THE PROFILE OF MALARIA AND INTESTINAL PARASITES AMONG REFUGEES ATTENDING THE DENIS HURLEY CENTRE IN CENTRAL DURBAN IN 2014.

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10TH December 2014

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Table of Contents

Acronyms and Abbreviations

CDC	Centre for Disease Control and Prevention
CMR	Crude Mortality Rate
DHC	Denis Hurley Centre
HIS	Health Information Data Capturing System
HIV	Human immunodeficiency virus
IDP	Internally displaced people
IMR	Infant Mortality Rate
MSF	Medicine's Sans Frontiers'
RDT	Rapid Diagnostic Test
SARS	Severe Acute Respiratory Syndrome
SPSS	Statistical Programme for Social Sciences
UKZN	University of KwaZulu-Natal
UNHCR	United Nations High Commission for Refugees
WHO	World Health Organisation

Abstract

Background

The majority of the refugee population congregate and live in major South African cities, some in overcrowded housing without access to basic health care and social services. These conditions put them at risk of transmission and spread of communicable diseases both amongst themselves and in the population they come into contact with. Therefore, knowledge of the burden of communicable diseases among them is crucial. In South Africa, there is limited data available on the prevalence of malaria and intestinal parasites in refugee populations.

Aim

The aim of the study was to determine the prevalence of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in South Africa in 2014.

Methods

Three articles analyse 303 participants, who attended the Denis Hurley Centre, Emmanuel Cathedral Parish in Central Durban, aged 18 years and above, provided written consent and responded to a questionnaire on their demographic details. The presence of malaria, intestinal parasites and haematological profiles of the participants were analysed using Rapid test detection kits, microscopy and the Sysmex XE 5000 automated haematology analyser.

Results

The results confirm the presence of asymptomatic malaria (prevalence 3.8%) in the refugee population living in the city. The majority of those infected originated from the Democratic Republic of Congo, followed by Burundi and Rwanda. More than 90% of the infections were due to *Plasmodium falciparum*. The prevalence of intestinal parasite infection among 270 participants was 18.8%. Common parasites identified were hookworm and *A. lumbricoides*. The results showed eosinophillia in 40.2% of 92 participants who were screened for haematological parameters. The mean absolute haemoglobin (Hb) level was reduced in 6.5% of the malaria positive patients (9.2 g/dl) with an extremely low packed cell volume (PCV) of 28.3%. While the total non-malaria infected cases 93.5% had a normal mean absolute Hb value of 12.6 g/dl and a slightly low packed cell volume value of 38 %.

Conclusion

Results from the present study confirm the presence of and provided useful information on the prevalence of asymptomatic malaria and chronic intestinal parasites in the refugees attending the Denis Hurley Centre in Central Durban.

CHAPTER 1

1. Introduction

1.1 Background

The World Health Organization (WHO) defines refugees as “persons fleeing to a place of safety, especially those who flee to a foreign country or power to escape danger or persecution in their own country of habitual residence because of race, religion, or political belief.” [1] While a refugee flees their country, an immigrant is defined as “a person who takes up permanent residence in a country other than their homeland. [2] Illegal immigrants and refugees face similar challenges and therefore leave their countries of origin under similar conditions. For the purpose of this study, the terms refugees, illegal immigrants and the externally displaced will be used interchangeably.

The WHO requested the United Nations High Commission for Refugees (UNHCR) to incorporate the topic of health and well-being of refugees globally as part of its agenda. [1] Thirty per cent of the 9.2 million refugees and 25 million displaced persons in the world are found in sub-Saharan Africa. [3] Estimates have indicated that South Africa harbours the highest number of asylum seekers globally. [4] Of this estimate, approximately thirty thousand thereof are in possession of legal documentation. In 2010, it was tentatively estimated that around 58 000 individuals live as refugees in South Africa and hail from countries, such as Zimbabwe, Mozambique, Somalia, Burundi, Rwanda and the Democratic Republic of Congo (DRC). The exact number of illegal immigrants in South Africa remains an unknown figure .[4]

As a result of long journeys and pre-existing condition or illnesses that could have been contracted from previous refugee settlements throughout South Africa, refugees on their arrival in Durban are in need of medical care. [5] These communicable diseases are usually asymptomatic and may not present with clinical signs that are common to the local population. This is due to the different strains of parasites that refugees may carry and that could result in severe disease when transmitted to the local population. However they encounter challenges at health

care facilities and are denied their basic rights due to language barriers and xenophobic attitudes from the staff.
As refugees compete for jobs and improved social services with the local South Africans, they are often subjected to brutal discriminatory attacks from the locals. [1,5]

Those that are able to obtain work permits still face several challenges. The incidence and spread of communicable diseases is probably elevated in South Africa, due to the free interaction of locals and refugees who have not received adequate health screening. [5]

There are a multitude of refugees from various regions of Africa presently living in crowded conditions in Central Durban. The danger exists of individuals, within this transient and polyglot community of infection by particular tropical infectious diseases. It is possible that they could be infected with a variety of tropical infections. Their lack of acceptance and the indifference or even hostility from locals frequently results in a lack of acceptance at health care facilities and there exists an incipient health threat to all residents of the city. They inadvertently face lack of acceptance at health care facilities and discrimination therefore posing a health threat to both fellow refugees and the general population they mingle with on a daily basis. Absence of allocation of accommodation to these refugees in an overcrowded Central Durban results in their seeking shelters that lack adequate water and sanitation. A health threat exists if refugees cannot access necessary health care. [5]

Differences in language were regarded as the main reason for being refused public health care services. Often, they were denied access to see doctors in public health facilities and receive the necessary and essential treatment, due to their inability, as a black person, to speak *IsiZulu*, the local black language. This also posed a problem when admitted to a hospital to receive appropriate treatment and follow up. Their refugee status influenced their rights to be treated fairly and adequately by the hospital staff. [5]

1.2 Aim of the study

The aim of the study was to determine the prevalence of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014 and to ascertain whether there is a risk of transmission of these diseases within the city.

1.3 Objectives

1. To measure the prevalence of symptomatic and asymptomatic malaria carriers and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

2. To measure the blood eosinophil count as an indicator of parasitosis in participants infected with malaria and (or) intestinal parasites**.**

3. To identify the types and intensity of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

4. To determine risk factors for transmission of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

The research will be presented in form of publication research papers.

1.4 Malaria

To date, malaria has proven to constitute one of the most severe causes of morbidity amongst refugee populations worldwide and the UNHCR reported that this disease affects more than a million cross-border migrants, a figure which includes women and children. [1]

It has been estimated that approximately ten per cent of South Africa's entire population continues to live in locations that have been considered as malaria endemic areas. [6] Research has shown that malaria infection and transmission in South Africa occurs mostly in low altitude border regions. These regions include: Mpumalanga, Limpopo and the northern parts of KwaZulu-Natal. In these regions malaria transmission is highly elevated between September and May annually. [6]

Factors favouring malaria transmission within South Africa are; parasitological factors and drug resistance .*Plasmodium falciparum* has been and remains the causative agent in more than 90% of malaria cases in South Africa. Drug resistance patterns to chloroquine were first observed between 1980 and 1987 in South Africa's malaria endemic areas. Between 1999 and 2000.there was a development of sulphadoxine-pyrimethamine resistant parasites in KZN. [7] Currently *P. falciparum* parasites in South Africa appear to be susceptible to artemisinin derivatives and most of their partner drugs. Entomology is another factor that influences malaria transmission in South Africa. *Anopheles gambiae* and *Anopheles funestus* complexes belong to the predominant malaria vectors in South Africa. *An arabiensis* mosquito is zoophilic, feeding on humans and cattle and is both endo and exophagic. This vector has modified its behaviour to be more exophillic .Also the *Anopheles merus* mosquito that has played a limited role in malaria transmission has been found in increasing rates and could become an increasingly important vector. [7] Another important factor is the climate, which imposes distinct biological constraints on the mosquito and the malaria parasite. [7]

Climatic factors like decreased temperatures interfere with the life cycle (sporogony stage) of the malaria parasite, thereby causing low survival rates of the infected mosquitoes to be decreased. Low rainfall restricts thriving of breeding sites, as a result of this, vector population and vectorial capacity of the malaria parasite is negatively affected. In Kwan-Zulu Natal spatial heterogenicity in the incidence of malaria locally has been partly explained by variations in average local rainfall and temperatures. [7]

In a study conducted by Maharaj et al, which spanned over a period of 10 years from the year 2000 to 2010, it was observed that, among the three malaria endemic areas in South Africa, KwaZulu-Natal served as the province that noted the greatest decrease in total number of malaria incidence cases, and that the number of such cases reduced from more than sixty six thousand in 2000 to less than seven thousand in 2010. [6] Local transmissions occur in the three endemic provinces only, while all provinces report imported malaria cases. Approximately 64% of all cases reported in South Africa in 2011 were imported cases. Imported cases of malaria are due to mosquito borne transmission and are acquired outside the country. The origin of imported cases can be traced to a known malarious area outside the country to which the case has travelled. [8]

The mosquito breeds in a warm and humid climate where pools of stagnant water provide the perfect breeding ground .As a result of this is important to emphasize the need to provide hygienic living conditions with adequate access to clean water and sanitation. Malaria proliferates in areas where awareness of the disease is low and where the health care systems are weak. The burden of malaria among the refugee population in Durban needs to be ascertained and assessed and be used to inform health policy for the appropriate delivery of health services to the refugee population, where access to health care remains a challenge, as the health status of the majority of refugees is unknown. [9]

There has occurred only a relatively few studies which have investigated malaria transmission due to imported cases of malaria being imported as a result of refugee migration into KwaZulu-Natal. Most of these refugees originate from areas that are rife with malaria, usually due to the tropical nature of the environment in most areas in sub-Saharan Africa. The, spread of malaria can be ascribed to the unsuitable locationing of most refugee settlements and the fact that the vast preponderance of refugees had to travel through malaria endemic areas, or countries who have experienced minimal success in curbing the spread of malaria. [1]

1.5 Intestinal parasites

Another communicable disease that could be spread easily through refugee movement is intestinal parasitosis, a condition which is caused either by nematodes or protozoa. More than 25% of the world population is infected with nematodes, and protozoan infections, and the burden of this disease fails to be accurately estimated because of persistent under-diagnosis. [10] Populations that migrate over long distances and are on the move, lack access to balanced food combinations and basic services, particularly such as sanitation and water, thus making them more susceptible to these types of diseases. [10]

Refugee populations often serve as ideal targets for increased incidence rates of infestation with intestinal parasites. [11] Factors such as overcrowding, an undrinkable water supply, inadequate sanitary measures, and poor sewage systems constitute challenges to life in refugee settlements, as the diseases are rife in such forced congregations of people, creating the perfect environment for transmission of intestinal parasites. There has been limited research undertaken in these settings, but a variety of cross-sectional surveys have indicated a high burden of intestinal parasites in displacement camps in Sierra Leone. [11]

1.6 Research problem statement

South Africa is host to the highest number of refugees in the world. [4]

Ref. Sowetan. Refugees overwhelm South Africa. 2012. http://www. Sowetanlive. co.za/ news/2012 /05/ 8 /refugees-overwhelm - South Africa (accessed 5 August 2012).

Estimates have shown that, at the start of 2010 Sub-Saharan Africa was home to more than two million refugees; of this number, four hundred and twenty thousand were registered asylum seekers. Statistics have also demonstrated that more than 50% of this number lives in South Africa. Most of the refugees in South Africa are from hyper endemic malaria areas. [4, 12]

The majority of the refugee population congregate and live in major South African cities, some in overcrowded housing without access to basic health care and social services. These conditions put them at risk of transmission and spread of communicable diseases both amongst themselves and in the population they come into contact with. Therefore, knowledge of the burden of communicable diseases among them is crucial. In South Africa, there is limited data available on the prevalence of malaria and intestinal parasites in refugee populations.

Refugees are frequently denied basic health care, specifically due to their inability to communicate with health staff. Several refugees urgently need proper health care and, although primary health care access has been guaranteed through a number of national directives, there continue to remain serious real barriers preventing them from accessing public health facilities. Those barriers or obstacles include language and an inability to pay for consultations. Many refugees at the Medicine's Sans Frontiers'(MSF) clinic indicate that they are made to feel unwelcome in public health facilities because they do not speak the local language. Since they have limited access to health screens, there is a need to determine the burden of communicable diseases amongst refugees. [5, 13]

Access to health care remains a challenge and thus the health status of the majority of refugees remains an unknown factor. Results from this study will be

used to describe the prevalence of malaria and intestinal parasites amongst the refugees attending the DHC in Central Durban.

Reported increased mortality rates of malaria and intestinal parasites are matters of particular concern among refugees. Due to the fact that a large number of malaria cases reported within South Africa occur as a result of or cross border transference from neighbouring countries, the issue of malaria and migration must be tackled urgently. [6] Most refugees that go to the centre for medical treatment come from African countries that are highly endemic to malaria Due to the fact that the effects of both malaria and intestinal parasites are severe if not treated, most refugee clinics, including the DHC clinic, treat the refugees for suspected cases of malaria and intestinal parasitosis without proper diagnosis due to restricted funding.

The public health significance of the study is to lobby that diagnostic services (screening programmes) which are recommended for refugees in other countries will be provided at the clinic and will form part of the national malaria surveillance programme in South Africa.

1.7 Format of Dissertation

This dissertation will be presented in the form of publication articles. There are three articles that were derived from this study, on published, one published abstract and one draft manuscript,

The 3 papers are presented in Chapter 3:

1. Asymptomatic malaria in refugees living in a non endemic South African city.

2. Parasitaemia and haematological alterations in malaria among refugees in Durban, South Africa.

3. Prevalence of intestinal parasites among adult refugees living in Central Durban.

CHAPTER 2

2.0 Literature review

It has been estimated that at the onset of 2011, the UNHCR was caring for more than 10 million refugees globally .Almost 5 million refugees were sustained and looked after by either private or United Nations relief agencies. [12] Twenty per cent of such refugees live in Africa .This specific number represents refugees that have been registered. They are faced with the following options: resettlement, integration into the communities or repatriation. [12] The WHO acknowledges that refugees are classified as vulnerable persons, a designation due to several reasons, which include their relocation, poverty and their demographic make-up or profile with 80% being women and children. [3] The vulnerability of refugees often stems from the fact that these individuals arrive in a foreign land with absolutely no basic knowledge of the way of life or the language of the people within the host countries. Owing to these factors, they bear the brunt of discrimination, a hostility which frequently results in violent xenophobic attacks being directed against them.

Towards the end of 2010, more than 58,000 individuals whose countries of origin mostly involved Angola, the DRC, Burundi, Rwanda and Somalia, had been recognized as refugees in South Africa. [1] They are permitted to find jobs and have a right to access basic social services. These refugees encounter various challenges due to the difficulties that are associated with acquiring proper documentation. The aforementioned violent xenophobic attacks have arisen due to the perceived competition between indigenous South Africans and the refugee population for improved social services, business ventures and job opportunities. [1]

In an article released in June 2009, by Doctors without Borders/Medicines Sans Frontières (MSF) titled" No refuge, access denied", refugee health was a major concern. This report narrated the dangers refugees were exposed to when crossing the border into South Africa, the daily dangers they confront , while residing in South Africa, and their inadequate access to basic health care .At the MSF clinic, refugees suffered from gastro intestinal ailments, skin diseases and respiratory disease. [13]

Several diseases were ascribable to the unhygienic and overcrowded living conditions that these refugees were subjected to. The article further stated that if there is no immediate intervention, refugee health will not improve.

The South African government has enacted several attempts to introduce and maintain domestic standards and ensure protection of the rights of refugees within South Africa .Irrespective of the success or inadequate nature of these attempts, asylum seekers and refugees remain a vulnerable migrant population in South Africa today. [12] Several refugees within South Africa daily confront many challenges. Accessing primary health care continues to remain one of the few rights that are being denied to them. Durban has centralised refugee networks in particular areas that allow refugees to interact with other asylum seekers and established refugees. Within this network, they gain a wider knowledge and understanding concerning South Africa and the culture of the host country, and how to make a living in this new environment Thus there exist several identifiable areas within central Durban that are recognised as being particularly populated by foreigners. [12] Most of these foreigners reside and make a living in the city centre of Durban. These areas include: Albert Park, Point, Victoria Street area and the Warwick Junction areas. In spite of existing national legislation within South Africa to uphold refugee rights and grant adequate health services to this vulnerable group, health service providers have often denied refugees the constitutional right to proper health care due to the inherent existing xenophobic attitudes, or the perception thereof, of local members of the staff. [12]

With their health status remaining un-investigated and unchecked, the burden of disease is probably high in these refugees due to the sometimes inherent strains of more virulent pathogens in these individuals. In most instances, the time required for refugees to relocate from one country to another is of a much smaller duration than the period required for the pathogen to infect the host, propagate and infect another individual. [14] The correlation that exists between the movement of a group of people and the spread of disease is now widely being recognised. The high risk of infection with intestinal parasites and the spread of malaria amongst refugees in settlements are epidemics that now simply cannot be ignored. [14]

2.1 Malaria and intestinal parasites

The increased health burden that is posed by malaria infection worldwide cannot be overemphasized. Thirty six per cent of the global population live in high malaria burden areas. [15] Irrespective of the fact that malaria is a serious health issue for refugees in sub Saharan Africa and taking into consideration the long period of time that most refugees spend in overcrowded conditions, limited research has been conducted in these settings to provide accurate data that can assist in gaining insight into, and greater clarity concerning the transmission patterns of the disease, or attaining success in curbing the spread of malaria. [16]

Malaria is transmitted exclusively through the bites of the anopheles mosquito. Some refugees from non- or very low malaria endemic areas have to travel through malaria endemic areas in order to get to South Africa. Non-immune refugees are at a greater risk and have higher mortality rates. Non- immune refugees like children under five or none immune/semi-immune pregnant women are at risk. Refugees that have HIV also fall within this high risk category. [9]

2.2 Malaria in Africa

Historically, the sudden mass movement of people from one country to another has unleashed the development of a marked elevation of malaria incidence in countries that once had recorded a low malaria incidence rate. Despite the fact that one third of the global population reside in low malaria endemic areas a re-emergence of the disease has been detected. [15, 16] In an Australian study, it was reported that in a sample of over three quarters of refugees who arrived from Africa, it was recorded that those who originated from sub-Saharan Africa suffered from the highest prevalence of most diseases, malaria 8%, 7% schistosomiasis, 5% hookworm, and 2% strongyloidiasis. [17] Using the Health Information Data capturing system (HIS), data were collected from several African countries between 2006 and 2009 in UNHCR designated refugee sites. The countries included the following: Uganda, Kenya, Ethiopia, Burundi, Cameroon, and Tanzania.

Information on malaria incidence rates and mortality were investigated, it was established that a rate of 300 per 1000 refugees died. The highest malaria incidence was confirmed from refugee sites in Tanzania, with a recorded incidence in excess of more than 399 cases per 1000 refugees. [18]

2.3 Malaria in South Africa

Among the three malaria endemic provinces in South Africa, it was noted that there was a discernible difference in the proportion of malaria infections that were acquired locally, compared with those that had originated as a result of cross border transfers. It was observed that in both Mpumalanga and KwaZulu-Natal, the number of malaria cases that had occurred as a result of imported malaria (cross border malaria) were of a far greater magnitude or dimension than the cases of the disease that were acquired locally. [6]

There also occurred unclassified cases of malaria in both Limpopo and KwaZulu-Natal. Intestinal parasite infestation has been shown to exhibit extremely high incidence rates amongst refugees on the move, especially those that hail from low income countries. [6]

2.4 Co infection of malaria and intestinal parasites

Significant numbers of studies pertaining to infectious diseases in humans have revealed the means whereby co-infection of parasites adopts or exerts an important role in the infection process through effects on the immune response of the host. [18]

Few studies in the past have linked the dynamics between co-infecting parasites to epidemiological disease. Co-infection is relevant for studies in refugee populations. Due to the presence of a wide variety of parasite species and strains that affect this population, in conjunction with the phenomenon of wide spread parasitism, there forms, or is established, a platform for concurrent infection. [19]

Human hosts have been known to be infected with several parasites at once. This co-infection of parasites within the host can influence parasite density, distribution and dynamics of one another. Studies have shown that infection with one parasite will influence the host's response to infection with other parasites. [19]

Studies have investigated the effect of helminthic infestation (macro-parasites: which are parasites that complete only part of their life cycle within the host, and micro-parasites: which can complete their life cycle within a single host) for example, the malaria parasite. Parasitic infestation will result in two possible immune responses:

(a). During an infection with helminths, release of the T helper cell type 2 (Th2) is induced .This response involves the release of cytokines which include the interleukins: (IL)-4, (IL)-5, and (IL)-13. These interleukins elicit effectors mechanisms which are effective in fighting big intracellular parasites. [19]

(b). Several helminths shield themselves against host immunity by taking advantage of the hosts immune-regulatory pathway. They do this by enhancing T regulatory cells (T reg), which have the ability to suppress both Th1 and Th2 immune responses. This means that helminthic infection can generally lower immune responsiveness which includes protection against micro-parasites.

As a result of this, an initial helminthic infection can result in several micro-parasitic infections at individual level. [19] Limited research has been carried out pertaining to the spread of disease amongst refugees once they have migrated from areas with high intestinal parasites infection incidence rates to none or low endemic areas. [20] Usually a combination of worms and protozoa are detected during stool analysis.

In the case of malaria, research has shown that refugees that resettle from countries of increased endemnicity could possibly act as asymptomatic reservoirs for malaria infection: ***P falciparum*** is of greatest concern due to the severity of disease .

Even though sustained malaria transmission would be unlikely, single cases or small outbreaks would be possible, with the potential for fatal outcomes. [21]

A blood film with a high eosinophil count, could most of the time prove intestinal parasite infection. [20] In refugees, the most ubiquitous parasitic infections that are associated with eosinophillia, are infections with soil transmitted helminths, as well as tissue invasive parasites (the malaria parasite).While the presence of an increased absolute eosinophil count is highly indicative of a recent or current infection with a tissue invasive parasite, the absence of eosinophillia does not indicate the absence of parasitic infection. Eosinophillia is also lowered in persons with chronic infections as the host adjusts to the presence of parasites. An eosinophil count greater than 400 cells/µl is considered as high by most refugee health experts. The lower threshold is used because it will increase sensitivity to the test as the majority of refugees have a high probability of parasitic infection. [22] A limitation to this is that test specificity decreased.

2.5 Status of refugees within South Africa.

There are several identifiable benefits in giving adequate healthcare services to refugee populations .These services will aid in curbing the spread of disease, not only among the refugees but also among the local population with whom they come in contact with. [.5, 20] According to the Refugee Act of 1998 refugees are by law entitled to several rights, which include legal immigration documentation, the right to work, access to social and health services. [12] The areas where refugees settle are characterised by a large number of people that cluster together in a particular sector of their host country area, after an extended and often dangerous journey with no access to clean water, adequate nutrition or sanitary conditions. These conditions pose a health threat to the lives of these refugees .As these individuals continue to move from one place to another without adequate health care, they consequently alter the health status of the communities they come in contact with. [13, 14] This can also result in a financial and economic burden on the society.

Due to their inability to communicate in the local vernacular language and their total lack of cultural and social knowledge in the new environment that they now call

their home, refugees, face several challenges. As previously alluded to, they often find themselves at the centre of violent xenophobic attacks; as a result, thereof, they flee the local areas to the inner cities in search of jobs and a better life. As a result of long journeys and pre-existing conditions or illnesses that could have been contracted from previous refugee concentrated areas throughout South Africa, refugees need medical attention on their arrival in Durban and are in need of further health care. In spite of foreign and domestic guidelines stating the need to provide adequate health services to migrants under the 1998 Refugee Act, refugees still face difficult challenges in accessing health care services. [12]

In a study carried out by Kaplan, it was observed that less than fifty per cent of refugees utilized health care services within South Africa. However, almost thirty per cent who were residing within Durban, acknowledged setbacks when they tried to access health services. The most common reasons cited for being denied health care included communication problems, insufficient legal documentation, verbal abuse by health staff, more difficulty in accessing health care than their South African counterparts and expensive fee payment for these health services. Most of these factors feature prominently in the continued under-utilization of health services by refugees and asylum seekers in Durban.[12] As a result of this, many refugees live in crowded environments and living quarters that allow for the unchecked propagation of various communicable diseases. [5, 14] The threat of the spread of malaria and intestinal parasite infections as a result of these unhygienic living conditions needs to be urgently addressed.

The spread of malaria depends on the changes in the correlation that exists between refugees, the parasite and nature. Whenever a group of people move from a high to low malaria endemic area, a strong likelihood exists it is likely that this will result in an increase of malaria infections .Complications arise when parasites originate from an area where the individuals are drug resistant to anti-malaria drugs. Due to these dynamics, the drug efficacy of the country of destination will be affected. [15]

Reported increased mortality rates of malaria and intestinal parasites are matters of particular concern among refugees. Due to the fact that a large number of malaria cases reported within South Africa occur as a result of or cross border transference from neighbouring countries, the issue of malaria and migration must be tackled urgently. [6]

References

1. The United Nations High Commission for Refugees. UNHCR. UNHCR: Country operations profile. South Africa: 2012.http://www.unhcr.org/egi-bin/texts/vtx/page (accessed 12 July 2012).

2. Webster M. Merriam Webster: online dictionary 2012. Available from: www.merriam-webster.com/dictionary/immigran. (accessed 4 June 2012).

3. Seicean A. Refugee health making a difference in Sub-Saharan Africa: A literature review 2008. www://cwru.edu/med/epidbio/mphp 439 (accessed 7 March 2012).

4. Sowetan. Refugees overwhelm South Africa. 2012. http://www. Sowetanlive. co.za/ news/2012 /05/ 8 /refugees-overwhelm - South Africa (accessed on 5 August 2012**).**

5. Apalata T, Kibiribiri J, Knight S, Lutge E. Refugee perceptions of their health status and quality of health care services in Durban South Africa. A community based survey. Health Link 2007. http://Health link.org.za (accessed 23 February 2012).

6. Maharaj J, Morrison N, Seocharan I, Kruger P, Moonar D, Mabuza A, *et al*. The feasibility of malaria elimination in S Afr. Mal J 2011; 11: 423-428.

7. Maharaj R, Raman J, Moonsar D, Durrheim D, Seocharan I, Kruger P, Shandukani B, Kleinschmidt I. Epidemiology of malaria in South Africa: from control to elimination. S Afr Med J 2013; 103: (10) 779 -788.

8. Misiani E, Shandukani.M, Nawn B, Ramalawa.N, VanShalkwyk C, Bester E, et al. Malaria directorate: Malaria elimination in South Africa. North west malaria meeting. November 2012.

9. World Health Organization. WHO media centre/Fact sheet. Modes of transmission in low endemic areas.http://www.who.int/media centre (accessed 23 November 2013).

10. Chris B, Singh S, Surdashi D. Neglected tropical diseases, conflict and the right to health: National academy of science 2011.http://www.ncbi.nlm.gov/books/NBK 6258 (accessed 4 June 2012).

11. Gabakima A, Konteh R, Kallon M, Mansaray H, Sahr F ,Bah Z. Intestinal protozoa and intestinal helminthic infections in displacement camps in Sierra Leone. J Med Sci 2007; 36: 1-9.

12. Kaplan C. Migration vulnerability and xenophobia: central african refugee and asylum seekers access to health services in Durban South Africa independent study project (ISP) collection 2011 http://www.migration vulnerability and xenophobia _ central African .Refugee (accessed 11April 2013).

13. Doctors without borders. The lives of survival migrants and refugees in South Africa. 2012 http://www.Doctors without borders.org/publications/article (accessed 15 July 2012).

14. Wilson E. Travel and the emergence of infectious diseases. Emerg infect dis 1995; (1):2 (http://www.ncbi.Nlm.Gov/pmc/articlrs/pmc2626831/pdf/8903157 (accessed 6 March 2012).

15. Montavlo G, Renal-Querron M. Fighting against malaria: prevent wars while waiting for the malaria vaccine 2007 http://www.econ.upf.edu/ (accessed 5 June 2012).

16. Bayoh N, Akhwale W, Ombok M, Sang D, Engoki S, Koros D, *et al.* Malaria in Kaukuma refugee camp, Turkana Kenya facilitation of *Anopheles arabiensis* vector population by installed water distribution and catchment systems. Mal J 2011; 10 (149):1475-2875.

17. Martin A, Mack D. Changing faces: A review of infectious disease screening of refugees by the migrant health unit, Western Australia 2003 and 2004. Aust Med J 2007; 11: 607 - 610.

18. Anderson J, Doocy S, Hskew C, Spiegel P, Moss W. The burden of malaria in post -emergency refugee sites: a retrospective study. conflict and health 2011;5:11.http://conflict and health.com/content/5/1/17 (accessed 5 June 2012).

19. Ezenwa V, Jolles A. From host immunity to pathogen invasion: The effect of helminthic co-infection on dynamics of microparasites. Integ Comp Bio 2011; 51(4):540-51.

20. Centre for Disease control and Prevention (CDC). Overseas refugee health guidelines: Intestinal parasites2012 24th April 2012. http://www.cdc.gov/immigrant.refugees.com. (accessed on 24 April 2012).

21. Centres for Disease Conrtol and Prevention. (CDC). Presumptive treatment of P.falciparum malaria in refugees relocating from Sub Saharan Africa to the United States. http://www.cdc.gov/immigrant/refugeehealth/malaria guidelines-domestic.html (accessed 23 November 2013).

22 .Staufner W. Eosinophilia:Refugee health technical assistance centre.http:www.refugee.healthta.org. (accessed 20 November2013).

23. Cheesbrough M, Mac Arthur M. A Laboratory manual for rural tropical hospitals. New York: Churchill Livingstone: Longman Group; 1976; 209.

CHAPTER 3.

Article1: Asymptomatic malaria in refugees living in a non endemic South African city

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Asymptomatic Malaria in Refugees Living in a Non-Endemic South African City

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Abstract

Background: Asymptomatic malaria infection in refugees is both a threat to the lives of the individuals and the public in the host country. Although South Africa has been experiencing an unprecedented influx of refugees since 1994, data on malaria infection among refugees is lacking. Such information is critical since South Africa is among the countries that have planned to eliminate malaria. The objective of this study was to determine prevalence of asymptomatic malaria infection among a refugee population living in a city of KwaZulu-Natal province, South Africa.

Methods and Findings: A survey was conducted on adult refugee participants who attended a faith-based facility offering social services in a city of KwaZulu-Natal province, South Africa. The participants were screened for the presence of malaria using rapid diagnostic tests and microscopy. Demographic data for the participants were obtained using a closed ended questionnaire. Data was obtained for 303 participants consisting of 51.5% females and 47.5% males, ranging from 19 to 64 years old. More than 95% of them originated from sub-Saharan African countries. Two hundred and ninety participants provided a blood sample for screening of malaria. Of these, 3.8% tested positive for rapid diagnostic test and 5.9% for microscopy. The majority of malaria infections were due to Plasmodium falciparum.

Conclusions: The study confirms the presence of asymptomatic malaria infections among a refugee population residing in a city of KwaZulu-Natal province that is not endemic for malaria. The results have important implications for both public health and malaria control in South Africa, particularly since the country has decided to eliminate malaria by 2018. To achieve this goal, South Africa needs to expand research, surveillance and elimination activities to include non-endemic areas, particularly with high refugee populations. We further recommend use of powerful diagnostic tests such as PCR for these interventions.

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Introduction

Malaria infection in refugees poses a health risk for both the infected individual and the public in the host country [1]. Studies conducted in countries with a high influx of refugees like US, Canada and Australia have reported prevalence rates of 3–50% among refugees that come from malaria endemic countries particularly Sub-Saharan Africa (SSA) [2–6]. For this reason, these countries have mandatory programmes and guidelines for screening and treatment of malaria in refugees either pre-departure from their own countries or on arrival in the host countries [5,7–10].

Since 1994 South Africa is among the top countries that experience a high influx of asylum seekers mainly from SSA. It is reported that the number of applications for refugee status that are processed by the Department of Home Affairs annually exceed 100 000. By law, refugees in South Africa are free to move around, work and access basic social services [11]. However, many are now confined to the poor areas in cities and towns due to challenges of unemployment, language, xenophobia, costs of private health care services and attitude of staff at public health facilities [12]. As a result they rely on faith-based or non-government organizations which offer limited social and health care services [13–14]. Screening for surveillance and treatment of malaria, and other communicable diseases is not included in health programmes offered by these aid organizations, particularly since they are mainly located in malaria-free areas.

South Africa is reported to have exceeded the target for MDG 6 and it is among 34 African countries that have planned to eliminate malaria by 2018 [15–16]. Although it is known that 20–80% of malaria cases in South Africa are imported, the available data on malaria comes only from malaria endemic areas [17–18] which are targeted for the elimination programme. Data on prevalence of malaria, and in particular on asymptomatic infections, among refugee populations living in South Africa is lacking. For South Africa to be successful in its efforts to eliminate malaria, research on malaria among refugees, is needed to support implementation of active surveillance (regular screening for

malaria) and management of malaria. Such information is critical for various reasons. First, refugees originate from many African countries with stable malaria transmission and have developed some immunity to malaria. It is likely that some would harbour asymptomatic infections, as has been observed in other studies [2,4]. If transmitted, these infections could be more virulent to local populations who would have acquired little immunity under the hypo-endemic seasonal malaria transmission that prevails in South Africa. Second, the areas where these refugees live are non-endemic to malaria and therefore are not targeted by malaria control and elimination activities of the malaria control programmes that are available in endemic areas [19]. Third, unconfirmed reports of malaria cases in non-endemic areas of KwaZulu-Natal have been cited, mainly in the major urban cities. This study sought to determine prevalence of asymptomatic malaria infection among a refugee population living in a city of KwaZulu-Natal province, South Africa. In this paper we share how this aim was achieved.

Materials and Methods

A cross-sectional survey was carried out during September 2012 and September 2013. The participants recruited into the survey included all adult refugees over the age of 18 years who sought social and health services for various reasons at a faith-based centre located in the city of Durban, KwaZulu-Natal Province, South Africa. Ethical approval to conduct the survey was granted by the University of KwaZulu-Natal Biomedical Research Ethics Committee (REF 122/ 11). Each participant signed a written informed consent to answer a closed ended questionnaire that incuded demographic details and malaria history. The participants also provided a peripheral blood sample to screen for malaria using SD Bioline rapid diagnostic tests (Standard Diagnostics Bioline, Korea) and microscopy thick and thin smears. All participants that tested positive for malaria were treated on site with a recommended antimalarial as per national treatment guidelines. Data entry and analysis was carried out in Microsoft Excel and frequency tables were generated to show demographic information of the study participants and prevalence of malaria.

Results

Data was obtained for 303 participants consisting of 51.5% females and 47.5% males aged 19 to 64 years old. Of these participants, 289 originated from 12 different SSA countries, excluding South Africa. More than half of them came from DRC followed by Burundi, Rwanda and Zimbabwe. When asked about previous infections with malaria 89.1%% of participants responded that they had previously been infected with malaria prior to entering South Africa (Table 1). Two hundred and ninety participants provided a peripheral blood sample for screening of malaria. The prevalence of asymptomatic malaria was 3.8% for RDT, 5.9% for thin blood smear and 4.5% for thick blood smear. The majority of malaria infections were due to P. falciparum (88.2%) and the remainder resulted from mixed infections of P. falciparum/ P. vivax (5.9%) and P. falciparum/P. ovale (5.9%). A higher prevalence of malaria was observed in participants that were male, from DRC and Burundi, in the age group 21–30 year olds and had a secondary level of education (Table 1). It was not possible to indicate a statistical difference due to low numbers of infected participants.

Discussion

The results show that all, but two participants originated from SSA countries which are highly endemic for malaria transmission and carry a heavy burden of malaria [1,20]. Similar to reports by the UNHCR [11] a large number of refugees come from the DRC, Burundi, Rwanda and Zimbabwe. Unlike in many studies conducted on refugees, particularly those from Asian countries, there is a slightly higher number of females than males in this population. The fact that many participants come from countries that experience stable malaria transmission explains why the participants did not present with symptoms of malaria when they were recruited into the survey. This is suggestive of some level of immunity acquired in the countries of origin. The possibility that they will thereby harbour undetected asymptomatic infections, makes them likely to act as a reservoir for transmission of malaria parasites to the South African population, who are generally non-immune because of the low seasonal transmission [16] putting them at risk of developing severe malaria.

The results confirm the presence of asymptomatic malaria (prevalence 3–5.2%) in a refugee population living in the city. This concurs with research from Canada, US, Australia and European countries that reported prevalence rates of 3–50% asymptomatic malaria among refugees entering these countries [2,4,7,10]. It is possible that the prevalence in our sample could be under-estimation due to the low sensitivity of RDT and microscopy used to detect asymptomatic malaria infection. We believe that PCR tests would have yielded a much higher prevalence as was the case in other studies [21–24].

The presence of malaria transmission in an area classified as non-endemic for malaria raises two major concerns. First, this poses as a public health threat because malaria transmission may be re-introduced in the city. Malaria in South Africa is known to occur in the areas bordering Mozambique, Zimbabwe and Swaziland which lie in the north-eastern parts of KwaZulu-Natal, Limpopo and Mpumalanga provinces located mainly in rural areas [19]. Second, the presence of malaria transmission in the city is a major threat to tourism in the province and country as they are regarded as South Africa's premier tourist destinations. The Durban city is known as a popular tourism destination and a big event venue particularly for its warm sub-tropical climate even during winter. The vision of the tourism sector in KwaZulu-Natal province is to make the city globally renowned as Africa's top holiday destination by 2030 [25]. The city is currently marketed globally as a malaria-free tourist destination and the aim is to keep it under prevention of re-introduction (zero cases/ 1000 people) within the malaria elimination continuum [26–28]. In South Africa the most common vector is Anopheles arabiensis, but Anopheles merus has been on the increase. Anophele funestus funestus is another vector which re-appeared in the 2000's but was eliminated [17]. Maharaj et al agrees that the presence of these vectors in areas with malaria could lead to local transmission of malaria [17].

The results further demonstrate that two-thirds of infections are due to P. falciparum. This is expected given that almost all participants originated from SSA region which is highly endemic to P. falciparum malaria. Despite large numbers of SSA refugees that enter South Africa and the known fact that 20–80% of malaria in South Africa is imported, the national malaria elimination strategy is silent on how asymptomatic malaria will be dealt with [15,26,27]. Other countries known to have high influx of refugees from SSA have implemented mandatory screening and treatment programmes and guidelines of refugees when entering the country for the first time [7,8,10]. In South

Table 1. Malaria infection and participants characteristics.

Variable	Malaria status (%) *n = 17*	Total (%) *n = 303*
Age		
# 20	3 (17.6)	9 (3.0)
21–30	6 (35.3)	148 (48.8)
31–40	3 (17.6)	101 (33.3)
41–50	3 (17.6)	33 (10.9)
$ 50	1 (5.9)	6 (2.0)
Unknown	1 (5.9)	6 (2.0)
Gender		
Female	5 (29.4)	156 (51.5)
Male	11 (64.7)	144 (47.5)
Unknown	1 (5.9)	3 (1.0)
Education		
None	1 (5.9)	15 (5.0)
Primary	2 (11.8)	67 (22.1)
Secondary	11 (64.7)	191 (63.0)
Tertiary	3 (17.6)	27 (8.9)
Marital status		
Married	8 (47.1)	177 (58.4)
Single	9 (52.9)	117 (38.6)
Other	0 (0)	8 (2.6)
Occupation		
Employed	8 (47.1)	180 (59.4)
Unemployed	9 (52.9)	114 (37.6)
Unknown	0 (0)	9 (3.0)
Country of origin		
DRC	8 (47.1)	154 (50.8)
Burundi	7 (41.2)	98 (32.3)
Rwanda	0 (0)	12 (4.0)
Zimbabwe	0 (0)	10 (3.3)
Other countries	2 (0)	14 (4.6)
Unknown	0 (0)	15 (5.0)
Previous malaria infection		
Yes	17 (100)	270 (89.1)
No	0 (0)	27 8.9)
Unknown	0 (0)	6 (2.0)

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Africa it remains a challenge to introduce malaria screening at border areas and previous efforts proved unsuccessful [18,27].

Though limited to a single setting, the results fulfill criteria used in other countries for enforcing treatment of asymptomatic malaria among refugees to prevent severe illness and transmission to local non-immune populations and re-introduction of malaria in areas declared malaria-free. We believe that our choice of the setting was appropriate because the city is one of the most populous areas and among the largest in the country with more than 3.5 million people and a high concentration of refugees [29–30]. Furthermore, given the challenges faced by refugees in South Africa especially the recent xenophobic attacks, refugees have limited access to public health facilities [12,31]. They rely heavily on health care services offered by faith- and community-based organisations. We regard these facilities as reliable sources of data on refugees.

Conclusions and Recommendations

To our knowledge the study is the first in South Africa to document the prevalence of asymptomatic malaria in a refugee population, residing in an urban area of KwaZulu-Natal province that is not endemic for malaria. These findings have important implications for both public health and malaria control in South Africa, particularly since the country has decided to eliminate malaria by 2018. To achieve this goal, South Africa needs to expand research, surveillance and elimination activities to include non-endemic areas and marginalized communities. The findings further emphasize the importance of integrating services such as

malaria surveillance into other public health intervention programmes, and provide refugees with full access to public health services as prescribed by the law. It is envisaged that this study will serve as a basis for a comprehensive research on the burden of asymptomatic malaria among refugee populations residing in non-endemic areas of South Africa. Such research should include children and pregnant women, as well as using screening tests with high sensitivity for detection of low parasitaemia (PCR). The study presents further opportunities for research on the level of resistance to anti-malarials among refugee populations.

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Author Contributions

Conceived and designed the experiments: JMTG UO. Performed the experiments: UO. Analyzed the data: JMTG UO. Contributed reagents/ materials/ analysis tools: JMTG. Wrote the paper: JMTG UO. Data collection and laboratory processing: UO. Data interpretation and drafi manuscript: JMTG UO. Finalise manuscript: JMTG.

References

1. Anderson J, Doocy S, Haskew C, Spiegel P, Moss WJ (2011) The burden of malaria in post-emergency refugee sites: a retrospective study. Conflict and Health 5: 17. doi:10.1186/ 1752-1505-5-17.
2. Matisz CE, Naidu P, Shokoples SE Grice D, Krinke V, et al (2010) Post arrival screening for malaria in asymptomatic refugees using real-time PCR. Am. J. Med. Hyg 84: 161–165. doi:10.4269/ ajtmh.2011.10-0494.
3. Monge-Maillo B, López-Vélez R (2011) Is screening for malaria necessary among asymptomatic refugees and immigrants coming from endemic countries? Expert Rev Anti Infect Ther 9(5):521–524. doi:10.1586/ eri.11.37.
4. Monge-Maillo B, Jiménez BC, Pérez-Molina JA, Norman F, Navarro M, et al (2009) Imported infectious diseases in mobile populations, Spain. Emerg Infect Dis 15(11):1745–1752. doi:10.3201/ eid1511.090718. PMCID: PMC2857245.
5. Paxton G (2013) Malaria Screening: Immigrant Health Service. Immigrant health clinic protocols. Melbourne: The Royal Children's Hospital. Available: http:/ / www.rch.org.ua/ immigranthealth/ clinical/ malaria-screening/. Accessed 6 November 2013.
6. Wickramage K, Premaratne RG, Peiris SL, Mosca D (2013) High attack rate for malaria through irregular migration routes to a country on verge of elimination. Malaria J 12: 276. doi:10.1186/ 1475-2875-12-276.
7. Stauffer WM, Weinberg M, Newman RD, Causer LM, Hamel MJ, et al (2008) Pre-departure and post-arrival management of P. falciparum malaria in refugees relocating from sub-Saharan Africa to the United States. Am J Trop Med Hyg 79(2): 141–6.
8. US Department of Health and Human Services (2012a) Domestic refugee health guidelines: malaria. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases. Division of Global Migration and Quarantine.
9. US Department of Health and Human Services (2012b) Overseas refugee health guidelines: malaria. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases. Division of Global Migration and Quarantine.
10. Young MK, McCall BJ, Heel K (2010) The impact of pre-departure screening and treatment on notifications of malaria in refugees in south-east Queensland. CDI 34(1): 37–40.
11. UNHCR (2013) UNHCR Global Appeal 2013 Update- South Africa. Available: http:/ / www.unhcr.org/ 50a9f825b.html. Accessed 11 December 2013.
12. Apalata T, Kibiribiri J, Knight S, Lutge E (2007) Refugee perceptions of their health status and quality of health care services in Durban South Africa. A community based survey. Health Link. Available: http:/ / Healthlink.org.za. Accessed November 2013.
13. Medecins Sans Frontieres (2010) Lives of vulnerable migrants in South Africa. Available: http:/ / www.msf.org.za/ msf-publications/ lives-vulnerable-migrants-south-africa. Accessed 11 December 2013.
14. Medecins Sans Frontieres (2011) Nowhere else to go: survival migrants in South Africa caught between evictions and policy vacuum. Available: http:/ / www.msf.org.za/ msf-publications/ nowhere-else-to-go-survival-migrants-south-africa-caught-between-evictions-and. Accessed 11 December 2013.
15. Moonasar D, Nuthulaganti T, Kruger PS, Mabuza, Rasiswi ES, et al (2012) Malaria control in South Africa 2000–2010: Beyond MDG6. Malar J 11: 294. [http:/ / dx.doi.org/ 10.1186/ 1475-2875-11-294].
16. Moonasar D, Morris N, Kleinschmidt I, Maharaj R, Raman J, et al (2013) What will move malaria control to elimination in South Africa? S Afr Med J 103 (10) Suppl 2: 801–806. doi:10.7196/ SAMJ.7445.
17. Maharaj R, Morris N, Seocharan I, Kruger P, Moonar D, et al (2012) The feasibility of malaria elimination in South Africa. Malaria Journal 11: 423–428. [http:/ / dx.doi. org/ 10.1186/ 1475-2875-11-423].
18. Maharaj R, Raman J, Morris N, Moonasar D, Durrheim D N, et al (2013) Epidemiology of malaria in South Africa: From control to elimination. S Afr Med J 103 (10) Suppl 2: 779–783. doi:10.7196/ samj.7441.
19. Morris N, Frean J, Baker L, Ukpe I S, Barnes K I, et al (2013) Re-defining the extent of malaria transmission in South Africa: Implications for chemoprophylaxis. S Afr Med J 103(11):861–864. doi:10.7196/ SAMJ.7533.
20. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, et al (2009) A world malaria map: Plasmodium falciparum endemicity in 2007. PLoS Med 6: e1000048. doi:10.1371/ journal.pmed.1000048.
21. Dal Blanco MP, Koster KB, Kombila UD, Kun JF, Grobusch MP, et al (2007) High prevalence of symptomatic Plasmodium falciparum infection in Gabonese adults. Am J Trop Med Hyg 77: 939–942.
22. Ndao M, Bandyayera E, Kokosin E, Gyorkos TW, MacLean JD, et al (2004) Comparison of blood smear, antigen detection, and nested-PCR methods for screening from regions where malaria is endemic after a malaria outbreak in Quebec, Canada. J Clin Microbiol 42(6): 2694–2700.
23. US Department of State (2006) Proposal refugee admission guidelines for fiscal year 2006 report to Congress. Available: http:/ / www.state.gov/ g/ prm/ refadm/ rls/ rpts/. Accessed 11/ 12/ 2013.
24. Stauffer WM, Newberry AM, Cartwright CP, Rosenblatt JE, Hanson K, et al (2006) Evaluation of malaria screening in newly arrived refugees to the United States by microscopy and rapid antigen capture enzyme assay (Binax-NowÒ). Pediatr Infect Dis J 25(10).
25. KZN Department of Economic Development & Tourism (2013) KwaZulu-Natal Tourism Master Plan. Pietermaritzburg: KZN DEDT.
26. Shandukani B, Moonasar D, Misiani E, Benson FG (2011) Progress towards malaria elimination in South Africa. Malaria Directorate. Pretoria: National Department of Health.
27. South African National Department of Health (2012) Malaria Elimination Strategy for South Africa 2012–2018. Pretoria: National Department of Health.
28. World Health Organization (WHO) (2007) Malaria Elimination: A Field Manual for Low and Moderate Endemic Countries. Geneva: WHO. Available: Available: http:/ / whqlibdoc.who.int/ publications/ 2007/ 9789241596084_eng.pdf. Accessed 11 August 2013.
29. KZN Top Business (2013) Durban Tourism: The Warmest place to be. http:/ / kzntopbusiness.co.za/ site/ top-business-sector/ Durban-Tourism/ page/ 251. Accessed 16 October 2013.
30. Statistics South Africa (2011) Census 2011. Pretoria: StatsSA.
31. Kaplan C (2011) Migration vulnerability and xenophobia: Central African refugee and asylum seekers access to health services in Durban South Africa Independent study project (ISP) Collection. Available: http:/ / www.migration vulnerability and xenophobia _ central African.Refugee. Accessed 11 April 2013.

Article 2: Parasitemia and Haematological Alterations in malaria-infected refugees in South Africa

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Abstract.

Background

Haematological alterations associated with malaria are well recognised, but specific changes may vary with level of malaria endemnicity, background, haemoglobinopathy, nutritional status, demographic factors and malaria immunity. Though malaria in South Africa has been reduced dramatically in endemic areas, little is known about haematological changes associated with malaria infection among refugee populations that live in the cities.

Objective

The objective of the study was to describe haematological alterations among malaria infected refugees that live in Durban, South Africa.

Materials and Methods*:*
A cross-sectional study was conducted in 2013 at refugee centre located in central Durban, South Africa. The blood samples of 102 adult African refugees were examined for infection with the malaria parasites and haematological profiles were compared with standard normal values.

Results
Amongst the 102 participants, malaria infection was detected in 15.7% participants. The mean absolute haemoglobin (Hb) level was reduced in 6.5% of the malaria positive patients (9.2 g/dl) with an extremely low packed cell volume (PCV) of 28.3%. While the total non malaria infected cases of 93.5% had a normal mean absolute Hb value of 12.6 g/dl and a slightly low PCV value of 38%.

Conclusions
Anaemia was common among participants with malaria infections than among un-infected participants. Other haematological alterations were common in both malaria infected and uninfected participants, suggesting that infections other than malaria or other underlying factors that cause haematological alterations may be present. This research needs to be expanded to include a large sample, other areas and infections.

Introduction

The increased health burden that is posed by malaria infection worldwide cannot be overemphasized. [1, 2] To date, malaria has proven to constitute one of the most severe causes of morbidity amongst refugee populations worldwide and the UNHCR reported that this disease affects more than a million cross-border migrants, a figure which includes women and children. [2] Thirty per cent of the 9.2 million refugees and 25 million displaced persons in the world are found in sub-Saharan Africa. [3] Irrespective of the fact that malaria is a serious health issue for refugees in sub Saharan Africa and taking into consideration the long period of time that most refugees spend in overcrowded conditions. Limited research has been conducted in these settings to provide accurate data that can assist in gaining insight into, and greater clarity concerning the transmission patterns of the disease, or attaining success in curbing the spread of malaria. [4, 5] It has been estimated that approximately ten per cent of South Africa's entire population continues to live in locations that have been considered as malaria endemic areas. [6] Approximately 64% of all cases reported in South Africa in 2011 were imported cases. [6]

Malaria is transmitted exclusively through the bites of the anopheles mosquito. Some refugees from non-malaria or very low malaria endemic areas have to travel through malaria endemic areas in order to get to South Africa. Non-immune refugees are at a greater risk and have higher mortality rates. Non- immune refugees like children under five or none immune/semi-immune pregnant women are at risk. Refugees that have HIV also fall within this high risk category. [7]Haematological alterations associated with malaria are well recognised, but specific changes may vary with level of malaria endemnicity, background, haemoglobinopathy, nutritional status, demographic factors and malaria immunity. [1]

Despite the high numbers of refugees in South Africa and the fact the majority of refugees come from high malaria endemic areas, little is known about their health status, particularly malaria infection and its complications. This study aimed to evaluate and determine the frequency of various haematological alterations among refugees infected with malaria and those not infected.

Materials and methods

This study was an observational, cross-sectional study of refugees living in central Durban. The study was conducted at a refugee centre between2012-2013 run by a church-based non-profit organization. In total 102 participants who attend the centre during the data collection period for the study, irrespective of clinical signs, previous tests for malaria were included in the study. Data on demographic details and malaria infection were collected from the participants.

Malaria in the patients was confirmed by blood film examination, or by rapid diagnostic test (RDT) or both. Patients that did not display any clinical symptoms of malaria but were confirmed positive by the blood film examination or the rapid diagnostic test were included in the study. Thin and thick blood films were made and stained with Giemsa stain. Malaria parasitaemia was determined by using high power magnification in oil immersion. A manual counter was used to count infected red blood cells in 5 - 10 fields that have approximately 1000 red blood cells, all cells that contained parasites in this field were counted. The RDT used was an Antigen (pLDH) based card test; SD pf /pan Ag card, manufactured by Standard Diagnostic Inc.

The haematological profile of the patient's blood samples was analysed using the Sysmex XE 5000 Automated Haematology Analyser.

The analyser determined the eosinophil count as a percentage of the total differential white blood cell count. In the study, eosinophil levels (1-6%) were defined within normal range , while a percentage value greater than 6% was considered as an elevated eosinophil count (Eosinophilia >6%).

Results

Epidemiological and clinical features

The study included 102 participants, who were enrolled between September 2012 and July 2013. The majority (61.8%) of the participants were male, while 38.2% were female**.** The participants originated from African countries that are known to be malaria endemic. Of these, 89.1% reported to have been infected at least once with the malaria parasite in their countries of origin.

Malaria infection and parasitaemia

The malaria rapid test kit (SD Bioline) detected 11 positive malaria cases amongst the102 study participants. Investigation of thin and thick Giemsa-stained blood films with microscopy indicated five more malaria positive samples, bringing the total number of malaria positive samples to 16 (15.7%). Ten test slides indicated infection with the *Plasmodium falciparum* species only. The other six test slides indicated infection with two plasmodium species inclusive of *P falciparum and P ovale*. None of the 16 malaria positive cases presented with classical symptoms of malaria in the form of fever, rigor, chills, sweating or splenomegaly. The remaining 84.3% tested negative for malaria infection. The overall prevalence of malaria infection was 15.6%, out of this number, 3.9 % of participants were co-infected with intestinal parasites.

Out of the malaria positive samples 13 (76%), were adequate for further parasitaemia analysis. Of these, four participants had a percentage parasitaemia grading level of 0.002% with an average value of 0.1%. At this level of grading the average number of parasites was 4,074 parasites per/µl. The clinical correlations to these values indicate that participants may be asymptomatic below this level. Nine participants had parasitaemia grading levels of 0.2% with an average of 0.68%. This means that immune participant will exhibit symptoms above this level. None of the malaria positive participants exhibited levels of percentage parasitaemia greater 2% which is the maximum parasitaemia levels for ***P.vivax*** and ***P.ovale*** or hyperparasitaemia levels which are above 10% (Table 1).

Table1: Determination of parasitaemia protocol. (Garcia 2001).

Grading levels of % Parasitemia	Grading levels of % Parasitemia	Parasites/ µl range	No of participants Parasites/µl	Clinical correlation
0.0001 - 0.000.4%	----	5-20	---	**------**
0.002%	4(0.10%)	100	4 (4,075)	Patients may be symptomatic below this level
0.2%	9 (0.68%)	10,000	9 (22,294)	Level above which immune patients will exhibit symptoms
2%	---	100,000	---	Maximum parasitemia of P.vivax and P.ovale
2-5%	---	100,000-250,000	---	Hyperparasitemia ,severe malaria, increased mortality
10%	----	500,000	---	High mortality, may need exchange transfusion
Absolute degree of parasitemia (G/l)	Participants 13 (16,342 G/l)			

Garcia ,L.S.2001.Diagnostic Medical Parasitology,4th Ed.,ASM press,Washington ,DC.

Haematological alterations

The haematological parameters of 102 refugees was evaluated (n=102), of these (84.3%) tested negative for malaria and were only 6 samples (15.7%) malaria positive cases were available for further haematological analysis. Table2: the mean absolute haemoglobin (Hb) level was reduced in 6.5% of the malaria positive patients (9.2 g/dl) with an extremely low packed cell volume (PCV) of 28.3%. While the total non-malaria infected cases 93.5% had a normal mean absolute Hb value of 12.6 g/dl and a slightly low PCV value of 38.0%. A low normal total white blood count (WBC) was observed in the malaria positive participants ($4.1x10^9$/L), while the non-malaria positive participants had a normal total WBC count of $5.6x\ 10^9$/L (Table 2).

The mean corpuscular volume (MCV), was within normal range in both malaria infected participants with mean of 84.3 fl and non-malaria infected participants with mean of 82.0fl respectively. The mean corpuscular haemoglobin (MCH) was low in malaria positive participants.

Table 2: Haematological parameters of study participants

Parameters	**Malaria infected n=6 (6.5%) %**	**Malaria negative n= 86 (93.5%) %**	**Reference range**
MA Hb g/dl	9.2 (2.05)	12.6 (2.3)	13 ± 2 g/dl
Total WBC $x10^9$/l	4.1(0.46)	5.6 (1.89)	7 ± 3 $x10^9$/l
Platelet count $x10^9$/l	175(140.0)	227 (78.9)	150-400 $x10^9$/l
PCV%	28.3 (12.4)	38.0 (5.9)	45 ±5 %
MCV	84.3(5.71)	82.0 (8.4)	85 ± 9 fl
MCH	26.1(4.17)	27.0 (3.8)	29.5 ± 2.5 pg
MCHC	31.1(5.21)	32.4 (1.75)	33 ± 2 g/dl

MA Hb- Mean absolute haemoglobin level WBC=White blood cells, MCV = Mean corpuscular volume, MCHC= Mean corpuscular Haemoglobin, MCHC=Mean corpuscular haemoglobin concentration PCV =Packed cell Volume

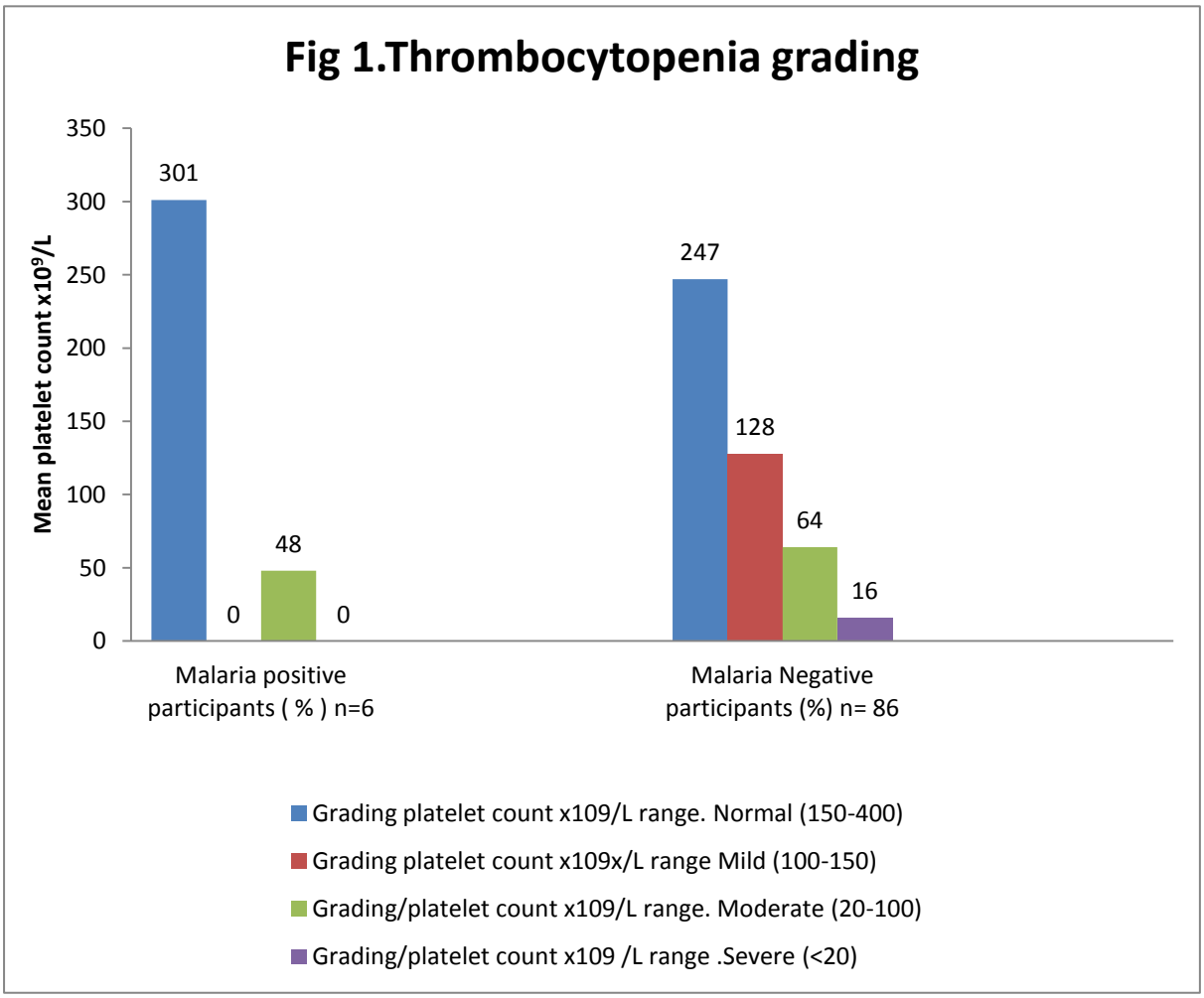
The MCH mean average value in malaria positive participants was 26.1 pg, while the malaria negative participants presented with an MCH value of 27.0 pg. The MCH concentration showed a low mean value of 31.1.g/dl in malaria infected participants and the malaria negative participants presented with a MCH concentration value of 32.4. g/dl.

Table 3. Anaemia classification table

	No of female partic. N=35	**Mean**	**SD**	**No of Male Part. N=57**	**Mean**	**SD**
Normal Hb levels >12.0g/dl	19	13.6	1.42	15	15.4	2.0
Moderate anaemia 7-11.0g/dl	11	11.4	0.535	37	11.7	0.73
Severe anaemia <7g/dl	5	8.6	1.25	5	6.62	3.4

Out of the 92 participants that had their Hb levels evaluated, (38.1%) were female while (61.9%) were male. The Hb levels were graded as normal, moderate anaemia and severe anaemia. The level of Haemoglobin among 4 co-infected participants was 9.02 g/dl.

Fig 1: Thrombocytopenia grading



Twelve (13.9%) out of the 86 malaria negative participants, presented with mild to severe thrombocytopenia, while 74 (86.0%) of the malaria negative participants presented with normal platelet levels with a mean platelet count 247 $x10^9$/L. Three (50%) of the malaria positive participants presented with moderate thrombocytopenia (Figure1).

Both malaria infected participants and malaria negative participants presented with an elevated eosinophil counts of (24.9%) and (19.6%) respectively), but slightly higher in malaria infected. The majority of participants presented with a normal eosinophil count (Table 4).

Table 4: Eosinophil counts of participants

Eosinophils levels	Malaria infected n=6	Non malaria infected n=86
High (>6%)	1(24.9%)	36 (19.6%)
Normal (1-6%)	5(1.8%)	50 (2.5%)

The odds ratio for the 2x2 table below is 0.2778. This low value indicates that the odds of eosinophillia are negatively associated in malaria infection. The chi square value could not be evaluated due to the fact that not all cell frequencies are up to or greater than 5.

Table 5. Odds ratio table on the presence of malaria and eosinophillia

	Malaria Negative Participants N=86	**Malaria positive participants N=6**	**Total**
Group 1 Eosinophillia (>6%)	**36**	**1**	**37**
Group 2 Normal Eosinophil levels (1-6%)	**50**	**5**	**55**
Total	**86**	**6**	

Discussion

Plasmodium falciparum has been and remains the causative agent in more than 90% of malaria cases in South Africa. [8] The present study has shown that *P. falciparum* infection is more common than *P .ovale* infection or mixed infections in the study participants. None of the malaria positive patients presented with any classical symptoms of malaria disease. Previous studies have linked haematological alterations with malaria infection. Differences in haematological analytes are often affected by several other factors like diseases that affect the haemopoetic physiology at different levels. [9] This the case with an endemic disease like malaria which affects the body's homeostasis at different levels.

According to the World Health Organization (WHO) anaemia is defined as Hb levels of less than 13g/dl in men and less than 12g/dl in women. The basis of this is the average Hb levels of Healthy individuals. [10] Results from this study have shown that malaria infected participants are anaemic with a low Hb level mean value of at 9.2 g/dl which is in agreement with other studies. [1] While the non malaria population presented with more stable haemoglobin with a mean value of 12.6g/dl. However the results for the blood indices for the participants of both malaria infected participants show abnormal results, that is, low MCH values in the malaria positive group and low normal values in the malaria negative group.

The nutritional status of participants should be considered even in the absence of malaria. The morphology of the red cell in the presence or absence of malaria could be influenced by their nutritional status. Participants could be deficient in vitamin B 12, iron or folic acid. [8] In our study, both malaria positive and negative groups presented with a low packed cell volume (PCV). The decreased (PCV) values in all participants could be an indicator of nutritional deficiency. This was also observed by Bhawana et al in a study that investigated haematological alterations in malaria. [1]

Helminthic co-infection with malaria could also be a cause of anaemia. Across the continent a number of helminth species share the same spatial extents as P.falicparum the most common of these are soil transmitted helmints (STH:*A. Lumbicoides,T. Trichiura and Hookworms)*. [11]Several studies have linked co-infection of P.falciparum and hookworms in a number of populations in Africa.

The effects of infection with one helminth species on the risk of anaemia are well documented. With risk correlated with infection intensity. Hookworm causes iron deficiency anaemia through the process of intestinal blood loss. Schistosomes also cause blood loss as eggs penetrate the intestinal wall. Like malaria, anaemia due to schistosomiasis can also arise from destruction of red blood cells and diserythropoiesis. [11] Based on the distinct mechanisms by which malaria, hookworms and schistosomes reduce haemoglobin levels ,it can be speculated that their combined presence might interact to increase the risk of anaemia. [11]

Even though several controversies seem to exist in several studies[1], there have been findings of leucocytosis and leucopenia. [1, 9] In the present study both malaria positive and negative groups presented with a normal mean white blood count (WBC) count. This is in line with other studies. [1] The normal WBC counts are however not enough to create a clear picture of underlying disease. A differential analysis of the individual white blood cells would have created a more precise blood picture. The elevated eosinophil counts in both groups could be indicative of intestinal parasite infestation of study participants. Few studies have investigated parasitic infestation of refugees in these settings.

All malaria positive participants did not present with any common malaria symptoms ,which is indicative of asymptomatic malaria The clinical correlations to malaria parasitaemia grading values indicate that participants may be asymptomatic below the observed levels. This explains why none of the participants presented with any clinical signs of malaria.

The results also confirm that the malaria positive participants presented with thrombocytopenia, which is in line with several other studies from other African settings endemic for malaria. [6]

Study limitations

Face validity

The physiological measure instrument has face validity due to the fact that the physiological measures like the presence of the malaria was analysed by known and tested laboratory analytical methods, both manually and with the aid of a laboratory multi- analyser. It is possible however that an error could have been introduced by the calibration of the multi-analyser.

Predictive validity

Both the physiological measures, and data collection instruments possess predictive validity. Because previous literature pertaining to this research finds evidence of low incidence of malaria in low endemic areas but does not rule out the possibility of asymptomatic cases among migrants to low or non malaria endemic areas.

Conclusion

This study has shown that all malaria positive participants were asymptomatic carriers as all presented with low grade parasitaemia and none presented with any obvious malaria symptoms. The results also confirm that the malaria positive participants, presented with severe anaemia and thrombocytopenia. The malaria negative participants presented with normal haemoglobin levels even though their packed cell volume was low presented with a normal platelet count. The decreased (PCV) values in all participants could be an indicator of nutritional deficiency. Similarly, the low MCH confirms anaemia, which could be an indicator of nutritional deficiency. The elevated eosinophil counts in more than 35% of all participants could be as a result of infection with malaria, intestinal parasites, or other undiagnosed health burdens of the participants. Like in high income countries, we recommend

regular health screening and adequate treatment of refugee populations to reduce disease burden and transmission in KwaZulu-Natal and South Africa.

Authorship and Disclosure

Prof Joyce Tsoka Gwegweni was the principal investigator and takes primary responsibility for the paper. U.E.Okafor performed the laboratory work of this study. Mr Kimoto kungwa and U.E.Okafor, were responsible for recruiting participants into the study. Statistical analysis was performed by U.E. Okafor and Prof Joyce Tsoka Gwegweni. The paper was written by U.E.Okafor, J.T.Gwegweni, Andrei Bibirigea, Andrei Cucuianu and Ciprian Tomuleasa. The authors report no conflict of interest.

References

1. Bhawana S, Bharthi A, Yogesh K, Reena A. Parasitemia and hematological alterations in malaria: A study from the highly affected zones. Iran J Path 2013; 8(1).1-8.

2. UNHCR. UNHCR: Country operations profile. South Africa: 2012.http://www.unhcr.org/egi-bin/texts/vtx/page (accessed 12 July 2012).

3. Seicean A. Refugee health making a difference in Sub-Saharan Africa: A literature review. 2008 www://cwru.edu/med/epidbio/mphp 439 (accessed 7 March 2012).

4. Bayoh N, Akhwale W, Ombok M, Sang D, Engoki S, Koros D, *et al.* Malaria in Kaukuma refugee camp, Turkana Kenya facilitation of *Anopheles arabiensis* vector population by installed water distribution and catchment systems. Mal J. 2011; 10. (149):1475-2875.

5. Montavlo G, Renal-Querron M. Fighting against malaria: prevent wars while waiting for the malaria vaccine. 2007 http://www.econ.upf.edu/ (accessed 5 June 2012).

6. Maharaj J, Morrison N, Seocharan I, Kruger P, Moonar D, Mabuza A, *et al*. The feasibility of malaria elimination in S Afr. Mal J 2011;11: 423-428.

7. World Health Organization. WHO media centre/Fact sheet. Modes of transmission in low endemic areas.http://www.who.int/media centre (accessed 23 November 2013).

8. . Maharaj R, Raman J, Moonsar D, Durrheim D, Seocharan I, Kruger P, Shandukani B and Kleinschmidt I. Epidemiology of malaria in South Africa: from control to elimination. S Afr Med J 2013;103:(10) 779 -788.

9. Maina R,Walsh D,Gaddy C,Hongo G,Waitumbi J,Otieno L,Jones D, Ogutu R, Malar J. The impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. 2010; 9(Suppl doi: 10.1186/1475-2875-9-S3-S4).

10. Aina B, Suman V, Fairbanks V et al. Incidence of anaemia in older people: an epidemiological study in a well defined population. Journal of American Geiatric Society. 1997:45:825-831.

11. : Brookers S,Willis A,Pullan R, Estambale B, Clarke S, Snow R, Hotez P. Epidemiology of plasmodium helminth co-infection in Africa: populations at risk , potentialimpact on anaemia and prospects for combining a control. Am J Trop Med Hyg 2007. 77 (66 Supp):88-98. http:www.ncbi.nlm.nih.gov/pmc/articles/pmc2637949 (accessed 7May 2015).

Article 3:Prevalence of intestinal parasites in adult refugees living in Central Durban, South Africa

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Abstract

Background

It is reported that more than 25% of the world population is infected with nematodes, and protozoan parasitic infections, and the burden of this disease fails to be accurately estimated because of persistent under-diagnosis. [1] Limited research has been carried out pertaining to the spread of intestinal parasites amongst refugees once they have migrated from areas with high disease infection incidence rates to none or low endemic areas. [2]

Methods

A cross-sectional study was conducted in 2013 at a refugee centre located in central Durban KwaZulu-Natal, South Africa. Data collection included socio-demographic details of 303 study participants. The patients were screened for the presence of intestinal parasites, urinary parasites and blood eosinophil levels.

Results

88% of the 303 study participants had previously been infected with intestinal parasite infection in their countries of origin. Of the 270 participants that were infected with intestinal parasites, 45% were infected with hookworms, 21.6% with *A. lumbricoides*, 15.7 % with *S. mansoni*. Infection with other soil transmitted helminths (STH) and intestinal protozoa was minimal. The overall prevalence of intestinal parasite infection among 270 participants was 18.8%. Out of this number, only 1.48%of participants were co-infected malaria. The results showed eosinophillia in 40.2% of 92 participants who were screened for blood eosinophil levels.

Conclusion

The study provides new findings and confirms soil transmitted helminths parasitic infection in refugees that live in South Africa. High prevalence of hookworm indicates that infections were not acquired locally. The overall prevalence below 20% justifies deworming once per annum as per WHO recommendation. A comprehensive study of the refugees in the province will provide a better understanding of the burden and transmission of intestinal parasites.

Key words: EOSINOPHILS, HELMINTHS, KWAZULU-NATAL, PARASITES, PREVALENCE, REFUGEES, SOUTH AFRICA, TRANSMISSION,

Introduction

There are a multitude of refugees from various regions of Africa presently living in Central Durban, some in crowded conditions. The danger exists of individuals, within this transient and polyglot community of infection by particular tropical infectious diseases. It is possible that they could be infected with a variety of tropical infections. Their lack of acceptance and the indifference or even hostility from locals frequently results in a lack of acceptance at health care facilities and there exists an incipient health threat to all residents of the city. They inadvertently face lack of acceptance at health care facilities and discrimination therefore posing a health threat to both fellow refugees and the general population they mingle with on a daily basis. Absence of allocation of accommodation to these refugees in an overcrowded Central Durban results in their seeking shelters that lack adequate water and sanitation. A health threat exists if refugees cannot access necessary health care. [3]

With their health status remaining un-investigated and unchecked, the burden of disease is probably high in these refugees due to the sometimes inherent strains of more virulent pathogens in these individuals. In most instances, the time required for refugees to relocate from one country to another is of a much smaller duration than the period required for the pathogen to infect the host, propagate and infect another individual. [4] The correlation that exists between the movement of a group of people and the spread of disease is now widely being recognised. The high risk of infection with intestinal parasites and the spread of malaria amongst refugees in settlements are epidemics that now simply cannot be ignored. [4]

In a study carried out by Kwitshana et al, in KwaZulu-Natal (KZN), the third poorest province in South Africa, among teenagers and the middle aged adult population, reported the overall prevalence of parasites in 5733 screened stool samples of 20.4% with *A.lumbricoides* (10.7%) and *T.trichiuria* (6.7%) being the most common followed by hookworm and *S mansoni* infections. [5]

There was high rate of infection in Jozini, the coastal region (30.3%) to (11.2%) inland in New castle. Hookworm and *S. mansoni* infections were high in the coastal regions of Durban. Portshepstone ,Empangeni and Jozini [5]

The objective of this study was to determine the prevalence of intestinal parasites among adult refugees in the Central City of Durban, South Africa and to measure the participant's blood eosinophil count as an indicator of parasitosis.

Materials and Methods

A cross-sectional survey was carried out during September 2012 and September 2013. The 303 participants recruited into the survey included all adult refugees over the age of 18 years that attended a refugee centre, located in the inner city of Durban, KwaZulu-Natal Province, South Africa.

Ethical approval to conduct the survey was granted by the University of KwaZulu-Natal Biomedical Research Ethics Committee (REF BEO48/14).

Each participant signed an informed consent to answer a closed ended questionnaire that included demographic details and intestinal parasite history. A qualified research nurse assisted with collection of stool and urine samples for the detection intestinal parasites, and blood samples for blood eosinophil analysis. Stool samples of 270 adult refugees were examined for infection with intestinal parasites (STH, schistosomiasis and protozoa). Intestinal parasites in stool samples were detected by using the Formol-saline ether sedimentation method. Slides of wet preparation were made and viewed microscopically using a 10x and x40 lens. [6] Similarly, urine samples were analysed using the sedimentation method and observed under microscope for the presence of parasites [5]

Only 92 participants provided their blood samples for eosinophil analysis. The blood eosinophil count was determined using the Sysmex XE 5000 Automated Haematology Analyser.

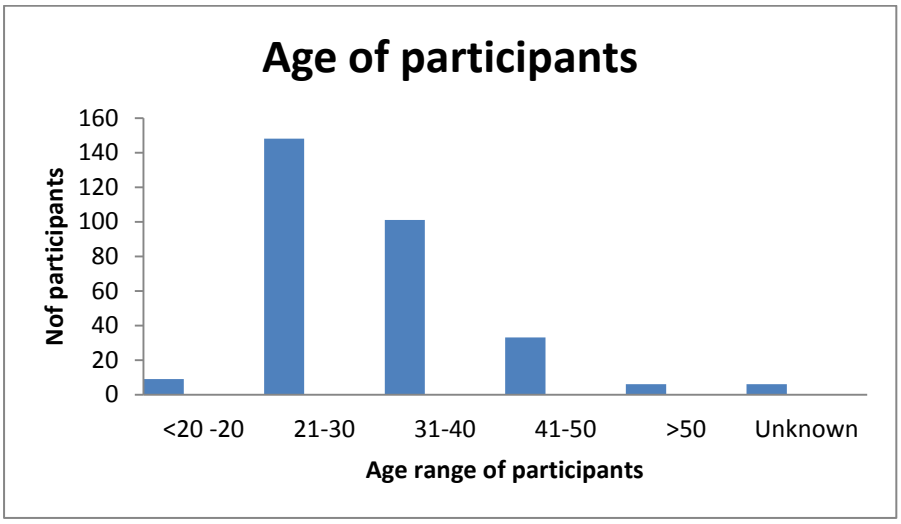
The analyser determined the eosinophil count as a percentage of the total differential white blood cell count. In the study, eosinophil levels (1-6%) were defined within normal range , while a percentage value greater than 6% was considered as an elevated eosinophil count (Eosinophilia >6%).

Results

Patient socio-demographic characteristics

The results show the socio-demographic profile of 303 participants that were recruited into the study during September 2012 and July 2013. Of these, 45% were male, while 51% were female. The average age was 31.6%, with the highest number of participants being in the 21-30 years age category.

Figure 1: Age of participants



The highest qualification of the majority of participants was a 12th grade qualification (63%) with few in other educational categories (Table 1). The majority of the participants (59.4%) were employed compared to the unemployed and those studying. More than 90% of the participants had access to clean water inside their residence, while 2.31% obtained their water from a source outside their house.

The highest number of the study participants were from the DRC (50.8%) followed by (32.3%) Burundi (Table 1).

Prevalence of intestinal parasites

Table:1. Intestinal parasite infection by participants' characteristics

Variable	Intestinal parasite status	Percentage
Age		
≤20	2	4.6
21-30	20	46.5
31-40	12	27.9
41-50	6	13.9
≥50	1	2.3
Unknown	2	4.6
Gender		
Female	21	44.7
Male	26	55.3
Unknown		
Education		
None	2	3.7
Primary	7	13.2
Secondary	38	71.6
Tertiary	6	11.3
Marital status		
Married	27	56.2
Single	21	43.8
Occupation		
Employed	25	51.0
Unemployed	21	42.8
Student	3	6.1

Country of origin		
DRC	19	26.0
Burundi	21	28.7
Rwanda	12	16.4
Zimbabwe	10	13.6
Malawi	5	6.8
Other	6	8.14

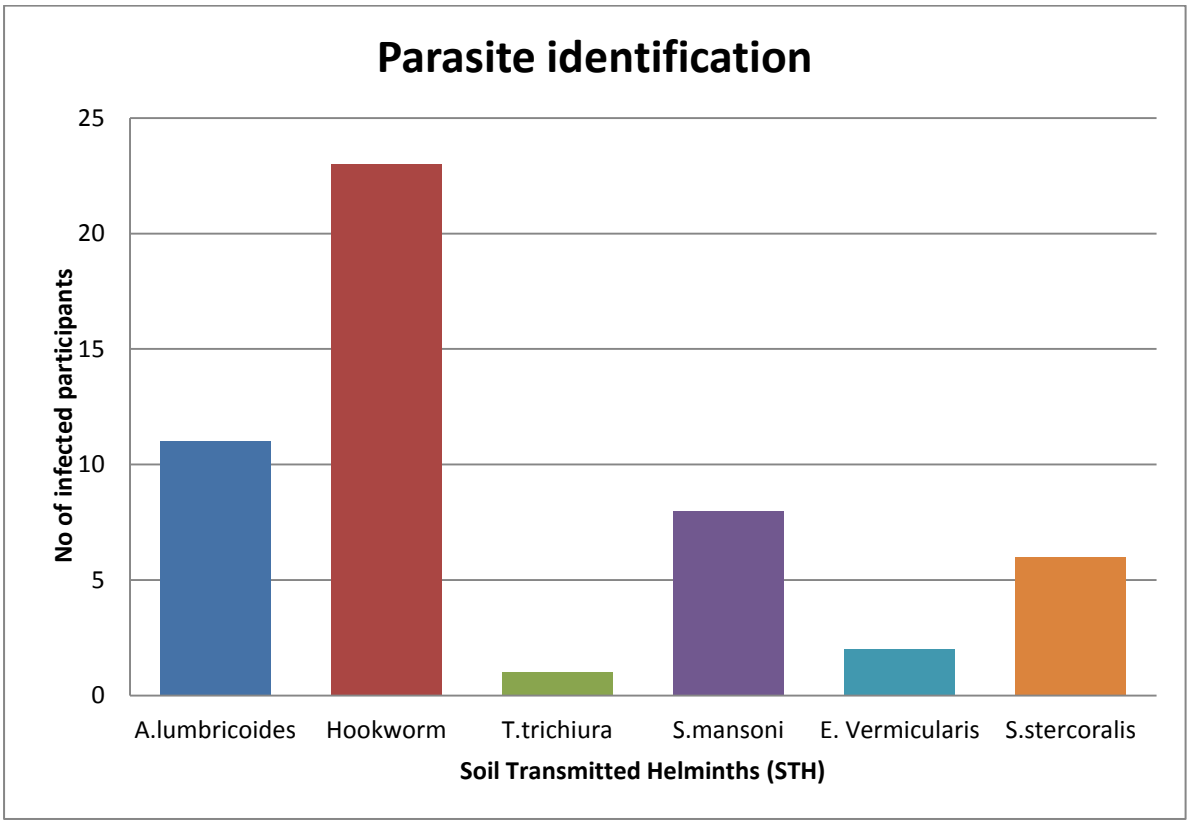
Table 2: Living Conditions of participants (n=303).

No of rooms in participants accommodation	**Participants**	**%**
1	**106**	**35.0**
2	**153**	**50.4**
3	**29**	**9.6**
4	**2**	**0.7**
5 and>5	**4**	**1.3**
Unknown	**9**	**3.0**
No of People living with participants	**Participants**	**%**
<5	93	30.7
5	55	18.2
>5 -10	137	45.2
>10	9	3.0
unknown	9	3.0

The living conditions of the majority of the participants can be regarded as overcrowded because they resided in 1- or 2- roomed apartments with 5 to 10 other members. In South Africa, the maximum number of people allowed to stay in a 1-roomed apartment is two including children, while only four people are allowed to stay in a 2-roomed apartment. [7] This is a concern because the over-crowded living conditions could be portals for the easy transmission and the spread of disease among this population, particularly the intestinal parasites.

When asked about previous infection with intestinal parasites, 88% of study participants indicated that they had previously been diagnosed with intestinal parasite infection in their countries of origin.

Figure 2 Parasite identification



Of the 270 participants that provided a stool sample for screening of intestinal parasites, 45% were infected with hookworms, 21.6% with *A. lumbricoides* and 15.7% with *S. mansoni*. Only 2.3% of the participants that provided urine samples were infected with *S. haematobium and* all of the infected presented with haematuria. Infection with other soil transmitted helminths (STH) and intestinal protozoa were minimal.

The overall prevalence of intestinal parasite infection was 18.8%. Out of this number, only 1.48 % of participants were co-infected with both intestinal parasites and malaria.

The results showed eosinophillia in 40.2% of 92 participants who were screened for blood eosinophil levels. Table 3.

Table 3: Eosinophil counts of participants.

Eosinophils (1-6%)	**N=92**	**%**
High	37	40.2%
Normal	55	59.7%

Out of the 92 participants screened for blood eosinophils, 87 provided their stool. 58.8% of the participants that were infected with intestinal parasites, presented with eosinophillia, while 41.2% of the infected participants presented with normal eosinophil levels. Table 3.

Table 4: Eosinophil counts and intestinal parasite infection of participants.

Eosinophil levels (1-6%)	Infected with intestinal parasites N=17	%	Non infected parasite participants N=70	%
High (>6%)	10 (19.8)	58.8	27 (19)	38.5
Normal (1-6%)	7 (3.5)	41.2	43 (2.4)	61.4

Table 5. Odds ratio table to test association between Eosinophillia and intestinal parasite infection

.

	Non infected parasite participants N=70	Infected with intestinal parasites N=17	
Group 1 Eosinophillia (>6%)	**27**	**10**	**37**
Group 2 Normal Eosinophil levels (1-6%)	**43**	**7**	**50**
Total	**70**	**17**	**87**

The odds ratio of 2.2751 Suggests that the odds of eosinophillia are positively associated with intestinal parasitaemia. It concludes that those that had eosinophillia were 2.7 times more likely to be infected with intestinal parasites.

Table 6.Chi square evalauation.

Phi	yates	Pearson
+ 0.16	1.54	2.3
	0.214618	0.129374

A higher prevalence of infection with intestinal parasites was observed in participants that were male, came from Burundi and the DRC, and were aged 21-30 years (Table 1). There was also an elevated prevalence among participants that had a secondary level of education. Due to insufficient numbers, statistical analysis was not performed on the results.

Discussion

We confirm the presence of intestinal parasitosis in a refugee community that lives in Durban, South Africa. The participants have admitted to being previously infected in their countries of origin prior to migrating to South Africa. However, moderate prevalence rates were observed in participants from Burundi and the DRC which is contrary to other studies that have shown that African refugees bear a very high burden of intestinal parasites. [8, 9, 10] The overall prevalence of 18% is much lower than that reported in north-eastern KwaZulu-Natal province by Appleton et al [10], more than 20 years ago in Mozambican refugees. According to the WHO, areas that experience prevalence below 20% should receive deworming programme at least once per year. [11]

The prevalence of hookworm reported in our study is high suggesting that infections were acquired outside the study, which does not support local transmission of hookworm due to low altitude. [12, 13, 14]

In a study carried out by Mabaso et al which investigated environmental factors influencing distribution of hookworm infection, data revealed that higher hook worm prevalence are limited to areas 150 meters above sea level (The coastal plains), which are characterised by sandy soils ,warm temperatures and relatively high rainfall and low prevalences to areas above this level. [15]

The majority of the refugees in this study were asymptomatic. This is in line with observations of several other studies that have shown that African refugees in Europe who reported gastrointestinal symptoms were less often infected than those without symptom. [8, 9] Even though most parasitic infections are asymptomatic, data from several other studies have indicated that helminthic infections may alter the course of other life threatening communicable diseases like HIV and tuberculosis. [10] Though very few of the participants were infected with *Schistosoma haematobium*, all infected participants presented with haematuria.

Helminthic infections may bring about several health conditions that include iron deficiency anaemia, malnutrition, mal-absorption syndrome and intestinal obstruction. Garg et al [10], recommends that immune responses to other disease conditions would be better understood if knowledge of parasitic infections amongst refugees is not only used to guide in implementation of health programs but in-depth study into the immune responses of these populations.

Significant numbers of studies pertaining to infectious diseases in humans have revealed the means whereby co-infection of parasites adopts or exerts an important role in the infection process; through effects on the immune response of the host. [16] Few studies in the past have linked the dynamics between co-infecting parasites to epidemiological disease. Co-infection is relevant for studies in refugee populations [17] This includes South Africa, where there is a large influx of asylum seekers annually and the burden of other infections such as HIV and TB are high. [3]

Studies have shown that infection with one parasite will influence the host's response to infection with other parasites. [17] Helminthic infections are well known to elicit Type 2 (TH2) non inflammatory T-cell responses, a hallmark of which is IgE elevation and eosinophillia, which may be strong enough to exert a biasing effect on concomitant infection as well as Type 1 (TH1) mediated chronic infections including

intracellular parasites. [9, 18] The diversity in host immune response has important implications in the clinical management of populations from different parts of the developing world.

Refugee populations often serve as ideal targets for increased incidence rates of infection with intestinal parasites. [19] Factors such as overcrowding, an undrinkable water supply, inadequate sanitary measures, and poor sewage systems constitute challenges to life in refugee settlements, as the diseases are rife in such forced congregations of people, creating the perfect environment for transmission of intestinal parasites. [19]

Countries that receive large numbers of refugees like US, Canada, France and Australia have policies that enforce screening and treatment of intestinal parasites in newly arrived refugees. [2, 11] Though limited to a single setting, this study provides South Africa with an opportunity to include coverage of refugees in its 2025 national strategy to control STH and Schistosomiasis.

Study limitations

Measurement error

Errors could have been introduced during the analytical phase of counting and identification of parasites when using microscopy because microscopy is not a highly sensitive method of parasite detection.

B. Systematic errors (Validity of Data Source)

The quality of the data collected during this study depended on efforts to increase the validity and reliability of the data collection instruments. However several challenges to the study could have affected the validity of the study.

The predictive validity of this research is based on the fact that the result of this research should have some correlation with previous research made in this area. Also the demographic data of refugees is predictive due to previous studies carried out on refugee populations. However, South Africa is a different setting with muti-faceted conditions, populations, culture and systems.

Conclusion

This study confirms the presence of intestinal parasitic infections in refugee population living in South Africa. The presence of hookworm suggests that infections could have occurred outside the study area. Overall prevalence of less than 20% justify deworming once per year as stipulated by the WHO and regular screening of refugees as practiced in high income countries. Before implementation of any deworming programme, a comprehensive study will provide more insight into the problem of parasites in other areas in KwaZulu-Natal and South Africa inhabited by refugee populations. Such study must be supported by more sensitive methods of parasite detection like Kato Katz and PCR.

Authors statements

The protocol was approved by the scientific review board of the department in December 2013. The ethical approval was obtained from the Biomedical Research Ethics Committee of the Nelson R Mandela School of Medicine South Africa (Reference number: BEO/48) (see Appendix). In May 2014 permission was also obtained from the management of the Denis Hurley Centre in central Durban .Study participants were only be included into the study if they provided written informed consent, participation in the study was voluntary, and refusal to participate did not compromise the services received at the DHC Clinic. The authors would like to thank and acknowledge the college of health sciences of the University of KwaZulu-Natal for the funding of this research.

Author's contributions

This article was co - written by Prof Joyce Tsoka Gwegweni and Uchenna E Okafor of the department of Public health medicine of the University of KwaZulu-Natal South Africa.

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References

1. Chris B, Singh S, Surdashi D. Neglected tropical Diseases, conflict and the right to health. National academy of science 2011 http://www.ncbi.nlm.gov/books/NBK 6258 (accessed 4 June 2012).

2. Centre for Disease control and Prevention (CDC). Overseas refugee health guidelines: Intestinal parasites 2012 http://www.cdc.gov/immigrant.refugees.com. (accessed 24 April 2012).

3. Apalata T, Kibiribiri J, Knight S, Lutge E. Refugee perceptions of their health status and quality of health care services in Durban South Africa. A community based survey. Health Link 2007. http://Health link.org.za (accessed 23 February 2012).

4. . Wilson E. Travel and the emergence of infectious diseases. Emerg infect dis. 1995; 1 (2). http://www.ncbi.Nlm.Gov/pmc/articlrs/pmc2626831/pdf/8903157 (accessed 6 March 2012).

5. Kwitshana Z, Tsoka-Gwegweni J, Mabaso L. Intestinal parasitic infections in adult patients in KwaZulu-Natal. S Afr Med J 2007;98:9. http://www.scielo.org.za/scielo.php (accessed 8 March 2015).

6. . Cheesbrough M, Mac Arthur M. A Laboratory Manual for Rural Tropical Hospitals. New York: Churchill Livingstone: Longman Group; 1976. 209 p.

7. Housing Development Agency: South Africa informal settlement status.www.thenda.co.za/upload/images/HDA_informal_settlement_status_south_africa.pdf. (accessed 9 December 2014).

8. Bengiuzer A, Caparu T, Aust-Kettis, Bjorkman A.1999.High Frequency of Gastrointestinal Parasites in refugees and asylum upon arrival in Sweden.1999; 31,(1): 79-82 http://informahealthcare.com/doi/abs/10.1080/00365549950161934 (accessed 10 july2014)

9. Elliot D, Segal U. Refugees worldwide Volume 1:Global perspective. http:books.google.co.za. (accessed 10 July2014).

10. Garg P, Perry S, Dorn M, Hardcastle L and Parsonet J. Risk of intestinal helminth and protozoan infection in a refugee population. Am J Trop Med Hyg 2005; 73(2): 386- 391 http.www.ajtmh.org/content/73/2/386. (accessed 16 July 2014).

11. Appleton C, Ngxongo.S, Braack L and Le Suer D. Schistosoma mansoni in migrants entering South Africa from Mozambique. A threat to public health in north eastern KwaZulu-Natal. S Afr Med J 1996; (86):350-353.
12. World Health Organisation (WHO).Fact Sheet: Soil Transmitted Helminth Infections 2014 http://www.who.int/mediacentre/factsheet/fs366/en (accessed 7th December 2014).

13. Mabaso M, Appleton C, Hughes J, Grows E. Hookworm (Necator Americanus) transmission in inland areas of sandy soils of KwaZulu-Natal ,S Afr. Trop Med Inte Health 2005; 9 (4):471-476.

14. Saathof E, Olsen A, Kavalsvig J. Appleton C, Sharp B. Kleinschmidt. Ecological co-variates of Ascaris Lumbricoides infection in school children from rural KwaZulu-Natal, South Africa. Trop Med Inte Health 200; 10 (5): 412-422.

15. Mabaso M, Appleton C, Hughes J and Gouws E.
The effect of soil type and climate on hookworm (*Necator americanus*) distribution in KwaZulu-Natal South Africa. Trop Med Health 2003;8(8): 722-7.http://www.ncbi.nlm.gov.publication. (accessed 6 March 2015).

16. Anderson J, Doocy S, Hskew C, Spiegel P, Moss W. The burden of malaria in post -emergency refugee sites: a retrospective study. Conflict and health. 2011;5:11.http://conflict and health.com/content/5/1/17 (accessed 5 June 2012).

17. Ezenwa V, Jolles A. From host immunity to pathogen invasion: The effect of helminthic co-infection on dynamics of microparasites. Integr Comp Bio. 2011; 51(4):540-51.

18. Staufner W. Eosinophilia:Refugee health technical assistance centre.http:www.refugee.healthta.org. (accessed 20 November2013).

19. Gabakima A, Konteh R, Kallon M, Mansaray H, Sahr F ,Bah Z. Intestinal protozoa and intestinal helminthic infections in displacement camps in Sierra Leone. J Med Sci 2007; 36: 1-9.

CHAPTER 4: CONCLUSIONS

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4.1 Conclusion

Malaria infection is globally recognised as the world's most important parasitic infection, as it presents with major health challenges. Malaria is not a disease of uniformity. In its complexity, it encompasses a variety of manifestations and makes an impact on epidemiological setting. [1] Limited studies have documented the prevalence of malaria in refugees that reside in a non malaria endemic setting. Several studies however have investigated the prevalence of intestinal parasites amongst refugee populations. [2, 3] but not in the South African context.

This study sought to document the prevalence of malaria and intestinal parasites in adult refugees that live around the Durban city centre. Refugees are marginalised communities in South Africa, disadvantaged by language barriers, limited access to public health services and other social needs. [4] The majority of the refugees in South Africa now rely on community and faith based organisations for health and social services. Due to limited funds available to these organisations, provision of health services is limited to treatment of minor ailments, HIV/AIDS counselling and testing and TB screening.

Though most of these refugees originate from countries with hyper-endemic malaria and high parasite burdens, these communicable diseases are not prioritised particularly since they present mainly asymptomatically.

The Aim of the study was achieved through four Objectives:

1.To measure the prevalence of symptomatic and asymptomatic malaria carriers and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

2. To measure the blood eosinophil count as an indicator of parasitosis in participants infected with malaria and (or) intestinal parasites

3. To identify the types and intensity of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

4. To determine risk factors for transmission of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

These Objectives were realized through a series of three manuscript papers presented in Chapter 3, including one published.

Article1: Asymptomatic malaria in refugees living in a non endemic South African city

The first article titled “asymptomatic malaria in refugees living in a non -endemic South African city,” was aimed at determining the prevalence of malaria and among refugees attending the Denis Hurley Centre in Central Durban in 2014 and to ascertain whether there is a risk of transmission of these diseases within the city This research publication addresses objectives 1, part of 3 and 4 above that is; to measure the prevalence of symptomatic and asymptomatic malaria carriers among refugees attending the Denis Hurley Centre in Central Durban; to identify the types of malaria among refugees attending the Denis Hurley Centre in Central Durban and to determine risk factors for transmission of malaria parasites among refugees attending the Denis Hurley Centre in Central Durban.

In article one, it was shown that 90% of the study participants had previously been infected with malaria prior to entering South Africa. All malaria positive participants were asymptomatic and none had any classical symptoms of malaria disease. The prevalence of asymptomatic malaria was 3.8 for RDT, 5.9% for thin blood smear and 4.5% for thick blood smear. The majority (88.2%) of malaria infections were due to *P. falciparum* only and the remainder resulted from mixed infections of *P falciparum* with *P.ovale.* A high prevalence of malaria was observed in participants that were male. This is in line with studies carried out by Bhawna et al, [1] which showed male participants to have a slightly higher risk of infection with the malaria parasite.

High prevalence of malaria infection was shown among participants from DRC and Burundi, in the age group 21-30 year olds and had a secondary level of education. The level of association could not be proven statistically due to insufficient numbers of study participants.

The results confirm the presence of asymptomatic malaria in an area that is non-endemic for malaria, that is Durban, KwaZulu-Natal in South Africa. This has important implications for the city, province and South Africa in terms of malaria elimination strategy planned for 2018. [5] and tourism which regards Durban and KwaZulu-Natal as the favourite tourist attraction places in the country. [6] To avoid putting the malaria elimination plan in jeopardy and introduction of malaria in the city, it is important to introduce a malaria screening and treatment programme for refugees in facilities where they seek health care.

Article 3: Prevalence of intestinal parasites in adult refugees living in Central Durban, South Africa

The third article manuscript focussed on the prevalence of intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014. This paper addresses objectives 1, part of 2, 3 and 4.

The results in article 3, show that 88% of study participants had previously been diagnosed with intestinal parasite infection in their countries of origin. Of these 78.6% were infected with soil transmitted helminths, while infection with intestinal protozoa was minimal. The prevalence of intestinal parasites among the participants that provided their stool samples was 18.8%. Compared with data on refugee populations from other African countries, the prevalence rates for both malaria and intestinal parasitosis among this population was low. These studies carried out elsewhere in refugee populations from Sub Saharan Africa, reported high prevalence of soil transmitted helminths (STH) and protozoa. [7]

Another objective of this study was to determine the types and intensity of intestinal parasites among the study population. Intestinal parasite infection among the infected participants was: 41.8% (hookworms), 20% with *A. lumbricoides,* 14.5% with *S. mansoni* and 2.3%with.*S.haematobium*. All participants presented with haematuria which is a clinical manifestation of infection with *S .haematobium*

High prevalence for infection with intestinal parasites was also shown among males (55.3%), participants in the 21-30 age range, and originated from Burundi and the DRC. It was not possible to indicate a statistical difference for these factors due to low numbers of infected participants. It is possible that the prevalence reported in this study is an underestimation because more sensitive methods such as the Kato Katz or PCR were not used to detect the presence and intensity of parasites.

Article 2 Article 2: Parasitemia and Haematological Alterations in malaria-infected refugees in South Africa

The article manuscript titled " Parasitaemia and haematological alterations in malaria among refugees in Durban, South Africa" addresses objectives 2 and part of 3.

Four participants had a percentage parasitaemia grading level of 0.002% with an average value of 0.1%. At this level of grading the average number of parasites was 4,074 parasites per/µl. The clinical correlations to these values indicate that participants may be symptomatic below this level. Nine participants had parasitaemia grading levels of 0.2% with an average of 0.68%. This means that the immune participants exhibited symptoms above this level. None of the malaria positive participants exhibited levels of percentage parasitaemia greater 2% which is the maximum parasitaemia levels for P. vivax and P. ovale or hyperparasitaemia levels which are above 10%. Twelve out of the 86 malaria negative participants

Results from this study have shown that malaria infected participants were anaemic. This is in agreement with other studies. [1, 7, 8] However, the results for the blood indices for the participants of malaria infected participants show abnormal low MCH values in both groups. The reasons for this could be as a result of nutritional deficiencies among the study participants.

The results confirm the presence of asymptomatic malaria in the refugee population living in the city and prevalence of intestinal parasite infection below 20% recommended by the WHO for deworming of once per year. The results showed the presence of eosinophillia in more than 40% of the participants. Both malaria infected participants and malaria negative participants presented with an elevated eosinophil count. Eosinophillia and anaemia are ubiquitous haematological alterations in the tropics. [9]

Few studies have used these haematological parameters for diagnosing parasitic infections. A blood film with a high eosinophil count, could most of the time prove intestinal parasite infection. [7] Anaemia, leucocytosis and thrombocytemia are associated with several disease conditions. [9] To make predictions based on the values of these haematological parameters, one has to put into consideration their occurrance, distribution and the degree of correlation with a particular intestinal helminth. [9]

High prevalence for infection with intestinal parasites and malaria was shown among males (55.3%), participants in the 21-30 age range, and originated from Burundi and the DRC. It was not possible to indicate a statistical difference for these factors due to low numbers of infected participants. The risk factors that were determined in research articles 1 and 3 address objective 4

4.2. Study limitations

A. Data collection errors

The questionnaire in this study which was a data collection instrument was administered in the form of a structured interview due to the fact that many refugees could not communicate in English or read the questionnaire .There was only one trained qualified nurse who collected data using a specific data collection sheet designed for the study and assisted with translation into French and other common African language dialects. Errors could have been introduced during the collection of data.

Biological variations

The nurse also assisted with the collection of blood samples for malaria detection and stool and urine samples for the detection of the presence, intensity and types of intestinal parasites. If stool or urine samples could not be provided on the same day by the patients were required to bring in the samples later. The samples after collection from the participants were transported, stored and handled according to good clinical and laboratory practices and analysed at the School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal. Sometimes there was a delay if the participants brought the stool samples from their homes.

This could have affected the quality of samples and resulted in an under-estimation of prevalence.

C. Bias in Epidemiological studies (issues of trustworthiness related to the study).

(i) Selection bias

The selection bias could have been introduced during the sample selection phase. This sampling bias encountered during the sampling phase of this study could be as a result of volunteer bias .The samples of the volunteers in this study could be actually more healthier than the general population of refugees , that attend the centre thereby giving a lower estimate of the actual prevalence of malaria and intestinal infestation among this population. The investigators tried to control this error by using the type of study design which is cross-sectional in nature, this helped to ensure that the samples are a random selection of the target population that attends the DHC on a daily basis. One of the objectives of the study is to screen refugee participants both for asymptomatic and symptomatic malaria. This removes the healthy volunteer bias.

(ii) Information bias

This error might have been introduced into the study during sample selection. The problem of communication between the refugees and the researcher may have brought about the collection of erroneous details of the participant. Also the clinical measure of the analytes could have been compromised due to an error in measurement.

Attempts to control information bias in the study, was made by providing for the furnishing of an interpretation service between the English and French languages to be available for the study participants who have need of such a service. Also, all variables were measured in the same way on all participants in order to avoid misclassification of results.

(iii) Other confounders

The majority of the participants both those infected with parasites and the healthy participants were anaemic and suffering from low MCH values which are not only indicators of parasitic infections but also indicators of nutritional deficiencies.

4.3 Additional limitations:

The scope of this study faced several limitations. As many of the refugees come from African countries where local South African languages are not spoken, language may act as a barrier to recruiting study participants who do not speak *IsiZulu* or English. Refusals to join the study may also have occurred due to fear of stigmatisation or for being identified as a refugee or illegal immigrant. As a result of this, only people who were willing to participate were recruited into the study. A limitation of this study in regards to the analysed low prevalence could be an underestimation because more sensitive methods such as the Kato Katz kit or PCR analysis were not used.

Also, though refugees are more concentrated in the centre of the city, there are pockets of other refugee groups that reside outside of the city which the study did not reach.

References

1. Bhawana S, Bharthi A, Yogesh K, Reena A. Parasitemia and hematological alterations in malaria: A study from the highly affected zones. Iran J Path 2013; 8 (1):1-8.

2. Bengiuzer A, Caparu T, Aust-Kettis, Bjorkman. A.high Frequency of gastrointestinal parasites in refugees and asylum seekers upon arrival in Sweden. Information health care.1999; 31 (1): 79-82. http://informahealthcare.com/doi/abs/10.1080/00365549950161934 (accessed 10 July 2014).

3. Garg P, Perry S, Dorn M, Hardcastle L, Parsonet J. Risk of intestinal helminth and protozoan infection in a refugee population. Ame J Trop Med Hyg 2005; 73(2): 386- 391 http.www.ajtmh.org/content/73/2/386 (accessed 16 July 2014).

4. Apalata T, Kibiribiri J, Knight S, Lutge E. Refugee perceptions of their health status and quality of health care services in Durban South Africa. A community based survey. Health Link 2007 http://Health link.org.za (accessed 23 February 2012).

5. Khoza E, Kuonza L, Kruger P, Maimela E. Toward elimination of malaria in South Africa:a review of surveillance data in Mutale Mnicipality,Limpopo province,2005-2010. Mal J 2013; doi.1186/1475-2875-12-7. http://www.ncbi.nlm.nih.gov/pmc. (accessed 9 December 2014).

6. Tsoka-Gwegweni J, Okafor E. Asymptomatic malaria in refugees living in a non-endemic South African city. PLOS/one. Doi 10. 1371/journal.pone.0107693 2014. www.plosone.org/article/info. (accessed 10 December 2014).

7 .Gabakima A, Konteh R, Kallon M, Mansaray H, Sahr F ,Bah Z. Intestinal protozoa and intestinal helminthic infections in displacement camps in Sierra Leone. J Med Sci 2007; 36:1-9.

8. Maina R,Walsh D,Gaddy C,Hongo G,Waitumbi J,Otieno L,Jones D, Ogutu R. The impact of *Plasmodium falciparum* infection on haematological parameters in children

living in Western Kenya . Mal J 2010; 9 (Suppl 3): S4. http//www. 13, 2010. doi: 10.1186/1475-2875-9-S3-S4

9. Pilger D, Heukelbach J, Diedrichs.A, Schlosser.B,.Araujo.C, Keysersa.A .liesenfeld. O, Feldmeir. H. Anaemia, leucocytosis and eosinophillia in a resource poor population with helminto-ectoparasitic coinfection. J infect Dis 2011; 5 (4): 260-269 (accessed 30 August 2014).

CHAPTER 5: ANNEXURES

5.1 Research ethics approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

21 May 2014

Ms Uchenna E Okafor
P O Box 4921
Empangeni
3880
Uchenna77@yahoo.com

Dear Ms Okafor

PROTOCOL: The Profile of malaria and intestinal parasites amongst refugees attending the Denis Hurley Centre in central Durban in 2013. REF: BE048/14

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 15 January 2014.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 29 April 2014 to queries raised on 26 February 2014 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 21 May 2014.

This approval is valid for one year from **21 May 2014.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **10 June 2014.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

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Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

5.2 Registration of degree acknowledgement

5.3 Acknowledgement of role played by student in the research study.

My role in the research study was to assist in the collection of data in the form of administering the questionnaire to the English speaking refugees. My role involved the supervision of sample collection and transportation from the Dennis Hurely Centre in Central Durban to the laboratory for analysis of blood, urine and stool samples collected from the participants. The samples were analysed by me in the laboratory, the data collected was captured in Microsoft Excel software package. Quantitative data analysis was carried out, to describe the communicable disease profile of the participants by measuring the prevalence of malarial and intestinal parasites and the risk factors for their infection. Subsequently, the data will be analysed using the SPSS version 18 or other advanced statistical package.

5.4 Technical appendices

Appendix 1.

Laboratory analysis of blood samples for detection of malaria parasites.

SD Bioline rapid test for malaria

Using the 5 micro litre disposable specimen loop provided, the circular end is dipped into the blood specimen. Carefully placing the circular end of the loop into the round sample well, 4 drops of assay diluents will be added into the square assay diluents well. The result will be read within 15 minutes.

Test interpretation

1. One band: negative- the presence of one colour band (‘C’ control line) within the test result window indicates a negative result.

2.Two bands: C’ and *P .f –P. falciparum* positive.(The presence of two colour bands P.f and test line ‘C’ control line) within the test window, no matter which band appears first indicates a P .f positive result.

3.Three bands :’C’ ‘,P.f,’ and’ Pan’- The presence of three colour bands, ’C’, ’P .f’ and ‘pan’ within the test result window, no matter which band appears first indicates a P. positive or mixed infection of P. Falciparum .P. vivax, P. malariae or P. Ovale.

4. Invalid result: If the control line fails to appear within the test window the test is considered invalid.

Appendix 2

Microscopic examination of blood films

Thin blood film

Thin blood films will be prepared from collected blood by placing three drops of anti - coagulated blood on glass slide near the edge of the slide.

Another smooth edged slide will be used to spread the blood evenly across the first slide by collecting a small amount 1cm from the first big drop at a 45 degree angle. The thin blood smear slides will be air dried and labelled.

Thick blood films

Tick blood films will be prepared by adding a drop of blood of about 3mm in diameter in the middle of the slide and spreading it with the corner of another slide.

The film is allowed to air dry and placed in a staining rack. Do not fix the slide.

Preparation of Giemsa stain.

The Giemsa stain will be prepared by mixing 3.8 grams of Giemsa powder with methanol (250ml) and glycerol (250ml) The Giemsa stain is then filtered and will be diluted 1 in 10 with pH 7.2 buffered water for use daily

The malaria parasite in the patient sample, will be identified by staining the thin and thick films and viewing them microscopically. Thin and thick blood film will be stained in the following ways:

Staining of blood films

The thin films will be fixed with methyl alcohol for about 30 seconds before being stained with the Giemsa stain. Thick films are not fixed.

The smears will be placed face down in a staining tray of diluted Giemsa stain and left for 15 minutes for thin stain and 20 minutes for the thick stain.

The staining tray will then be flooded with pH 7.2 buffered water for thin film 5 minutes and thick films few seconds respectively.

The thin blood film will be washed gently in water for 2-3 seconds. The slides will then be allowed to air dry in the slide rack.

Strains of the malaria parasite will be evaluated by examining the stained thin and thick film slides with the oil immersion using x 100.At least 50 fields of the thin smear and the thick film is used as a confirmatory slide.

The Giemsa stain for thin films will then be used on a thin blood film to determine the malaria parasite count. [23]

Parasitaemia determination (counting of malaria parasites)

Malaria parasitaemia will be determined by using high power magnification. Choosing a field where the red cells are separated and evenly spread.

A manual counter, will be used to count infected red blood cells in 5 - 10 fields that have approximately 1000 red blood cells, all cells containing parasites in this fields will be counted. This excludes several parasites in one cell. (Multiple parasites within one red cell will be counted as one parasite).

Calculations for the determination of %parasitaemia and the absolute parasite count will be as follows:

% parasitaemia = No of infected RBC's x 100 =%

1000

Absolute count = parasitaemia% x total red cell count

Appendix 3

Estimation of eosinophil % blood count

The eosinophil count will be determined using the Sysmex XE 5000 Automated Haematology Analyser. The analyser will determine the eosinophil count as a percentage of the total differential white blood cell count.

Appendix 4

Laboratory analysis of stool samples for detection of intestinal parasites.

Intestinal parasites in stool will be detected by the Formol-saline ether sedimentation method:

A small quantity of stool will be mixed in about 10ml of 10% formol saline in a tube. Sieving the suspension into a beaker through a strainer with small holes.

6ml of the sieved suspension will be poured into a centrifuge tube.

Adding 3.0 ml of ether to the tube, the contents will be mixed well and centrifuged for 3,000 rev/minutes for 1minute.

The middle layer will be separated from the sides of the tube.with an applicator stick. The ether and formol -saline will be poured away.

Transferring the re-suspended deposit to a slide with a Pasteur pipette., a wet preparation will be made of the deposit and one drop of iodine and is covered with a cover glass. View microscopically using a 10x and x40 lens. [23]

Appendix 5

Laboratory analysis of urine samples for detection of intestinal parasites:

Intestinal parasites will be detected from collected urine samples by placing 10mls of the urine into a centrifuge tube and centrifuging to obtain a sediment or deposit.

Pouring off the supernatant fluid, the deposit will be mixed by tapping the bottom of the tube.

Transferring the deposit to a slide and covering with a cover glass. The deposit on the slide will then be examined microscopically Malaria parasite using the 10x and 40 x objectives, for the presence of parasites. [23]

Appendix 6

Participant information sheet

The profile of malaria and intestinal parasites among refugees attending the Denis Hurely Centre in Central Durban in 2014. (BEO 48/14)

GOOD DAY!

Who we are?

We are researchers from the Nelson R .Mandela School of Medicine, University of KwaZulu-Natal .Research is just a process to learn the answer to a question.

Why we are here?

We are doing research on malaria and intestinal parasites, urine parasites or worms and eosinpophils. These are diseases that make people in Africa and South Africa to fall sick and if they are not detected early ,treated and managed properly they can result in severe illness or even death .These diseases are called communicable diseases because they are caused by organisms that may be carried from a person, animal or the environment to another vulnerable person either directly or indirectly .Our aim is to learn whether and how many people that visit the Denis Hurley Centre are affected by these diseases.

Invitation to participate

We invite you to participate in the research to enable us to achieve the aim of the research.

Why choose the Denis Hurley Centre (DHC)?

The reason the Denis Hurley Centre is chosen as the place to do the research is because some people who visit the centre come from other African countries that also have a problem with these diseases, but may be slightly different from the ones that are seen in South Africa. This may require different types of treatment and management. When an individual is infected with one of these diseases it is

important that the disease is detected and the individual receives treatment early to prevent severe illness, passing the disease on to other people or even death.

What is involved in the study

We need to select 344 study participants from all who visit the Denis Hurley Centre to take part in the research. Once you agree to join the research we will require you to assist us in the following manner:

Answer a few questions

a). Free testing for all the diseases.
b). Your age, gender, whether you work or not, whether or not you have been ill with one or more of these diseases before and other information.

c) Provide us with a small amount of blood (about 5ml) to be tested for malaria parasites .The nurse will draw blood from your arm

d) Provide us with urine and stool samples in the bottles supplied, to test for worms.

This will only take about 30 minutes of your time .The results of the test will be available after one week and if you wish to know the results, please come and collect them at Usizo Lwethu Clinic after a week .You will be reimbursed R50 for your transport costs.

What risks are involved in the research?

There are no risks involved in this study are minimal except that you will experience a little discomfort when the nurse draws blood from your arm because the nurse will be using a needle. This will last for a few seconds and afterward the needle prick will heal.

You may feel uncomfortable to bring the bottles of urine and stool samples in the presence of other people; this will be done in private with no other person present except the nurse.

What are the potential benefits of being part of the research?

1. You will receive no incentives for participating in the research but you will receive:

b) If you have tested positive for any of these diseases you will be treated for malaria and intestinal parasites at the Usizo Lwethu Clinic.

2. Long term, the risk of transmission and infection among the community will be reduced and thereby reducing this problem of disease and improving the quality of life.

Voluntary participation

You are not forced to participate in the research. We will respect your wish if you do not want to participate in the research or should you decide to withdraw at any time during the research. Your refusal or withdrawal from the research will not result in any penalty or loss of benefits or services provided by the Denis Hurley Centre to which you are entitled.

Results

Should you wish to know the results of your tests, you are welcome to return after seven days (one week) from the day you gave your samples to be tested. You will receive R50 towards your travel expenses when you come back to check your test results.

Confidentiality

Data that we collect from you will collect from you will be confidential .When the data from the research is being analysed, your names will not be used to identify you ,only numbers will be used .Personal information may not be disclosed only if required by law. The primary investigator will hold a master sheet linking names and study ID's to enable them to provide results to those who require them. The master sheet will be locked away and will be available to the PI.

Organizations that may inspect and/or copy research records for quality assurance and data analysis include groups such as the University Biomedical Research Ethics Committee (BREC).

If results are published, no individual or cohort identification will be used (results will be published anonymously).

Contact details of researcher/s-for further information/reporting of study related adverse events:

1. Prof Joyce Tsoka Gwegweni

Nelson R. Mandela School of Medicine University of KwaZulu-Natal.

Tel:0312604386

E-mail:tsokagwegweni@ukzn.ac.za

2. Uchenna .E. Okafor

Discipline: Public Health Medicine University of KwaZulu-Natal.

Cell phone Number: 083868585.

E-mail:uchenna77@yahoo.com

Contact details of BREC Administrator or Chair- for reporting of complaints/problem: Biomedical Research Ethics ,Research Office UKZN .Private bag x 54001,Durban 4000

Telephone: +27 (0)31 260 469/260 1074

Fax: +27(0) 31 260 4609

E- mail: BREC @ukzn.ac.za.

Appendix 7

Informed consent.

The profile of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014.

I, ----------have been informed about the research and its purpose by the research team

Their contact details are

1. Prof Joyce Tsoka Gwegweni

Nelson. R. Mandela School of Medicine University of KwaZulu-Natal.

Tel:0312604386

E-mail:tsokagwegweni@ukzn.ac.za

2. Uchenna .E. Okafor

Discipline: Public Health Medicine University of KwaZulu-Natal.

Cell phone Number: 083868585.

E-mail:uchenna77@yahoo.com

I understand what my involvement in the study means and I voluntarily agree to participate. I have been given the opportunity to ask any questions that I may have in the participation of this study. I will be given a signed copy of the document and the participant information sheet which is a written summary of the research.

I may contact the Biomedical Research Ethics Office on 031-2604769 or2601074 or E-mail BREC@ukzn.ac.za if I have any questions about my right as a participant.

Signature of participant---------- Date----------

Signature of witness/translator----------Date----------

Appendix 8

Questionnaire

The profile of malaria and intestinal parasites among refugees attending the Dennis Hurley Centre in Central Durban in 2014.REF:BEO48/14.

April 2013

Instructions: Please mark your choice by putting a circle in the appropriate box and answering the questions in the space provided.

1. General information.

1.1Date of the interview----------

1.2 Name of the interviewer----------

1.3 Respondents Unique No----------

2. Demographic information

2.1. Sex :----------(1). Male :----------(2). Female: ----------.

2.2 Date of birth: -------d d/mm/y y-/---------- Age ----------

2.3 Country of origin ----------

2.4. Marital status:-------(1).Single----- (2). Married ---------- (3) Widowed----------

4. Casual relationship----------

2.5. Highest educational qualification achieved ? (1). None (2).Grade1-7 (3).Grade 8-12 (4). Other----------

2.5a Did you complete your education?

2.6. What is your mother tongue (language)?

(1). *IsiZulu*------- (2) .English. .------- (3). *Sesotho*. ----- (4). *Swahili* ------- (5). Other/specify----------

2.7Residential address ----------

2.8a. Are you residing in South Africa with your family/family members? Yes / no.

2.8b. How many people live with you? ----------

(a). 1 ----------(b). 2-5 . ---------- (c). More than 5---------- (e) Other----------

2.9 How many bed rooms does your house have? (a). 1 -----(b). 2----- (c) .3.------- (d).more ----------

2.10 What type of housing do you live in? (a). A flat ---------(b) .A brick house-------- (c). Other----------

2.11 Where do you get your water from? (a). Tap inside the house (b) .Tap outside the house (c) Other

2.12What is your occupation? (a). Unemployed (b). Employed. (c). Student (d). Other/specify**----------**

2.13 Which clinic/ Hospital do you go to for your health needs? (a).Usiso Lwethu (b). Addington (c). None

3. Malaria information

3.1Have you ever suffered/diagnosed with malaria before in your country of origin?(A). Yes (b).No (c) Don't know/N/A.

(Malaria is a parasitic infection caused by the bite of the ?*Anopheles mosquito*)

3.2. If yes, did you receive treatment for the malaria infection in your country of origin? (a).Yes (b). No (c). DK

4. Intestinal parasite information

4.0 What is the nature of your toilet system? (a)Pit (b).Water system (Flushing) (c) Other /specify

4.1. Have you ever suffered from any kind of intestinal parasite infection? When you were younger (worm infestation) (a).Yes (b).No (c). DK/NR

4.2 Has any of your family members suffered from an intestinal parasite infection?. (a)Yes (b). No (c). DK / NR.

4.3. Did you submit specimen for laboratory diagnosis (a).urine (b). Stool (c) .Blood (d). None

THANK YOU FOR YOUR COOPERATION

Appendix 9.

Detailed budget

	Reagents	**Specification**	
	Laboratory consumables and Reagents		
	1.Gloves	10 boxes for collection /Analysis	
	2.Microscopic Slides	7 Boxes (100) 25 x76mm Glass Microscope Slides	
	3. Microscope Cover glasses	7 Boxes (100) of no 1 size thickness cover Glasses	
	4.Plastic Centrifuge Tubes	350pcs	
	5.Vaccutainer blood collection Tubes	400 pcs	
	6.Plain Sterile stool containers	400 Containers	
	7.Counting chambers	2	
	8.Sample rack	2 Racks.	
	9.Micro pipette	1	
	10.Distilled Water	20 Litres	
	11.Giemsa Powder	20g	

	12.Glycerine (Glycerol)	1 Litre	
	13.Methyl Alcohol (Methanol)	1Litre	
	14.Sodium citrate powder	60g	
	15.Formaldehyde (40%w/v) solution	500 mls	
	16 .NaCl Powder	1 Packet	
	17.Glass beads		
	18.Potassium Dihydrogen Phosphate	50grams	
	19.Disodium Hydrogen Phosphate	50grams	
	20. Ether	2 litres	
	21.Centrifuge		
	22.Stool Applicator sticks	500 pcs	
	23.Immersion oil	2	
	24.Cotton wool		
	25.SterileGauze		
	26.Adhesive Labels		
	27.Grease pens	2 packets	
	28.Pens		
	29.Soap ,Detergents ,Disinfectants		
	30.Disposable Pasteur pipettes	400	
	31.Iodine	10g	
	32.Rapid Test identification Slides	400	
	33. Potassium Iodide	20gram	
	34.Beakers Glass	2 of each	
	35.Reagent storage containers	6 4	
	36.Laboratory cover coats	2	

	37.Filter paper	5 Boxes	
	38.pH papers Narrow range	2 rolls	
	Total		R80,000

A	Stationeries	10,000
B	Nursing Assistant	20,000
C	Laboratory processing of samples	40,000
	GRAND TOTAL	**R 150,000**

5.5 Research protocol

5.5 PROJECT PROPOSAL

TITLE: THE PROFILE OF MALARIA AND INTESTINAL PARASITES AMONG REFUGEES ATTENDING THE DENIS HURLEY CENTRE IN CENTRAL DURBAN IN 2014.REF:BEO48/14

NAME: UCHENNA .E.OKAFOR

STUDENT NO: 212561704

DISCIPLINE: PUBLIC HEALTH MEDICINE

SCHOOL OF NURSING AND PUBLIC HEALTH

Table of content

Abbreviations

CDC	Centre for Disease Control and Prevention
CMR	Crude Mortality Rate
DHC	Denis Hurley Centre
HIS	Health Information Data Capturing System
HIV	Human immunodeficiency virus
IDP	Internally displaced people
IMR	Infant Mortality Rate
MSF	Medicine's Sans Frontiers'
RDT	Rapid Diagnostic Test
SARS	Severe Acute Respiratory Syndrome
SPSS	Statistical Programme for Social Sciences
UKZN	University of KwaZulu Natal
UNHCR	United Nations High Commission for Refugees
WHO	World Health Organisation

Abstract

Background

The majority of the refugee population congregate and live in major South African cities, some in overcrowded housing without access to basic health care and social services. These conditions put them at risk of transmission and spread of communicable diseases both amongst themselves and in the population they come into contact with. Therefore, knowledge of the burden of communicable diseases among them is crucial.

Aim

The aim of the study is to determine the prevalence of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014.

Objective

The objectives of the study are: to measure the prevalence of asymptomatic and symptomatic malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban, to measure the blood eosinophil count as sign of acute malarial infection and as an indicator of intestinal parasitosis, to identify the types and intensity of malaria and intestinal parasites among refugees and to determine risk factors for transmission of malaria and intestinal parasite infections.

Methods

Study design and setting*:* This is an observational cross-sectional descriptive study with an analytical component, which will be conducted at the Denis Hurley Centre, Emmanuel Cathedral Parish in Central Durban. The majority of refugees that attend the centre come from African countries.

Population*:* The population for this study will comprise of adults aged 18 years and above.

Data collection*:* Data collection will include socio-demographic details of the study participants and risk factors for infection. A qualified research nurse will

collect blood, stool and urine samples for the detection of malaria and intestinal parasites.

Conclusion

Results from the proposed study will provide useful information on the prevalence of malaria and intestinal parasites amongst the refugees attending the Denis Hurley Centre in Central Durban. It is critical to know the species and intensity of parasites that affect the refugee population in Durban, more particularly so because there have been unconfirmed reports of malaria cases in non-endemic areas. Such a development has serious implications for malaria control in the province of KwaZulu-Natal. (195 words).

The profile of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014

1. Introduction and background

1.1 Definition

The World Health Organization (WHO) defines refugees as “persons fleeing to a place of safety, especially those who flee to a foreign country or power to escape danger or persecution in their own country of habitual residence because of race, religion, or political belief.” [1] While a refugee flees their country, an immigrant is defined as “a person who takes up permanent residence in a country other than their homeland. [2] Illegal immigrants and refugees face similar challenges and therefore leave their countries of origin under similar conditions. For the purpose of this study, the terms refugees, illegal immigrants and the externally displaced will be used interchangeably.

The WHO requested the United Nations High Commission for Refugees (UNHCR) to incorporate the topic of health and well-being of refugees globally as part of its agenda. [1] Thirty per cent of the 9.2 million refugees and 25 million displaced persons in the world are found in sub-Saharan Africa. [3] Estimates have indicated that South Africa harbours the highest number of asylum seekers globally. Of this estimate, approximately thirty thousand thereof are in possession of legal documentation. In 2010, it was tentatively estimated that around 58 000 individuals live as refugees in South Africa and hail from countries, such as Zimbabwe, Mozambique, Somalia, Burundi, Rwanda and the Democratic Republic of Congo (DRC). The exact number of illegal immigrants in South Africa remains an unknown figure. [4]

Those that are able to obtain work permits still face several challenges. The incidence and spread of communicable diseases is probably elevated in South Africa, due to the free interaction of locals and refugees who have not received adequate health screening. [5]

Violent xenophobic attacks directed against refugees by the local South African population have resulted in a mass movement of refugees from the local areas to the inner cities of South Africa. [5]

There are a multitude of refugees from various regions of Africa presently living in crowded conditions in Central Durban. The danger exists of individuals, within this transient and polyglot community of infection by particular tropical infectious diseases. It is possible that they could be infected with a variety of tropical infections. Their lack of acceptance and the indifference or even hostility from locals frequently results in a lack of acceptance at health care facilities and there exists an incipient health threat to all residents of the city. They inadvertently face lack of acceptance at health care facilities and discrimination therefore posing a health threat to both fellow refugees and the general population they mingle with on a daily basis. Absence of allocation of accommodation to these refugees in an overcrowded Central Durban results in their seeking shelters that lack adequate water and sanitation. A health threat exists if refugees cannot access necessary health care. [5]

Differences in language were regarded as the main reason for being refused public health care services. Often, they were denied access to see doctors in public health facilities and receive the necessary and essential treatment, due to their inability, as a black person, to speak *IsiZulu*, the local black language. This also posed a problem when admitted to a hospital to receive appropriate treatment and follow up. Their refugee status influenced their rights to be treated fairly and adequately by the hospital staff. [5]

1.2 Malaria

To date, malaria has proven to constitute one of the most severe causes of morbidity amongst refugee populations worldwide and the UNHCR reported that this disease affects more than a million cross-border migrants, a figure which includes women and children. [1]

It has been estimated that approximately ten per cent of South Africa's entire population continues to live in locations that have been considered as malaria endemic areas. [6] Research has shown that malaria infection and transmission in South Africa occurs mostly in low altitude border regions. These regions include: Mpumalanga, Limpopo and the northern parts of KwaZulu-Natal. In these regions malaria transmission is highly elevated between September and May annually. [6]

Factors favouring malaria transmission within south Africa are; parasitological factors and drug resistance .Plasmodium falciparum has been and remains the causative agent in more than 90% of malaria cases in South Africa. Drug resistance patterns to chloroquine were first observed between 1980 and 1987 in South Africa's malaria endemic areas. Between 1999 and 2000.there was a development of sulphadoxine-pyrimethamine resistant parasites in KZN. Currently P. falciparum parasites in South Africa appear to be susceptible to artemisinin derivatives and most of their partner drugs. Entomology is another factor that influences malaria transmission in South Africa. Anopheles gambiae and Anopheles funestus complexes belong to the predominant malaria vectors in South Africa. Recently Anopheles merus mosquitoes have been found in increasing rates and could become an increasingly important vector. Another important factor is the climate , which imposes distinct biological constraints on the mosquito and the malaria parasite [7]

In a study conducted by Maharaj et al, which spanned over a period of 10 years from the year 2000 to 2010, it was observed that, among the three malaria endemic areas in South Africa, KwaZulu-Natal served as the province that noted the greatest decrease in total number of malaria incidence cases, and that the number of such cases reduced from more than sixty six thousand in 2000 to less than seven thousand in 2010. [6] Local transmissions occur in the three endemic provinces only, while all provinces report imported malaria cases. Approximately 64% of all cases reported in South Africa in 2011 were imported cases.

Imported cases of malaria are due to mosquito borne transmission and are acquired outside the country. The origin of imported cases can be traced to a known malarious area outside the country to which the case has travelled [8]

The mosquito breeds in a warm and humid climate where pools of stagnant water provide the perfect breeding ground .As a result of this is important to emphasize the need to provide hygienic living conditions with adequate access to clean water and sanitation. Malaria proliferates in areas where awareness of the disease is low and where the health care systems are weak. The burden of malaria among the refugee population in Durban needs to be ascertained and assessed and be used to inform health policy for the appropriate delivery of health services to the refugee population, where access to health care remains a challenge, as the health status of the majority of refugees is unknown.[9]

There has occurred only a relatively few studies which have investigated malaria transmission due to imported cases of malaria being imported as a result of refugee migration into KwaZulu-Natal. Most of these refugees originate from areas that are rife with malaria, usually due to the tropical nature of the environment in most areas in sub-Saharan Africa. The, spread of malaria can be ascribed to the unsuitable locationing of most refugee settlements and the fact that the vast preponderance of refugees had to travel through malaria endemic areas, or countries who have experienced minimal success in curbing the spread of malaria. [1]

1.3 Intestinal parasites

Another communicable disease that could be spread easily through refugee movement is intestinal parasitosis, a condition which is caused either by nematodes or protozoa. More than 25% of the world population is infected with nematodes, and protozoan infections, and the burden of this disease fails to be accurately estimated because of persistent under-diagnosis.[10]

Populations that migrate over long distances and are on the move, lack access to balanced food combinations and basic services, particularly such as sanitation and water, thus making them more susceptible to these types of diseases. [10]

Refugee populations often serve as ideal targets for increased incidence rates of infestation with intestinal parasites. [11] Factors such as overcrowding, an undrinkable water supply, inadequate sanitary measures, and poor sewage systems constitute challenges to life in refugee settlements, as the diseases are rife in such forced congregations of people, creating the perfect environment for transmission of intestinal parasites. There has been limited research undertaken in these settings, but a variety of cross-sectional surveys have indicated a high burden of intestinal parasites in displacement camps in Sierra Leone. [11]

1.4 Research problem statement

South Africa is host to the highest number of refugees in the world: Estimates have shown that, at the start of 2010 Sub-Saharan Africa was home to more than two million refugees; of this number, four hundred and twenty thousand were registered asylum seekers. Statistics have also demonstrated that more than 50% of this number live in South Africa .Most of the refugees in South Africa are from hyper endemic malaria areas [4, 12.]

Refugees are frequently denied basic health care, specifically due to their inability to communicate with health staff: Several refugees urgently need proper health care and, although primary health care access has been guaranteed through a number of national directives, there continue to remain serious real barriers preventing them from accessing public health facilities. Those barriers or obstacles include language and an inability to pay for consultations. Many refugees at the MSF clinic indicate that they are made to feel unwelcome in public health facilities because they do not speak the local language .Since they have limited access to health screens, there is a need to determine the burden of communicable diseases amongst refugees [5, 13.]

Access to health care remains a challenge and thus the health status of the majority of refugees remains an unknown factor. Results from the proposed study will be used to describe the prevalence of malaria and intestinal parasites amongst the refugees attending the DHC in Central Durban.

2.0 Literature review

It has been estimated that at the onset of 2011, the UNHCR was caring for more than 10 million refugees globally .Almost 5 million refugees were sustained and looked after by either private or United Nations relief agencies. [12] Twenty per cent of such refugees live in Africa .This specific number represents refugees that have been registered. They are faced with the following options: resettlement, integration into the communities or repatriation. [12] The WHO acknowledges that refugees are classified as vulnerable persons, a designation due to several reasons, which include their relocation, poverty and their demographic make-up or profile with 80% being women and children.[3] The vulnerability of refugees often stems from the fact that these individuals arrive in a foreign land with absolutely no basic knowledge of the way of life or the language of the people within the host countries. Owing to these factors, they bear the brunt of discrimination, a hostility which frequently results in violent xenophobic attacks being directed against them.

Towards the end of 2010, more than 58,000 individuals whose countries of origin mostly involved Angola, the DRC, Burundi, Rwanda and Somalia, had been recognized as refugees in South Africa. [1]They are permitted to find jobs and have a right to access basic social services. These refugees encounter various challenges due to the difficulties that are associated with acquiring proper documentation. The aforementioned violent xenophobic attacks have arisen due to the perceived competition between indigenous South African locals and the refugee population for improved social services, business ventures and job opportunities. [1]

In an article released in June 2009, by Doctors without Borders/Medicines sans Frontières (MSF) titled" No refuge, Access denied", refugee health was a major concern. This report narrated the dangers refugees were exposed to when crossing the border into South Africa, the daily dangers they confront , while residing in South Africa, and their inadequate access to basic health care .At the MSF clinic, refugees suffered from gastro intestinal ailments, skin diseases and respiratory diseases. [13]

Several diseases were ascribable to the unhygienic and overcrowded living conditions that these refugees were subjected to. The article further stated that if there is no immediate intervention, refugee health will not improve.

The South African government has enacted several attempts to introduce and maintain domestic standards and ensure protection of the rights of refugees within South Africa .Irrespective of the success or inadequate nature of these attempts, asylum seekers and refugees remain a vulnerable migrant population in South Africa today.[12] Several refugees within South Africa daily confront many challenges. Accessing primary health care continues to remain one of the few rights that are being denied to them. Durban has centralised refugee networks in particular areas that allow refugees to interact with other asylum seekers and established refugees. Within this network, they gain a wider knowledge and understanding concerning South Africa and the culture of the host country , and how to make a living in this new environment Thus, ,there exist several identifiable areas within central Durban that are recognised as being particularly populated by foreigners. [12] Most of these foreigners reside and make a living in the city centre of Durban. These areas include: Albert Park, Point, Victoria Street area and the Warwick Junction areas. In spite of existing national legislation within South Africa to uphold refugee rights and grant adequate health services to this vulnerable group, health service providers have often denied refugees the constitutional right to proper health care due to the inherent existing xenophobic attitudes, or the perception thereof, of local members of the staff. [12]

With their health status remaining un-investigated and unchecked, the burden of disease is probably high in these refugees due to the sometimes inherent strains of more virulent pathogens in these individuals. In most instances, the time required for refugees to relocate from one country to another is of a much smaller duration than the period required for the pathogen to infect the host, propagate and infect another individual .[14] The correlation that exists between the movement of a group of people and the spread of disease is now widely being recognised. The high risk of infection with intestinal parasites and the spread of malaria amongst refugees in settlements are epidemics that now simply cannot be ignored. [14]

2.1 Malaria and intestinal parasites

The increased health burden that is posed by malaria infection worldwide cannot be overemphasized. Thirty six per cent of the global population live in high malaria burden areas. [15] Irrespective of the fact that malaria is a serious health issue for refugees in sub Saharan Africa and taking into consideration the long period of time that most refugees spend in overcrowded conditions, limited research has been conducted in these settings to provide accurate data that can assist in gaining insight into, and greater clarity concerning the transmission patterns of the disease, or attaining success in curbing the spread of malaria. [16]

Malaria is transmitted exclusively through the bites of the anopheles mosquito .Some refugees from non or very low malaria endemic areas have to travel through malaria endemic areas in order to get to South Africa. Non-immune refugees are at a greater risk and have higher mortality rates. Non- immune refugees like children under five or none immune/semi-immune pregnant women are at risk. Refugees that have HIV also fall within this high risk category [9].

Historically, the sudden mass movement of people from one country to another has unleashed the development of a marked elevation of malaria incidence in countries that once had recorded a low malaria incidence rate. Despite the fact that one third of the global population reside in low malaria endemic areas a re-emergence of the disease has been detected. [15,16] In an Australian study, it was reported that in a sample of over three quarters of refugees who arrived from Africa, it was recorded that those who originated from sub-Saharan Africa suffered from the highest prevalence of most diseases, malaria 8%, 7% schistosomiasis, 5% hookworm, and 2% strongyloidiasis. [17] Using the Health Information Data capturing system (HIS), data was collected from several African countries between 2006 and 2009 in UNHCR designated refugee sites. The countries included the following: Uganda, Kenya, Ethiopia, Burundi, Cameroon, and Tanzania. Information on malaria incidence rates and mortality were investigated .It was established that a rate of 300 per 1000 refugees died. The highest malaria incidence was confirmed from refugee sites in Tanzania, with a recorded incidence in excess of more than 399 cases per 1000 refugees. [18]

Among the three malaria endemic provinces in South Africa, it was noted that there was a discernible difference in the proportion of malaria infections that were acquired locally, compared with those that had originated as a result of cross border transfers. It was observed that in both Mpumalanga and KwaZulu-Natal, the number of malaria cases that had occurred as a result of imported malaria (cross border malaria) were of a far greater magnitude or dimension than the cases of the disease that were acquired locally. [6]

There also occurred unclassified cases of malaria in both Limpopo and KwaZulu-Natal. Intestinal parasite infestation has been shown to exhibit extremely high incidence rates amongst refugees on the move, especially those that hail from low income countries. [6]

Significant numbers of studies pertaining to infectious diseases in humans have revealed the means whereby co-infection of parasites adopts or exerts an important role in the infection process through effects on the immune response of the host.[18]

Few studies in the past have linked the dynamics between co-infecting parasites to epidemiological disease. Co-infection is relevant for studies in refugee populations. Due to the presence of a wide variety of parasite species and strains that affect this population, in conjunction with the phenomenon of wide spread parasitism, there forms, or is established, a platform for concurrent infection. [19] Human hosts have been known to be infected with several parasites at once. This co-infection of parasites within the host can influence parasite density, distribution and dynamics of one another. Studies have shown that infection with one parasite will influence the host's response to infection with other parasites. [19]

Studies have investigated the effect of helminthic infestation (macro-parasites: which are parasites that complete only part of their life cycle within the host, and micro-parasites: which can complete their life cycle within a single host) for example, the malaria parasite. Parasitic infestation will result in two possible immune responses:

(a). During an infection with helminths, release of the T helper cell type 2 (Th2) is induced .This response involves the release of cytokines which include the interleukins: (IL)-4, (IL)-5, and (IL)-13. These interleukins elicit effector mechanisms which are effective in fighting big intracellular parasites. [19]

(b). Several helminths shield themselves against host immunity by taking advantage of the hosts immune-regulatory pathway. They do this by enhancing T regulatory cells (T reg), which have the ability to suppress both Th1 and Th2 immune responses. This means that helminthic infection can generally lower immune responsiveness which includes protection against micro-parasites. As a result of this, an initial helminthic infection can result in several micro-parasitic infections at individual level. [19]

Limited research has been carried out pertaining to the spread of disease amongst refugees once they have migrated from areas with high intestinal parasites infection incidence rates to none or low endemic areas. [20] Usually a combination of worms and protozoa are detected during stool analysis.

In the case of malaria, research has shown that refugees that resettle from countries of increased endemnicity could possibly act as asymptomatic reservoirs for malaria infection :P falciparum is of greatest concern due to the severity of disease .Even though sustained malaria transmission would be unlikely, single cases or small outbreaks would be possible, with the potential for fatal outcomes .[21]

.A blood film with a high eosinophil count, could most of the time prove intestinal parasite infection. [20] In refugees, the most ubiquitous parasitic infections that are associated with eosinophillia, are infections with soil transmitted helminths, as well as tissue invasive parasites (the malaria parasite).While the presence of an increased absolute eosinophil count is highly indicative of a recent or current infection with a tissue invasive parasite ,the absence of eosinophillia does not indicate the absence of parasitic infection .Eosinophillia is also lowered in persons with chronic infections as the host adjusts to the presence of parasites. An eosinophil count greater than 400 cells/µl is considered as high by most refugee health experts. The lower threshold is used because it will increase sensitivity to the test as the majority of refugees have a high probability of parasitic infection. [22]

There are several identifiable benefits in giving adequate healthcare services to refugee populations .These services will aid in curbing the spread of disease, not only among the refugees but also among the local population with whom they come in contact with .[5, 20] According to the Refugee Act of 1998 refugees are by law entitled to several rights, which include legal immigration documentation, the right to work, access to social and health services. [12]

As a result of long journeys and pre-existing conditions or illnesses that could have been contracted from previous refugee concentrated areas throughout South Africa, refugees need medical attention on their arrival in Durban and are in need of further health care. In spite of foreign and domestic guidelines stating the need to provide adequate health services to migrants under the 1998 Refugee Act, refugees still face difficult challenges in accessing health care services. [12]

In a study carried out by Kaplan, it was observed that less than fifty per cent of refugees utilized health care services within South Africa. However, almost thirty per cent who were residing within Durban, acknowledged setbacks when they tried to access health services. The most common reasons cited for being denied health care included communication problems, insufficient legal documentation, verbal abuse by health staff, more difficulty in accessing health care than their South African counterparts and expensive fee payment for these health services. Most of these factors feature prominently in the continued under-utilization of health services by refugees and asylum seekers in Durban. [12]

The areas where refugees settle are characterised by a large number of people that cluster together in a particular sector of their host country area, after an extended and often dangerous journey with no access to clean water, adequate nutrition or sanitary conditions. These conditions pose a health threat to the lives of these refugees .As these individuals continue to move from one place to another without adequate health care, they consequently alter the health status of the communities they come in contact with. [13, 14] This can also result in a financial and economic burden on the society.

Due to their inability to communicate in the local vernacular language and their total lack of cultural and social knowledge in the new environment that they now call their home, refugees, face several challenges. As previously alluded to, they often find themselves at the centre of violent xenophobic attacks; as a result, thereof, they flee the local areas to the inner cities in search of jobs and a better life.

As a result of this, many refugees live in crowded environments and living quarters that allow for the unchecked propagation of various communicable diseases. [5, 14] The threat of the spread of malaria and intestinal parasite infections as a result of these unhygienic living conditions needs to be urgently addressed.

The spread of malaria depends on the changes in the correlation that exists between refugees, the parasite and nature. Whenever a group of people move from a high to low malaria endemic area, a strong likelihood exists it is likely that this will result in an increase of malaria infections .Complications arise when parasites originate from an area where the individuals are drug resistant to anti-malaria drugs. Due to these dynamics, the drug efficacy of the country of destination will be affected. [15]

Reported increased mortality rates of malaria and intestinal parasites are matters of particular concern among refugees. Due to the fact that a large number of malaria cases reported within South Africa occur as a result of or cross border transference from neighbouring countries, the issue of malaria and migration must be tackled urgently. [6]

2.2 Aim of the study

The aim of the study is to determine the prevalence of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014 and to ascertain whether there is a risk of transmission of these diseases within the city.

2.3 Objectives

1. To measure the prevalence of symptomatic and asymptomatic malaria carriers and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

2. To measure the blood Eosinophil count as an indicator of parasitosis

3. To identify the types and intensity of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

4. To determine risk factors for transmission of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban.

3.0 Methodology

3.1 Research design and Research setting

This is an observational - cross sectional descriptive study with an analytic component, amongst refugees in Central Durban in 2014.The study will be conducted at Usizo Lwethu Clinic at the Denis Hurley Centre (DHC), Emmanuel Cathedral Parish in central Durban. The Centre provides a range of social services, including food and clothing, as well as health services provided by the Usizo Lwethu Clinic .Staff are able to speak a number of international languages such as French, Swahili and Portuguese, and the centre specifically caters to the needs of refugees and the homeless .The clinic provides primary health care services and refers patients to health services within the public health services for more specialised care.

3.2 Study population

The Denis Hurley Centre serves approximately 3000 people, of whom 70% are refugees and the rest live as homeless individuals on a monthly basis .The refugees will be identified based on their country of origin and refugee status . The majority of refugees come from African countries and speak very limited English or *IsiZulu.* The population for this proposed study comprises of male and female adults aged 18 years and above.

3.2.1 Inclusion and exclusion criteria

1. All the refugees who attend the Denis Hurley Centre during the data collection period for the study, irrespective of clinical signs, previous tests for malaria and intestinal parasites will be included in the study.

2. The following will be specifically excluded from the study: Individuals under the age of 18 years and non -refugees.

3.3 Sampling and Sampling size

. At the advice of a biostatistician, no hypothesis will be tested. Therefore this is a descriptive study, and the sample size was calculated as follows: The Denis Hurley Centre serves approximately 3000 people per month. For a descriptive study, a 10% of these 3,000 people is needed as a sample population. Therefore the sample was estimated at 300 participants and adjusted to 350 based on the fact only 70% seen at the Centre are refugees, the rest are homeless.

3.4 Data collection

The researcher and a trained qualified nurse will collect data using a specific data collection sheet designed for the study. The study will be pilot tested under similar conditions on 35 participants .Data collection will commence once ethical clearance from the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee and permission from the Denis Hurley Centre to proceed with the research have been obtained .All study participants will be required to provide written informed consent to participate. Data collection will include socio-demographic details of the study participants and risk factors for infection. A qualified nurse will assist with translation into French and the collection of blood samples for malaria detection. The nurse will also collect stool and urine samples for the detection of the presence, intensity and types of intestinal parasites. If stool or urine samples cannot be provided on the same day by the patient, they will be required to bring in the samples later. The samples will be transported, stored and handled according to good clinical and laboratory practices and analysed at the School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal.

3.5 Laboratory analysis of samples

Details of the laboratory analysis are included in the Appendices.

3.6 Data analysis

The variables will include demographic data such as age, gender, area of residence in Durban, education level and country of origin. Further, employment status, number of family members per household when possible, access to clean water and sanitation will also fall in this category. This data is crucial as they have been shown to be risk factors and for malaria and intestinal parasite infection.

Other variables will include:

1. Prevalence of malaria: The presence of malaria will be detected by using rapid diagnostic tests and microscopy.

2. The presence of intestinal parasites in positive smears will be counted under the microscope and identify types of intestinal parasites.

3. Blood eosinophil counts will be estimated as indicators of parasitosis.

3.61 Planning and Statistical Analyses

The data collected will be captured in Microsoft Excel software package. Quantitative data analysis will be carried out, as the study seeks to describe the communicable disease profile of the participants by measuring the prevalence of malarial and intestinal parasites and the risk factors for their infection. Subsequently, the data will be analysed using the SPSS version 18 or other advanced statistical package. Descriptive statistics such as mean, standard deviation, frequencies percentages will be used to summarize data .Pearson chi square test will be used to test the association between country of origin and strain of the parasites. Logistic regression will be used to identify possible risk factors for malaria and intestinal parasite infection

3.7 Limitations of the study

As many of the refugees come from African countries where local South African languages are not spoken, language may act as a barrier to recruiting study participants who do not speak *IsiZulu* or English. Provision has been made for the furnishing of an interpretation service between the English and French languages to be available for the study participants who have need of such a service. Refusals may also occur due to fear of stigmatisation or for being identified as a refugee or illegal immigrant. Should this happen, other people who are willing to participate will be recruited.

4.0 Ethical considerations

Study participants will only be included if they provide written informed consent, and participation in the study will be voluntary, and refusal to participate will not compromise the services received.

Those who agreed to be part of the study can withdraw from the study without any penalty or loss of services offered at the Denis Hurley Centre. Privacy and confidentiality will be maintained at all times during the study period. Furthermore, data will be stored locked in a private room.

The study will be anonymous, using unique identifiers during data collection, capturing, interpretation and dissemination. All study participants who wish to know their results will be supplied therewith. .As such results may not be available on the day of sample collection, those returning to collect them will be reimbursed for travel costs at R50 per person .Those that tested positive for malaria and intestinal parasite, will be referred to be treated at Usizo Lwethu Clinic at the Denis Hurley Centre.

5.0 Study period

Activity	Target dates
Proposal Development	May- September 2012
Application for funding	February – May 2012
Order consumables and Equipment	November 2012
Submission for scientific Review	January 2013- September 2013
Submission for Ethics Review	Oct – Dec 2013
Data collection	Jan – Mar 2014
Laboratory sample analyses	Jan-Mar 2014
Data Capturing and analyses	Apr 2014
Write up and Dissertation preparation	Mar-Apr 2014
Submissions for examinations	May 2014.

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6. Budget

NO	**Item**	**Quantity**	**Cost (Rands)**
1	Reimbursement of participants	350	R17500
2.	Laboratory consumables	Appendix 4	R 32,300
3.	Laboratory reagents	Appendix 4	R 100,000
	Total		R 150,000

References

1. The United Nations High Commission for Refugees. UNHCR. UNHCR: Country operations profile. South Africa: 2012.http://www.unhcr.org/egi-bin/texts/vtx/page (accessed 12 July 2012).

2. Webster M. Merriam Webster: Online Dictionary 2012. Available from: www.merriam-webster.com/dictionary/immigran. (accessed on: 4 June 2012).

3. Seicean A. Refugee health making a difference in Sub-Saharan Africa: A literature review 2008. www://cwru.edu/med/epidbio/mphp 439 (accessed 7 March 2012).

4. Sowetan. Refugees overwhelm South Africa. 2012. http://www. Sowetanlive. co.za/ news/2012 /05/ 8 /refugees-overwhelm - South Africa (accessed on 5 August 2012).

5. Apalata T, Kibiribiri J, Knight S, Lutge E. Refugee perceptions of their health status and quality of health care services in Durban South Africa. A community based survey. Health Link . 2007. http://Health link.org.za. (accessed on: 23 February 2012).

6. Maharaj J, Morrison N, Seocharan I, Kruger P, Moonar D, Mabuza A, *et al*. The feasibility of malaria elimination in South Africa. Malaria Journal. 2011;11: 423-428.

7. Maharaj R, Raman J, Moonsar D, Durrheim D, Seocharan I, Kruger P, Shandukani B and Kleinschmidt I. Epidemiology of malaria in South Africa: from control to elimination. S Afr Med J 2013;103:(10) 779 -788.

8. Misiani E, Shandukani.M, Nawn B, Ramalawa.N, VanShalkwyk C, Bester E, et al. Malaria directorate: Malaria elimination in South Africa. North West Malaria meeting. November 2012.

9. World Health Organization. WHO media centre/Fact sheet. Modes of transmission in low endemic areas.http://www.who.int/media centre.(accessed on 23 November 2013).

10. Chris B, Singh S, Surdashi D. Neglected tropical Diseases, conflict and the right to health: National academy of science. 2011.http://www.ncbi.nlm.gov/books/NBK 6258. (accessed on 4 June 2012).

11. Gabakima A, Konteh R, Kallon M, Mansaray H, Sahr F ,Bah Z. Intestinal protozoa and intestinal helminthic infections in displacement camps in Sierra Leone. Journal of Medical Science. 2007; 36 :1-9.

12. Kaplan C. Migration vulnerability and xenophobia: Central African refugee and asylum seekers access to health services in Durban South Africa Independent study project (ISP) Collection 2011 http://www.migration vulnerability and xenophobia _ central African .Refugee (accessed 11April 2013).

13. Doctors Without Borders. The lives of survival migrants and refugees in South Africa. 2012 http://www.Doctors without borders.org/publications/article (accessed 15 July 2012).

14. Wilson E. Travel and the emergence of infectious diseases. Emerging infectious diseases [Internet]. 1995 6th March 2012; 1 (2). http://www.ncbi.Nlm.Gov/pmc/articlrs/pmc2626831/pdf/8903157. (accessed on 6 March 2012).

15. Montavlo G, Renal-Querron M. Fighting against malaria: prevent wars while waiting for the malaria vaccine. 2007. http://www.econ.upf.edu/.(accessed 5 June 2012).

16. Bayoh N, Akhwale W, Ombok M, Sang D, Engoki S, Koros D, *et al.* Malaria in Kaukuma refugee camp, Turkana Kenya facilitation of *Anopheles arabiensis* vector population by installed water distribution and catchment systems. Malaria Journal. 2011; 10. (149):1475-2875.

17. Martin A, Mack D. Changing faces: A review of infectious disease screening of refugees by the migrant health unit, Western Australia 2003 and 2004. The Australian Medical Journal 2007; 11:607 - 610.

18. Anderson J, Doocy S, Hskew C, Spiegel P, Moss W. The burden of malaria in post -emergency refugee sites: a retrospective study. Conflict and Health. 2011;5:11.http://conflict and health.com/content/5/1/17 (accessed 5th June 2012

19. Ezenwa V, Jolles A. From host immunity to pathogen invasion: The effect of helminthic co-infection on dynamics of microparasites. Integrative and Comparative Biology. 2011; 51(4):540-51.

20. Centre for Disease control and Prevention (CDC). Overseas refugee health guidelines: Intestinal parasites2012 24th April 2012. http://www.cdc.gov/immigrant.refugees.com. (accessed on 24 April 2012).

21.Centres for Disease Conrtol and Prevention. (CDC). Presumptive treatment of P.falciparum malaria in refugees relocating from Sub Saharan Africa to the United States. http://www.cdc.gov/immigrant/refugeehealth/malaria guidelines-domestic.html. (accessed on 23 November 2013).

22.Staufner W. Eosinophilia:Refugee health technical assistance centre.http:www.refugee.healthta.org. (accessed on 20 November2013).

23. Cheesbrough M, Mac Arthur M. A Laboratory Manual for Rural Tropical Hospitals. New York: Churchill Livingstone: Longman Group; 1976. 209 p.

Appendix

Appendix 1

Laboratory analysis of blood samples for detection of malaria parasites.

1 SD Bio line rapid malaria test procedure:

Using the 5 micro litre disposable specimen loop provided, the circular end is dipped into the blood specimen. Carefully placing the circular end of the loop into the round sample well, 4 drops of assay diluents will be added into the square assay diluents well. The result will be read within 15 minutes.

Test interpretation

1. One_band: negative- the presence of one colour band (‘C’ control line) within the test result window indicates a negative result.

2.Two bands: C’ and *P .f –P. falciparum* positive.(The presence of two colour bands P.f and test line ‘C’ control line) within the test window, no matter which band appears first indicates a P .f positive result.

3.Three bands :’C’ ‘,P.f,’ and’ Pan’- The presence of three colour bands, ’C’, ’P .f’ and ‘pan’ within the test result window, no matter which band appears first indicates a P. positive or mixed infection of P. Falciparum .P. vivax, P. malariae or P. Ovale.

4. Invalid_result: If the control line fails to appear within the test window the test is considered invalid

Appendix 2

Microscopic examination of blood films

Thin blood film

Thin blood films will be prepared from collected blood by placing three drops of anti - coagulated blood on glass slide near the edge of the slide.

Another smooth edged slide will be used to spread the blood evenly across the first slide by collecting a small amount 1cm from the first big drop at a 45 degree angle. The thin blood smear slides will be air dried and labelled.

Thick blood films

Tick blood films will be prepared by adding a drop of blood of about 3mm in diameter in the middle of the slide and spreading it with the corner of another slide.

The film is allowed to air dry and placed in a staining rack Do not fix the slide.

Preparation of Giemsa stain.

The Giemsa stain will be prepared by mixing 3.8 grams of Giemsa powder with methanol (250ml) and glycerol (250ml) The Giemsa stain is then filtered and will be diluted 1 in 10 with pH 7.2 buffered water for use daily

The malaria parasite in the patient sample, will be identified by staining the thin and thick films and viewing them microscopically. Thin and thick blood film will be stained in the following ways:

Staining of blood films

The thin films will be fixed with methyl alcohol for about 30 seconds before being stained with the Giemsa stain. Thick films are not fixed.

The smears will be placed face down in a staining tray of diluted Giemsa stain and left for 15 minutes for thin stain and 20 minutes for the thick stain.

The staining tray will then be flooded with pH 7.2 buffered water for thin film 5 minutes and thick films few seconds respectively.

The thin blood film will be washed gently in water for 2-3 seconds. The slides will then be allowed to air dry in the slide rack.

Strains of the malaria parasite will be evaluated by examining the stained thin and thick film slides with the oil immersion using x 100.At least 50 fields of the thin smear and the thick film is used as a confirmatory slide.

The Giemsa stain for thin films will then be used on a thin blood film to determine the malaria parasite count. [23]

Parasitaemia determination (counting of malaria parasites)

Malaria parasitaemia will be determined by using high power magnification. Choosing a field where the red cells are separated and evenly spread.

A manual counter, will be used to count infected red blood cells in 5 - 10 fields that have approximately 1000 red blood cells, all cells containing parasites in this fields will be counted. This excludes several parasites in one cell. (Multiple parasites within one red cell will be counted as one parasite).

Calculations for the determination of %parasitaemia and the absolute parasite count will be as follows:

% parasitaemia = No of infected RBC's x 100 =%

1000

Absolute count = parasitaemia% x total red cell count

Appendix 3

Estimation of eosinophil % blood count

The eosinophil count will be determined using the Sysmex XE 5000 Automated Haematology Analyser. The analyser will determine the eosinophil count as a percentage of the total differential white blood cell count.

Appendix 4

Laboratory analysis of stool samples for detection of intestinal parasites

Intestinal parasites in stool will be detected by the Formol-saline ether sedimentation method:

A small quantity of stool will be mixed in about 10ml of 10% formol saline in a tube. sieving the suspension into a beaker through a strainer with small holes.

6ml of the sieved suspension will be poured into a centrifuge tube.

Adding 3.0 ml of ether to the tube, the contents will be mixed well and centrifuged for 3,000 rev/minutes for 1minute.

The middle layer will be separated from the sides of the tube.with an applicator stick. The ether and formol -saline will be poured away.

Transferring the re-suspended deposit to a slide with a Pasteur pipette., a wet preparation will be made of the deposit and one drop of iodine and is covered with a cover glass. View microscopically using a 10x and x40 lens. [23]

Appendix 5

Laboratory analysis of urine samples for detection of intestinal parasites:

Intestinal parasites will be detected from collected urine samples by placing 10mls of the urine into a centrifuge tube and centrifuging to obtain a sediment or deposit.

Pouring off the supernatant fluid, the deposit will be mixed by tapping the bottom of the tube.

Transferring the deposit to a slide and covering with a cover glass. The deposit on the slide will then be examined microscopically Malaria paraby using the 10x and 40 x objectives, for the presence of parasites. [23]

Appendix 6

Participant information sheet

The profile of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014.REF:BEO48/1

GOOD DAY!

Who we are?

We are researchers from the Nelson R .Mandela School of Medicine, University of KwaZulu-Natal .Research is just a process to learn the answer to a question.

Why we are here?

We are doing research on malaria and intestinal parasites, urine parasites or worms + eosinpophils. These are diseases that make people in Africa and South Africa to fall sick and if they are not detected early ,treated and managed properly they can result in severe illness or even death .These diseases are called communicable diseases because they are caused by organisms that may be carried from a person, animal or the environment to another vulnerable person either directly or indirectly .Our aim is to learn whether and how many people that visit the Denis Hurley Centre are affected by these diseases.

Invitation to participate

We invite you to participate in the research to enable us to achieve the aim of the research.

Why choose the Denis Hurley Centre (DHC)?

The reason the Denis Hurley Centre is chosen as the place to do the research is because some people who visit the centre come from other African countries that also have a problem with these diseases, but may be slightly different from the ones that are seen in South Africa. This may require different types of treatment and management. When an individual is infected with one of these diseases it is

important that the disease is detected and the individual receives treatment early to prevent severe illness, passing the disease on to other people or even death.

What is involved in the study

We need to select 344 study participants from all who visit the Denis Hurley Centre to take part in the research. Once you agree to join the research we will require you to assist us in the following manner:

Answer a few questions

a). Free testing for all the diseases.
b). Your age, gender, whether you work or not, whether or not you have been ill with one or more of these diseases before and other information.

c) Provide us with a small amount of blood (about 5ml) to be tested for malaria parasites .The nurse will draw blood from your arm

d) Provide us with urine and stool samples in the bottles supplied, to test for worms.

This will only take about 30 minutes of your time .The results of the test will be available after one week and if you wish to know the results, please come and collect them at Usizo Lwethu Clinic after a week .You will be reimbursed R50 for your transport costs.

What risks are involved in the research?

There are no risks involved in this study are minimal except that you will experience a little discomfort when the nurse draws blood from your arm because the nurse will be using a needle. This will last for a few seconds and afterward the needle prick will heal.

You may feel uncomfortable to bring the bottles of urine and stool samples in the presence of other people; this will be done in private with no other person present except the nurse.

What are the potential benefits of being part of the research?

1. You will receive no incentives for participating in the research but you will receive:

b) If you have tested positive for any of these diseases you will be treated for malaria and intestinal parasites at the Usizo Lwethu Clinic.

2. Long term, the risk of transmission and infection among the community will be reduced and thereby reducing this problem of disease and improving the quality of life.

Voluntary participation

You are not forced to participate in the research. We will respect your wish if you do not want to participate in the research or should you decide to withdraw at any time during the research. Your refusal or withdrawal from the research will not result in any penalty or loss of benefits or services provided by the Denis Hurley Centre to which you are entitled.

Results

Should you wish to know the results of your tests, you are welcome to return after seven days (one week) from the day you gave your samples to be tested. You will receive R50 towards your travel expenses when you come back to check your test results.

Confidentiality

Data that we collect from you will collect from you will be confidential .When the data from the research is being analysed, your names will not be used to identify you ,only numbers will be used .Personal information may not be disclosed only if required by law. The primary investigator will hold a master sheet linking names and study ID's to enable them to provide results to those who require them. The master sheet will be locked away and will be available to the PI.

Organizations that may inspect and/or copy research records for quality assurance and data analysis include groups such as the University Biomedical Research Ethics Committee (BREC).

If results are published, no individual or cohort identification will be used (results will be published anonymously).

Contact details of researcher/s-for further information/reporting of study related adverse events:

1. Prof Joyce Tsoka Gwegweni

Nelson R. Mandela School of Medicine University of KwaZulu-Natal.

Tel:0312604386

E-mail:tsokagwegweni@ukzn.ac.za

2. Uchenna .E. Okafor

Discipline: Public Health Medicine University of KwaZulu-Natal.

Cell phone Number: 083868585.

E-mail:uchenna77@yahoo.com

Contact details of BREC Administrator or Chair- for reporting of complaints/problem: Biomedical Research Ethics ,Research Office UKZN .Private bag x 54001,Durban 4000

Telephone: +27 (0)31 260 469/260 1074

Fax: +27(0) 31 260 4609

E- mail: BREC @ukzn.ac.za.

Appendix 7

Informed consent

The profile of malaria and intestinal parasites among refugees attending the Denis Hurley Centre in Central Durban in 2014.

I, ----------have been informed about the research and its purpose by the research team

Their contact details are

1. Prof Joyce Tsoka Gwegweni

Nelson. R. Mandela School of Medicine University of KwaZulu-Natal.

Tel:0312604386

E-mail:tsokagwegweni@ukzn.ac.za

2. Uchenna .E. Okafor

Discipline: Public Health Medicine University of KwaZulu-Natal.

Cell phone Number: 083868585.

E-mail:uchenna77@yahoo.com

I understand what my involvement in the study means and I voluntarily agree to participate. I have been given the opportunity to ask any questions that I may have in the participation of this study. I will be given a signed copy of the document and the participant information sheet which is a written summary of the research.

I may contact the Biomedical Research Ethics Office on 031-2604769 or2601074 or E-mail BREC@ukzn.ac.za if I have any questions about my right as a participant.

Signature of participant---------- Date----------

Signature of witness/translator----------Date----------

Appendix 8

Questionnaire

The profile of malaria and intestinal parasites among refugees attending the Dennis Hurley Centre in Central Durban in 2014.REF:BEO48/14.

April 2013

Instructions: Please mark your choice by putting a circle in the appropriate box and answering the questions in the space provided.

1. General information

1.1Date of the interview----------

1.2 Name of the interviewer----------

1.3 Respondents Unique No----------

2. Demographic information

2.1. Sex :----------(1). Male :----------(2). Female: ----------.

2.2 Date of birth: -------d d/mm/y y-/---------- Age ----------

2.3 Country of origin ----------

2.4. Marital status:-------(1).Single----- (2). Married ---------- (3) Widowed----------

4. Casual relationship----------

2.5. Highest educational qualification achieved ? (1). None (2).Grade1-7 (3).Grade 8-12 (4). Other----------

2.5a Did you complete your education?

2.6. What is your mother tongue (language)?

(1). *IsiZulu*------- (2) .English. .------- (3). *Sesotho*. ----- (4). *Swahili* ------- (5). Other/specify----------

2.7Residential address ----------

2.8a. Are you residing in South Africa with your family/family members? Yes / no.

2.8b. How many people live with you? ----------

(a). 1 ----------(b). 2-5 . ---------- (c). More than 5---------- (e) Other----------

2.9 How many bed rooms does your house have? (a). 1 -----(b). 2----- (c) .3.------- (d).more ----------

2.10 What type of housing do you live in? (a). A flat ---------(b) .A brick house-------- (c). Other----------

2.11 Where do you get your water from? (a). Tap inside the house (b) .Tap outside the house (c) Other

2.12What is your occupation? (a). Unemployed (b). Employed. (c). Student (d). Other/specify**----------**

2.13 Which clinic/ Hospital do you go to for your health needs? (a).Usiso Lwethu (b). Addington (c). None

3. Malaria information

3.1Have you ever suffered/diagnosed with malaria before in your country of origin?(A). Yes (b).No (c) Don't know/N/A.

(Malaria is a parasitic infection caused by the bite of the ?*Anopheles mosquito*)

3.2. If yes, did you receive treatment for the malaria infection in your country of origin? (a).Yes (b). No (c). DK

4. Intestinal parasite information

4.0 What is the nature of your toilet system? (a)Pit (b).Water system (Flushing) (c) Other /specify

4.1. Have you ever suffered from any kind of intestinal parasite infection? When you were younger (worm infestation) (a).Yes (b).No (c). DK/NR

4.2 Has any of your family members suffered from an intestinal parasite infection?. (a)Yes (b). No (c). DK / NR.

4.3. Did you submit specimen for laboratory diagnosis (a).urine (b). Stool (c) .Blood (d). None

THANK YOU FOR YOUR COOPERATION

Appendix 9.

Detailed budget

	Reagents	**Specification**	
	Laboratory consumables and Reagents		
	1.Gloves	10 boxes for collection /Analysis	
	2.Microscopic Slides	7 Boxes (100) 25 x76mm Glass Microscope Slides	
	3. Microscope Cover glasses	7 Boxes (100) of no 1 size thickness cover Glasses	
	4.Plastic Centrifuge Tubes	350pcs	
	5.Vaccutainer blood collection Tubes	400 pcs	
	6.Plain Sterile stool containers	400 Containers	
	7.Counting chambers	2	
	8.Sample rack	2 Racks.	
	9.Micro pipette	1	
	10.Distilled Water	20 Litres	
	11.Giemsa Powder	20g	

	12.Glycerine (Glycerol)	1 Litre	
	13.Methyl Alcohol (Methanol)	1Litre	
	14.Sodium citrate powder	60g	
	15.Formaldehyde (40%w/v) solution	500 mls	
	16 .NaCl Powder	1 Packet	
	17.Glass beads		
	18.Potassium Dihydrogen Phosphate	50grams	
	19.Disodium Hydrogen Phosphate	50grams	
	20. Ether	2 litres	
	21.Centrifuge		
	22.Stool Applicator sticks	500 pcs	
	23.Immersion oil	2	
	24.Cotton wool		
	25.SterileGauze		
	26.Adhesive Labels		
	27.Grease pens	2 packets	
	28.Pens		
	29.Soap ,Detergents ,Disinfectants		
	30.Disposable Pasteur pipettes	400	
	31.Iodine	10g	
	32.Rapid Test identification Slides	400	
	33. Potassium Iodide	20gram	
	34.Beakers Glass	2 of each	
	35.Reagent storage containers	6 4	
	36.Laboratory cover coats	2	

	37.Filter paper	5 Boxes	
	38.pH papers Narrow range	2 rolls	
	Total		R80,000

A	Stationeries	10,000
B	Nursing Assistant	20,000
C	Laboratory processing of samples	40,000
	GRAND TOTAL	**R 150,000**