work of the Parasitology Department in the monitoring of vector control interventions in Ghana, the development of transgenic parasite-resistant *An. gambiae* and various vector-borne

diseases research with US Navy Medical Research Unit 3 (NAMRU-3). In 2013, NVRL conducted investigations into the molecular basis of insecticide resistance in mosquito vectors of malaria in Ghana, Chad, Ethiopia, Liberia, and the Democratic Republic of Congo. NMIMR, Vestergaard is also actively involved in the Malaria Vector Control Oversight Committee of the National Malaria Control Programme in Ghana. A partnership with the African Regional Postgraduate Programme in Insect Sciences was established in 2013 to provide colonies of *Sitophilus zeamais* and *Prostephanus truncatus* for bioassays and students' research projects.

DEPARTMENT OF VIROLOGY

Prof. William K. Ampofo

During 2012 to 2014, research activities of the Virology Department were mainly concentrated on viral infections such as HIV, Influenza and Poliovirus with attention also on viruses of public health importance such as Viral Hemorrhagic Fevers. There were efforts to establish methods for the detection of new viral infections and some work also focused on interactions/co-infections with other sexually transmitted infections. There was also research on the animal-human interphase which sought to determine the prevalence of some high risk pathogens associated with zoonotic infections.

Activities in the area of key HIV infections included monitoring of anti-viral interventions for HIV/AIDS by biomarkers such as immune status, viral load and genotypic drug resistance studies whilst data was also generated on behavior patterns among high risk groups for HIV. Investigations of traditional plants continued to search for substances with activity against HIV replication and the ability of plant extracts to stimulate HIV expression in latently infected cells was also done. An examination of human leucocyte antigen polymorphisms in HIV and HTLV-1 infections has begun to characterize genome diversities in Ghana for a better understanding to inform the design of potential vaccine immunogens.

A project to obtain comprehensive data on the disease burden of influenza was launched to enable the determination of the relative importance of influenza and other respiratory pathogens as causes of ill health in Ghana. The surveillance for influenza infections via sentinel health facilities across the country was maintained to provide weekly profiling of influenza virus activity for the Ghana Health Service and the Global Influenza Surveillance and Response System.

The laboratory surveillance of Polioviruses in stool samples from acute flaccid paralysis (AFP) cases in Ghana, Togo and Benin was maintained to support the Global Polio Eradication Program. There were also studies to characterize human enteroviruses isolated from cases of AFP and healthy children under 5 years.

RESEARCH PROGRAMS

MALARIA

Despite recent falls in the incidence and prevalence of malaria worldwide, the burden of the disease is still at unacceptable levels, especially in endemic countries such as Ghana. Current estimates are that malaria mortality is in excess of 500,000 deaths and approximately 207m cases occur annually. The majority of these (90% of deaths and 80% of cases) occur in Africa and more than 3 out 4 deaths occur in children under 5 years of age [1]. In Ghana, malaria accounted for 44% of all outpatient clinic visits, 37.6% of all admissions and about 22.3% of all deaths in children under five in 2013. Given this, malaria research at the Institute constitutes one of the largest portfolios with studies on all aspects of the disease and carried out in 5 of the 9 departments, viz; Animal Experimentation, Clinical Pathology, Epidemiology, Immunology and Parasitology.

Despite the high mortality associated with acute childhood malaria, its pathogenesis and the factors which determine the survival or death of children during an attack of malaria have not been fully identified. The sequestration of infected malaria parasite to the endothelium as well as damage to the cerebral vasculature are important features of cerebral malaria. Circulating endothelial progenitor cells are required for microvascular repair. Prof. Ben Gyan and colleagues in the Immunology Department are leading studies to understanding the mechanisms of disease in children in Ghana.

Antibodies are an important component of the adaptive immune response and play a critical role in the control of many infectious agents including Plasmodium, the parasite that causes malaria. Antibodies have very wide applications including their use for disease diagnostics, immunological assay development as well as for the potential treatment and management of acute disease. Prof Daniel Dodoo, Dr Asamoah Kusi and Dr Michael Ofori in the Department of Immunology are leading studies in these areas in collaboration with several external partners.

Malaria control and eradication requires a multi-faceted approach involving all available tools, and an effective vaccine would be an important addition to currently available control tools. The development of an effective malaria vaccine depends largely on the identification of targets of protective immunity in Plasmodium species, and a better understanding of the parasite-host interactions that are relevant for the acquisition of immunity. The 5300 proteins expressed by *Plasmodium falciparum* during its multistage life cycle has however hampered these efforts as only a handful of these antigens have so far been characterized and evaluated as vaccine candidates.

The transmission of malaria within a community can be measured by evaluating the levels of transmission blocking immunity (TBI) individuals posses. Antibodies against pfs48/45 have been shown in mosquito membrane feeding assays to directly correlate with TBI as individuals with higher levels of anti pfs48/45 are able to alter the development of sexual stage malaria parasites within their mosquito host. The sexual stage of the malaria parasite begins its development as a gametocyte and is responsible for the transmission of the disease. Dr Linda Amoah in the Department of Immunology is studying the biology of the sexual forms of the malaria parasite.

Chemotherapy remains one of the mainstays for the control and management of *Plasmodium falciparum* malaria since there is no efficacious vaccine. However, chemotherapy has suffered a setback caused by the emergence and spread of strains of *P. falciparum* resistant to available antimalarial drugs. Continuous monitoring of the effectiveness of antimalarial drugs in disease-endemic areas is therefore crucial for early detection of reduced parasite susceptibilities to the drugs. Parasite genetic diversity may affect the clinical outcome of malaria, multiplicity of infection (MOI), transmission, drug response and naturally acquired or vaccine induced immunity. As a result (parasite) diversity information is useful for malaria control activities (monitoring of drug resistance, and transmission dynamics).

Working with the National Malaria Control Program, researchers in the Epidemiology and Parasitology Departments are monitoring the clinical and parasitological responses to treatment across the country. In addition, the Epidemiology Department is also monitoring the prevalence of malaria parasitaemia among febrile cases in more than 40 health facilities in the country. Finally, the department has carried out impact evaluation of intervention programs in selected districts in the country.

Malaria control by IRS aims to reduce the reservoir of infection by blocking malaria transmission. Traditionally, the reservoir of infection has been measured in population surveys by microscopic visualization of the parasite in blood smears (1). This method is not sensitive and does not capture within species variation to accurately assess the true reservoir of *P. falciparum*. Micro-epidemiological variations in malaria transmission exist at all levels of endemicity and this constitutes a major bottleneck for malaria elimination efforts. Human biting rates and mosquito infection rates vary across space and time. Mosquito populations also vary temporally, driven by climatic variables such as rainfall, temperature, and humidity. These sources of heterogeneity in the distribution of mosquito populations generate variability in the risk of human infection.

Other studies on malaria include the identification and pre-clinical development of Ghanaian medicinal plants as alternatives to treatment. This includes studies on the in vitro efficacy of the identified plants and toxicological studies on some Ghanaian herbal plants in the departments of Animal Experimentation and Clinical Pathology.

Research Projects

Malaria Vaccine Research and Capacity Building in Ghana (MAVARECA)

Investigators:	
NMIMR	
Department of Immunology	*Michael Fokuo Ofori, PhD,*
Department of Epidemiology	*Kwadwo Koram, MB ChB,; PhD,*
University of Copenhagen:	
Centre for Medical Parasitology	*Lars Hviid PhD*
	Jorgen Kurtzhals, MD, PhD
	Anja TR Jensen, PhD
	Lea Barfod, PhD
Hohoe Government Hospital:	*Nicholas Opoku, MD*
	Margaret Kweku, MD, PhD
Funding	*DANIDA*

Background

This is a Large Strategic Research Project, submitted in response to the Danish Ministry of Foreign Affairs' 2012 Call for Applications regarding Development Research. It is directly aimed at the funds allocated for the fight against malaria, HIV and TB with special focus on the development of vaccines. As a capacity building project, it has a number of work packages with one of them being the support of clinical research aimed at establishing a well-equipped clinical laboratory at the Hohoe Municipal Hospital. The upgraded facilities will be used for studying the profile of clinical malaria and supplement patient management as well as provide support for the PhD training component of the project.

Objectives:

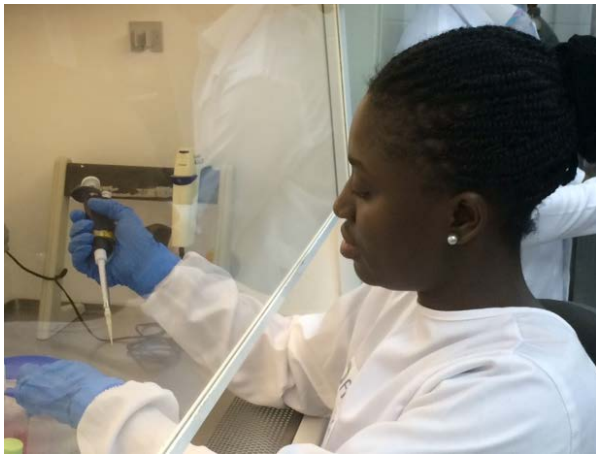
The overall objective of the project is to integrate creation of new state-of-the-art scientific knowledge with research capacity building that in combination, can accelerate the development of second-generation malaria vaccines for people living in areas with transmission of P. falciparum parasites. Specifically we will;

- develop and manage (within a five year period) well-equipped clinical research laboratory at Hohoe Municipal Hospital.
- investigate the profile of clinical malaria cases within the Hohoe district
- collect and characterize malaria parasite antigens implicated in severe malaria as possible vaccine candidate antigens.
- develop malaria vaccine candidates targeting the asexual blood stages of the *P. falciparum* parasites.

We will be doing this by collecting specimens and data aimed at establishing a direct connection between parasite antigens and patient immunity versus clinical signs of severe malaria and central events in malaria pathogenesis.

Four PhD students were recruited after a competitive selection in 2013 and have already began work on the project. These are Ms. Gertrude Ecklu-Mensah, Ms. Betty Bandoh, Ms. Frederica Partey and Mr. William Van der Puije. They are all registered for their PhD with the University of Ghana

Investigators on the MAVERECA Project after inception meeting, May 2013



Ms Bandoh, PhD candidate working at bench

Anti-sporozoite antibodies as alternative markers for malaria transmission intensity estimation

Investigators:

NMIMR

Department of Immunology	*Kwadwo A. Kusi, PhD.*
	Daniel Dodoo, PhD.
Department of Epidemiology	*Kwadwo A. Koram, MBChB; PhD*
US Naval Medical Research Centre	*Martha Sedegah, PhD*
Funding	*NMIMR Postdoctoral Programme funded by the Bill and Melinda Gates Foundation*

Effective monitoring of malaria transmission is an important pre-requisite for on-going efforts to eliminate the disease in many parts of Africa. Available standard monitoring tools such as the entomological inoculation rate (EIR) is however not very sensitive. Sero-epidemiological models, mostly based on seroprevalence of antibodies to the blood stage parasite are currently being explored for transmission monitoring, but these are limited by long-term persistence of antibodies to blood stage antigens. The aim of this study is therefore to develop tools based on the seroprevalence of antibodies to the sporozoite stages of the parasite, which are expected to be short-lived compared to those against blood stage parasites, for the effective monitoring of malaria transmission dynamics, especially in areas with very low levels of transmission.

To achieve this we (i) developed and compared transmission intensity estimation models based on the seroprevalence of antibodies against CelTOS, CSP and AMA1; (ii) Assessed the effect of transmission intensity on the quality (avidity and *in vitro* inhibitory capacity) of humoral responses elicited against asexual blood stage parasites.

Transmission estimation models based on seroprevalence of antibodies against three malaria antigens (CelTOS, AMA1, CSP) have been developed using archived plasma samples collected for a cohort study in Asutuare in the Dangme West district of the greater Accra Region, Ghana. The data confirms the hypothesis that anti-sporozoite antibodies do not persist for very long periods in comparison with antibodies to blood stage antigens, (see Fig 1) meaning that models based on anti-sporozoite antigens may indeed be important for predicting short term changes in malaria transmission. Additionally, models based on antibodies to the sporozoite antigen CSP predicted a 13-fold decrease in malaria transmission intensity about four years prior to the time of sampling (*published in Malaria Journal, 2014; 13: 103*).

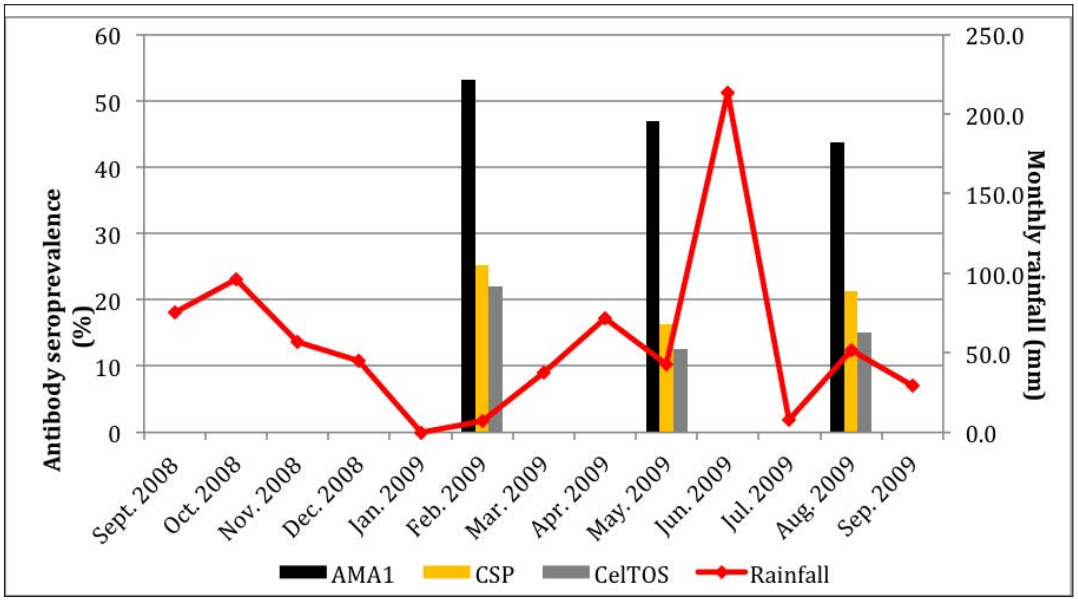


Fig. 1: Seroprevalence of 3 malaria antigens, AMA1, CSP & CelTOS and rainfall in Asutuare, 2008 - 2009

Future work

We hope to confirm these findings using a cohort from the Bongo district of the Upper East region of Ghana. Samples from two cross-sectional surveys (end of rainy season and end of dry season) will be used for model development and assessment of transmission intensity difference between moderate/high and low transmission periods. In addition, determination of the avidity of IgG from these samples, affinity purification of plasma IgGs on protein G columns and subsequent testing of purified IgGs against *Plasmodium* parasites *in vitro,* all in an effort to compare the functional quality of anti-malarial IgGs between the rainy and dry seasons, are on-going. PCR-based determination of sub-microscopic gametocytaemia in these samples is also on-going and involves developing constructs of cDNA from blood cell pellet-derived *P. falciparum* sexual stage RNA for estimation of submicroscopic gametocytaemia.

Circulating endothelial cells and the pathogenesis of malaria

Investigators:

NMIMR

Department of Immunology — *Ben Gyan, PhD.*

John Tetteh, MPhil, (PhD candidate)

Weill Medical College, Cornell University — *Linnie Golightly, MD*

Princess Marie Louise Children's Hospital — *Mame Yaa Nyarko, MBChB; MWACP*

Korle-Bu Teaching Hospital — *Richard Doe, MBChB*

Funding: — *NIAID /NIH*

Background

Despite the high mortality associated with acute childhood malaria, its pathogenesis and the factors which determine the survival or death of a child during an attack of malaria have not been fully identified. The sequestration of infected malaria parasite to the endothelium is an important feature of cerebral malaria. Damage to the cerebral microvasculature is a suggested feature of cerebral malaria. Circulating endothelial progenitor cells are required for microvascular repair. It is now known that microvascular damage induces the expression or activation of a series of molecules such as stromal cell derived growth factor 1 (SDF-1) and the matrix metalloproteinase-9 (MMP-9) which mediate the mobilization and release of EPCs from the bone marrow. We have shown in an earlier study that cerebral malaria may occur due to a dysequilibrium between malaria induced microvascular damage and host mediated repair. These results uncover a potentially novel role for EPC mobilization in the pathophysiology of cerebral malaria. Therapies that mobilize and improve the function of circulating endothelial progenitor cells (cEPC) which mediate microvascular repair, such as the HMG-CoA reductase inhibitor Lipitor, may therefore be of utility in the treatment of cerebral malaria.

Objectives

We have initiated a follow up study to determine the kinetics of the host response to microvascular damage, its relationship to the development of and recovery from cerebral malaria. The study is designed to examine the kinetics of the host cEPC response to microvascular damage.

Work Done So far

We have recruited cerebral malaria, uncomplicated malaria and healthy controls subjects in a case control study. These patients and subjects were followed up on recovery and 7 and 14 days post recovery. Flow cytometry analyses to enumerate the levels of endothelial cells marker CD34/VEGFR2 as well as chemokines/proteases associated with their release (SDF-1, and MMP-9) using ELISA, have been done.

Future Work to be Done

In a cross-sectional study we will use qRT-PCR to determine if mRNA levels are different in patients with CM as compared to controls and correlate it with cEPC numbers as determined by flow cytometry. Preparations are underway to determine transcript levels of VEGFR2, CD34, VE-cadherin, CD133, E-selectin, c-kit ligand, Tie-2, eNOS. Using the migration index to determine whether each of the assessments of cEPC function correlates with the number of cEPCs as determined by flow cytometry in each of the groups CM, UM, AP and healthy controls.

Developing immuno-epidemiological tools for the assessment of malaria transmission intensity:

Investigators:

NMIMR:

Immunology Department	*Kingsley Badu, PhD*
	Ben Gyan, PhD
Parasitology Department	*M Appawu PhD,*
Kumasi Centre for Collaborative Research (KCCR)	
Kwame Nkrumah University of Science and Technology:	*Ellis Owusu-Dabo, PhD*
University of California, Irvine, USA:	*G. Yan, PhD*

Funding — *NMIMR Postdoctoral Programme funded by the Bill and Melinda Gates Foundation*

Background

Micro-epidemiological variations in malaria transmission exist at all levels of endemicity and this constitutes a major bottleneck for malaria elimination efforts [Bousema *et al.*, 2012]. Human biting rates and mosquito infection rates vary across space and time. Mosquito populations also vary temporally, driven by climatic variables such as rainfall, temperature, and humidity [Galardo *et al.*, 2009]. These sources of heterogeneity in the distribution of mosquito populations generate variability in the risk of human infection. Assessing exposure to malaria vectors is critical to our understanding of disease transmission risk, and will facilitate planning of interventions. However, the gold standard for direct detection of exposure to infectious bites and mosquito population monitoring [EIR] suffers from well recognized limitations (James *et al.*, 2014). The use of antibodies to *Anopheles* salivary proteins as surrogates for human exposure to vector bites and risk of transmission is a promising endeavor. *Anopheles gambiae* salivary peptide (gSG6-P1) has been designed to enhance its specificity and immunogenicity to detect human exposure to malaria vectors. The salivary peptide gSG6-P1 has been recognized as specific to the *Anopheles* genus and highly conserved. Antibody response to this peptide offers promising characteristics as a biomarker for human biting; increases in these specific antibody levels correlated with increased rainfall in a region of very low mosquito exposure.

Objectives of study

The overall aim of the study is to determine field applicability of seroepidemiological tools in estimating malaria transmission in comparison to the gold standard entomological inoculation rate in terms of sensitivity to seasonal variations in holo-endemic northern Ghana and meso-endemic southern Ghana. Specifically, develop parameters for the immunoassays on archive samples from Asutuare and translate this to the field samples. Determine various entomological

indexes such as Human biting rates, MBR, sporozoite rate, (SR) and the annual entomological inoculation rate (EIR). This will be followed by the evaluation of human antibody responses to *An. gambiae*, and *P. falciparum* specific antigens: gSG6-P1, *PF11-0394/ PFE_ 056W*, CSP and MSP-1_{19} and finally develop age-dependent state transition model in order to predict malaria transmission intensity (MTI).

Work done so far:

Two cross-sectional serological surveys were conducted during October –November 2012 and April –May 2013 at Bongo/Soe in the North of Ghana and Odumase/ Agbetokope near Dodowa where an additional survey was carried out in September-October 2013 to allow for kinetic investigation of antibody response. Demographic data including fever, antimalarial drug intake and ITN usage were also obtained. In parallel, entomological surveys were also carried out in the study communities. A total of 779 sera from the North (Bongo and Soe) and 776 sera from Dodowa comprising both dry and rainy seasons have been analysed and data analysis is currently ongoing. In addition to the above, we have evaluated total IgG responses to gSG6-P1 and two malaria antigens (*Pf*CSP, MSP-1_{19}) in a longitudinal cohort from Asutuare, South-western Ghana, which is an area of relatively low but perennial transmission. 300 randomly selected sera were analyzed from archived samples belonging to a cohort that were followed at 3 contact times (n = 900) as follows; February, the dry season, May at the peak of the rainy season and August a dry period before the minor rainy season, representing snap shots of the perennial transmission in the year. Again as part of this seroepidemiological studies, malariometric indices such as parasite prevalence, fever, ITN coverage as well as entomological parameters in these study communities will be reported.

Preliminary findings

Entomological parameters: Preliminary findings suggests that currently only *Anopheles gambiae* s.s is involved in malaria transmission in the Dodowa area, where the majority (55%) belongs to the s molecular form, followed by 30% belonging to the m molecular form and 25% had both the s/m forms. Additional 250 *Anopheles* samples are being tested to confirm these preliminary findings. The entomological inoculation rate **(**EIR) has been determined for Dodowa area: Agbetokope 18.3 and Odumase 26.1 infectious bites per person per year based on the minor rainy season based on a total of 2,406 *Anopheles* mosquitoes collected on human baits. A second set of 2,500 mosquitoes has just been analysed with data entry and analysis ongoing.

Serological parameters: We explored the utility of the gSG6-P1 in identifying temporal exposure to infectious bites in comparison with *Pf*CSP and MSP-1_{19} which are well known malaria antigens. Seropositivity above threshold of negative group to the 3 antigens was detected in the cohort at all contact points across age groups. All antigens showed differences in proportion of seroprevalence in the seasonal trends similar to rainfall and mosquito exposure patterns and significantly so, particularly in the *Pf*CSP and the MSP 1_{19}, as shown in Figs 2a & 2b.

Fig. 2a

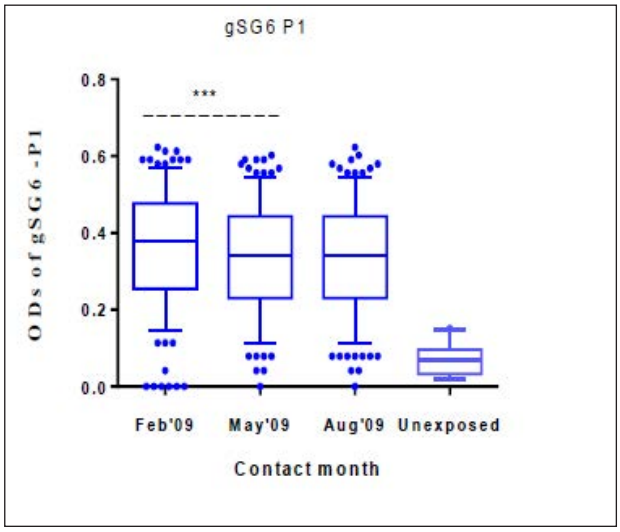
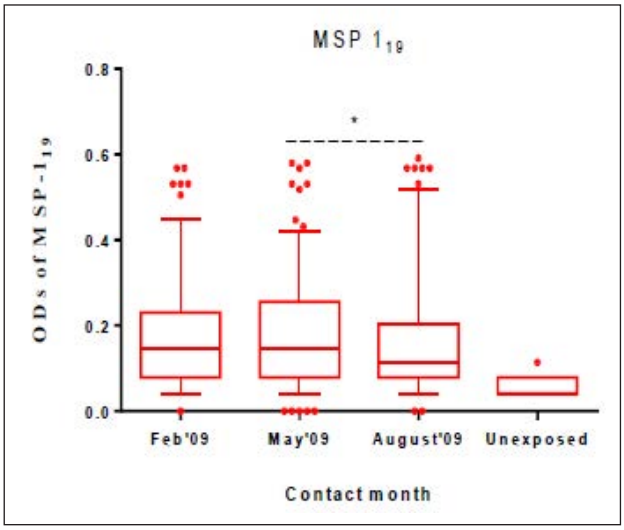


Fig. 2b



*** indicate P value < 0.0001, ** indicate P <0.01 and * indicate p value < 0.05

Comparison of median antigen specific antibody levels within the study cohort

Repeated measures, ANOVA as well as post hoc Tukey multiple comparison test of median antibody levels to gSG6-P1 showed significant difference in antibody levels in mosquito exposure between the peak rainfall and dry period preceding minor rainy season, detecting temporal variations in vector exposure among the cohorts at different time points with a two-month lag effect. Likewise median Ab responses to *Pf*CSP showed significant seasonal differences, but there was no significant difference observed in antibody titers to MSP1-$_{19}$ confirming its cumulative nature which is less affected by seasonal fluctuations [Badu *et al*., 2012].

It appears that the value of gSG6-P1 in assessing seasonal changes in vector exposure lies in the magnitude of antibody response and not in the proportions of seroprevalence. Because individuals remain exposed all year round, seroprevalence may not be able to differentiate short-term changes in transmission. However, antibody levels tend to be higher in actively infected people with a concomitant decline as infections are cleared [Drakeley & Cook, 2009], thus the antibody levels (IgG titers) can reflect fluctuations in recent exposure to salivary proteins. In general the longevity of Ab response generates a seroprevalence that is higher than equivalent parasite rates, making it a more sensitive measure in hypo-endemic areas like Asutuare. Thus Ab level of antibody, rather than seroprevalence of gSG6-P1 is robust and sensitive to detect seasonal changes in human exposure

Future studies

At this point, we are unable to distinguish between biting by different *Anopheles* species, but this would be important for estimating the relative contribution of each species to transmission. It will also be important to know the level of exposure (or the number of bites) required to eliciting detectable response additionally; there is a need to understand the duration of these responses more fully to determine their use as biomarkers for recent transmission patterns which, will be relevant deducing the sensitivity of the assay. Detection of these markers in human saliva and urine will be very convenient and it is critical to know how this will work out in less invasive samples.

Tracking Malaria Disease Prevalence in Ghana.

Investigators:

NMIMR

Department of Epidemiology — *Benjamin Abuaku, PhD.*; *Collins S. K. Ahorlu, PhD.*; *Nancy Quashie, PhD.*; *Kwadwo A. Koram, MBChB; PhD.*

Centre for Tropical Clinical Pharmacology and Therapeutics (CTCPT). UGMS — *Neils Quashie, PhD*

Funding — *NMCP /Global Fund*

Background

Key to the provision of effective case management in Ghana is adequate diagnosis, which is necessary for the assessment of the impact of preventive strategies such as Insecticide Treated Net (ITN) use and Indoor Residual Spraying (IRS). Malaria diagnosis has largely been presumptive over the years leading to poor case management as well as poor data on prevalence of disease in the country. It is in this light that the Noguchi Memorial Institute for Medical Research, in collaboration with the National Malaria Control Programme, is monitoring malaria parasite positivity rates across the country.

Objectives

The overall objective of the study is to assess progress of interventions towards reduction of malaria prevalence in Ghana. The specific objectives of the study are: to set up a surveillance system for generating data on malaria parasite positivity rates across the country, using Rapid Diagnostic Tests and by microscopy in selected health facilities across the country.

Approach

This is a surveillance activity being conducted in twenty-four (24) selected health facilities across the country by teams in each facility. Ten of the facilities are in urban areas and the remaining 14m rural areas. All persons presenting with a history of fever within the previous 72hrs are tested with a rapid diagnostic test according to national guidelines for malaria case management. To allow for monthly monitoring of malaria parasite positivity rates by both RDT and microscopy, thick and thin blood smears (for microscopy) are obtained for every third patient with fever. The RDT cassettes for these patients are labeled and stored in Ziplock bags for later molecular studies on malaria parasite diversity across the country. The blood slides are stained, dried, and stored for reading at NMIMR.

Preliminary findings and implications

Implementation of surveillance started between July and August 2013 after training of surveillance teams in June 2013. Preliminary data gathered between July and December 2013 show that the national parasite positivity rate was highest in September (43.4%; 95% CI: 42.2, 44.4) and lowest in

December (30.0%; 95% CI: 29.1, 30.9). During the peak month of September, Brong-Ahafo region showed the highest rate of 65.9% (95% CI: 54.7, 75.6) whilst Greater Accra region showed the lowest rate of 22.1% (19.1, 25.4). During December, Western region showed the highest rate of 54.6% (95% CI: 47.6, 61.5) whilst Greater Accra region showed the lowest rate of 10.4% (95% CI: 8.7, 12.4) (Fig 3). These findings suggest that Greater Accra remains the region with the least burden of malaria even during the peak month of September.

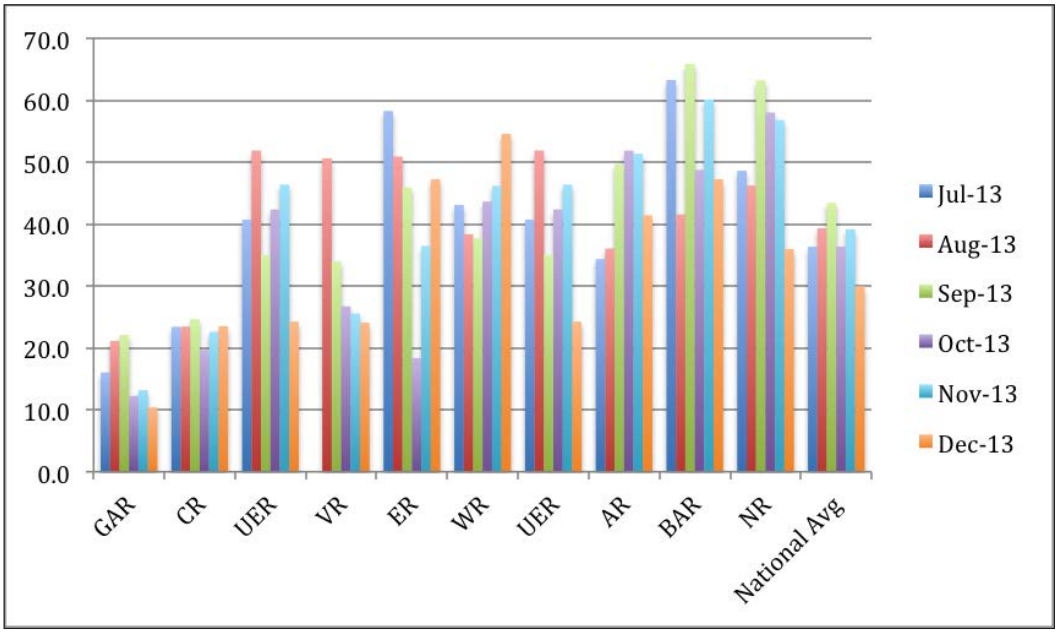


Fig. 3: Malaria test positivity rates in Ghana by region, July – Dec. 2013

Monitoring the therapeutic efficacy of antimalarial drugs in the treatment of uncomplicated malaria in Ghana.

Investigators:

NMIMR

Epidemiology Department	*Kwadwo A. Koram, MB; ChB; PhD,*
	Benjamin Abuaku, PhD.
	Nancy Quashie, PhD.
	Lydia Quaye, MSc.
National Malaria Control Program	
(NMC)	*Constance Bart-Plange, MBChB; MPH*
Funding	*Global Fund for AIDS, Malaria and TB (thru the NMCP)*

Background

Chloroquine and sulphadoxine-pyrimethamine had been first-line and second-line antimalarial drugs, respectively, for the treatment of uncomplicated malaria in Ghana until 2005. Evidence of progressively high treatment failure rates of these drugs necessitated the change of policy to artemisinin-based combinations (ACTs) because of their rapid effects on fever and parasite clearance. They also reduce gametocyte carriage rates thereby slowing down the spread of drug resistance. Since 2005, the Noguchi Memorial Institute for Medical Research (NMIMR), in collaboration with the National Malaria Control Programme (NMCP), has been coordinating surveillance activities to generate data on the therapeutic efficacy of currently used first-line antimalarials (i.e. amodiaquine-artesunate and artemether-lumefantrine combinations) in Ghana. The 2013/2014 surveillance year is the fifth round since 2005. The studies have sought to generate data on the clinical and parasitological efficacy of artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) in children aged 6 months to 9 years with uncomplicated *P. falciparum* malaria. In addition changes in haemoglobin concentration subsequent to treatment are also monitored. Putative molecular markers of parasite resistance to chloroquine, sulfadoxine-pyrimethamine and artemisinin are also monitored and the data is fed to the National Malaria Control Program to inform malaria drug treatment policy.

Approach

This is a one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment for uncomplicated malaria using the WHO 2009 protocol for the assessment of therapeutic efficacy of anti malarial drugs. Children with uncomplicated malaria who meet the study inclusion criteria are enrolled, treated on site with AA or AL and monitored for 28 days. The follow-up consists of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the children are classified as having therapeutic failure (early or late) or an adequate response. The proportion of patients experiencing therapeutic failure during the follow-up period is used to estimate the efficacy of the study drugs. PCR analysis is used to distinguish between a true recrudescence due to treatment failure and episodes of re-infection.

Preliminary findings and implications

A total of 353 children participated in the 2013 study in seven sentinel sites across the country. Preliminary data suggest that therapeutic efficacy of AS-AQ and AL in Ghana have remained over 85% since their introduction in 2005 and 2008, respectively (Fig 4). ACTs should therefore remain the drug of choice for treating uncomplicated malaria in Ghana whilst monitoring their therapeutic efficacy. Testing before treating should be promoted among prescribers and the general public to reduce over-diagnosis and over-treatment thereby preserving the efficacy of ACTs in the country.

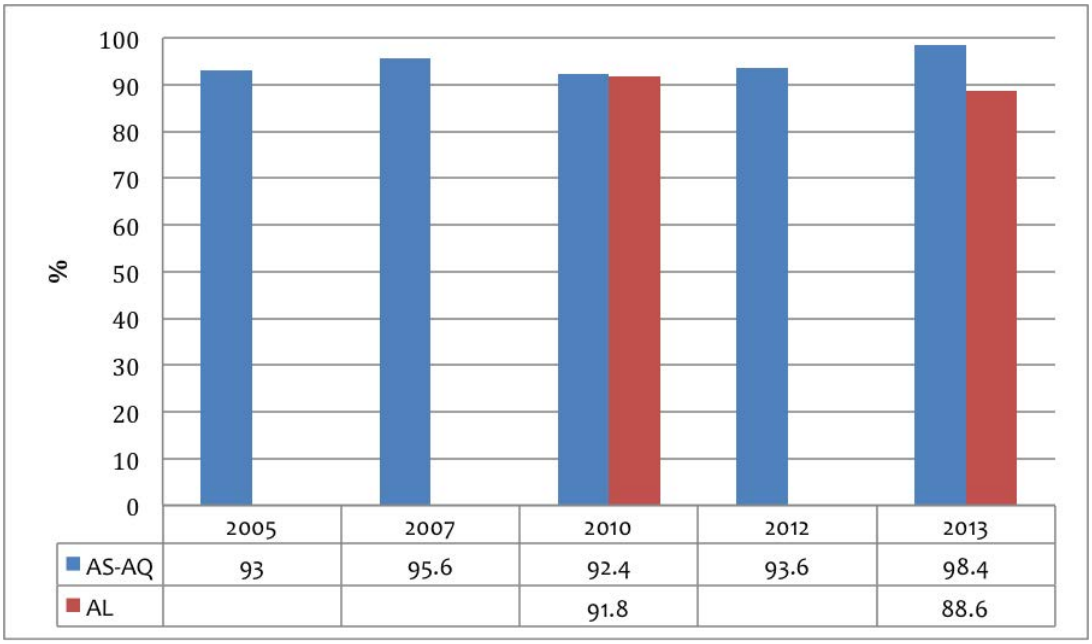


Fig 4. Day 28 pcr-uncorrected cure rates for AS+AQ and AL in Ghana (2005 – 2013)

Other Studies

Immunological memory B-Cell memory to variant *Plasmodium falciparum* antigens

Investigators:

NMIMR

Department of Immunology	*Michael Fokuo Ofori, PhD.*
University of Cape Coast	*Paulina Ampomah, MPhil*
University of Copenhagen	
Centre for Medical Parasitology (CMP)	*Lars Hviid, PhD*
	Lea Barfod, PhD
Funding	*DANIDA GETFUND, Ghana*

Mother-Child health in Southern Ghana – Malaria in pregnancy and in childhood

Investigators

NMIMR

Department of Immunology	*Michael F. Ofori, PhD*
University of Copenhagen	*Lars Hviid, PhD*
Centre for Medical Parasitology (CMP)	*Lea Barfod, PhD*
Funding	*DANIDA*

Functional analysis of selected *Plasmodium falciparum* Oocysts/Sporozoite stage genes.

Investigators	
NMIMR	
Department of Immunology	*Michael F. Ofori, PhD*
Department of Parasitology	*Daniel A. Boakye, PhD*
	Irene Ayi, PhD
	Samuel Dadzie, PhD
Tokyo Medical and Dental University	*Takashi Suzuki, PhD*
	Mitsuko Suzuki, PhD
Funding	*JICA*

Studies on the Roles Of 5-Ht Receptor Subtypes in the Sporogonic Life Cycle of *Plasmodium falciparum*

Investigators	
NMIMR	
Department of Immunology	Michael Fokuo Ofori, PhD
Department of Parasitology	*Michael Wilson, PhD*
	Anita Ghansah, PhD
UG Department of Biochemistry, Cell & Mol. Biology Marian	Nyako, *PhD*
	Samuel Yeboah, PhD
Funding:	*University of Ghana,*

The impact of anti-malarial drugs and naturally acquired immunity on *Plasmodium falciparum* asexual stage development in a malaria endemic area in Ghana

Investigators	
NMIMR:	
Department of Immunology	*Michael F. Ofori, PhD.*
Helena Nartey (PhD candidate)	
University of Ghana	
School of Public Health	*Isabella Quakyi, PhD*
University of Copenhagen	*Michael Alifrangis,*
Funding:	*BSU, DANIDA*

Study of immune correlates of protection against malaria after vaccination with RTS,S/AS01E: a comprehensive immunological arm of a Phase III double-blind, randomized, controlled multicenter trial

Investigators	
NMIMR	
Department of Immunology	*Ben Gyan, PhD*
Kintampo Health Research Center	*Seth Owusu Agyei, PhD*
	David Dosoo, MSc
CRESIB, Barcelona	*Carlota Dobano, PhD*
Funding:	*MVI /NIAID /NIH*

Immortalization of B cells from *P. falciparum*-exposed individuals for the production of parasite-specific monoclonal antibodies

Investigator:	
NMIMR	
Department of Immunology	*Kwadwo A. Kusi, PhD:*
Funding	*The World Academy of Sciences (TWAS), Trieste, Italy*

Association of protectiveHLA class I and II phenotypes with the risk of *P. falciparum* infection

Investigators:	
NMIKMR	
Department of Immunology	*Kwadwo A. Kusi, PhD*
	Daniel Dodoo, PhD
Naval Medical Research Centre (NMRC)	
US Military Vaccine Research Programme	*Martha Sedegah, PhD*
Funding:	*University of Ghana Research Fund (UGRF)*
	Naval Medical Research Center (NMRC), USA

Relationship between antibody recognition by *P. falciparum* pre-erythrocytic vaccine candidate antigens and clinical and parasitological protection against malaria in an endemic area

Investigators	
NMIMR	
Department of Epidemiology	*Kwadwo A. Koram, MB, ChB.; PhD*
Department of Immunology	*Kwadwo A. Kusi, PhD*
	Daniel Dodoo, PhD
NAMRU-#3	*Nehkonti Adams, MD, PhD*
	Naiki Puplampu, MPhil
Naval Medical Research Centre (NMRC)	
US Military Vaccine Research Programme	*Martha Sedegah, PhD*
	Eileen Vilasante, PhD
Navrongo Health Research Centre	*Frank Atuguba, MBChB. MPH*
Funding:	*US congressional Funds (NAMRU-3)*

Contributions of Pfs48/45 to malaria transmission blocking immunity

Investigators:	
NMIMR	
Department of Immunology	*Linda Amoah, PhD*
	Daniel Dodoo, PhD
Department of Epidemiology	*Kwadwo A. Koram, MBChB.; PhD.*
Department of Parasitology	*Samuel Dadzie, PhD*
Loyola University	*Kim Williamson, PhD*
University of Copenhagen	
Centre for Medical Parasitology	*Michael Theisen, PhD*
Funding:	*US National Institute of Health*

Alternative tools for identifying, measuring and monitoring malaria transmission in Ghana

Investigators:	
NMIMR	
Department of Immunology	*Linda Eva Amoah, PhD.*
	Kwadwo A. Kusi, PhD.
Funding:	*University of Ghana Research Fund*

Study of ex vivo production of infectious gametocytes

Investigator:	
NMIMR	
Department of Immunology	*Linda Amoah, PhD.*
Funding:	*BSU-PHH, University of Ghana*

Prevalence of *Plasmodium falciparum* parasitaemia and anaemia in children under five years of age at baseline and following annual vs. biannual indoor residual spraying (IRS) in Bunkpurugu-Yunyoo district, northern Ghana.

Investigators	
NMIMR	
Department of Epidemiology	*Benjamin Abuaku, PhD*
	Collins S. K. Ahorlu, PhD
	Kwadwo A. Koram, MBChB; PhD
Centres for Disease Control and Prevention (CDC)	*Paul Psychas*
	Philip Ricks
Ghana Africa IRS Project, Abt Associates	*Peter Mumba*
Funding	*US President's Malaria Initiative (PMI)*

Capacity building to prepare West African sites for clinical trials on HIV, TB and Malaria. West African Network of Excellence for TB, AIDS and Malaria (WANETAM): Assessment of malaria transmission in a potential site for clinical trials in Ghana.

Investigators	
NMIMR	
Department of Epidemiology	*Kwadwo A. Koram, MBChB; PhD.*
	Benjamin Abuaku, PhD
	Nancy Quashie, PhD
Centre for Tropical Clinical Pharmacology &	
Therapeutics, UGMS	*Neils Quashie, PhD*
Ghana Health Service, Hohoe	*Margaret Kweku, MD, PhD*
Funding	*EDCTP*

Characterization of molecular markers of drug resistance in *Plasmodium falciparum* from ten regions of Ghana over a 3 year period.

Investigators	
NMIMR	
Department of Epidemiology	*Nancy Quashie, PhD*
	Kwadwo A. Koram, MB; ChB; PhD
	Benjamin Abuaku, PhD
Centre for Tropical Clinical Pharmacology &	
Therapeutics, UGMS	*Neils Quashie, PhD*
US NAMRU-#3, Cairo	*Peter Sebeny, PhD*
	Jeff Villinski, PhD
	CDR Karl Kronmann, MD, PhD
	LCDR Christopher Duplessis
Funding	*Global Emerging Infections Surveillance & Response System, NAMRU-#3*

Genetic Dynamics of *Plasmodium falciparum* strains in Ghana

Investigators	
NMIMR	
Department of Parasitology	*Anita Ghansah, PhD.*
	Michael D. Wilson, PhD
Department of Epidemiology	*Kwadwo A. Koram, MB; ChB; PhD*
Funding	*NMCP (Global Fund)*

Association between allelic variations of the human genes Y11306 and Z35491 and resistance to severe malaria

Investigators	
NMIMR	
Department of Parasitology	*Anita Ghansah, PhD*
	Michael D. Wilson, PhD
Department of Epidemiology	*Kwadwo A. Koram, MB, ChB, PhD*
Funding	*NMIMR*

A study of the circadian periodicity of *Plasmodium falciparum* gametocytes in human peripheral blood, the role of biogenic molecules

Investigators	
NMIMR	
Department of Parasitology	*Anita Ghansah, PhD*
	Michael D. Wilson, PhD
Department of Immunology	*Michael F. Ofori, PhD*
UG, Department of Biochem., Cell & Mol. Biology	*Marian Nyako, PhD*
Funding	*NMIMR*

Impact of Distinct Eco-epidemiology on Malaria Drug Resistance in Ghana

Investigators	
NMIMR	
Department of Parasitology	*Anita Ghansah, PhD*
Department of Epidemiology	*Benjamin Abuaku, PhD*
	Kwadwo A. Koram, MBChB; PhD
Department of Immunology	*Michael F. Ofori, PhD*
Harvard School of Public Health	*Dyann Wirth, PhD*
	Sarah Volkman, PhD
	Clarissa Valim, PhD
Broad Institute	*Dan Neafsey, PhD*
Funding	*NIAID /NIH Grant # 1RO1 AI099527-01A1*

Measuring changes in reservoir of malaria infection in northern Ghana using molecular diagnostic methods

Investigators	
NMIMR	
Department of Epidemiology	*Kwadwo A. Koram, MBChB; PhD*
Department of Parasitology	*Anita Ghansah, PhD*
Navrongo Health Research Centre	*Abraham Oduro, MBChB; PhD*
University of Melbourne	*Karen Day, PhD*
	Kathryn Teidje, PhD
Funding	*Howard Hughes Medical Institute*

Exploring *Plasmodium falciparum* genome to understand the genetic diversity, emergence of drug resistance and vaccine efficacy

Investigators	
NMIMR	
Department of Parasitology	*Anita Ghansah, PhD*
Oxford University	
Wellcome Trust Centre for Human Genetics	*Dominic Kwiatkowski, PhD*
Funding	*Medical Research Council, (MRC) Centenary Fellowship Award*

Polymorphisms in genes implicated in artemisinin resistance: implications for artemisinin-based combination therapy for *falciparum* malaria in Ghana

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Functional analysis of selected *Plasmodium falciparum* Oocysts/Sprozoite stage genes

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MYCOBACTERIAL INFECTIONS

TUBERCULOSIS

Tuberculosis (TB) is the leading cause of adult deaths by a single infectious disease globally. About nine million cases of TB and 1.4 million deaths were recorded in 2012 with nearly 30% of the global burden of tuberculosis cases occurring in Africa though it houses only 11% of the world's population. The continuous recorded high cases of TB have been attributed to socio-economic factors such as urbanization, as well as synergies with other morbidities such HIV/AIDS and diabetes and the rise in drug resistance cases. Even though TB is a treatable disease, if drug resistance (DR) is not controlled, this may eventually result in TB becoming untreatable. Regular resistance surveillance is important to understand the level of resistance and also to guide retreatment regimen.

The majority of TB cases in humans are due to *Mycobacterium tuberculosis* and *M. africanum.* While *M. tuberculosis* has a global importance *M. africanum* is restricted to West Africa where in some of the countries it causes about 50% of all TB cases. Africa carries a disproportionally large share of the global TB burden and harbours the largest genetic diversity of the human-adapted MTBC lineages. Large amounts of resources are being invested into the development of new TB diagnostics, drugs and vaccines for TB infection and disease, but it is unknown how strain-specific diversity of the human-adapted MTBC in West Africa will affect the effectiveness of these new control tools. *M. africanum* has rarely been isolated in other regions of the world. Although it exist as 2 separate distinct lineages known as West African type 1 (MAF1) and West African type 2 (MAF2), both have been defined as an "ancient" clade while, the "modern" clade, *M. tuberculosis* (dominated in West Africa by *M. tuberculosis* "Cameroun genotype") of the human-adapted MTBC lineages also affect West African TB patients in significant proportions. Though, the "ancient" clade (MAF1 and MAF2) is genetically distinct from the "modern" clade (*M. tuberculosis* "Cameroun genotype"), the possibility of this genetic diversity being translated into phenotypic variation including virulence and immunogenicity has not been exploited. Ghana represents one of the few countries within Central West Africa known to habour all three genetic variants causing TB cases in significant proportions.

Our research activities focus on understanding disease epidemiology, genetic diversity within the causative agent, drug resistance mechanisms and elucidation of biomarkers.

Phenotypic and Genotypic Analyses of Drug Resistance in Tuberculosis in Ghana

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	Royal Society /Leverhulme Trust

Even though TB is a treatable disease, if drug resistance (DR) is not controlled, this may eventually result in TB becoming untreatable. Regular resistance surveillance is important to understand the level of resistance and also to guide retreatment regimen. The main objective of work within this area is to determine DR using phenotypic and sequencing of targets and to compare findings with that of one of the recommended rapid molecular kits (MTBDR*plus*, Hain lifescience). Analysis of more than thousand isolates identified 0.5% and 6.3% RIF and INH mono resistance respectively, while MDR accounted for 2.1%. Preliminary findings from on-going target sequence analysis of rpoβ, katG genes and the promoter region of *inhA* indicates that a specificity and sensitivity of more than 90% for RIF using the MTBDRplus kit.

On the other hand, when we screened isolates in a similar way with the MTBDR*sl* kit indicated for rapid screening of second line anti-TB drugs, we found poor correlation between phenotypic resistance and both the kit and mutations in the two resistance conferring genes (*rrs* and the *rpsL)* that are implicated for streptomycin resistance. These findings suggest that the MTBDR*sl* is not a good diagnostic kit for detection of streptomycin and other aminoglycoside resistance in Ghana. This finding thence paves the way for speculation of a possible involvement of other genes or epigenetic factors or the combination of both in the drug susceptibility of Mtb to streptomycin. Nevertheless, further work is required to identify and substantiate the exact mechanism for streptomycin resistance.

Phenotypic Characterization of Host-Pathogen Interaction In *Mycobacterium Africanum*

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	Damien Portevin, PhD
Funding:	*The Leverhulme-Royal Society Africa Award (2009) and Afrique-One Consortium*

Background

Africa carries a disproportionally large share of the global TB burden and harbours the largest genetic diversity of the human-adapted MTBC lineages. Large amounts of resources are being invested into the development of new TB diagnostics, drugs and vaccines for TB infection and disease, but it is unknown how strain-specific diversity of the human-adapted MTBC in West Africa will affect the effectiveness of these new control tools.

M. africanum subtype 1 rarely been isolated in other regions of the world is known to causes up to half of TB cases in West Africa only. Although it exist as 2 separate distinct lineages known as West African type 1 (MAF1) and West African type 2 (MAF2), both have been defined as an "ancient" clade while, the "modern" clade, *M. africanum* subtype II (*M. tuberculosis* "Cameroun genotype") of the human-adapted MTBC lineages also affect West African TB patients in significant proportions. Though, the "ancient" clade (MAF1 and MAF2) are genetically distinct from the "modern" clade (*M. tuberculosis* "Cameroun genotype"), this genetic diversity could translate in their phenotypic variation including virulence and immunogenicity. Ghana represents one of the few countries within Central West Africa known to have this unique genetic diversity of MAF1 and MAF2 that cause up to half of TB cases in significant proportions.

Aim and Objectives

This study proposes to take advantage of this unique TB epidemiology to understand the host - pathogen relationship of the phenotypic differences using immunological and microbiological assays, to evaluate the immunogenicity of recombinant early secretory antigenic target 6 and culture filtrate protein 10 (rESAT-6-CFP10) fusion protein in individuals infected with *M. africanum* subtype I and compared with individuals infected with *M. africanum* subtype II. strains; assess the virulence of MAF1, MAF2 and MTBss "Cameroun genotype" isolates infected in human monocyte-derived macrophages and also assess inflammatory phenotypes of MAF1, MAF2 and MTBss "Cameroun genotype" isolates infected in human monocyte-derived macrophages.

Work Done So far

Archived cryopreserved peripheral mononuclear cells of individuals infected with either *M. africanum* subtype I or subtype II have been stimulated with rESAT-6-CFP10 for six days, supernatants havested and stimulated cells stained for surface and intracellular cytokine markers to determine the frequencies of rESAT-6-CFP10 specific CD4 and CD8 phenotypic expression of interferon gamma IFN- (Th1) and IL-4 (Th2) levels. Analyses will be done by FlowJo Vx10.7. Th1 (IFN-, IL-17, sIL-2Ra, TNF-) and Th2 (IL-10) cytokines released into the supernatants will be measured using Luminex bead-based assay. Concentrations of each genotyped MAF1, MAF2 and MTBss "Cameroun genotype" isolates have been determined and cryopreserved in -80 ^{OC} freezer. Multiplicity of infection of 1:1 ie isolates with human monocyte - derived macrophages will be done after which levels of pro-inflammatory cytokines of innate response in the harvested supernatants will be determined. Expected findings may show distinct patterns of cytokine induction (by T subsets and infected human monocyte-derived macrophages) and intracellular growth rate of the strains.

Future Work to be Done

Future work will explore the genome-wide gene expression profiling of the differentially cytokine messenger ribonucleic acid (mRNA) expressed by infected human monocyte-derived macrophages with MAF1, MAF2 and MTBss "Cameroun genotype" isolates.

Micro-epidemiology: genotypic characterisation of *Mycobacterium* isolates

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Funding	*Wellcome Trust (Grant # 097134/Z11/Z*
	Royal Society /Leverhulme Trust

The conventional indicators used for assessing TB control programmes under the directly observed short course strategy (DOTS) focus on the proportion of patients with new, sputum smear positive pulmonary disease that is cured by the end of treatment or whose sputum microscopy becomes negative after the first 2 months of treatment. Such indicators ignore equally important aspects of TB control which includes the duration of infectivity, the frequency of reactivation, and the risk of progression among the infected contacts or the risk of transmission. However, understanding the patterns and dynamics of transmission is useful for the implementation of public health measures to reduce source of infection.

Using spacer-oligonucleotide typing (spoligotyping) and single nucleotide polymorphism typing (SNP typing) we confirmed that *M. africnaum* is a very important pathogen in Ghana and it is the cause of 20% of all TB cases. Lineage 4 was identified as the most prevalent *M. tuberculosis* lineage (62.0%) with the sub-lineages Cameroun and Ghana predominating around 58.0% and 25.0% respectively. Based on *in silico* analysis from our collaboration with Prof. Sebastien Gagneux and results of our *in vitro* work (figure 4), we have proposed 8 loci set of mycobacterium interspersed repetitive units- variable number of tandem repeats (MIRU-VNTR) for typing isolates from Ghana for epidemiological studies. With a unit cost of $11.24, the cost of performing standard MIRU-15 on one sample was $168.60, while the proposed customized MIRU-8 set for one sample being $89.2. Hence, by screening for only the relevant loci, did not only maximize discriminatory

power but also minimize genotyping costs. We are now applying this minimal MIRU-VNTR set in a molecular epidemiological investigation of MTBC transmission in a population based study in Accra Metro and Mamprusi East district to understand problem of re-infection vrs reactivation and risk factors aiding transmission. This project is also studying the impact of co-morbidities, such as HIV/AIDS and type 2 diabetes, on the biology and epidemiology of TB. In addition the on-going study has sent DNA samples for genome sequencing to analyse for genomic differences between *M. africanum* and *M. tuberculosis* and implications on targets being used for design of novel control tools.

Host immunological profiling from exposure to Mtb to active TB disease, in response to recently discovered differentially expressed Mtb proteins: a tuberculosis case-conduct study in Ghana

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London School of Hygiene and Tropical Medicine, UK	*Jackson Sillah, MBChB; PhD*
Funding	*Wellcome Trust (Afrique One [One Health] Consortium)*

Background

Most people infected with MTB have a latent TB infection (LTBI) and 5-10% of these individuals, if HIV negative will develop tuberculosis during their lifetime. Currently it is difficult to predict exactly who among the latently infected individuals will develop the disease and when, posing a major challenge to TB control. Current assays such as the quantiferon TB gold or T-Spot test based on IFN-γ production do not distinguish LTBI and active disease; this distinction is important to identify biomarkers of protection and cure that are useful as endpoints for clinical trials of preventive and therapeutic vaccines respectively.

Study Objectives

The aim of this study is to identify *in vitro* ESAT-6/CFP-10 Fusion protein and DosR regulon-encoded and Rpf antigen- induced expression of type 1 and type 2 cytokines in active TB patients (before and during treatment) and in LTBI individuals (longitudinally for 4 years) using the luminex bead-based assay and intracellular cytokine staining of specific T cell subsets. This is

to assist in the identification of more specific protective immunological responses in TB patients (after undergoing treatment) and in latently infected contacts.

Study population/sample size:

A total of 500 volunteers were originally planned (100 index cases and 400 contacts based on our assumption that 1 TB patient will have an average of 4 contacts). So far we have recruited 216 participants (104 index cases and 112 contacts) from Achimota, Maamobi and Legon Hospitals in Accra. This is because a good number of TB patients did not have contacts to be included in the study. Having lower numbers of contacts than originally anticipated does not affect the final results of the study. For inclusion, index cases are newly-diagnosed sputum smear positive and 16 years or older (with or without HIV). Contacts must be 6 months or older to be included. All participants are recruited using written informed consent, and questionnaires are administered to acquire demographic and other information.

Sample collection and laboratory analysis:

For index cases a sputum sample and 30mls of blood are taken before start of anti-TB therapy and again blood samples (30mls) are taken after 2 weeks, 2 months and 6 months of treatment. For contacts, 2mls of blood is taken for quantiferon gold test and up to 30mls (depending on age) of blood taken for further laboratory analysis. Blood samples are sent to the Immunology laboratory and sputum to the P3 laboratory (bacteriology) of the Noguchi Memorial Institute for analysis.

Isolation culture: Sputum samples are decontaminated with 5% oxalic acid and cultured on Lowenstein Jensen slants for up to 10 weeks for isolation of mycobacterium species. AFB positive isolates are tested with the Capilia TB-Neo test for confirmation of *Mycobacterium tuberculosis* complex and the Hain test will used for differentiation as *Mycobacterium tuberculosis* or *Mycobacterium africanum*.

Cell culture with antigens: Blood samples are processed to separate peripheral blood mononuclear cells (PBMC's). The PBMC's are then cultured with study antigens (ESAT6/CFP10, Rv1733c, Rv2029, Rv2628, and Rv1119) as well as a positive control SEB (staphylococcus enterotoxin B). Cultures are incubated for 6 days at 37^0C with 5% CO_2 and then the supernatant is harvested to be used for Luminex assay (IFNγ, TNFα, IL10, IL17, sIL2Rα, Granzyme B) while the cells are stained for intracellular cytokine assay to detect IFNγ, FoxP3, and IL4 expression of T cells.

Preliminary Analysis:

Participants: Male participants are 70.4% and females 29.6% among the TB cases. The HIV prevalence is 14.4% (15/104). The median age is 34.6 (range 16-78 years) with majority of the participants being within the 20-40 age range. All TB patients recruited have completed treatment and all their available contacts have been recruited.

Sixty-eight of the 112 TB contacts (60.7%) have latent TB infection, diagnosed as being quantiferon TB-Gold assay positive. Of the latently infected contacts, 30 have undergone CXR for identification of chest lesions. Seven had abnormal radiographs and have been referred to the NTP for further clinical evaluation and for possible treatment for TB as secondary cases. The remaining 38 latently infected contacts are yet to undergo CXR testing.

Isolation culture: All samples have been cultured with the following results: Isolate confirmed as MTC (65) and NTM (2). Hain genotyping confirmed 8 *M. africanum* and 57 *M. tuberculosis*.

PBMC culture:

a. ***Flow cytometry:*** the project continues to test the immune responses to the study antigens using PBMC's from TB patients and contacts. PBMCs are being processed and cryo-preserved and used for cell culture with antigens and subsequent intracellular cytokine staining assay by flow cytometry. Preliminary analysis on 30 patients with complete follow up samples using their surface and intracellular cytokine staining results indicated a decline in IL-4 and an increasing IFN-g recall responses to rESAT-6-CFP10 fusion protein as early as 2 weeks of treatment in both CD4 and CD8 T cells (Figs 5, 6 & 7) and it will be interesting to assess if a similar response will be observed with the other antigen panel. This preliminary result has been published in PLoS ONE 8(6): e68121. doi:10.1371/journal.pone.0068121. The remaining intracellular cytokine staining results are currently being analysed.

b. ***Multiplex assay:*** Culture supernatants are frozen for multiplex bead-based assay at a later date. A selected sample has been processed in an external laboratory abroad and the results being analysed is showing significant changes in Granzyme B and IL-10 by two weeks of treatment in response to ESAT-6/CFP-10 fusion protein but not to Rv2029, Rv1733 and Rv2628. This analysis is ongoing. We are also awaiting further processing of the bulk of our culture supernatants either at Noguchi or externally.

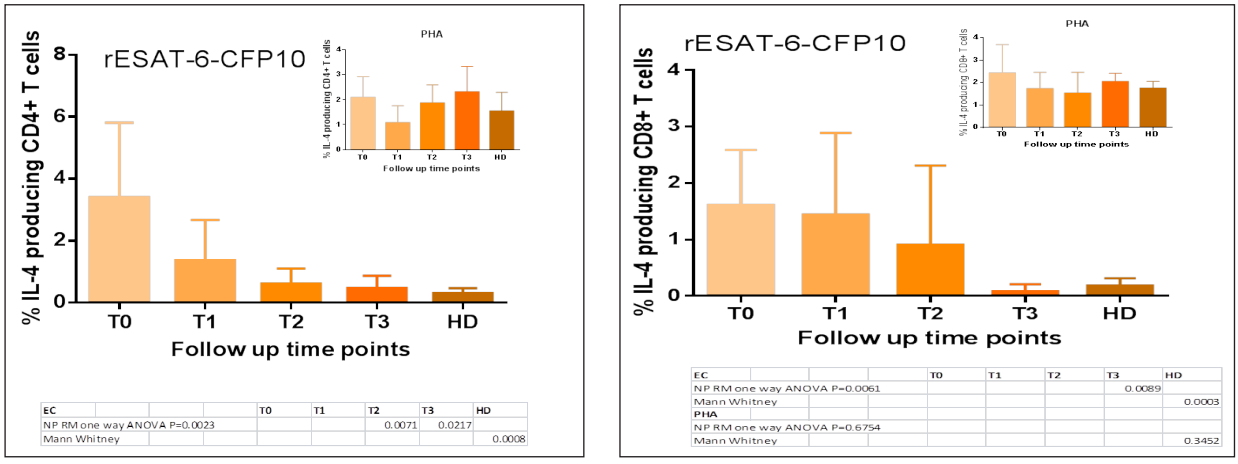

Fig. 5: (A&B) Decline in IL-4 recall response to ESAT-6-CFP10 as early as two weeks of treatment

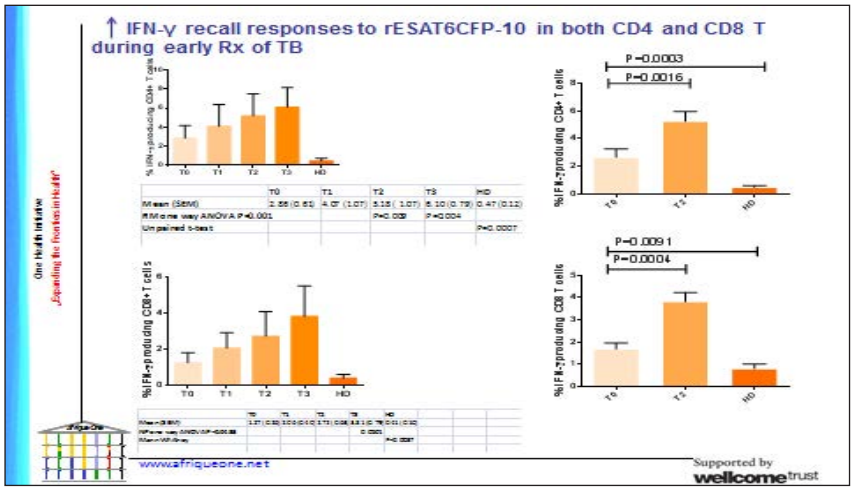

Fig 6: Increase in IFN-g recall response to ESAT-6-CFP10 as early as two weeks of treatment

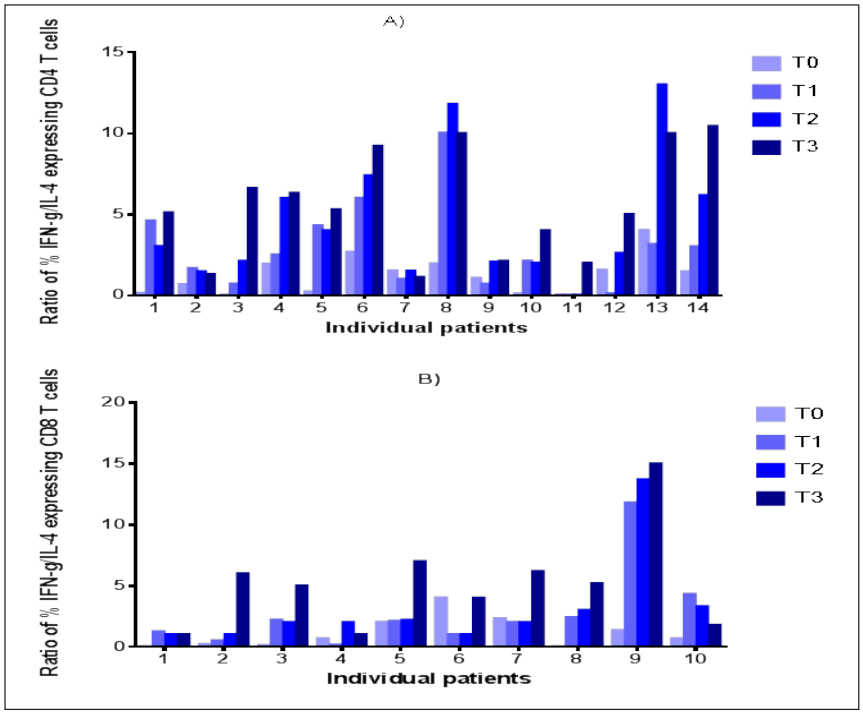

Fig 7: Comparison of esat-6/cfp-10 induced IFN-g/IL-4 ratios in individual patients during early treatment

Discussion

Interestingly, our initial findings from the longitudinal follow up of TB cases for two months suggest that effective treatment can lead to a switch in immunological responses to rESAT6CFP-10 fusion protein by 1 - 2 weeks of treatment. In vitro restimulation and intracellular cytokine analysis showed that the mean IFN-γ expressing CD4+ and CD8+ T cells increased significantly by 2 weeks of treatment with a concomitant reduction in IL-4. Individual results also suggested a significant increase in the ratio of IFN-γ/IL-4 producing T cells during the first 2 months of treatment. This finding could have implications in the early detection of MDR-TB as it is geared towards development of diagnostic assays for monitoring early treatment response.

Assessing tuberculosis disease prevalence in Ghana through a population-based survey Investigators

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Funding	*Global Fund /WHO /USAID – TB CARE 1*

Background

TB prevalence is the TB related MDG indicator that can be directly measured (given the challenges of near impossibility of directly measuring incidence and the absence of comprehensive vital registration data to measure TB mortality in Ghana). While trend analysis of programmatic data provides useful information to indirectly assess progress there are limitations. Firstly, we are unable to entirely rely on the routine surveillance system owing to its coverage, completeness and accuracy. Secondly, the current WHO estimates may be unrealistic; since the last well conducted prevalence survey based on TB disease in Ghana was in 1957. In 2000 TB burden measurement was done, but was based on TB infection, which is less reliable and accurate.

Objectives

The overall goal of this survey was to gain much better understanding of the burden of disease caused by TB and to identify ways in which TB control can be improved.

The General Objective of the survey was to obtain a direct measurement of the absolute burden of diseased caused by TB in Ghana in 2013.

The Primary Objective was to measure the prevalence of bacteriologically (smear or culture) confirmed TB cases among the adult population.

The secondary objectives were to determine among study participants with symptoms suggestive of TB, prevalence of sputum smear positive, and sputum culture positive TB, and to identify the extent to which people with TB or with symptoms suggestive of Pulmonary TB sought care. Finally, the study explored health seeking behaviors and exposure to some risk factors for TB and updated all population based estimates of the burden of disease (measured as TB incidence, prevalence and mortality, in combination with in-depth assessment of surveillance and programmatic data.

Study Methods

The methods used focused on measuring TB in adults who were at least 15 years old. 64,000 study participants were screened for TB in the population. Individual interview and Chest X-Ray screening were used to screen study participants, in order to include individuals with the highest risk of contracting TB and to reduce false positive results.

Those presenting with no symptoms and normal chest X-Ray were not considered TB suspects, and did not have to submit sputum samples. Individuals with TB suggestive symptoms and/or abnormal Chest X-Ray were considered as "suspects" and were asked to provide two sputum specimens for smear microscopy and culture. Smear microscopy was performed both with AFB and Fluorescence microscopy; culture was performed both with solid and liquid media. GeneXpert analysis was performed on all samples with positive microscopy. A component of the survey used cross sectional descriptive study to collect data on health seeking behavior to explore the reasons why some patients were diagnosed and treated for TB while others were not and described exposure to some risk factors for TB to gain some understanding of the TB burden.

Study Outcome

Although the survey outcomes are now being analyzed, the survey will provide a platform for exploring the interactions between patients and the health system. It will also have an additional advantage of using the generated data to evaluate the quality of TB disease surveillance in the country by identifying which patients with active disease have already been captured by the routine reporting system. With new insights and, diagnostic technologies, the study will help to establish a fairly accurate burden of TB in Ghana, whose results can be compared internationally. The final result will provide a good baseline against which subsequent surveys can be compared. Also, interpretation of programmatic data could be done within context of the burden of the disease.

BURULI ULCER

Buruli ulcer, caused by *Mycobacterium ulcerans*, is the third most important mycobacterioses globally after tuberculosis and leprosy. However, in West-Africa it is the second after tuberculosis. In the worst affected communities in countries like Ghana, Ivory-Coast and Benin, BU is the leading mycobacterial disease that affects immune-competent individuals. The disease which mainly affects the skin and sometimes the bone usually starts as a painless nodule in the less severe forms, but other forms such as plaque and oedema also occur as early forms in some individuals. If these early forms are not treated, extensive necrosis activity of the causative agent mediated by the potent toxin, mycolactone, leads to wide tissue damage resulting in the hallmark of BU, large ulcers. Serious permanent disabilities that may result include contractures, amputations and loss of vital organs such as the eye. The disease affects both sexes equally. All age groups can be affected, but currently children below age 15 account for close to 50% of all reported cases in the country. Many features of this debilitating disease including mode of transmission, immune protection mechanisms are not known and importantly there is no simple point of care diagnostic tool. The work at the bacteriology department focuses on understanding disease epidemiology and transmission, improvement of wound care, development of new simpler diagnostic tools and improving existing tools to offer services to the national control programme.

Epidemiology and Transmission of BU in Ghana

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National Buruli Ulcer Control Program, GHS	*Edwin Ampadu, MD*
Funding	*UBS Optimus Foundation (Stop Buruli Consortium)*
	VW Foundation (Contract # I/81308)

The epidemiology of BU in Ghana is not clear due to the focal distribution of cases where endemic and non-endemic communities are separated by few kilometres. The bacteriology department within the context of the Stop Buruli Consortium is studying the transmission of *M. ulcerans* (causative organism of Buruli ulcer) using conventional epidemiology, molecular epidemiology, sero-epidemiology tools and analysis of environmental samples to identify major transmission pathways and environmental reservoirs of *M. ulcerans.*

We profiled the disease burden along the Densu River by a house to house active case search and BU case mapping. We recorded no endemicity upstream the river and an increase in the disease burden as we moved downstream (Fig 8). Based on experience gained, we are carrying out similar but more detailed study along the Offin and preliminary results show similar pattern of distribution; upstream in Bedomase, no case was detected, but mid- stream (communities like Achiase and Ntobroso) and lower stream (communities like Mfantseman and Dominase) recorded higher burden with suspicion of active transmission within these portions of the river.

Within the Densu study, we investigated serological response against *M. ulcerans'* immuno dominant 18kDa small heat shock protein (shsp). Findings from our study show that the prevalence of the 18kDa shsp sero positive individuals was greater than 30% along the river. In addition, differences in anti-18KDa shsp IgG sero positivity rate (or titers) between inhabitants of BU endemic and non-endemic communities, was not significant. These findings imply that a large proportion of healthy individuals living in endemic regions do not develop overt disease though they show immunological evidence of exposure to *M. ulcerans*. It also implies that unknown host genetic, behavioural or socio-economic factors may trigger the development of sub clinical *M. ulcerans* infection, to clinical disease. Currently, we are carrying out similar studies along the Offin River to characterize exposure dynamics along the Offin and also assess age of sero conversion as a reflection of the onset of exposure to *M. ulcerans.*

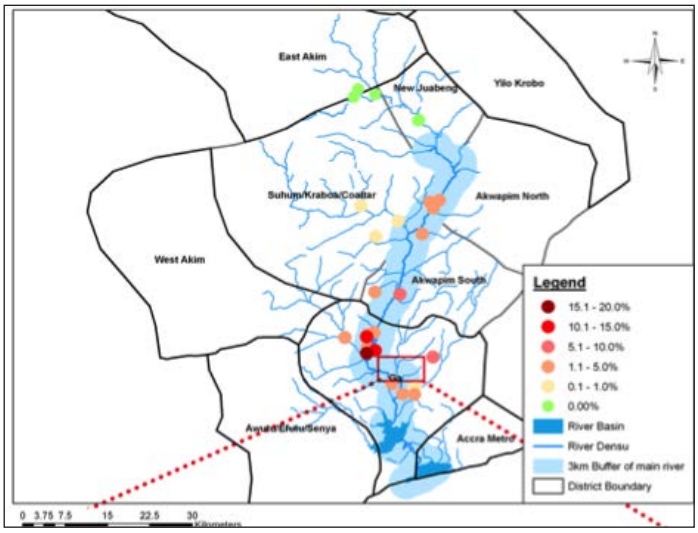

Fig 8: Map showing the prevalence of Buruli ulcer along the Densu River. Upstream no case detected, a few cases detected midsteam and disease burden highest downstream the river.

Micro-epidemiological studies in Buruli ulcer have also been hampered by the exceptional lack of genetic diversity among *M. ulcerans* strains. In collaboration with the Swiss TPH, we conducted whole genome sequencing of 7 isolates representative of the 3 earlier identified VNTR types in Ghana. This led to the detection of a comprehensive set of SNP markers suitable for genotyping studies and using real-time PCR assays, we identified 10 haplotypes among the 75 strains. However to routinely carry out strain we developed a Temperature-Switch PCR SNP typing tool. This tool was designed by replacing the 89 real-time PCR based assays by 10 strategically placed canonical SNPs (canSNPS) that enabled differentiation of the 10 haplotypes described earlier. The TSP- SNP typing PCR is agarose gel-based which makes it cheaper, and requires less expertise in operation. Using this assay in our laboratory we found it has a comparable discriminatory ability when compared with the real-time PCR assay. Findings of these studies indicate that water may not actively be involved in *M. ulcerans* transmission.

Fig 9: Environments that were sampled for analysis: left picture is a sand winning site in a BU endemic community while the right picture is stream for domestic use.

A major hindrance to the understanding of *M. ulcerans'* ecology and transmission is the inability to isolate *M. ulcerans* from the environment by culture, which is the only foolproof method to confirm the viability. Until now, only one cultivated *M. ulcerans* from an aquatic insect which was achieved through several passages using mouse footpad has been reported. The cultivation of *M. ulcerans* from the environment is particularly difficult for several reasons: environmental

specimens (Fig. 9) are often contaminated by other microorganisms causing overgrowth in the culture media since the contaminating organisms generally grow much faster than *M. ulcerans* which has extremely slow growth rate. A major focus of our activity is to improve decontamination step to remove unwanted fast growing organism and formulation of selective growth media to inhibit growth of unwanted bacteria. With this approach we have been able to isolate very important slow growing mycobacterial species from the environment including an *M. ulcerans*, *M. avium*, *M. gordonae* and *M. malmoense*. The isolated *M. ulcerans* strain has been genome sequenced and it is being analysed; in addition studies will be conducted to determine its virulence in animal model and to determine the type of mycolactone it produces. With this positive result we are doing more cultivation of real time PCR positive samples.

Analysis of causes of wound healing delay and Improvement of Wound care

Since 2006, BU is managed by an antibiotic treatment protocol which various reports have confirmed its effectiveness, however BU patients' general wound management has been grossly neglected. The focus has been largely on *M. ulcerans* specific anti-mycobacterial treatment. Problems of wound healing due to local immune reconstitution (paradoxical reactions) and intervening secondary bacterial infections have been misinterpreted and underestimated. In a cross sectional study involving 86 patients at different time points of antibiotic treatment we found that BU wounds can be infected despite mycolactone secretion by *M. ulcerans*. More importantly we found that the bacterial burden was highest in wounds that were sampled after antibiotic treatment. Microbiological and clinical evidence of infection was confirmed by histopathological analysis (Fig. 10) and the main pathogens were *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

To have a better understanding of the evolution of the wounds and effect of bacterial burden on the clinical course of wound healing we initiated a longitudinal study to follow patients before treatment until wound healing. The preliminary finding was that, before treatment, BU wounds usually have a moderate bacterial burden which goes down during antibiotic treatment but drastically increases after treatment. The clinical signs indicative of secondary infection observed included: localised pain, cellulitis, viscous/purulent discharge and oedema, discolouration of tissues both within and at the wound margins and offensive odour. Both microbiological and histopathological analysis confirmed secondary infections as one of the main causes of negative outcomes such as skin grafting failures and wound deterioration after anti-mycobacterial treatment. Moreover most of the pathogens were resistant against the tested antibiotics, including MRSA. In addition to secondary infection, co-morbidities with HIV/AIDS and diabetes as well as low haemoglobin levels were identified as other factors that could lead to negative treatment outcomes.

Our findings indicated the need for a clear guideline on wound care in BU case management as we observed general need for improvement in nursing practices. Thus together with the national control program and other collaborators a second draft of yet to be finalised guide manual and posters have been developed for training health workers managing BU. The obtained clinical isolates and that from the facility environment are being genotyped to analyse for nosocomial transmission.

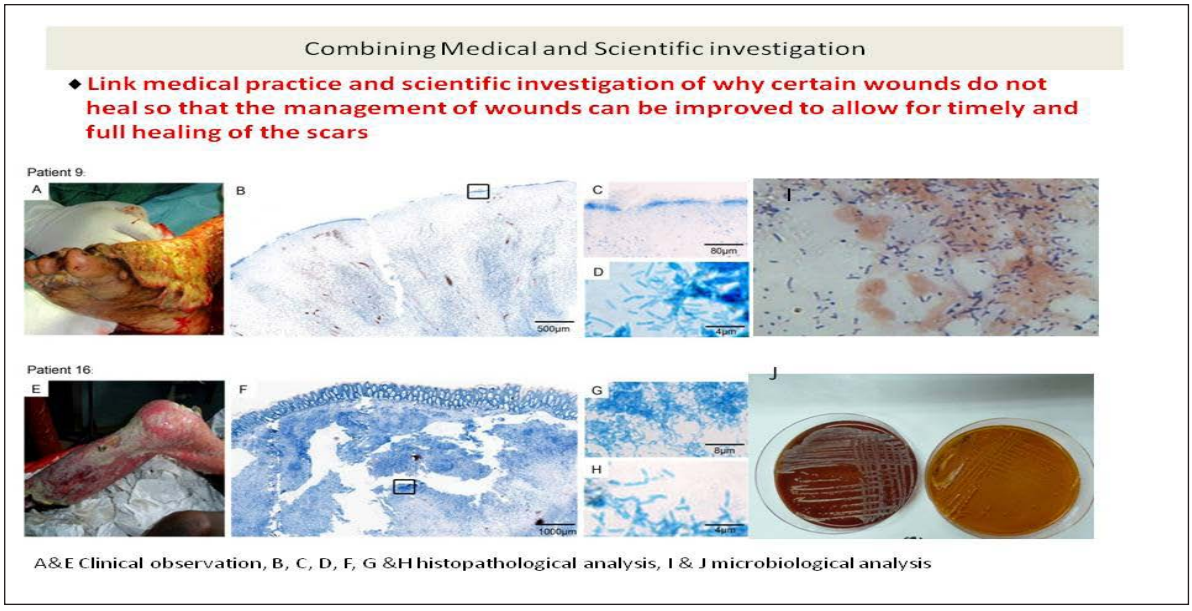

Fig 10: A&E Clinical presentation of two patients with secondary infection; B-D, F-H histological sections confirming infection and I and J are direct smear and culture results.

Development of a point of care test for the diagnosis of Buruli ulcer disease Investigators

Investigators	
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Department of Bacteriology	*Anthony Ablordey, PhD*
University of Melbourne	
Department of Microbiology	*Timothy Paul Stinear, PhD*
Program for Appropriate Technology in Health (PATH)	*Robert Burton*
Funding	*Grand Challenges Canada*

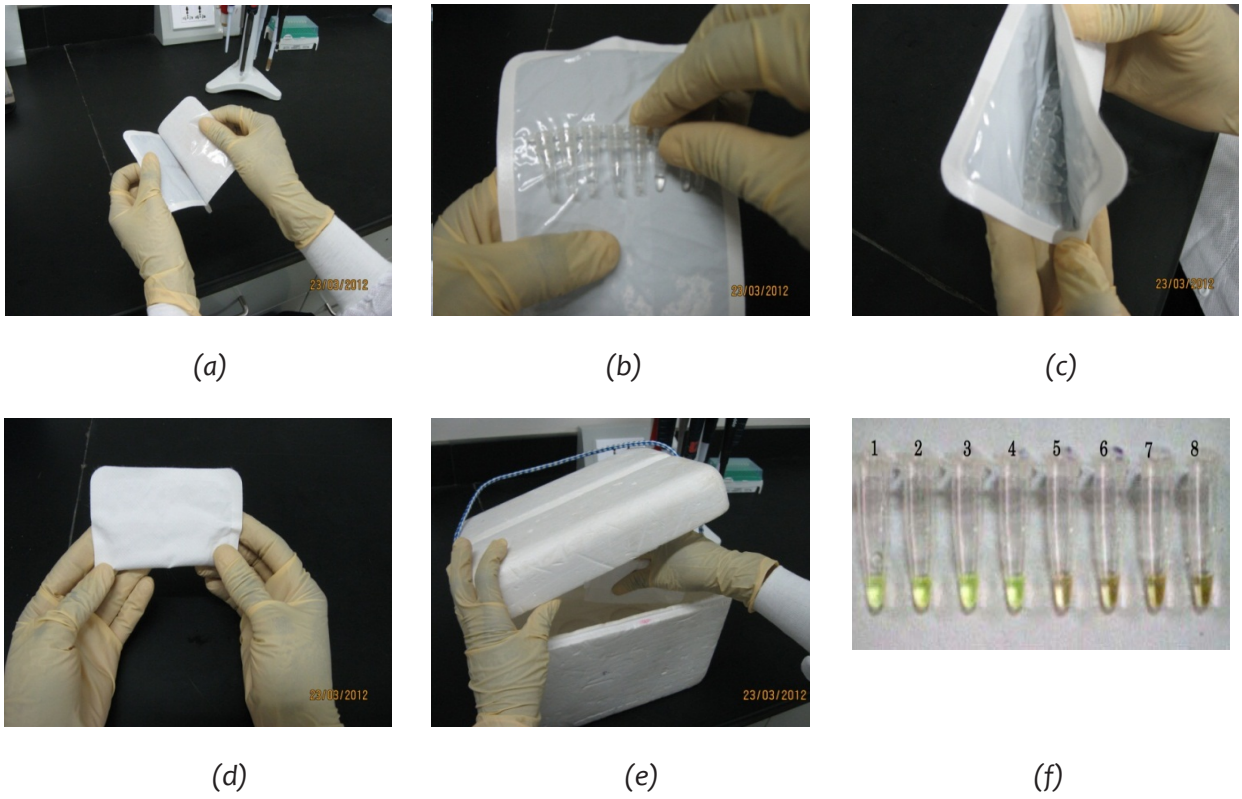
The absence of a simple and rapid test that is appropriate for early diagnosis and use in low resource settings where the disease is most prevalent remains a major challenge to Buruli ulcer control. There is therefore the need to bring a simple, yet accurate and cost effective diagnostic test to the doorsteps of communities endemic with Buruli ulcer disease. This will facilitate active case findings and significantly improve early case detection leading to better management of the disease and prevention of complications associated with late stages of the disease.

We have successfully carried out proof of principle for the detection of *M. ulcerans* by the Loop Mediated Isothermal Amplification (LAMP) test in which we identified six primer sets and optimal amplification conditions capable of detecting >1 genome of *M. ulcerans*. This level of sensitivity compares with the reference IS*2404* PCR.

In parallel with the development of the LAMP assay we have also formulated a simple and rapid DNA extraction protocol to provide a suitable DNA template for the LAMP assay.

We are studying two prototypes of the LAMP assay. In one prototype a disposable pocket warmer and a Styrofoam box are used to provide isothermal conditions for the reaction as shown in figure 1 below. This system has been found to be efficient in providing 60-62 °C for the isothermal incubation of the reaction.

We are collaborating with PATH (Programme for Appropriate Technology in Health, Seattle, USA) to evaluate the NINA (Non Instrumented Nucleic Acid Amplification) device for generating optimal incubation temperature for the IS*2404* BU LAMP assay. Preliminary results shows that the NINA device is capable of sustaining isothermal amplification of *M. ulcerans* DNA and the results compare well with the reference IS*2404* PCR achieving 98% sensitivity and 100% specificity.



(a) *(b)* *(c)*

(d) *(e)* *(f)*

Fig 11. The pocket warmer (PW) test. (a) Opening a pocket warmer; (b) Applying reaction tubes on PW; (c) Folding PW over reaction tubes; (d) Reaction covered by PW; (e) PW incubated in styrofoam box; (f) Readout – Positive test – green tubes, Negative test – yellow tubes

The current one tube closed system of amplification and fluorescence product detection precludes post amplification analysis and also has added advantages of non requirement of sophisticated reading devices and prevention of carry over contamination.

Since fluorescence readouts potentially results in ambiguities in the interpretation of test results, we are carrying out further testing to investigate the extent to which non-specific binding affects our LAMP assays. A lateral flow readout will be developed as an alternative detection system to address ambiguities of fluorescence.

Socio-cultural determinants of effective Buruli ulcer early case detection, treatment and control at the Obom sub-district of the Ga South Municipality in the Greater Accra Region of Ghana.

Investigators

NMIMR

Department of Epidemiology — *Collins S. K. Ahorlu, PhD*

Eric Koka (PhD candidate)

Department of Bacteriology — *Dorothy Yeboah-Manu, PhD*

School of Public Health, UG — *Philip B. Adongo, PhD*

National Buruli Ulcer Control Programme, GHS — *Edwin Ampadu, MD*

Funding — *UBS Optimus Foundation, Switzerland*

Background

Ghana reports an average of 1000 cases of Buruli Ulcer annually, mainly among impoverished populations mostly in rural areas. Buruli ulcer has a lot of social, psychological and economic implications for the infected and affected persons. It leads to long years of suffering, pain, loss of productivity, disrupted education, impaired mental and physical development, stigma and distress.

Aims and Objectives

The aims of this study are to: evaluate the effectiveness of social interventions for early case detection, timely treatment and adherence; understand cultural beliefs and practices associated with buruli ulcer wound care; determine the relationship between local wound care practices and microbial contamination of wound and determine patients' perceptions of wound management at health facilities and assess whether it meets their expectations in terms of socially acceptable and culturally appropriate wound handling in study communities.

Approach

The study employed both qualitative and quantitative research designs. A descriptive study was chosen to gain more information about characteristics within a particular field of study with the purpose of providing a picture of situations as they naturally occur. Data was generated through in-depth interviews, focus group discussions, surveys and patients' clinical record reviews to identify barriers to effective early case detection, treatment seeking behaviour and wound care practices in the study area.

Preliminary findings

The study demonstrated that social interventions such as the provision of breakfast and transportation to patients could help keep patients in treatment for the 56 days required to receive daily injections. It was also demonstrated that community mobilization through the use of a documentary film on Buruli ulcer coupled with information, education and communication could

enhance BU case findings and reporting at health facilities. The project also showed that it was possible to collaborate with traditional healers, former patients and community-based volunteers by training them to search, find and refer cases to health facilities. It was also demonstrated that socio-cultural factors rather than economic factors were influencing the decision to seek health for BU at the health facilities. Cultural practices and beliefs affect patients' wound care and help seeking behaviour. Patients believe that some wounds were caused by charms or spirits and therefore, required the attention of traditional healers or faith healers. In instances where patients' wounds were dressed in the hospital by a clinician who is considered culturally unfit, for example pregnant or lactating mother, patients often redressed the wounds later at home for fear of the wound not healing. Some of the materials often used for such wound dressing include urine and concoctions made of charcoal and gun powder. Some of these items were believed to have an innate power to drive away evil spirits from the wounds, a practice that may cause secondary infection of wounds.

Future directions

The PhD student is finalizing his thesis for submission to the School of Public Health, University of Ghana. Also, work is being done to determine why some patients do resist hospital admission despite the severity of their ulcers. The project is the social science aspect of the Ghana component of Optimus Foundation (United Bank of Switzerland) funded Stop Buruli Consortium project.

Elucidating the Transmission of Buruli ulcer in the mouse model by simulating six probable transmission scenarios

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	Maxwell Quartey, HD MIST-UK
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Funding	*Departmental Research*

Introduction

Buruli ulcer (BU) is an emerging skin disease caused by *Mycobacterium ulcerans*, a toxin-producing bacterium with predilection for the skin and its deeper tissues. BU is endemic in many developing countries in Africa. It is an indolent condition that manifests as papule, plaque, nodule, oedema, undermined ulcer and contracture, as well as hydrocele and osteomyelitis as a result of metastasis. The exact mode of its transmission is unclear; the pathogenesis is also not well understood and

surgery is the main treatment option because antimicrobials have not been very effective. This situation has necessitated further elucidation through animal studies. Studies on the transmission of Buruli ulcer (BU) and the effectiveness of some medicinal plant preparations as alternate medicines are reported.

Objectives

1. To compare effectiveness of BU transmission through intact and traumatized skin Possible routes/modes of BU transmission were investigated in the laboratory by simulating 6 probable scenarios for BU transmission. The scenarios were: (i) co-habitation of *Mycobacterium ulcerans* infected mice and non-infected mice; to simulate transmission in humans by direct contact; (ii) ingestion of *M. ulcerans* contaminated water and (iii) feed; to simulate transmission in humans through drink and food; (iv) contamination of intact or traumatized skin through swimming or (v) sand bathing in *M. ulcerans* contaminated water and sand respectively; to simulate children contracting the disease by playing in *M. ulcerans* contaminated rivers or sand/playground, and (vi) spraying of *M. ulcerans* contaminated water droplets directly into nose of mice, to simulate inhalation of *M. ulcerans* contaminated aerosols/air in BU endemic areas.

Method

BALB/c mice were exposed to various inoculum doses of *M. ulcerans* by the enumerated routes and monitored for 9 months. The mice were monitored 24hrs after experimental infection for confirmation of the presence of *M. ulcerans* in the gastrointestinal tract (GIT), lungs and skin, to confirm successful entry of the organism into the targeted parts of the body. Thereafter, the mice were maintained for 9 months and monitored daily for development of *M. ulcerans* associated lesions or death. Development of Buruli ulcer lesions were confirmed by necropsy, Ziehl Neelsen staining, culture, histopathology and PCR.

Preliminary Findings and Implications

Buruli ulcer lesions successfully developed in the traumatized skin of mice in the swimming and sand bathing scenario groups; all other scenarios failed to produce any BU lesion. The findings (i) confirm the predilection of *M. ulcerans* for the skin; (ii) suggest that persons with broken skin are more likely to get infected than those with intact skin and (iii) that contact of the skin with *M. ulcerans* contaminated water or sand/ playground could be likely routes of transmission, and therefore could be one of the plausible reasons for the higher incidence in children.

Future Directions

The study needs to be repeated with a larger number of mice to be statistically acceptable. Thereafter, the study should be repeated using water and sand from BU endemic areas in order to test the hypothesis, using realistic contamination levels, which would enable us to confirm or debunk the idea of possible transmission by the laboratory methods tested.

ANNUAL REPORT – NEGLECTED TROPICAL INFECTIONS NTDS

Epidemiology and molecular mechanisms of anthelminthic treatment failure in Kintampo North Municipality, Ghana

Investigators

NMIMR:

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Department of Immunology — *Ben Gyan, PhD*; *Patrick Ampomah, MSc*

Yale University Medical School — *Michael Cappello, MD, PhD*

New Haven, CT — *Debbie Humphries, PhD*

Funding: *NIAID/NIH, Project ID 1RO1AI099623-01*

Background

Initial results from mass drug administration (MDA), according to World Health Organization recommendations for the control of four neglected tropical diseases (NTDs) suggest that while MDA decreases intensity and prevalence of helminths infection in some communities, elimination of hookworm in endemic areas has not been demonstrated. Moreover, in order to make a lasting impact, chemotherapy may need to be repeated at least three times per year, the effect of which has not been carefully evaluated with regard to long term toxicity and the potential emergence of parasite resistance. Reports of documented or suspected hookworm resistance to commonly used anthelminthic agents suggest that currently available treatments may soon be ineffective, with significant implications for healthcare providers and Ministries of Health officials responsible for developing NTD control policies in endemic countries.

It was previously shown in a study in children from Kintampo North that serum IgG responses to adult *A. ceylanicum* excretory/secretory proteins correlate with infection status as determined by faecal microscopy. The study also shown that in Kintampo, *N. americanus* is the predominant hookworm species, present as a single or mixed infection in more than 90% of infected subjects. Whilst infected individuals from endemic areas exhibit strong humoral responses to various hookworm antigens, certain IgG subtypes may be more closely related to infection status.

This work was aimed at measuring serum IgG antibody against the novel adult hookworm ES protein (Aces ES), with the aim of identifying correlation with hookworm infection status (as represented by faecal egg counts). We will carry out immuno-epidemiologic screening of serum samples collected in the longitudinal study against soluble protein extracts from third stage larvae of hookworm.

The specific objectives were to;

- Identify hookworm egg positive samples from the cohort for correlation of serum anti-hookworm antibody levels
- Determine possible cases of cross-reactive IgGs.

Work done so far

IgG antibody against hookworm for population recruited at baseline has been analysed. Preliminary results did not show any correlation of on-going infections with antibody levels. Further analyses that will take into consideration age effect among others will be done.

Future work to be done

We are planning to carry out studies that would measure specific IgG subtypes as well as cross reactive IgGs.

The targeted development of a new generation Vaccine for Schistosomiasis (The SchistoVac Project)

SchistoVac is a FP7-HEALTH-2009 Collaborative project running from Feb. 2010 - July 2014, and coordinated by Leiden University Medical Centre

Investigators	
NMIMR	
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Department of Immunology	*Ben A. Gyan, PhD*
Leiden University Medical Centre	
Department of Parasitology	*Maria Yazdanbakhsh, PhD*
Funding	*European Commission*

Background

The SchistoVac project is searching for exposed proteins and/or glycans of the vulnerable skin stage schistosomula, as safe and effective vaccines. It is a multi-national consortium project involving eleven laboratories in ten countries including Ghana. Ghana is one of four study sites in Africa with the main responsibility of selecting a cohort of participants through parasitological screening whose serum samples and PBMCs will be included in the study.

Objectives

The aim of this project is to develop a new generation vaccine for schistosomiasis. The vaccine will be based on exposed proteins and/or glycans of the vulnerable skin stage schistosomula. The life stage-specific vaccine target selection strategy is based on state-of-the-art schistosomal transcriptomics and glycomics technologies and data. Unique serum and sample libraries from endemic areas will be the key to identifying protective immune responses and effective targets.

Approach

Major baseline screening and sampling at The SchistoVac Project site: The main parasitological screening and blood sampling in accordance with the requirements of the project, and following the granting of consent by individuals/volunteers, was done at the Abodom study site during the 1st and 2nd quarters of 2012. Following the parasitological screening of urine and stool samples from participants, blood draws were conducted for individuals who consented, after which free treatment for schistosomiasis (Praziquantel) and soil transmitted helminths (Albendazole) were administered to all who took part in the study. Blood samples from donors were checked for malaria parasites by blood smear microscopy and then transported to the laboratory at Noguchi for further processing- specifically for full blood count measurements and peripheral blood mononuclear cell (PBMC) isolation and storage. The PBMC and aliquots of the plasma were subsequently transported to the coordinating institution of TheSchistoVac, Leiden University Medical Centre (LUMC), by dry shipper and dry ice respectively, for further cellular and serological investigations.

Preliminary findings

Participants at pre-treatment were 221, aged 6 to 55 years (mean 17.14 ± 10.45) and consisted of 123 males and 98 females. Of these, children were 176 (79.6%) with BMI range of 12.9 to 24.8 (mean 16.8 ± 2.5) and adults, 45 (20.4%) with BMI range of 16.8 to 31.8 (mean 23.2 ± 3.4). Prevalence of parasites detected is presented in Fig 12. No Hookworm was detected. Prevelence of *S. haematobium* infection by age group is shown in Fig 13. At 5 to 7 weeks post-treatment, *S. haematobium* infection prevalence among participants was 4/114 (3.5%).

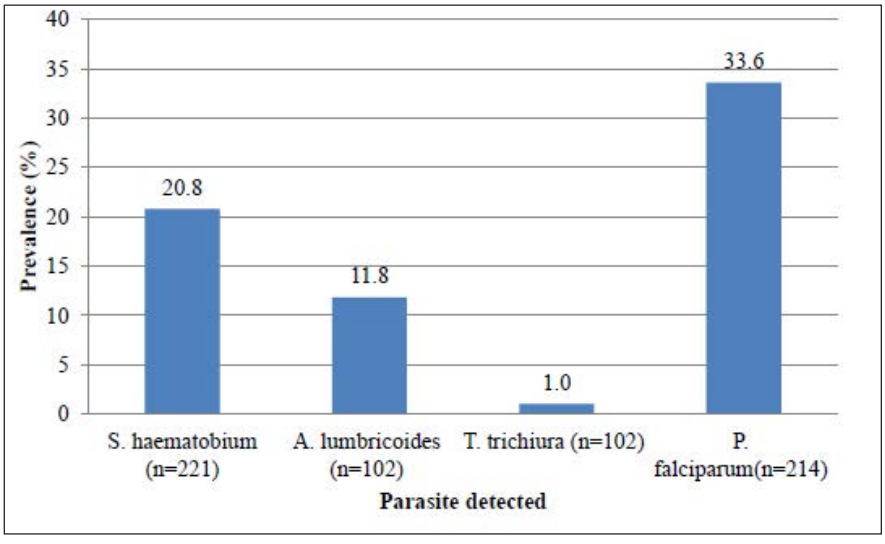

Fig. 12: Prevalence of parasites detected among study participants pre-treatment

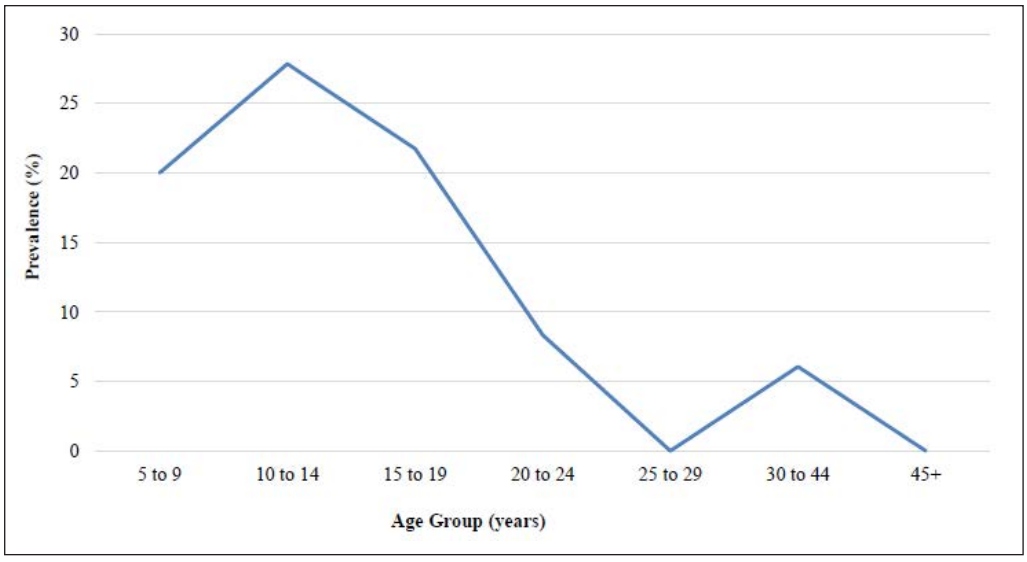

Fig. 13: Prevalence of S. haematobium infection by age group pre-treatment

Identification of possible biomarkers for estimating the intensity of schistosomiasis infection before and after praziquantel treatment

Investigators	
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	Emmanuel Adongo
	Emmanuel Awusah Blay, MPhil
Ghana Health Service	
Parasitic Diseases Division	*Lawrence Yelfari*
KNUST School of Medical Sciences	*S. C. K. Tay, PhD*
Funding	*Ghana Health Service*

Background

The large-scale administration of Praziquantel to school-aged children is the main-stay of current programmes focusing on morbidity control of schistosomiasis. Since the MDA program focuses mainly on reducing intensity of infection, egg count by microscopy using Kato-Katz technique, has been the standard method of measurement. However, this method comes with limitations which include difficulty in processing diarrhoeal stools, lack of sensitivity, poor reproducibility and counting eggs in Kato-Katz smears can also be a tedious and time consuming process, often leading to technical errors. Eosinophil cationic (ECP) level measurement by ELISA is very sensitive and may present a better alternative to egg count by microscopy.

Objectives

To compare intensity of *Schistosoma* spp infection by egg count and measurement of eosinophil cationic protein levels in infected human samples before and after treatment with Praziquantel (PZQ).

Approach

Urine, stool and blood samples were collected from study participants before Mass administration of PZQ and 8 weeks post-treatment from the same participants. Microscopic diagnosis of schistosomiasis was done on the stool and urine samples and intensity of infection expressed as egg per gram (epg) of stool or eggs per 10 ml of urine. Egg cationic protein (ECP) levels in serum samples were measured by ELISA using MESACUP ECP Test Kit (MBL Co. Ltd, Nagoya, Japan). The absorbance from each well was measured as optical densities (ODs) at 450 nm wavelength using an ELISA micro plate reader (BIOTEK). The results were interpreted in accordance with the manufacturer's instructions and designated as positive or negative for ECPs and their respective levels. ECP levels were compared with infection intensity by egg counts.

Preliminary Results

Participants pre-treatment were 217 (Male = 106; Female = 111; aged 6 to 76, mean age 19.2 ± 1.049). Post-treatment studies involved 95 (Male = 38; Female = 57; aged 8 to 37, mean age 11.95 ±4.36) participants. Table 2 shows *Schistosoma haematoium* and *S. mansoni* infection prevalence as categorized by infection intensity, pre- and post-treatment with PZQ. Table 3 shows comparison between geometric mean egg counts and ECP mean OD levels at 95% CI and significance level of 0.05.

Table 2. Prevalence of Schistosoma spp infection by microscopy as categorized by infection intensity pre- and post-treatment with Praziquantel

	Prevalence of S. haematobium (%)		*Prevalence of S. mansoni (%)*		*Prevalence of Mixed infections (%)*	
Infection intensity	*Pre-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*
Light infection (1-100 egg/10 ml or epg)	*14.1*	*2.1*	*10.1*	*2.1*	*1.4*	*0.0*
Moderate infection (101-400 egg/10 ml or epg)	*1.2*	*2.1*	*5.1*	*0.0*	*0.0*	*1.1*
Heavy infection (>400 egg/10 ml or epg)	*3.6*	*0.0*	*0.5*	*0.0*	*0.0*	*0.0*
Total	*18.9*	*4.2*	*15.7*	*2.1*	*0.0*	*1.1*

Table 3. Comparison between Egg counts and Eosinophil Cataionic Protein levels in estimating intensity of infection

Infection intensity	*S. haematobium*			*S. mansoni*		
	Egg count (egg/10mls)	*ECP[2] (ng/ml)*	*P value (α =0.05; CI=95%)*	*Egg count (epg)*	*ECP[1] (ng/ml)*	*P value (α =0.05; CI=95%)*
Light infection (1-100 egg/10 ml or epg)	*15.67*	*77.62*	*0.081*	*27.77*	*78.19*	*0.024*
Moderate infection (101-400 egg/10 ml or epg)	*70.4*	*77.27*	*0.017*	*163.2*	*104.32*	*0.014*
Heavy infection (>400 egg/10 ml or epg)	*502.86*	*90.06*	*0.039*	*480*	*94.00*	*0.034*

[1] ***Mean Optical Densities***

The level of ECP in sera of schistosomiasis patients by microscopy was significantly higher than diagnostic levels (according to manufacturer's instructions). ECP OD levels, however, did not show a consistent increase with increase in intensity by egg count.

Toxoplasmosis Research

Toxoplasmosis, the disease of which *Toxoplasma gondii* is the causative agent, is usually asymptomatic and self-limiting but can have serious or even fatal effects on a ***foetus*** whose mother first contracts the disease during pregnancy or on an ***immune-compromised*** human such as HIV infected and AIDS affected people. Infection with *T. gondii* is either congenitally transmitted or acquired through ingestion of environmentally sporulatedoocysts from cat faeces, contaminated unwashed vegetables and fruits, tachyzoites in unpasteurized goat milk, and bradyzoites encysted in muscles of domestic animals such as pigs, sheep and goats. Other means of tachyzoites transmission are through blood transmission and organ transplant, although they are rare. Cockroaches and filth flies may also carry oocysts from soil to food and water that can be ingested (Wallace 1971). Consequences of congenital transmission occurring early in pregnancy are severe and involve the central nervous system causing hydrocephalus or intrauterine death. Congenital transmission late in pregnancy has consequences involving the eye such as chorioretinitis and manifests in the second decade of child's life.

In Ghana, toxoplasmosis is less studied despite delivery of hydrocephalic babies in our hospitals. Preliminary studies conducted by researchers at NMIMR have established moderate to high serum anti-*T. gondii* antibody positivity of 30.5% to 39.4% in animals across the country (van der Puije*et al.*, 2000; Arko-Mensah *et al.*, 2000) but infection to humans through domestic animals has not been established. Sporadic studies on humans has involved screening of different groups of individuals including eye patients (85.2%), pregnant women (51.2 to 73.6%), HIV/AIDS affected people (84.0%), blood donors (78.9%) and children 0-5 years old (58.5%) (Ayi *et al.*, 2005, 2009; Akwovia, 2008; Sowah, 2010) establishing that the Ghanaian populace is exposed to *T. gondii* infection. Further research is therefore being conducted on the epidemiology of toxoplasmosis in Ghana to establish a database that can serve as evidence for advocacy to influence policy

formulation in the management of the infection, especially in women of childbearing age and among immune-competent (potential blood donors) in the healthcare system in Ghana. The following studies are currently ongoing at the Institute

Studies on the Mother-To-Child Transmission of *Toxoplasma gondii*Infection among Pregnant Women in the Greater Accra Region

Investigators	
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	Kofi Dadzie Kwofie, BSc
UGMS	
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Tokyo Medical and Dental University	
Environmental Parasitology, Faculty of Medicine	*Nobuo Ohta, PhD*
Funding	*J-GRID /MEXT Japan*

Background

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* which can be acquired by ingestion of infective stages of the parasite or congenitally from mother to child. Infection can be acute with tachyzoites in circulation or chronic with formation of cysts in muscle and organ tissues. Acute infection which may be either primary or as a result of re-activation of chronic infection can lead to congenital transmission of the disease. Congenital infection of infants is known to result in several neurological and brain disorders including ophthalmic disorders later in life. Recent research in Ghana revealed high sero-prevalence among pregnant women and eye patients with eye lesions. Toxoplasmic eye lesions in adults have been known to be a consequence of congenital infection.

Objective

To investigate the risk of foetal infection of *T. gondii* through mothers at a hospital facility in Ghana.

Method

Consented third trimester pregnant women from parts of Greater Accra Region were recruited from ante-natal clinic at the Korle-Bu Teaching Hospital. At delivery, placental tissue and maternal blood samples were taken after expulsion of each placenta. Nested-PCR (nPCR) was run on genomic DNA extracted from placental tissues to detect *T. gondii* using SAG3 and GRA6 primers. ELISA was used to detect anti-*T. gondii*IgG and IgM in matched maternal blood samples. Data were analyzed using SPSS version 16.

Results

Eighty-eight women aged 18 to 45 years participated, from whom 88 placental tissues and 87 blood samples were obtained. Overall, 39.8% (35/88) of placental tissues was positive for *T. gondii* DNA and 40.2% (35/87) blood samples were positive for anti-*T. gondii* IgG. Sero-prevalence among age groups is presented in Fig 14. All the blood samples were negative for anti-*T. gondii* IgM. Out of the anti-*T. gondii* IgG positive blood samples, 88.6% (31/35) had corresponding placental tissues testing positive for *T. gondii*DNA by nPCR for SAG3 and/or GRA6 markers. Thirty-one (31) placental tissues and corresponding maternal blood samples tested positive for both nPCR and ELISA (Fig. 15).

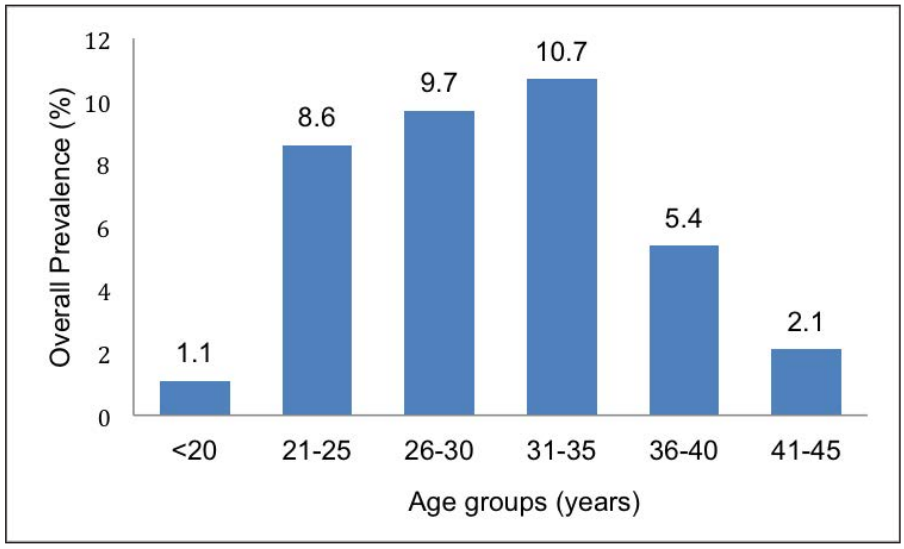

Fig 14: Prevalence of serum anti-T. gondii IgG among mothers as categorized by age groups

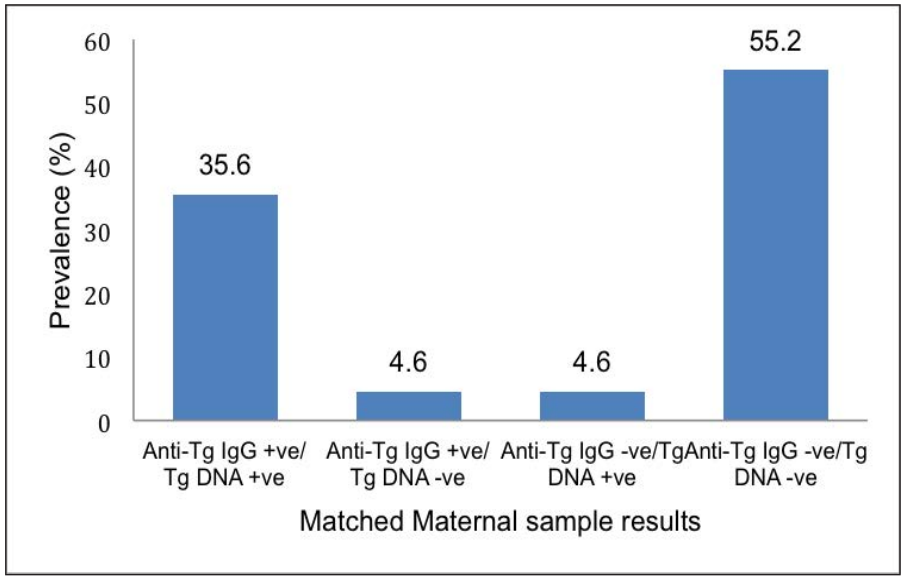

Fig 15: Prevalence of T. gondii infection infection by serum anti-parasite IgG using ELISA and parasite DNA from placental tissues by nPCR in matched maternal samples

Conclusion

Toxoplasma gondii DNA detected in placenta which is formed during pregnancy may be largely from cysts and is indicative of infection of the mother during gestation. Detection of *T. gondii* in placental tissues indicates exposure of the foetus to the risk of infection which implies that almost 40% of the infants were at risk of congenital infection. Further studies need to be done to determine the rate of mother-to-child transmission of *T. gondii* in Ghana.

Prevalence of *Toxoplasma gondii* in HIV Patients and Blood Donors

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Department of Infectious Diseases *Korle-Bu Teaching Hospital* *Tokyo Medical and Dental University*	*Margaret Lartey, MB ChB; MPH, FGCP*
Environmental Parasitology, Faculty of Medicine	*Nobuo Ohta, PhD* *Takashi Suzuki, PhD*
Funding	*J-GRID /MEXT Japan*

Background

Toxoplasma gondii is categorized into 3 clonal lineages denoted as types I, II, and III. Primary infections in humans are usually asymptomatic or they are characterized by non-specific symptoms and are followed by latent chronic infections, which are normally harmless to the host. The most common clonal lineages found in immune-compromised and immune-competent individuals are assessed.

Objectives

In Ghana the extent of *Toxoplasma* infection in humans is, relatively, unknown. This study aimed at detecting and genotyping *T. gondii* clones in healthy blood donors and HIV/AIDS clients in Accra.

Approach

This cross sectional study was conducted among attendants at the Korle-Bu Teaching Hospital from May to December 2011. It involved 148 HIV/AIDS patients and 149 healthy blood donors. Informed consent was obtained from pre anti-retroviral therapy HIV-positive individuals with 0≥CD4+T-cell count/mm3 blood≤200, and healthy blood donors. Genomic DNA was extracted from

whole blood samples of participants. Nested PCR and restriction fragment length polymorphism analysis were employed to detect and genotype *T. gondii* using SAG3 and GRA6 markers.

Preliminary Results

Overall, 54.7% (81/148) HIV-positive samples were positive for SAG3 and/or GRA6 *T. gondii* markers. Prevalence of *T. gondii*i nfection among HIV-seropositive individuals distributed by their CD4+ T-cell counts is presented in Fig 16. Overall, 93.8% (76/81) positives were of clonal type II, 1.2% (1) type I, 1.2% (1) both type I and II whiles the genotype of 3.7% (3/81) could not be determined. For blood donors, 3.4% (5/149) were positive for the markers and 2 were type I and 3, type II. No type III was detected. There was a high prevalence of *T. gondii* clonal type II in both HIV-positive and healthy individuals.

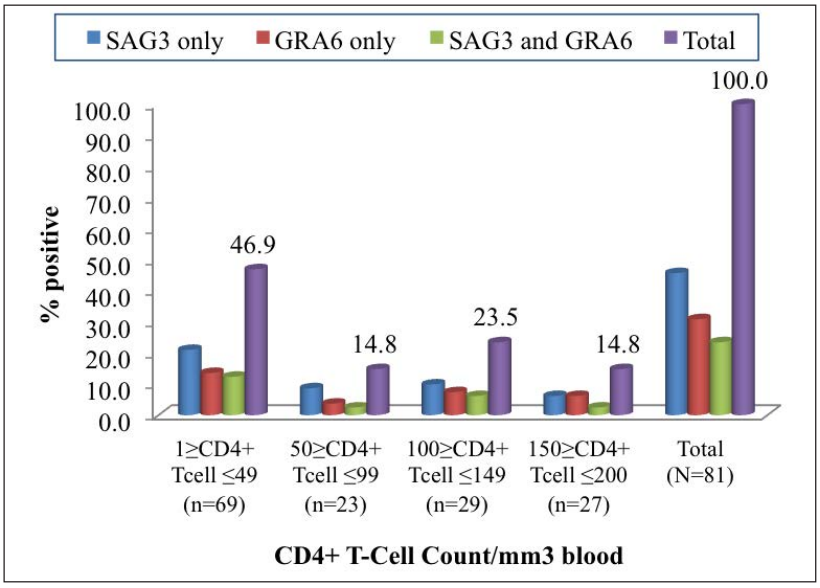

Fig 16: Prevalence of T. gondii infections in HIV seropositive individuals by nPCR using SAG3 and GRA6 gene markers as categorized by CD4+T-cell count/mm3 blood

Implications: This indicates more animal source infections among the participants. This information may be helpful in the effective management of *T. gondii* infections in HIV/AIDS clients and the general prevention and control of toxoplasmosis in Ghana

Toxoplasmosis and malaria co-infection in pregnant women and the impact on birth outcomes

Investigators

NMIMR

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UGMS
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& Conservation Science — *Langbong Bimi, PhD*
Funding — *JGRID /MEXT, Japan*

Background

Malaria and congenital toxoplasmosis have all been individually reported to cause severe negative outcomes in pregnancies. Recent studies in Ghana show high prevalence of Malaria and Toxoplasmosis in pregnant women (Ofori *et al.*, 2009; Ayi *et al.*, 2009). There is, however, no reported information on the outcome of combined effect of malaria and toxoplasmosis co-infection and related health conditions such as pregnancy induced hypertension in expectant mothers and their infants.

Objectives

- To investigate the prevalence of *Plasmodium falciparum* and/or *Toxoplasma gondii* co-infection on birth outcomes in women
- To investigate the impact of *Plasmodium* species and *Toxoplasma gondii* co-infection on pregnancy outcomes in women in Accra.

Approach

Maternal and cord blood samples were collected into labelled tubes after expulsion of the placenta and placental tissue samples into tubes containing physiological saline. Samples were transported in a cool box to the laboratory for processing. DNA was extracted from portions of the placenta and whole blood using QIAGEN kit and the remaining span for plasma. DNA was amplified by Nested PCR and products ran on agarose gel to detect *Plasmodium falciparum* and *T. gondii* in the maternal, cord blood and placental samples using the appropriate primers. Anti *T. gondii* IgG antibodies were detected from plasma using commercial ELISA kits. Demographic data and medical history of participants were obtained from hospital folders. Differences in obstetric characteristics by co-infection status were assessed by chi square (CI=95%, $p<0.05$) to determine the effect of *Plasmodium falciparum* and/or *Toxoplasma gondii* co-infection on pregnancy outcomes.

Results

The sero-prevalence of anti-*T. gondii* IgG for the mothers was 75.9% (60/79) and the IgG sero-prevalence of their infants was 48.8% (40/82). 51.4% (36/70) of the matched mother and infant pairs tested sero-positive for anti-*T. gondii* IgG (Table 4). Prevalence of single and co-infection of *Toxoplasma gondii* and *P. falciparum* in mothers as detected by PCR on placental and/or peripheral blood DNA is presented in Table 5. The overall prevalence of *T. gondii* and *P. falciparum* detected in placental tissues only by PCR is shown in Table 5.

Analysis between single and co-infections in women revealed no significant associations with the adverse outcomes of low birth weight (LBW), anaemia, still birth and hypertension (Table 6). Women infected with *P. falciparum* only seemed to show higher frequencies of all adverse factors, with *T. gondii* only infected women accounting for the least frequencies although the differences were not statistically significant.

Table 4: Prevalence of anti-Toxoplasma gondii IgG detection in women and their matched babies

		Infant	
	Anti-*T. gondii* IgG results (%)	Positive	Negative
Mother	Positive	36(51.40)	25(35.71)
	Negative	0	9(12.86)
	Total	36(51.40)	34(48.57)

Table 5: Prevalence of Toxoplasma gondii and P. falciparum single and co-infection in women detected by PCR on placental and/or peripheral blood DNA

		Toxoplasma gondii			
	Test result (%)	Positive	Negative	X^2	*P* value
Plasmodium falciparum	Positive	14(17.72)	25(31.64)		
	Negative	31(39.24)	9(11.39)	13.9	<0.01
	Total	45(56.96)	34(43.03)		

Table 6: Relationship between different birth defects and P falciparum and/or T. gondii infection

Parameter	All (%)	*P. falciparum* only	*T. gondii* only	*P. falciparum* and *T. gondii* co-infection	χ^2	P value
LBW	15(18.29)	9(11.39)	1(1.26)	3(3.79)	3.51	0.74
Anaemia	29(36.7)	11(13.92)	2(2.53)	7(8.86)		
Still Birth	10 (12.1)	6(7.59)	-	1(1.26)		
Hypertension	32(40.5)	14(17.72)	4(5.06)	7(8.86)		

The result is *not* significant at $p < 0.05$

Other Studies

Prevalence and molecular epidemiology of trypanosomes found in different ecological zones in Ghana

Investigators

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Centre for Plant Medicine Research, Mampong — *Daniel Boamah, PhD*

Tokyo Medical and Dental University

Environmental Parasitology, Faculty of Medicine — *Nobuo Ohta, PhD*

Funding — *J-GRID /MEXT Japan*

Relationship between Eosinophil Cationic Protein and Infection Intensity by egg count in a schistosomiasis-endemic community in Ghana

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Leiden University Medical Centre	*Maria Yazdanbakhsh, PhD*
Funding	*European Commission*

Impact assessment surveys to define the factors determining the successful implementation of MDA to eliminate LF in Sierra Leone.

Investigators	
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National NTD Program,	*Santigie Sesay*
Mercy Hospital, Bo, Sierra Leone	*Rashid Ansumana*
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School of Community Health Sciences	*Abu Conteh*
Funding	*Centre for Neglected Tropical Diseases (CNTD), Liverpool School of Tropical Medicine, Liverpool, UK*

Consortium on Asthma among African-ancestry Populations in the Americas' (CAAPA) study

Investigators	
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	Abena S. Amoah, MSc
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Funding	*European Commission*

Eco-Health approach to the control of onchocerciasis in the Volta Basin of Ghana Investigators

Investigator	
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Funding:	*DFID/International Development and Research Center, Canada*

MEDICINAL PLANT RESEARCH

SATREPS* Project: Studies of anti-Viral and anti-Parasitic Compounds from Selected Ghanaian Medicinal Plants

Co-Principal Investigators	
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Nagasaki International University	*Osamu Morinaga, PhD*
Funding	*JICA /JST*

Background

Medicinal plants have been used as "Natural Pharmacy" for decades and phytomedicine is increasingly being identified as a common form of alternative medicine (Ogbonnia *et al*., 2011), especially in resource poor settings such as Ghana. It has been estimated that about 70- 80% of the world's population rely on non-conventional medicines, mainly of herbal origin (Chan, 2003). In Ghana, there is increasing optimism for the use of medicinal plants in all forms, including being developed as precursors for synthetic drugs. Two diseases selected for study under this project were HIV and trypanosomiasis.

The fight against HIV/AIDS is a global challenge requiring urgent action. Suppressing the progression of HIV to prevent the onset of AIDS is one approach that is becoming established, but it would be desirable to develop drugs that are effective against persistent, latent HIV infections. In addition, finding novel therapies for some neglected tropical infections such as trypanosomiasis is also a priority because the disease affects animals as well as humans and in Africa, as many as 50 million people and 25 million domestic animals are infected. The available treatments have either serious adverse effects or are relatively not very efficacious and are also difficult to use.

In 2010 the Institute in collaboration with the Mampong Centre for Plant Medicine Research (CRPM), The Tokyo Medical and Dental University (Tokyo) and the Nagasaki International University (NIU) with sponsorship from JICA and Japan Science and Technology Initiative (JST) began a project to study the anti-viral and anti-parasitological (trypanosocidal) effects of selected Ghanaian medicinal plants. The basic design of the project was as follows; CRPM in Mampong undertook the identification of potential medicinal plants from their collection, performed the initial fractionation and then passed the fractions to NMIMR for primary bioassays in the Departments of Virology (HIV) and Parasitology (Trypanosomiasis) and Chemical Pathology (toxicological studies). See Fig 17.

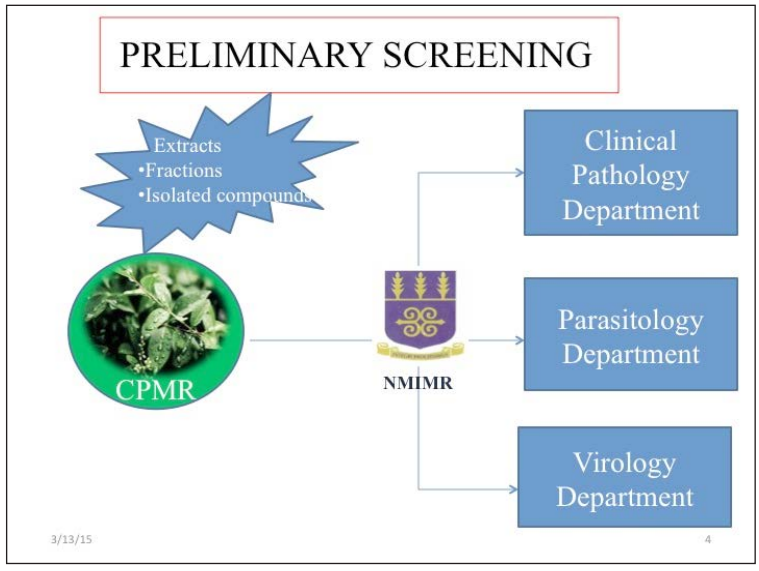

Fig 17: Scheme of Work in the SATREPS project

The project had the following objectives;

a. To screen and identify active compounds in selected Ghanaian medicinal plants against *T. b. Brucei* and characterise their mechanisms of action
b. To screen herbal extracts from selected Ghanaian medicinal plants for their ability to induce expression of the provirus in latently HIV-1-infected cells.
c. To screen all the crude plant extracts fractions and compounds derived for cytotoxicity and to determine non-cytotoxic concentrations that will be useful for bioassays.

Overall, 113 extracts were obtained from 89 plants and were all tested and subjected to toxicity studies to determine non-cytotoxic concentrations useful for conducting the bio-assays.

a) Screening and identification of active compounds in selected Ghanaian Medicinal plants against *T. b. brucei*

Investigators

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Nobuo Ohta, PhD

A 3-step high throughput bio-assay system was established for the detection of anti-trypanosomal activity in crude plant extracts, fractions and compounds. Crude extracts/compounds from selected Ghanaian medicinal plants were screened for trypanocidal activity against the GUTat 3.1 strain of *T. b. brucei* using the 3-step in vitro system. Effects of the extracts on parasites viability and proliferation were determined using Alamar blue® assay (Invitrogen). Subsequently parasites were subjected to FACS analysis to investigate extracts' ability to induce apoptosis and/or cell cycle alteration. Then, immunohistochemistry was performed to detect any extract-induced morphological and marker expression changes in parasites. Crude extracts with activity in the first round 3-step screening were fractionated and re-screened. Further purification of active fractions into compounds was then done. The purified compounds were also taken through this 3-step screening for identification of compounds with anti-trypanosomal activity.

Results

Eight of the crude extracts showed strong trypanocidal activities (IC_{50} 0 – 10 µg/ml) and induced apoptosis. In addition, some extracts showed G2/M phase alteration during cell cycle in trypanosomes. Upon further analysis, a total of five including two novel active compounds were identified (ML-2-1, IC_{50} 518 µM; ML-2-2, IC_{50} 1.27µM: novel; ML-2-3, IC_{50} 3.75µM: novel; ML-2-4, IC_{50} 15.37µM; ML-2-5, IC_{50} 13.68 µM). ML-2-2 and ML-2-3 caused flagellum deficiency, as well as, nuclear disintegration and fragmentation, respectively, in the parasites (Figs 18 & 19). Toxicity assays using mammalian cells showed commendable selective index (SI) values for ML-2-3 to merit further *in vivo* studies for efficacy in mice.

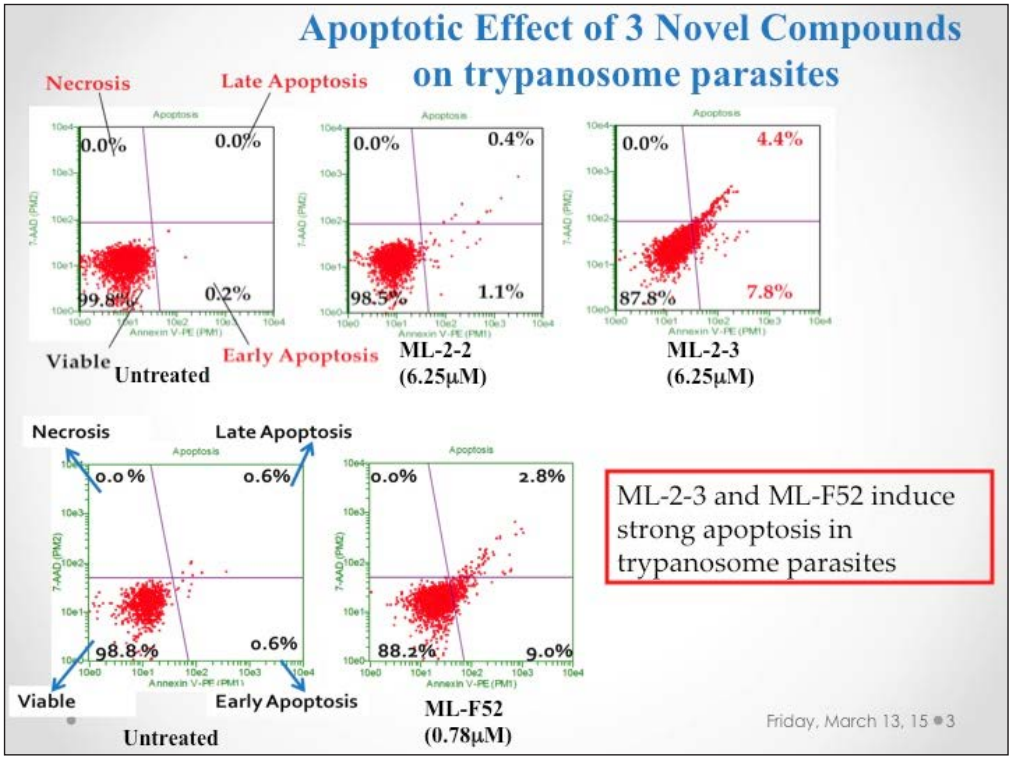

Fig. 18: ***FACS analysis of Nexin Assay for ML-2-2 and ML-2-3 showing marked ML-2-3-induced apoptosis in trypanosomes. N.C. is for compound-naive trypanosomes***

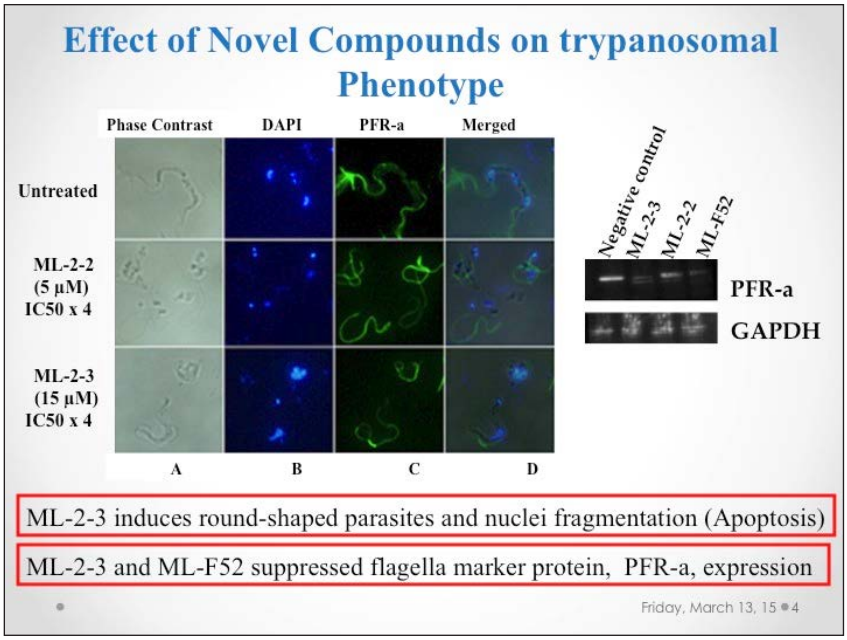

Fig. 19: Immunohistochemistry analysis showing ML-2-3-induced nuclear round-shaped parasites with fragmented nuclei and no flagella

In vivo experiments are continuing at the Section of Environmental Parasitology of the Tokyo Medical and Dental University, Japan. An application has been filed for a US patent for the two novel compounds, ML-2-2 and ML-2-3.

Studies of anti-HIV compounds from selected Ghanaian medicinal plants

Investigators:	
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	Jerry Ndzinu, M Phil
	Angela Frimpong, BSc
	John Odoom, PhD
	J. Kofi Bonney, PhD
	William K. Ampofo, PhD
Tokyo Medical & Dental University	*Takanori Hori, PhD*
	Shoji Yamaoka, PhD
Funding:	*JICA / Japan Science and Technology Agency (JICA /JST)*

Background

HIV/AIDS is difficult to manage because of the capacity of the virus to replicate rapidly and develop resistance to antiretrovirals due to its high mutation rate. Nevertheless, the use of plant medicine has a long tradition among Ghanaians and speculations of the effectiveness of some plants extracts to cure HIV/AIDS abounds.

Objectives

This study examined some plants used as herbal treatments to identify compounds with activity against HIV.

Approach

A dual luciferase reporting assay system was adopted to screen plant extracts to determine ability to suppress replication of HIV-1. In addition we induced expression of provirus in latently HIV-1-infected cells for targeting and degradation by antiretroviral drugs.

Findings & Implications

One hundred and thirteen plant extracts have been screened and two plant extracts showed anti-HIV activity, while 7 other plant extracts had HIV pro-virus stimulatory activity. Candidate extracts have been subjected to biochemical purification to identify fractions with anti-HIV activity. Among these 7 plant extracts, it is worth noting that both crude and partial fractions from *Theobroma Cacao*, persistently exhibited high level dose-dependent HIV pro-virus stimulatory activity.

Efficacy has been further analyzed by the conduct of various biochemical assays. Safe levels of the crude extracts were established on susceptible cell lines with measurements of level of infectivity and/or expression of HIV pro-virus in specially engineered cells.

Future Directions

These studies are expected to lead to the establishment of new therapeutic interventions for the management of HIV-1 infections.

b) Screening herbal extracts for their ability to induce expression of the provirus in latently HIV-1-infected cells.

Investigators

NMIMR

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Tokyo Medical and Dental University	*Takanori Hori, PhD*
	Shoji Yamaoka, PhD

Background

HIV/AIDS is difficult to manage because of the capacity of HIV to rapidly replicate and the quick development of resistance to antiretroviral therapy. The fight against HIV and AIDS is a global challenge requiring urgent action. Suppressing the progression of HIV to prevent the onset of AIDS is one approach that is becoming established, but it would also be desirable to develop drugs that are effective against persistent, latent HIV infections. The Virology department, under the SATREPS project therefore undertook experiments to identify Ghanaian medicinal plants with activity against HIV, especially those that are effective against persistent latent infections as well as those that can suppress replication of HIV-1.

Approach

A dual luciferase reporting assay system was adopted to determine the ability of the extracts to suppress replication of HIV-1. Another approach used was to induce expression of provirus in latently HIV-1-infected cells for targeting and degradation by antiretroviral drugs.

Findings & Implications

One hundred and thirteen plant extracts were screened and two extracts showed anti-HIV activity, while seven other plant extracts had HIV pro-virus stimulatory activity. Both the crude and partial fractions from *Theobroma cacao*, consistently exhibited high-level dose-dependent HIV pro-virus stimulatory activity.

Results of the 1st-line Screening

- Ten out of 113 extracts induced the expression of proviral reporter gene in JLR-2 cells
- Plant extracts were subjected to fractionation and further purification

NO	SAMPLE ID	Trial 1	Trial 2	Trial 3
1	**CVP001A**	++	++	+++
2	**CVP003B**	++	+++	+++
3	**CVP012A**	+++	+++	+++
4	CVP025A	+++	+++	+++
5	CVP026A	+++	+++	+++
6	**CVP028A**	+++	+++	+++
7	CVP020C	+++	+	+
8	CVP049A	+++	++	+++
9	CVP058A	+++	+	+
10	**CVP057B**	+++	++	+++

+ : 0 < x <= 50 +++: 100 < x
++: 50 < x <=100 (x= fold induction of control)

Fig. 20: Results of the 1st-Line Screening

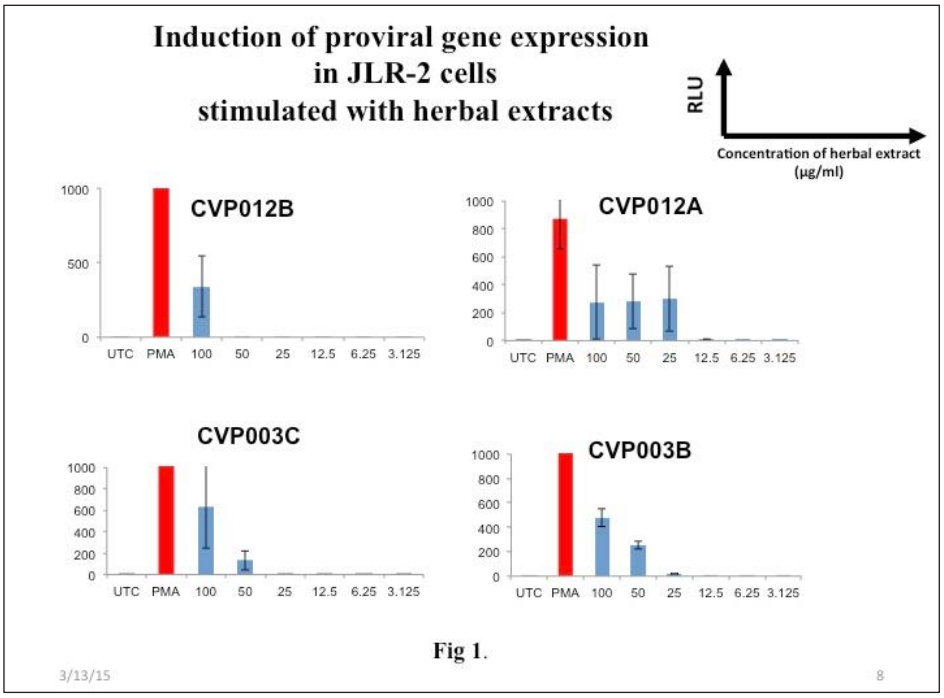

Fig. 21: Inducton of proviral gene expression in JLR-2 cells stimulated with herbal extracts

c) Toxicity studies

Investigators:

NMIMR

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	NiiAyiAnkrah, PhD
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Nagasaki International University	*TakuhiroUto, PhD*
	Yukihiro Shoyama, PhD
Centre for Plant Medicine Research	*Dominic Edoh, PhD*
Funding	*JICA /JST*

The aim of this study is to identify anti-HIV and anti-trypanosomal Ghanaian medicinal plants. All the crude plant extracts, fractions and compounds were screened to determine non-cytotoxic concentrations that will be useful for the bioassays. A total number of one hundred and thirteen (113) plant extracts were screened for cytotoxicity to Jurkat (*human* T-lymphoblast-like leukemia) and CEM (human T cell leukemic) cell lines, using MTT assay. Experiments were performed for 48h and 24h to facilitate use of the data for parasitology and virology assays, respectively. The results showed that 22% and 30% of the extracts tested at 24h and 48h, respectively were highly cytotoxic to the Jurkat cells, with IC_{50} values <100 µg/ml. A similar trend was observed in experiments with the CEM cells (see Figs 22 and 23). Bioassay-guided fractionation was performed using extracts with significant anti-parasitic and anti-HIV activities and these fractions were also

tested for cytotoxicity. Fractions showing strong cytotoxicities were identified and re-tested at lower concentrations. Four pure compounds isolated from the most bioactive fractions were also tested for cytotoxicity. All four compounds tested had IC_{50} values <100µg/ml, and two of them had IC_{50} values <10µg/ml (Fig 24). In summary, cytotoxicity profile of all crude extracts in two cancer cell lines have been obtained for the determination of non-cytotoxic concentration for bioassays.

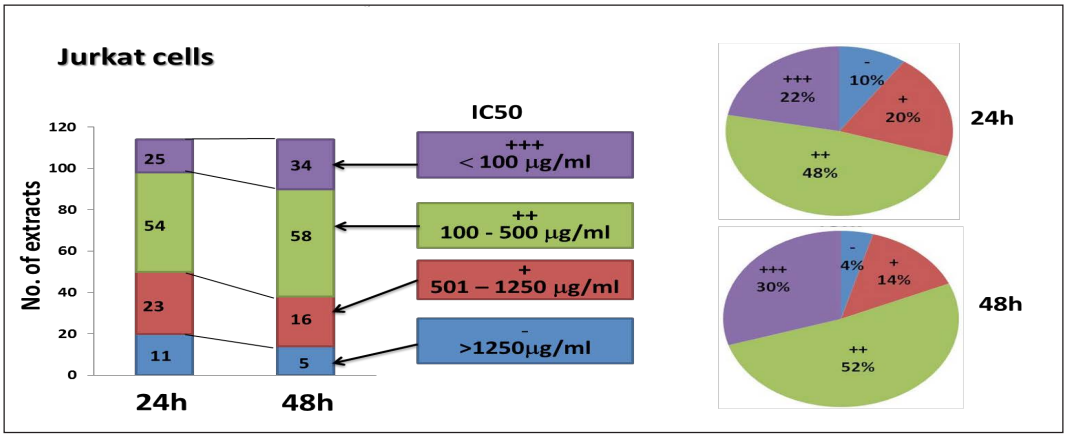

Fig. 22: Toxicity profile of extracts from Ghanaian medicinal plants in Jurkat cells

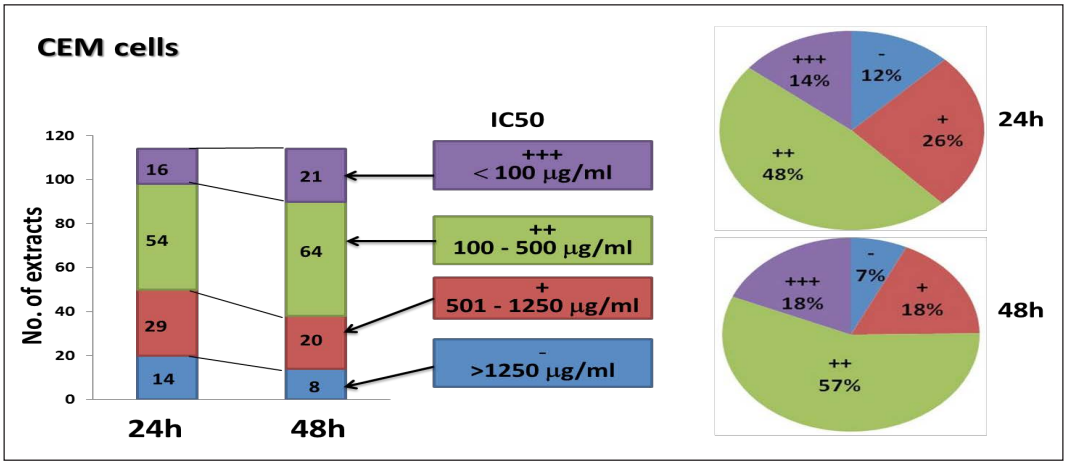

Fig. 23: Toxicity profile of extracts from Ghanaian medicinal plants in CEM cells

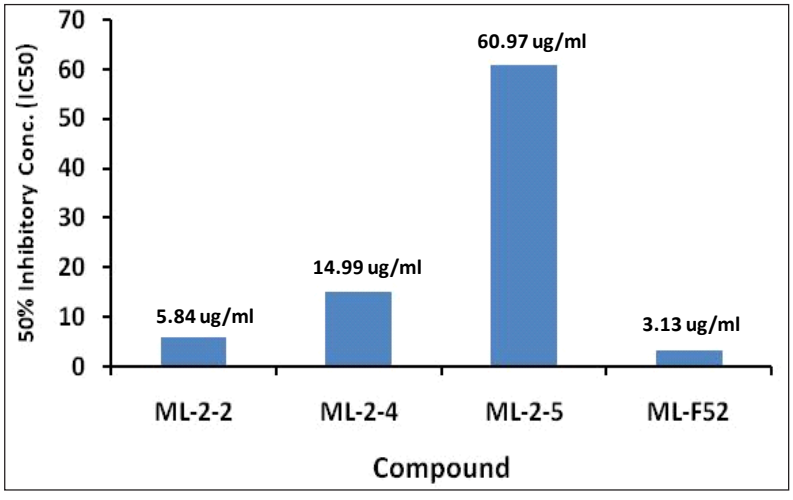

Fig. 24: Toxicity profiles of compounds from Ghanaian medicinal plants in Jurkat cells

Developing an Alternate Anti-Epileptic Product from Medicinal Plants

Investigators:

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Department of Pharmacology & Toxicology — *Patrick Amoateng, PhD*

Funding — *UG Research Fund*

Background

Epilepsy is one of the most common and serious brain disorders in the world and the World Health Organization (WHO) estimates that it affects more than 50 million people worldwide with 80% of these living in developing countries. This study seeks to develop a polyherbal product with potent anti-epileptic effect and minimal side-effects.

Objectives

1. To compare the anti-epileptic effects of a polyherbal extract consisting of three plant materials with extracts from individual plants constituting the polyherbal product
2. To determine the anti-epileptic activityof the polyherbal extract in comparison to phenobarbital
3. To determine the effect of combination of the polyherbal extract with phenobarbital for synergism or drug-herbs interaction
4. To determine the acute, sub-acute and chronic toxicity of the polyherbal extract
5. To formulate a polyherbal product using the polyherbal extract as the active ingredient through the use of appropriate pharmaceutical technology

Approach

Plant collection and extraction. Samples of the three plants selected for the project, *Synedrella nodiflora* whole plant, *Leea guinensis* leaves and *Ficusexaxperata* leaves were collected from the botanical gardens, UG, Legon, botanical gardens, KNUST, Kumasi and close to Kwabenya, Accra respectively. The samples were air-dried, pulverized and cold macerated with 70% ethanolic extract in water. The filtrate was subjected to concentration under reduced pressure and the syrupy mass obtained dried on a water bath. The extracts obtained were designated as SNE, LGE and FEE for *Synedrella nodiflora*, *Leea guinensis* and *Ficus exasperata,* respectively.

Anti-epileptic testings. Two conventionally accepted screenings for anti-epileptic drugs were employed namely: Pentylenetetrazole (PTZ) and maximal electroshock (MES) induced seizures. These methods characterise both chemically and electrically induced seizures which typify convulsions in humans.

PTZ-induced seizures test :The PTZ-induced seizures test was done as described by Vellucci et al, 1984. Briefly, clonic seizures were induced in drug/vehicle pre-treated male ICR mice (20-30g) by an intrapertoneal injection of 75 mg/kg^{-1} pentylenetetrazole (PTZ) into the mice. For each group,

a minimum of five animals were used. The animals were pre-treated with all the extracts (100, 300 or 1000 mg/kg^{-1}, p.o), or phenobarbitone (PHB) sodium (10, 30,100 mg kg^{-1} i.p) 1 hr or thirty minutes prior to the injection of PTZ. The control animals received 0.9 % saline solution orally (0.01 ml kg^{-1}).

The ED_{50} (a measure of antiepileptic potency) of the extracts and the reference antiepileptic were calculated from the dose-response curves of the percent seizure inhibition by the drug/extracts to the vehicle-treated group.

MES-induced seizures: The MES-induced seizures as described by Schmutz *et al*., (1990) was used. Male ICR mice were treated with SNE (100-1000 mg kg^{-1}, p.o), carbamazepine (CBZ) (3, 10 30 mg kg^{-1}, *p.o.*) or 0.9 % saline solution orally (0.01 ml kg^{-1}). For each group, a minimum of five animals was used. One hour after the treatments, tonic convulsions of the hind extremities of mice were induced by passing alternating current (50 Hz, 60 mA, 0.2 s) from the maximal electroshock apparatus via ear electrodes. The current used was predetermined before experimentation and was the maximal current that caused hind limb extension in all mice in the trials. Mice were restrained by gripping the loose skin on their back. The total duration of tonic hind limb extension were determined in each group

Preliminary findings and Implications

The extracts and PHB significantly ($p< 0.05$-0.01) decreased the frequency and duration of PTZ-induced seizures (Figs 25 and 26). The order of increasing potency as deduced from the ED50 values is as follows: LGE<SNE<FEE<PHB for ED_{50} values of 928, 261.5, 107.6 and 11.71 mg/kg respectively. In the MES-induced seizures test, the extract SNE significantly decreased the duration of hind limb extension, as did CBZ in comparison to the vehicle-treated control group (Fig. 27).

Preliminary anticonvulsant activity of the hydro-ethanolic extracts of Synedrella nodiflora, Leea guineensis and Ficus exasperata in pentylenetetrazole-induced and maximal electroshock- induced seizure tests in mice were done to ascertain the repeatability of activity and confirm early reports of the anticonvulsant activity of the extracts. Our findings for the first time revealed anticonvulsant activity of the extract of Synedrella nodiflora in maximal electroshock convulsions.

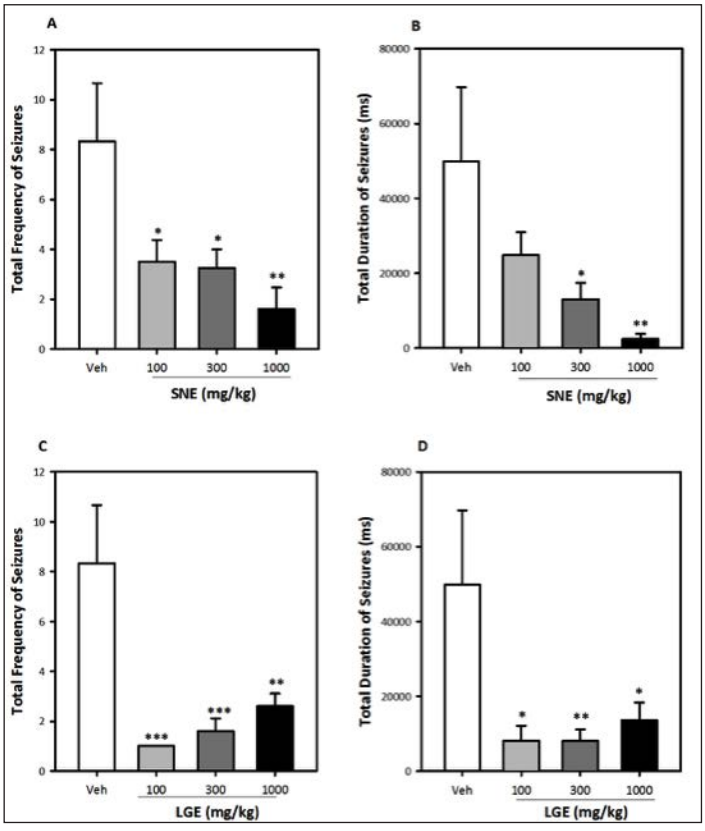
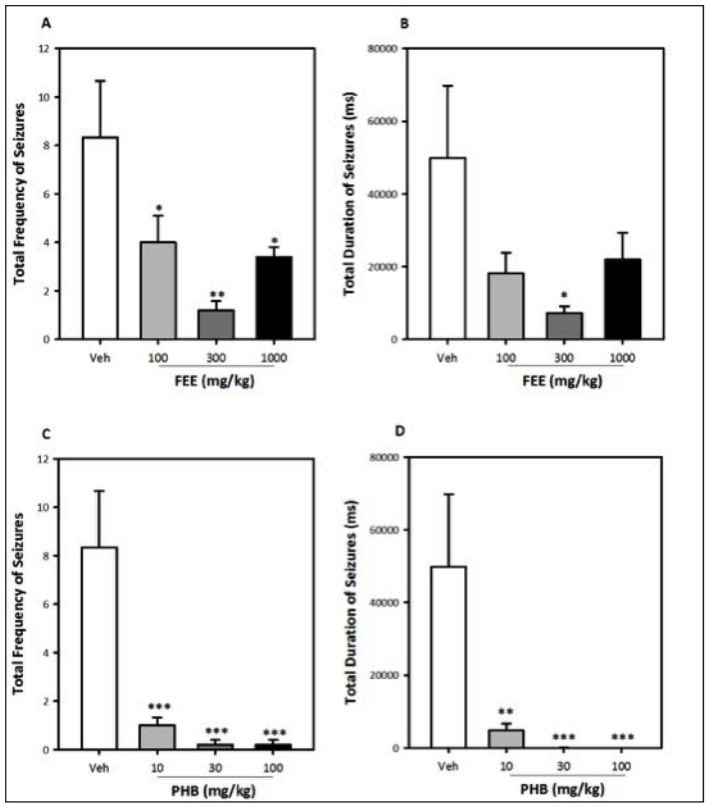


Fig.25: Effect of different doses of SNE and LGE on the total frequency (A, C) and duration (B, D) of PTZ-induced seizures in mice. Data are shown as Mean±SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 compared to control group (vehicle-treated) by One-way ANOVA followed by Dunnett's Multiple Comparison Test.

Fig. 26: Effect of different doses of FEE and PHB



*on the total frequency (A, C) and duration (B, D) of PTZ-induced seizures in mice. Data are shown as Mean±SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 compared to control group (vehicle-treated) by One-way ANOVA followed by Dunnett's Multiple Comparison Test.*

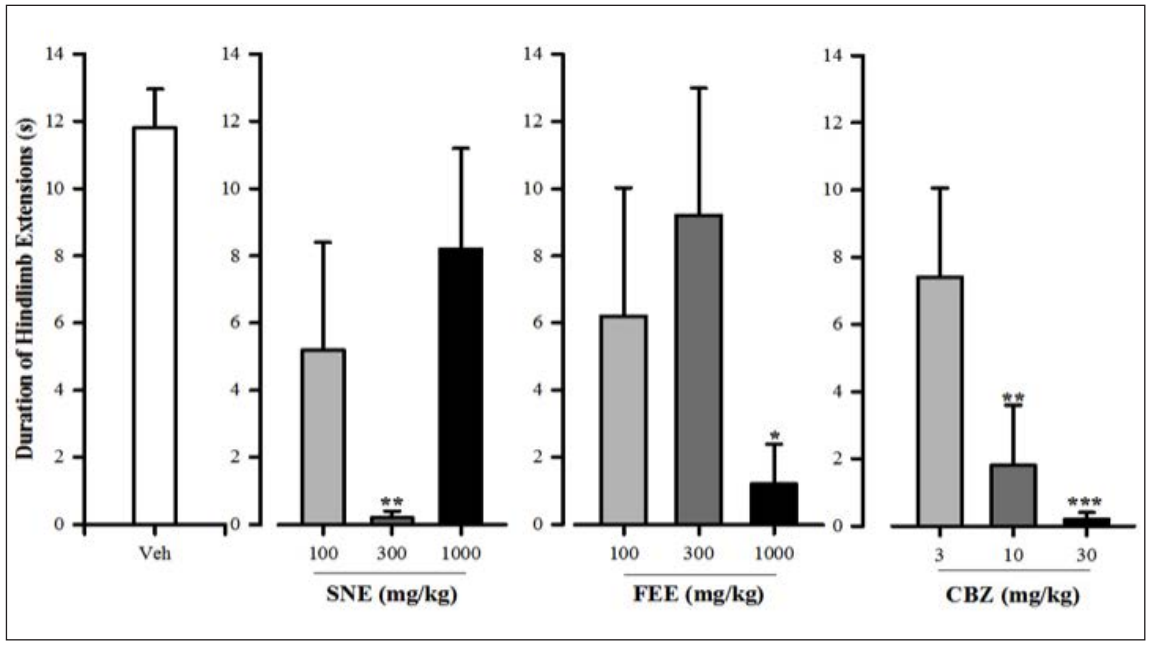


*Fig. 27: Effect of different doses of SNE, FEE and CBZ on the duration of hindlimb extensions in the MES-induced seizures in mice. Data are shown as Mean±SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 compared to control group (vehicle-treated) by One-way ANOVA followed by Dunnett's Multiple Comparison Test.*

Evaluation of *Croton membranaceus root extract* for Anti-Diabetic Activity

Investigators:

NMIMR

Department of Animal Experimentation — *Samuel Adjei, PhD*

Phyllis G. Addo, DVM, PhD

School of Allied Health Sciences, CHS — *George Asare, PhD*

Funding — *NMIMR*

Introduction

In folk medical practice around the world, many plants have been used to treat diabetes (Barbosa-Filho *et al*., 2005; Agra *et al*., 2007). However most of these medicinal plants are not scientifically validated, for their therapeutic efficacy and safety. There are reports of diverse medicinal properties of different species of *Croton* worldwide, including their anti-diabetic activity. However, there is paucity of information on the anti-diabetic potential of the species of Croton found in Ghana, *C. membranaceus*. The study investigated the hypoglycemic potential of the aqueous root extract of *Croton membranaceus*.

Methods

A total of twenty-eight (28) male mice of the *BKS.Cg/BomTacm*$^{+/+}$*Lepr*$^{db/+}$ strain, used for type 2 diabetes studies, were divided into four groups of seven (7) mice. All mice were fasted overnight and fasting blood glucose levels were determined using a glucometer (One Touch Ultra®), after which animals were allowed to eat. Extract, metformin and water were administered one (1) hour after animals were allowed to eat. The first three groups (diabetic groups, db/db) were dosed orally with *Croton membranaceus* root extract (250mg/kg body wt); Metformin (250 mg/kg body wt) as the reference drug, and water as positive control, respectively. The fourth group (non-diabetic, db/+) serving as negative control group was also given water. Blood Glucose Levels were determined after 1, 2 and 3 hours of administration of the test substances.

Preliminary Findings and Implications

Results show that *Croton membranaceus* reduced the blood glucose levels of diabetic mice by 34.57% while the reference drug metformin reduced their levels by 80.45% (Fig 28). The findings show that *Croton membranaceus* has anti-diabetic property but needs to be investigated further.

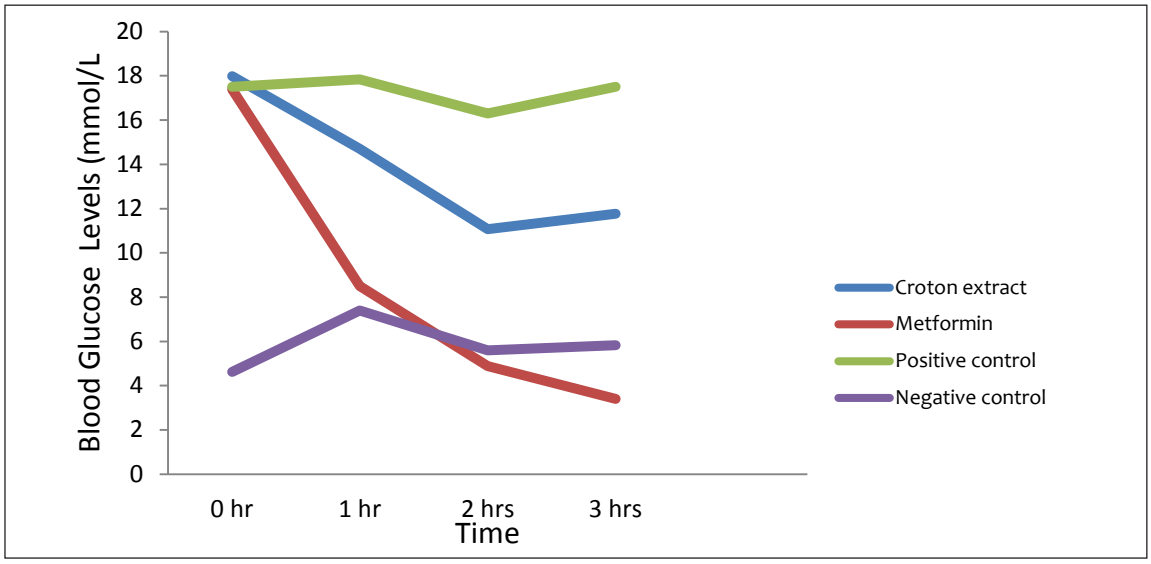


Fig. 28: Effect of Croton membranaceous root extract on blood glucose levels in mice

3. Evaluating utility of the Eker Rat model in uterine leiomyoma phytotherapy

Investigators	
NMIMR	
Department of Animal Experimentation	*Samuel Adjei, PhD*
	Phyllis Addo, DVM, PhD
Department of Immunology	*Ben Gyan, PhD*
School of Allied Health Sciences	*George Asare, PhD*
Funding	*UG Research Fund*

Background

Uterine leiomyomas (fibroids) are the most common type of reproductive tract tumor of women. Uterine fibroids are non-cancerous tumors consisting of fibers or fibrous tissue that arise in the uterus. Modern imaging techniques and careful pathological examination of specimens indicate that the incidence of uterine fibroids is as high as 70%, suggesting that these tumors are far more prevalent than previously estimated by clinical cases (20 to 50%) (*Berry et al., 2006*). In Nigeria 60.6% of uterine fibroid was reported in a retrospective clinicopathological analysis of uterine leiomyomata over a five-year period (1996 – 2000) (*Mohammed et al., 2005*). Regardless of their generally benign neoplastic characteristics, uterine fibroids are responsible for significant morbidity in a large segment of the female population.

Current treatments for uterine leiomyoma are far from satisfactory and leave much to be desired. This is particularly relevant for women with symptomatic uterine fibroids who want to preserve their procreation potential and would not accept the fertility precluding option of hysterectomy. Clearly, the development of a safe, effective, non-surgical and localized method of treatment for uterine fibroids would greatly benefit many women, especially with more women delaying childbirth into the age range when symptomatic fibroids are most prevalent.

The Eker rat is the only animal model that develops spontaneous uterine leiomyomas and these tumours share many characteristics with those found in humans. Eker rats harbour a germ line mutation in the tuberous sclerosis complex-2 (Tsc-2) tumor suppressor gene, and develop uterine leiomyoma (Fibroid) at a rate of 65% (Everit et al., 1995).

For purposes of breeding and to ascertain its use in fibroid research, the presence or absence of the Tsc-2 mutation in the Eker rat was determined by PCR, hormonal and oxidative stress marker assays were performed to ascertain their association with uterine leiomyoma, as reported previously (Massart et al., 2003; Forster et al., 1989), and the effect of ***Phyllanthus niruri extract*** on fibroids was determined in this study.

Approach

i. Genotyping of Eker rat

Method

Genomic DNA was extracted from the cut tail tip of the Eker rats according to the manufacturer's protocol (Qiagen, USA). The Ts-2 gene region was amplified by polymerase chain reaction and viewed by agarose gel electrophoresis under UV light. The assay was scored for the presence or absence of the mutant band only in a total of 80 (31 males and 49 females) v Eker rats.

Preliminary Findings and Implications

All samples (n = 80) has the wild type band (Fig 29). To date, presence of the mutation in the Eker rat colony established in the Department of Animal Experimentation has been confirmed and its occurrence is on average, 43% and 36% in females and males respectively. Mutant males and non-mutant females are used as breeders while mutant females are used for uterine leiomyoma research.

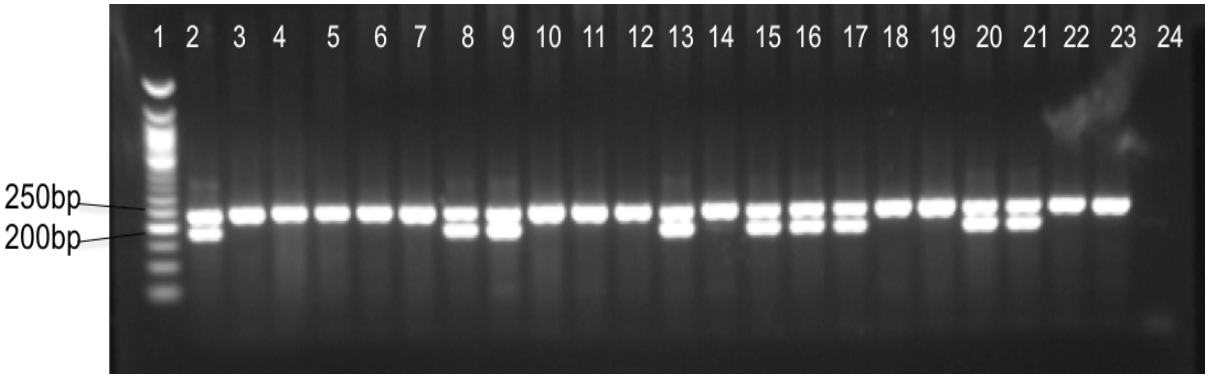


Fig. 29: Electropherogram showing wild type allele (upper band) and mutant allele (lower band). Lane 1 is a 50bp DNA ladder, lanes 2 and 3 are positive controls whilst lane 24 is the negative control.

ii. Reproductive hormones assay

Method

Two reproductive hormone (oestrogen and progesterone) serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA). The respective immunoassay reagent kit for rat samples was obtained from Cayman Chemical Company (Michigan, USA). The assays were carried out according to the manufacturer's instruction.

Preliminary Findings and Implications

The mean progesterone concentrations for the mutant (positive, n = 7) and non-mutant (negative, n = 4) groups were 115.5 pg/ml and 79.6 pg/ml, respectively. Estradiol concentrations were 256.5 pg/ml and 152.2 pg/ml for the positive and negative groups, respectively (Fig 30). These results show a strong association between uterine fibroids and the reproductive hormones assayed, as reported in previous studies (Forster *et al*., 1989).

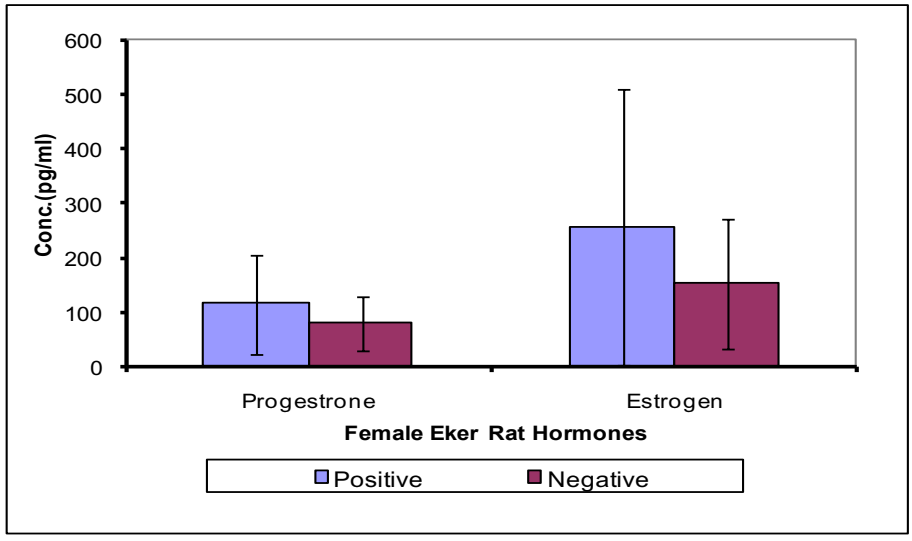


Fig. 30: Mean serum concentrations of progesterone and estradiol in mutant and non-mutant Eker rats

iii. Oxidative stress marker assay

Method

Two oxidative stress markers, 8-Hydoxy-2'-deoxyguanosine (8-OHDG) and Tumour necrosis factor-alpha (TNF-α), found to be associated with uterine fibroids (Marek *et al*., 2000), were assayed using the ELISA method. Eker rats that tested positive for the Tsc-2 gene mutation by PCR (n = 4) and those that tested negative (n = 4), were used.

Preliminary Findings and Implications

Results showed high serum levels of 8-OHDG and significantly low serum levels of TNF-α in the test animals compared to the control group (Table 7), implicating the two analytes in the etiology of uterine fibroids.

Table 7: Serum levels of oxidative stress markers analysed in mutant and non-mutant Eker rats

ANALYTES	GROUP	N	MEAN± SEM	T-VALUE	P-VALUE
(ng/ml)					
8-OHdG (ng/ml)	Mutant	4	172.0±71.6	1.062	0.316
	Non-mutant	4	132.1±22.9		
TNF-α (pg/ml)	Mutant	4	359.1±62.1	-5.911	0.001
	Non-mutant	4	753.8±16.2		

iv. Effect of Phyllanthus niruri extract on Uterine Leiomyoma

Method

To provide scientific evidence to prove or disprove claims by herbal practitioners that *Phylanthus niruri* cures uterine fibroids, female Eker rats (n = 7) confirmed by PCR as carrying the *Tsc-2* mutation and further proved by hormonal and immuno-reactive assays, were orally dosed with aqueous leaf extract of the plant over a period of three months. Control animals (n = 3) were given water. At the end of the three months both test and control animals were culled and uteri were examined for the presence or absence of fibroids.

Preliminary Findings and Implications

Preliminary data shows no significant effect of the extract on fibroids since fibroids were found in both test and control rats. However, one notable effect is the absence of gallstones in test animals compared to the control group. Further work need to be done to confirm the physical presence of fibroids by laparoscopy or other procedures. This will enhance Eker rat utility in uterine leiomyoma research in general and phytotherapy research in particular.

In-vivo evaluation of medicinal plants with previously demonstrated antimicrobial activity against *Mycobacterium ulcerans in-vitro*

Investigators

NMIMR

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	Mrs. Mona Abbas, HD, Mist-UK
	Mr. Kenneth Agyeman-Badu, HND
Funding	*WHO-Global Buruli Ulcer Initiative, NMIMR*

Background

Surgery is the main treatment option for Buruli ulcer because antimicrobials have not been very effective. The World Health Organization (WHO) has provisionally recommended the use of selected anti-mycobacterials in combination with surgery, while the search for effective anti-mycobacterials continues. There are unconfirmed reports of successful treatment with herbal preparations in Ghana and other BU endemic African countries; therefore, the *in-vitro* inhibitory activities of 44 herbal preparations with antimicrobial and wound healing properties were tested against 7 *M. ulcerans* isolates from two BU endemic communities in Ghana. Twenty-five of the 44 herbal preparations demonstrated anti-*M ulcerans* activity *in-vitro*; subsequently, 11 of them were evaluated *in-vivo* because they also demonstrated broad-spectrum antimicrobial activity against fast growing organisms with predilection for the skin; which makes them desirable BU herbs because of the secondary infections known to accompany and complicate BU. Five of the 11 herbal preparations were BU remedies provided by traditional herbal practitioners (THPs).

Objectives

1. To determine if medicinal plants with antimicrobial and wound healing properties have anti-BU activity *in-vitro*
2. To determine if anti-BU medicinal preparations of local herbalists have anti-BU activity *in-vitro*
3. To determine if medicinal plants and preparations which have demonstrated anti-BU activity *in-vitro* would also demonstrate anti-BU activity *in-vivo*

Method

One hundred and thirty BALB/c mice were experimentally infected in the footpad with *M. ulcerans* and randomly assigned to 11 herbal treatment groups and 2 control groups (1 positive control and 1 diluent (negative) control group). The herbal preparations were administered daily for 8 weeks by gastric intubation after onset of BU lesion (congestion/swelling of inoculated footpad). The footpads and general condition of the experimental and the control groups were monitored daily.

The effectiveness of the 11 herbal preparations were assessed on two levels by: (i) scoring the clinical condition of treated and non-treated groups, and (ii) enumerating and categorising *M ulcerans* AFBs in footpad homogenates into solid and fragmented bacilli, which was based on the morphological index method (MI) used for evaluating the efficacy of treatment in leprosy. According to the MI index, solid AFBs represent viable bacteria at the time of the preparation of the smear, and therefore served as indicators of non-response to treatment; fragmented AFBs represent non-viable bacteria at the time of the preparation of the smear; therefore, served as indicators of effective treatment (Mcrae & Shepard, 1971; Cheesbrough, 1984). All the animals were euthanized after the 8th week; their infected footpads were homogenised, ZN-stained and examined for AFBs, which were categorized according to the MI index, described above.

Preliminary Findings and Implications

All the 11 herbal preparations yielded a preponderance of fragmented bacilli in the footpad homogenates, which suggest that they are effective against BU. The mean percentage of

fragmented AFBs (non-viable bacteria) in the herb-treated group was 85.36 ± 4.72%; while that of the non-treated group was 4.1 ± 1.66%. The mean percentage of solid AFBs (viable bacteria) in the herb-treated group was 14.64 ± 4.72%; while that of the non-treated group was 95.9 ± 1.66%.

The findings of the study principally suggest that apart from antibiotics, there are medicinal plants with *in-vitro* and *in-vivo* antimicrobial activity against *M. ulcerans*. These medicinal plants are locally available and herbalists are already using some for the treatment of BU. In view of these findings, attention should be given to development of herbal therapy as a BU treatment option.

Future Directions

The study would be repeated with a larger number of animals to facilitate evaluation of relapse, a feature of BU. The results would help select the best preparation for further study and possible identification of a lead for drug development.

Decontamination of *Vibrio cholerae* and *Salmonella typhi* contaminated foods with medicinal plants – *in-vitro* and in a food model

Investigators

NMIMR

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	Innocent Afeke, MPhil
	Believe Ahedor, BSc
	Kenneth Agyemang-Badu, HND
Funding	*British Council*

Background

Some medicinal plants have antimicrobial properties that may help to prevent the outbreak of certain diseases of public health importance, such as cholera and typhoid fever. The antimicrobial effectiveness of 3 medicinal plants (HJ112, HJ212, and HJ312) against *Salmonella typhi* and *Vibrio cholerae* were investigated.

Objectives

1. To determine the *in-vitro* inhibitory activities of herbal preparations HJ112, HJ212 and HJ312 against *Salmonella typhi* and *Vibrio cholerae*
2. To determine the *minimum inhibitory* (MIC) and *minimum bactericidal* (MBC)concentrations of herbal preparations HJ112, HJ212 and HJ312 against *S. typhi* and *V. cholerae*
3. To determine the decontaminating potential of HJ112, HJ212 and HJ312 of *S.typhi* and *V.cholerae* contaminated foods

Method

The three herbal preparations were assayed for antimicrobial activity against *S.typhi* and *V. cholerae* using the agar dilution method. Based on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results, HJ212 and HJ312 were assayed further in a food model study to evaluate their food decontaminating potential. Two sets of sterile maize porridge were contaminated with twice the infectious doses of *S. typhi* and *V. cholerae* and later decontaminated with HJ212 and HJ312. The two sets of porridge were sampled intermittently (5min, 15min, 30min and 60min) and their microbial loads, expressed as colony forming units (CFU) were determined and subsequently compared with the CFU of the original contaminated porridge.

Preliminary Findings and Implications

Herbal preparation HJ212 reduced *V. cholerae* from 1.7 x 10^9 CFU/ml to zero after 5min of contact (Fig 31), and reduced *S. typhi* from 1.2 x 10^9 CFU/ml to 9 x 10^1 CFU/ml after 30min of contact (Fig 32). HJ312 on the other hand, showed moderate activity by reducing *V. cholerae* from 1.7 x 10^9 CFU/ml to 1.65 x 10^3 CFU/ml after 60min (Fig 31) and *S. typhi* from 1.7 x 10^9 CFU/ml to 1.85 x 10^7 CFU/ml after 60min (Fig 32). In conclusion, HJ212 is a better decontaminant of the salmonella and cholera contaminated porridges and therefore should be studied further for its possible use in the household prevention of typhoid fever and cholera.

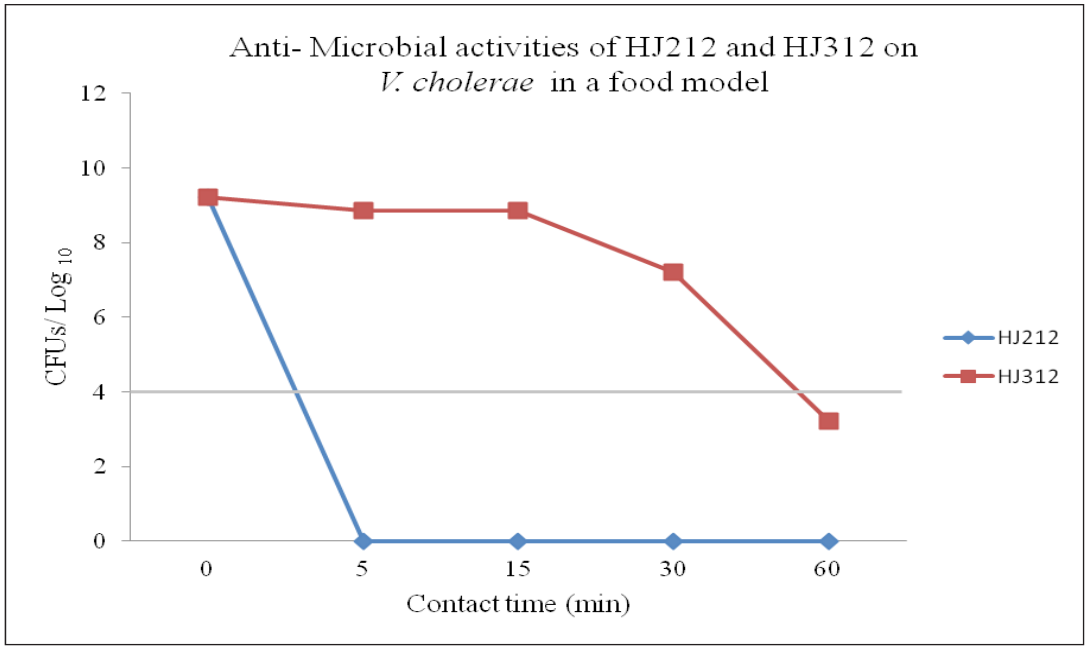

Fig. 31: Anti-microbial activities of HJ212 and HJ312 on V. cholera in a food model

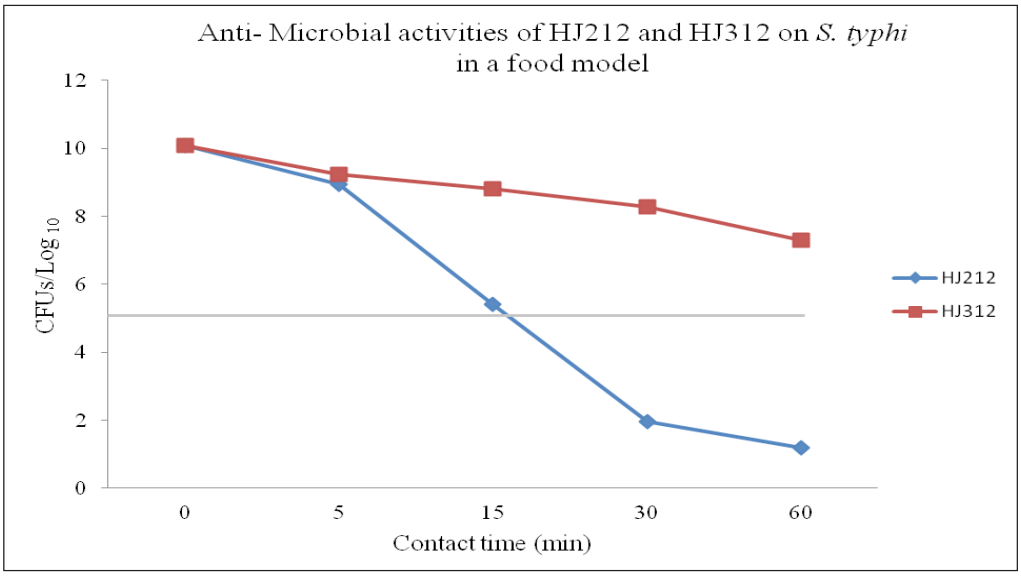

Fig 32. Anti-microbial activities of HJ212 and HJ312 on S. typhi in a food model

Future Directions

Animal models of *S.typhi* and *V.cholerae* would be established and the study conducted *in-vivo*. A successful outcome would call for safety (toxicity) evaluation of the successful herbal preparation, followed by a small scale human study in typhoid fever and cholera endemic communities in Ghana.

Identification of medicinal plants for the management of diabetes and obesity

Investigators

NMIMR

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	Shirley Adu-Poku, BSc
	Constance Forson, BSc
Funding	*British Council*

Background

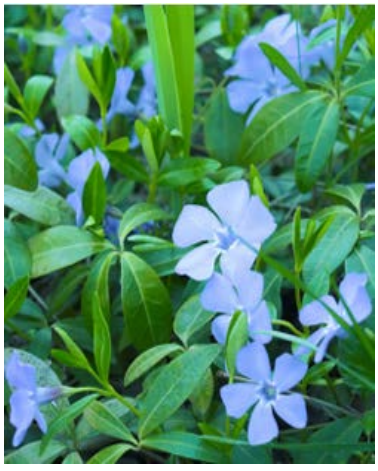
According to the World Health Organization, at least 285 million people worldwide suffer from diabetes (WHO, 2010) and about 70% live in developing countries (International Diabetes Federation, 2009). Its incidence is increasing rapidly and it is estimated that by the year 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild *et al.*, 2004). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet, which also leads to obesity. Many traditional plant treatments for diabetes exist (Gray and Flatt., 1999), however, few have received scientific or medical scrutiny and the World Health Organization has recommended that traditional plant treatment for diabetes warrant further evaluation (WHO, 2002, 2010).

Objectives

1. To screen 13 medicinal plants for anti-diabetic activity
2. To compare the performance of the medicinal plants with metformin, an orthodox anti-diabetic treatment drug
3. To determine the effect of metformin and the anti-diabetic medicinal plants on body weight

Method

Thirteen medicinal plants (Fig 33) and an orthodox anti-diabetic drug – metformin (reference drug; Table 8) were tested for demonstration of anti-diabetic property, in BKS.Cg/BomTacm$^{+/+}$Lepr^{db} mice, made up of diabetic (db/db) and non-diabetic (db/+) littermates. Seventy-five of the diabetic mice served as the treated group (test group or diabetic treated group) while 5 served as untreated diabetics (positive control or diabetic control group); the non-diabetic (db/+) littermates served as untreated non-diabetics (negative control group). The reference drug and plant products were administered daily for 4 weeks and at the same time each day. The One Touch Ultra Glucometer was used to determine blood glucose levels, while a weighing scale for laboratory mice was used for weekly weight determinations.



Periwinkle

Coconut

Noni

Fig 33: Three of 13 medicinal plants evaluated for anti-diabetic activity.

Table 8. List of Plants Screened for Anti-Diabetic Activity

1. Periwinkle (*Vinca major*),	6. Aloe vera (*Aloe vera*)	11. Bitter Leaf (*Vernoniaamygdalina*)
2. Tridax (*Tridaxprocumbens*)	7. Cactus (*Opuntia*)	12. Garlic (*Allium sativum*):
3. Noni (1) - fruit (*Morindacitrifolia*)	8. Coconut Oil (*Cocosnucifera*)	13. Prekese (*Tetrapleuratetraptera*)
4. Noni (2) - juice (*Morindacitrifolia*)	9. Momordica (Yayara) (*Momordicacharantia*)	14. Cocoa (*Theobroma cacao*)
5. Dandelion (*Taraxacumofficinale*)	10. Guava (*Psidiumguajava*)	15. Metformin (Ref. Drug)

Preliminary Findings and Implications

All the plant extracts that were tested demonstrated anti-diabetic property and some quite impressively (Fig. 34). Most of the plant extracts also demonstrated weight-reducing property (Fig. 35). Some of the plants investigated are known to have anti-inflammatory properties, which is an important attribute in the management of diabetes since inflammation plays a key role in diabetes. In view of all the above observations, further pharmacological studies have to be conducted on the plants.

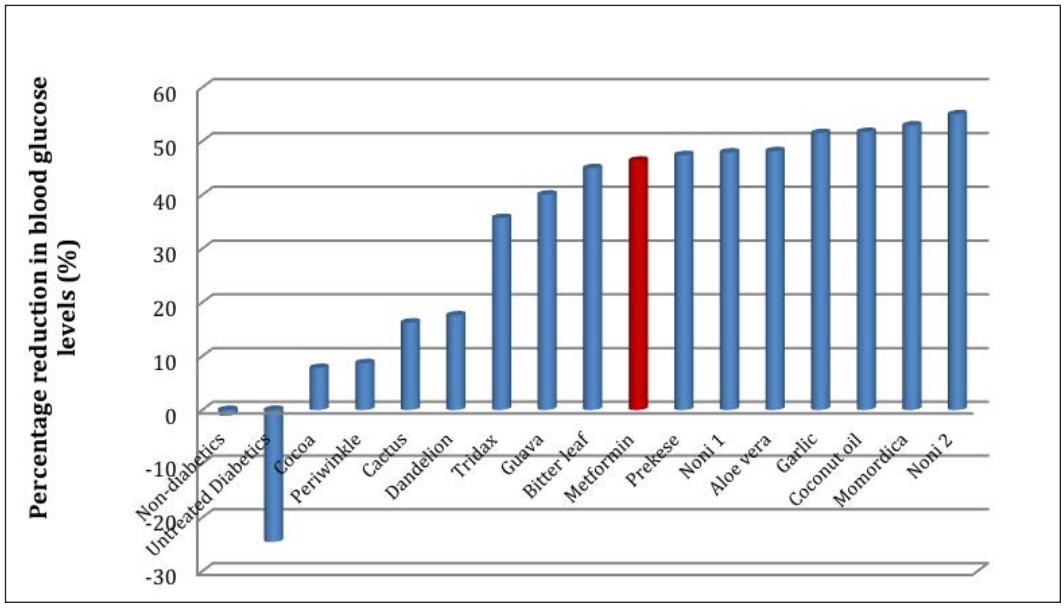


Fig. 34: Percentage reduction in blood glucose levels after treatment with Metformin and herbal extracts

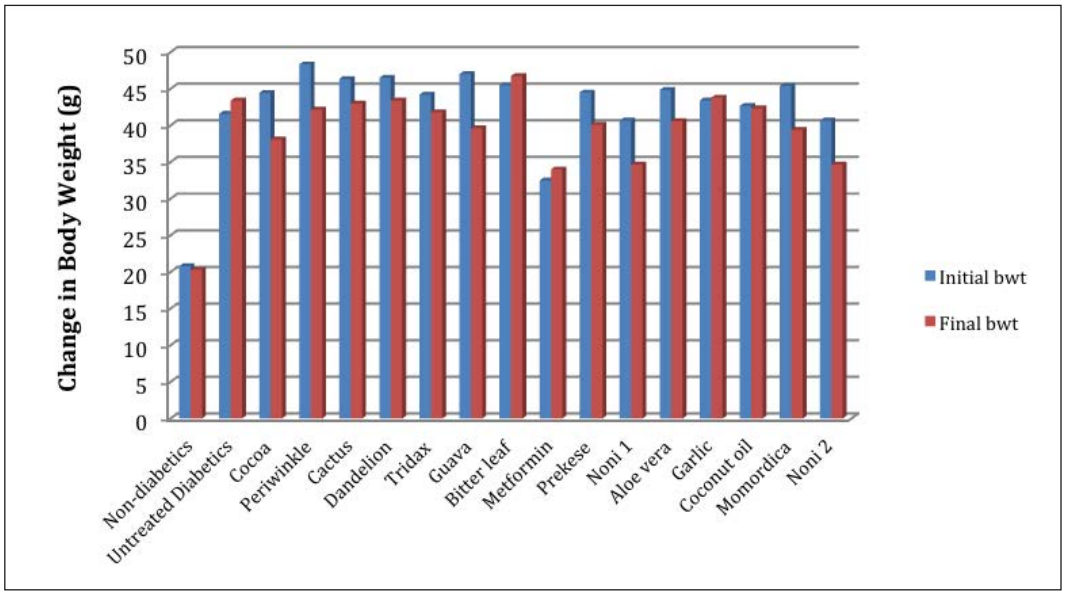


Fig. 35: Change in body weight after treatment with Metformin and herbal extracts

Non-clinical safety evaluation of *Morinda citrifolia* (noni), a popular herbal drink in Ghana

Investigators

NMIMR

Department of Animal Experimentation — *Phyllis Addo, DVM, PhD*

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Shirley Adu-Poku, BSc

Funding — *British Council*

Introduction

In recent times in Ghana, a medicinal fruit juice popularly called Noni (*Morinda citrifolia*) is being used extensively and planted on a large scale for export. The juice of the noni fruit is used without restraint across the socio-economic strata; raising concerns about its safety and implications for its use as medicine. Noni has been used in folk medicine for over 2000 years; every part (i.e. roots, stem, bark, leaves, flowers and fruit) is utilized in various combinations (Tabrah and Eveleth, 1966). The fruit juice is in high demand for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcer, sprains, mental depression, senility, poor digestion, blood vessel problems, and drug addiction. Noni juice is said to have no known side effects apart from few reported cases of minor discomfort (bloating or mild digestive trouble), among first-time users. Notwithstanding such 'good' reports, since noni is being used extensively, information on its safety and efficacy should go beyond hearsay or anecdotal evidence; scientific assessment of its toxicity and efficacy are needed in order to protect its numerous patronisers.

Aim

To determine the acute and subchronic oral toxicities of *Morinda citrifolia (Noni)* in Sprague Dawley rats

Specific Objectives

1. To determine the toxicity of Noni fruit extract at three dose levels after one time dosing of Sprague Dawley rats, with special reference to the gross morphology of the liver and kidney.
2. To determine the toxicity of Noni fruit extract at three dose levels after 90 days of repeated dosing of Sprague Dawley rats, with special reference to haematology and clinical chemistry parameters, and gross/histopathology of all organs.

Acute toxicity – one time dosing

Generally, the liver, kidney, spleen, intestine, CNS, brain and reproductive organs play a part in drug metabolism depending on the drug in question. Since the metabolism of Noni is not known, our immediate interest was on the two most prominent organs in drug metabolism namely; the liver and kidney.

Method

Noni fruits were washed and blended in sterile distilled water. The blended noni was administered by gastric intubation to 30 (5/dose /sex), 8 week-old Sprague Dawley rats at a dose of 5000, 2000 and 500mg/kg body weight. A control group of five males and five females received the vehicle (sterile distilled water) alone, under the same experimental conditions. All the animals were observed for 14 days following one-time oral dosing. The general wellbeing, and feed and water consumption of both experimental and control groups of animals were monitored daily; their body weights were recorded weekly. On the 15th day both experimental and control groups of animals were euthanized, exsanguinated and subjected to necropsy, during which body and individual organ weights were recorded.

Preliminary findings and implications

The acute oral toxicity study ended with none of the rats dying; an indication that the extract may have some margin of safety. However, there were adverse findings with respect to body weight, percentage kidney to body weight and percentage liver to body weight, which are suggestive of some level of toxicity.

The extract resulted in a decrease in the body weights of all animals in the high (5000mg/kg bwt) and medium dose (2000mg/kg bwt) groups within the first week; however, there was a recovery in the second week. The changes in body weight were not statistically significant. Conversely, there was a continuous increase in body weights of all animals in the 500mg/kg bwt (low dose) group; the trend was similar to that observed with the control group (water only). The similarity between the 500mg/kg bwt group and the control group suggest that 500mg/kg bwt may be the safest dose of the noni extract; the situation was similar in both sexes.

The percentage of kidney to body weight indicated that there was an adverse effect on the kidney at 5000mg/kg bwt and 2000mg/kg bwt; however, at 500mg/kg bwt the effect was very minimal and somewhat similar to that of the control group; suggesting that 500mg/kg bwt may be the safest dose; the situation was similar in both sexes (Figs 36 & 37).

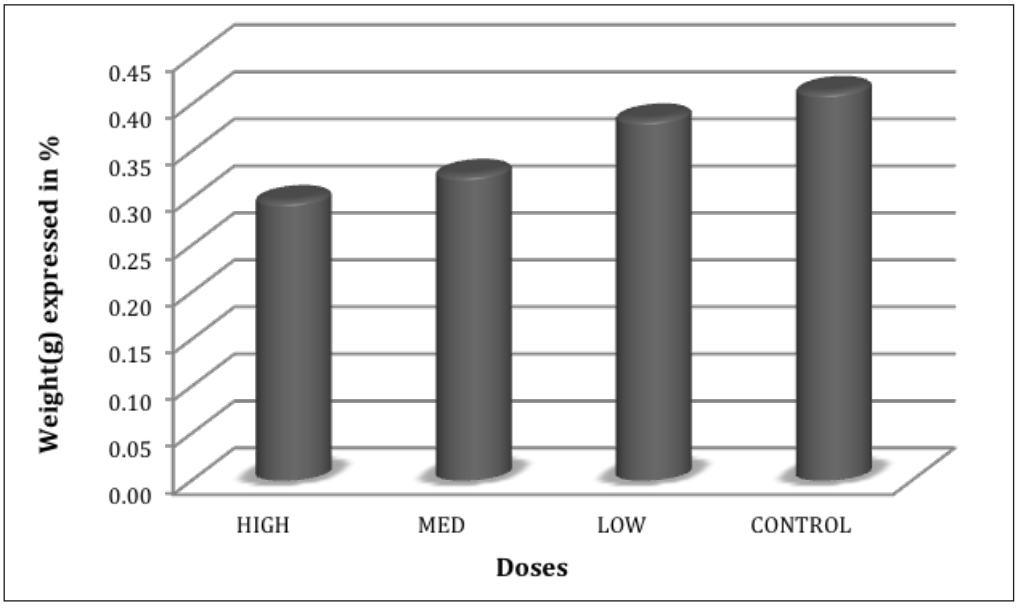

Fig. 36: Percentage of kidney to body weight in males

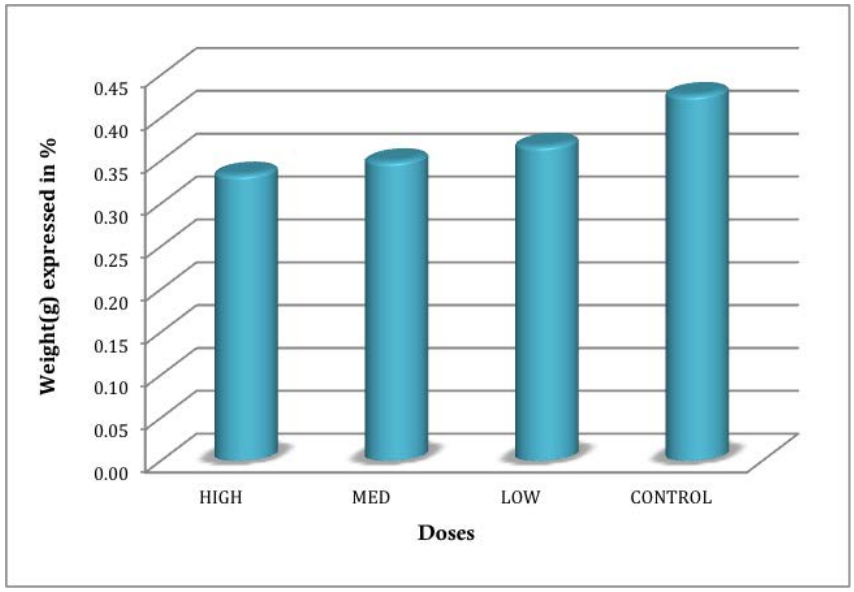

Fig 37: Percentage of kidney to body weight in females by dose

Effect of Noni on percentage liver to body weight

The effect of noni on percentage liver to body weight was generally similar in both sexes but with slight differences. In the males, the 5000mg/kg bwt and 500mg/kg bwt groups registered comparatively lower % liver to body weight than that of the control group. That of the 2000mg/kg bwt group was unexpectedly closer to that of the control group (Fig. 38); it was however due to pathological enlargement of the liver. With regard to the females, the 5000mg/kg bwt group had a % liver to body weight comparatively lower than that of the control group; similar to that observed with the males. On the other hand, at 500mg/kg bwt and 2000mg/kg bwt the % liver to body weight was closer to that of the control group (Fig. 39). From the overall findings it could be concluded that Noni has a predilection for the liver; and should be used with caution; especially by those with liver impairment.

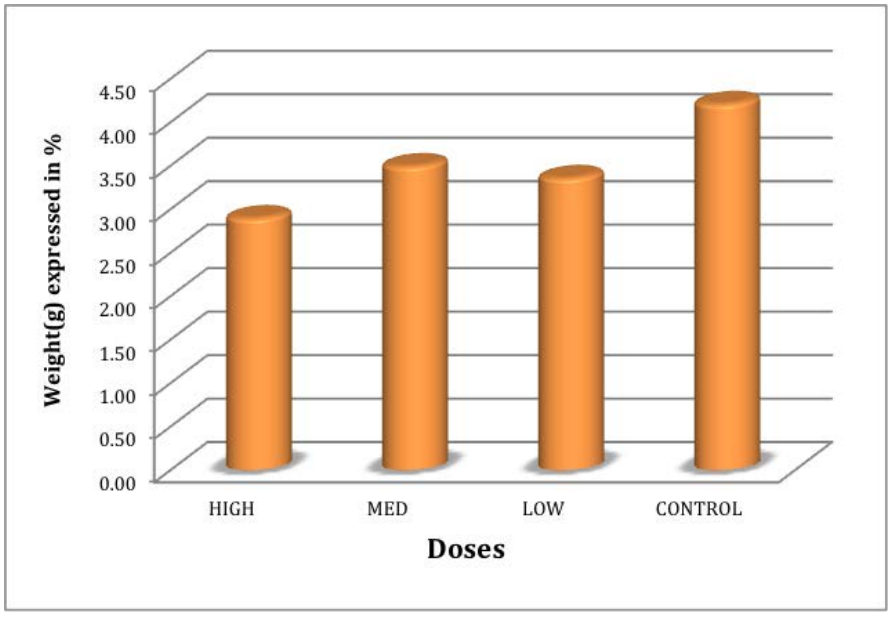

Fig 38: Male: Percentage of Liver to body weight

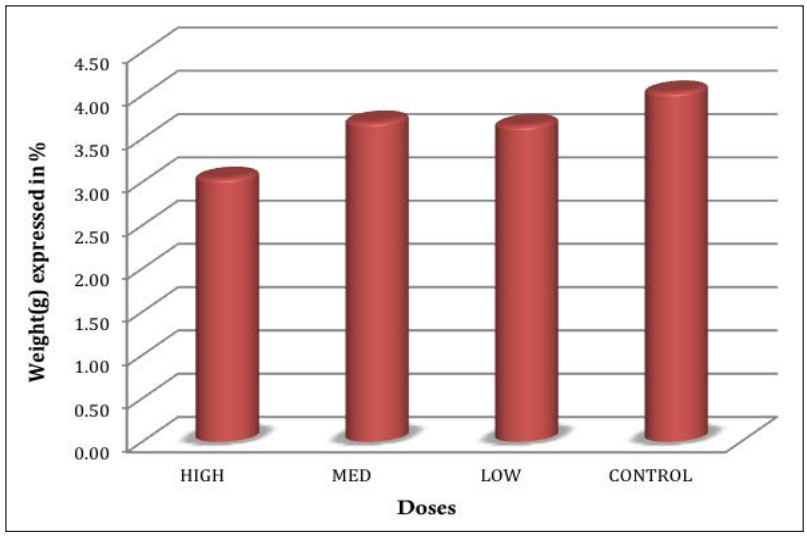

Fig 39: Female: Percentage of Liver to body weight

The use of noni extract as a safe food revealed adverse effects on the kidney and liver at the three doses tested; irrespective of sex. This outcome of the study raises concerns about the indiscriminate and continuous use of noni as a food supplement, bearing in mind that the amount taken by users (40-52g/day/70kg bwt = 571 - 742mg/kg bwt) is above the lowest dose of 500mg/kg bwt, which was observed to be slightly safe with respect to body weight and kidney but not to liver. This notwithstanding, the situation is still of great concern because 500mg/kg bwt is only safe for the kidney at one time use; not for continuous use as currently practised by users.

Treatment of hypercholesterolemia: screening of *Solanum macrocarpon* Linn (Solanaceae) as a medicinal plant in Benin

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Introduction

Despite remarkable advances in medicine and research, there is an increase in cardiovascular diseases. It is one of the leading causes of death worldwide. They constitute 30% of mortality in the world, with three out of four occurring in countries with low and middle incomes. Twenty-five million deaths due to cardiovascular diseases are expected in 2020. Hypercholesterolemia is the greatest risk factor for cardiovascular diseases. It is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis, and it is characterized by the elevation of cholesterol and lipid parameters (LDL-cholesterol, triglycerides). Hypercholesterolemia is estimated to cause about 4.4 million deaths (7.9%) in the world. Scientists often cite lifestyle: unhealthy diet, physical inactivity, lack of exercise, stress, smoking, and obesity as predisposing factors to hypercholesterolemia. Pharmacologic treatment of hyperlipidemia in conjunction with therapeutic lifestyle changes are used for both primary and secondary prevention of cardiovascular disease. Other cholesterol-lowering medications used for primary or secondary prevention of cardiovascular disease have not been shown to consistently improve patient-oriented outcomes. *Solanum macrocarpon* is traditionally used in Nigeria to treat hypercholesterolemic disorders; its purported efficacy as an anti-hypercholesterolemic was evaluated in this study.

Objective

To evaluate the lipid lowering activity of leaves and fruits of *Solanum macrocarpon*, a vegetable, on Wistar rats experimentally rendered hypercholesterolemic by Triton X-100.

Method

The effects of powdered leaves and fruits of *S. macrocarpon* on hypercholesterolemia induced in Wistar rats were assessed and then compared with those of a reference chemical product, Atorvastatin. Wistar rats were rendered hypercholesterolemic by intraperitoneal administration of Triton X-100 at a single dose of 150 mg/kg body weight. The Triton X-100 was chosen because of its convenience, availability, and especially the reproducibility of the animal model created (Kothiyal and Gupta, 2011). Aqueous extracts of the leaves and fruits of *S. macrocarpon* were administered orally to the rats at 400 and 800 mg/kg of body weight for 7 days. Atorvastatin was used as reference treatment drug at 10 mg/kg body weight. The livers of all groups of rats were evaluated histopathologically. The data were analyzed by the Brown-Forsythe ANOVA, Dunnett's T3 multiple comparison test, and Dunnett's t test. All tests were done at the 5% significance level.

Findings and Implications

Hypercholesterolemia was successfully established in all of the groups treated with Triton X-100. All the hypercholesterolemic groups treated with Atorvastatin or *S. macrocarpon* showed a reduction in all hypercholesterolemia parameters (total cholesterol, LDL, VLDL, and triglyceride) and an increase in HDL. The mean differences were all statistically significant ($p<0.05$) with the exception of LDL ($p=0.157$). Hepatic disorders due to the Triton were corrected by *S. macrocarpon*.

Conclusions

Solanum macrocarpon effectively suppresses experimental hypercholesterolemia in Wistar rats, suggesting a protective role in cardiovascular diseases. A diet rich in vegetables such as *S. macrocarpon* should be recommended. Its use by individuals at risk should be promoted.

Other Studies

Characterization of two medicinal plant species, *Croton membranaceus* and *Cryptolepissanguinolenta*

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Funding	*Volkswagen Foundation*

The effect of the aqueous root extract of *Croton membranaceus* on the treatment of Benign Prostate Hyperplasia and Prostate Cancer

a) Determination of the effect of CMARE on BPH in experimental animal models

b) Observation of socio-scientific markers of the effect of sub-chronic use of the lyophilized Croton membranaceus root extract on BPH patients.

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Developing an alternate anti-epileptic product from medicinal plants: exemplifying utilization of natural resources for quality health.

a) A study of the analgesic properties of Synedrellanodiflora extract in vincristine-induced neuropathic pain in rats

b) Safety assessment of the hydro-ethanolic whole plant extract of Synedrellanodiflora in rats

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The Search for New Anti-Plasmodial Agents: Fate of *Mitragyna inermis*, *Pseudocedrela kotschyi and Moringa oleifera*

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Elucidating the Transmission of Buruli ulcer in the mouse model by simulating six probable transmission scenarios

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Screening Ghanaian traditional medicinal plants for bioactive anti-cancer agents

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Viral Infections

Research on viral infections is centered in the Departments of Virology and Electron Microscopy and Histopathology. These include research on viral infections such as HIV, Influenza and Poliovirus and also with attention to viruses of epidemic potential such as those responsible for Viral Hemorrhagic Fevers (e.g. Ebola, Marburg, Lassa etc). There was research on the animal-

human interphase to determine the prevalence of some high-risk pathogens associated with zoonotic infections. There were efforts to establish methods for the detection of new viral infections and some work on interactions/co-infections with other sexually transmitted infections.

Research in the area of key HIV infections included monitoring of anti-viral interventions for HIV/AIDS by biomarkers such as immune status, viral load and genotypic drug resistance studies whilst data was also generated on behavior patterns among high-risk groups for HIV. Investigations of traditional plants continued to search for substances with activity against HIV replication and the ability of plant extracts to stimulate HIV expression in latently infected cells was also done. An examination of human leucocyte antigen polymorphisms in HIV and HTLV-1 infections was begun to characterize genome diversities in Ghana for a better understanding to inform the design of potential vaccine immunogens.

The surveillance for influenza infections via sentinel health facilities across the country was maintained to provide weekly profiling of influenza virus activity for the Ghana Health Service and the Global Influenza Surveillance and Response System. A project to obtain comprehensive data on the disease burden of influenza was launched to enable the determination of the relative importance of influenza and other respiratory pathogens as causes of ill health in Ghana.

The laboratory surveillance of Polioviruses in stool samples from acute flaccid paralysis (AFP) cases in Ghana, Togo and Benin was maintained to support the Global Polio Eradication Program. There were also studies to characterize human enteroviruses isolated from cases of AFP and healthy children less than 5 years. Tests for the presence of viral pathogens in clinical cases of unknown etiology (especially suspected viral hemorrhagic fever) were done throughout the year for the Ministry of Health / Ghana Health Service.

The studies on viral etiological agents of infantile diarrhea continued and the focus on Rota virus has been expanded to include investigations on the role of Noro virus in episodes of diarrhea in Ghanaian children.

HIV Studies

Longitudinal study of HIV/AIDS for emergence of HIV drug resistance mutations

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Background

The main objective of antiretroviral therapy (ART) is to reduce death and morbidity and improve the quality and lifespan of persons living with HIV/AIDS disease. Patients on ART in Ghana are supported to remain on first line therapy for as long as practicable.

Objectives

The aim of ART is to maintain the efficacy of the therapy for as long as possible in patients.

Approach

A cohort of HIV patients was established by recruiting with informed consent in 2010, 300 patients on ART at the Koforidua Government Hospital. These patients were followed up between October 2010 and June 2013. Blood samples were processed to obtain cellular genomic material, which were retrotranscribed and sequenced in the HIV *pol* gene region. The sequence data of reverse transcriptase (RT) and protease (PRT)-coding regions of the gene were investigated for mutations with the potential to develop resistance to ARVs.

Findings & Implications

Viral load measurements were undetectable for 77% of 300 patients in 2011, 81% in 2012 and 92% of 270 patients in 2013 indicating successful benefit from ART. In the same period, CD4 count levels were 69% (2011), 68% (2012) and 62% (2013). Improvement in CD4 counts in the same patients however did not necessarily correlate with undetectable viral loads. Immune responses were poorer, suggesting that viral loads may be a better surrogate of the efficacy of ARVs compared with CD4 counts. Moderate success and failure could be ascribed to serious adverse reaction or stigma leading to non-adherence, poor immune response.

Future Directions

Further studies will be conducted to improve the number of HIV/AIDS disease persons who can benefit from ART.

Human leukocyte antigen (HLA) typing and associated human immunodeficiency virus (HIV) and human T cell lymphotrophic virus type 1 (HTLV-1) genome mutations in Ghana

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	Taku Nakane, PhD
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Background: The human leukocyte antigen (HLA)-restricted cytotoxic T-lymphocyte (CTL) immune response is one of the major factors determining the genetic diversity of HIV and HTLV-1. There are few studies on the amino acid variations associated with the host HLA type and their clinical relevance in patients from Africa. This study therefore seeks to investigate HLA associated polymorphisms in HIV-1 gag protein and HTLV-1 core protein in HIV/HTLV-1 infected Ghanaian individuals, and also examine the CTL escape mutations of HIV-1.

Approach: One hundred and four HIV-infected persons, naïve to antiretroviral therapy (ART) were recruited from the Eastern Regional Hospital, Koforidua. Written informed consent was obtained from eligible patients and venous blood was collected and transported to the NMIMR, Legon. Blood samples were processed into peripheral blood mononuclear cells (PBMC) and plasma. Serology, to determine presence of HTLV-1 antibodies, HIV type, viral load estimations and DNA extractions were done at the NMIMR, Legon, while HLA typing and sequencing of the gag gene of HIV and core genes of HTLV-1 was performed at the AIDS Research Center, National Institute of Infectious Diseases (ARC - NIID), Tokyo, Japan.

Findings & Implications: From 104 blood samples obtained from HIV patients after informed consent, 85 patients (82%) were confirmed as HIV-1, two subjects (2%) as HIV-2, fourteen patients (13%) had HIV-1/2 dual infections and 3 cases (3%) were negative for HIV infection. Three HIV patients (3%) were also were positive for HTLV-1 antibodies. Typing and sequencing of the HLA are underway at the ARC-NIID in Tokyo, Japan. The clinical team at Koforidua Hospital reviewed the clinical status of three patients who were determined to be negative for HIV infection.

Serological profiles of HIV infected patients on antiretroviral therapy in Ghana.

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Background

Since 1986 when the first case of HIV infection in Ghana was detected by the NMIMR, the AIDS epidemic in Ghana has been characterized by the presence of both HIV-1 and HIV-2 infections with a dominance of HIV-1 since the mid 1990s. The National AIDS/STI Control Program established a survey to monitor the emergence of drug resistance to first-line antiretroviral therapy in Ghana.

Objectives

As the national HIV drug resistance (HIVDR) survey protocols target HIV-1 infections, it was deemed necessary to determine the type of HIV infection prior to genotypic analyses. Serological studies on patients enrolled into the HIVDR survey were therefore conducted.

Approach

One thousand, two hundred and twenty seven HIV seropositive samples, collected from 10 antiretroviral therapy centres for an HIV drug resistance survey were analyzed at the NMIMR, Legon. These samples had been previously tested at the collection sites with rapid assays for HIV. Serotyping was performed with Inno-lia HIV-I/II Score, a line immunoblot assay from Innogenetics, Belgium.

Findings

Out of the 1227 samples tested, 95.7% had antibodies to HIV-1, 1% had antibodies to HIV-2 and 3.3% had antibodies to both HIV-1 and HIV-2. The prevalence of HIV-1 infection ranged from 89.1% in Agomanya to 100% in Sunyani and Tamale while that of HIV-2 ranged from 0% in Tamale and Sunyani to 3.5% in Koforidua.

Implications

The results confirmed the co-circulation of HIV-1 and HIV-2 in Ghana. It further corroborated data from the 2011 HIV Sentinel Survey and emphasized the predominance of HIV-1 in the country.

Future Direction

Although genotyping assays for HIV-1 will provide vital information on HIV drug resistance in the larger proportion of HIV-infected patients, it is important to also monitor the emergence of drug resistance mutations in dual HIV-1/-2 and HIV-2 only infected patients.

Influenza Virus Surveillance

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Influenza virus surveillance in Ghana is carried out by the National Influenza Center (NIC), which is housed in the Virology Department. This activity is supported by the U.S. Naval Medical Research Unit No. 3 (NAMRU-3), the U.S. Centers for Disease Control and Prevention (CDC) in conjunction with the Ghana Health Service (GHS) and the World Health Organization (WHO). In addition, the Ghana NIC provides technical assistance to neighboring countries for influenza surveillance. Sentinel sites was operated in all ten regions of Ghana with 24 influenza-like illness (ILI) and 4 severe acute respiratory illness (SARI) sites. The NIC also collaborated with the Ghana Armed Forces Health Directorate and US NMARU3 to maintain influenza surveillance in nine military medical facilities in five regions.

Approach

Sentinel sites submit respiratory samples from suspected cases weekly to the NIC for influenza virus testing, which enabled weekly updates to the Ghana Health Service and the WHO. The NIC also provided technical assistance to Sierra Leone and Burkina Faso and tested samples that enabled influenza surveillance reports to WHO by Sierra Leone in 2013.

Findings

Over the 3-year period 2012 to 2014, a total of 8,601 respiratory specimens collected from 33 sites (civilian and military) across the country were tested for influenza and 1041 were positive for the presence of influenza virus. Representative Ghanaian influenza virus isolates were shared with the WHO Collaborating Centre (CC) in London for a contribution to the global antigenic analysis of circulating influenza viruses. The Ghana NIC also tested 241 respiratory samples (9 influenza A(H3N2) viruses were detected) on behalf of the Central Public Health Laboratory, Sierra Leone. The table below shows the total number of respiratory samples received and processed by the NIC over the three year period under review with influenza A virus dominant over influenza B .

Table 9. Total number of respiratory samples received at the NIC from 2012 – 2014

YEAR	SPECIMEN TESTED	FLU A	FLU B	TOTAL FLU CASES
2012	3065	334	166	500
2013	2778	211	49	260
2014	2758	113	168	281
TOTAL	**8601**	**658**	**383**	**1041**

The graph below (Fig 40) presents a clear profile of influenza virus sub types in circulation in Ghana during the period as per weekly data submitted into the WHO global influenza surveillance and response system FluNet online program.

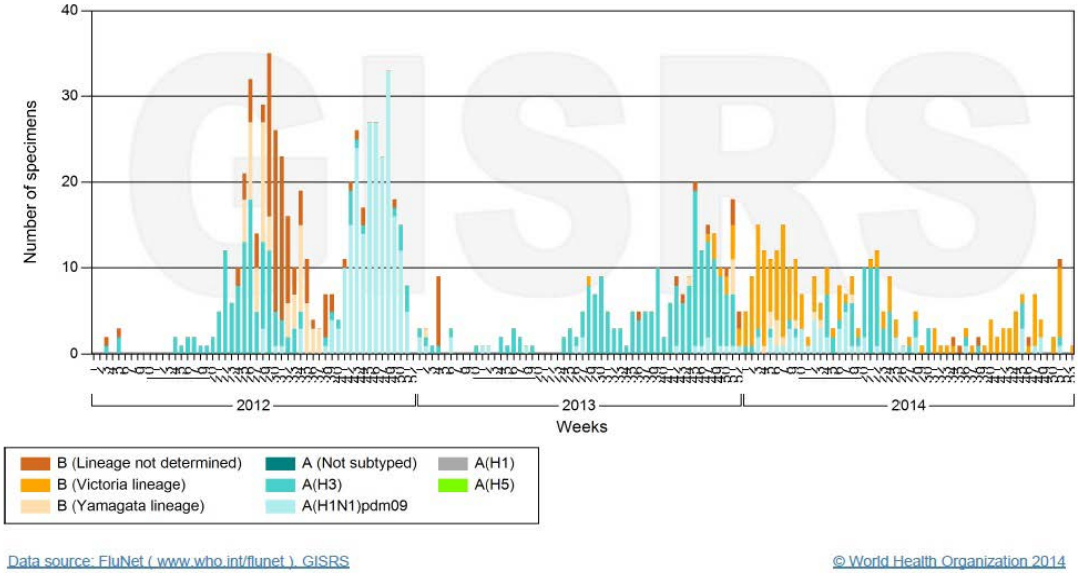

Fig. 40: Profile of circulating influenza virus sub types in Ghana, 2012 - 2014

Under capacity building, the NIC carried out the following:

- Trained Ghana Health Service staff members from selected district and regional hospitals with the Integrated Management of Adolescent and Adult Illnesses Guidelines to improve health care capacity for severe acute respiratory illnesses.
- Conducted a one-day meeting of Deputy Directors of Public Health of the Ghana Health Service and discussed national preparedness and response to the emergence of Influenza A (H7N9) and the Novel Coronavirus.
- Updated Regional and District Surveillance/Disease Control Officers from 3 regions in Ghana on surveillance, outbreak investigations and implementation of the IHR 2005 with special emphasis on respiratory infections such as pandemic influenza.
- Conducted a 5 day training workshop on laboratory diagnosis of influenza and other respiratory viruses for 10 West African countries under a US CDC collaborative agreement with technical assistance from the WHO.

To strengthen the collaboration with Ghana Armed Forces, a mid-year meeting was held at the NMIMR to review progress and explore mechanisms to integrate influenza surveillance into routine medical services.

Fig. 41: Pictures of the meeting and participants at the NMIMR conference hall

Surveillance For Other Respiratory Viruses

The National Influenza Center and the Ghana Health Service Port health unit, screened about 5,000 Hajj pilgrims who had returned from Saudi Arabia for respiratory viral infections in 2013 (Fig 42).

A semi-structured questionnaire was used to collect bio data; name, date of birth, sex, nationality, place of residence and date of onset of symptoms from symptomatic pilgrims.


Fig. 42: NIC staff collecting throat swabs during the screening exercise

Nasopharyngeal and oropharyngeal swabs were collected from persons with cough and sore throat and tested for influenza and the Middle East respiratory syndrome coronavirus (MERS-CoV.) A total of 518 pilgrims were tested for MERS-CoV and all were negative for the presence of this virus. However, 29 persons were positive for influenza virus with 14 A(H1N1)pdm09, 3 A(H3N2) and 12 Influenza B. The screening of Hajj pilgrims is likely to be conducted annually as MERS-CoV is regarded as a potential public health threat in addition to pandemic influenza.

Poliovirus Research

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Since 2003, the World Health Organization (WHO) and the Noguchi Memorial Institute for Medical Research (NMIMR) have maintained a technical service agreement. Under this mandate, a WHO accredited Regional Reference Poliovirus laboratory (RRL) at the Virology Department, supports the Global Poliomyelitis Virus Eradication Initiative in the West African sub-region.

Poliovirus Regional Reference Laboratory (RRL) activities (2012-214)

Background

The Virology Department as Poliovirus RRL, provides the critical role of laboratory detection and confirmation of poliovirus infection in acute flaccid paralysis (AFP) surveillance in Benin, Ghana and Togo.

Approach

Polio Virus Isolation: From 2012 to 2014, isolation and characterization of Polioviruses in stool specimens from a total of 1,805 new acute flaccid paralysis (AFP) cases were performed for Ghana, Togo, and Benin by the RRL.

Intratypic Differentiation: The polioviruses isolated were subjected to intratypic differentiation (ITD) by rRT-PCR, while Sabin isolates were further screened using rRT-PCR VDPD. Wild poliovirus confirmation was referred to the South Africa WHO poliovirus laboratory for sequencing.

Findings

The total number of stool samples processed from 2012-214 was 3,600 and the results were reported to the WHO within the stipulated 14 days for 100% timeliness. The details of samples processed were as follows: Ghana (1851), Togo (759), and Benin (990) as shown in Table 10, Two hundred and ten (210) poliovirus isolates were obtained with 621 non-polio enteroviruses (NPEV) isolated. There was a high percentage of virus isolation indicating optimal sensitivity of the cell cultures used.

The types of polioviruses isolated were as follows: Ghana - type one Sabin strains (72), type two Sabin strains (36) and type three Sabin strains (36); Togo - type one Sabin strains (11), type two Sabin strains (11), type three Sabin strains (30); and Benin - type one Sabin strains (25), type two Sabin strains (9) and type three Sabin strains (25). No wild poliovirus was isolated from any of the 3 countries in 2013 (Table 11).

Table 10: Polio Virus Isolates Obtained From Afp Stools: 2012 To 2014

Country	2012					2013					2014				
	Cases	Specimen Number	Results in 14 days n(%)	Polio isolates	NPENT n (%)	Cases	Specimen number	Results in 14 days n(%)	Polio isolates	NPENT n (%)	Cases	Specimen Number	Results in 14 days n(%)	Polio isolates	NPENT n (%)
BENIN	153	306	306 (100%)	17	52 (17.2%)	181	362	362 (100%)	19	61 (16.9%)	161	322	322 (100%)	15	62 (19.3%)
GHANA	199	396	396 (100%)	20	84 (21.2%)	342	682	682 (100%)	46	128 (18.7%)	389	773	759 (98.2%)	51	115 (14.9%)
TOGO	90	179	179 (100%)	13	32 (17.3%)	156	312	312 (100%)	19	45 (14.4%)	134	268	268 (100%)	10	43 (16.0%)
TOTAL	442	881	881 (100%)	50	168 (19.1%)	679	1356	1356 (100%)	84	233 (17. 2%)	684	1363	1349(99%)	76	220 (16.1%)

Table 11: Poliovirus Types Detected; 2012 to 2014

	2012					2013					2014				
Country	No. of Cases	no. of specimen	P1	P2	P3	No. of Cases	no. of specimen	P1	P2	P3	No. of Cases	no. of specimen	P1	P2	P3
BENIN	153	306	9	2	8	181	362	9	5	11	161	322	7	2	6
GHANA	199	396	10	2	10	342	682	30	26	12	389	773	32	8	14
TOGO	90	179	4	0	11	156	312	4	5	13	134	268	3	6	6
TOTAL	442	881	23	4	29	679	1356	43	36	36	684	1363	42	16	26

Sequencing: WHO has supported the RRL to upgrade a genetic analyzer machine with onsite training of staff in poliovirus sequencing. This has equipped the RRL to perform sequencing of any wild type strains identified in the sub-region.

Reporting: Weekly laboratory reports were sent to Ghana Health Service EPI and Surveillance teams, WHO country office and WHO HQ to up-date all partners on laboratory operations.

Data Cleaning and Reconciliation: The RRL is a member of the national polio team that met regularly for data cleaning and data reconciliation. The Polio laboratory maintained full accreditation status due to excellent performance during the period 2012 to 2014. Future work plans include the conduct of field sampling from healthy school children to investigate both polio and non-polio enteroviruses.

Characterization of non-polio enteroviruses - human enteroviruses isolated during acute flaccid paralysis surveillance in Ghana

Background

Studies on the prevalence and distribution of non-polio enteroviruses (NPEV) in Ghana, have included virus isolation from acute flaccid paralysis cases and healthy children in Ghana. This is necessary to complement efforts on the eradication of poliomyelitis in Ghana.

Approach

Stool suspension was prepared from 308 samples received in 2009 from the surveillance activities throughout the country and inoculated on both RD and L20B cell lines. Isolates that showed growth on L20B were selected for real-time RT-PCR using degenerate and non-degenerate primers and probes. RD isolates were characterized by micro-neutralisation technique with antisera pools. Viruses that were un-typable were subjected to neutralization assay using antibodies specific for E71

Findings

Of the 308 samples processed, 17 (5.5%) grew on both L20B and RD cells while 32 (10.4%) grew on RD only. All 28 isolates from L20B were characterized by rRT-PCR as Sabin-like polioviruses. No wild poliovirus or VDPV was found. Six different enteroviruses were identified and Coxsackie B was most predominant, followed by Echovirus. Three children from whom non-polio enteroviruses were isolated had residual paralysis.

Implications

The study showed the absence of wild or vaccine-derived poliovirus circulation in the country. However, the detection of three non-polio enteroviruses and one Sabin-like poliovirus with residual paralysis calls for continuous surveillance even in the post-polio eradication era.

Viral Haemorrhagic Fevers

Laboratory investigations of clinical cases for hemorrhagic fever viruses

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Background

Viral hemorrhagic fevers (VHF) are acute diseases characterized by bleeding, organ failure, and shock. These VHF cases are difficult to distinguish clinically from other diseases including viral hepatitis. In West Africa including Ghana, VHFs such as Yellow Fever, Lassa Fever, Dengue are known to be endemic with infrequent reports of Ebola, Marbug, West Nile and Crimean Congo hemorrhagic fever viruses.

Methods

We previously conducted a three-year study to determine the involvement of hemorrhagic fever and hepatitis viruses in morbidity and mortality in the Central and Northern parts of Ghana. Under this study, protocols based on molecular diagnosis for direct detection of viral genomes were established to test blood specimens from suspected cases for Yellow Fever, Marburg, Lassa, Ebola, West Nile and Dengue viruses. In 2013, blood samples from 53 patients from various health facilities with VHF symptoms were referred either via the national Public Health Reference Laboratory (NPHRL) in Accra or directly to the NMIMR for VHF tests. These specimens were subjected to polymerase chain reaction for the direct detection of Lassa, Ebola, Marburg, Yellow fever, Dengue fever and West Nile viruses.

To further strengthen diagnostic capacity for VHFs in Ghana, a collaboration was established with the US Centers for Disease Control and Prevention, Atlanta. Staff from the NPHRL and the Virology department were trained at the NMIMR P3 facility, on the use of enzyme linked immunosorbent assays (ELISA) to detect the presence of Lassa fever antibodies in serum specimens. The programme was a hands-on training to detect human anti-Lassa IgG, IgM and also reviewed Lassa virus RT-PCR SOPs at the department. The training also included troubleshooting to remedy errors in test procedures and/or results generation.

Findings & Implications

We did not confirm any of the cases from the two geographical areas for Lassa, Ebola, Marburg, Yellow fever, Dengue fever and West Nile using the molecular assays. However, we detected

Immunoglobulin M class antibodies against Lassa fever in a contact of a confirmed case from Liberia. This indicates that, screening of suspected VHF cases for Yellow fever infection only, is insufficient and that tests for other hemorrhagic fever viruses need to be integrated into the national surveillance system.

Laboratory investigations of suspected cases of Ebola virus disease

From March 2014, a team was established to determine the presence of Ebola virus in specimens from patients suspected of Ebola virus disease (EVD) in Ghana. The laboratory investigations were conducted in partnership with the Ministry of Health / Ghana Health Service. The NMIMR is the designated national center by the Ministry of Health for the laboratory investigations of suspected cases of Ebola virus disease (EVD) in Ghana. It is a member of the EVD national technical coordinating committee and the Emergency Operations Center for EVD. A viral haemorrhagic fever (VHF) laboratory team of 8 persons was established with training on all related standard operational protocols (SOPs). This team was charged with the special responsibility to receive and rapidly process all suspected cases submitted even over weekends or holidays. These SOPs included the use of personal protective equipment (PPE), safe blood sampling, shipment of specimens, testing for ebola virus and other VHFs, data management and results reporting. Strict enforcement of these SOPS was ensured with receipt and processing of blood samples from suspected EVD patients only in the Pathogen level 3 facility (Fig 43).

Fig 43

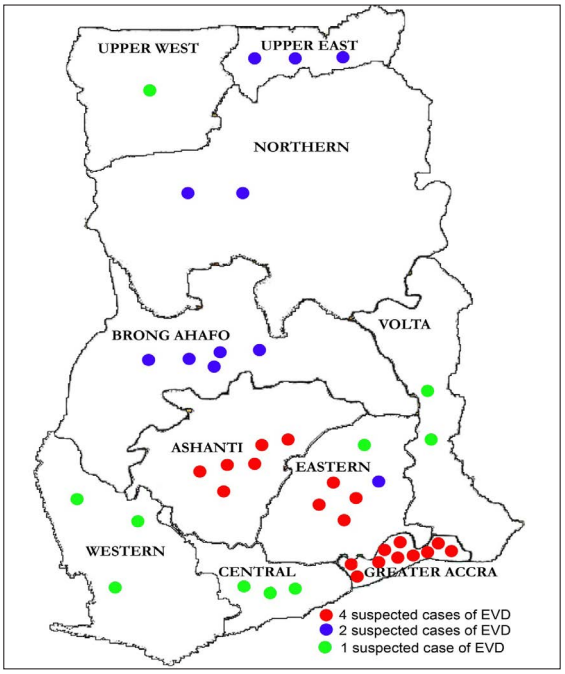

Fig 44.

Conventional and real time RT- PCRs for the detection of Ebola, Marburg, Lassa fever, Yellow fever, Dengue and West Nile viruses were conducted on 140 suspected EVD cases received in 2014 from health facilities across the country (Fig 44). Various agencies including the private sector (financial institutions and telecommunications service companies) supported this activity, supplementing funds from the Ministry of Health, WHO and JICA.

Human-animal interphase study on zoonotic pathogens

Investigators

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Background

The Integrated human-animal-vector surveillance project investigated endemic and emerging vector-borne and zoonotic pathogens in targeted high-risk populations. The specific aim was to investigate potential cross transmission of pathogens between sheep, goats and their human handlers.

Methods

This study scrutinized the prevalence of some high-risk pathogens among animals and their human handlers in the Kumasi abattoir, within a dense human/animal interphase environment. Field samples were collected between September 2010 and September 2012 from humans, sheep, goats and cattle to the Department of Virology, NMIMR. The samples were processed for the presence of vector-borne and zoonotic pathogens.

Findings

Both humans and animals (sheep and goats) were reactive for antibodies to the pathogens tested, namely Rift Valley Fever (RVF), West Nile (WNV), Dengue Fever (DF), Crimean-Congo Hemorrhagic Fever (CCHF), Hepatitis E Virus (HEV) and Sandfly Fever viruses (SFV). In addition, *Coxiella burnetti* (Q fever, QF), *Leptospira* sp. (LPT) and *Brucella* spp (BRC) infections were detected. The results provide an indication of agents of neglected diseases circulating in Ghana, which lack active surveillance. Infection with Sandfly Fever Virus (SFV) was detectable simultaneously in humans and animals.

Future Direction

There is a need to investigate the possibility of cross transmission of SFV between sheep, goats and their human handlers in subsequent studies. Further molecular epidemiology studies are planned to investigate zoonotic transmission modes between the animals and human handlers.

Effectiveness of the Monovalent Rotavirus Vaccine (Rotarix TM) against Severe Rotavirus Diarrhoea in Ghana

Investigators:

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	K. Amponsa-Achiano, MB,ChB; MPH
University of Health and Allied Sciences	*Fred N. Binka, MB, ChB; MPH; PhD*
Centres for Disease Control, (CDC) Atlanta	*Ben Loopman, PhD*
	Umesh Prashar, PhD
	Manish Patel, PhD
Funding	*CDC Foundation; PATH*

Background

Diarrhea disease is the second leading cause of morbidity and mortality in children under five years of age with more than 1.3 million deaths occurring globally every year of which nearly 800,000 are in Africa. (1) Rotavirus is the most common cause of severe diarrhea in children <5 years of age globally, accounting for an estimated 2.4 million hospital admissions and 450,000 deaths each year (2). Ghana introduced the Rotarix vaccine into the EPI and for the past year had an aggressive programme to vaccinate all eligible children with this vaccine. As part of the vaccine introduction process, there was the need and request from the WHO and the Ghana Health service to set up a phase 4 study to monitor the impact of the vaccine introduction as well as the rare disease of intussusception. With funding from PATH through the CDC and support from the WHO, Noguchi is collaborating with the Ghana Health Service to set up a national surveillance to monitor the effectiveness of the rotavirus vaccine.

Methodology

Children born on or after April 1, 2012, hospitalized for diarrhea or who received intravenous hydration in the emergency department and tested positive for rotavirus by ELISA were enrolled as case patients in this study. All case-patients were identified through an ongoing active surveillance system that is measuring rotavirus disease burden and strain distribution in 5 out of the 10 regions of Ghana.

Amongst rotavirus cases, a random selection of fully vaccinated (i.e., those receiving 2 doses of Rotarix with routine EPI vaccines at approximately 2 and 4 months of age, with maximum upper age limit of 24 weeks) and unvaccinated children will be selected and their homes visited and their household contacts tested for evidence of rotavirus infection. This is to help estimate the secondary spread and asymptomatic infection in the households and community.

Objectives:

- The objective of this study was thus to measure the impact of rotavirus vaccination on rotavirus and all-cause diarrhea morbidity in Ghana using a case control design.
- The study also seeks to measure directly the role and contribution of young children in the spread of rotavirus and the impact of vaccination in this process providing important scientific/public health answers regarding how vaccination impacts the spread of rotavirus.
- Finally, this evaluation will strengthen support for the continued procurement of vaccines locally and provide important information for other low and middle-income countries considering a similar vaccine introduction strategy.

Work progress

The recruitment started first at the Navrongo War Memorial Hospital in the Upper East and then gradually expanded to Greater Accra, Ashanti, Volta and Western Regions by the end of February 2013. The study had enrolled 433 participants of which 423, more than 95%, had received at least one dose of the Rotarix rotavirus vaccine. The vaccination status of participants was verifiable by the examination of EPI weighing/vaccination cards in 80% of respondents. Of the 433 diarrhoea stool samples tested for rotavirus antigens, 111 (26%) of the children were found to be shedding rotaviruses in their stools. For the household surveys, 48 households have been visited and 74 household members interviewed. A total of 74 stool samples have been obtained from household members of children shedding rotaviruses and tested. The rotavirus positivity rate amongst household members is 15%.

Preliminary Results

There was a general reduction in diarrhea hospitalizations observed in all the study sites. (Fig. 45). We observed a marked reduction in hospitalizations in the vaccination target group after the first year of vaccine introduction. Although a reduction in hospitalization was observed in older children, it was not as dramatic as that in the children less than 12 month of age. We hope to have a fuller picture of the trend at the end of the study.

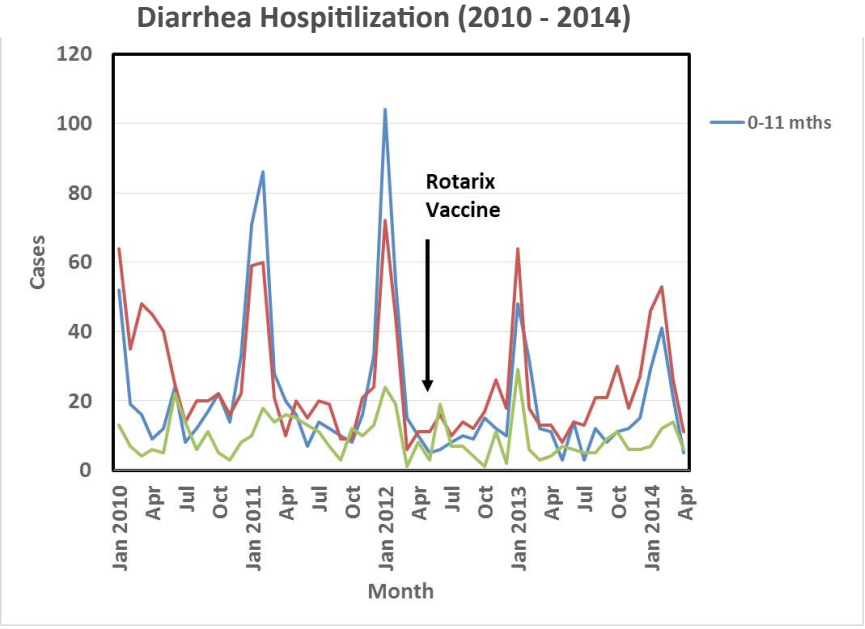

Fig. 45: Pre and post vaccine introduction temporal trend of diarrhoea distribution by age group

Post-marketing Intussusception Monitoring after Introduction of Oral Rotavirus Vaccines

Investigators	
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Centres for Disease Control (CDC)	*Manish Patel, PhD*
	Umesh Parasher, PhD
Funding	*CDC Foundation; PATH*

Background

Rotavirus infection is the leading cause of severe dehydrating gastroenteritis responsible for >500,000 annual deaths worldwide in children <5 years of age [1]. Based on this tremendous disease burden and associated costs, the World Health Organization (WHO) recommends two new rotavirus vaccines -- RotaTeq and Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) – for all infants worldwide. These vaccines have demonstrated good efficacy against severe rotavirus disease with an efficacy of 85%-98% in the middle and high-income countries and 39%-77% in African and Asian countries [2]. As rotavirus vaccines are introduced into immunization programmes, monitoring their safety is a high priority because of possible differences in factors such as age at immunization and the characteristics of infants receiving the vaccine. In view of the past experience with rotavirus vaccines [3], both with respect to the risk of adverse effects and varying efficacy in different settings, countries planning to introduce rotavirus vaccines or which have introduced rotavirus vaccines are encouraged by the WHO's Global Advisory Committee on Vaccine Safety (GACVS) to develop a system of post-marketing surveillance for these vaccines using a standardized approach to address potential safety issues at the population level to accompany the introduction and implementation of rotavirus vaccines.

A study was initiated in July 2013 at the two largest hospitals in Ghana, the Korle Bu Teaching Hospital in Accra and the Komfo Anokye Teaching Hospital in Kumasi to collect and document information on ISS in Ghana both retrospectively and prospectively.

Objectives

The primary goal of this evaluation is to monitor the safety of rotavirus vaccines under routine public health use among infants in Ghana. The specific aim of this evaluation is to monitor

vaccine safety by assessing for any potential association between oral rotavirus vaccines and intussusception, after national introduction of the vaccine in the routine childhood immunization schedule. The procedures will include 3 general steps: 1) surveillance for intussusception case-patients at sentinel paediatric hospitals; 2) verification of vaccination status; 3) determining by the use of a self-controlled case-series (SCSS) whether an association exists between oral rotavirus vaccines and intussusception.

Methods

Intussusception following rotavirus vaccination was monitored using the SCCS method. The SCCS is a simple method that uses data on case-patients alone (i.e. without external control group) gathered through a sentinel surveillance system to assess if a safety risk exists in defined time intervals after vaccination. This allows the comparison of the relative incidence of intussusception within the risk window of interest (1-7 days after vaccine receipt) with the incidence of risk outside the immediate post vaccination period. The risk window is the period during which the infant is likely to be at highest risk for developing intussusception on the basis of peak period of viral replication in the intestine. Active surveillance was conducted through the review of admission logs on the Paediatric Ward to track all children aged under 12 months who are admitted to the hospital for suspected intussusception. All children who met the Level 1 Brighton Collaboration criterion were enrolled in the study. The training programme for study site coordinators and site personnel involved in the study was conducted between January and June 2013. This was capped with an investigators meeting at Noguchi attended by collaborators from the Centre for Disease Control, Atlanta, USA. Active surveillance begun in August 2013 in the Departments of Paediatric and Surgery of the participating hospitals - Komfo Anokye Teaching Hospital in Kumasi and the Korle Bu Teaching Hospital in Accra.

Preliminary Findings

The study has enrolled a total of 181 cases, 35 retrospectively and 146 prospectively at the end of 2014 (Table 12). More than 85% of the children had been vaccinated and received at least a dose of the Rotarix© vaccine. Most of the intussusception cases recorded were in children between 6 -9 months of age and the majority were females. Fig. 46 shows the number of intussusception cases within 90 days of receiving one or two doses of the Rotarix© rotavirus vaccine (RV1 and RV2).

Table 12: Enrolled Intussusception (IS) cases (2013 to 2014)

	Korle Bu	KATH	Total
Number of IS cases <12 months of age	99	82	181
Number (%) of IS cases with card confirmed vaccination status	94 (95%)	62 (76%)	156 (86%)
N (%) of IS cases that died	2	6	8

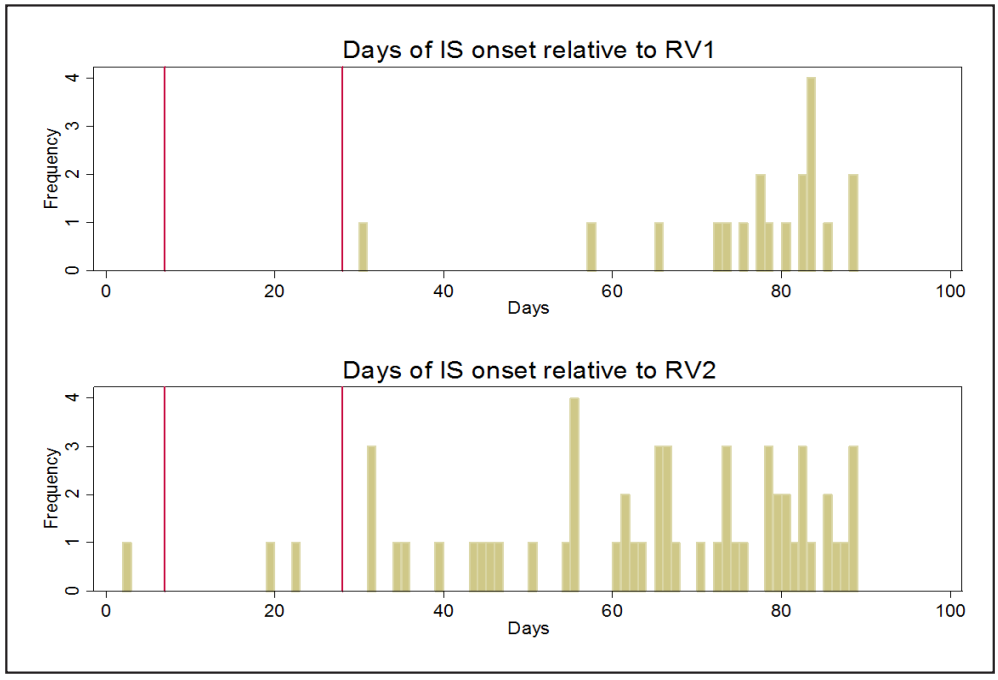

Fig. 46: Intussusception Cases within 90 days of vaccination

A Randomized, Double-Blind, Placebo-Controlled Evaluation of the Efficacy, Immunogenicity, and Safety of Two Single Doses of RRV TV in Neonates/Infants (Trial Registration Number: PACTR201110000333955)

Investigators

NMIMR,

Department of Electron Microscopy and Histopathology — *George E. Armah, PhD.*

University of Health and Allied Sciences — *Fred N. Binka, MB, ChB; MPH; PhD*

Navrongo Health Research Centre — *Abraham Hodgson, MB,ChB; MPH; PhD*

Funding — *Inter Medica Foundation, USA*

Introduction

Diarrheal diseases are responsible for high death rates in children below 5 years of age and account for high mortality below 2 years of age in developing countries. Of all diarrheal illnesses globally, more than 125 million cases of diarrhea can be attributed to RV, which causes an estimated 25% of all deaths due to diarrheal disease and 6% of all deaths in children younger than 5 years of age in developing countries. Two licensed rotavirus vaccines; Rotarix and RotaTeq are presently licensed for use in developed countries and have showed a marked impact in reducing the burden of rotavirus diarrhoea in the Americas, Australia, South Africa, and some European countries. Surveillance studies in Africa indicate an earlier exposure of African infants to rotavirus infection with 20% of infants infected before the age of one month. However, neither of the presently licensed vaccine is indicated for administration in the neonatal period, leaving a protection gap in

the first weeks or months of life. RRV TV is an orally administered vaccine being retested by the sponsor for the prevention of GE caused by rotavirus in neonates/infants. RRV TV was previously marketed as RotaShield® and was previously administered as three oral doses. A neonate/infant vaccine-dosing schedule would optimize delivery to protect the majority of children early in life.

Objectives

The primary objective of this study was to evaluate the efficacy of two doses of RRV TV in neonate/infant subjects against gastroenteritis with presence of rotavirus (RV GE). The study also sought to evaluate the efficacy of the vaccine against severe RV GE, in which the stool sample contained at least one of the serotypes present in the RRV TV vaccine. Additional secondary objectives included the evaluation of the immunogenicity and safety of 2 doses of RRV TV study participants.

Methodology

The study was conducted in Kassena Nankana and Bongo Districts of the Upper East Region This was a randomized, double blind, placebo controlled study in neonates/infants. The study consisted of a treatment period of two scheduled visits where participants received single doses of the vaccine or placebo (ratio 1:1) at 0 to 29 days, and the second dose at age 30 to 59 days and a follow up period. The dosing schedule was selected to ensure that no dose of vaccine was administered on or after 60 days of age. A subset of neonates/infants within the enrolled cohort was evaluated for immunogenicity to the RRV-TV vaccine. Immunogenicity was evaluated by measurement of serum anti RV Immunoglobulin A (IgA) antibody titers. The primary endpoint for efficacy evaluation was the occurrence of RV GE (defined as episodes of RV GE that occurred more than 2 weeks after the subject's last dose of treatment and for which a stool sample, taken no more than 7 days after the start of the subject's diarrhea, was available. All participants were evaluated for safety by monitoring all adverse events post immunization

Results

A total of 1030 subjects were screened and 998 subjects who met the eligibility criteria were randomized to treatment receive the RRV-TV vaccine (500) or placebo (498). A total of 949 (94.8%) subjects completed the study: (RRV-TV, 474 94.8%; Placebo, 475, 95.4%). Twenty subjects (2.9%) were lost to follow up and 29 (15 RRV-TV and 14 Placebo) died. The major causes of death were malaria, bronchopneumonia and respiratory tract infections.

Vaccine efficacy was 60.68% against any RV GE, 52.82% against serotype G1, 88.93% against serotype G3, and 64.63% against pooled vaccine serotypes. Consistently fewer RRV-TV subjects experienced RV GE with serotypes contained in the RRV-TV vaccine (serotypes G1, G2, G3, and G4) compared to Placebo group. Data analysis is still in progress.

Conclusions (interim)

RRV-TV was effective and well tolerated in this two-dose double-blind, placebo-controlled study in 998 neonate/infant subjects in rural Ghana. RRV-TV demonstrated consistently high levels of efficacy comparable to efficacy levels seen in a recent three-dose study with a different rotavirus vaccine in a similar subject population in the same locality in Ghana. Vaccine efficacy against

any RV GE experienced in the study was 60.68%, with efficacy against pooled vaccine serotypes of 64.4%. RRV-TV was well tolerated in this study population and severe adverse events post immunizations were similar between the RRV-TV and Placebo groups. The most common cause of severe adverse events was malaria, bronchopneumonia and respiratory tract infections. There were no documented cases of confirmed or suspected intussusceptions or any pattern of increased incidence of fever as has been observed in earlier trials of the Rotashield® vaccine.

Conclusion

These study results suggest that RRV-TV administered in two doses between the ages of 0 and 60 days provides safe and effective protection against RV GE, offering a new tool in the fight against a major cause of infant mortality.

ENTOMOLOGY

Entomological studies are based in the Department of Parasitology and have largely been on malaria vectors. The studies could be classified under 3 broad categories; monitoring and evaluation of insecticide susceptibility of malaria vectors in the country, field testing of the effectiveness of LLINs under the WHO Pesticides Evaluation Scheme (WHOPES) and assessment of EIRs in malaria control programs. Some studies have sought to identify vectors of VHFs in the country and lately some studies on the vectors of cutaneous leishmaniasis identified in the Volta Region of the country have been undertaken in collaboration with NAMRU-3.

Monitoring of Indoor Residual Spraying (IRS) Program in Northern Ghana

Investigators	
NMIMR	
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	Maxwell Appawu, PhD
	Samuel Dadzie, PhD
Funding	*USAID/President Malaria Initiative*

Background

Ghana is a beneficiary of a five-year President's Malaria Initiative indoor residual spraying (IRS) program being implemented by USAID through the Africa Abt IRS project in collaboration with the Ghana National Malaria Control Programme (NMCP). As part of the programme, (IRS) was started in Northern Ghana in 2008 and entomological monitoring has been carried out in the past four years in three IRS districts; Tolon Kumbungu (TKD), Savelugu /Nanton (SND) and Bunkpurugu-Yunyoo (BY), and in Tamale, a non-IRS district. Under the program, Abt Associates has enlisted the expertise of Noguchi Memorial Institute for Medical Research to provide oversight and quality assurance for the entomological monitoring activities in three (3) of the IRS districts in Northern Ghana.

Approach

As part of the scope of work for the year 2013 (FY13), Noguchi carried out some monitoring activities in the operational areas. These activities include monitoring the impact of IRS on vector behavior and insecticide susceptibility as well as on malaria transmission in the operational areas.

Key findings

The project after five years of monitoring has shown that the implementation of Indoor Residual Spraying programme in the operational areas has led to significant reduction of malaria transmission (Fig 47). The prudent management of insecticide resistance in the area has enabled that mosquitoes remain susceptible to some of the approved insecticides for IRS.

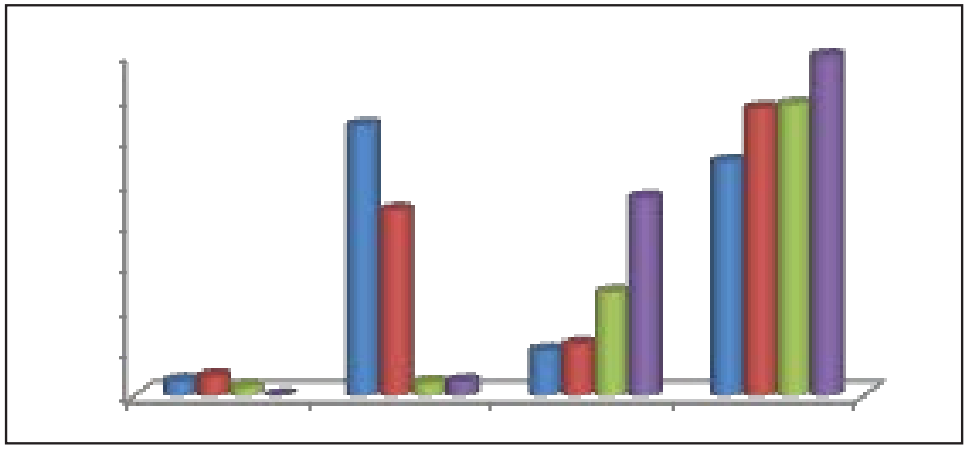

Fig. 47 Entomological inoculation rates in IRS and Non-IRS areas

Monitoring of insecticide resistance profile of malaria vectors in Ghana

Investigators

NMIMR

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Maxwell Appawu, PhD

Daniel A. Boakye, PhD

Funding — *USAID/President Malaria Initiative*

Background

The use of Insecticide treated nets (ITN) and indoor residual spraying (IRS) form the main vector control tools in the country. Pyrethroids are the insecticide of choice for ITNS/Long lasting Nets (LLNS). Currently, universal coverage for LLNS has been reached in the country. This makes pyrethroid use most widespread. IRS is also being carried in some areas in the country and this intervention uses pyrethroids. However, non-pyrethriods have been used due the detection of resistance to pyrethroids in some areas in the country. Resistance of malaria vectors to pyrethroids and other insecticides have been reported in many parts of Ghana.

Key expected outcomes

In collaboration with the Ghana Health Service, Malaria Vector Control Oversight Committee (MAvCOC) of the National Malaria Control Programme, the department has established the National Insecticide Resistance Monitoring Partnership (NIRMOP). This concept is to develop nationwide insecticide susceptibility maps from the sentinel sites and identify mechanisms involved in the resistance development to assist in its management. In 2013, training programmes have been organized for 56 Ghana Health Service staff selected across the 20 sentinel sites and logistics distributed to all the sites (Figs 48 and 49).

Fig 48: Training of Ghana Health Service Staff

Fig 49: Laboratory work at NMIMR

Phase III evaluation of long-lasting insecticidal nets:

(a) Large-scale (Phase III) evaluation of efficacy, fabric integrity and community acceptability of Olyset Plus long-lasting insecticidal nets compared with Olyset Net in Ghana (2013-2016)

Investigators	
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Department of Epidemiology	*Collins S. K. Ahorlu, PhD*
Funding	*WHO, Pesticide Evaluation Scheme (WHOPES)*

Background

The development of the technology of insecticide-treated materials (ITMs) such as insecticide treated nets (ITNs) has been one of the major innovations in the field of malariology during the past two decades. The importance of ITMs on malaria transmission in experimental trials in sub-Saharan Africa has been proven as shown by reduction of various entomologic indices http://www.ajtmh.org/cgi/content/full/68/4_suppl/16 - R4#R4 and more importantly, by reduction of morbidity and mortality due to malaria, especially in children. Removing the need for re-treatment of these nets can eliminate a major operational problem faced by net owners and ITN projects. Currently, some manufacturers in collaboration with WHO have developed long-lasting insecticidal nets (LLNs) that in principle, retain effective concentrations of insecticide after long-term use and repeated washings.

Approach

This study is a Phase III household single blinded randomized, equivalence trial of Olyset Plus and Olyset LLNs according to standard WHO guidelines and procedures at Asutsuare, in the Greater Accra Region of Ghana. Household randomized trial would be undertaken in Osudoku District of Ghana to evaluate and compare the efficacy, fabric durability, community acceptance and washing methods as well as adverse events of the two set of nets under field conditions.

(b) Phase III evaluation to compare insecticidal efficacy, longevity, fabric integrity and community acceptance of long-lasting insecticidal net PermaNet® 3.0 with PermaNet® 2.0 in Ghana (*2013-2016*)

Approach

This is a Phase III household single blinded randomized, equivalence trial of Permanet 3.0 and Permanet 2.0 LLINs according to standard WHO guidelines and procedures at Asutsuare, in the Greater Accra Region of Ghana.

Objectives

To evaluate and compare the efficacy, fabric durability, community acceptance and washing methods as well as adverse events of the two set of nets under field conditions.

Assessment of public attitudes to the potential use of genetically modified mosquitoes as a malaria control tool in Ghana

Investigators	
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Department of Epidemiology	*Collins S. K. Ahorlu, PhD*
School of Allied Health Sciences, CHS	*Charles A. Brown, PhD*
Funding:	*J-GRID, MEXT, Japan*

Background

The challenges posed by current control strategies of bednet usage, indoor residual spraying and larviciding, as well as the development of insecticide resistance to all the major classes of insecticides used against the vectors have led to a renewed interest in the use of GMMs. While various studies on attitudes to GMM and open releases have been undertaken in some developed countries, this information is missing in Africa and other developing countries. Thus, we carried out a pilot questionnaire study, aimed at understanding the needs, requirements and factors necessary for the acceptance of GM mosquitoes as a potential malaria control tool in Ghana.

Approach

The study was conducted in and around the University of Ghana (UG) Campus, since this provides an assembly of people from different backgrounds and ethnicity who work at the UG or are students. A total of 210 questionnaires were administered to individuals 18 years and above.

Findings

This was a pilot survey of young, mostly educated adults (18–29yrs) in an urban setting to determine attitudes to GMMs. Despite their background, several identified concerns that they would like to see addressed in any programme of GMMs in malaria control. These include; safety to humans and the environment (45.8%), effectiveness of the technology (27.1%), resistance of GMMs to control measures (18.8%). Respondents identified the following as required information prior to deployment; evidence from successful laboratory trials (58.6%), extensive public awareness and educational campaign on the technology (53.5%), provision of safeguards against adverse effects (56.7%) and a dialogue and approval by the majority of the community (48.5%). Approximately a third of respondents (34.3%) would want to be able to stop the program if need be.

Sources that respondents would trust for credible information on GMMs included local scientific organizations (70%), the United Nations (44.3%), and the Ghanaian government (35.5%). Less than a fifth of respondents would trust the local media (15.8%) and religious groups (8.4%) as sources of credible information on GMMs. In this urbanized educated community, only a quarter of respondents (24.8%) would agree to the release of GMMs while almost one in five respondents would never agree to a release under any circumstances.

Despite the achievements in developing GMMs, there are risks, benefits and public acceptance challenges that must be addressed in every country before any field trials and eventual release of the GMMs could be undertaken. In this pilot study in an urbanized mostly educated community, we identified social and ethical concerns among other issues that will need to be explored before any deployment of the technology.

Insecticide susceptibility and the transmission risk of viral hemorrhagic fevers on the University of Ghana Campus in southern Ghana

Investigators

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	Samuel Dadzie, PhD
	J H N Osei, MPhil
	A. M Adimazoya, MPhil
Tokyo Medical and Dental University	
Section of Environmental Parasitology	*Nobuo Ohta, PhD*
	A. Sasaki
	Takashi Suzuki, PhD
Funding	*J-GRID /MEXT, Japan*

Background

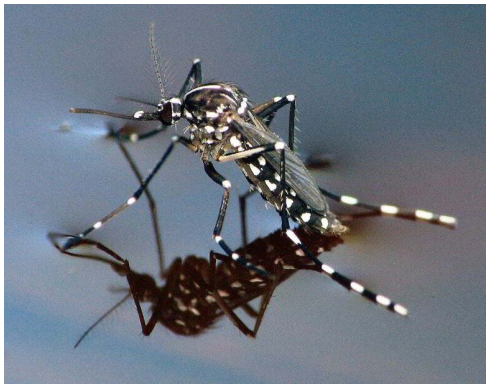
In order to control viral hemorrhagic fever (VHF) such as dengue and yellow fever, it is necessary to control the vector, *Aedes* mosquitoes.

Approach

Entomological survey to understand the species composition, VHF risk assessment and WHO insecticide susceptibility test were conducted on *Aedes* mosquitoes sampled from Legon campus, Accra. A total of 170 households were surveyed.

Preliminary findings

Nine hundred and eighty five (985) *Aedes* mosquitoes were morphologically identified. *Aedes aegypti*, and *Ae. vittatus* formed 75.5% and 23.9 % respectively with the rest being *Ae. albopictus* and *Ae. granti*. This is the first report of *Ae. albopictus* in Ghana and needs further studies to confirm (Fig 50). From the larval surveys, household index, Breteau index, and container index were calculated as 8.2%, 11.2% and 10.3% respectively. The mortalities recorded from the WHO insecticide susceptibility test for DDT (4%), deltamethrin (0.05%), lambdacyhalothrin (0.05%) and permethrin (0.75%) were 88%, 94%, 80% and 99% respectively. The man-vector contact rate was 0.42. From this survey, the density of *Aedes* mosquitoes was considered to be sufficient to promote an outbreak of VHF. *Aedes* mosquitoes were revealed to be resistant to DDT, but susceptible to Permethrin.



Aedes albopictus

Aedes aegypti

Fig 50: Aedes mosquitoes (source: http://www.cirrusimage.com/fly_mosquitoes.htm)

Nationwide Investigation of the Risks of Dengue and Yellow Fever and the Pyrethroid Susceptibility of *Aedes* Mosquitoes in Ghana

Investigators

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Section of Environmental Parasitology — *Takashi Suzuki, PhD*; *Nobuo Ohta, PhD*

Nagasaki University, Institute of Tropical Medicine — *Hitoshi Kawada, PhD*; *NoburoMinakawa, PhD*; *Yukiko Higa, PhD*; *Kyoto Futami, PhD*

Funding — *MEXT, Japan*

Background

Aedes aegypti is the most important vector of dengue, dengue haemorrhagic fever (DF) and yellow fever (YF). YF is endemic in Ghana. Although DF has not been reported in Ghana, it has been detected in two adjacent countries, Côte d'Ivoire and Burkina Faso. Increasing migration of people across borders and the absence of organized *Aedes* mosquito control in Ghana might change the status of DF transmission. In addition, pyrethroid-resistance mosquitoes are becoming a major problem for the vector control program since at present there are no suitable chemical substitutes for pyrethroids. While pilot studies have been conducted in Northern region and Greater Accra region of Ghana, nation-wide screen of *Aedes* mosquito-distribution and pyrethroids-resistant information are not available.

Approach

Thus in this study, mosquito larvae collection in breeding sources from North to South and West to East along national roads and species identification of each mosquito species is being performed. The study started four months ago and simplified susceptibility tests are being carried out using the fourth instar larvae of *Aedes* mosquitoes in order to clarify the possible risks of DF and YF in the country.

Evaluation of spatial repellent (SR) products under semi-field conditions in Liberia and profiling hybrid molecular form of *Anopheles gambiae* in Ghana.

Investigators
NMIMR
Department of Parasitology — *Samuel Dadzie, PhD*; *Mba-ThissommahMosore, MPhil*

Vestergaard/NMIMR Research Labs,
Vector Biology Research Program, — *Melinda Hadi, PhD ?*

U.S. Naval Medical Research Unit # 3, Cairo, Egypt — *Joseph Diclaro II, PhD*; *Jennifer Curry, PhD*

Funding — *NAMRU-3*

Background

Mosquito-borne diseases are global health threats for people residing in, and military personnel deployed to sub-tropical and tropical regions. Presently vaccines are not available for malaria and dengue, the two leading causes of mosquito-borne diseases. Alternate disease control and prevention strategies, such as control of the disease vectors, are needed to supplement and enhance drug and vaccine programs. Vector control research has focused on identifying new active ingredients (AI) and/or innovative methods to reduce human-mosquito interactions. These efforts include the evaluation of spatial repellents (SR), compounds capable of altering mosquito behaviour without direct contact with the chemical source. It has been demonstrated that airborne SR in the parts per billion range, well below levels shown to be toxic in humans, can be measured within test systems and that these low levels are sufficient to repel mosquitoes.

Objective

To evaluate commercial and advanced developed SR products in semi-field conditions for the control of mosquito disease vectors in Liberia. This project will leverage existing expertise within the Navy Medicine Research Enterprise and seek to establish product testing capabilities with host nation partners at the Liberia Institute for Biomedical Research (LIBR) and the Armed Forces of Liberia Preventive Medicine Unit (AFL PMU). The study will also profile M/S molecular forms of *An. gambiae* for insecticide resistance (IR) utilizing IR bioassays and PCR and evaluate M/S molecular forms of *An. gambiae* behaviour to spatial repellents in semi field conditions.

Other Studies

Development of an Integrated Push-Pull System for the Control of Biting Flies.

Investigators	
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	Mba-ThissommahMosore, MPhil
Vestergaard/NMIMR ResearchLabs.	*Melinda Hadi, PhD ?*
Vector Biology Research Program,	
U.S. Naval Medical Research Unit # 3, Cairo, Egypt	*Joseph Diclaro II, PhD*
	Jennifer Curry, PhD
Funding	*NAMRU-3*

Nutrition and Food Security

TELFUN (Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition) Research Programme.

Investigators

NMIMR

Department of Nutrition — *Margaret Armar-Klemesu, PhD*

Funding — *The Nestle Foundation*

University of Wageningen, Netherlands

Background

TELFUN (Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition) is an interdisciplinary multi-country research project funded by INREF (Interdisciplinary Research and Education Fund). The main interest of the TELFUN research project is food sovereignty. The project is designed to investigate how innovative scientific and technological practices tailored to the needs of local food networks can be used to enhance food sovereignty and improve the nutritional status of resource-poor people.

In Ghana, the project was a nutrition intervention study aimed at assessing the efficacy of iron-fortified cowpea-based diet to improve diet quality and iron and nutritional status of school children (6 – 8 yrs) as well as its contribution to food sovereignty in Tolon/Kumbungu district in Northern Ghana.

Objectives

1. To assess food and nutrient intake, and nutrient adequacy among school children
2. To determine the contribution of cowpea to nutrient intake.
3. To assess nutritional status (including iron and zinc) of school children
4. To assess the impact of school feeding programme on nutrient intake and local food sovereignty
5. To assess the efficacy of an iron fortified cowpea-based meal in improving the iron status of school children.

Approach

An interdisciplinary approach comprising four different but linked disciplines. These disciplines are: plant breeding, food science and technology, human nutrition and social science.

Findings

The results from the intervention trial showed that fortification of whole cowpea flour with NaFeEDTA resulted in improvement of haemoglobin ($p<0.05$), serum ferritin ($p<0.001$) and body iron stores ($p<0.001$) and reduction of transferring receptor concentration ($p<0.001$). Overall the study showed that in a malarious region with high iron deficiency consumption of iron-fortified

cowpea resulted in 30% and 47% reduction in the prevalence of iron deficiency (ID) and iron deficiency anaemia (IDA) ($p<0.05$), respectively.

Effect of fish meal and vitamin C on the anaemia and nutritional status of Ghanaian school children consuming cowpea-based food

Investigators

NMIMR

Department of Nutrition — *Godfred Egbi, PhD*

Funding — *The Nestle Foundation*

College of Health Sciences, UG

African Population & Health Research Centre (APHRC) IDRC

Background

Malnutrition (under nutrition, over nutrition and hidden hunger) is an issue of public health concern among Ghanaian school children. One appropriate and innovative strategy to alleviate this problem is to promote dietary diversification and modification using locally available food groups such legumes to improve the nutritional status of school children. Cowpea has high protein content (20% – 30%) and an appreciable level of non-haeme iron (8 mg – 9 mg).

Objectives

This project sought to investigate the effectiveness of cowpea-based food containing fishmeal and served with vitamin C-rich drink to improve non-haeme iron bioavailability iron status, nutrients intake and growth of Ghanaian school children.

Approach

The study was undertaken in a school in the Adaklu-Anyigbe district of Volta Region, Ghana. The project was a pre-test, post-test experimental design that involved a cross-sectional baseline and a six month nutrition intervention. Study participants were 143 school children, 6 – 12 years of age, not on any iron supplement, not allergic to cowpea-based foods, not severely anaemic and not participating in any school feeding programme. Baseline data collected included dietary data by 24 hour recall method and food frequency questionnaires (FFQ), anthropometric data (height and weight measurements and socio-demographic data collected by interview questionnaires. Blood samples were analysed for haemoglobin, serum ferritin and complement reactive protein concentrations as well as malaria parasitaemia. Stool samples were examined for soil transmitted helminths.

Findings and implications

The prevalence of anaemia among the study groups at baseline was 30.1%, higher than the global prevalence of anaemia among school children; 25%. Most of the study participants had mild to moderate anaemia (haemoglobin levels between 90 and115 g/L) at baseline and end-line.

Cowpea-based food containing 3% fishmeal served with 33 mg vitamin C/100ml vitamin C-rich drink improved iron stores non-significantly but increased haemoglobin concentration significantly ($p<0.05$) leading to appreciable reduction in prevalence of anaemia. Haemoglobin levels increased and anaemia prevalence decreased for both experimental groups and control and so the role of other nutrients cannot not be ruled out. However, the significant increase observed in haemoglobin levels with greater reduction in anaemia prevalence among the experimental group were likely attributable to the haeme iron content of the diet in addition to bioavailability of non-haem iron in the presence of fishmeal and vitamin-C. Both fishmeal and vitamin-C are enhancers of non-haem iron bioavailability.

Our findings confirm and give support to suggestions by an earlier study that mere addition of iron to staple foods in developing countries, without providing an enhancer of iron absorption, is unlikely to have significant effect on iron status (2).

Study participants had very low (5%) iron bioavailable staple diets comprising cereals, roots and legumes. All the children had Nutrient Adequacy Ratio (NAR) >1 for dietary iron intake both at baseline and end-line. The intervention and control diets contributed at least half of the total dietary iron intake. No significant difference was observed for the contribution of the various cowpea-based diets to total dietary iron intake of the participants in the three study groups. In view of this, it is suggested that fishmeal be purposefully served as an enhancer of non-haem iron bioavailability in the diet.

Randomized controlled trial of a Home Grown School Feeding Pilot Project

Investigators	
NMIMR	
Department of Nutrition	*Gloria K. Folson, PhD*
Department of Parasitology	*Kwabena M. Bosompem, PhD*
	Irene Ayi, PhD
Department of Epidemiology	*Daniel K. Arhinful, PhD*
UG, Institute of Social Statistical & Economic	
Research (ISSER)	*Felix Asante, PhD*
University of Sussex	*Eduardo Masset, PhD*
Partnership for Child Development, Imperial	
College, UK	*Leslie Drake, PhD*
	Aulo Geli
Funding	*Dubai Cares*
	Bill & Melinda Gates Foundation

Background

The Ghana School Feeding Programme (GSFP) was piloted in 10 schools in late 2005. By the end of 2009, GSFP had progressively grown to serve 1,695 public schools with 656,624 pupils in all the 170 districts in Ghana. As of 2011, the programme was reaching over 1.6 million primary school

children in all 170 districts in Ghana. Co-ordination and implementation are undertaken by a National Secretariat, with programme oversight provided by the Ministry of Local Government and Rural Development (MoLGRD).

School food programmes are popular interventions to support the education, health and nutrition of school children. Home-grown school feeding has the potential to link the increased demand for school feeding goods and services to community stakeholders, including small-holder farmers (mainly subsistence farmers) and women groups.

Although the GSFP was designed as a strategy to increase domestic food production, household incomes and food security in deprived communities, programme delivery is provided through private caterers who are awarded contracts by the GSFP to procure, prepare and serve food to pupils in targeted schools. Each caterer is responsible for procuring food items from the market, preparing school meals and distributing food to pupils. The caterers are not restricted or guided in their procurement and are able to procure on a competitive basis without commitment to purchasing from small-scale farmers even though the GSFP project document prioritises procurement from the communities surrounding the assisted schools, broadening the focus to the district and national levels when food items are not locally available.

There is limited evidence of the impact of providing a reliable market for small-holder farmers through "home-grown" school feeding approaches and despite the large investment in the national programme, to date, there are no rigorous impact evaluations of the GSFP.

Objectives

This study is designed as a randomized control trial which aims to evaluate the impact of school feeding sourced from small-holder (mainly subsistence) farmers on school children's nutrition, health and education as well as on small-holder food security in Ghana.

Approach

This study involves a field experiment around the scale-up of the national Ghana School Feeding Programme, including 120 primary schools in 60 Districts. The randomly assigned interventions are:

1) School feeding programme group, where the standard Ghana School Feeding Programme is implemented;

2) "Home-grown" school feeding and social accountability group (HGSF+), including schools and communities where the Ghana School Feeding Programme is implemented in addition to training of community based organizations and local government; and

3) Control group, including schools and household from communities where the intervention will be implemented in two years (preferably without informing schools and households of impending intervention).

A sub-study within this trial is evaluating the use of a multi-nutrient powder (MNP) as part of school meals. The study was initiated in October 2012 and a community based baseline study covering 2,626 households across the country was completed in August 2013. Data cleaning, analyses have been completed and report writing is on-going.

OTHER STUDIES

Environmental studies

Crude oil drilling: On shore environmental assessment of the Jubilee oil field of the Western region of Ghana

Investigators:

NMIMR

Department of Clinical Pathology — *Regina Appiah-Opong*, PhD*; *Alexander K. Nyarko, PhD*; *Mark Ofosuhene, PhD*

Department of Epidemiology — *Daniel K. Arhinful, PhD*

UG, Department of Marine & Fisheries Sciences — *Francis K. E. Nunoo, PhD*

Institute of Environment and Sanitation — *Benedicta Y. Fosu-Mensah*

Funding — *UG Research Fund /ORID*

** Principal Investigator*

Industrialization of our society, including drilling of oil, mining of coal and some metals has led to increased production of xenobiotic and natural chemical substances. Constant leaching of heavy metals from mining or drilling activities leads to bioaccumulation in plants and animals, creating the danger of toxicity. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and alter/damage the blood composition and vital organs. In Ghana, oil drilling is ongoing at the Jubilee oil fields in the Western region, however, on shore baseline environmental assessment required for monitoring of pollution of districts bordering the oil fields have not yet been performed. In this study, we are performing an environmental assessment of some communities bordering the oil drilling field to establish (1) levels of heavy metals in soil, plants, water and fishes, (2) levels of polycyclic aromatic hydrocarbons in soil, plants, water and fishes, (3) levels of fish hepatic enzymes and determine DNA adduct formation in fishes, (4) physicochemical properties of water samples. The outcome of this study will provide a baseline for future assessment of the impact of oil drilling activity on the districts and also provide a basis for similar studies in mining areas prior to the mining activity.

Six (6) communities bordering the Jubilee oil field were selected. These are Secondi-Takoradi, Shama, Nzema East, Jomorro, Ellembelle and Half-Assini. Water, soil, plant and fish samples were collected. Blood of fish samples collected have been processed and examined for the presence of DNA adducts. Measurement of physicochemical properties such as Conductivity, Total Dissolved Solids, Biological Oxygen Demand, pH and Temperature of all water samples have been done. Measurement of levels of heavy metals in the water samples using an Atomic Absorption Spectrophotometer (AAS) has been completed. Processing of soil, plant and fish samples have been done and AAS analysis is ongoing.

H3Africa Chronic Kidney Disease Project

Investigators:	
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Department of Parasitology	*Anita Ghansah, PhD*
UG School of Medicine & Dentistry	*Dwomoa Adu*, MB, ChB; FRCP*
	Charlotte Osafo, MB, ChB
University of Michigan	*Akinlolu Ojo, MD, PhD*
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Obafemi Awolowo University	*Fatiu Arogundade, MB, ChB*
Duke University	*Rasheed Gbadegesin, PhD*
University of Toronto	*Rulan Parekh, PhD*
Loyola University	*Richard Cooper, PhD*
	Bamidele Tayo, PhD
University of Western Cape	*Nicki Tiffin, PhD*
Funding	*NIH*

** Principal Investigator*

The H3A initiative aims to study the genetic and environmental contributors to disease and health in Africa. The chronic kidney disease genomics laboratory, is one of such initiatives established to apply genomic approaches to assess monogenic-mutation kidney disorders in affected families, genetic variants of single genes (APOL1), associated with kidney diseases in the populations and genome wide association studies. The research collaborative encompasses research projects conducted in a cohort of 4000 participants with chronic kidney disease and 4000 general population controls, with a nested case-control approach to carry out four genomics based research projects on kidney disease of varying etiology.

A total of 2,492 samples have been received by our lab from 7 Clinical Centres so far. Each participant provides whole blood and a mouth rinse sample. DNA is isolated from each of the two sample types from each participant and subjected to PCR amplification and an Amelogenin-XY gender determination assay, as well as, quantitation via NanoDrop. In addition, samples are run on 1% TBE, agarose gel as an additional QC to determine presence or absence of isolated DNA. Isolated samples are currently stored in a -80 freezer pending their transfer to a bio repository. A total of 50 isolated DNA samples from 6 Ghanaian groups with tribal affiliation have been shipped to the University of Michigan for whole genome sequencing and further analysis.

Our major constraints are centered on shipping and logistics with respect to procurement. Maintaining sample integrity especially after shipment from clinical centers is also an area we hope to improve and maintain, as it directly affects DNA yields for downstream processes. The laboratory has taken receipt of several equipment and apparatus including a tabletop centrifuge, Thermocycler and NanoDrop to help streamline processes. In addition, the lab has recently appreciated in personnel with the hope that once recruitment commences in all 9 clinical centres,

samples can be effectively and efficiently processed. A manuscript on DNA sample integrity based on the practical results is in preparation. Efforts are being made to establish a functional data storage and retrieval database for samples.

Studies on Sickle Cell Disease: Newborn Screening for Sickle Cell Disease in Ghana

Investigators:

NMIMR Department of Epidemiology	*Francis K. Nkrumah, MD, MPH*
Department of Clinical Pathology	*Regina Appiah-Opong, PhD*
Children's Hospital of Philadelphia	*Kwaku Ohene-Frimpong*, MD*
Komfo Anokye Teaching Hospital	
Funding	*Ministry of Health; NIH; Sickle Cell Foundation, Ghana*

** Principal Investigator*

Sickle cell disease (SCD) is a common inherited disease of people with African origin. About 80% of children born with SCD worldwide are born in Africa, and about 400,000 are born with SCD each year. There are four common types of SCD, these are SS, SC, S/beta-plus thalassaemia and S/beta-zero thalassaemia. The most common of these types found in Ghana is SS, affecting about 60% of the people with SCD, followed by SC which affects about 38%. Health problems caused by sickle cell are commonly moderate to severe anaemia, severe pain (crisis), silent and visible stroke, chronic damage to lungs, kidneys, joints, eyes and other organs.

The Sickle cell laboratory of the Clinical Pathology Department, NMIMR, has been designated as the National Laboratory for screening for SCD and other haemoglobin disorders in newborn and young infant. Haemoglobin analyses of dried blood spots (DBS) collected from newborns using isoelectric focusing technique has been ongoing. By December 31 2014, a total of 428,139 newborns had been screened for SCD. Out of this number 7,229 have been diagnosed with SCD. In 2014, a total of 29,488 newborns were screened for SCD and out of this number 445 were diagnosed with SCD. It is expected that beginning this year 2015, newborn screening would be established in all districts in Ashanti and other regional capitals.

Prevalence of diseases in captive grasscutter (*Thryonomys swinderianus*) colony maintained at the Department of Animal Science of the University of Ghana

Investigators

NMIMR	
Department of Animal Experimentation	*Phyllis Addo, DVM, PhD*
Bangladesh Agricultural University	*Mohammed Habibur Rahman DVM, PhD*
	IqbalHossain, PhD
	MokbulHossain, PhD
UG School of Veterinary Medicine	*K. G. Aning, DVM, PhD*
UG Department of Animal Science	*B. K. Ahunu, PhD*
Funding	*UG*

Introduction

Grasscutter (*Thryonomys swinderianus*) production serves as a major tool for improved household income generation, nutrition and bio-diversity conservation in Ghana. It provides a quick turnover; serves as a foreign exchange earner and helps reduce unemployment rate of the country. Additionally, it provides employment avenues for the physically challenged and people suffering from HIV/AIDS. Environmentally, grasscutter rearing is very friendly since it helps to meet the demand for bush meat without placing undue pressure on the environment in many rural areas of Nigeria and other West African countries like Benin, Ghana, Togo and Ivory Coast. In sub-urban Accra, grasscutter farming in captivity is very popular and therefore the Department of Animal Science of the College of Basic and Applied Sciences is very active in participating in its development through breeding.

Objective

To determine the prevalence of diseases in a captive breeding colony of grasscutters, of various age groups in relation to the different seasons of the year 2014.

Method

An observational study on causes of deaths among captive grasscutter colonies maintained at the Department of Animal Science of the College of Basic and Applied Sciences, University of Ghana. The diseases were diagnosed based on clinical history, clinical signs, and symptoms prior to death, lesions observed after postmortem examination of dead animals and isolation and identification of causal agents. A total of 39 dead and sick animals were examined.

Findings and Implications

Among the diseases diagnosed, wound infection due to fights was found to be the most fatal and caused 44% of total deaths, followed by those found dead in cages due to acute congestion of lung. Acute congestion of lung constituted 33% of deaths and was related to *Enterobacter cloacae*, which was found to be the main causal agent of deaths in the cages. Of the dead in cages, 50% was found to be males. 10% of the animals were found to be lethargic and were unable to move before death and 5% had malocclusion. Five percent of females found dead in cages were pregnant. In general, the highest number of deaths was recorded from January to April and then decreased from May to August. There were no deaths recorded in the month of September; however, there was a steady increase from October to December. It was observed that fighting and cannibalism declined in the months of May to August, which are cooler due to rainfall accompanied by build-up of moisture in the air. Therefore, the high mortality recorded during the dry season could be reduced by fogging the animal rooms with cold water vapour. In view of this, the moisture level in grasscutter houses must be monitored on a regular basis. Distribution and proportionate incidence of grasscutter diseases reveals that high level of ammonia build-up may predispose the animals to respiratory problems. In conclusion, the results suggest that there is a probable relationship between the deaths of grasscutters and the ambient temperature on one hand, and ammonia build up in the animal house on another hand to predispose these animals to respiratory problems.

Acanthamoeba polyphaga Experimentally Transmits *Mycobacterium ulcerans* and Enhances its Virulence in ICR Mice

Investigators

NMIMR

Department of Animal Experimentation — *Phyllis Addo, DVM, PhD*; *Bright Azumah, MPhil*

Department of Parasitology — *Michael D. Wilson, PhD*; *Daniel Boakye, PhD*

Department of Electron Microscopy & Histopathology — *Alfred K. Dodoo, MPhil*

UG Department of Biochemistry, Cell &Mol. Biol. — *Gordon Awandare, PhD*; *Lydia Mosi, PhD*

Funding — *ORID/UG*

Background

The natural transmission of *Mycobacterium ulcerans* is poorly understood although a number of possible routes, including bites of aquatic insects have been proposed. Since *Acanthamoeba* species is capable of harbouring the closely related *M. leprae*, it was hypothesized that *Acanthamoeba* species may be natural reservoirs and possible vectors in buruli ulcer (BU) transmission.

Approach

To test this hypothesis, intact or shaved pinpricked rumps of ICR mice were treated with: (i) *M. ulcerans* alone; (ii) a co-culture of *A. polyphaga* and *M. ulcerans* or (iii) *A. polyphaga* only. To investigate differences in virulence, two additional groups of mice were injected in their footpads with identical concentrations of *M. ulcerans* alone or *A. polyphaga* infected with *M. ulcerans*. All groups were observed daily for development of lesions. Fine needle aspirations were performed on lesions from live animals, while those of dead animals excised and homogenized, followed by culture on Lowenstein-Jensen (LJ) plates, Zeihl-Neelsen acid-fast (ZN)-stained and examined for acid fast bacilli.

Findings and Implications

Both *M. ulcerans* and *A. polyphaga-M. ulcerans* elicited inflammation when topically applied (Day 31), oedema (Days 45 and 44 respectively) and ulcers (Day 49) at sites on pinpricked skin (Fig. 51 A, B, C & D). Also, the infected *A. polyphaga* enhanced the virulence of *M. ulcerans* by causing early inflammation in mice footpads by day 3 compared to day 14 with the same concentration of *M. ulcerans* only. The aspirates and homogenized tissues of ulcers were all positive for acid-fast bacilli (*M. ulcerans*) and showed growth of *M. ulcerans* when cultured. This study lends credence to the hypothesis that *A. polyphaga* may be a possible reservoir and a vector in BU transmission. The study also confirms the possibility of passive infection by *M. ulcerans* as a likely transmission route and also provides another mouse model, ICR other than BALB/c for BU studies.

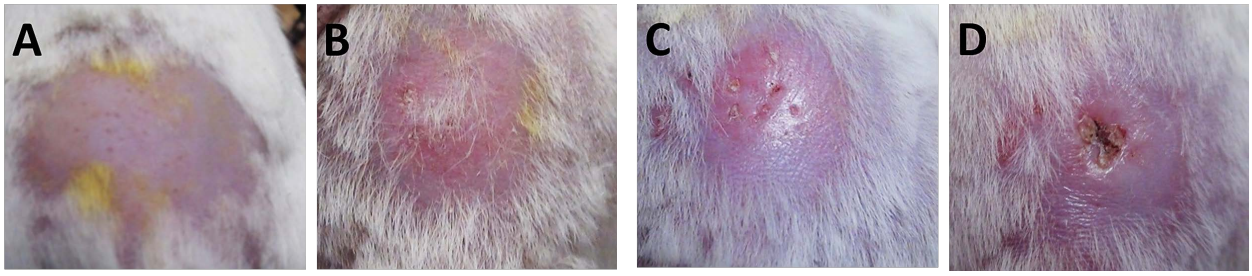

Fig. 51: The progressive development of BU in mouse topically treated with M. ulcerans-infected A. polyphaga at the punctured skin of the rump (lower back). Panel A shows site of inoculation 1 day post-inoculation (dpi). Panel B shows inflammation (erythema) at site of inoculation 31 dpi. Panel C shows an oedema at site of inoculation 44 dpi. Panel D shows ulcer at the site of inoculation 49 dpi.

Assessment of Faecal Exposure Pathways in Low-Income Urban Settings – the SaniPath Study

Investigators:	
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Emory University, Centre for Global Safe Water	*Christine L. Moe, PhD*
	Claire Null, PhD
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	Eugene Larbi, PhD
Funding	*Bill & Melinda Gates Foundation*
	Center for Global Safe Water, Rollins School of Public Health, Emory University

Background

Rapid urbanization has led to a growing sanitation crisis in urban and peri-urban areas of low-income countries. Currently, over half of the global population resides in urban areas and this proportion continues to grow. In contrast to urban development in developed countries, rapid population growth in developing countries has outpaced existing infrastructure, including water and sanitation systems. For example, in Accra, the average annual population growth rate of 4.4% and up to 7% in some informal settlements, has led to increasing numbers of urban dwellers living in very polluted environments. Peri-urban populations face the greatest risk of exposure to faecal contamination due to inadequate access to safe drinking water and sanitation as is illustrated by high rates of enteric infection and illness. Unfortunately, water and sanitation services are often regarded as a "public good" and as a government responsibility. Policy-makers who face trade-offs when balancing policies/regulations intended to protect health against policies that promote economic activity and growth, suffer from a paucity of data to inform these decisions. Few data exist to inform strategies to mitigate risks of faecal exposure in developing countries. In particular, there is an absence of exposure assessment methods to appropriately describe and quantify the magnitude of faecal exposure associated with transmission pathways.

The development of efficient, cost-effective, and accurate environmental health indicators will enable data-driven prioritization of sanitation investment strategies to mitigate faecal exposure. Such indicators may form the basis for rapid assessments of exposure to faecal contamination which could function as important decision-making tools for government and donor agencies (e.g. DFID, Accra Waste Project) to set priorities, monitor progress, and shape policies.

This project consists of an in-depth, interdisciplinary assessment of exposure to human faecal contamination in low-income neighborhoods of Accra. The major faecal exposure pathways in the public domain include: (a) contact with surface water, (b) use of wastewater irrigation or faecal sludge fertilizer for food crops, (c) contact with open drainage or sewers, (d) practice of open defecation or use of public latrines, and (e) contact with soil that as a result of flooding of drains and/or open defecation is contaminated.

Objectives

1. Address the scarcity of data available to sanitation policy makers and implementers, identify and describe:
    - Sources and movement of human faecal contamination in low-income urban environment
    - Behaviour of adults and children that leads to exposure to various faecal contamination pathways
2. To Use WHO QMRA approach to determine which exposure pathways pose the greatest risk

Methodologically

The faecal sources of the study neighbourhoods were mapped and assessed for human behaviour associated with risk of exposure to faecal contamination. Data was collected using unstructured/ structured observations as well as key informant interviews, focus group discussions and household interviews and surveys.

Future work

The environmental samples will be tested for enteric bacteria and viruses as well as helminths. In order to characterize exposures during different seasons, we will collect data for approximately 18 months

Fig. 52: Study sites in Accra

Estimating the Economic Burden of Gastroenteritis in Ghanaian Children:

Investigators	
NMIMR. Department of Electron Microscopy & Histopathology	*George E. Armah, PhD*
School of Public Health	*Moses K. Aikins, PhD*
UGMS Department of Surgery	*Hope Glover Addy, MB, ChB*
PATH, Seattle	*Deborah Atherly, PhD*
	Lauren Gazley
Funding	*PATH*

Background

Diarrhea disease is the second leading cause of morbidity and mortality in children under five years of age with more than 1.3 million deaths occurring globally every year of which nearly 800,000 are in Africa. Rotavirus has been identified as the single leading cause of diarrhoea deaths in children under-five years of age with an estimated 265,000 rotavirus associated deaths. According to recent estimates, over 5,000 Ghanaian children die of diarrhea disease each year. The only intervention for rotavirus infection is by vaccination. Presently, two widely tested vaccines are available, and have shown good efficacy in clinical trials in Africa and Asia. In Ghana the Rotarix vaccine has been introduced into the Expanded Programme for Immunization (EPI). Understanding the economic burden associated with gastroenteritis is important for effective planning and resource allocation of both existing and future interventions designed to combat the disease. Little is however known about the economic burden associated with diarrhea disease in Ghana. This study sought to provide a comprehensive assessment of the economic burden of moderate to severe rotavirus gastroenteritis in Ghanaian children less than 5 years of age by estimating the direct medical, non-medical, and indirect costs associated with diarrhea illness for children (rotavirus and non-rotavirus, gastroenteritis) in both out-patient and in-patient settings.

Methodology

The study tagged on the ongoing rotavirus disease surveillance in three hospitals in Ghana (Korle Bu Teaching hospital, Agogo Presbyterian hospital, and the Navrongo War Memorial Hospital) to collect economic and demographic information after consenting from mothers and care givers of children on the episode of diarrhoea they had presented to the health facility. Data collected included the cost of drugs, procedures and laboratory tests, out of pocket expenses and lost household income and other events related to the diarrhoea episode. A stool sample was also collected for testing for rotaviruses.

Work Progress

The study begun in October at the Navrongo War Memorial Hospital in the Upper East Region of Ghana and in the Agogo Presbyterian Hospital (Ashanti Region) and the Korle Bu teaching Hospital in December 2011. The study has achieved 81% (142/176) of its enrollment target in Navrongo: 88% outpatients (70/80) and 75% inpatients (72/96). At Agogo Presbyterian Hospital, the enrollment rate has been 60% (133/220): 100% outpatients (120/120) and 13% inpatients (13/100) whilst at the Korle Bu Teaching Hospital we achieved an enrollment rate of 59% (153/260): 25% Outpatients (35/140) and 98% inpatients (118/120). The low inpatient enrollment rate at Agogo has been due primarily to the referral of very sick children to the nearby teaching hospital in Kumasi, the regional capital and hence low admissions in the hospital. The opposite was observed at the Korle Bu Hospital where the newly introduced Ghana Health Service (GHS) referral system ensures that mild disease is seen at the peripheral clinics and only very sick children get to the tertiary health care facilities. The expected outpatient and inpatient diarrhoea cases from participating sites are 2095. The participant enrollment of 428 (Table 1) achieved however exceeds the minimum sample size of 420 required to provide a 10% precision and 0.5% coefficient of variation.

Future work

Data cleaning and check has just been completed. The analysis and final report is in preparation.

Sexual and reproductive resilience of adolescents in Ghana and Tanzania

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Background

Teenage pregnancy is high in developing countries and in Ghana, as various estimates put the rate between 9% and 14%. Adolescent pregnancy exposes female adolescents to medical, social and economic risks. In Ghana, young mothers are more likely to experience complications during pregnancy and delivery compared to older mothers. This study examined the competency of adolescent girls to either proactively avoid pregnancy or reactively deal effectively with it.

Aims and Objectives

The aim of the study is to examine how actors like family and peers, and institutions/ organizations, such as schools and health services, influence adolescent girls' competence in preventing or coping well with teenage pregnancy and childbirth.

Approach

The study used a mixed method approach to generate data from urban and rural sites in Ghana and Tanzania, using a structured questionnaire and interview guides. A peer-to-peer data collection approach was adopted to remove any age-related social barriers to enhance rapport and create mutual trust between the interviewees and interviewers. Field workers were thoroughly trained and supervised during data collection. Data were analyzed using simple descriptive statistics, chi-square and logistic regression.

Preliminary findings

Out of 820 adolescents interviewed, 128 (15.6%) were pregnant/mothers. Adolescents in both groups (62.0% never pregnant girls and 68.0% pregnant/mothers) have access to social support, especially from their parents, in terms of advice on how to avoid or deal with teenage pregnancy. More pregnant/mothers (79.0%) compared to never pregnant girls (38.0%) (P=<0.001) have access to economic support. Access to social, economic and cultural capitals is associated with high competence to either avoid or deal with pregnancy among adolescent girls. Parents are taking the place of aunts and grandmothers in providing sexual education to their adolescent girls due to changing social structure where extended families are no longer living together in most cases. A key finding was that most adolescent girls who became pregnant did not become vulnerable victims but rather used their new status to rediscover themselves and develop new competencies to cope well with pregnancy and childbirth. Existing resilience studies tend to focus on social and economic capitals as the main factors influencing competence, however, findings presented here showed that cultural capital is also important for competence development among adolescent girls to either proactively avoid teenage pregnancy or reactively cope with it.

Rapid Mortality Monitoring Births and Deaths Registration Plus (RMM-BDR+) project

Investigators

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Background

This collaborative project seeks to develop, assess and implement alternative methods for producing and interpreting measurements of under-five mortality in the Northern Region of Ghana. The effort is being undertaken in the context of increased support for the achievement of MDG-4 and measuring the resulting changes and lessons in mortality among children less than five years of age.

Approach

Community based volunteers (CBVs) collect on-going births, deaths and pregnancy data with supervision by district and regional births and deaths registration officers.

Preliminary results

- From January 2012 to September 2013, CBVs reported 2,765 births, which was above the expected births for the northern region (2,487) based on MICS 2007 estimates. During the same period CBVs reported 50% of expected under five deaths (139) as against the expected number of 276.
- Limited capacity at BDR creates a challenge for improving community based vital event registration in Ghana
- Engaging community volunteers to record vital events in their community is feasible, but requires significant time and effort
- Notwithstanding challenges, community based vital event registration significantly improves birth registration

Enhancing and sustaining health insurance participation in Ghana through improved client-oriented quality of care (2011-2015).

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Funding	*Netherlands Organisation for Scientific Research WOTRO, Science for Global Development*

Background

The study aims to design and evaluate interventions to improve service quality for a client centred and sustainable health insurance in Ghana.

Approach

The interventions involve monitoring the services of health providers with community representatives and providing feedback in some cases as a proof of concept to improve client-centeredness of services. The interventions is from March 2013 to June 2014 with a post intervention study in July 2014.

Preliminary findings

Clients associate quality of services to relational factors while providers relate quality to medical technical aspects, indicating a gap between perceptions of clients and providers. The studies undertaken using two different assessment tools show that clients perceive quality care standards as generally low at the health care facilities, while providers perceive them as adequate.

The Health Inc. ("Financing health care for inclusion") research project

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Funding	*7th Framework Program, European Commission*

Background

The project is exploring the hypothesis that social exclusion restricts access to health services despite recent health financing reforms and how social health protection can be increased. The research has been conducted in Ghana, Senegal and the Indian states of Maharashtra and Karnataka.

Approach

The project uses both quantitative and qualitative approaches to examine whether social exclusion can explain why majority of Ghanaians are enrolling (or not re-enrolling) into NHIS, and those that do enroll are not accessing healthcare services.

Preliminary findings

Initial analysis indicate that, the currently insured are more likely to be economically well endowed and live closer or in the district capital with better access to educational, health and transport infrastructure and services. Among the reasons for social exclusion are weakening extended family system, perceived poor quality of care and the mentality of some non-insured that they "don't get sick". Cultural exclusion barriers include large family size, preference for alternative care such as herbal and self-medication as well as lack of trust in the scheme leads to a low desire to enroll in the scheme.

Reaching the poor in Ghana's National Health Insurance-(SHINE Ghana project)

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The aim of the SHINE-Ghana project was to provide insights into why the poor are excluded and design an intervention to solve the problem of sub-optimal enrolment in the National Health Insurance Scheme (NHIS) in Ghana.

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92. Patrick Tshibangu

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4. John Odoom, *PhD*
5. Kofi Bonney , *PhD*
6. Mrs. Evelyn Y. Bonney, *PhD*
7. Ndahwouh Talla Nzussouo, *PhD*
8. Edward Maina, *PhD*
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10. Ivy A. Asante, *MSc*
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12. Jacob Arthur-Quarm, *BSc*
13. Joseph Asamoah Frimpong, *MPhil*
14. Prince Parbie, *MPhil*
15. Mildred Amoah-Bosompem, *MSc*
16. Awo Eve-Marie Benson, *MB ChB*
17. Deborah Pratt, *BSc*
18. Linda Boatemaa, *BSc*
19. Ernestina Agbenyo, *BSc*
20. Gifty Mawuli, *BSc*
21. Keren Minta-Asare, *BSc*
22. Rosemary Ardayfio, *BBA*
23. Wilma Couston , *BSc*
24. Collins Amoako Addae, *BSc*
25. Gladys Mamle Ayiku, *BSc*
26. Gloria Nyankumah, *BSc*
27. Elijah Edu-Quansah, *BSc*
28. Angela Frimpong, *BSc*
29. Esinam Agbosu, *BSc*
30. Dzigbordi Aziati , *BSc*
31. Christopher Abana, *BSc*
32. Ewurabena Oduma Duker, *BSc*
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35. Miriam Eshun, *HND*
36. Sampson Kwame Dumedah
37. Thomas Antwi
38. Peter Odoi Anang
39. Isaac Boakye
40. David Asimah

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28. Hudson Odoi
29. Abdul Razak
30. Isaac Nyamekye
31. Edward Ansah
32. Frank Cole
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78. Julius Tsitsi
79. Thomas Kaka
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81. John Apuing
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