

FROM PARASITES TO PARASITES: A PARASITOLOGIST'S EXPEDITION

Olaoluwa Pheabian Akinwale BSc, MSc, PhD
Director of Research (Neglected Tropical Diseases)
Head, Molecular Parasitology Research Laboratory
Public Health Division
Nigerian Institute of Medical Research
Yaba, Lagos State
Nigeria



Protocol

Dedication

Preamble

Family life

- Husband – General (Dr) Abayomi Akinwale FMLSCN, MPA, MSc, PhD, MSS, DSS
- Children – Ayobami, Ifeoluwa and Oluwadamilola



My primary research objectives

- Provide evidence that will aid policy makers in formulating appropriate disease control strategies
- Alleviate the pains of victims of parasitic infections
- Enrich the body of knowledge in the academics

To achieve these objectives I competed for and won 22 international training and travel scholarships

- Infectious Diseases Institute, University of the Witwatersrand Johannesburg, South Africa - Reproductive Health (2005)
- Smith College, Northampton, Massachusetts, USA – Molecular Biology (2006)
- Institute Pasteur Tunis, Tunisia – Bioinformatics (2007)
- Wellcome Trust/Kenya Medical Research Institute, Kilifi, Kenya – Genomics (2007)
- Research Institute of Rio de Janeiro, Rio de Janeiro, Brazil – Phylogenetics (2009)
- National Institute for Health and Welfare (THL), Helsinki, Finland – Public Health (2010)
- KIT Royal Tropical Institute, Amsterdam, The Netherlands – Disease Control (2015).

International research grant awards - 8

Established the Molecular Parasitology Research Laboratory in NIMR

Current research grant awards - 3

- Principal Investigator - 2
- Co-Investigator - 1

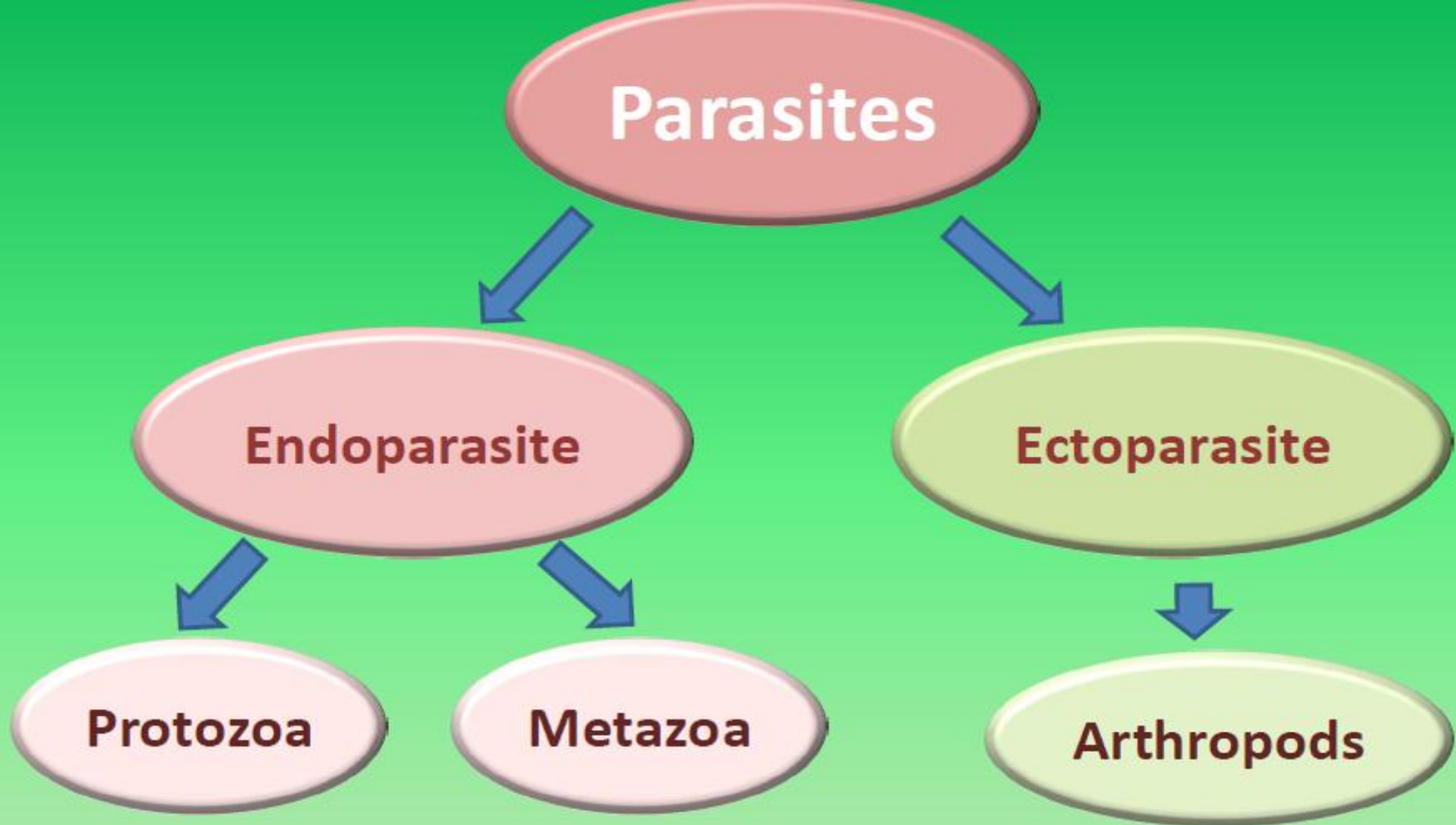


WHAT IS A PARASITE?

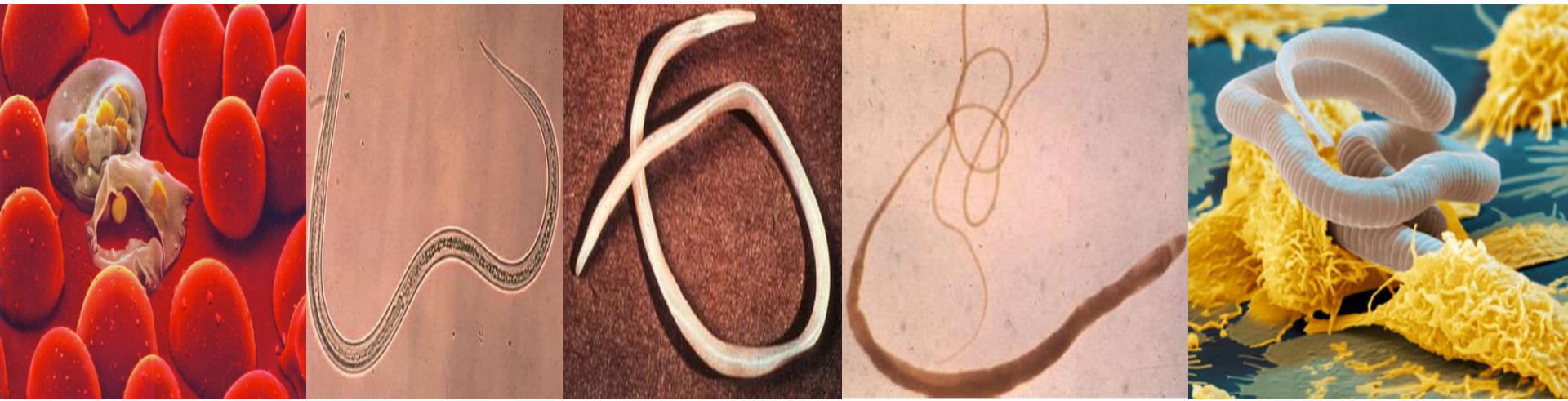
Carl Zimmer - a columnist at the *New York Times*

"Every living thing has at least one parasite that lives inside it or on it. Many, like leopard, frogs and humans, have many more. There's a parrot in Mexico with thirty different species of mites on its feathers alone. And the parasites themselves have parasites, and some of those parasites have parasites of their own. Scientists have no idea just how many species of parasites there are, but they do know one dazzling thing: parasites make up the majority of species on earth. According to one estimate, parasites may outnumber free-living species by ratio four to one. In other words, the study of life is, for the most part, parasitology."





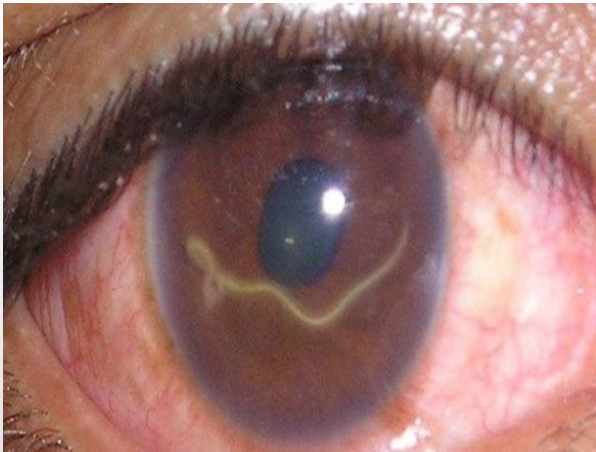
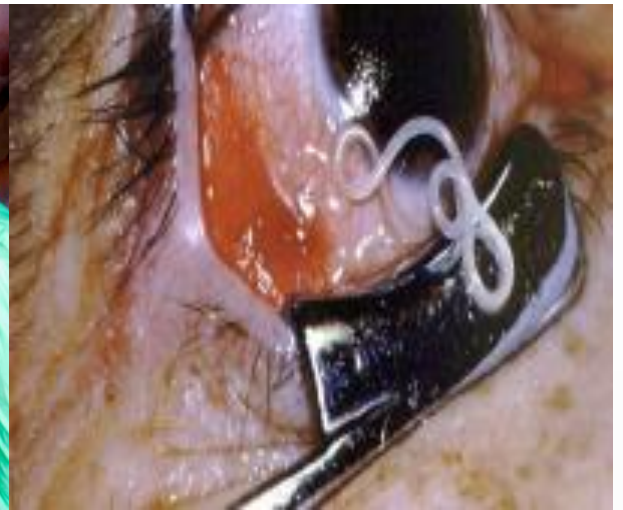
Some common medically important parasites in Nigeria



General characteristics

- Living organisms
- Live in or on the living tissue of other organisms
- Cause harm to their hosts without immediately killing them
- Metabolically dependent on other organisms for survival
- Always smaller than their hosts
- May or may not cause diseases
- Exhibit highly specialized adaptations, hence they exploit host resources to their benefit.





Common signs and symptoms of parasitic infections

- Diarrhea
- Vaginal irritation
- Joint pain
- Chronic fatigue
- Anemia
- Headaches
- Restlessness
- Arthritis
- Foul-smelling stools
- Mucous in stools
- Abdominal cramps and gas
- Vomiting
- Fever
- Loss of appetite
- Distended abdomen
- Coughing
- Weight loss
- Swollen lymph nodes



Table 1. Common parasitic infections in Nigeria and their prevalence

| Disease | Prevalence |
|--------------------------------------|---------------------|
| Hookworm | 38 million infected |
| Schistosomiasis | 29 million infected |
| Ascariasis (Round worm) | 55 million infected |
| Trichuriasis (Whipworm) | 34 million infected |
| Lymphatic filariasis (elephantiasis) | 106 million at risk |

General characteristics

- Constitute major human health problems throughout the world
- Usually chronic sometimes mimic non-communicable diseases
- Endemic in rural areas
- Highly prevalent among the poor
- Promote poverty
- Cause cognitive impairment in children
- Disfigure
- Cause permanent disability
- Stigmatization
- Many are transmitted from animals to humans - zoonotic infections.



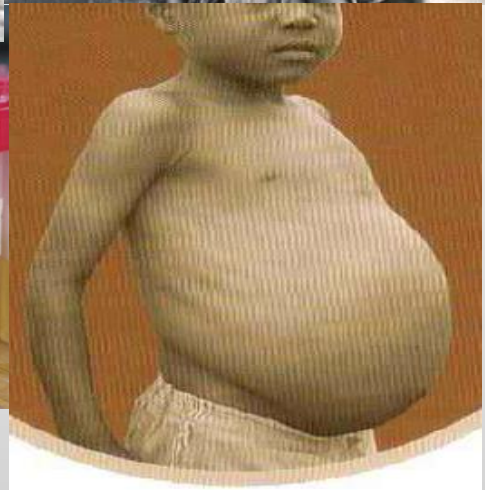
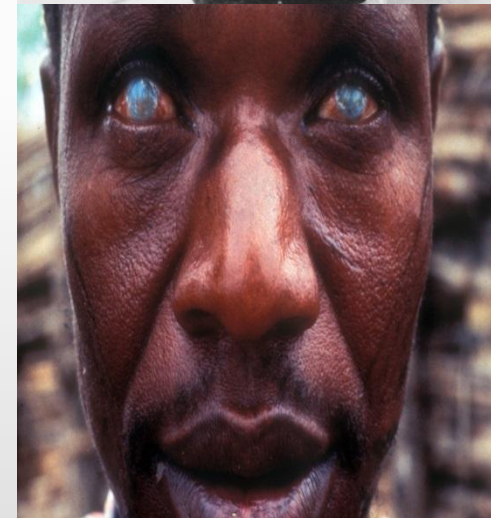
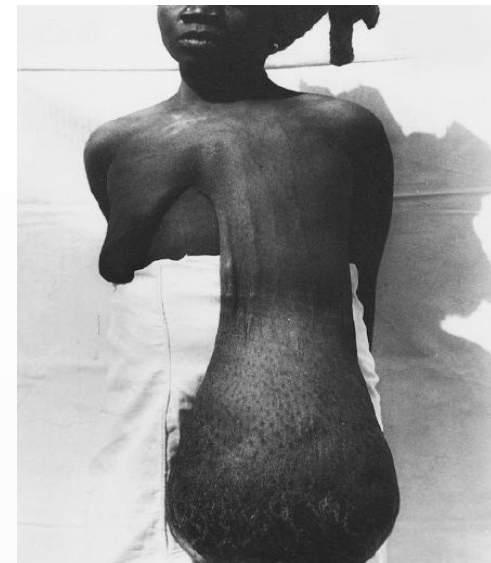
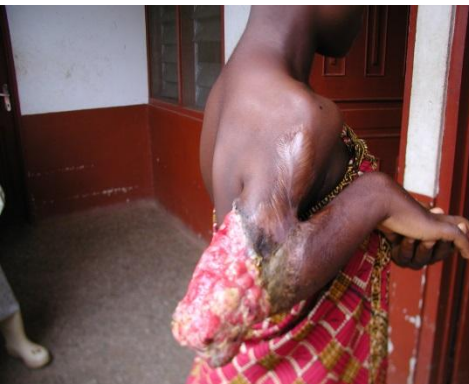


Table 2. Estimated burden of major parasitic infections in Sub-Saharan Africa

| Disease (Number of Cases in SSA) | Country with Highest Prevalence | Country with Second Highest Prevalence | Country with Third Highest Prevalence | Country with Fourth Highest Prevalence |
|--|---|---|--|---|
| Hookworm infection (198 million) | Nigeria 38 million | DR Congo 31 million | Angola and Ethiopia 11 million cases each | Cote d'Ivoire 10 million |
| Schistosomiasis (192 million) | Nigeria 29 million | Tanzania 19 million | DR Congo and Ghana 15 million cases each | Mozambique 13 million |
| Ascariasis (173 million) | Nigeria 55 million | Ethiopia 26 million | DR Congo 23 million | South Africa 12 million |
| Trichuriasis (162 million) | Nigeria 34 million | DR Congo 26 million | South Africa 22 million | Ethiopia 21 million |
| Lymphatic filariasis (382–394 million at risk) | Nigeria 106 million at risk | DR Congo 49 million at risk | Tanzania 31 million at risk | Ethiopia 30 million at risk, Kenya 29 million at risk |
| Trachoma (30 million) | Ethiopia 10.3 million | Sudan 3.6 million | Tanzania 2.1 million | Kenya and Niger 2.0 million cases each |
| Yellow fever (180,000) | Cote d'Ivoire 16 reported cases in 2006 | Mali 5 reported cases in 2006 | Cameroon, CAR, Ghana, and Guinea 1 case each in 2006 | |
| Human African trypanosomiasis (50,000–70,000) | DR Congo 10,369 | Angola 2,280 | Sudan 1,766 | Congo 839 |
| Leprosy (30,055) | DR Congo 6,502 | Nigeria 5,381 | Ethiopia 4,611 | Mozambique 1,830 |
| Leishmaniasis (visceral) (19,000–24,000 new cases) | Sudan 15,000–20,000 new cases | Ethiopia 4,000 new cases | Kenya and Uganda not determined | |
| Dracunculiasis (9,585) | Sudan 5,815 | Ghana 3,358 | Mali 313 | Nigeria and Niger <100 cases each |
| Buruli ulcer (>4,000) | Cote d'Ivoire 2,000 | Benin and Ghana 1,000 each | | |

Source: Hotez and Kamath (2009), *PLoS NTD* 3(8). e412. DOI:10.1371/journal.pntd.0000412



Parasitology

The study of the parasite itself - All disciplines of biology

Host-Parasite relationships - Ecology

Immunology of parasitic infections - Humoral and cellular immunology

Chemotherapy of parasitic infections - Medicine

How diseases spread amongst the population - Epidemiology



MY RESEARCH REPORTS ON PARASITIC INFECTIONS OF PUBLIC HEALTH IMPORTANCE IN NIGERIA

African trypanosomiasis

Human African trypanosomiasis (HAT) - Sleeping sickness

African animal trypanosomiasis (AAT) – Nagana

- Caused by protozoan parasites –Trypanosomes
- Mostly transmitted by tsetse flies - vector
- Prevalent mainly in African regions where the vector exists
- Cause serious economic losses
- *Trypanosoma brucei gambiense* and *T. brucei rhodesiense* - HAT
- *T. congolense*, *T. vivax*, *T. brucei*, *T. simiae* and *T. godfreyi* - AAT
- Domestic animals are the animal hosts -- Cattle, water buffalo, sheep, goats, camels, horses, donkeys, pigs, dogs, cats etc



Tsetse fly Stages

Human Stages

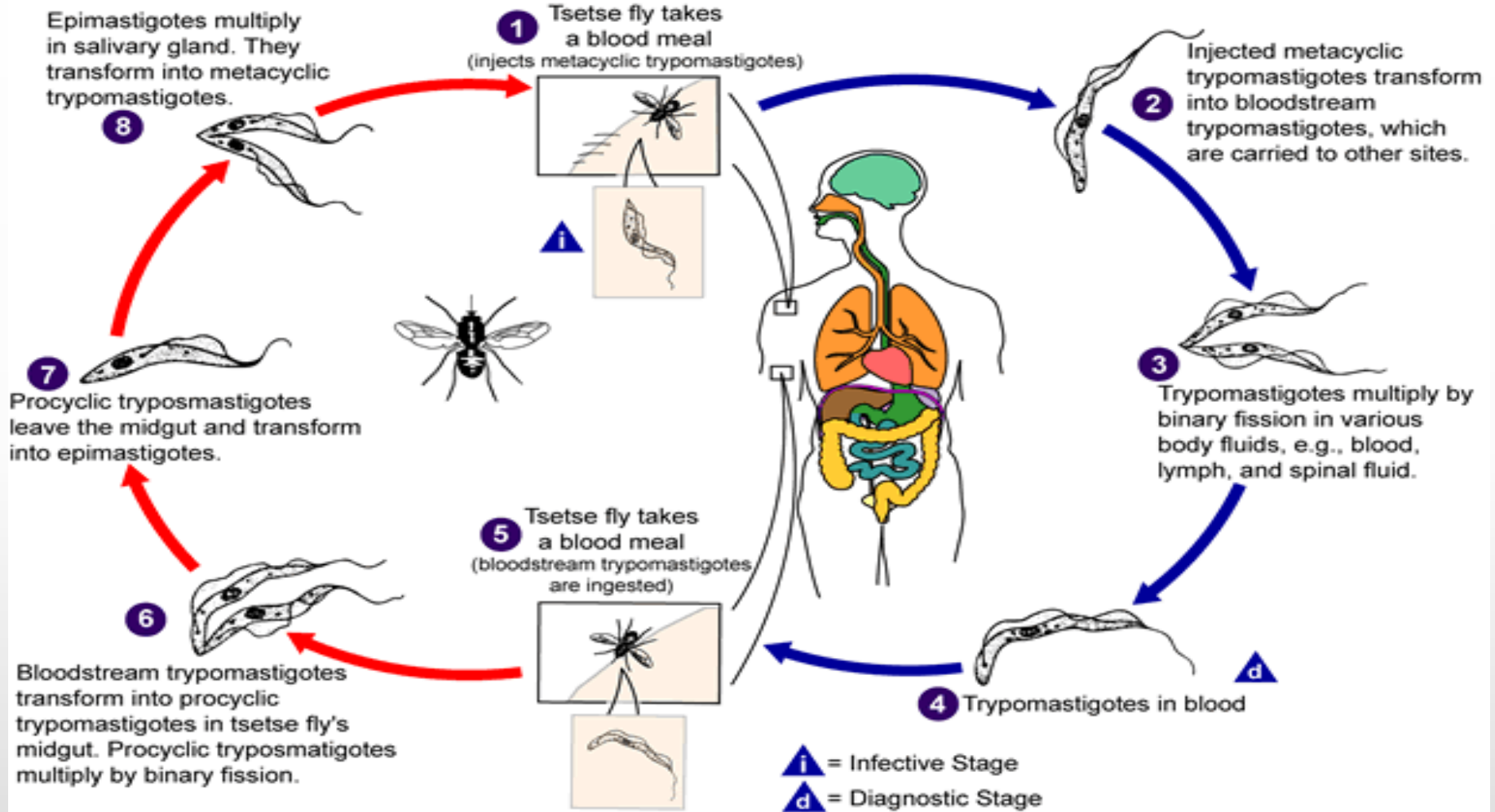


Figure 1: Life cycle of *Trypanosoma* species (Source: Division of Parasitic Diseases CDC, USA)



Study on the susceptibility of Sahel goats to experimental *Trypanosoma vivax* infection

Olaoluwa P. Akinwale*, Ishaya H. Nock, King A.N. Esiebo,
Henry U. Edeghere, Yetunde A. Olukosi

Public Health Division, Nigerian Institute of Medical Research, PMB2013 Yaba, Lagos, Nigeria

Received 11 January 2005; received in revised form 13 January 2006; accepted 17 January 2006

Abstract

Sahel goats, also known as Borno whites are found in the northern semi-arid, tsetse free Sahel region of Nigeria. They are transported alongside cattle from this zone to all other zones in the country, including the tsetse-infested zones, for commercial purposes and are kept for some time in these tsetse-infested zones until they are sold. This study therefore assessed the susceptibility of this breed of goats to trypanosome infection and its response to treatment with Berenil. Six bucks were inoculated intravenously with *Trypanosoma vivax* through the jugular vein while two served as uninfected control. The mean pre-patent period was 4.5 days and increasing parasitaemia followed the establishment of infection. Onset of parasitaemia was associated with increase in rectal temperature in all the infected goats and the temperature peak coincided with the only parasitaemic peak second week post-infection. The infected goats were treated with Berenil (Hoechst, Germany) 3.5 mg/kg body weight at 4 weeks post-infection. The packed cell volume (PCV) continued to fall from a mean $30.73 \pm 0.01\%$ pre-infection to a mean $13.21 \pm 0.18\%$ at 1 week post-treatment. Deaths were recorded for 4 of the infected goats 1 week post-treatment while the remaining two died 2 weeks post-treatment, not responding to treatment.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Susceptibility; Experimental; *Trypanosoma vivax* infection; Sahel goats

1. Introduction

African trypanosomiasis, caused by infection with any of several members of the genus *Trypanosoma*, both as sleeping sickness in man and as the livestock disease called nagana, is currently resurgent across much of tropical Africa, reaching epidemic levels in many

places (Pepin and Meda, 2001; Stanghellini and Josenando, 2001; Stich et al., 2003). *Trypanosoma vivax* is a parasite of major importance in West and East Africa, particularly in the former and is about the most important cause of animal trypanosomiasis by virtue of its predominance, compared to *Trypanosoma congolense* and *Trypanosoma brucei* (Anosa et al., 1995).

In the large majority of African countries affected by animal trypanosomiasis, trypanocidal drugs remain the principal method of control. This is due to lack of

* Corresponding author. Tel.: +234 1 4811590.
E-mail address: pheabian@yahoo.co.uk (O.P. Akinwale).



Human schistosomiasis

- Caused by digenetic blood flukes - Schistosomes
- Three main species - *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*
- Adults are dioecious - distinct male and female individual organisms
- Inhabit blood vessels - bladder (*S. haematobium*); small intestine (*S. mansoni* and *S. japonicum*)
- Produce non-operculate eggs
- Larvae invade the human hosts through the skin
- Distributed worldwide particularly in Africa, South America, the Middle East, Southern China and Southeast Asia
- Imported cases reported in Europe and the United States of America
- Snail intermediate hosts – *Bulinus*, *Biomphalaria* and *Oncomelania* species.



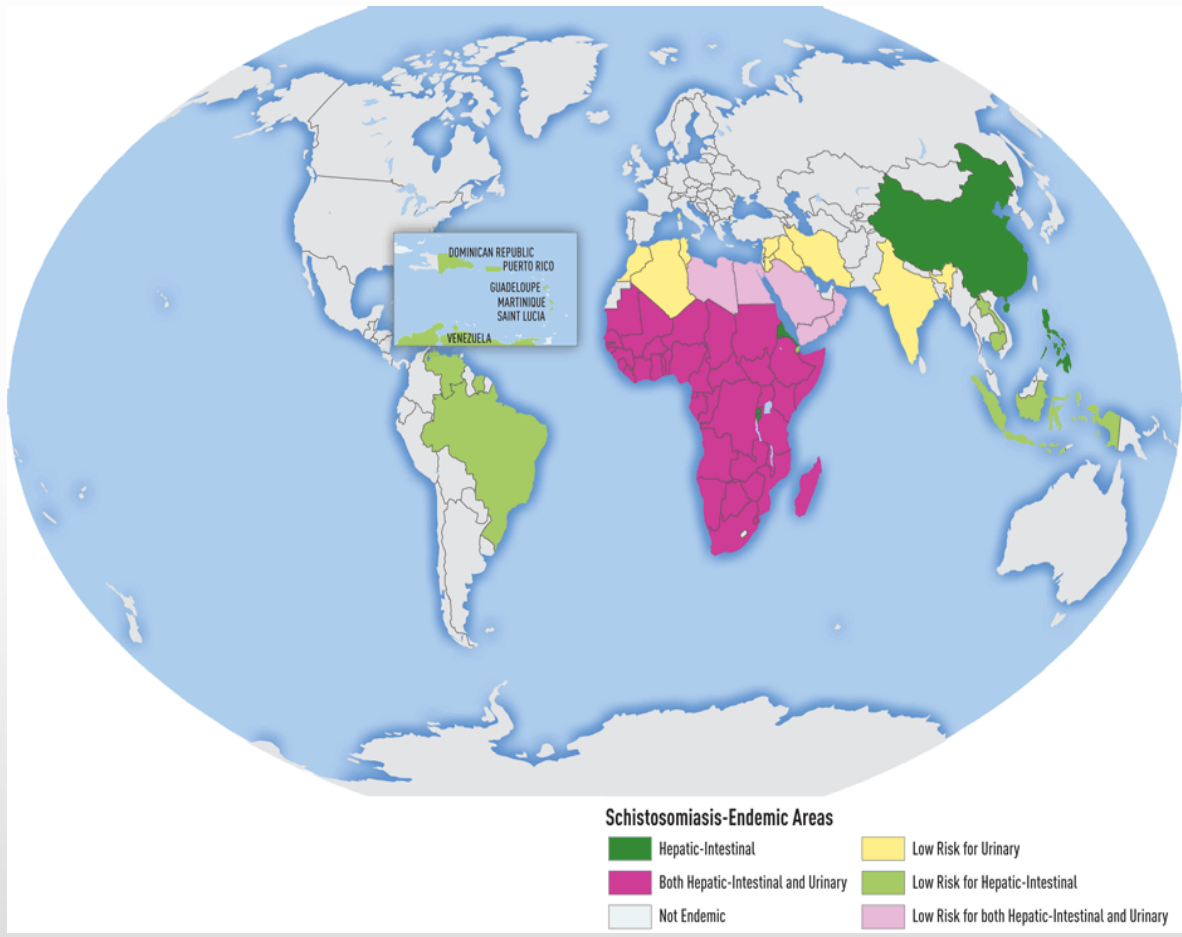


Figure 2: Geographic distribution of human schistosomiasis (Source: Division of Parasitic Diseases CDC)



Schistosomiasis

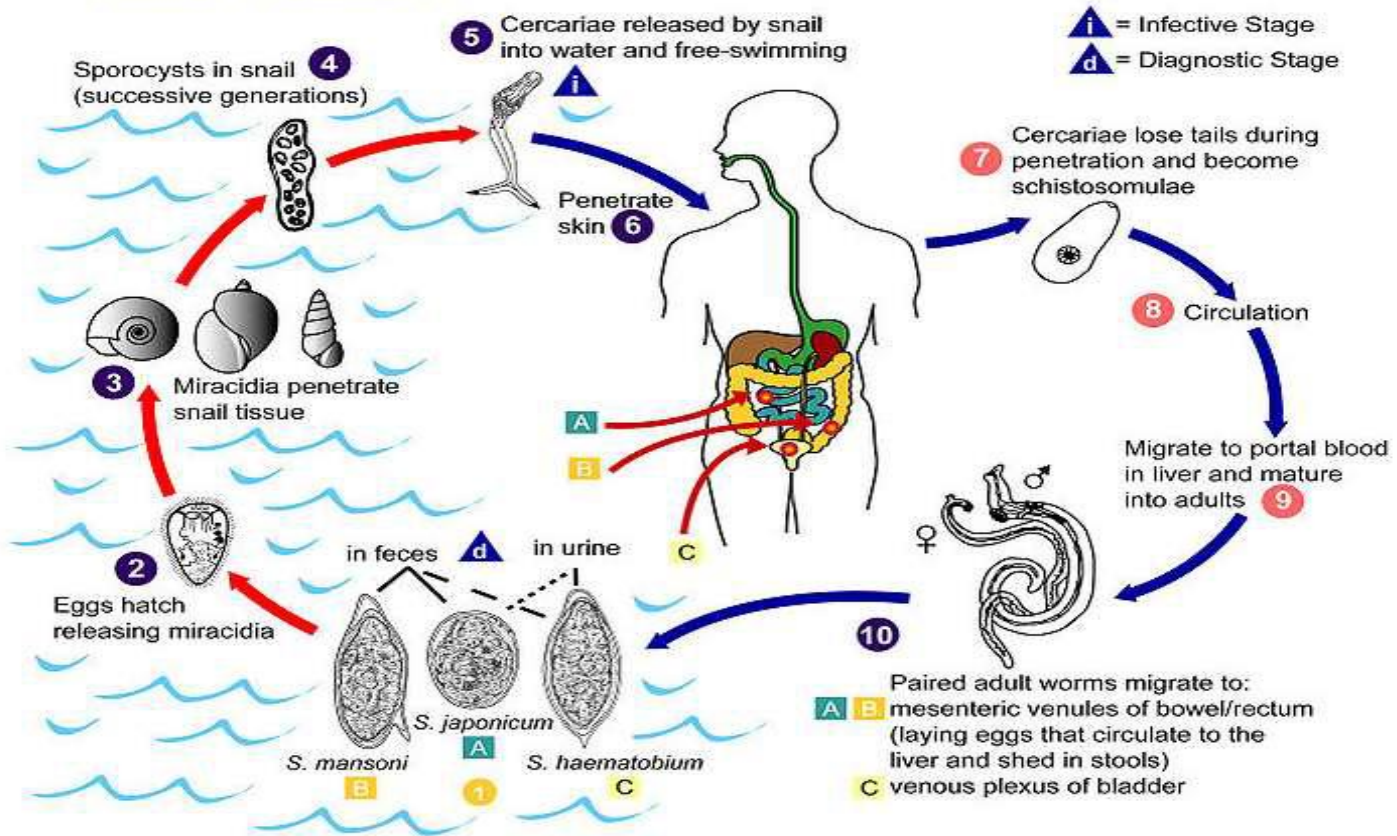


Figure 3: Life cycle of Schistosoma species (Source: [http:// www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx))



Original Research Article

Open Access
Online Journal

Prevalence of *Schistosoma haematobium* Infection in a Neglected Community, South Western Nigeria

Received: 16-Feb-09

Revised: 04-May-09

Accepted: 05-May-09

Abstract

Purpose: Schistosomiasis ranks second to malaria among parasitic diseases of socio-economic and public health importance. In Nigeria, urinary schistosomiasis caused by *Schistosoma haematobium* is endemic. This study aimed at producing an accurate data on the prevalence of urinary schistosomiasis in Apojula, a neglected community located around Oyan Dam, southwest Nigeria, using parasitological and molecular techniques.

Methods: Parasitological examinations were carried out on urine samples from 63 participants whose ages ranged between 7 and 63 years. Matched blood and urine samples were also screened for *S. haematobium* infection by polymerase chain reaction (PCR) amplification of the schistosome Dra1 repeat.

Results: Of the 63 participants, 33 (52.4%) were positive for haematuria while 6 (9.5%) had *S. haematobium* ova in their urine. PCR amplification of *S. haematobium* Dra1 repeat from their urine and blood samples showed that 59 (93.65%) and 62 (98.4%) were infected respectively.

Conclusion: There was a high prevalence of *S. haematobium* infection as detected by PCR amplification of schistosome Dra1 repeat from the urine and blood samples of the study participants. In addition, the PCR was able to detect schistosome infection in cases otherwise shown to be negative by parasitological examinations thereby making them also to receive chemotherapy.

Keywords: *Schistosoma haematobium*, haematuria, urine, blood, PCR, Dra1.

Olaoluwa P Akinwale*
Morakinyo B Ajayi
David O Akande
Monsuru A Adeleke
Pam V Gyang
Adeniyi K Adeneye
Angelina A Dike

Molecular Parasitology Laboratory,
Public Health Division, Nigerian
Institute of Medical Research,
P.M.B 2013, Yaba, Lagos, Nigeria

*For Correspondence:

Tel: +234 805 514 6173.
E-mail: pheabian@yahoo.co.uk



Urinary Schistosomiasis around Oyan Reservoir, Nigeria: Twenty Years after the First Outbreak

**OP Akinwale, MB Ajayi, DO Akande, PV Gyang, MA Adeleke, AK Adeneye, MO Adebayo, AA Dike*

Public Health Division, Nigerian Institute of Medical Research, P.M.B 2013, Yaba, Lagos, Nigeria

(Received 27 Sep 2009; accepted 8 Dec 2009)

Abstract

Background: Oyan reservoir, South-West Nigeria was constructed in 1984 to generate electricity but has altered the existing physical, biological and socio-economic environment of the people. This study, carried out between October 2006 and March 2008, aimed at investigating the current status of *Schistosoma haematobium* infection around the reservoir.

Methods: Urine samples from 536 participants in five communities were examined for haematuria using reagent strips and *S. haematobium* ova was detected using sedimentation by gravity method. The participants were drawn from Abule Tuntun (n= 115), Ibaro (n= 156), Imala Odo (n= 88), Imala (n= 103) and Apojula (n= 74) communities.

Results: Prevalence rates by haematuria were (Abule Tuntun- 33.04%; Ibaro- 73.07%; Imala odo- 60.22%; Imala- 7.77%; Apojula - 39.19%) and by presence of parasite ova were (Abule Tuntun- 39.13%; Ibaro- 83.97%; Imala Odo- 62.5%; Imala- 20.39%; Apojula- 54.05%).

Conclusion: *S. haematobium* transmission has been sustained in the reservoir since the outbreak was first reported in 1988. Mass treatment with praziquantel was conducted 8 years ago (2001) in two of the communities. However, the infection has persisted due to lack of pipe borne water and safe waste disposal system.



Squamous Cell Abnormalities in Exfoliated Cells from the Urine of *Schistosoma haematobium*-Infected Adults in a Rural Fishing Community in Nigeria

¹O. P. Akinwale, ²G. C. Oliveira, ¹M. B. Ajayi, ¹D. O. Akande, ³S. Oyebadejo and ⁴K. C. Okereke

¹ Public Health Division, Nigerian Institute of Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria

²Centro de Pesquisas René Rachou - Fundação Oswaldo Cruz, Av. Augusto de Lima 1715, Belo Horizonte, MG, 30190-002, Brazil

³Department of Medical Microbiology, College of Medicine, University of Lagos Teaching Hospital, Idi Araba, Lagos, Nigeria

⁴Department of Medical Microbiology, College of Medicine, University of Lagos Teaching Hospital, Idi Araba, Lagos, Nigeria.

Abstract

Schistosoma haematobium infection is endemic in Nigeria, with substantial transmissions in all the states of the federation and a high prevalence rate in schools. Literature has linked bladder cancer, mostly squamous cell type, with long-term *S. haematobium* infections. The objective of this descriptive study was to screen exfoliated cells in the urine of *S. haematobium*-infected patients for squamous cell abnormalities through cytopathological examinations. Study participants were drawn from Imala Odo, a community near Oyan Dam in Abeokuta North Local Government Area, Ogun state, Southwest Nigeria. Due to a considerable day-to-day variation of *S. haematobium* eggs in urine, 3 rounds of 200 ml of urine samples were collected on 3 different days from 32 infected patients and 10 uninfected controls and examined. Cytological preparations of the infected 15 males and 8 females and 10 controls (5 males and 5 females) were screened for squamous cell abnormalities. Severely dysplastic to frankly malignant squamous cells were observed in 1 (3.1%) male and 2 (6.3%) females, while no abnormality was observed in the controls.



Urinary schistosomiasis transmission in Epe, an urban community of Southwest Nigeria

OP Akinwale, VN Akpunonu¹, MB Ajayi, DO Akande, MA Adeleke, PV Gyang, MO Adebayo, AA Dike

Molecular Parasitology Research Laboratory, Public Health Division, Nigerian Institute of Medical Research, Lagos State, ¹Department of Medical Microbiology and Parasitology, School of Medical Laboratory Sciences, Lagos University Teaching Hospital, Idi Araba, Lagos State, Nigeria

KEY WORDS

Molecular, survey, Nigeria, urinary schistosomiasis

ABSTRACT

Background: A survey of *Schistosoma haematobium* infection in Epe, an urban community in Lagos State, Southwest Nigeria, was carried out to ascertain the possibility that schistosomiasis, otherwise considered a rural disease, could reach urban populations. **Materials and Methods:** About 100 ml of voided urine samples from 200 pupils aged 6–13 years [109 (54.5%) males and 91 (45.5%) females], attending an Anglican primary school, Ebute Afuye, and a community primary school, Erepeto, were examined parasitologically for hematuria and *S. haematobium* ova following informed consent obtained from their parents/guardians. All samples were screened using polymerase chain reaction (PCR) amplification of the schistosome *Dra1* gene. Fourteen *Bulinus* snails collected from the two sites, Ebute Afuye (6) and Erepeto (8), were screened for schistosome infection by the PCR amplification of the schistosome *Dra1* gene. PCR-RFLP of the snails' *its* region was analyzed for species identification and a subregion of the *cox 1* gene from four infected snails (two from each site) was amplified and sequenced. **Results:** In the Anglican primary school, Ebute Afuye, and community primary school, Erepeto, 16% and 29% were positive for hematuria, and 16% and 17% had schistosome ova, respectively. PCR analysis showed that 57% and 40% were positive for the infection in Anglican primary school, Ebute Afuye, and community primary school, Erepeto, respectively. PCR screening of the snails confirmed that four from Ebute Afuye and three from Erepeto were infected with schistosomes. PCR-RFLP showed that all the 14 snails were *Bulinus truncatus* while phylogenetic analysis of the sequenced partial *cox 1* gene corroborated the PCR-RFLP results. **Conclusions:** There was a high prevalence of *S. haematobium* infection among the participants detected by PCR, which was able to detect infection in cases otherwise shown to be negative by hematuria. We also observed that *B. truncatus* is one of the snail species responsible for the transmission of urinary schistosomiasis in the Epe community. For national control programs, it is very important that trends in the prevalence and intensity of schistosomiasis in urban cities be monitored.





Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology



Short technical report

Detection of schistosomes polymerase chain reaction amplified DNA by oligochromatographic dipstick

O.P. Akinwale^{a,*}, T. Laurent^b, P. Mertens^b, T. Leclipteux^b, D. Rollinson^c, R. Kane^c,
A. Emery^c, M.B. Ajayi^a, D.O. Akande^a, T.W. Fesobi^d

^a Public Health Division, Nigerian Institute of Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria

^b Coris BioConcept, Gembloux, Belgium

^c Wolfson Wellcome Biomedical Laboratories, Department of Zoology, The Natural History Museum, London, UK

^d Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria

ARTICLE INFO

Article history:

Received 21 December 2007

Received in revised form 3 April 2008

Accepted 5 April 2008

Available online 12 April 2008

Keywords:

Diagnostic test

Schistosomes

DNA

Chromatography

Dipstick

ABSTRACT

The applications of highly specific and sensitive molecular techniques based on polymerase chain reaction (PCR) have constituted a valuable tool for the diagnosis of schistosomiasis and also for the detection of schistosome infections in the snail intermediate hosts. The common method of detecting PCR amplicons is gel electrophoresis in the presence of ethidium bromide, a carcinogen, which is followed by UV transillumination. Other methods, which are available for detecting PCR products, are real-time PCR, PCR–enzyme-linked immunosorbent assay (PCR–ELISA) and mass spectrometry but they are cumbersome while they are sometimes complex and expensive. Therefore, a simple method of PCR product detection would be a welcome idea and a most valuable tool particularly in disease endemic countries with limited research facilities and resources. In this study, we applied a simple and rapid method for the detection of *Schistosoma haematobium* and *Schistosoma mansoni* PCR amplified DNA products using oligochromatographic (OC) dipstick. The amplicons are visualized by hybridization with a gold conjugated probe, while a control for the chromatographic migration is incorporated in the assay. The lower detection limit observed was 10 fg of genomic DNA from each of the two species, while the dipstick was also specific for each of the species used in this study.

© 2008 Elsevier B.V. All rights reserved.



Differentiating *Schistosoma haematobium* from *Schistosoma magrebowiei* and other closely related schistosomes by polymerase chain reaction amplification of a species specific mitochondrial gene

Olaoluwa P Akinwale¹, Tang T Hock², Fan Chia-Kwung³, Qi Zheng⁴, Shen Haimo⁴, Charles Ezeh⁴, Pam V Gyang^{1,3}

¹Public Health Division, Molecular Parasitology Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria, ²Infectious Disease Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia, ³Department of Parasitology, College of Medicine, Taipei Medical University, Taipei 1101, Taiwan, ⁴National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 20025, PR, China


KEY WORDS

Differential identification, nicotinamide adenine dinucleotide subunit 3 gene, polymerase chain reaction assay, *Schistosoma haematobium*, *Schistosoma magrebowiei*

ABSTRACT

Introduction: *Schistosoma haematobium* infection afflicts about 150 million people in 53 countries in Africa and the Middle East. In many endemic areas, *S. haematobium* is sympatric with *Schistosoma bovis*, *Schistosoma mattheei*, *Schistosoma curassoni*, *Schistosoma intercalatum* and *Schistosoma magrebowiei*, its closely related species. In addition, they also develop in the same intermediate snail hosts. Since these schistosome species often infect snails inhabiting the same bodies of water, examining cercariae or infected snails for estimating transmission of *S. haematobium* is always confounded by the need to differentially identify *S. haematobium* from these other species. Recently, differentiating *S. haematobium* by polymerase chain reaction (PCR) from *S. bovis*, *S. mattheei*, *S. curassoni* and *S. intercalatum*, but not from *S. magrebowiei* was reported. However, to be able to evaluate residual *S. haematobium* transmission after control interventions in areas where *S. haematobium* may be sympatric with *S. magrebowiei*, a differential tool for accurate monitoring of infected snails is needed. **Materials and Methods:** Thus in this study, we developed a new PCR assay using a pair of primers, ShND-1/ShND-2, to amplify a target sequence of 1117 bp (GenBank accession number KF834975) from *S. haematobium* mitochondrion complete genome (GenBank accession number DQ157222). Sensitivity of the assay was determined by PCR amplification of different concentrations of *S. haematobium* gDNA serially diluted from 10ng to 0.1pg. For assay specificity, different concentrations of gDNA from *S. haematobium* and the other

schistosome species, 20 positive urine samples and five controls as well as 20 infected snails were subjected to PCR amplification, while some of the PCR products were sequenced. **Results:** The assay detected up to 1pg of *S. haematobium* gDNA, while a differential identification of *S. haematobium* DNA content from other closely related species was achieved when applied to urine and naturally infected snails. When a protein-protein blast search was carried out using Blastp, the amplified sequence was found to encode a protein that shows a 100% similarity with *S. haematobium* nicotinamide adenine dinucleotide dehydrogenase subunit 3 (GenBank accession number YP_626524.1). **Conclusion:** The PCR assay was sensitive, specific and was able to successfully differentiate *S. haematobium* from *S. magrebowiei*, in addition to its other closely related animal infective schistosome species.

Tropical Parasitology 

Address for correspondence

Dr. Olaoluwa P Akinwale,
Public Health Division, Molecular Parasitology Research Laboratory,
Nigeria Institute of Medical Research, P.M.B. 2013,
Yaba, Lagos State, Nigeria.
E-mail: pheabian@yahoo.co.uk

Access this article online

| | |
|---|--|
| Quick Response Code:  | Website: www.tropicalparasitology.org |
| | DOI: 10.4103/2229-5070.129163 |



Molecular approaches to the identification of *Bulinus* species in south-west Nigeria and observations on natural snail infections with schistosomes

O.P. Akinwale^{1*}, R.A. Kane², D. Rollinson², J.R. Stothard²,
M.B. Ajayi¹, D.O. Akande¹, M.O. Ogungbemi¹, C. Duker¹,
P.V. Gyang¹ and M.A. Adeleke¹

¹Molecular Parasitology Laboratory, Public Health Division, Nigerian Institute of Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria: ²Wolfson Wellcome Biomedical Laboratories, Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK

(Accepted 10 August 2010)

Abstract

The current study considers the distribution of a small sample of 138 *Bulinus* snails, across 28 localities within eight Nigerian states. Snails were identified using a combination of molecular methods involving both DNA sequencing of a partial cytochrome oxidase subunit 1 (*cox1*) fragment and restriction profiles obtained from ribosomal internal transcribed spacer (*its*) amplicons. The results showed that the majority of *Bulinus* samples tested belonged to the species *Bulinus truncatus* while only two were *Bulinus globosus*. The use of *RsaI* restriction endonuclease to cleave the ribosomal *its* of *Bulinus*, as a method of species identification, was adopted for the majority of samples, this being a quicker and cheaper method better suited to small laboratory environments. Polymerase chain reaction (PCR) amplification of the schistosome *Dra1* repeat within each of the collected *Bulinus* samples was employed to determine the extent and distribution of infected snails within the sample areas. Successful amplification of the *Dra1* repeat demonstrated that 29.7% of snails were infected with schistosomes. Sequencing of the partial schistosome *its* from a small subset of snail samples suggested that some snails were either penetrated by both *Schistosoma haematobium* and *Schistosoma bovis* miracidia or hybrid miracidia formed from the two species.



MOLECULAR CHARACTERISATION OF *BULINUS* SNAILS – INTERMEDIATE HOSTS OF SCHISTOSOMES IN OGUN STATE, SOUTH-WESTERN NIGERIA

OLAOLUWA AKINWALE^{1,*}, OPEYEMI OSO², OYETUNDE SALAWU³, ALEXANDER ODAIBO², PETRUS TANG⁴, TING-WEN CHEN⁵, PAM GYANG¹

¹ Molecular Parasitology Research Laboratory, Public Health Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria (e-mail: pheabian@yahoo.co.uk, gyangvince@yahoo.com)

² Parasitology Research Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria (e-mail: holyopeforchrist@yahoo.com, alexodaibo@yahoo.com)

³ Department of Biosciences and Biotechnology, Babcock University, Ilishan, Ilishan-Remo, Nigeria (e-mail: zootund@yahoo.com)

⁴ Molecular Regulation and Bioinformatics Laboratory, Department of Parasitology, College of Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan (e-mail: petang@mail.cgu.edu.tw)

⁵ Bioinformatics Core Laboratory, Molecular Medicine Research Centre, Chang Gung University, Kweishan, Taoyuan, Taiwan (e-mail: afratw@gmail.com)

*corresponding author

ABSTRACT: Freshwater snails of the genus *Bulinus* O. F. Müller, 1781 are intermediate hosts for schistosomes, trematode parasites which cause schistosomiasis. The genus includes closely related species complexes with restricted gene flow between populations of each taxon. Despite their importance as intermediate hosts, unambiguous identification of these snails remains challenging. We applied molecular approach to their identification to achieve a better understanding of the epidemiology of schistosomiasis in an endemic region, south-western Nigeria. A total of 149 snails were collected and their genomic DNA was screened for schistosome infection using PCR amplification of schistosome *DraI* repeat sequence. The snails were identified by PCR-RFLP and/or sequencing of an amplicon of their entire ITS region including the 5.8S ribosomal RNA (rRNA) gene. Four *Bulinus* species, namely *B. globosus* (Morelet, 1866), *B. forskalii* (Ehrenberg, 1831), *B. camerunensis* Mandahl-Barth, 1957 and *B. senegalensis* O. F. Müller, 1781 were identified, and 34.9% (n = 52) of the 149 snails were infected: *B. globosus* 25.5% (n = 38), *B. forskalii* 5.4% (n = 8), *B. camerunensis* 2.7% (n = 4) and *B. senegalensis* 1.3% (n = 2). Restriction fragment profiles of the ribosomal ITS region for *B. globosus* closely matched those obtained in our previous study thus confirming the view that ribosomal ITS region of these snails could be well suited for taxonomic studies. The use of sequencing for species identification was costly and time-consuming, but it was effective in resolving true identities of snails whose restriction profiles were similar and inconclusive.

KEY WORDS: species identification, *Bulinus*, ITS, rRNA gene, schistosome, schistosomiasis, Nigeria



MOLECULAR SURVEY OF FRESHWATER SNAIL INTERMEDIATE HOSTS OF *Schistosoma malayensis* IN NORTHERN PENINSULAR MALAYSIA

Akinwale, P O.^{1*}, Tang, T.H², Doblin, A.S² and Cheah, H.L².

¹Molecular Parasitology Research Laboratory, Public Health Division, Nigerian Institute of Medical Research, Yaba, Lagos state, Nigeria, ²Infectious Disease Cluster, Advanced Medical & Dental Institute, Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

ABSTRACT

This study aimed at incorporating current molecular techniques into epidemiologic surveillance for schistosomiasis, particularly in reportedly low transmission areas of peninsular Malaysia. Snail sampling was conducted in five locations in Kampung Kaki Bukit, Baling, Kedah State, northern peninsular Malaysia. A total number of 481 snails were collected: Kampung Tanjung, Mukim Pulau (n = 85), Kilang Kampung (n = 102), Kampung Sekolah, Pulau (n = 125), Carok Belantek, Mukim Pulau Kampung (n = 93) and Carok Teluk Teduri, Mukim Pulau Kampung (76). Out of which 104 snails [Kampung Tanjung Mukim Pulau (n = 6), Kilang Kampung (n = 4), Kampung Sekolah, Pulau (n = 2), Carok Belantek, Mukim Pulau Kampung (n = 80) and CarokTelukTeduri, MukimPulaiKampung (n = 12)] were subjected to PCR amplification for further identification and also screening for *S. malayensis* infection. Twenty one snails from three locations; Kampung Tanjung, Mukim Pulau (n = 3), Carok Belantek, Mukim Pulau Kampung (n = 14) and Carok Teluk Teduri, Mukim Pulau Kampung (n = 4) were identified as *Robertsias* *ilvicola*, the snail intermediate host of *S. malayensis*, based on PCR amplification of the snails' cytochrome oxidase 1 (*cox1*) gene fragments. None of the 21 snails was positive for *S. malayensis*. More *R. silvicola* species were found in Carok Belantek, Mukim Pulau Kampung than in other locations sampled in this study, because the stream was shallower and more gently flowing, thus making human water contact with such streams very likely.

KEYWORDS: Molecular, survey, snail, intermediate host, *Schistosoma malayensis*

Received for Publication: 22/04/13

Accepted for Publication: 26/06/13

Corresponding Author: E-mail: pheabian@yahoo.co.uk





Thursday, November 19, 2015



Research for National Health

It is not all about parasites

- There was very few research on non-communicable diseases in NIMR at a time
- Took up the challenge and constituted a team of researchers from within and outside NIMR
- Competed for and won a seed grant awarded by the International Association of National Public Health Institutes (IANPHI)
- Conducted a study titled "Intervention study on non-communicable diseases and preventable lifestyle risk factors in three urban slums of Lagos Nigeria"

The following are publications from the study

Akinwale OP, Oyefara JO, Adejoh PE, Adeneye AA, Adeneye AK, Musa ZA, Oyedeji KS, Sulyman MA. (2013). Benefits of using community-engaged research approach to promote healthy lifestyles in three Nigerian urban slums. *Southern African Journal of Epidemiology and Infection*. 29(1):48-50(ISSN 1015-8782).

Olaoluwa Pheabian Akinwale, Lekan John Oyefara, Pius Adejoh, Adejuwon Adewale Adeneye, Adeniyi Kazeem Adeneye, Zaidat Adesola Musa, Kolawole Solomon Oyedeji, Medinat Ayobami Sulyman (2013). Survey of hypertension, diabetes and obesity in three Nigerian urban slums. *Iranian Journal of Public Health* 42(9): 972-979.

Akinwale OP, Adeneye AK, Musa ZA, Oyedeji KS, Sulyman MA, Oyefara JO, Adejoh PE, Adeneye AA. (2013). Living conditions and public health status in three urban slums of Lagos, Nigeria. *South East Asia Journal of Public Health (SEAJPH)* 3(1): 36-41.

Akinwale, O. P., Adeneye, A. K., Oyefara, J. O., Adejoh, P. E., Musa, Z. A., Oyedeji, K. S. O., Sulyman, M. A., and Adeneye, A. A. (2015). Behavioral risk factors for non-communicable diseases in three popular Nigerian urban slums using WHO STEPwise approach. *Iranian Journal of Public Health*. In press.



MY MENTORSHIP PROGRAM FOR YOUNG RESEARCHERS

PRINCIPLES

- Give kudos to distinguished researchers for their accomplishments and successful careers
- Spare a thought for younger researchers who can do the same but need a little encouragement
- Never underestimate the power of potentials of my mentees

CORE OBJECTIVES

- Inspire and build the research capacity of next generation of researchers
- Assist them to gain international exposures and acquire skills in up-to-date and cutting edge research
- Secure scholarships for them to undertake Masters, PhD and Post-doctoral programs in reputable institutions overseas
- Strengthen their grant writing capabilities

ACCOMPLISHMENT

Some of my mentees in and outside NIMR have

- completed their masters, doctoral and post-doctoral programs abroad and are back home
- published their research findings in international journals with high impact factors
- presented their results in national and international conferences
- and are mentoring others



- **O. Hassan**, A. O. J. Amoo, O.P. Akinwale, **M. A. Adeleke** and **P.V. Gyang**. Molecular Characterization and Detection of Infection in Vector Snails of Urinary Schistosomiasis around Erinle and Eko Ende Dams in South West Nigeria. *British Microbiology Research Journal*. In press.
- **Vincent P. Gyang**, Ting-Wu Chuang, Yueh-Lun Lee, Olaoluwa P. Akinwale, Akwaowo Orok, Olusola Ajibaye, Chien-Wei Liao, Po-Ching Cheng, Chia-Mei Chou, Ying-Chieh Huang, Kuo-Hua Fan, Chia-Kwung Fan. Intestinal parasitic infections: current status and associated risk factors among schoolchildren in an archetypal African urban slum in Nigeria. *African Health Sciences*. In press.
- **Vincent Pam Gyang**, Olaoluwa Pheabian Akinwale, Yueh-Lun Lee, Ting-Wu Chuang, Akwaowo Orok, Olusola Ajibaye, Chien-Wei Liao, Po-Ching Cheng, Chia-Mei Chou, Ying-Chieh Huang, Kuo-Hua Fan and Chia-Kwung Fan. (2015). Toxoplasma gondii infection: seroprevalence and associated risk factors among primary school children in Lagos City, Southern Nigeria. *Revista da Sociedade Brasileira de Medicina Tropical* 48(1): 56-63. DOI.org/10.1590/0037-8682-0310-2014.
- **Pam V. Gyang**, Olaoluwa P. Akinwale, Yueh-Lun Lee, Ting-Wu Chuang, Akwaowo B. Orok, Olusola Ajibaye, Chien-Wei Liao, Po-Ching Chen, Chia-Mei Chou, Ying-Chieh Huang, Ursula Barghouth , Chia-Kwung Fan. (2015). Seroprevalence, disease awareness, and risk factors for Toxocara canis infection among primary school children in Makoko, an urban slum community in Nigeria. *Acta Tropica* 146: 135–140.
- **AO Hassan**, AOJ Amoo, OP Akinwale, AM Deji-Agboola, **MA Adeleke**, **PV Gyang** (2014). Current status of urinary schistosomiasis in communities around the Erinle and Eko-Ende Dams and the implications for schistosomiasis control in Nigeria. *Southern African Journal of Infectious Diseases* 29(4):137-140.
- **Adeleke, M. A.**, Sam-Wobo, S. O., Akinwale, O. P., Olatunde, G. O., Mafiana, C. F. (2012). Biting on human body parts of Simulium vectors and its implication on the manifestation of Onchocerca nodules along Osun River System, Southwest Nigeria. *Journal of Vector Borne Diseases* 49 (3): 140-142.



- **Hassan, A. O.**, Amoo, A. O. J., Akinwale, O. P., Deji-Agboola, A. M., **Adeleke, M. A.**, **Gyang, P. V.** and Oluwadun, A. (2012). Human water contact activities and urinary schistosomiasis around Erinle and Eko-ende dams. *Global Advanced Research Journal of Medicine and Medical Sciences*. 1(4): 77 – 84.
- **Adeleke, M. A.**, Sam-Wobo, S. O., Olatunde, G. O., Akinwale, O. P. and Mafiana, C. F. (2012). Attraction of *Simulium damnosum* Complex To *Pterocarpus santalinoides*: A preliminary study. *Munis Entomology and Zoology* 7(1): 368-371.
- **Adeleke, M. A.**, Sam-Wobo, S. O., Olatunde, G. O., Akinwale, O. P., Ekpo, U. F. and Mafiana, C. F. (2011). Bioecology of *Simulium damnosum* Theobald Complex along Osun River, Southwest Nigeria. *Journal of Rural and Tropical Public Health* 10: 39 - 43.
- **Monsuru Adebayo Adeleke**, Chiedu Felix Mafiana, Sammy Olufemi Sam-Wobo, Ganiyu Olatunji Olatunde and Olaoluwa Pheabian Akinwale (2010). Morphotaxonomic studies on *Simulium damnosum* Theobald complex (Diptera: Simuliidae) along Oshun River, southwestern Nigeria. *Acta Entomologica Sinica* 53(11): 1319 - 1324.
- **M. A. Adeleke**, C. F. Mafiana, S. O. Sam-Wobo, O. P. Akinwale, G. O. Olatunde, S. M. Sanfo, A. Adjami and L. Toe (2010). Molecular characterization of the *Simulium damnosum* complex (Diptera: Simuliidae) found along the Osun River system, in south–western Nigeria. *Annals of Tropical Medicine and Parasitology* 104(8): 679 - 683.
- **Monsuru A. Adeleke**, Chiedu F. Mafiana, Sammy O. Sam-Wobo, Ganiyu O. Olatunde, Uwem F. Ekpo, Olaoluwa P. Akinwale and Laurent Toe (2010). Biting behaviour of *Simulium damnosum* complex and *Onchocerca volvulus* infection along the Osun River, Southwest Nigeria. *Parasites and Vectors*. 3: 93 doi:10.1186/1756-3305-3-93.
- **Ude, E. A. G.**, Akinwale, O. P., Ukaga, C. N., Ajayi, M. B., Akande, D. O., **Adeleke, M. A.**, **Gyang, P. V.** and Dike, A. A. (2009). Prevalence of urinary schistosomiasis in Umuowele, Agulu community, Anambra State, Nigeria. *International Journal of Health Research*. 2(4): 347 - 353.



ONGOING RESEARCH PROJECTS FUNDED BY INTERNATIONAL GRANTS

- Building laboratory diagnostic capacity and strengthening existing laboratory system for Buruli ulcer control and research in Nigeria.
Funded by - UNICEF/UNDP/World Bank/WHO/Special Programme for Research and Training in Tropical Diseases (TDR).
- Evaluation of skills for life project of youth empowerment and development initiative for youth with intellectual disabilities in Nigeria.
Funded by - the Special Olympics Incorporated, USA.
- Node Regional Pilot – a Smartphone Application for Real-time Infectious Diseases Monitoring.
Funded by - the United States National Science Foundation.



MY VISIT TO A BIOSAFETY LEVEL 4 (BSL-4) LABORATORY

- BSL-4 laboratories - for research and diagnosis of dangerous and exotic diseases such as Ebola, Lassa fever and Marburg
- Visited the National Bio containment Facility - Galveston National Laboratory (GNL), University of Texas Medical Branch, Galveston, Texas, USA
- GNL is a high security National Bio containment Laboratory that houses several BSL-4 research laboratories
- Run by the University of Texas Medical Branch in Galveston
- Is one of only two such facilities in the United States and the largest in the world located on an academic campus
- Has special engineering and design features to prevent microorganisms from being disseminated into the environment
- Viruses handled to BSL-4 laboratories include – Ebola, Crimean-Congo hemorrhagic fever, Junin, Lassa fever, Machupo, Marburg, and tick-borne encephalitis virus complex including Absettarov, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian Spring-Summer encephalitis.





SERVICE TO MY INSTITUTE AND COMMUNITY

Time consuming but a very valuable experience

Membership and sometimes chairmanship of committees such as

- National Reproductive Health Research Committee in Nigeria
- NIMR Senior Management Committee
- Finance Committee, NIMR
- Appointment and Promotions Committee (Scientific), NIMR
- Appointment and Promotions Committee (Technical and Administration), NIMR
- Due Process Monitoring Committee (Scientific supplies), NIMR
- NIMR Institutional Review Board
- Committee for Compilation of Management Guidelines for the Human Virology Laboratory, NIMR
- Research and Training Assessment Committee, NIMR
- Scientific Committee, 2nd Annual NIMR Scientific Conference on Health Research in Nigeria
- Staff Remunerations Evaluation Committee, ASURI NIMR
- Head of Public Health Division, NIMR
- Matron, Biological Science Students' Association, University of Agriculture, Abeokuta, Nigeria
- Reviewer of postgraduate seminars in the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos
- Member of Editorial Boards of many international scientific journals



PROFESSIONAL MEMBERSHIP

- Member, Nigerian Society for Parasitology and Public Health
- Fellow, Commonwealth Science Council (CSC)
- Fellow, Royal Society of Tropical Medicine and Hygiene (RSTMH), UK
- Member, Third World Organization of Women in Science (TWOWS)
- Member, International Network of Women Engineers and Scientists (INWES)
- Member, International Society for Urban Health (ISUH)
- Member/Author, Cochrane Collaboration Depression, Anxiety and Neurosis Group (CCDAN), the Cochrane Collaboration, UK
- Member, the Systematics Association, UK
- Member, the International Society for Neglected Tropical Diseases (ISNTD)
- Member, African Research Network for Neglected Tropical Diseases (ARNTD)
- Member, World Intellectual Property Organization (WIPO | Re: Search).



CONTROLLING PARASITIC INFECTIONS - THE WAY FORWARD

Adequate health is a legitimate right of every Nigerian

However, factors resulting in parasitic diseases expand beyond the scope of the health sector

Such factors include socio-economic and environmental indices such as

- Poverty
- Illiteracy
- general social deprivation
- poor sanitary condition
- unsafe drinking water
- human perception and behavior that encourage parasite transmission

Parasitic diseases of poverty can be controlled by improving these indices

In addition, technology and chemotherapy will lessen their negative effects

But chemotherapy alone cannot prevent new infections and re-infections

Improving the health of the poor is therefore not through technology alone, but by ensuring that the basic needs of all are met through intervention that is liberal in action

But our problem in Nigeria is not about resources but equity



Need to change unhealthy behaviors that promote parasitic infections

Behaviors such as

- enjoying undercooked meat and meat products
- eating with dirty hands
- eating unwashed fruits and vegetables with a strong assumption that 'germs don't kill black man'
- keeping dogs as 'baby nurse' to eat fecal wastes and wipe the anuses of babies
- dumping wastes indiscriminately
- urinating and defecating at every available place
- allowing domestic animals such as pigs, goats, fowls, cats and dogs to roam freely in homes and on the streets

Option of behavioral change activities attracts minimal financial demand from government and the people

This option will successfully complement disease control efforts

In addition, education can improve knowledge about safe hygiene practices and uptake of health-related information thus leading to sustained healthy behavior.



CONCLUSION

To achieve a holistic control of these infections, government should

- see the occurrence of parasitic diseases in the present century as a social defect
- formulate appropriate policies and demonstrate the political will to address them
- develop a broad based strategy that combines
 - good planning
 - policy consistency
 - a progressive improvement guideline supported by strong structure for its implementation

Remember, healthy planning, healthy city, healthy people



ACKNOWLEDGEMENT

- Prof Samuel Ashaolu of the Zoology Department, Faculty of Science, University of Ife (now Obafemi Awolowo University)
- Prof David Rollinson, former President, World Federation of Parasitologists and Individual Merit Researcher in Parasitology as well as the Director of WHO Collaborating Centre, Natural History Museum, currently a Professor of Life Sciences, University of Aberdeen, UK
- Dr David Johnston, currently of the School of Medicine, University of Southampton, Southampton, United Kingdom
- Prof Steven Williams of Smith College, Northampton, Massachusetts, USA
- Dr Guilherme Oliveira of the René Rachou Research Center, Minas Gerais, Brazil



- My loving parents-in-law, late Papa George and late Mama Emily Akinwale - When counting my blessings, I count my mother-in-law twice
- My Air Force General
- I acknowledge my wonderful children, both biological and non-biological
- My sisters, brothers, uncles, aunties, cousins, nieces, nephews, in-laws, pastors, brethren and friends, home and abroad
- The entire NIMR community for giving me this wonderful opportunity to showcase my work, I will cherish it forever
- All my colleagues outside NIMR
- In the course of my life journey, I also came across people who treated me wrong. To them I say thank you for making me strong
- Finally, I give thanks to God through whose grace I have reached this level in my career



THANK YOU FOR YOUR ATTENTION

