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REVIEW ARTICLE

Health related vulnerabilities and enabling institutions to facilitate responses to climate change in East Africa

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ABSTRACT

It is now accepted that climate change is having and will continue to have a direct or an indirect impact on human health and in most cases, it will be negative. In this review the links between climate and climate sensitive diseases is established. The review goes further to examine what it will take for health research institutions to address adaptation to climate change, while reducing institutional vulnerability and improving their response to climate change. Evidence has emerged that range expansion of climate sensitive diseases such as malaria, meningitis, Rift Valley Fever, chikungunya and cryptosporidium is occurring and this will increase pressure on the health systems across Africa. Climate related risks in health will require a proactive approach in order to prevent rather than manage health disasters. Diseases epidemic predictive models will enable early detection of the risks and intervention. The health system is dependent on several national and foreign partners forming a critical network. If these networks malfunction then the health systems will be highly susceptible to failure. Governance and leadership of the institutions will determine the rate of adaption to climate change. There is need to strengthen research institutions in Africa because they can run long term programs addressing climate change. Furthermore these institutions must expand their research agenda to include a multidisciplinary approach to solving problems. A closer collaboration between departments of meteorology, remote sensing and mapping and medical research institutions is now more urgent than ever. Development partners and national governments must invest in infrastructure that will enable adaptation with the aim of increasing the institutional capacity to cope and minimize the potential impacts of climate driven diseases.

Keywords: Climate change human health zoonosis institution vulnerability adaptation

INTRODUCTION

Climate change will have a direct and indirect impact on human health and well-being through different pathways. The frequency and intensity of extreme events associated with negative impacts is increasing and this trend will continue [1].

Institutions are organizations with a formal governance structure that are instituted through a legal framework to carry out specific mandates.

African health research institutions will need to develop adaptation options to reduce the vulnerability and impacts. These public owned institutions and in partnership with civil society institutions will need to carry out an analysis of their strengths, weaknesses, opportunities and threats that may limit or promote the adaptation process. The threats from climate change will range from relatively benign diseases outbreaks to complex multi-disease epidemics that will be difficult to deal with. African populations expect that their research institutions will develop, test and deploy strategies to detect threats and prevent them. This paper intends to identify infectious diseases and other health conditions that are climate sensitive and that show clear seasonality. Thereafter insritutional research capacity fo adaptation research is explored. Lessons are derived from previous regional climate and health research projects.

Climate and health analytical framework

Climate change is an evolving process that is expected to intensify Many infectious and non-infectious diseases and health conditions have different sensitivities to climate. Indeed the distribution of disease in time and space is a function to climate. Climate change which is a change in the mean climate state will have a direct impact on diseases spatial – temporal distribution. Climate change will effect diseases range expansion and contraction. Climate variability will affect the transmission intensity in time and space and this includes the evolution of epidemics 2. Climate change may facilitate the emergence and reemergence of new and old infections 3. In general climate change has a large scale effect in diseases distribution and incidence affecting large populations that can easily overwhelm health services in a country. Besides a change in spatial-temporal changes in disease distribution, a change in the frequency of outbreaks and epidemics can be expected. In addition spontaneous and multiple diseases epidemic may occur with severe consequences in the health system. Health research institutions will need to address the challenges of the new threats arising from climate change. These include tools and strategies for anticipating and minimizing risks. Such tools include early warning systems and mapping of risk in space and time. They could also include ways of targeting interventions to population that are vulnerable.

In the first part of this review an exploratory literature search has been carried out to determine if there is any evidence of the expected changes in diseases epidemiology under climate change.

Some of the major infectious and non-infectious diseases and how they are linked to climate and weather are reviewed. The limitations of the response of these institutions in reducing the impacts to climate change in health is explored.

Health related vulnerabilities

Vector-borne, water-borne, air-borne, food-borne infections

Infectious diseases include simple or complex life cycles that have many environmental determinants including climate. Each element of the life cycle has its degree of sensitivity to meteorological parameters and interaction with other environmental attributes such as topography, hydrology, land use and land cover. The meteorological parameters modulate the seasonality of the diseases and their distribution in space while some geographic features such as hydrology and topography may restrict the distribution of the diseases only in space. Climate change is expected to be associated with changes in some infectious diseases distribution in space while climate variability will affect the intensity of disease transmission in time. It is critical to assess the potential risk of epidemiological changes associated with climate change in preparation to counter the emerging public health threats whose impacts can be devastating. The risks are higher when the transmission range expands in areas where human population have low immunity and where the public health sector is unprepared to prevent and even treat the diseases. Already the epidemiology and impacts of the climate sensitive diseases has been observed in Africa and elsewhere in the world in infectious diseases such as malaria, meningitis, cholera, arbovirus infections and diarrhoea.

Malaria

Malaria is the most important infectious and vector borne disease in Africa. It is estimated that 74% of the population in Africa live in highly endemic areas for the disease and 19% in endemic prone areas 4. Most of the epidemics occur in the highlands and desert fringes and are driven by anomalous temperatures and rainfall 5. Many countries in Eastern Africa for example Kenya, Tanzania, Uganda, Eritrea, Burundi, Rwanda and Ethiopia have suffered from these epidemics. During these epidemics the disease incidence can increase 4-8-fold and mortality by as much as 5-fold 6, 7, 8.There is emerging evidence that malaria is spreading to higher altitudes in East Africa 9 and to new highland areas that were previously free of the disease 10. Warming occurring at the rate of 0.21°C per decade 3 in western Kenya has been associated with permissive conditions for increased malaria transmission 11. Recently malaria control activities across Africa have reduced the number of infections by as much as 50% 12. While this is a positive development it will be accompanied by reduced exposure to the disease and subsequent reduction of the development of immunity resulting in increased vulnerability to severe disease unless the control efforts are sustained 13. Low immunity and increased climate driven risk would reverse the gains made in the control of the disease.

Rift Valley Fever

Rift Valley Fever (RVF) is a zoonotic disease that mainly affects livestock but is also infectious to humans. The disease represents a serious human and

animal health threat and an economic burden. The pathogenic agent is a member of the genus Phlebovirus and family Bunyaviridae virus that cause haemorrhagic fevers. The disease is widespread in Africa and extending from Egypt to South Africa 14.

The primary vectors of the virus are Aedes mosquitoes while Culex, Mansonia and Anopheles species are also major vectors. The pioneer vector species in a habitat is the Aedes and within an ecological succession sequence is anopheles, followed by culex and finally Mansonia species. This virus can also be transmitted by aerosols of viremic blood during haemorrhage and by contact with infected animal and consumption of milk from infected animals. A devastating outbreak in Egypt in 1977–79 was associated with more than 200,000 human infections and 600 deaths and losses in livestock >$100M at that time 15. More recently (1997–1998) a RVF outbreak occurred in eastern Africa affecting Kenya, Tanzania, and Somalia resulting in an estimated 27,500 human cases, and about 170 deaths 16.

Rift Valley Fever epidemics are associated with a sudden increase of Aedes mosquitoes caused by extensive flooding 17. The virus survives the dry season in the drought resistant eggs of the Aedes mosquito species which then infects the larvae and finally the adult mosquito. The Culex and some Anopheles mosquitoes can then join the cycle and transmit the virus from one animal to the other. The mosquito infection rates can be very high as reported in Kenya where 5.9% *Anopheles squamosus*, 30% *Aedes ochraceus*, 42% *Aedes mcintoshi* were infected 18.

In Eastern Africa RFV epidemics have been associated with heavy flooding caused by the El Nino Southern Oscillation (ENSO) and the Indian Ocean Dipole 19.

Rift valley fever enzootics (epidemic in animals) have been expanding their range in Africa for the last several decades. For example in Kenya, incidence has increased from one province in the 1960s to six provinces by 2007 20. Enzootics have occurred when rainfall increases by over 50% of the normal amounts 20. Climate change is expected to increase extreme events such as floods and this is highly likely to increase the intensity and range of RVF enzootics. Livestock vaccination is currently in use in Kenya 21. however the vaccination is not licenced for human use.

Yellow Fever

Yellow Fever is caused by a flavivirus (family Flaviviridae) transmitted by the *Aedes aegypti* mosquito. The disease has two transmission cycles one occurring in urban areas and the other in the jungle (Sylvatic). The urban type is transmitted from man to man by the *Ae. aegypti* mosquito while in the jungle transmission occurs between monkeys and is transmitted by other Aedes species such as *Ae. simpsoni* and *Ae. africanus*. The severe form of the disease has a fatality rate of ranging from 15 to over 50% (CDC 2007).

Thirty-two African countries are now considered at risk of Yellow fever, with a total population of 610 million people, among which more than 219 million live in urban settings 22. The disease occurs in epidemic forms particularly in Central and West Africa. Unpublished reports indicate that in 2008 Africa saw 13 Yellow fever outbreaks compared to the average of 2-5 outbreaks per year. The possible causes of the outbreaks range from lack of vaccination, accelerated urbanization and climate change. While Yellow fever has largely been controlled using vaccines, significant risks exist in unvaccinated populations. Furthermore climate change is likely to expand the range of the vectors while uncontrolled urbanization will increase the availability of breeding habitats for *Ae. aegypti.* Africa accounts for 90% of global Yellow fever cases 23. The heterogeneity of Yellow fever spatial-temporal distribution in Africa can be largely explained by temperature suitability of the vectors and the virus in addition to rainfall 23. This suggests that climate change and variability will affect the epidemiology of the disease 24.

West Nile Fever

West Nile Fever (WNV) virus,which was first isolated in Uganda in 1937 25 is widely spread in Africa and it belongs to the genus Flavivirus. The virus mainly affects birds and equines and occasionally humans. In Africa horses are at a particularly high risk of the infection. The virus which was first isolated from Uganda in a human case has traditionally caused mild febrile illness in the past. However since the 1990s a more severe form of the disease was observed in Algeria, Morocco and Tunisia 26. Unlike RVF, WNF is mainly transmitted by culex species that primarily feed on birds. Human are considered a dead end host because they are highly unlikely to infect the mosquito vectors. The major vector of WNFV is *Culex pipiens*, however because of vector species succession, there could be several bridging vectors such as *Cx.*

antennatus and *Cx. univittatus* have been implicated 27.

Chikungunya

Chikungunya (CHKV) is a viral disease caused by an alphavirus genus and belonging to the family togaviridae. It was first discovered in Tanzania in 1952 and causes symptoms similar to dengue fever 28. The virus occurs in Africa with a wider distribution in the Eastern Africa and it is transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. Although the disease has had low prevalence for a number of decades, recent outbreaks in Democratic Republic of Congo in 1999-2000 and Gabon in 2007 have caused concern. A more recent outbreak (2011) was reported in the Congo DR involving at least 8,000 people 29. It has been shown that the virus has mutated 30 and it's now able to infect *Aedes albopictus* and cause severe disease and mortality while in the past the virus was only transmitted by *Ae. aegypti* and caused mild to acute disease symptoms. In 2004 serological studies indicate that the prevalence of antibodies to CHKV in western Kenya was 60% and at the Kenyan coast 24% suggesting that there was active viral transmission 31. In 2005-06 an outbreak in La Re-union Island the Indian Ocean involved 244,000 people and with severe clinical cases 32. The new vector *Aedes albopictus* in Africa which is originally from Asia is spreading fast on the continent. In Brazil it has been found that arbovirus infections transmitted by *Ae. aegypti* correlate with precipitation and temperature 33 thus climate variability and change is expected to affect Chikungunya transmission.

Dengue

The Dengue (DENV) causing virus belongs to the genus flavivirus and family flavivaridae. The disease is endemic in 110 countries in the world. The main vector is *Ae. aegypti* but *Ae. albopictus* is an important vector. Dengue has four closely related viruses referred to as DENV 1, 2, 3 and 4. The disease has two forms these being dengue fever (DF) and dengue haemorrhagic fever (DHF) with the former having febrile illness and the latter being severe and associated with mortality. During the period 1960-2010 a total of 22 countries in Africa reported sporadic cases of outbreaks of dengue and in the 12 other countries dengue was reported in travellers who had visited the areas 34. Dengue has similar symptoms to malaria and is most likely misdiagnosed and under reported in Africa. As many as 70% of fevers clinically treated for malaria in Africa are not attributable to the disease but may be caused by flavivirus 17. Dengue is mainly an urban disease and in some countries it has been associated with both excessive rainfall and drought. During droughts, *Ae. aegypti* breeds prolifically in water storage containers and during heavy and prolonged rainfall in discarded water containers 17. Dengue infections exhibit seasonality often associated with rainfall and temperature suggesting that the disease is climate sensitive 35, 36.

In general rainfall increases vector abundance, while temperature shortens the vector and virus development period and increases their blood feeding frequency 37 (With regard to the parasites and viruses temperature reduces the extrinsic incubation period subsequently increasing the vector's infectious life. In combination the climatic factors increase disease transmission.

Water-borne/food-borne diseases

Many of the waterborne diseases such as cholera, and typhoid are also food borne diseases. However cholera, typhoid fever and hepatitis A are considered as the major water-borne diseases. Cholera is caused by the bacterium *Vibrio cholerae* which is found in marine and freshwater ecosystems 38.

Between 1970-79, 19.7% of global cholera cases were reported from Africa and by 2005, 94.8% of these cases came from Africa. However the absolute number of reported cases in Africa has not increased significantly between the two periods but the number of countries reporting the disease has increased 39, 40. Cholera has been associated with temperature and rainfall anomalies 41. In Zanzibar it was shown through modelling that a 1°C increase in temperature at 4 months lag resulted in a 2-fold increase of cholera cases, and an increase of 200 mm of rainfall at 2 months lag resulted in a 1.6-fold increase of cholera cases 42. The disease has a complex physiochemical and biotic drivers that resonate with climate variability. These factors include salinity, iron content, phosphates, nitrates, organic matter, algae, zooplanktons and temperature 43. Cholera epidemics in East Africa have been associated with El Nino years 44, 45. In the Western Kenya Lake Victoria region several discreet waves of cholera outbreaks and epidemics occurred in 1971-72, 1980-84, 1992-93, 1997-1998 and 2008 periods associated with El Ninos 46. In Kenya severe epidemics occurred in 1982 and 1997-8 years with strong and very strong El Nino events. Rift Vallley Fever epidemics have been reported in 2018/9 in Mayotte 47.

Other enteric diseases

Enteric diseases (shigellosis, typhoid fever and cryptosporidiosis) are driven by a combination of human and environmental factors including, poverty, water resource and climate 48. Shigella is a family of bacteria that infect humans through contaminated water and food. Of the several types, Shigella dysenteriae can cause severe epidemics. The disease that mainly affects children under 5 years causes bloody diarrhoea and can be fatal if not treated. Lack of water leading to poor hygiene during droughts can increase the risk of Shigella 49.

Typhoid fever is a bacterial disease, caused by *Salmonella typhi* and is transmitted through contaminated food and water. Highest incidence usually occurs where water supplies serving large populations are contaminated by faecal matter 50.

The estimated incidence of the disease in sub-Saharan Africa is 50 cases per 100,000 persons 51. Floods in areas of poor sanitation can increase the risk of faecal contamination of public water supplies.

In Africa cryptosporidium is a diarrhoea disease caused by the *Cryptosporidium parvum,* and *Cryptosporidium hominis* which are protozoan parasites. These parasites infect both man and animals and are transmitted through contaminated drinking water. The disease exhibits seasonality with the peak incidence being observed in the rainy season. However increased incidence has been observed in the dry season most likely due to the use of the few available contaminated water sources 52. In sub-Saharan Africa the disease is most prevalent in children 6-12 years old. The prevalence of the diseases is 5-25% in the region.

The seasonality of diarrhoea diseases has a complex relationship to weather and climate. In some instances the infections may be driven by drought while in others rainfall may be the driving factor 53. Diarrhoea incidence in Botswana is associated with La Nina conditions that are characterised by above normal rains 54.

Non- infectious health conditions

Malnutrition and under-nutrition

Malnutrition is a condition mainly seen in children that do not consume a balance diet and leads to high risk of mortality, lowered resistance to infection such as diarrhoea and respiratory diseases, retarded physical and mental growths. A balanced diet should include proteins, carbohydrates and micronutrients such as vitamins and mineral. The World Health Organizarion (WHO)regards hunger and malnutrition as the gravest threat to public health, and climate change threatens to further destabilize already fragile food-production systems 55. In the year 2000, 32% of children fewer less than 5 years of age in-sub-Saharan Africa suffered various degrees of malnutrition 56 though this proportion has reduced in some countries notably Senegal and Uganda 57. Coupled with high fertility rates and shrinking arable land, high food prices and political instability, climate change induced droughts and reduced agricultural productivity will exacerbate the situation 58.

While malnutrition refers to the quality of food, under-nutrition refers to the quantity consumed. Under-nutrition leads to wasting, underweight and stunting 59. Under-nutrition has been strongly associated, with shorter adult height, less schooling, reduced economic productivity, and for women, lower offspring birth weight 60.

Heat waves and heat stress

According to the World Meteorological Organization definition, a heat wave is when the daily maximum temperature of more than five consecutive days exceeds the average maximum temperature by 5^{o}C degrees the normal period being 1961–1990 17. Excessive heat and high humidity are associated with heat waves. The most vulnerable are young children, the elderly and those in poor health. Heat waves cause heat oedema, heat rash, heat crump, syncope, stroke, heart attacks and death 61. While heat waves have occurred in the United States, Europe Australia and parts of Asia, no documented events have been reported from Africa. However in Burkina Faso the mean monthly temperature was significantly related to mortality in old ages and cardiovascular mortality varied by season, with higher mortality rates occurring in the hot dry season 62.

Air-borne diseases

Meningococcal meningitis (MC)

Meningitis is an air-borne disease caused by the bacteria *Neisseria meningitides.* The disease occurs in the meningitis belt (Senegal –Ethiopia) which is defined as areas of West Africa limited mainly by rainfall, being bound by isohyets with the northern limit set at 300 mm rainfall per year and a southern limit at an 1,100 mm rainfall 63. Epidemics are associated with humidity below 30% for a period of five days. It has also been linked to the speed of the

Harmattan winds 64. Currently the meningitis belt is expanding eastwards to Ethiopia and Uganda most likely due climate change 65. During epidemics, the incidence can approach 1,000 per 100,000, or 1% of the population 66. Improved MC vaccines are a promising investment that could substantially contribute to reduction of child meningitis mortality world-wide 67.

Ecosystem vulnerability

Highlands

The highlands were previously free of any major vector and water-borne diseases due to low temperatures. However in the recent past malaria epidemics have occurred in the highlands and an increase in temperature has been recorded. This trend is likely to continue leading to an increase of the human population at risk of severe disease and epidemics.

Arid areas

Arid areas are characterized by low rainfall and frequent droughts. Many of them are plains that are prone flooding during extreme events. Health threats range from famine to Rift Valley Fever and malaria epidemics.

Aquatic systems

Aquatic systems include lakes, oceans and rivers and they are directly affected by climate change. Extreme precipitation can cause flooding affecting human settlements and their health. Lakes and coastal areas can also be a reservoir for cholera. Strong El Ninos and flooding have been associated with large scale cholera epidemics 68. Many rapidly expanding cities are located in deltas that are at high risk of flooding as a consequence of sea level rise.

Emmerging threats

There is evidence of range expansion in malaria, Rift Valley Fever Chikungunya, cryptosporidium and meningitis. However there is evidence that current interventions such as the use of insecticide impregnated bed nets for malaria control , vaccines for RFV and meningitis have reduced the incidence of diseases and the occurrence of epidemics. However, some of the interventions such as insecticide impregnated bed nets may lose their efficacy in time while the climate risk is increasing. New viral strains could affect the efficacy of the meningitis vaccines.

Climate change and variability impacts

Climate change includes the change in the mean ambient temperature and also the departure from the mean. In Kenya for example the rate of change in the mean temperature has been 0.2°C/decade. This change has led to the increasing the suitability of malaria transmission in the highlands.

Climate variability refers to the departure of the ambient temperature from the mean value and this change can lead to diseases epidemics. For example during the 1997/8 El Nino event a temperature of 5.9°C above normal was observed in western Kenya and this was associated with the malaria and cholera epidemics in East Africa.

Extreme dry conditions and low humidity have been associated with meningitis epidemics in the Sahel region.

Social economic vulnerability: poverty and culture

Poverty leads to lack of access to information and other basic human needs including access to health care, safe drinking water and nutrition. A significant proportion of human populations in Africa lives in poverty and in environments referred to as pathogenic landscapes. These populations live in areas that have poor communication and infrastructure and are thus hard to reach in times of climate disasters. These populations have very low access to safe drinking water and are thus highly vulnerable to waterborne disease. Food insecurity leads to severe malnutrition and under nutrition during drought and famine.

Certain cultures can increase the exposure of populations to diseases. These include behaviour and practices that can increase the risk of encounter with pathogens. For example drinking raw milk and blood by pastoralist communities can increase the risk of Rift Valley Fever following severe flooding. Failure to use latrines by some communities contaminates streams and rivers during the rainy season and this increases the risk of diarrheal diseases.

Importance of public and civil institutions to facilitating adaptation

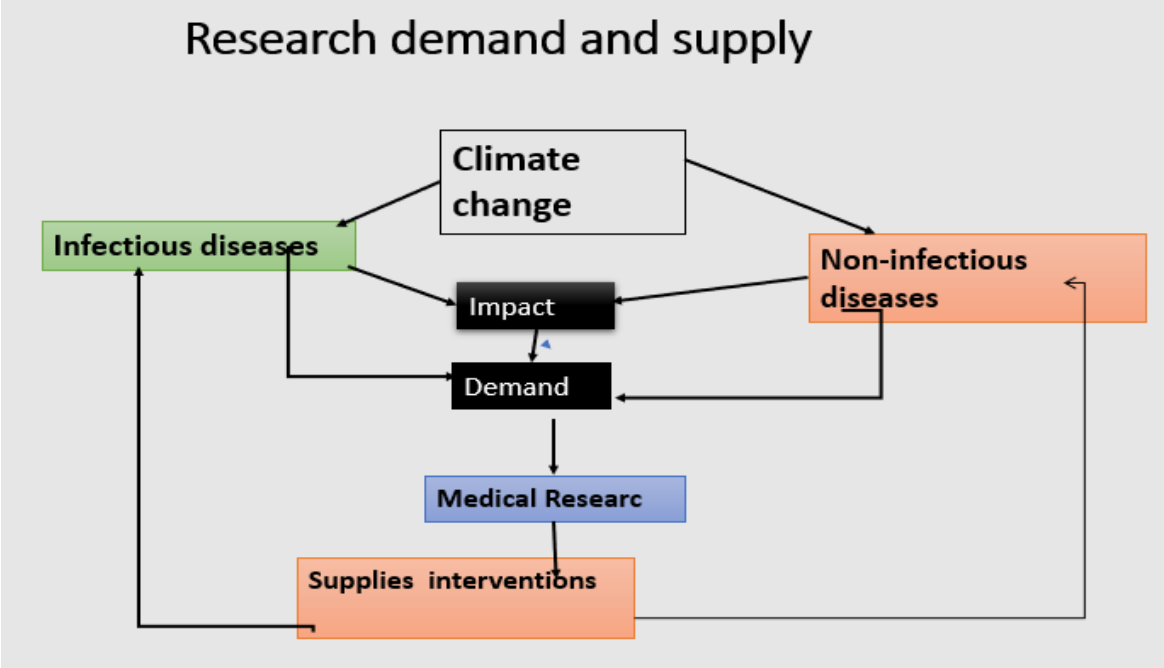
Institutional Vulnerability

Institutions are human organizations created or developed to address specific activities geared towards the production of goods and services for the common good of society. In many cases they have a legal mandate, stated goals, performance targets and

societal values. Their organizational structure and governance are geared to achieving these stated goals and performance targets. A Pressure-State-Response (PSR) framework is required to assess the performance of an institution given certain levels of demands or pressures 69 In order to provide an objective assessment of an institution a set of indicators are required. In the health sector there are several players that provide policy, knowledge, services, interventions, training and financial support. Linkages between these players and their institutions are critical to the performance of the sectors and in particular their response to pressures and demands such as would be caused by climate change. Pressure from climate change is characterized by uncertainties and increasing risks arising from the dynamic state of the climate. Such a state increases the institutions vulnerability to external and internal shocks and failure to meet the set targets of minimizing disease incidence and poor health.

A number of factors can affect the performance of an individual institution. These include governance, human capacity, capital, linkages and relationships with partners and end users. A failure in any of these attributes can reduce the capacity of an institution in meeting its performance targets and in particular under the pressure of climate change. For example can health institutions prevent instead of manage climate related health risks? Are the institutions proactive or reactive?

FIGURE 1: Research dema under and supply under climate stress



Stakeholder engagement and institutional vulnerability

There are a number stakeholders involved in adaptation to climate change and these include knowledge generating institutions, policy makers, implementers of policy and supporters of policy implementation (development partners). Policy may evolve from a number of events such as new or emerging knowledge, a political or development vision or a need to address a public need e.g. public health. These stakeholders represent a supply chain for the development of policy and their implementation. However in some cases there is a disconnect in the supply chain and some stakeholders are not aware of what the others are doing. For example a university may make a breakthrough in technology but the technology may remain in academia. In some other cases good technologies may be developed however in

the absence of donor support this technology may never see the light of the day. These types of disconnections among stakeholders represent substantial institutional vulnerability. In this respect institutions are not able to respond to their stated mandates due to failures or weakness in the adaptation process supply chain. For example while the malaria early epidemic prediction model 70 was published in 2001 its testing and validation did not take place until 2007 when funds become available from the International Development and Research Centre (IDRC)

funded Climate Change Adaptation in Africa Program (CCAA). Local research institutions were unable to carry out the validation despite the fact that the skills existed in the region.

In the next section, attributes that could increase institutional vulnerability are addressed.

Health Institutions

The role of governments in the health sector is to provide disease prevention, maternal and child healthcare, and curative services. The great majority of illnesses are caused by infectious diseases and which in many cases are climate sensitive. Climate change is increasing the risk of infectious diseases and epidemics associated with these diseases and there is a need to develop strategies for addressing these new threats. In addition there are emerging and re-emerging diseases that may be associated with climate and environmental change. Research institutions are expected to address the new health threats and develop preventative strategies and reduce the public health impacts. Weak public health institutions are highly vulnerable to the impacts of climate change as they may be unable to cope with large numbers of cases involved in disease outbreaks, epidemics and new populations are at risk. There is a need for the health institutions to address the threat of climate change on health in Africa. Many of these institutions are fragile and may not be able to cope with this emerging threat that is expected to increase.

Health institutions in Africa have the capacity to use climate information to identify climate associated risks particularly in infectious diseases and other direct impacts of extreme weather events. This has led to disaster prevention rather than disaster management. The health authorities have recognized the need for early warning systems in epidemic prevention but until recently these systems were not developed. Furthermore it is critical to use climate data and disease surveillance systems to identify populations at risk from diseases whose transmission range is increasing or where the environments are becoming receptive to transmission.

Research Institutions

In Africa medical research is carried out by national medical research institutes, universities non-governmental organizations and foreign institutions. Each of these institutions has its own mandates and research priorities which may or may not correspond to the national research priorities. Where they exist, the national research institutes have the mandate to address existing, emerging and re-emerging health concerns. However, many of these institutions are to a large extent dependent on external supports to carry out their research and in many cases research focus is dependent upon the priorities of the funding agency 71. In addition research may place priority on health conditions with the greatest burden and urgency such as Aquired Immuno-deficiency Syndrom (AIDS) / Human Immunodeficiency Virus (HIV), Corona Virus Disease (COVID-19) and Tuberculosis (TB)

Even in the absence of climate change Africa has a great disease burden driven by the tropical climate and low socioeconomic development. Research institutions are engaged with improving the control of diseases that have existed for millennia . While some countries have dedicated medical research institutes others do not have them and mainly rely on academic institutions to carry out health research. In other instances research is carried out by foreign entities that focus on very specific research programs.

Climate change increases the burden of infectious and non-infectious diseases. Some of the diseases such as viral infections require specialized laboratories to handle the often highly infectious pathogens. Very few of these facilities exist in Africa and quite often foreign assistance is sought in diagnostics of such infections. In additional there are few active surveillance programs to track the incidence of transmission in the inter-epidemic and enzootic periods.

Climate variability is largely responsible for large scale epidemics of malaria, Rift Valley Fever, chikungunya, dengue fever, cholera and meningitis. The capacity to accurately predict the risk of these epidemics is critical to epidemic prevention. Until recently climate based early warning systems were not developed and quite often health authorities were caught unaware, resulting in ineffective and late response and subsequently high morbidity and mortality. Recently

early malaria epidemic prediction models have been developed and have been shown to have high positive predictive values, sensitivity and specificity 7. In addition they have a lag of 2-4 months between the climate signals and the onset of the epidemic thus providing ample time for interventions and prevention. Similarly RVF epidemic can now be predicted with considerable accuracy using sea surface temperatures (S STs) in the Pacific and Indian Ocean. Research and development of climate based prediction models for meningitis is in progress.

Research institutions in Africa are often constrained by lack of funding, low capacity for research and retention of personnel. Climate change is putting pressure on health and there is need to undertake research projects that provide tools for climate risk management and indeed new strategies for immediate and long term disease control. For example swamp reclamation in many highlands regions has led to a significant increase in malaria transmission 72 by alteration of the microhabitat and microclimate. However, it has been shown that restoration of the swamps with Napier grass could reduce water temperature and reduce the breeding of malaria vectors by about 80% 73.

In some instances there is a weak link between health providers and research institutions. Yet health providers collect vital health data that can indicate trends in climate sensitive diseases. Lack of strong linkages between the research institutions and their data generating partners weakens their capacity for a proactive management of climate related health risks. Historically health management information systems (HMIS) in Africa have been very poor with the exception of South Africa. Reasons for this state of affairs have been the lack of "data culture" very low data recording and storage capacity, poor diagnoses and low prioritization of morbidity data. Priority has been placed on recording births and deaths (DFID Health Resource Centre Eldis). Since the mid 1990s there has been some improvement on data management following the introduction of computers to the HMIS. This has greatly contributed to allocation of resources and improved management of the health systems. Whereas this data is useful in tracking disease trends, quite often there is a question of data quality for research purposes as quality assurance may be absent. In times of climate change and rapidly changing disease landscapes there is a great need to access high quality health data in the development of predictive models and their validation. In addition such data can identify new trends and emerging or remerging infections and other health conditions. It is time to change from a leisssez-faire approach to a proactive one. Decisions on preventative interventions of diseases are dependent on reliable data on diseases trends and climate data that indicate trends in the risk of a diseases outbreak. Predictive models are required for routine use in climate risk management but as of now even where models are available the health systems have not made use of them.

Funding agencies (including development banks)

It is a well-established fact that funding agencies determine the priority funding areas among the many health research needs which may reflect global or regional health concerns. In many cases funding agencies will depend on health statistics indicating the incidence of disease in prioritizing areas that need research. Significant changes in disease incidence may call the attention of funding agencies to determine the cause of the changes. This process will lead to attribution of the drivers of the incidence. Climate change is a slow process that may not cause dramatic increase in diseases incidence. However, climate variability may cause alarming epidemics that warrant concern by the whole health system and this could lead to funding research that attempts to solve the puzzle. This has been the traditional approach to funding medical research. It is important to recognize that range expansion of infectious diseases can occur quite rapidly once certain climatic thresholds have been exceeded. For example in the Central Kenya highlands malaria transmission took place after the annual mean temperature permanently exceed the 18^{o}C threshold temperature for malaria transmission 74. This recent spread in malaria transmission has put an additional 4 million people at a risk. A similar process may be taking place in the expansion of the meningitis belt. Research institutions should be carrying out passive and active case surveillance to map out new areas that are at risk. In addition they should be monitoring changes in climate that will support the spread to disease. Failure to undertake these types of studies will leave populations exposed to risk of emerging and re-emerging infections. Thus research institutions that suffer from various forms of funding and technical and leadership shortfall will be vulnerable to climate change shocks.

IPCC reports have played a big role in showing the linkages between diseases and health conditions with climate change and variability. Nevertheless there has been dissent by a section of the scientific community, which has slowed down the response of funding agencies due to the uncertainty in attribution of the causes of epidemic such as malaria in the East African

highlands. For example it has been reported 75 that the widespread increase in resistance of the malaria parasite to drugs and the decrease in vector control activities may be more likely driving forces behind the malaria resurgence in the western Kenya highlands. However other researchers attributed the evolution of epidemic to climate change 76, 77, with drug resistance and lack of vector control acting as secondary amplifying factors.

In recent years, new climate change funding mechanism has facilitated research on the impact of climate change on diseases. For example the Assessment of Impacts and Adaptation to Climate Change (AIACC) project was an outcome of the IPCC third assessment report funded projects on the impacts of climate sensitive malaria and cholera in the Lake Victoria Basin in East Africa. Thereafter, the Climate Change Adaptation in Africa (CCAA) supported the development, validation and transfer of the climate based early malaria epidemic prediction models.

There are indications that traditional funding agencies and new funding mechanism are beginning to address the impacts of climate change on health. For example funds from Google were made available to carry out research on Rift Valley Fever. (RFV) in Kenya. In the process it was discovered that other arbovirus co-exist with the RVF namely chikungunya, West Nile Fever and dengue fever in eastern Africa. Following the initial manifestations of global climate change on human health, United States government is in response to expanding the focus of its research and development activities to include an increased emphasis on understanding, predicting, and responding to climate change impacts 78 The National Institutes of Health (NIH) in the United States are perhaps the biggest funding agency for health related research. Nevertheless, these funding opportunities are extremely competitive and few African researchers have the capacity to access this funding and this raises another point in the vulnerability of African health research institutions.

The International Development and Research Centre (IDRC) of Canada and the British Department of International Development (DFID) founded a special funding model for adaptation in Africa by making funds available to the Climate Change Adaptation in Africa (CCAA) a program that was fully managed by Africans and African institutions. This model encouraged African research institutions to collaborate in solving common adaptation problems in several climate sensitive sectors. This model is unique and seems to have provided Africans with opportunities to make critical research and management decisions adding a sense of ownership to the process of adaptation.

Within the development banks domain, the World Bank is examining how public finance can catalyse climate action by piloting innovative ways to leverage both climate and development finance, such as combining resources and instruments to maximize synergies, exploring new opportunities to expand the scope for market mechanisms, and strengthening the capacity to facilitate access to resources and their effective use 79. In addition the bank has in a small step started funding health pilot projects through the Global Environmental Facility. The African Development Bank recognizes that health problems in Africa are integral to other development problems 80 It is hoped that the banks will in the near future invest in building capacity in climate related research in health.

United Nations Institutions, World Meteorological Organization (WMO), Intergevernmental Panel on Climate change (IPCC), World Health Organization (WHO), United Nations Environmental Program (UNEP) and United Nations Children Fund (UNICEF).

The United Nations (UN) agencies have been very instrumental in handling matters of climate change and building international consensus on the impacts of climate change and health.

The World Meteorological Organizations has the role of providing weather services and preventing weather related disasters. Moreover WMO has a leading role in monitoring global climate change and variability.

The IPCC reports have identified where there are gaps in knowledge that requires inputs from research. For example the Assessment of Impacts and Adaptation to Climate Change in Africa (AIACC) project was a spin off from the Third Assessment Report. The World Health Organization acknowledged that climate change will, particularly in Africa, have a negative impact on vulnerable populations. According to WHO areas with weak health infrastructure – mostly in developing countries – will be the least able to cope without assistance to prepare and respond 81. Many African countries- mainly in the sub-Saharan region fall in this category. Again this raises the institutional vulnerability of African health infrastructure on handling climate change impacts.

The United Nations Environmental Program (UNEP) has an important role in monitoring environmental

changes including climate change and proposing interventions. In particular UNEP pays special attention to vulnerable ecosystem that will be affected by climate change.

According to UNICEF 2007 an estimated 9.2 million children worldwide under the age of five died from largely preventable causes among these malaria, diarrhoea and malnutrition, all climate sensitive diseases. A significant proportion of these children came from Africa. Weak and fragile health institutions in Africa will not be in a position to reverse this trend. Climate change will increase demand from these institutions.

Non-Governmental Organizations (Local and International)

Non-governmental organizations play a critical role in research and development in Africa. They play an important role on the development and deployment of adaptation strategies and in accessing research funding in a very competitive environment. Among the major players in health is the International Centre for Insect Physiology and Ecology (ICIPE) which has a focus on crop, animal, human and environmental health. The International Livestock Research Institute (ILRI) has a focus on animal health and has projects in several countries in Africa. Its research affects peoples livelihoods particularly pastoralists living in fragile ecosystems and who are very vulnerable to climate change and variability.

While the former are examples of international NGOs there are myriads of local NGO that are deeply embedded in communities across Africa and they play an important role in embedding adaptation strategies in rural and urban communities in Africa. Many of the local NGO are involved in distribution of insecticide impregnated bed nets for malaria control while others are involved in the provision of safe drinking water. Thus both international and local NGOs have an important role in the development of adaptation strategies and their deployment.

Universities

Universities play a dual role in training and research. It is the duty of universities to address the impacts of climate change and develop adaptation strategies. African universities must engage in research that can be translated into adaptation options. In the past universities have been referred to as "ivory towers" that were more concerned with academia rather than addressing practical solutions in society. Many of the African experts on climate change are based in national universities and being the most knowledgeable members of society they must lead in finding solution to adaptation in health and other climate sensitive sectors.

In recent times special funds have become available to climate change and higher education 82.

Governments

Health services are provided by governments and the private sectors but with the governments providing most of the services to the majority of the population. Planning of the capital and recurrent expenditure while dependent on the availability of funds must take into account trends in health care demands. The health of African populations is expected to improve as the economies improve. However climate change may reverse some of the gains made in population health. It should be noted that the health sector talks of interventions instead of adaptation. These could be short and long term interventions.

Governments have several mechanism and options in facilitating interventions. For example they are responsible for policy development and financing and coordinating intervention programs. Furthermore government can access special international funds for adaptation projects that aim at developing strategies to reduce or avoid the impacts of climate change. Governments are signatories to international agreements such as the Millennium Development Goals and which have a direct link to adaptation to climate change.

The ministries of health are responsible for interventions such as national malaria control programs and disease control by for example vaccination. Primary health programs in the ministries of health aim to prevent disease at the community levels through public health education, vaccination and enforcement of sanitation. These measures can for example significantly reduce the risk of cholera and other climate sensitive infectious diseases. The level of investment in the health sector will determine the degree of vulnerability of the populations to the impacts of climate change.

Governance, research and capacity building

Leadership

Leaders drive institutions to success or to peril. Leadership should be earned and not conferred. There are a number of leadership styles but three of them will suffice. The first type is transformational

leadership that leads the institution to achieve its goals and missions by a positive transformation of the teams. The leader may completely change the attitude of the teams through a number of techniques and strategies. A second form of leadership is the transactional type where performers are rewarded and non-performers chided. The third type of leadership is the laissez-faire behaviour where a leader does not perform, that is, leadership absence. The leader abdicates responsibility, delays decisions, gives no feedback, and makes little or no effort to satisfy followers' needs 83.

Some leaders practice a mix of these forms and this could lead to uncertainties in the teams who may be unable to deal with the unpredictability of the leader. Institutions have a chain of leadership hierarchy whose dominant behaviour may draw its strength and direction from the prime leader.

In a research institution skills in project management are key to the success of the institution. The principle investigator must have the ability to write competitive grants, attract funding operationalize the grant and publish the results. This calls for intellectual and physical stamina that is not common. Obtaining such skills takes many years of practice and usually under a good mentor. Project management cannot practice a Laissez-faire behaviour but will need to practice a transformational and a transactional style.

To remain competitive institutions must have a high productivity of research results that are usually assessed through publications. The productivity of an individual can be determined though the internet based publication records. Even more important the general interest in ones work can be gleaned on the internet.

The prime leadership of an institution should exhibit unquestionable credibility given that research institutions received substantial amounts of funds which need to be accounted for. A high moral and ethical standard is required. Funding agency take a keen interest in the credibility of the institution and any weakness in this area could lead to high vulnerability to missed opportunities.

Visibility of an institution is of particular importance to its clients, that being the general public end-users and funding agencies. Highly successful but quiet institutions have received lesser attention than institutions that are more visible. Institutions must be seen to be proud of their achievements and they must never be seen to doubt their own products. They must embrace competition and its allied strategies.

Finally institution must remain highly relevant to their prime beneficiaries and these being the general public. There are instances where researchers have become the prime beneficiaries of a research institutions and this can lead to withdrawal of support by funding agencies.

Internal and external power and politics

In the functioning of institutions certain individuals or offices are mandated to perform defined roles and to execute decisions. These roles may include policy formulation, service provision and technical support. Appointments to these roles are dependent upon legal requirements, technical competence financial leverage and political affiliation. Much conflict can occur in the appointments to certain positions. For example an office requiring high technical competence may be occupied by a political appointee. Likewise the power hierarchy may be distorted by political affiliations. Offices with legal mandates may use this leverage to down-regulate technically competent subordinates and subjugate their role in the organization by denying them the means, for example financial support to perform their legitimate roles.

Organizations have internal and external power players that influence the decision making process such as policy formulation and implementation. The internal players include bureaucrats and technocrats while external players include policy advisors and even external technical advisors. Self-interest may dictate the decision making process and may also result in a power struggle within the institution and its partners. This situation can lead to poor implementation of policy, misdirection of resources and non-performance.

Such a scenario would cause the institution to become highly susceptible to failure under the pressures of climate change. For example superficially induced uncertainty about the cause of malaria epidemic can delay an early intervention. For example there are numerous assertions in literature that climate variability is not the key driver of malaria epidemics and by extension climate data cannot be used for early prediction of the epidemics. Such a scenario causes the responsible institutions to be highly vulnerable to inadequate or no responses to the health disasters. Likewise financial considerations may lead to the application of inappropriate interventions which leads to a loss of public confidence in the health institutions.

Key health intervention decisions are based on data. In particular climate and epidemiological data are very

important when dealing with climate change risk management. Data can become a powerful political tool that can be used for institutional or personal gains. An institution that is dependent on external data sources is highly vulnerable to underperformance and low decision making capacity.

Infrastructure and resource sharing

Increased research and development in adaptation will demand an increase in research infrastructure which includes laboratories, office space, equipment and transport. In some cases there will be need to share the existing research facilities due to the interdisciplinary nature of response to climate change impacts and adaptation. For example regional remote sensing facilities have greater application in disease mapping. Ground mapping using Geographic Information System (GIS) and Global Positioning System (GPS) will be used to map disease risks and populations at risk. Spatial modelling of disease risk will relay on these technologies. While in the past there was very little interaction between medical scientists and the mapping community this will have to change in the future. In addition health and meteorologist will need to work together in climate data collection and analysis. The health community will be called upon to take a greater role in the meteorological data collection using electronic devices and automatic meteorological station.

In general investments in infrastructure will be required but also a greater partnership with existing specialized facilities. There will be a need for collaboration between local and regional institutions to make research cost effective.

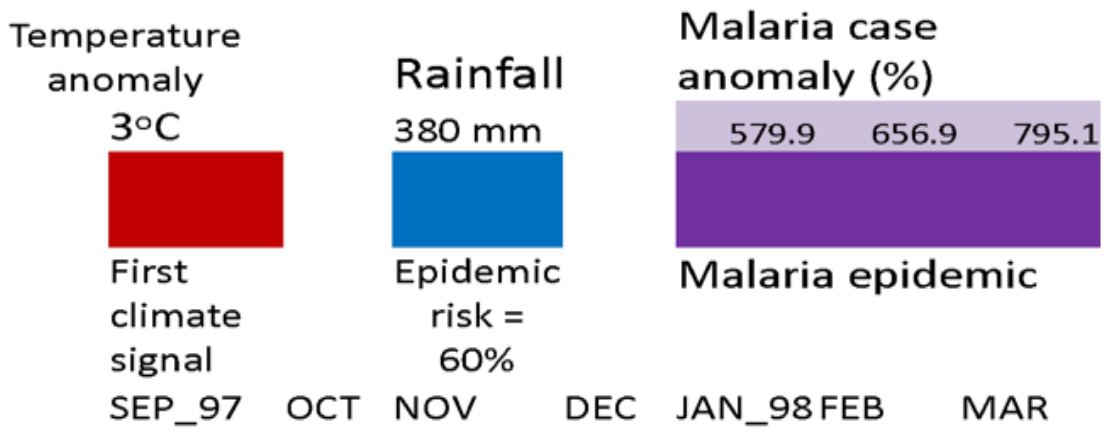
Some key areas that will need research include, diagnostic tools, disease surveillance, disease risk mapping, diseases range expansion and contraction (meningitis, malaria dengue etc.), active and passive diseases surveillance in vulnerable ecosystems and epidemic risk potential.

Model development

Models are critical tools for risk assessment as they can extrapolate temporal and spatial risks thus providing sufficient time for response. Three types of models are relevant to health. Many infectious diseases such as malaria, cholera, meningitis and Rift Valley Fever are climate sensitive prone to large scale epidemics that can affect significant proportions of human populations. In many cases morbidity and mortality can increase several-fold. The rate of transmission in some cases has an exponential trend which makes interventions very difficult and the disease spreads rapidly. Progress has been made in the use of climate information in identifying meteorological threshold that represent epidemic risks. This type of information can be used to develop early warning systems (EWS) with high sensitivity, specificity and positive predictive power. In the case of malaria the climate based early epidemic prediction model has a lead time of 2-4 months thus providing ample time and opportunity to intervene and prevent an

epidemic.

FIGURE 1: Evolution and prediction of malaria epidemic



Previously epidemics could only be detected when they were in progress and they could not be prevented. Models for other diseases such as cholera, Rift Valley Fever and meningitis have not been well developed.

Infectious diseases are spreading from their endemic areas to new areas due to climate change. Malaria in particular is spreading to highlands areas where it did not exist before. In Kenya the disease has spread from low altitudes to higher altitudes in central Kenya highlands putting about 4 million people at risk 9. Spatial models need to be developed to identify areas that risk of disease spread and also the rate of spread.

Adaptation in the agriculture and water sectors will involve irrigation and construction of dams and water reservoirs. This will have an impact on water related diseases such as malaria and schistosomiasis. Models need to be developed to assess the risk of establishment of these diseases in new areas.

The risk of disease transmission intensity in space is not uniform and it has been shown to vary depending on some geographic parameters such as topography and hydrology. In some cases transmission has been shown to be clustered while in other cases it is random. Such characteristics have implications for intervention strategies. Spatial modelling can make major contributions to guide intervention strategies 84.

Building partnerships

Climate change will call for building new institutional and sectorial partnerships to bring in effective adaptations that are cost effective. The traditional sectorial funding will need to change. For example there is very little interaction between the irrigation sector and the health sector or between the water and the health sector. Irrigation can be a major source of vector borne diseases such as malaria. House modification by medical scientists in a rice irrigation scheme in western Kenya has been shown to reduce the risk of mosquito bites by 80% 85. Water conservation strategies using micro-dams in Ethiopia caused a significant increase in malaria and schistosomiasis. These examples demonstrate the need for different sectors to collaborate so as to avoid mal-adaptation.

Knowledge sharing across regions can be very cost effective. Adaptation strategies obtained from one country may be applicable to other countries in the region. Currently there is little active knowledge sharing arrangements across the regions. It is noteworthy that while there are regional economic blocks such as Common Market for Eastern and Southern Africs (COMESA), Economic Community for West African States (ECOWAS), Southern Africa Development Community (SADAC) there are no common climate adaptation platforms where knowledge can be shared. It is time that regional blocks with similar adaptation challenges should initiate collaboration for more cost effective development of adaptation strategies.

Likewise it is desirable to develop international networks that can mobilize funding for adaptation research and development. Such networks can also marshal the necessary skills that may not be available in a region. Each partner in the network will bring in unique strengths that will synergize the network.

Mobilization of funding

Research and development requires sustained funding. This is an investment to avoid future damage by the impacts of climate change. African institutions engaged in adaptation in climate change must develop sustainable strategies to cope with a rapidly changing environmental that will largely have a negative impact on human health and wellbeing. The “wait and see”, laissez-affaire attitude and “business as usual” approach to adaptation will be ineffective against the forces of climate change. Adaptation to climate change will have to be integrated into development programs and the institution will be required to prioritize their development strategies while taking into account the need to reduce the impacts of climate change.

In many cases national governments require the support of external development partners for budgetary support. In order to determine areas of priority that needs support data is required to provide evidence that sectors are being impacted by climate change and that these impacts need to be addressed and reduced. Research institutions will therefore have to collect the relevant data to show the impacts. It is thus critical that links are developed between research institutions and development partners. In the past research institutions have had very little contact with development partners. Research institutions will need to address transitional research whereby research results are translated directly into utilities relevant to climate change adaptation.

Adaptation will also demand that more basic research is carried out in order to understand more complex phenomenon that affect human health. For example

how will extreme heat affect learning in African schools where there is no chance of air conditioning? How will malnutrition increase vulnerability to infectious and non-infectious diseases in semiarid areas? Climate change will be associated with complex health impacts that need to be addressed. This area of research is mainly funded by specialized agencies through competitive grants that need highly trained scientists with strong track records. These types of grants require a network of national and foreign institutions that can pool their human and capital resources. National research institutions are encouraged to explore ways of forming partnerships of this kind.

In most African countries there is very little support in terms of research grants from national governments. Even where grants are available the impacts of these grants is not visible. It may be a good idea to channel such funds into a network of junior and more experienced senior scientists in addressing an issue that is relevant to climate change impact in health. For example can environmental intervention reduce malaria 73 in the east African highlands? Non chemical interventions have been developed but they need to be tested and deployed. These are low budget projects that national governments can afford to support. Several private foundations have resources that they could invest in finding solutions to climate change adaptation in Africa, such as support for higher education with reference to climate change. Private foundations in the US invested $436 million to address climate change in 2007 86. Foundation giving for climate change has increased nearly 5-fold domestically since 1997 and nearly 8-fold internationally. African research institutions will need to develop strategies for accessing such funding through various mechanisms.

Training

Climate is a life supporting system that is fundamental to existence. Training and education must take cognizance of climate changes in order to increase skills required for the reduction of vulnerability to impacts from meteorological hazards. Training can occur at primary, secondary and tertiary levels and perhaps even more important at the community level. While training can increase the skill for adaptation, education will increase the public awareness on the hazards, vulnerability and adaptation. In addition various steps in mitigation will have to be undertaken to reduce the drivers of climate change. Failure to increase training and education programs will leave the human populations in a state of vulnerability 87.

Furthermore there is need to increase skills in adaptive capacity in all sectors. It is expected that demand for adaptation skills will increase and these skills could result in migration of highly skilled manpower. There is therefore need to retain these skills in the countries of origin. Incentives must be made available to encourage research into adaptation strategies and technologies. Low human resource capacity increases the vulnerability of the institutional functions to adaptation to climate change.

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EAHRC
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META ANALYSIS ARTICLE

A Review on *Serenoa serrulata*: A Potential Medicinal Plant for Prostatic Diseases

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ABSTRACT

Background: Prostatic diseases which include prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer are the benign or malignant disorders that affect the prostate. Phytotherapies have been adopted as the alternative treatment/ management option especially for BPH since the current modern methods of treatment presents a lot of adverse effects.

Methodology: The literature was searched using different databases including Medline/PubMed, Cochrane library, Scopus, Proquest library, Embase, EBooks and Google Scholar for relevant records for a period from 1988 to 2018 to identify all the published articles of *S. serrulata* regarding treatment of prostatic diseases. The key search terms were *Serenoa serrulata,* S. repens, Saw palmetto, Prostate cancer treatment with *Serenoa serrulata,* treatment of Benign Prostatic Hyperplasia with *Serenoa serrulata,* phytochemicals of *Serenoa serrulata,* ethnobotanical uses of *Serenoa serrulata,* toxicity of *Serenoa serrulata,* pharmacological activities of *Serenoa serrulata* and also traditional management and treatment of prostatic diseases using *Serenoa serrulata* and also clinical trials on treatment of prostatic diseases with *Serenoa serrulata.* The retrieved articles were reviewed, synthesized and analyzed qualitatively. The reference list of the retrieved articles was also reviewed and synthesized. The original research articles which reported an investigation of *S. serrulata* of any study design, original published research articles, any time of publication and grey literature (conference papers, reported articles, academic thesis) were included. The articles whose full texts were not freely available by the time of search and those without clear information about methodology and study design were excluded.

Results: This review reported that *Serenoa serrulata* belonging to the *Arecaceae* family commonly known as saw palmetto is used traditionally for treating prostatic disease conditions and other infertility conditions in both men and women. Phytochemical screening of hexanic and ethanolic extracts of *S. serrulata* comprised of free fatty acids and phytosterols which together contribute to their antiprostatic activities. These extracts of *S. serrulata* exhibited antiandrogenic, anti-inflammatory and anti-proliferative activities through inhibition of both isoenzymes 5**α**- reductase and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors. This is a similar mechanism exhibited by finasteride and Tamsulosin both antiprostatic conventional drugs though the plant phytochemicals do not interfere with PSA secretion. *S. serrulata* has also been reported to be non-toxic in both non-clinical and clinical trial studies. The medicinal plants reported by this review to be used in combination include; stinging nettle (*Urtica dioca*), *Zingiber officinalis, Echinacea angustifolia* and pumpkin (*Cucurbita pepo*). The antiprostatic conventional drugs reported include Finasteride and Tamsulosin.

Conclusion and Recommendation: The results showed that *S. serrulata* is effective in treating prostatic diseases. The potency and safety are improved when used in combination with *Urtica dioca, Cucurbita pepo, Zingiber officinalis* and *Echinacea angustifolia* as compared with anti-prostatic conventional drugs Finasteride and Tamsulosin alone. The plant combination has also been shown to have improvement in the quality of life and as well enhancing the synergy of Finasteride and Tamsulon and

their adverse effects. Effective medicinal plant combinations should be formulated into products and integrated into the usual treatment for prostatic diseases.

Key words: Prostatic diseases, Prostate Cancer, Benign Prostatic Hyperplasia, prostate tumors, Phytotherapy, anti-prostatic drugs, *Serenoa serrulata*, ethnobotanical, phytochemical, toxicity and pharmacological.

INTRODUCTION

Prostatic diseases are the benign or malignant disorders affecting the prostate. Common examples of prostatic diseases include Prostatitis, Benign Prostatic Hyperplasia (BPH) and Prostate cancer.[1] Prostatitis is an inflammatory disease mainly caused by infections or related health conditions affecting mainly younger and middle aged men characterised by symptoms of pain and discomfort around the anus, scrotum and the area in between.[1] This mainly occurs in men younger than 35 years. Benign Prostatic Hyperplasia (BPH), a non-cancerous growth of the prostate is so common in older men over the age of 60 years causing an enlargement of the prostate. Statistics show that 92% of men from the age of 31 to 40 years do not have symptoms of BPH and this increases with age to only 10% not showing symptoms of BPH from the age of 60 years and above.[2,3] This non-cancerous growth is mainly caused by proliferation of the stroma and epithelia cells of the prostate. However, it is a slow progressing and non-cancerous condition leading to an impediment of the urethra causing difficulty in urination. The treatment goal of BPH has always been to relieve irritative (urgency, frequency and nocturia) and obstructive (weak stream, hesitancy, intermittency and incomplete emptying) symptoms.[4] Prostate Cancer (PC), a slow growing cancer that occurs in the prostate is the second most common malignancy in adult male over the age of 65 worldwide.[4]. This slow growing cancer accounts for 7.1% of all cancers in men with about 1,276,106 new cases annually.[5,6] Though with variations in the statistics among countries and continents.[6] This is a fatal disease which grows slowly and do not spread beyond the prostate, however some are aggressive and spread quickly to other areas of the body.[1] Prostate cancer and Benign Prostatic Hyperplasia whose pathogenesis and progression are due to inflammation are chronic prostatic diseases with a long period of development and progression. The factors minimising cell apoptosis and stimulating proliferation create an imbalance existing
effects. The prostatic diseases could also co-exist with other health conditions, commonly referred to as comorbid.[2] Examples of comorbid diseases include diabetes, high blood pressure, cancer etc. Combinatory therapies also could indirectly have effect on the between prostate cell growth and apoptosis.[7] Prostate cancer and Benign Prostatic Hyperplasia are also found to form in different areas of the prostate with only 20% coexisting in the same zone.8 There is no clear molecular and genetic relationship between PC and BPH and they present 2 distinctive pathogenic pathways. Neither PC nor BPH is a single disease; both are hormonal dependent.[9] However, both PC and BPH if left untreated develop disease progression since they are progressive diseases.

The current method of treatment for both PC and BPH include modification in lifestyle especially avoiding consumption of highly protein animal related diet, smoking cigarettes and drinking alcohol; device and surgical therapies; pharmaceutical and phytotherapeutic therapies.[4,10, 11] These methods of treatment have a lot of adverse effects, however, some prove to be less effective. Phytotherapeutic agents have been most commonly used because of their proven potency, availability, cheap and present few or no adverse effects.

Serenoa serrulata (Hook. F.) Michx (syn. S repens [Bartram]) (*Arecaceae*) is one of the most common phytotherapies used for treatment or management of prostatic diseases. The safety and efficacy of the extracts of *S. serrulata* have been proven both in vitrally and in vivally. This highly rich anti-prostatic plant possesses an inhibitory effect of the 5-α reductase enzyme, anti-androgenic and estrogenic effect.[12] However, the anti-prostatic activity of *S. serrulata* have been proven with mixed reports with some manuscripts reporting that its extracts have no significant therapeutic effects even at high dose.[13]

The used of combination therapies are now days on the rise. Combination therapies are commonly preferred to aid in the reduction of the amount of dose of the drugs used, improving on the therapeutic effect of drugs by improving on the activity of the other, minimising the risk of adverse effects as well as provide synergistic

comorbid conditions thus supporting their potency.[2] The most commonly used combination therapies are between phytotherapies and modern therapy.

Medicinal plants are used in third world countries for management or treatment of various health conditions including cancer because of their availability or being cheap. Some of these medicinal plants have shown their potency either singly or in combination. *S. serrulata* is one of those medicinal plants highly used for prostatic diseases either singly or in combination with other medicinal plants or even with anti-prostatic conventional drugs. However, there are few reports especially on their combinatory use. Therefore, this review synthesised information on *S. serrulata's* botany, phytochemistry, pharmacology as well as it's used in combination with other medicinal plants and anti-prostatic conventional drugs either clinically or non-clinically.

METHODS

Literature Review

The literature was searched from different databases including Medline/PubMed, Cochrane library, Scopus, Proquest library, Embase, EBooks and Google Scholar for relevant records for a period ranging from 1988 to 2021 to identify all published articles on *S. serrulata* regarding treatment of prostatic diseases. The key search terms were *Serenoa serrulata*, *S. repens*, *Saw palmetto*, Prostate cancer treatment with *Serenoa serrulata*, treatment of Benign Prostatic Hyperplasia with *Serenoa serrulata*, phytochemicals of *Serenoa serrulata*, ethno botanical uses of *Serenoa serrulata*, toxicity of *Serenoa serrulata*, pharmacological activities of *Serenoa serrulata*, traditional management and treatment of prostatic diseases using *Serenoa serrulata* and also clinical trials on treatment of prostatic diseases with *Serenoa serrulata*. The retrieved articles were reviewed, synthesised and then quantitatively analysed. The reference list of the retrieved articles was also reviewed and synthesised. The original research articles which reported an investigation of *S. serrulata* of any study design, original published research articles, any time of publication and grey literature (conference papers, reported articles, academic thesis) were included. The articles whose full text were not freely available by the time of search and those without clear information about methodology and study were excluded.

RESULTS

A total of 220 articles were obtained from multiple databases. After a thorough review, a total of 80 articles were excluded because they were not related to the subject matter, 40 articles did not have freely available full text, 35 articles did not have clear research design and methodology and thus 65 were reviewed for this article.

Botany and Description of Serenoa Serrulata.

Serenoa serrulata (Hook.F.) Michx (syn. S repens [Bartram]) (*Arecaceae*) commonly known as *saw palmetto*, *sabal*, America dwarf palm tree, cabbage palm, fan palm, scrub palm is a rhizomatous plant with fan-shaped leaves and fragrant creamy flowers bearing ovoid blue-black fruits. This medicinal plant is native to South-eastern United States and grows between 3 and 6 feet in height, reaching up to 15 feet. This highly rich medicinal plant grows in sandy soil, producing fruits throughout summer.[9] The fruit is bluish-black when fully ripe with a distinct sweet aroma, peculiar with a taste that is slightly soapy and acrid.[9] The classification of *S. serrulata* is shown in ***Table 1***.

TABLE 1: Classification of *Serenoa serrulata* (Hook. F.) Michx.

Family;	***Arecaceae***
Genus;	*Serenoa*
Species;	*S. serrulata* (Michx) G. Nicholson
Scientific name (s);	*Serenoa serrulata* (Michx) G. Nicholson
Synonym (s);	*Brahea serrulata*, *Chamaerops serrulata* Michx, *Corypha repens*, *Sabal serrulata*, *Sabal serrulatum*, *Serenoa repens* (W. Bartram) Small, *Serenoa serrulata* (Michx) G. Nicholson
Common name (s);	American dwarf palm tree, Cabbage palm, Dwarf palmetto, Fan palm, *Fructus Serenoae Repentis*, *Sabal fructus*, *Sabal palm*, *Saw palmetto*, *Saw palmetto berry*, *Scrub palm*, *Scrub palmetto*, *Serenoa*

Habit;	Shrub tree
Habitat;	Flatwoods, Scrub, Swamps, acidic to alkaline sandy soil
Propagation;	Seeds

Ethnobotanical uses of S. serrulata.

S. serrulata is used traditionally in several forms as medicine for many ailments, mostly for treating benign enlargement of the prostate.[12] The use of *S. serrulata* can be traced back to the 18th century when it was first introduced into the Western medicine practice for prostate related health conditions and other urologic conditions.[12] It's other uses include; treatment of enlarged prostate, cystitis, gonorrhoea and irritation of the mucous membranes.[12] Apart from its medicinal uses, some communities use the fruits of *S. serrulata* as food for nourishment.[14] The fruits of *S. serrulata* provide it's medicinal properties. These fruits can be used as herbal tea to treat benign enlargement of the prostate, frequent urinary tract infections, enhancing hair growth, reducing cancer cell growth, boosting sexual drive and sperm production in men as well as reducing on the frequent or excessive night urination caused by the inflammation of the bladder or prostate.[15] It's fruits are also commonly used traditionally as treatment for infertility and underdeveloped breasts in women, increasing lactation and mitigating painful menstruation cycles.[9,12]

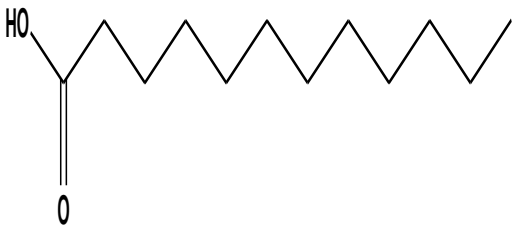
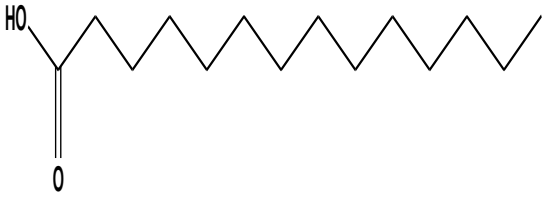
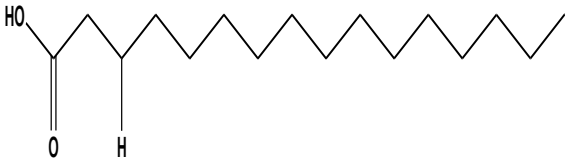
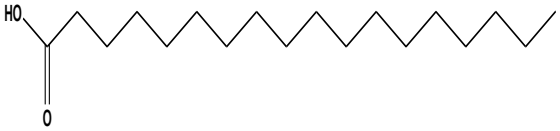
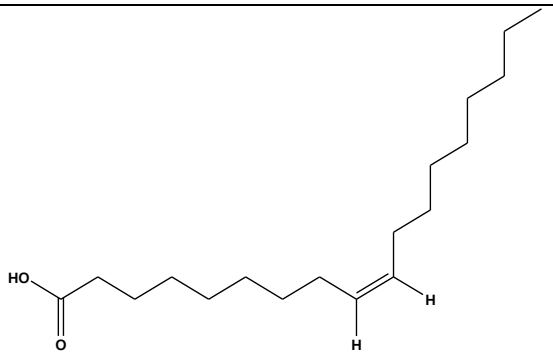
Phytochemicals of Serenoa Serrulata and their activities against Prostatic Diseases

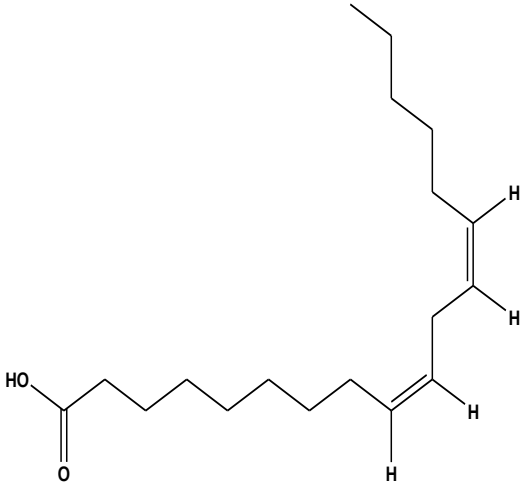
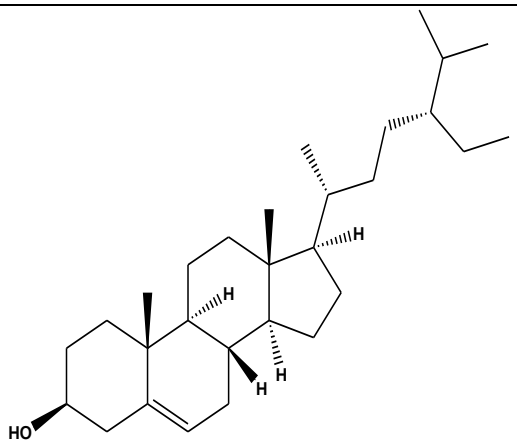
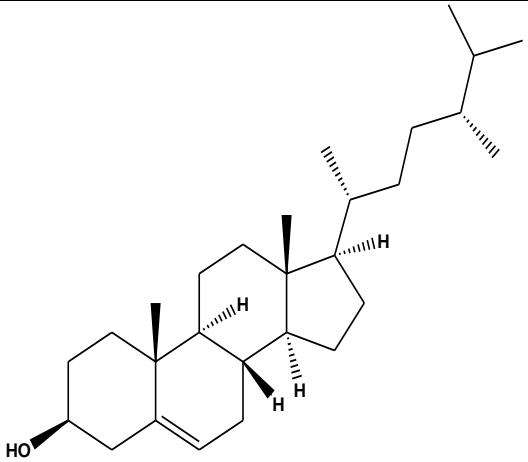
Phytochemicals are biologically active compounds or substances that are produced by plants, the phytochemicals give the plants their therapeutic activities. These chemicals are mainly extracted from the plant material by different solvents depending on the nature of their polarity. Examples of active compounds in the plant extracts include Alkaloids, Fatty acids, Sterols, Steroids, Coumarins, Flavonoids, Anthocyanin, Anthracenoside etc. These phytochemicals have been reported to have anticancer, anti-malarial, anti-bacterial, anti-fungal activities etc. and most of the pharmaceutical drugs in use are derived directly or indirectly from these compounds.

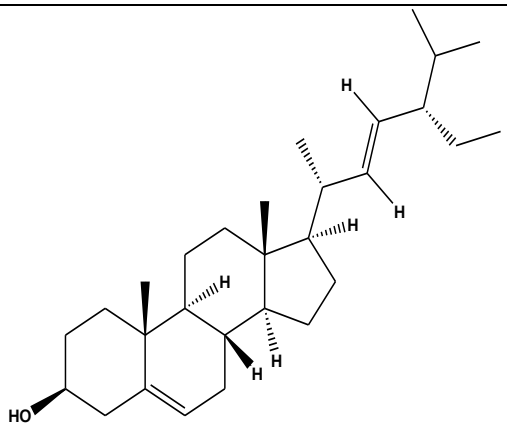
S. serrulata is one of the medicinal plants whose berries have been reported to be rich in a number of phytochemicals as shown in ***Table 2***. Phytochemical screening of *S. serrulata* have shown presence of fatty acids, phytosterols and other bioactive components which together contribute to the pharmacological activities of this medicinal plant.[9,16] The most commonly screened extracts of *Serenoa serrulata* (Hook. F.) Michx are the hexanic, ethanolic and the supercritical fluid carbondioxide.[9,16] Phytochemical screening of *S. serrulata* (Hook. F.) Michx showed that it contains high amounts of free saturated and unsaturated short chained fatty acids and their esters; phytosterols, triglycerides, aliphatic alcohols and various polyprenic acids.[9,16] The potent phytochemicals of *Serenoa serrulata* (Hook. F.) Michx, are best extracted with ethanol 90%, hexane and supercritical carbondioxide.[9] The fatty acids from *S. serrulata* include Lauric acid, oleic acid, Myristic acid and palmitic acid; of which Lauric and oleic acid are the majority and the phytosterols include β-sitosterol, campesterol, stigmasterol and cycloartenol; of which β-sitosterol has the highest content.[9] The fatty acids and phytosterols are collectively responsible for the reduction of the amount of dihydrotestosterone (an active form of testosterone), by blocking conversion of testosterone to dihydrotestosterone and inhibiting the actions of inflammatory substance by suppressing the production of prostaglandins resulting into the prevention of the swelling of the prostate, thus playing an important role in the management and treatment of prostate diseases.[13,17,18] *S. serrulata* is postulated to work by reducing androgenic activity through inhibition of 5-α reductase I & II and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors. *S. serrulata* also has anti-inflammatory activity, anti-proliferative activity and also binds to the receptors existing in the lower urinary tract.[19,20] The fatty acids are known for inhibiting 5-α reductase only while the phytosterols inhibit 5-α reductase, reduces
prostate tumour growth and ameliorate BPH symptoms however, none of the phytochemicals of *S. serrulata* is effective alone. [13,17,21,22]

Interestingly, some conventional anti-prostatic drugs also act by inhibiting conversion of testosterone to dihydrotestosterone through inhibition of 5-alpha reductase but some do not inhibit both type 1 and 2 isoenzymes of 5-alpha reductase.[23] A major undoing is that alpha blockers and 5-alpha reductase inhibitors are associated with major adverse effects like retrograde ejaculation and erectile dysfunction.[24, 25] (***Table 2***).

TABLE 2: Phytochemicals of *S.serrulata* and their activities.

Bioactive component	Group	Structure	Activity	Reference
Laurate (Lauric acid).	saturated medium-chain fatty acid.		-inhibition of isoenzymes (5α-reductase 1 and 2). -inhibition of prostate enlargement. -reduction in prostate weight. -decreases inflammation of the prostate.	[28, 49, 50]
Myristate (myristic acid)	saturated medium-chain fatty acid.		-inhibition of prostate enlargement. -reduction in prostate weight. -decreases inflammation of the prostate.	[13, 49,50]
Palmitate (palmitic acid).	fatty acid		-decreases inflammation of the prostate.	[13]
Stearate (stearic acid).	fatty acid		-decreases inflammation of the prostate.	[13]
Oleate (oleic acid).	fatty acid		-inhibition of 5α- reductase 1. -decreases inflammation of the prostate.	[13,28, 51, 52]

Linoleate (linoleic acid).	fatty acid		-inhibition of isoenzymes (5α-reductase 1 and 2). -decreases inflammation of the prostate.	13, 28, 51, 52,
Beta-Sitosterol.	Phytosterol		-inhibit 5α-reductase. -inhibit prostate cancer cell/growth, BPH symptoms.	9,53,54,55,56
Campesterol	Phytosterol		-inhibit 5α-reductase. -inhibit prostate cancer cell/growth, BPH symptoms.	18,53,54,55,56

Stigmasterol	Phytosterol		-inhibit 5α-reductase. -inhibit prostate cancer cell/growth, BPH symptoms.	[18,53,55,56,57]

Pharmacological activity of *Serenoa serrulata* (Hook. F.) Michx. *Serenoa serrulata* (Hook. F.) Michx have been reported to elicit its anti-prostatic effects through anti-androgenic, anti-inflammatory and pro-apoptotic, anti-edematous and anti-cancer activities.[19,20,26] These activities are attributed to the presence of the free fatty acids and the phytosterols.[9,16,27]

A study by Bayne et al., reported that phytosterols of *S. serrulata* inhibit both forms of 5-alpha reductase type 1 and 2 iso-enzymes[19] resulting in an anti-androgenic effect. Most of the synthetic drugs for prostate cancer like Finasteride and Furosteride inhibit only type 2 isoforms.[28] Finasteride is only a competitive inhibitor of type 2 5-alpha reductase[26] while *S. serrulata* (Hook. F.) Michx, inhibits both forms of 5-alpha reductase (1&2) and as well ensures greater control of the activity of the enzyme in the gland.[19] Dutasteride, a synthetic drug is an inhibitor of both type 1 & 2 5-alpha reductase[28] just like the phytosterol of *S. serrulata* (Hook.F.) Michx. However, S. serrulata (Hook. F.) Michx, does not only inhibit type 1& 2 5-alpha reductase, an ant androgenic effect2[6] but also inhibits the binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors. *S. serrulata* (Hook. F.) Michx, does not interfere with the cellular capacity of the prostate to secrete Prostate Specific Antigen (PSA) in vitro and in vivo[19]. This offers far much better therapeutic advantage over the other conventional 5 alpha reductase inhibitors since continuous screening and monitoring of the tumour progression in prostate cancer can be carried out through continuous measurement of PSA levels.

An in vivo study conducted using wistar rats in 2000 found out that the phytosterols of *S. serrulata* inhibits both androgenic and prolactin in lateral prostate hyperplasia.[29] The inhibition of prolactin and growth factor induces cell proliferation. A double blind placebo-controlled clinical study conducted using *S. serrulata* was also found to greatly lower the oestrogen receptors in the nuclear.[30] *S. serrulata* administered orally daily in an in vivo experiment using mouse model produced a significant potent anti-inflammatory activity.[31]

The phytochemicals of this plant just like other anti-prostatic conventional drugs do not interfere with cellular capacity of the prostate in secreting Prostate Specific Antigen (PSA), making it beneficial for continuous screening and monitoring of the tumour progression in prostate cancer by continuous measurement of PSA.[19] The activities exhibited by these phytochemicals support the potential activity of this plant extract in the management and treatment of prostatic diseases.[21, 13, 22, 17]

Summary of Studies Showing Effects of S. Serrulata on Prostatic Diseases

Inhibition of Isoenzymes 1& 2 Alpha Reductase

A study conducted by Iehle, C. et al., in 1995 described the independent expression of the type 1 and 2 isoforms of human 5-α reductase and compared the effects of finasteride, turosteride, 4-MA and lipido-sterol extract of S. repens. The study found out that finasteride and furosteride inhibited type 2 isoforms only but the lipido-sterol extract of S. repens inhibited both type 1 and 2 iso-enzymes.[28]

Inhibition of Prolactin and Growth Factor Induced Cell Proliferation

It has been established that prolactin and androgens influence the growth and development of prostate gland naturally.[29] In a study conducted by Van Coppenolle et al., in 2000 using wistar rats to compare

the effects of lipidosterolic extract of S. repens and finasteride both of which are 5-α reductase inhibitors, the lipidosterolic extract of S. repens was found to inhibit both androgenic and prolactin in lateral prostate hyperplasia while the finasteride only inhibited the effect of androgens on prostate enlargement.[29]

Antiestrogen Effects

A double blind placebo-controlled clinical study conducted by Silverio, F. et al., in 1992 using 35 BPH patients who had never been on any treatment, showed that the estrogen receptors in the nuclear were significantly lowered in groups treated with the extracts of S. repens than those which were not treated with *S. repens*.[32]

Anti-inflammatory effects

In an in vivo experiment conducted by Bernichtein, S. et al., in 1995 using unique pro-inflammatory mouse model of prostate hyperplasia with Permixon- a hexanic lipidosterolic extract of S. repens orally administered daily at a dose of 100mg/kg for 28 days, there was a significant potent anti-inflammatory activity in the group that were given the extract when compared to the group that were not.[31]

Toxicity of Serenoa Serrulata

There are a number of preparations from dried berries of *S. serrulata* in the market. The most commonly available, used and highly investigated remedy, clinically and non-clinically with most published reports on the toxicity profile of *S. serrulata* is Permixon (French Producer Pierre Fabre Medicament).[33] Permixon is a hexane lipidosterolic extract of the berries of *S. serrulata*.[33] The adverse effects of extracts of berries of *S. serrulata* are abdominal pain, diarrheal, nausea, fatigue, headache, rhinitis and decreased libido which are all mild, infrequent and reversible.[21,13, 22, 17] A clinical trial study conducted in 1997 on 132 patients suffering from Benign prostatic hyperplasia to determine the efficacy and safety of 2 dosage forms (160 mg b.i.d and 320 mg o.d) of the extract of *Serenoa* repens concluded that the extracts of the 2 dosage forms were safe and efficacious.[34] Meanwhile in an in vitro study to investigate the hepatotoxicity potential of saw palmetto in rats' liver function, it was reported that the extracts of saw palmetto exhibited no toxic effect on the laboratory animal.[35] Furthermore, an in vivo experiment to assess the tolerability and toxicity of lipidosterolic extract of America dwarf palm *Serenoa* repens in wistar rats concluded that there was no toxicological effect of the preparations in the experimental animals.[33]

Combination of *S. Serrulata* with other Medicinal Plants for the Treatment/Management of Prostatic Diseases

Combination therapy is an important treatment modality in many disease settings including cancer, cardio-vascular disease and infectious diseases.[36] Poly-herbal formulation is a common practice for exploiting the advantage of synergistic interaction for enhanced therapeutic efficacy.[36] Many chronic conditions have been treated with combination therapy for many years based on the phenomenon of resistance.[37] Resistance arises when an organism gains the ability to resist a drug which initially effectively slowed the growth or even killed the target organism. Treatment with a combined therapy reduces the chance of resistance especially if the 2 drugs have different mechanisms of reducing the organism's normal functions.[38] Combining drugs enhance the efficacy, minimises the adverse effects of drugs, improved therapeutic value, dose and toxicity reduction as well as to minimising or delaying the induction of drug resistance. Toxicity reduction and resistance minimisation benefits could also be the outcomes of synergism.[38,36,39] The 2 drug combinations might have effect on either one another or on the organism.

When drugs are combined, there are 3 possible effects: First is they act independently of one another, Secondly they increase each other's effect; this could happen because they affect the body in the same way or because one drug increases the concentration of the other in the body and thirdly, they decrease each other's intended effects; this could occur when one drug blocks or prevents another drug from working (combination-drugs).[36,39]

Phytotherapies comprise of many active constituents which enhance their activity synergistically. The effects of many constituents found in herbal medicinal products and their extracts are mainly explained by the term synergy and polyvalence especially when it is difficult to distinguish the active ingredient.[39] Drug synergy occurs when drugs interact in ways that enhance or magnify one or more effects or side effects of the drugs.[39] Synergy is used in a positive sense, that is, an increase in effect greater than that predicted. However, an unexpected decrease in activity referred to as negative synergy or antagonism may occur particularly in interactions between some modern medicines and herbal products.[39,40] Synergy often occurs when an extract of a plant gives a greater or

safer response than an equivalent dose of the compound considered to be the active one.[41,42] The choice of treatment or management methods of prostatic diseases have always been the use of a combination of therapies to prevent the spread to other places. The treatment modalities are radical prostatectomy for the localised tumours, radical radiotherapy and androgen deprivation therapy for non-confined tumours.[43,44] The presence of comorbidities which are highly prevalent among these patients are also some of the factors for the combination therapies. Comorbidities which contribute to the increase in mortality rates among the prostatic patients need to be managed through combination therapies because monotherapies treat or manage only one condition and yet a factor contributing to the death of the patient could be from the comorbidities.[45]

The rationale for combinatory treatment is to use drugs that work by different mechanism thus decreasing the possibility of building resistance. When drugs with different effects are combined, each drug can be used at its optimal dose without intolerable side effects. A combinatory treatment also reduces disease symptoms and prolongs life. Combination therapy can be between modern therapies, modern therapy with phytotherapy or between phytotherapies only. In this review, the combinatory therapies showed a better synergy, improvement in the quality of life and as well reduction in the adverse effects especially of the conventional drugs. The conventional drugs used in combination with *S. serrulata* in studies reported in this review include Finasteride and Tamsulosin while the medicinal plants reported include Stinging Nettle (*Urtica dioca*), *Zingiber officinalis*, *Echinacea angustifolia* and Pumpkin (*Cucurbita pepo*). The isolated compounds reported include β-sitosterol, Vitamin E, lycopene and Selenium; the extract of pollen grain- Cernitin. Finasteride is an anti-prostatic conventional drug which acts by inhibiting type 2 5-α reductase, a similar mechanism exhibited by *S. serrulata*, however, *S. serrulata* inhibits both type 1 and 2 isoforms of 5-α reductase; a mechanism which complements the other. The summary of studies showing combination between phytotherapies alone and then phytotherapies with conventional drug is shown in ***Table 2***. The combinatory therapy has exhibited high efficacy on prostatitis diseases as compared to monotherapies. This is shown in the ***Table 3***. The synergistic effect shown by the combinations as presented in ***Table 3*** proved to be better than the conventional monotherapy alone.

TABLE 3: Combination of *S. serrulata* with other medicinal plants

Study design	Combination	Prostatic disease	Observations	Conclusion	References
Randomized-controlled - double blind-placebo	-Cernitin (collection of pollens) -Saw palmetto (*S. serrulata* or *S. repens*) -Beta Sitosterol -Vitamin E (antioxidant)	-BPH	- the combination exhibited a decreased in the overall symptoms of BPH like Nocturia and frequency of urination. - the monotherapies exhibited very small change in the PSA measurement, maximal and average urinary flow rates and residual volume which was not significant.	-There was a general improvement in the symptoms of BPH with the combination compared to the monotherapy and the placebo.	58

			-The combination test group had no adverse effect.		
In vivo study	-Saw palmetto (extract and whole berry) -Cernitin	-Prostate growth	-The prostate size Reduced in all the treatment to the same size as the non-castrated rat. -the body weight reduced in all the treatment group too.	-significant reduction in the prostate size in all the treatment. -combination of Saw palmetto and Cernitin influences prostatic hyperplasia through the effects on androgen metabolism.	59
Randomized, double blind, placebo controlled trials clinical study	-Pumpkin seed oil -Saw palmetto oil	-BPH	-There was a little Improvement of Quality of Life in the saw palmetto oil group but much higher in the combination group within a short period of about 6months. -The PSA level was reduced in the saw palmetto oil group after 3 months. -All the group treatment had no improvement in prostate volume. -Maximal urinary flow rate had great improvement in saw palmetto and pumpkin seed oil group.	-Combination of saw palmetto and pumpkin seed oil had an insignificant improvement in all the parameters though its effect was higher symptomatically. -The combination was clinically safe. -Recommends the used of the combination for BPH.	60
Randomized double blinded clinical study	-*Serenoa repens* -Lycopene and selenium -Tamsulosin	-BPH -LUTS	-Much higher significant improvement in the combination therapy. -Increase in Qmax. -Changes in IPSS and Qmax was greater for the combination than	-Combination was more effective than single therapies in improving IPSS and increasing Qmax in patients with LUTS.	61

			for the monotherapies.		
Randomized double blind clinical study	-Tamsulosin (alpha blockers) or Finasteride (5alpha reductase inhibitor) -*Sabal repens* -*Urtica dioca*	-BPH (Nocturia in men with LUTS)	-There was a significant decreased in nocturnal voiding frequency by the plant combinations compared to Placebo and Tamsulosin or Finasteride. -A high decreased in total IPSS with plant combination than with the Placebo.	-The combination had a Significantly higher improvement in all the parameters than the Placebo.	54
Clinical trial	-Tamsulosin -*Serenoa repens*	-BPH	-The combination of Tamsulosin and *S. repens* had a great reduction in IPSS compared to the monotherapies. -The combination also exhibited Significantly higher improvement in the storage symptoms. -There was an insignificant improvement in the Voiding score, LUTS related QoL, Qmax, PVR, PSA and prostate volume by the combination. -The combination however showed some adverse effects like ejaculatory disorders, postural hypotension, dizziness,	-The combination of *Serenoa repens* and Tamsulosin had greater effect than the Tamsulosin alone and as well it reduced the BPH symptoms in patients within 6 to 12 months.	62

			headache, gastrointestinal disorders, rhinitis, fatigue, and Asthenia.		
Randomized double blind multi centre clinical trial	-*Sabal repens* -*Urtica dioca* -*Finasteride*	-Prostate volume	-Both the combined phytotherapies and Finasteride exhibited no statistically significant difference in the Maxima urinary flow. -The combination and Finasteride showed no statistical Improvement in the international prostate symptoms score. -The combination presented better results for voiding symptoms in the patients with prostate than finasteride. -More adverse effects cases were reported by the patients using Finasteride but minor effects reported by the group using plant therapy.	-The plant combination and Finasteride exhibited the same therapeutic effect on the prostate volume however, the phytotherapy had better tolerability than Finasteride with less or no adverse effects compared to Finasteride.	63
In vitro	-*Saw palmetto* -*Astaxanthin*	-BPH -Prostate cancer	-Astaxanthin had a greater inhibition of 5alpha reductase. -Combination had even much higher inhibition of the enzyme. -Reduction in prostatic carcinoma cells by the monotherapies and was much	-much increase in testosterone level by the combination. -the combination therapy was more effective in stopping the growth of cancer cells than the monotherapies.	64

			greater by the inhibition.		
Randomized study	-*Zingiber officinalis* -*Saw palmetto* -*Echinacea angustifolia*	-BPH	-the plant combination had a significant regression of urogenital symptoms in both men and women than each plant extract. -the combination as well exhibited reduction of inflammation in prostatism and pelvic pain.	-there was a high synergy exhibited by the combination compared to the individual plant.	65

DISCUSSION

Prostatic diseases include prostate cancer, prostatitis and benign prostatic hyperplasia. Prostate cancer and Benign Prostatic Hyperplasia are still common condition affecting men especially of older age while prostatitis affect men as young as 35 years mainly due to infections. There are quite a number of treatment options being used and most of them present adverse effects while others are not very effective even at higher doses which are not pleasant to the end user.[46] Medicinal plants have been used for decades for treatment or management of different ailments including prostatic diseases.[47] One of the medicinal plants used for treatment or management of prostatic diseases is *S. serrulata*. Both clinical and non-clinical studies have proven the used of *S. serrulata* either singly or in combination with anti-prostatic conventional drugs or with other medicinal plants.

The phytochemical and the pharmacological results for the investigation of *S. serrulata* as an antiprostatic remedy is in agreement with the ethnobotanical claims as shown *in* ***Tables 1*** and ***2***. The plant toxicity profile is also in agreement with its ethnobotanical claims. This medicinal plant when used in combination is proven to enhance its activity. ***Table 3***, further points out the synergistic effect of the combination of *S. serrulata* with other medicinal plants and even with conventional drug presenting minimal or no adverse effect compared to the conventional drugs Tamsulosin or Finasteride used alone. This study found out that the medicinal plants which are used in combination with *S. serrulata* include; *Urtica dioca, Cucurbita pepo, Zingiber officinalis* and *Echinacea angustifolia* and then the anti-prostatic conventional drugs include; Finasteride and Tamsulosin. This finding could also explain the variations in efficacy reported by some studies during the determination of the anti-prostatic activities of *S. serrulata*.[8] The review also noted that most of these studies were clinical, few were *in vivo* and the review didn't come across any *in vitro* study.

CONCLUSION AND RECOMMENDATION

This review has shown that *S. serrulata* is used as a remedy for different ailments but most commonly used for prostatic diseases with less or no adverse toxic effects. This medicinal plant has also shown a great potential when used as an anti-prostatic remedy when used in combination. *S. serrulata* has vital phytochemicals with promising pharmacological activities which could be developed into well standardised drugs either as a single plant or in their combinations with other medicinal plants. There is however need to investigate further their effects and safety on prostatic conditions when used in combination with other medicinal plants which might not have been mentioned here but have been reported to have anti-prostatic activities.

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REVIEW ARTICLE

Effectiveness of *Azadirachta indica* (neem tree) on prevention and treatment of clinical human malaria: A systematic review

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ABSTRACT

Introduction: Neem tree parts such as leaves, stem barks, and roots are known to have some medicinal values in both humans and animals. However, the evidence is scattered and vary with populations and regions. This systematic review sought to explore the effectiveness of neem as a therapeutic and prophylactic agent against malaria.
Methodology: The systematic review examined the effectiveness of neem using the pre-registered review protocol and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. The Population Intervention Comparator Outcome (PICO) question was: "What is the effectiveness of neem (*Azadirachta indica*) when used as a therapeutic and prophylactic agent for malaria infection?" It included all cross-sectional survey studies, qualitative studies, case-control studies, randomised controlled trials, quasi-experimental studies, and cohort studies with or without comparison groups. Studies that had both children and adult patients who were diagnosed with malaria were included in the survey. Malaria patients using traditional medications other than neem as well as those who did not use neem were excluded from this study. The search for articles, screening, and synthesis were conducted using the Rayyan software.
Results: Out of the total 1089 articles retrieved, only 3 fitted the inclusion criteria, 1 article could not be retrieved. A narrative synthesis was therefore done on 2 final research articles that were retrievable. The pooled evidence shows that *Azadirachta indica* is effective against malaria. The medicinal effects are more on symptoms and curbing development to clinical disease than ant parasitic effects.
Conclusion: Neem is potential traditional medicine for malaria symptoms' treatment, but evidence on ant parasitic effects is still not conclusive. The study recommends further primary studies to enhance the power of results to further recommend this plant for the prevention of or treatment of malaria symptoms.

Keywords: Malaria, *Azadirachta indica*, symptomatic treatment, Neem, herb

INTRODUCTION

Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Neem is cultivated in at least 30 countries worldwide, in Asia, Africa as well as Central and South America. Some small-scale plantations are also reportedly successful in Europe and the United States of America. The Latinised name for neem is *Azadirachta indica*. It is derived from the Persian, it means "the free tree of India".[1] According to Biswas et al.[2], the neem tree was described as *Azadirachta indica* as early as 1830 and its taxonomic position is as follows: Order-Rutales; Suborder: Rutinae; Family: Meliaceae (mahogany family); Subfamily: Melioideae; Tribe: Melieae; Genus: Azadirachta; and Species: indica.

Azadirachta indica is perhaps one of the most useful traditional medicinal plants to the extent that it is named "the village pharmacy" in some parts of the world. The therapeutic and prophylactic roles of neem cannot be underestimated in high malaria endemic areas. Anecdotal evidence suggests the potential benefit of each of the part of the neem tree; roots, trunk bark, leaves, and flowers, these traditionally have some medicinal value against various diseases.[3-5] Neem is said to variably relieve patients symptoms of malaria, stomach and intestinal ulcers, skin diseases, teeth and other pains, respiratory disorders, constipation, rheumatism, chronic syphilitic sores and fever.[3] It is also reported that neem can work as an anticancer and treatment agent for various disease via the regulation of its various biological and physiological pathways.[3] Consequently, the plant has been variably and widely used for various medicinal purposes with varying praises. Evidence on complete treatment varies and is not conclusive in available documented reports. Tests conducted at the King Institute of Preventive Medicine indicate that the Siddha neem preparation brought down symptoms and speeded up the recovery of patients affected by dengue.[6,7] In a similar context, the US National Academy of Science had published a report in 1992 entitled "Neem: A tree for solving global problems"[8] which was 10 years later declared by the United Nations as the "Tree of the 21st century".[9]

Neem is one of the most extensively researched trees of medicinal value.[10] The chemical investigations on the products of the neem tree have been undertaken since the middle of the twentieth century, and more than 135 compounds have been isolated from its different parts.[2] Experimental studies in mice using neem leaf and stem bark extracts showed a significant reduction in mouse malaria parasites – the *Plasmodium berghei*.[11] Among human beings, neem use resulted in a decline of *Plasmodium falciparum* culture asexual and the sexual forms by 50% compared to control cultures.[12] In addition, neem has insecticidal effects on malaria vectors.[13-15] Despite the wide range of potential benefits of neem, the eco-geographic and genetic variation of the plant may influence the concentrations of azadirachtin and other contents in the seeds.[16] The variations may influence the medicinal value and potential use of the plant for various purposes. The half-life of Azadirachtin varies in different situations. For example, formulations can retain over 59% of the azadirachtin content for about a year when stored at 10–15°C in the dark. The same formulations decompose at higher temperatures, in alkaline and strongly acidic media, particularly in the light.[17] This photosensitivity necessitates the need of carefulness in storage for uses especially when the extract is used as a pesticide.[18]

Neem plant and its parts are variably and safely used in different geographic locations. It is used as a pesticide as well as ovicidal, larvicidal and adulticidal agent in control of Anopheles mosquitoes, the malaria vector.[19] This systematic review focused on the effect of neem as a therapeutic and prophylactic agent, restricting information on *in vivo* studies not extract-based neither *in vitro, ex-vivo* nor experimental studies. Our focus was dictated by the pronounced use of neem for cure of malaria traditionally, the information that could create a base for proposing the use of neem for malaria control at the household level. Apart from informal use of neem for various purposes in different populations, information on neem as a therapeutic and prophylactic agent in its natural state is limited despite the reported neuroprotective effects in experimental cerebral malaria.[20]

In order to consolidate evidence to effect policy and implementation thereof, systematic collection and appraisal of evidence to refine recommendations are essential. During protocol development in preparation for this review, we could not find a systematic review previously conducted to examine the effectiveness of neem on prevention and treatment of malaria in human beings. This systematic review, therefore, aimed to explore the effectiveness of neem as a therapeutic and prophylactic agent for malaria infection.

METHODS

Study Design

This systematic review was conducted to address the following Population Intervention Comparator Outcome (PICO) question: "What is the effectiveness of neem when used as therapeutic and prophylactic agent for malaria infection?" The sample included studies with patients who were diagnosed with malaria. *Intervention* included studies that had analysed the use of *Azadirachta indica* singly and not in combination with other herbs for malaria treatment and prevention. The main outcome was the effectiveness of *Azadirachta indica* in enabling patients to recover from disease after use and impact on prevention, control and treatment of the disease.

The protocol for this study was registered at the PROSPERO database - an international prospective register of systematic reviews. This is an open access online database of systematic review protocols on health-related topics where researchers prospectively

register their reviews. The study was given a registration number **CRD42018097453**. We followed the PRISMA criteria and checklist to conduct this review.

Selection of Studies and Databases

We included all cross-sectional survey studies, qualitative studies, case-control studies, randomised controlled trials, quasi-experimental studies, and cohort studies with or without comparison groups. We excluded potential review articles, opinions, and editorials, even if addressing some of the PICO items. We conducted evidence search in medical databases. The databases included PubMed, The Cochrane Library, EBSCO, CINAHL, HINARI, SCOPUS and Web of Science via Hinari, Popline, lilacs, clinical trial registry, Eric, Science Direct. Moreover, we conducted a grey literature search from respective databases (***Appendix 1***) and conducted a hand search from relevant articles in the subject matter. We also searched Google scholar to augment results from other databases. Relevant literature and published reports were retrieved from other websites and organizations. We searched literature published in English only without restricting time period.

Inclusion and Exclusion Criteria

For participants or population, we included studies that had both children and adult patients who were diagnosed with malaria. We excluded studies in which malaria patients had used other traditional medications other than neem as well as those who had not used neem. We further assessed societal perception and attitude on the use of neem as a medicinal plant to control malaria in endemic areas. Additional outcomes included: the improved quality of health following the use of the product as medication, reduced visits to hospital/malaria clinics due to malaria bouts, improved quality in designing malaria studies, increased level of awareness about the usefulness of *Azadirachta indica* in treating malaria, cost-effectiveness compared to standard drugs, and reported side effect. The search terms were developed by information scientists in the team, as attached in Appendix 1.

Data Extraction and Management

3 reviewers (EVM, MR, and ETL) conducted the search and 2 reviewers (EVM and ETL) initially screened the studies by titles and abstracts only for possible inclusion. EVM and ETL conducted the full-text screening. Agreement on the inclusion of all studies was reached via consensus. Rayyan software was used to conduct the screening. Data extraction was carried out under the guidance of the PRISMA checklist by Moher et al.[21,22] EVM and ETL independently extracted data from included studies using a standardised data extraction form. Data were entered into an excel sheet by EVM and checked by ETL.

Search Result

The searches produced a total of 1,089 references, and after de-duplication, we ended up with 825 references (***Figure 1***). We excluded a total of 772 references basing on their title and abstract. A total of 53 ethno-botanical studies on the use of medicinal plants for the treatment of diseases in humans were retrieved from PubMed as per methods section. 50 publications were excluded from this review because either they dealt with the use of medicinal plants in the treatment of other human infections other than malaria or used other medicinal plants to treat malaria as shown in ***Figure 1***.

Data Analysis

The risk of bias of the included studies was assessed by using the quality of included articles through the scale for quantitative and qualitative studies developed by Kmet et al.[23] The tool for the quantitative studies has 14 items, which can be scored based on the degree to which the specific criteria were met ("yes" = 2, "partial" = 1, "no" = 0). The items that were not applicable to a particular study were scored as "n/a".[23] That means they were not included in the summary score. The tool for qualitative studies had 10 items, and the scores can be calculated in a similar fashion as for quantitative studies. The "not applicable" option is not allowed for quantitative studies. Therefore, the "summary score for each article was calculated by summing the total scores obtained across the 10 items and dividing by 20 (the total possible scores").[23] 2 raters (EVM & ETL) independently performed the critical appraisal and then resolved differences.

Risk of Bias

The risk of bias was assessed for the two quantitative studies that were selected. The overall scores (***Table 1***) assigned by both reviewers was 0.5. Both reviewers assigned the same overall score to 2 studies.

We expected to carry out a meta-analysis and therefore to deploy and even subgroup analysis by age, geographical location as well as other demographic characteristics. However, owing to fewer retrieved studies, heterogeneity among the studies, and differences in measurements of the outcome of interest, we could only conduct narrative synthesis.

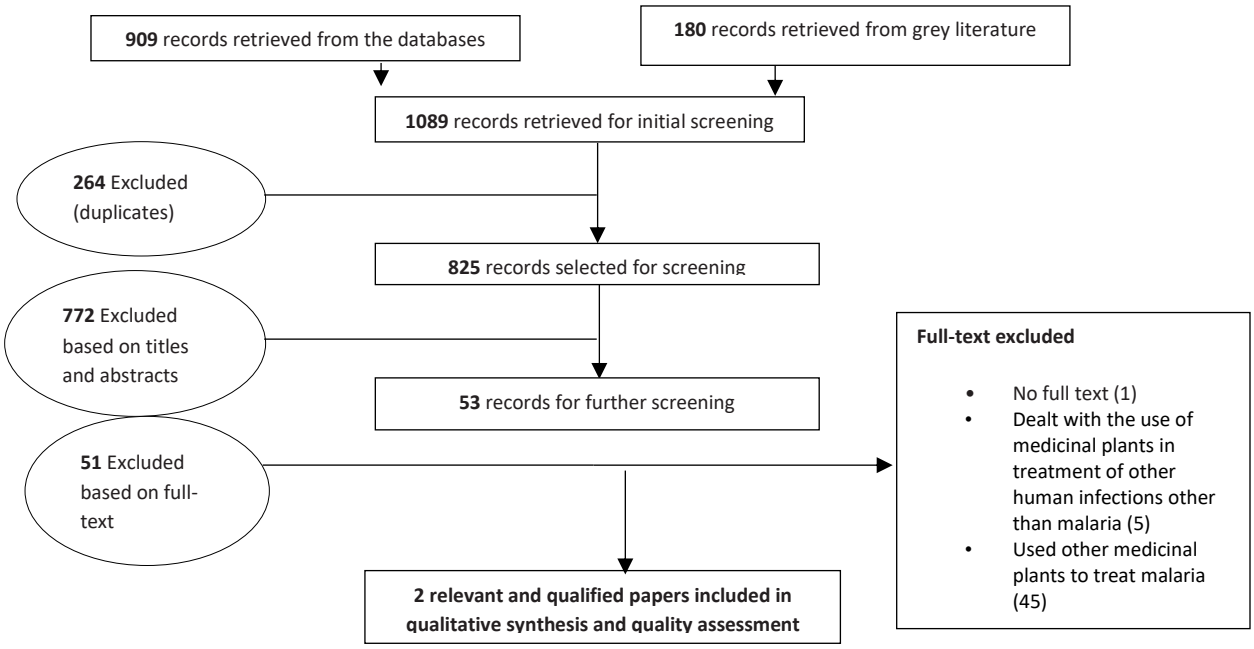
RESULTS

Effectiveness of Azadirachta Indica as a Therapeutic and Prophylactic Agent for Malaria Infection

Evidence from the 2 pooled studies suggests the potential effects of neem as a medicinal plant (***Table 1***). The first article[24] reported the ethno pharmacological information about the uses of neem, particularly the leaves, besides the insecticidal one, on the treatment of malaria. Information gathered indicated that treatment is based on the parts of the plant used and the way the plant is used. Identified plant specimens were collected and made into a herbarium voucher. The study considered the high variability and complex chemical constituent of neem, thus used a High-Performance Thin Layer Chromatography (HPTLC) analysis on neem leaves coming from both the Indonesian island of Bali and the Indian subcontinent.

This study produced data on the medical use of traditional preparations from leaves of neem that displayed a wide spectrum of applications. It was noted that in the Indian Subcontinent, neem leaves are used to treat a variety of conditions including dental and gastrointestinal disorders, malaria fevers, skin diseases and as an insect repellent. In the Balinese, neem leaves

FIGURE 1: Flow chart diagram: Publications screening process to get relevant articles that qualified for qualitative synthesis and quality assessment



Criteria	Iwalewa et al (1999)	Sujarwo et al (2016)
Objective	1	0
Design	1	0
Subject/comparison group selection or source of information/input variables	1	1
Subject characteristics	1	1
Interventional and random allocation	1	N/A
Interventional and blinding of investigators	N/A	N/A
Interventional and blinding of subjects	N/A	N/A
Outcome and (if applicable) exposure measure	1	1
Sample size	0	2
Analytic methods	1	1
An estimate of variance is reported for the main results	N/A	N/A
Controlled for confounding	N/A	N/A
Results reported in sufficient detail	1	1
Conclusions supported by the results	2	2
Average score	0.5	0.5

were used mainly as ant diuretic and anti-diabetes, but also for headache, heartburn, and stimulation of appetite. The authors suggest that the differences in utilisation are not related to chemical differences and other constituents other than limonoids and recommend that further investigations should be considered focusing on its observed multipurpose activity. Informants had good knowledge of neem use in the treatment of diseases with good attitude and practice in the use of neem and other traditional medicines for the treatment of malaria. The study concluded by revealing neem leaves to be believably useful in the treatment of diabetes in both Balinese and Indian communities. The study however had a reservation that limonoids cannot be considered the only factor responsible for digestive properties and recommended further research to validate this report by carrying out enzymatic tests and the identification of active constituents.

Similarly, the second article[25] explored the contributory pharmacological effects of *Azadirachta indica* leaf in the treatment of malaria with the aim of assessing the effect of crude extracts on various signs and symptoms of malaria infection in vivo and in vitro. The results showed that the methanolic and diethyl ether extracts have significant antipyretic, analgesic, anti-inflammatory and anti-aggregatory activities, while the chloroform extract did not show appreciable effect. The effects of crude extracts compared favourably with chloroquine in pyrexia, indomethacin in platelet aggregation and Acetylsalicylic Acid (ASA) in analgesia and inflammatory experimental models. The study concluded that the pharmacological effects of these extracts might explain the effectiveness of *Azadirachta indica* leaf in malaria therapy traditionally.

Screening of articles for qualitative synthesis and quality assessment

In the screening of retrieved articles for qualitative synthesis and quality assessment (***Figure 1***), 1 (1.96%) out of 51 records of publications was excluded as we could not find its full text. 5 (9.80%) articles comprised of medicinal plants other than *Azadirachta indica* used for the treatment of other human infections than malaria. 45 (88.24%) articles comprised of other ethno botanical medicinal plants than *Azadirachta indica* that were used to treat malaria. This observation indicates that apart from the believed use of *Azadirachta indica* in the treatment of malaria and other infections, there are several other medicinal plants variably used as medicines to treat malaria and other infections. This necessitates further investigation that may include extraction of ingredients from each plant species of interest and testing for its effectiveness to treat malaria and other infections. Most studies on medicinal plants and infection are regional with more studies coming from the Indian sub-continent, particularly India, Pakistan

and Indonesia. In Africa, most of the studies relating *Azadirachta indica* and other medicinal plants come from West African countries, namely Nigeria, Benin, Burkina Faso, and Ghana to mention a few. In Africa, south of Sahara, most of the studies seem to be concentrated in South Africa, with Kenya and Uganda dominating for the East African region. It is however worth noting that despite the limited number of reported studies on *Azadirachta indica* and other medicinal plants, regional informal use of these plants as medicines for variable medicinal values cannot be underestimated considering the variable cultures and traditions.

Pre-Clinical Studies

In the screening process, it was noted that most of the research on medicinal plants believed to be useful in treating malaria and other infections had been tried in vitro or in mice/rodents. The results from these trials provide clues on the role of these medicinal plants in human host. We look forward to seeing the next step in this research where the real impacts will be observed in humans. Only 2 publications working on *Azadirachta indica* and malaria were identified. This number is too small, however, it could not mean that the plant and its products are useless. This could be because most treatments are conducted by traditional healers or by individuals in their homes, thus, such treatments cannot be easily reported due to remoteness. This contributes to the limited information out of many in use.

Despite the novelty of this study and its potential for policy and guidance, evidence gathered should be discussed in light of the following limitations: There is no enough empirical research done around the neem plant. In this systematic review study, only 2 studies out of 53 recorded for further screening met the inclusion criteria and qualified for further analysis. This shows that the use of *Azadirachta indica* to treat diseases is an area worth further research in order to publicise the therapeutical effect of the neem plant for positive treatment outcome of diseases. Despite the rare publicity of neem as a therapeutic or prophylactic agent, various parts of the plant are informally and variably used in different societies to alleviate individuals from a diverse of disease conditions.

DISCUSSION

Azadirachta indica is a medicinal plant commonly used to treat various diseases in traditional medicine. It has also been knowingly or unknowingly used prophylactically or as a treatment for malaria in regions where the disease is endemic. *Azadirachta indica* has shown a wide range of pharmacological activities in traditional medicine, particularly the neem leaf and other non-wood parts of the plant.[5,26,27] This systematic review explored the effectiveness of *Azadirachta indica* in the treatment of malaria infection as a therapeutic or a prophylactic agent. The evidence collected was appraised to be of moderate quality, nevertheless showed that neem has potential to address malaria symptoms though not complete remission. *In vitro* studies involving the potential roles of diverse bioactive compounds from *Azadirachta indica*, mainly limonoids including nimbolide, azadarachtin, and gedunin are involved in modulation of multiple cells signalling pathways.[28] The proposed multiple cellular and molecular mechanisms of bioactive compounds include free radical scavenging, DNA repair, cell cycle alteration, programmed cell death (apoptosis) and autophagy. In addition, the bioactive materials are said to be involved in immune surveillance, anti-inflammatory, anti-angiogenic, anti-invasive and anti-metastatic activities as well as modulation of several dysregulated oncogenic signalling pathways.[29] The economic importance of neem, however, extends to its evidence-based additional diverse complementary and safe use as mosquitocide.[19, 30]

The ethno botanical uses of neem are historical where it has variably and believably been used to alleviate individuals from common diseases such as diabetes mellitus, malaria, various skin conditions and other symptoms such as fever and headaches.[24,31,32] Reports also show that neem leaf and stem bark extracts have ant malarial effects on Plasmodium berghei infected mice with a significant reduction in parasitaemia speculating its potential for malaria treatment.[11] Our review on the therapeutic effects indicated that Neem has high potential as antipyretic, analgesic, anti-inflammatory as well as anti-platelet aggregation.[25] Although the antipyretic effects of *Azadirachta indica* seem to be short-lived, its use and effectiveness can possibly be heightened by complementing other ethno botanical medicinal plants if available or modern drugs such as paracetamol where necessary. The effect however, seems to be symptom-based than curative by relieving patients from symptoms associated with the disease rather than acting on the malaria parasites. In this context, while the effects of *Azadirachta indica* is traditionally considered therapeutic against malaria, it is in fact, prophylactic in the sense that when used it may reduce the possibility of patient's progression to or persistence of clinical signs resulting from infection by the malaria parasites.

In this systematic review, the 2 studies[24,25] variably explored the effectiveness of *Azadirachta indica* as medicinal plant against malaria but looking at different angles and different approaches. While the Sujarwo et al. study[24] focused on ethno pharmacological information on uses of neem, in particular the leaves, besides the insecticidal one considering the historical background of their uses, the Lwalewa et al. study[25] had an interest on the *in vivo* and *in vitro* effects of the plant extracts as antipyretic, analgesic, anti-inflammatory and anti-aggregatory activities against malaria disease. The plant medicinal value ranges from fruits to roots.[24] The plant seemed to have variable uses such as to treat dental and gastrointestinal disorders, malaria fevers, skin diseases, as an insect repellent, as a diuretic, and treatment of diabetes, headache, heartburn and stimulating appetite. The antipyretic, analgesic, anti-inflammatory and anti-aggregatory activities were common findings[25] despite the different methods of testing the effectiveness of the plant leaf extracts on malaria. Hence, both studies reveal that *Azadirachta indica* is effective in the treatment of malaria and its effectiveness to be specifically from the use of leaves or leaf extracts of the plant. The role of *Azadirachta indica* in the prevention and treatment of diseases is via the regulation of various biological and physiological pathways.[3]

CONCLUSION

In conclusion, this systematic review shows that although *Azadirachta indica* can have variable believed medicinal applications such as relieving patients from symptoms of a diverse number of diseases, it is effective against malaria. However, its effects are more on the symptoms and development to clinical disease than acting on the parasite itself to highlight the proposed regulation role on various biological and physiological pathways. Given the historical and traditional belief on the use of *Azadirachta indica* in the treatment of various diseases, this study recommends further investigations to get more complementary value of the plant in alleviating floods of disease calamities in developing world, especially in malaria-endemic regions. In addition, further investigations on other traditional plants for their values in treating malaria and other infections should also be advocated.

Limitations

Restricted access to articles in some databases such as Web of Science and Embase because they are not accessible in our region.

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APPENDIX 1

Pubmed

#6	Search (#5 NOT #4)
#5	Search (#1 AND #2 AND #3)
#4	Search ((animals[mesh] NOT humans[mesh]))
#3	Search (((prevention and control [Subheading] OR "primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics)))
#2	Search (((Malaria[mesh] OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh")))
#1	Search (((azadirachta[mesh] OR azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia")))

CINAHL

S6	S4 NOT S5

S5	MH animals NOT MH humans
S4	S1 AND S2 AND S3
S3	MH malaria OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh"
S2	MH "Preventive Health Care+" OR "primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics
S1	MH azadirachta OR azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia"

SCOPUS through Research4Life programmes

ALL (azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia") AND ALL (malaria OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh") AND ALL ("primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics)

Popline

(azadirachta OR azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia") AND (malaria OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh") AND ("primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics)

Web of Science through Research4Life programmes

TS=(azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia") AND TS=(malaria OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh") AND TS=("primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics)

ERIC

(azadirachta OR azadirachta* OR "Neem tree*") AND (malaria OR "Remittent Fever" OR "plasmodium infection*") AND (prevention OR control)

ScienceDirect

All fields	(azadirachta OR azadirachta* OR "Neem tree*") AND (malaria OR "Remittent Fever" OR "plasmodium infection*")
limit to Title, abstract or keywords	(azadirachta OR azadirachta* OR "Neem tree*") AND (malaria OR "Remittent Fever" OR "plasmodium infection*")

Lilacs

(azadirachta OR azadirachta* OR "Neem tree*" OR "tree* Neem" OR "azadirachta indica*" OR "indica* azadirachta" OR "melia azadirachta*" OR "azadirachtas melia") AND (malaria OR "Remittent Fever" OR "Fever Remittent" OR paludism OR "plasmodium infection*" OR "infection* plasmodium" OR "Marsh Fever" OR "Fever Marsh") AND ("primary prevention" OR "secondary prevention" OR "tertiary prevention" OR prophylaxis OR "preventive therapy" OR "preventive measures" OR prevention OR control OR therapy OR treatment OR "disease management" OR therapeutics)

Google scholar

intitle:(azadirachta OR "Neem tree*") AND intitle:(malaria OR "Remittent Fever" OR "plasmodium infection*")

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Detectable viral load among HIV -1 positive pregnant women on ART (Option B +) in northern Tanzania: Baseline results from the HIVDR study.

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ABSTRACT

Introduction: In Tanzania, the *Ministry of Health*, Community Development, Gender, Elderly and Children (MoHCDEC) has implemented the Option B+ as one of the strategies to facilitate achievement of elimination of mother to child transmission of HIV. To prevent emergence of drug resistance mutations early identification of option B+ failure is critical. The emergence of drug resistance mutation and subsequent treatment failure poses a major concern for HIV program in low- and middle-income resource settings where treatment options are limited.

Methodology: We recruited treatment naïve, treatment experienced HIV-1 positive pregnant women and those who had prophylaxis in their previous pregnancy in Kilimanjaro, northern Tanzania August 2016 to February 2017. Whole blood (2ml) for biochemistry, viral load and drug resistance testing were taken at baseline. ARV drug resistance testing was done on women with VL ≥ 1000 copies/ml. We used descriptive statistic and logistic regression to determine the strength of association between virologic outcome (virologic failure) and independent predictors.

Results: One hundred and forty eight (148) pregnant HIV-positive women were enrolled in the study with mean age of 29.82 years (SD=6.17) from August, 2016 to February, 2017. Virologic failure was demonstrated in 34 (23%) with viral load ≥ 1,000 copies/ml. Genotyping results were available from 26 women, mutations associated with ARV resistance were detected in 23.1% (n = 6/26). Among the six women with ARV resistance mutation 4(66.7%) had high level resistance and 2(33.3%) had low level resistance. Among the 26 samples genotyped 15(58%) viruses were subtype A, while eight were subtype C (31%) and three subtypes D (11%). The most dominant drug resistance mutations against the reverse transcriptase inhibitors for the women with high level resistance were K103N, Y188L, D67N, K70R, M184V, T215F, K219EQ, and the low-level resistance was E138A. The older age was associated with virological failure compared to those who were < 20 year of age.

Conclusion: Viral load testing should be done on women who were already on antiretroviral treatment on their first antenatal visit to ensure early detection of virological failure and enable clinicians to take an appropriate course of action on their management.

Educational intervention on adherence should be targeted at an early stage to women with virological failure during pregnancy to reduce the emergence of HIV-1 drug resistance mutations.

Keywords: PMTCT, ART, HIV, pregnant women, viral load suppression, HIV drug resistance, Tanzania

INTRODUCTION

Since 2012, the World Health Organization (WHO) recommends using lifelong antiretroviral therapy (ART) for all pregnant and breastfeeding women regardless of CD4 counts and clinical stage, and provision of nevirapine or zidovudine to all HIV-exposed infants for four to six weeks regardless of the feeding method. These recommendations are known as "Option B+".[1] In 2013, the WHO consolidated antiretroviral (ARV) guidelines that endorsed this approach, particularly in HIV-endemic resource-limited settings.[2] These guidelines also promoted the

use of viral load (VL) testing as the preferred approach to monitoring ART and identifying treatment failure, recommending testing six months after starting ART and every 12 months thereafter.

In September 2013, the Tanzanian government initiated a policy of lifelong ART for all HIV-infected pregnant women for prevention of mother-to-child transmission (PMTCT) regardless of CD4 count or clinical status (also known as Option B+).[3] In the Kilimanjaro region, Northern Tanzania, Option B+ was deployed in April 2014. There are limited VL data available from programs implementing "Option B+," lifelong ART to all HIV-positive pregnant and postpartum women, in resource-limited settings. The emergence of drug resistance and subsequent treatment failure poses a major concern for HIV programs in low resource settings where treatment options are limited. The most challenging issue, particularly with Option B+, is combined antiretroviral therapy (cART) adherence because these women are relatively healthy and therefore less motivated to accept cART inconveniencies.

Suboptimal adherence may promote HIV drug-resistance (HIVDR) development.[4] Furthermore, the implementation of Option B+ would have major programmatic implications: scale-up of women receiving ART from pre-conception will rapidly increase with 2015 WHO recommendations for ART initiation in all HIV- positive pregnant and non-pregnant women regardless of their CD4 cell count.[5] However, empirical data to adequately inform policy are currently lacking, in particular as to what extent B/B+ could result in increased rates of ARV drug resistance. In this study, the overall objective was to assess the virological failure and drug resistance mutations in ART naïve and ART experienced HIV-1 pregnant women.

MATERIALS AND METHODS

Study Design

The HIVDR study was a descriptive cross-sectional study of 148 HIV- positive pregnant eligible women. We enrolled women falling in any of the following three categories: treatment naïve pregnant women who have initiated ART for the first time, secondly those who were previously enrolled through PMTCT program either through prophylaxis or treatment (option A& B) and lastly were pre-ART HIV-1infected pregnant women who had been initiated cART through care and treatment services before the current pregnancy.

Study Population

Between August 2016 and February 2017, eligible HIV- positive pregnant women were enrolled from three study sites, Kilimanjaro Christian Medical Centre (KCMC) and Pasua, and Himo Health centres. Eligibility criteria included documented HIV infection, women aged ≥18 years old, HIV-1 positive pregnant women with a first HIV-1 positive test during the current pregnancy, HIV- 1 positive pregnant women with previous history of ART exposure for PMTCT or for post-exposure prophylaxis (PEP), those who have been on cART through care and treatment centre (CTC) program and willing to provide informed consent for herself.

Data Collection

Women underwent an enrolment interview, which included demographic information, HIV and ART-related history, use of alcohol and concomitant drug use. Medical history and liver function tests were done. Responses were documented on a case report form (CRF). Whole blood (2ml) for biochemistry, VL and HIVDR testing were taken at baseline. Data were reviewed for completeness and quality, and entered into the study database. RNA-PCR testing for VL in women was done at the time of enrolment by the clinical laboratory at KCMC using COBAS AmpliPrep/COBAS TaqMan (manufacturer Roche Platform) HIV-1 Test, version 2.0 (v2.0) with a lower detection limit at 20 copies/ml. ARV drug resistance testing was conducted on women with VL ≥ 1000 copies/ml at National Health Laboratory. Quality Assurance and Training Centre in Dar es Salaam for HIVDR genotyping testing.

Informed consent was obtained and documented via signature and all eligible women were enrolled in the study. All study staff received training on data collection and on all ethical issues regarding research with human subjects. Ethical approvals were obtained from the KCMC College Research Ethics Committee, in Moshi, Tanzania and by the National Health Research Ethics Committee at the National Institute for Medical Research in Dar-es Salaam, Tanzania.

Statistical analysis

Descriptive statistics were used to summarize demographics. Logistic regression analysis was performed and an odds ratio with 95% confidence interval (CI) was used to determine the strength of association between virologic outcome (virologic failure) and independent predictors. All statistical tests were two sided and the level of statistical significance

was set at P < 0.05 (2-tailed). The crude analysis assessed the association between individual characteristics of the women and the outcome variable (HIV-1 VL). Then data was analysed using the statistical package for social science (SPSS version 20) IBM Cooperation by Armonk, New York, United States of America.

RESULTS

Demographic Characteristics

A total of 148 pregnant HIV-positive women were enrolled in the study with a mean age of 29.82 years (SD=6.17) from August 2016 to February 2017. The newly diagnosed HIV pregnant women were 82(55.4%) and previously diagnosed from CTC were 39(26.4%) and 27(18.2%) were those given prophylaxis in their previous pregnancy (***Table 1***). The median ART duration for women who were already enrolled in CTC was 4.5 years with a range of 3 months to 11 years and 6 months among those women who were previously diagnosed in CTC. Most (2–5%) HIV positive pregnant women with active TB were distributed across all the treatment arms.

Viral load

Detectable VL was >1000 copies/ml among newly diagnosed HIV -1 infected pregnant women compared to those who were previously on ART before the current pregnancy and those who had prophylaxis in their previous pregnancy (< 1000 copies/ml) and this was found to be statistically significant (P= 0.006) (***Table 1***). Virologic failure was demonstrated in 23% overall (***Table 2***).

TABLE 1: Characteristics of HIV positive pregnant women-initiated ART under option B+ by virologic outcome at study enrolment (N=148)

Variables	HIV viral load (copies/mls)		$\chi 2$ P-value
	<1000copies/ml (n=114)	>1000copies/ml (n=34)	
Hospital site			
Kilimanjaro Christian Medical Centre	53(77.9)	15(22.1)	
Himo Health Centre	27(84.4)	5(15.6)	.359
Pasua Health Centre	34(70.8)	14(29.2)	
Age (Years)			
<20	4(80.0)	1(20.0)	
20-24	22(84.6)	4(15.4)	.744
25-29	30(73.2)	11(26.8)	
30 and above	58(76.3)	18(23.7)	
Gestational age at screening			
1st trimester (6-12 weeks)	17(81)	4(19)	
2nd trimester (13-24 weeks)	50(70.4)	21(29.6)	.179
3rd trimester (25-40 weeks)	47(83.9)	9(16.1)	
Index pregnancy and ART initiation*			
During pregnancy	79(72.5)	30(27.5)	.02
Before pregnancy	35(89.7)	4(10.3)	
Anti TB*			
Yes	5(100)	-	.266
No	109(76.2)	34(23.8)	
Alcohol*			
Yes	25(80.6)	6(19.4)	.810
No	89(76.1)	28(23.9)	
Treatment arms			
Newly diagnosed	55(67.1)	27(32.9)	
CTC	35(92.1)	4(7.9)	.006
PMTCT	24(85.7)	3(14.3)	

*Fischer exact test

TABLE 2: Factors associated with virologic outcome among HIV pregnant women-initiated ART under option B+ (N=148)

Variable	Total	Viral load ≥ 1000copies/ml n (%)	Crude analysis	
			Odds ratio (OR) (95% CI)	P-value
Total	**148**	**34(23)**		
Age group				
<20	5	1(20.0)	Ref	
20-24	26	4(15.4)	0.73(0.06-8.32)	.85
25-29	41	11(26.8)	1.47(0.15-14.59)	.744
30 and above	76	18(23.68)	1.24(0.13-11.83)	.798
Gestational age				
1st trimester (6-12 weeks)	21	4(19.05)	Ref	
2nd trimester (13-24 weeks)	71	21(29.58)	1.79(0.54-5.94)	
3rd trimester (25-40 weeks)	56	9(19.07)	0.81(0.22-2.99)	.756
Alcohol				
No	117	28(23.93)	Ref	
Yes	31	6(19.35)	0.76(0.28-2.05)	.591
Exposed ART				
Yes	25	5(20)	Ref	
No	123	29(23.58)	1.23(0.43-3.58)	.699
ALT (normal)				
Yes	144	32(22.2)	Ref	
No	2	1(50)	3.5(0.21-57.53)	.380
AST (normal)				
Yes	120	27(22.5)	Ref	
No	26	6(23.08)	1.03(0.38-2.23)	.949

Antiretroviral Drug Resistance

There were 34 (23%) with VL ≥ 1,000 copies/ml for genotyping for ARV drug resistance mutations. The VL among these women with genotyping results was 1038 to 242208 copies/ml. Genotyping results were available from 26 women, mutations associated with ARV resistance were detected in 6(23.1%). Among the six women with ARV resistance mutation 4(66.7%) had high level resistance and 2(33.3%) had low level resistance. Three women were previously diagnosed in the CTC, one woman was previously exposed to ART during her previous pregnancy and two were newly diagnosed. Among the 26 samples genotyped 15(58%) viruses were subtype A, while eight were subtype C (31%) and three subtype D (11%). The most dominant drug resistance mutations against the reverse transcriptase inhibitors for the women with high level resistance were K103N, Y188L, D67N, K70R, M184V, T215F, K219EQ (***Figure 1***) and for the low-level resistance it was E138A.

Determinants of virological failure

In the crude analysis the following significant predictors for VL ≥1000copies/ml were identified: Age at ART initiation, gestational age at ART initiation, previous exposure to ART, alcohol use, use of anti TB medications and liver function test AST and ALT (Wald test, P-value<0.05). At bivariate analysis none of these variables were associated with VL ≥1000copies/ml. However, increase in age above 20 years was associated with the odds of viral load ≥ 1000copies/ml (ORcrude = 0.73, 95% CI=0.06 to 8.32) and (ORcrude = 1.47, 95% CI=0.15 to 14.59) compared to those who were less than 20 years of age but the differences were not statistically significant (***Table 2***).

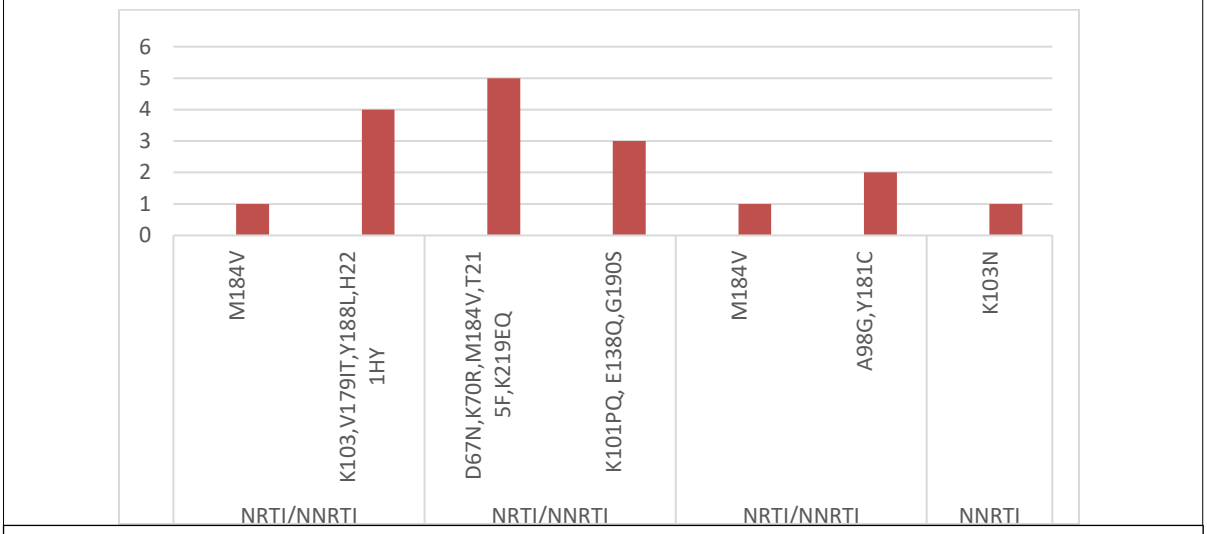
Virologic failure sub-group analysis

In a sub-group analysis of the factors associated with virologic failure among previously diagnosed HIV pregnant women in CTC, 7.7% (3/39) had VL ≥

1000copies/ml. All these women had dual high level of resistance mutations to the nucleoside reverse transcriptase inhibitors (NRTIs) and the non-nucleoside reverse transcriptase inhibitors

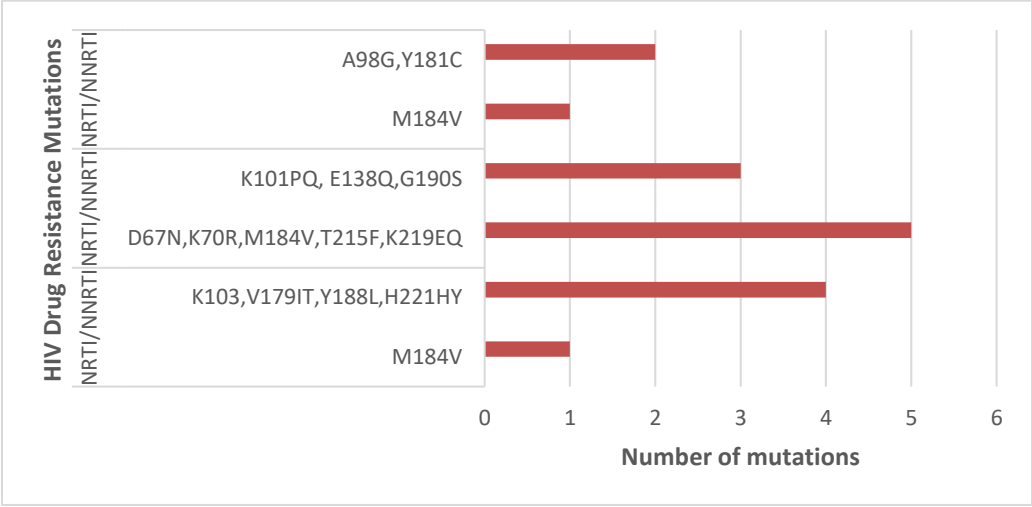
(NNRTIs). Intermediate drug resistance mutations were also present in two among the three women, one had etravirine and the other one had tenofovir drug resistance mutations. All these HIV positive pregnant women had high levels of resistance mutations to the NRTI's lamivudine and Emtricitabine. One woman had high levels of resistance mutations to another NRTIs Zidovudine. All the three women were found to have high levels of resistance mutations to NNRTIs Nevirapine and Rilpivirine, two women on Efavirenz and one woman on Etravirine. All these women had subtype A viral infection with both NNRTIs and NRTIs HIVDR mutations (***Figure 2***).

FIGURE 1: High level HIV drug resistance mutations for reverse transcriptase inhibitors



NRTI; Nucleoside reverse transcriptase inhibitor; NNRTI: Non- nucleoside reverse transcriptase inhibitors.

FIGURE 2: Antiretroviral medications with HIV drug resistance mutations



DISCUSSIONS

In this descriptive cross-sectional study conducted to estimate the proportion of HIV pregnant women with VL ≥1000copies/ml and to identify the clinical factors associated with virologic outcome, we found an overall proportion of VL ≥1000copies/ml of 23%. Amongst the three treatment arms the newly diagnosed HIV pregnant women had the highest proportion of VL ≥1000copies/ml (P= 0.006) compared to women who were previously initiated on ART and those who had taken prophylaxis for PMTCT in their previous pregnancy. We found six women with HIVDR mutation in the current study. Among the six women three of them who were previously on ART had virological failure and this poses a concern in our setting because in routine ANC pregnant women already receiving ART are continued on ART without VL testing. HIV- infected pregnant women with high VL are at risk of transmitting HIV and drug -resistant mutations to their infants.[6] Our findings showed that 8% of women had a VL ≥1000 copies/ml and were receiving ART at the time of their first ANC . A recent study done in Malawi showed 10% of women receiving ART for ≥6 months presenting to ANC were not virally suppressed.[7] The number of pregnant women receiving universal ART reporting for ANC for the first time is likely to increase. Targeted antenatal HIV RNA testing provides an opportunity to identify virological failure and minimize the risk of mother – to – child transmission.

The low percentage of women with treatment failure at the ANC suggests that Tanzania has made progress towards achieving the last 90% of the UNAIDS 90-90-90 targets among women for control of the HIV epidemic.[8] Although not statistically significant, we also observed a trend towards higher rates of viral load suppression in the younger age group (≤20 years). HIVDR mutations have been reported in Tanzania, some with thymidine analogue associated mutations which compromise susceptibility to NRTIs. Early ART failure and drug resistance were observed among two newly diagnosed treatment naïve HIV pregnant women. Switching to second-line ART without programmatic HIVDR drug resistance testing, which is a common practice, may transfer cross-resistance patterns to newly switched drugs and limit available treatment options. Initiating treatment early and ensuring optimal adherence are vital for the success and durability of first-line ART in these settings. The most common HIV drug resistance DR mutations in this study were against the reverse transcriptase inhibitors (K103N, Y188L, D67N, K70R, M184V, T215F, K219EQ and E138A) which are similar to those reported in resistance studies from other SSA and Tanzanian settings. 9-12

All the observed mutations are associated with the ARV drugs with a low genetic barrier to resistance (lamivudine, nevirapine and efavirenz) that are commonly used in SSA and Tanzania.

An important finding in this study, although limited by the small numbers, was pre HIVDR mutation in two women at baseline. Several studies have shown an association between pre-therapy resistance and an increased risk of ART failure. 13-16 This study has some limitations. Although the findings highlight several successful aspects of the program, all study facilities are high-volume where CTCs are integrated in the ANC sites located in the Kilimanjaro region. The study enrolled only women in the three sites in Kilimanjaro. However, our study fulfils a critical need for evaluation of VL suppression data among ART-experienced HIV positive pregnant women and ART-naïve women in ANC in an established Option B+ program. Future studies on HIV VL and drug resistance mutation testing for those with high VL should be planned as an optimal approach to limit transfer of cross-resistance patterns to newly switched drugs and limit available treatment options.

CONCLUSIONS

VL testing should be done on women who were already on ART on their first antenatal visit to ensure early detection of virological failure. This will enable clinicians to take an appropriate course of action on their management. Educational intervention on adherence among HIV-1 positive pregnant women on ART should be targeted at an early stage to those with virological failure during pregnancy to reduce the emergence of HIVDR mutations.

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Conflict of interest

There was no conflict of interest

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REVIEW ARTICLE

Chlamydia trachomatis and *Neisseria gonorrhoea* Co-Infection Among Patients Attending a Teaching Hospital in Nairobi County: A Retrospective Study

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ABSTRACT

Background: *Chlamydia trachomatis* and *Neisseria gonorrhoea* are microbes that have been associated with urethritis in both male and female genders, which often may lead to complicated conditions such as pelvic inflammatory disease (PID) and infertility globally among others health complications. In Kenya and other developing countries, sexually transmitted infections associated with *Chlamydia trachomatis* and *Neisseria gonorrhoea* still pose a challenge in public health.

Methods: A retrospective study was conducted by reviewing laboratory data from Jan 2018 to Dec 2018 to estimate the prevalence of *C trachomatis* and *N gonorrhoea* coinfections in patients attending a tertiary institution and its satellite clinics spread across the country. A total of 1228 patient's data aged 3-69 years was reviewed; with age, gender and *Chlamydia trachomatis* and *Neisseria gonorrhoea* status being analyzed.

Results: A total of 1228 patients who visited the hospital in 2018 had their urine samples being tested for *Chlamydia trachomatis and Neisseria gonorrhoea* by use of a PCR technique. Majority of the patients were males (63.7%). The patients who tested for *Chlamydia trachomatis and Neisseria gonorrhoea* had an average age of 34 years (range: 3–69 years). Of those 1.4% tested positive for both *Chlamydia trachomatis and Neisseria gonorrhoea* infections, and males were more infected than females (1.1% vs 0.3).

From the information gathered during the study period, the proportion of patients with *Chlamydia trachomatis* infection was (16.1 %) (95 % CI 9.5, 17.9), and with N. *gonorrhoea* infection was 5.4%. Coinfection was highest among sexually active group that is those aged between 21 years to 40 years.

Conclusion: The prevalence of *C. trachomatis* is significantly high among male patients. We recommend the implement a molecular screening for *Chlamydia trachomatis* and *Neisseria gonorrhoea* to identifying asymptomatic female cases. This study further provides evidence on the importance of contact tracing in the management of *Chlamydia trachomatis* and other STIs. There is an urgent need for studies designed to investigate the prevalence and risk factors of *Chlamydia trachomatis and Neisseria gonorrhoea* among female patients who are majorly asymptomatic in Kenya.

INTRODUCTION

Neisseria gonorrhoea and *Chlamydia trachomatis* are microbes that have been associated with urethritis in both males and females, which often may lead to complicated conditions such as pelvic inflammatory disease (PID) and infertility globally.[1] Therefore, early detection and clinical management are important in eliminating these complications. Gonococcal urethritis is caused by a gram-negative diplococci bacterium commonly associated with sexually transmitted infections (STI) in males and female patients that manifests either symptomatic or asymptomatic.[2] Early gonococcal infection in women is always asymptomatic which may further spreads to the upper part of the genital tract causing salpingitis. According to WHO, the estimated global prevalence of Chlamydia trachomatis

and gonorrhoea stands at 4.2% and 0.8% respectively. In 2014 for instance over 1 million sexually transmitted disease (STD) cases were thought to be reported per day.[3] A study by Kularatne *et al*[4] in South Africa estimated that the prevalence of *N. gonorrhoea* and *Chlamydia trachomatis* was at 6.6% and 14.7% among female patients respectively. The male had a prevalence of 3.5% and 6.0% for *N. gonorrhoea* and *Chlamydia trachomatis* respectively in 2017 among adult patients aged between 15–49 years.[4] Screening for STIs among patients is, therefore, an important way of identifying asymptomatic individuals earlier enough before any complications.[5,6] Indeed, studies have shown that earlier detection of STIs minimizes their sequelae effect in the affected individuals.[7,8]

Information currently available from surveillance data on most common STIs among patients in Kenya has been largely from studies done among high-risk groups[9,10] and special categories like expectant mothers.[11] There is however missing knowledge about the point prevalence of STIs among patients in the general population. This is because the surveillance of STIs, although recommended[12,13,14], has not been well implemented; with the major focus in Kenya in the last 10–15 years being on HIV, Tb, and Malaria. This is further complicated by the fact that surveillance guidelines exist for the screening of general patients for curable STIs and utilization of laboratory data to monitor disease patterns in the communities. Further, studies have demonstrated that a large number of infected people with no reported clinical symptoms act as a disease reservoir and act as a source of infection to susceptible groups if not detected on time.[6] Therefore, this study aimed to determine the point prevalence for *C. trachomatis, N. gonorrhoea* coinfection in the general patient population attending a tertiary teaching institution. Knowledge generated from this study is important for clinicians and health policy makers to aid them in coming up with comprehensive diagnostic guidelines.

METHODS

This was a retrospective descriptive observational study conducted at tertiary teaching and referral hospital in Nairobi, Kenya by reviewing laboratory data between January to December 2018. The hospital offers comprehensive laboratory services, with its main laboratory opened for 24 hrs. The laboratory offers molecular diagnostic services for the detection of infectious disease including *Chlamydia trachomatis and Neisseria gonorrhoea*. The study reviewed all patient's laboratory data that included the patient's age, gender and laboratory molecular results for *Chlamydia trachomatis and Neisseria gonorrhoea.* The study inclusion criteria were all the patients served during the selected review period. Patients with missing a laboratory report were excluded from the study. Data collected were entered into Microsoft Excel and statistically analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Chi-square and Fisher's exact tests were used to determining univariate social demographic factors associations of *C. trachomatis* and *N. gonorrhoea* co-infection. A p-value of <0.05 was considered to be statistically significant.

RESULTS

A total of 1228 patients had their urine samples tested for *Chlamydia trachomatis and Neisseria gonorrhoea* by use of a PCR molecular technique in 2018. The patients had an average age of 34 years with an age range between 3–69 years as shown in Table 1. The patients that were tested for *Chlamydia trachomatis and Neisseria gonorrhoea* were aged between 31-40 years (6.9%, 2.8%), followed by those between 21-30 (6.0%, 1.4%). The majority of the patients were men 781 (63.6 %) and 447 (36.4 %) were female as summarized in Table 2.

TABLE 1: Calculated mean age and distribution of the review participants

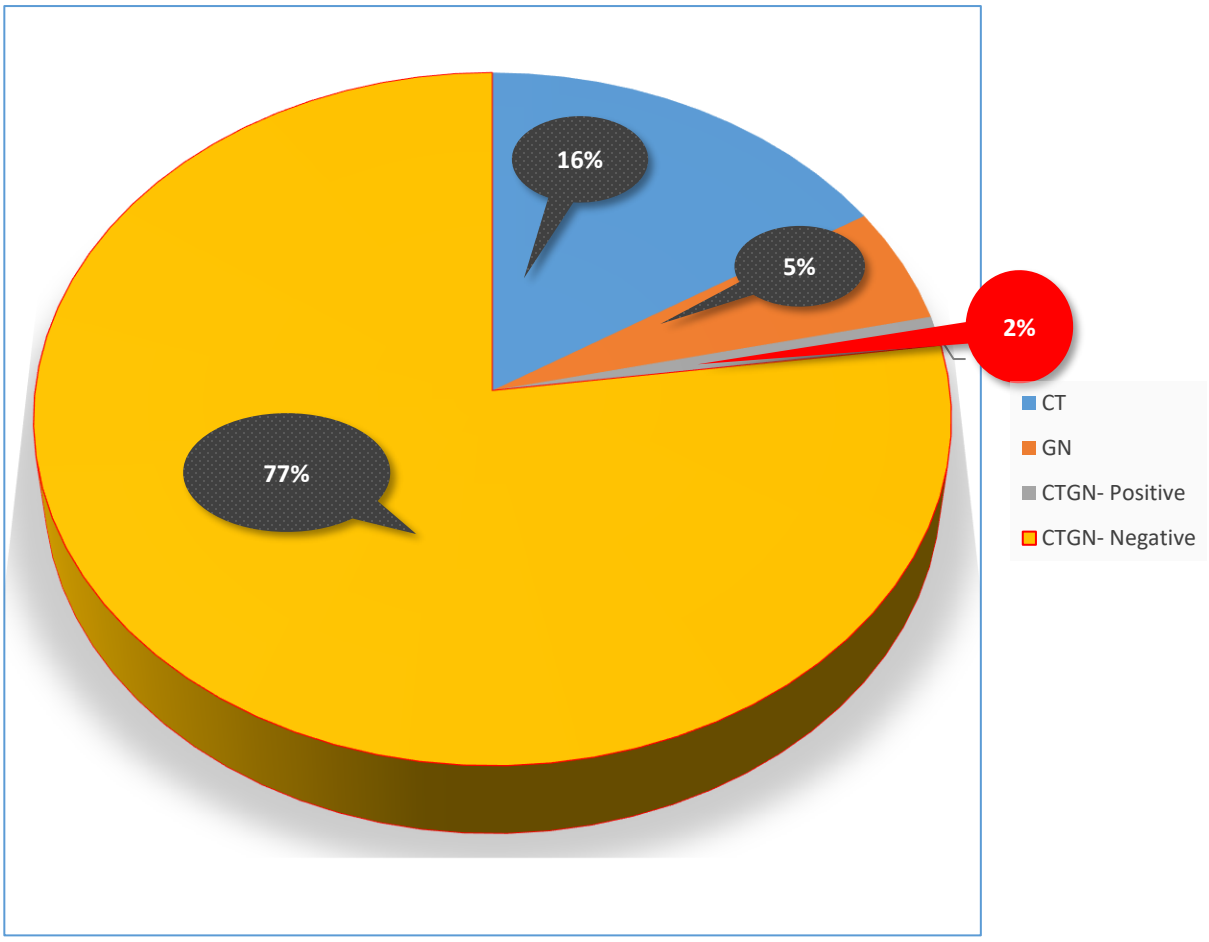
	Frequency
Mean Age	34
SD	9
Median	33
Max	69
Min	3
Age groups	
Below 20	52
21-30	421
31-40	502
41-50	187
Above 50	66

TABLE 2: Distribution of C trachomatis and N gonorrhea infection across age groups and gender.

	C trachomatis N gonorrhea				***n* (%)**
	CTNG-Negative *n* (%)	CT-Positive *n* (%)	NG-Positive *n* (%)	CTNG-Positive n (%)	
Under 20	39 (3.2%)	9 (0.7%)	4 (0.3%)	1 (0.08%)	53 (4.3%)
21-30	321 (26.1%)	74 (6.0%)	17 (1.4%)	9 (0.7%)	421 (34.3%)
31-40	378 (30.8%)	85 (6.9%)	34 (2.8%)	7 (0.6%)	504 (41.0%)
41-50	159 (12.9)	20 (1.6%)	9 (0.7%)	0 (0.0%)	188 (15.3%)
above 50	50 (4.1%)	10 (0.8%)	2 (0.2%)	0 (0.0%)	62 (5.0%)
Male	559 (45.5%)	148 (12.1%)	61 (5.0%)	13 (1.1%)	781 (63.6%)
Female	388 (31.6%)	50 (4.1%)	5 (0.4%)	4 (0.3%)	447 (36.4%)

From the information gathered during the study, *Chlamydia trachomatis had* the highest prevalence of 16 % (198/1228) (95 % CI 9.5, 17.9), and *N gonorrhoea* at 66/1228 (5 %) as shown in Figure 1. A co-infection of *Chlamydia trachomatis and N gonorrhoea* was found in 17/1228 (2 %) of the sampled data. Of all the patients 947 (77%) were found to have no *Chlamydia trachomatis and Neisseria gonorrhoea* infection during the review period Figure 1.

FIGURE 1. Chlamydia trachomatis and Neisseria Gonorrhea co-infection from the study sample



Higher frequency of *Chlamydia trachomatis* and *N gonorrhoea* were observed among patients aged 31–40 years which was at 6.9% and 2.8% respectively and lowest (0.7%, 0.3%) among patients aged below 20 years respectively. The male patients exhibited the highest prevalence of n=148/198 (74.7%) as presented in Figure 1. Coinfection was highest at 9/17 (52.2%) among patients aged between 21-30 years and among the male patients at 13/17 (76.5 %). Least prevalence was seen in the age groups above 40 years Table 2.

DISCUSSION

This study estimated point prevalence of *C. trachomatis* and *N. gonorrhoea* co-infection among patients whose urine sample were tested by a PCR technique. The point prevalence of *C. trachomatis* and *N. gonorrhoea* co-infection as reported by this study stood at 1.4%(in the year 2018. With the highest prevalence being seen in the male patients aged between 31-40 yrs. with a p-value of 0.31. However, patients above 40 years had the least prevalence was recorded. These study findings were comparable to 2.8 % reported in previously published studies among female patients at a US juvenile centre and with a lower prevalence as compared to 4.6 % reported among men how to have sex with men.[15,16]

A higher *Chlamydia trachomatis* prevalence of 16 % was also reported in this study among patients aged 30-41 years. Similar studies have found a prevalence of between 6 and 16 % among female patients attending family planning clinics in Nairobi- Kenya.[17,10,18] What we can learn from this is that the prevalence of *C. trachomatis* is of great concern among the male patients and individuals in their mid-years. This high numbers could be attributed to the symptomatic nature of *C trachomatis* among the male gender and increased sexual activities among individuals in their mid-years. Therefore, introducing a molecular screening procedure for routine screening of STIs will help in reducing the burden of the disease.[7]

However, a point prevalence of 5% for *N. gonorrhoea* is less than what is seen in initially published work by Marx et al[19] from a similar setting who found an *N. gonorrhoea* prevalence of 6 % among HIV-1 infected pregnant patients in Nairobi; in another study by Fonck et al.[20] he found a prevalence of 6 % for *Chlamydia trachomatis* and 4 % for *Neisseria gonorrhoea* among patients with complaints of vaginal discharge attending a sexually transmitted diseases (STD) referral clinic in Nairobi. While Daly et al.[21] found a *Neisseria gonorrhoea* prevalence of 3.2 % among patients seeking treatment in Nairobi which is lower as compared to what was found in this study.

It was not clear why this study recorded a lower prevalence of *Chlamydia trachomatis and gonorrhoea* among female patients as most studies have shown that a higher prevalence among female patients. However, the female patients were the minority in our study as compared to male patients. The higher *Neisseria gonorrhoea* and *Chlamydia trachomatis prevalence* could be attributed to a good precision of the molecular method as compared to traditional culture methods.[22,23]

All the patients that turned out to be positive *Chlamydia trachomatis* and *N gonorrhoea* were aged between the 21-50 and the male patients being the majority. These findings, therefore, suggest that the male patients were symptomatic as compared to the female gender. These further depict the importance of contact tracing, testing, and treatment to reduce re-infection.[24]

The high proportion of symptomatic patients who tested positive for *C. trachomatis* that is in agreement with other findings and further emphasizes the importance of molecular techniques in the diagnosis and management of curable STIs.[24] However, due to the high cost of molecular techniques and poorly equipped laboratory in most of the developing countries, the WHO guidelines recommend a syndromic approach in the management of STIs.[25] Syndromic management consists of a group of symptoms and easily recognized signs that are identified before treatment.[26]

CONCLUSION

The prevalence of *C. trachomatis* is significantly high among male patients. We recommend the implement a molecular screening for *Chlamydia trachomatis* and *Neisseria gonorrhoea* to identifying asymptomatic female cases. This study further provides evidence on the importance of contact tracing in the management of *Chlamydia trachomatis* and other STIs. There is an urgent need for studies designed to investigate the prevalence and risk factors of *Chlamydia trachomatis and Neisseria gonorrhoea* among female patients who are majorly asymptomatic in Kenya.

Ethical consideration

Ethics approval was not sorted for this retrospectively obtained and anonymized data non-interventional study.

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Conflict of interest

There was no conflict of interest

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REVIEW ARTICLE

Prophylactic Effects of ARTAVOL® on *Plasmodium berghei* Infected Mice

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ABSTRACT

Introduction: Despite the efforts of governments and health organisations to eradicate malaria, it is still endemic in sub-Saharan Africa and this could be due to cost of antimalarial drugs, resistance to these drugs and climate change among others. Traditional medicine practitioners and scientists have started developing antimalarial drugs from medicinal plants among which is ARTAVOL®. ARTAVOL® is a herbal product that is used to prevent malaria in some communities in Uganda, however, its prophylactic effects on *Plasmodium berghei* infected mice has not been established yet.

Methods: The infusion of ARTAVOL® powder was prepared, cooled, filtered, concentrated *in vacuo* at 55 ^{0}C and freeze-dried. The freeze-dried extract was reconstituted with distilled water for antimalarial using prophylactic model mice. Thirty-six mice were randomised into 6 groups of 6 mice each. Groups I to III mice were orally administered with the extract at 15 to 60 mg/kg/day while group IV received Pyrimethamine (1.25 mg/kg) while groups V and VI (uninfected) received 0.2 mL distilled water for seven days before the inoculation of *Plasmodium berghei* ANKA parasites on day 7 (D_7). The parasitaemia levels were examined after 72 hours using standard procedure and blood collected through cardiac puncture for haematological study.

Results: The Lethal Dose (LD_{50}) of the crude ARTAVOL extract was greater than 5000 mg/kg. Also, there was calmness and paw licking immediately after dosing which stopped after few minutes. Significant reduction in parasitaemia level was observed in all test doses when compared with negative control. At 30 mg/kg, the extract gave 62.9% suppression, which was not significantly different from that of 60 mg/kg (68.7%) on day 3. On day 5, the extract gave 62.3% and 66.4% Suppressions At 30 And 60 Mg/Kg That Were Not Significantly Different From Each Other. A Dose Dependent Reversal of Hematocrit (HCT) reduction was observed at the 3 dose levels but their parameters did not show any significant difference when compared to the normal group but significant when compared with negative control.

Conclusion: ARTAVOL® extract has shown a dose dependent reducing effect on the level of parasitaemia in *P.berghei* infected mice; it is acutely safe and has ability to increase RBC counts.

Keywords: ARTAVOL®, Parasitaemia, *Plasmodium berghei*, Prophylactic activity

INTRODUCTION

Malaria remains one of the Public Health challenges in Africa[1]. According to WHO malaria report (2018), an estimated 228 million cases of malaria occurred worldwide[2]. The WHO global malaria control programme strategy 2030 is aimed at reducing malaria case incidence by at least 90%, eradicating malaria in at least 35 countries and preventing a resurgence of malaria in countries that are malaria

free[3]. A number of protective interventions are being used in endemic areas to reduce the risk of malaria infection. These include chemoprophylaxis, using conventional medicines recommended by WHO[4, 5]. However, the designed eradication programs have had challenges such as poorly managed Vector Control Programs, emergence of antimalarial drug resistance, poverty and the effect of global warming and climate change among others[6, 7]. Antimalarial drug resistance also poses a challenge to malaria control measures[8-10].

Since 2018, Uganda recorded a surge in the incidence of malaria in some districts due to a number of factors such as climate change, a decline in the use of protective nets and high influx of refugees[11]. A number of strategies are being deployed to control the mosquitoes in Uganda. These include:- use of Long-Lasting Insecticidal Nets (LLINs), Indoor Residual Spraying of Insecticide (IRS), clearing bushes around homesteads and draining of waterlogged ditches around homesteads to prevent mosquito breeding[12].

Traditional medicines have been used to treat malaria for thousands of years and are part of the culture and tradition of African people[5]. It has been reported that up 80% of the human population in developing countries rely on herbal medicinal products as their primary source of healthcare[6]. Many developed and developing countries have also embraced the use of herbal remedies as complementary and alternative medicines[13].

The traditional medical practitioners of Uganda use symptoms like high temperature, shivering, among others in malaria diagnosis[14]. Some people in rural communities in Uganda use herbal plants and plant products to prevent and treat malaria. The most commonly used plants are: - *Aloe* species, *Vernonia amygdalina*, *Azadirachta indica* A Juss, *Moringa oleifera* Lam among others[14-17]. ARTAVOL® is one of the herbal products used in the prevention of malaria in Uganda and was developed by scientists at Natural Chemotherapeutical Research Laboratory and Makerere University[18]. ARTAVOL® is composed of extracts of dried *Artemisia annua*, avocado seed base and lemon grass extract. The artemisinin in the *A. annua* is first removed from the extract by partitioning with petroleum ether and using separating funnel to separate the two-immiscible layers formed. The aqueous layer is further concentrated, and then mixed with avocado seed extract and lemon grass extract for the production of ARTAVOL. The major phytochemical ingredients of ARTAVOL® are coumarins, sterols, triterpenes, flavonoids and lemon grass derivatives[18, 19]. The powder is dissolved in hot water, porridge or hot milk and taken as a beverage to prevent frequent fevers, worm infestation and malaria[18]. Although, ARTAVOL® is being used to prevent malaria, its ability to reduce parasitaemia levels in mice infected with *Plasmodium berghei* has not yet been established. Hence, this research studied the prophylactic effects of ARTAVOL® with a view to providing the missing information on its activity *in vivo* using *Plasmodium berghei*-infected mice.

MATERIALS AND METHODS

ARTAVOL® product was obtained from ARTAVOL LTD (ARTAVAL LTD. P.O. Box. 34 Ntinda, Kampala-Uganda) the manufacturer.

A picture of ARTAVOL® tin.

Malaria parasites

The chloroquine sensitive *Plasmodium berghei* ANKA was obtained from Biodefense and Emerging Infection Research Resources Repository (BEI Resources), United State of America (USA).

Laboratory animals

Adult male and female Swiss albino mice between 18 to 22 grams were obtained from the Animal Laboratory Research, Mbarara University of Science and Technology, Uganda. The mice were fed on grower pellets and had free access to water. The mice were allowed to acclimatise for 2 weeks before the experiments.

Ethical Considerations

Permission to use ARTAVOL® herbal product in the study was obtained from developers of the product. This study also received ethical clearance from Mbarara University Research and Ethics Committee and Uganda National Council for Science and Technology registration number HS465ES. All the animals in the experimental study were treated humanely according the Organization for Economic Co-operation and Development (OECD) guidelines and American Psychological Association[20, 21]

Preparation of Crude ARTAVOL Extracts

The ARTAVOL® extract was prepared using infusion method in which distilled water was boiled at 600c, powered into 173 g powdered ARTAVOL®, and

allowed to stand for 15 minutes. Thereafter, the extract was filtered, concentrated in vacuo at 55 0C and freeze-dried. The aliquot of the stock solution of 100mg/mL concentration was prepared and stored in a fridge 5-8^{0}c. The test doses of 15,30 and 60mg/Kg was separately determined from the stock solution and administered orally to already grouped mice.

Acute toxicity study of the Crude ARTAVOL Extracts

The median Lethal Dose (LD_{50}) of ARTAVOL was determined *in vivo* using the Lorke[22] method. In the first phase, 9 mice were randomly divided into 3 groups of 3 mice each and each group received the extract at 10, 100 and 1000 mg/kg body weight orally (via a feeding cannula). The mice were then observed for signs of adverse effects and mortality for the first 48 hours and then for 12 more days. In the second phase, 4 mice were divided into 4 groups of 1 mouse each and similarly treated but at doses of 1000, 1600, 2900 and 5000 mg/kg orally. The animals were then monitored for any sign of toxicity like stretching, rubbing of nose on the floor and wall of cage, change in body weight and mortality over a period of 24 hours and then 14 days. LD_{50} was calculated using the formula:

$LD_{50} = [A \times B]^{1/2}$ Where A= highest non-lethal dose and B= the lowest lethal dose.

Preparation of malaria parasite Inoculum

Chloroquine (CQ)-sensitive *Plasmodium berghei* ANKA parasites were activated in the mice according to BEI Resources procedures. The vial containing the parasites was defrosted in water bath at 35^{0}C and the lid was wiped with 70% ethanol before opening. About 0.2 mL of inoculum was injected into the donor mouse intraperitoneally and the parasites growth was monitored after 72 hours by preparation of smear from the blood taken from the tail of infected mice. The parasites were maintained by continuous blood passage of the blood collected from the donor mouse into a new group of mice. A standard inoculum of 1×10^7 parasitized erythrocytes was prepared by dilution of blood collected through cardiac puncture from a donor mouse (> 30% parasitaemia) with normal saline and administered intraperitoneally (200 µL) to each test mouse.

Calculation of the inoculum

The percentage parasitaemia of the donor mouse was determined as follows:

Percentage parasitaemia= P.RBC/T.RBC * 100 [23, 24]

Where P.RBC= parasitized red blood cells and T.RBC =Total number of red blood cells counted.

The total number of RBC counted on different fields should be 1000 cells. Using $C_1V_1 = C_2V_2$ and having known the volume of infected blood from the donor, the collected volume from donor mouse was diluted to obtain 2% parasitaemia in infected blood (2% parasitaemia is the recommended concentration of the inoculum). Assuming the percentage of parasitaemia in the donor mouse is 30% and volume of blood collected is 0.5mL; then using the above formula $C_1V_1 = C_2V_2$. C_1 = 30%, V_1= 0.5mL, C_2%, V_2. $C_1V_1 = C_2V_2$= 30% * 0.5/2% * V_2 therefore V_2= 30% * 0.5/2= 7.5mL. Top up 0.5mL to 7mL of PBS to make 7.5mL. The volume needed for all the animals to be inoculated = no of animals x volume needed for each animal. Volume for 30 mice is 30 * 0.2 mL = 6.0 mL.

Prophylactic Test and Determination of Parasitaemia levels.

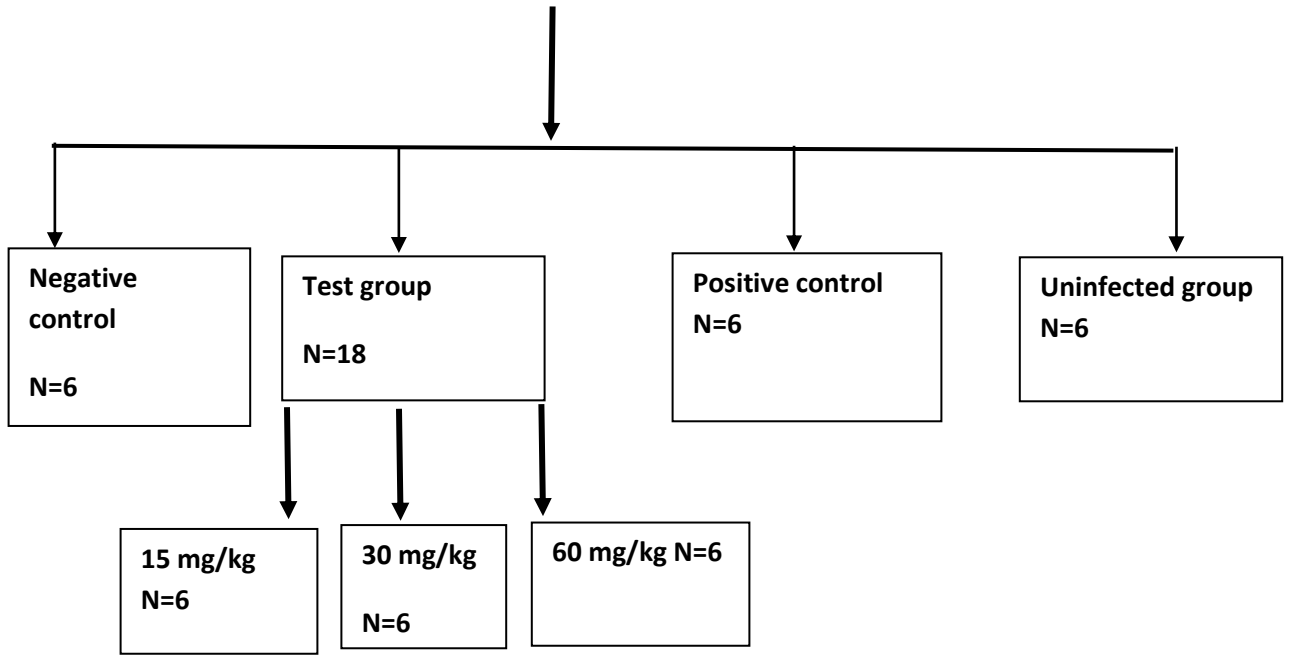
The prophylactic antimalarial potential of ARTAVOL® was carried out according to Peter's method 2002[25]. Thirty six (36) mice were grouped into 6 groups of 6 mice each; Mice in Groups I –III were orally administered with ARTAVOL® by using oral cannula while the animals in Group IV were given Pyrimethamine at 1.25 mg/kg/day (positive control), group V (negative control) and group VI (not infected) received 0.2 ml/mouse/day distilled water for 7 consecutive days ($D_1 - D_7$) respectively. On day 7 (D_7), the mice in group I-V were inoculated with *P. berghei* parasite. On day 3 (72 hours) and day 5 (120 hours) after inoculation, blood samples were collected from the tail vein of the mice and thin blood films on microscope slides were prepared, air-dried and fixed with methanol. The fixed thin smears of blood were stained with 10% Giemsa stain diluted with buffered water (pH = 7.2) for 20 to 30 minutes. The percentage parasitaemia was determined by counting the number of parasitized red blood cells out of 1000 blood cells in 10 randomly selected microscopic fields using oil immersion lens (×100). Percentage parasitaemia level was determined using the formula: Parasitaemia level= $(Np/Nt) \times 100$, where N_p is the number of parasitized red blood cells and N_t is the total number of the red blood cells.

The percentage suppression was determined by:

$$PS = \left\{\frac{X-Y}{X}\right\} \times 100$$[26].

Where 'X'= mean parasitaemia of negative control group, 'Y'= parasitaemia of test group.

FIGURE 1: Schematic diagram of mice grouping for prophylactic study



Determination of Packed Cell Volume, RBC & WBC

On day 6 post inoculation, the mice were sacrificed, blood was collected through cardiac puncture and kept in EDTA tubes to prevent dotting. A hematology analyser (Beckman Coulter AC.T 5Diff – CP by Beckman Coulter Inc, California, USA) was used to measure the PCV, RBC and WBC values.

Statistical analysis

Data was analysed using Graph Pad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Results obtained from the study were expressed as mean ± standard error of mean[27]. The variation in a set of data was analysed through One-way Analysis of Variance while the difference among the means was considered at 95% confidence level using the post-hoc method of Tukey's Multiple Comparison Test.

RESULTS

The acute oral toxicity test of aqueous ARTAVOL® extract caused no gross behavioural changes such as loss of appetite, paw licking, body temperature, calmness, locomotion, etc., and there was no mortality within 24 hours as well as in the next 14 days, indicating that the LD_{50} values of the aqueous ARTAVOL® extract were greater than 5000 mg/Kg in mice.

On day 3, the ARTAVOL® extract gave a dose dependent activity by reducing the growth of the parasite at 30 mg/kg to 60 mg/kg from 8.11% to 5.35%, which was significantly different from negative control with 14.4 % (Table 1). In addition, the ARTAVOL® extract gave 62.9% suppression at 30 mg/kg, which was not significantly different from 60 mg/kg with 68.7% suppression on day 3 (Fig.2). On day 5, the extract gave 62.3% suppression at 30 mg/kg, which was not significantly different from 60 mg/kg with 66.4% suppression (Fig 2).

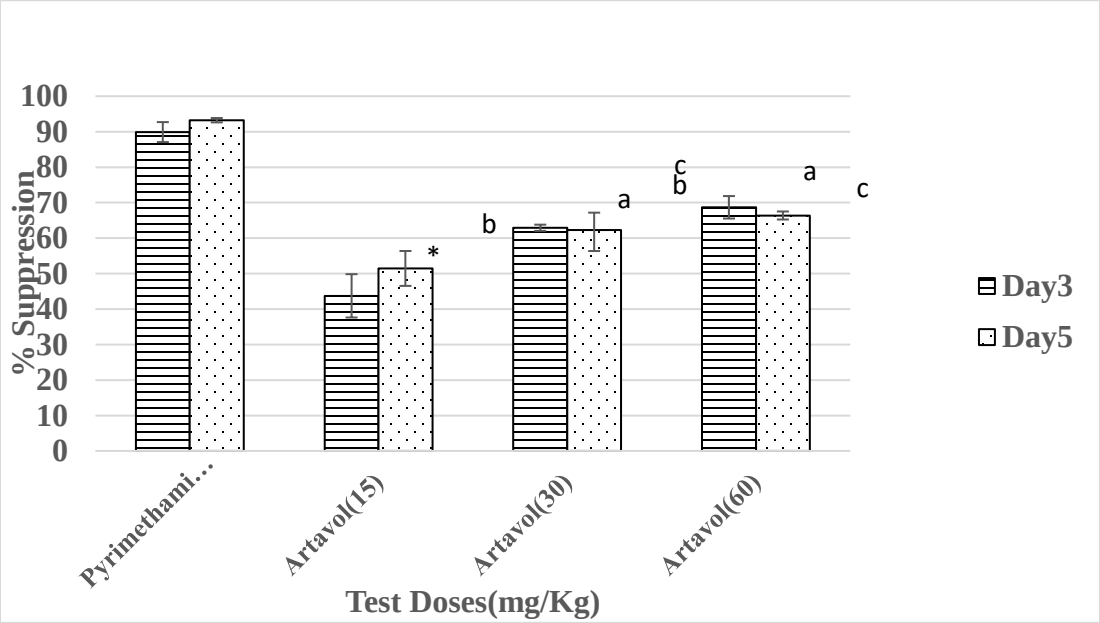
Table 1 shows the parasitaemia level on day 3 and Day 5, Figure 2 shows the percentage parasitaemia suppression in the treated (ARTAVOL® 15-60mg/kg) groups in comparison to positive control group on day 3 and day 5 and Table 2 shows the effect of ARTAVOL® prophylaxis on Monocytes, HCT, and RBC values of P. berghei infected mice.

TABLE 1: The level of Parasitaemia in treated (ARTAVOL® at 15-60mg/kg) and control groups

	Parasitaemia (%)			
	Day 3 after infection		Day 5 after infection	
Test doses (mg/kg)	Parasitaemia	CI	Parasitaemia	CI
Negative Control (0.2mL distilled H_2O)	14.42±0.63	-	23.60 ±2.37	-
Pyrimethamine (1.25)	1.46±0.41***	10.64 -15.28	1.60 ±0.14 ***	16.50 --27.51
ARTAVOL(15)	8.11±0.88***	3.983- 8.624	11.46 ±1.16 ***	6.633 -17.65
ARTAVOL(30)	5.35±0.13**	6.750-11.39	8.91 ±1.40***	8.918- 20.47
ARTAVOL(60)	4.51±0.46**	7.582 -12.22	7.94 ±0.27***	10.16- 21.17

*Values are presented as Mean ± SEM, ***means P<0.0001, ** means P<0.001 when compared to Negative control for parasitaemia*

FIGURE 2: The Percentage parasitaemia suppression in the treated (ARTAVOL® 15-60mg/kg) groups in comparison to positive control group on day 3 and day; Superscripted letters implies P>0.05



All the extract doses significantly reduced parasitaemia when compared to negative, however, the effect was still significantly lower when compared to the positive control drug. The percentage parasitaemia of negative control dramatically increased after Day 3 and Day 5 post treatment with parasitaemia level of 14.42% and 23.60 % respectively (Table 1). The extract showed a relative suppression activity but it was not significant when compared to positive control.

TABLE 1: Effect of ARTAVOL® prophylaxis on Monocytes, HCT, and RBC values of P. berghei infected mice

Treatment Groups	HCT (%)	CI	RBCx10^{12}/L	CI	Monocytes (%)	CI
Normal group	41.80±0.98	-	9.03±0.37	-	12.18±1.62	-
Negative control	19.00±3.73***	9.96-35.63	3.84±0.56***	2.31-8.09	4.37±0.82***	3.62-11.99
Positive control	33.98±2.16[a]	-6.24-21.88	7.68±0.58[a]	-1.82-4.52	5.17±0.75***	2.84-11.19
15mg/Kg	33.15±3.03[a]	-4.19-21.48	6.63±0.90[a]	-0.49-5.29	4.32±0.69***	3.67-12.04
30mg/Kg	39.47±3.77[a]	-12.85-17.52	7.54±0.42[a]	-1.93-4.91	6.77±0.96*	0.46-10.36
60mg/Kg	37.55±0.51[a]	-9.81-18.31	7.43±0.46[a]	-1.56-4.78	8.22±0.86[a]	-0.23-8.14

***P< 0.0001, **P<0.001, *P<0.05, [a]P >0.05 compared to normal group

The 3 test doses of extract in a dose dependent way and positive control were able to reverse the HCT reduction (Table 2) which could have been caused by infection and their parameters did not show any significant difference when compared to the normal group but significant when compared with negative control.

All ARTAVOL® test doses prevented destruction of RBCs (Table 2) due to infection by *P. berghei* and this was not significantly different as compared to normal group but significant when compared to negative control. There was no significant difference in red blood cell count (Table 2) in ARTAVOL® extracts doses when compared positive control but significant when compared to negative control.

The ARTAVOL® extract doses at 15mg/Kg and 30mg/Kg showed a significant rise in the level of monocytes (Table 2) compared to negative control group.

DISCUSSION

The prophylactic anti-plasmodial activity of the aqueous ARTAVOL® extract on *Plasmodium berghei* infected mice produced a dose-dependent activity. The extract elicited suppression activity at the 3 doses investigated. The test doses of ARTAVOL® extract was able to inhibit the growth in the parasitaemia levels and this reduction was significant when compared with the negative group. Despite the overall less suppression effect compared to the positive control group, all test doses of ARTAVOL® extract produced a dose related significant changes in parasitaemia suppression as compared to the negative control group. It has been reported that a compound/product is active when its percentage chemosuppression is more than 30%[28].In this study, the aqueous extract of ARTAVOL® produced chemosuppression in the prophylactic activity with over 62%.

All the test doses of the ARTAVOL extract caused significant increase in monocytes count when compared to negative control group and was not different from the normal group. Widiyantoro[29] reported that secondary metabolites including alkaloids, flavonoids, terpenoids, steroid, phenolic, and saponins stimulate extra release of monocytes in circulation. It was also reported that monocytes are one of the innate immune cells that play a significant role in eliminating parasites through the mechanism of phagocytosis[30-32].

Hematocrit (HCT) values were measured in this study to determine the effectiveness of ARTAVOL® extract in inhibiting hemolysis of the red blood cells (RBCs) and further infection of normal RBCs. The ARTAVOL® extract in this study was able to inhibit further destruction of RBCs in tested mice to level that was significant when compared to the negative control. Many studies have reported that *Plasmodium* infections causes anaemia and this may be due to rapid destruction of infected erythrocytes[33, 34]. The slight increase in HCT values in this study demonstrated a progress in disease regression, which suggests

ARTAVOL® extract could have prevented destruction of RBCs during infection. There was no significant effects of ARTAVOL® extract on RBC counts when compared with the normal group. This implies that ARTAVOL® extract inhibited destruction of red blood cells. This could be probably due to the presence of secondary metabolites including alkaloids, coumarins, flavonoids, sterols, triterpenes, tannins, volatile oils, fatty acids and reducing compounds which were reported to have anti-parasitic effects[18].Flavonoids have also been reported to have antioxidant, anti-inflammation properties and inhibit the parasites protein synthesis[35, 36].

CONCLUSION

In this study, the aqueous ARTAVOL® extract did not show any lethal effect in the tested mice up to 5000mg/kg dose and therefore could be acutely safe. Also, the extract has displayed a dose dependent reducing effect on the level of parasitaemia in the tested mice and could therefore be recommended as an antimalarial prophylaxis. Further investigation on its sub-chronic effect and its immuno-modulatory effects is recommended.

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Competing Interests

None declared.

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ORIGINAL ARTICLE

Survey of Urinary Aflatoxin Levels Among Residents of Makueni County, Kenya: A Follow-Up Study

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ABSTRACT

Although fungi are known to be less pathogenic and mostly saprophytic in their nature as compared to other groups of microbes, those that produce aflatoxin have been associated with severe human disease. An example of such disease is Aflatoxicosis caused by soil-borne pathogenic fungi of the species *Aspergillus parasiticus* and *Aspergillus flavus*. They produce a mycotoxin substance that is carcinogenic to the human liver with severe outcomes. The objective of this study was to determine urinary aflatoxin levels among the residents of Makueni County, previously affected by Aflatoxicosis.

This was a cross-sectional study that involved the use of primary data collected from 106 participants. The method for data collection included a structured questionnaire and the collection of the urine samples for aflatoxin M1 analysis at Bora Biotech Laboratories LTD. The urinary levels of AFM1 were detected by use of an ELISA kit. Data was entered in SPSS and analysed through Chi-Square for the association.

The study participants, including both male and female, had an age of between 15 and 91 years and with an average age of 41±18. Out of the 106 study participants, n=68 (72%) were females and n=26 (28%) were males. Majority of the study participants were with a median age of 24 years old. AFM1 levels were detected in 99.1% % of all urine samples at a range of 25-2337 pg./ml. The mean and median concentration of AFM1 in urine was 637.6 ± 512.7and 525 pg./mL, respectively.

The results of this study provide information on the current situation of aflatoxin exposure. From what is evident from our study a lot needs to be done to mitigate on the long-term effect of this high exposure. Therefore, the study encourages the concerned ministry to have a broader focus on the extent of aflatoxin food contamination from this region plus other regions across the country.

Keywords: Urinary Aflatoxin Levels in Makueni County, Kenya

INTRODUCTION

Fungi are a group of microbes found everywhere in the environment and have been associated with human illness. Although fungi are known to be less pathogenic and mostly saprophytic in nature as compared to other groups of microbes, those that produce aflatoxin have been associated with severe human disease. An example of such disease is Aflatoxicosis caused by soil-borne pathogenic fungi of the species *Aspergillus parasiticus* and *Aspergillus flavus*. These two species are known to contaminate foodstuffs such as maize, rice, groundnuts, sorghum, wheat, millet and cassava among others. They produce a mycotoxin substance that is toxic to the human liver with severe outcomes. For those exposed to this carcinogenic Aflatoxin, the condition may range from acute to chronic state. The severity of the condition is related to the host factors, which include; age, diet, nutrition quality, the extent of the exposure, other underlying diseases condition and gender of the affected individual. Clinical manifestation in Aflatoxicosis include; severe jaundice, liver cirrhosis and imminent liver failure. Other organs affected by Aflatoxin B1 (AFB1) apart from the liver include; oesophagus causing oesophagial carcinoma which is the 6th most prevalent type of cancer globally

Historically Aflatoxin discovery was in Great Britain in the 1960s, after an outbreak of a disease in turkeys that was referred to as Turkey X disease by then. During this outbreak over 100.000 people lost their lives. After investigation, this mortality was discovered to be caused by a fungal metabolite called Aflatoxin common in mouldy cereal feeds. [1-3] This was followed by numerous outbreaks that were reported globally. Aflatoxins are poisonous molecules produced by certain kinds of fungi (mould) that are found naturally all over the world. They can contaminate food cereals and present a harmful effect on man and domesticated animals.

In Kenya, a first local case of Aflatoxicosis poisoning was reported in the late 1970s in the former eastern province. However, the worst aflatoxin outbreak happened in 2004 in Kitui and Makueni districts of the then eastern province of Kenya. [4, 5] Ever since, the eastern region remains to be an aflatoxin prone area.[6] By this time nobody knew what was happening until when samples collected from the affected patients to investigate for possible known causes of hepatitis returned negative results for known viral infections (Environ Health Perspect, 2005). Further evaluation, gave a similar clinical picture and resembles tthat seen in aflatoxin poisoning from symptomatic cases reported in Machakos in 1981. [7] This was followed by maize sampling from the affected areas for analysis which confirmed the presence AFB1 with as high as 4400 µg / kg. This on average was far above the minimum required standard of 10 µg/kg of aflatoxin levels recommended for food meant for human consumption. [8-10] This outbreak was attributed to widespread aflatoxin contamination of maize grown locally characterized by poor drying, storage under damp conditions and exposure to humid conditions. [6]

Several preventive strategies are available with most of them focusing on proper pre and post-harvesting storage, adequate processing before consumption, of food cereals and many others. Aflatoxins contamination depends on various factors ranging from; how crops are planted, harvested, stored, and processed for human and animal consumption. However irrespective of having adequate measures to combat aflatoxins poisoning what we need to remember is that they are not destroyed by heating at the time of food preparation. The same is still applicable to manufactured products like peanut butter and other industrial processed products and their potency would still affect the consumer depending on the quantity of food available. [11-16] The focus of this study was therefore to determine the prevalence rates and compliance to aflatoxin preventive measures among the residents of Makueni County, previously affected by Aflatoxicosis.

METHODS

Study Area

This study was purposefully carried out in Makueni County, which had the highest number of fatalities following Aflatoxin poisoning in 2004. The County is made up of five Sub-Counties namely; Mbooni, Kaiti, Kilome, Kibwezi West and Kibwezi East. The County has a total population of 987,653 communities (Census, 2019). Its annual rainfall ranges between 800-1200 mm. Majority of the County is arid with temperature ranging between 20^0 C to 24^0 C. Farming in the area is largely for subsistence crops like maize, beans, peas, cassava sweet potatoes, millet, and sorghum. They also do fruit farming of watermelons, pawpaw, oranges, mangoes and lemons. Their main domesticated animals include cows, goats, sheep, and donkeys.

Study Design

This was a cross-sectional study and using primary information collected randomly from the patients attending Makueni County Referral Hospital through the collection of urine samples for laboratory analysis,

interviews and hospital records for those undergoing treatment or have completed treatment and information on any death.

Study Population, Inclusion and Exclusion Criteria

Sample Size

The sample size was 100 participants determined based on the incidence rate due to infected maize consumed at the time of the 2004 outbreak of aflatoxin in which Makueni County had the highest deaths. [17]

Sampling Procedure

Urine samples were collected by participants in clean containers at Makueni county referral hospital in the month of May 2020. The samples were frozen at –20°C until analysis.

Analyzing Urine for the Presence of Aflatoxin

The urine samples were collected in sterile urine containers and transported in a cool box to Bora Biotech Labs in Nairobi for aflatoxin M1detection. Before the samples were analyzed, they were allowed to thaw at room temperature. Then 5 ml of thawed urine from each participant was centrifuged at 4000 rpm for at least 10 min. Skatron assay tubes were labeled with the participant's sample identification number. Then 950 ml of distilled water were pipetted into skatron (SKATRON AS LIER, Norway. CAT. No 7071) tubes and 50 µl of standards or supernatant-urine was added into 950 µl of distilled water in the skatron tubes and mixed by priming pipetting at least five times. 200 µl of the assay buffer was added into the mixing well per plate and 100 µl of the diluted standards (ranging from 0 to 40 ppt) and urine samples were added into the wells.

The contents of the mixing well were shaken using a micro-shaker (DYNATECH) for 2 min. One hundred microliters of the mixture were transferred to the antibody-coated Reaction-Assay Plate (aflatoxin M1 assay for urine, Helica Biosystems Inc, 1527 W. Alton Santa Ana, California, USA). The samples were mixed by shaking for 1 min and incubated at 18-28°C in the dark for 1 h. The plate was washed three times using phosphate-buffered saline–Tween-20 (0.05%) using the well-wash (Thermo Scientific, Finland) machine with 3-min intervals between the washes. After drying, 100 µl of the conjugate was added into each well, mixed gently by tapping, and incubated at room temperature for 15 min in the dark. The plate was then washed and 100 µl of substrate reagent (tetramethylethidine) was added into each well, mixed gently by tapping, and incubated at room temperature for 15 min in the dark. The reactions were stopped by adding 100 µl per well of stop solution and the optical density (OD) read at 450 nm within 15 min of stopping the reaction. The level in each sample was determined using the program from the kit manufacturer, which allowed calculations of the levels based on the absorbance readings.

Ethical Consideration

Ethical approval was obtained from the Kenyatta University's Ethical review committee. Other authorizations sought before the commencement of the study include informed written consent from the research participants, approval from NACOSTI and authorization from the hospital administration.

Data Analysis

Collected data were entered in a computer excel spreadsheets and statistically analyzed using SPSS Version 20.0. Exploration of the data was by numerical summaries together with the use of graphics. Social demography characteristics of the participants were presented in contingency tables as mean ± standard deviation. The level of significance was accepted as $p < 0.05$. The strength of association between variables was determined using chi-square test.

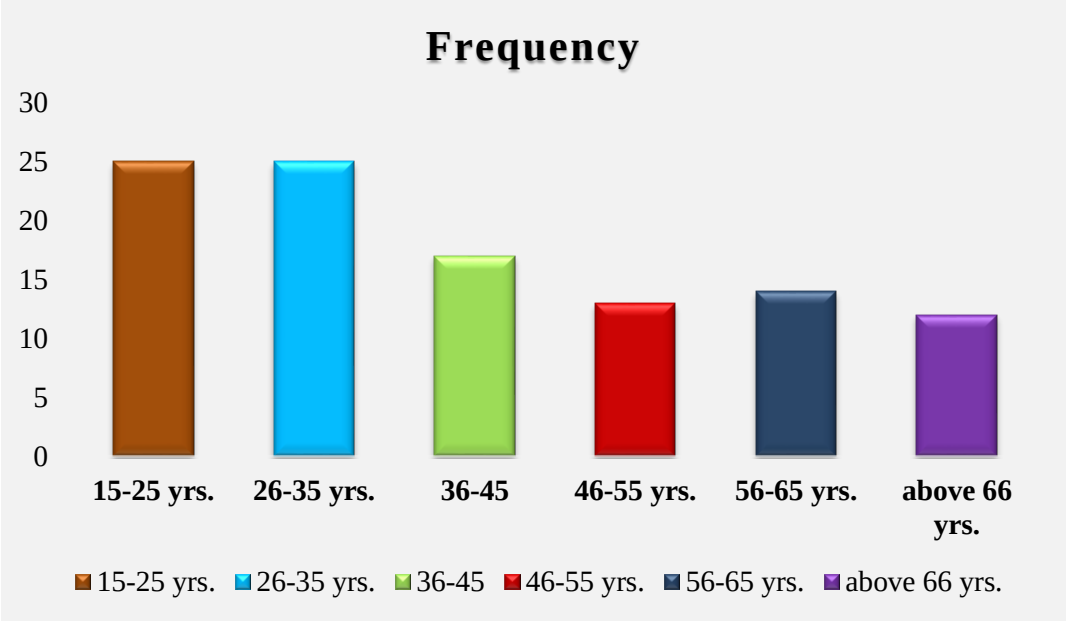
RESULTS

Sociodemographic Characteristics of the Participants

Age distribution of studies of participants

The study participants, including both males and females, had an age range of between 15 and 91 years. On average the participants were between 41±18 of age. Majority of the study participants were with a median age of 24 years old. Half of the selected participants were aged between 15 and 35 years n= 50 (47%) (Figure 1). A total of 106 study participants underwent an initial examination that included a medical history taking. Of the 106 screened study participants, all were to be of sound health and were nonsmokers. For the analyses conducted in the current study, no study participants were excluded.

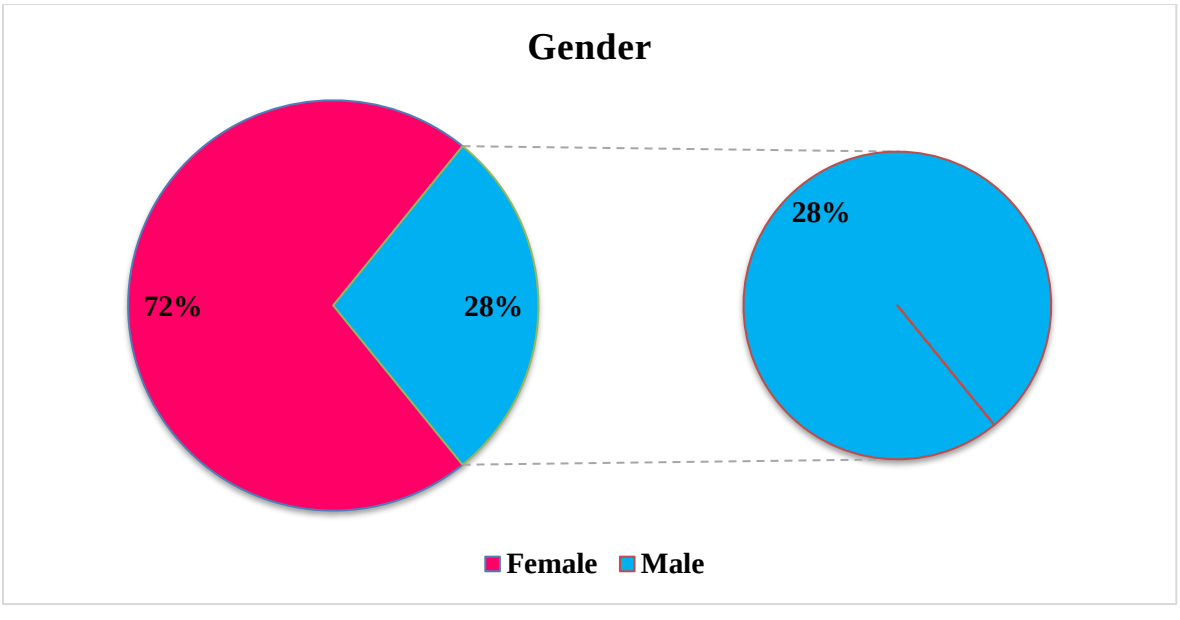
FIGURE 1: Age distribution of the research participants



Gender distribution of studies of participants

Figure 2 gives a representation of the gender distribution of study participants. Out of 106 study participants, n=76 (72%) were females and n=30 (28%) were males.

FIGURE 2: Gender distribution of the study participants



The current prevalence rates of Aflatoxicosis

The study had an aim of establishing whether there was a reduction in the prevalence rate in Makueni County and this after some intensive prevention and control strategies after the initial outbreak of 2004. To achieve this aim urine aflatoxin levels were used as a parameter to measure the exposure rates of resident of Makueni county. At the time of the study samples with AFM1 concentration below 0.0 pg/ml were categorized as negative samples and those with detectable level above 1 pg/ml of AFM1 through the extrapolation from the standard curve were categorized as positive samples. Of 106 urine samples analyzed during the study period, 105 had AFM1 urine concentration levels of above 1 pg/ml which were categorized as a positive finding. Only one sample had an assay value reading of 0.0 pg/ml and this was categorized as a negative value. From those with positive AFM1, 39 had Aflatoxins value below 300 pg/mL. The rest had a value greater than 300 pg/ml, where the majority fall. The positive 105 samples had urinary AFM1 concentration ranging from 25 to 2375 pg/mL, with an average of 637.6±512.8 pg/mL. The urinary levels of AFM1 were detected by enzyme-linked immunosorbent assay. AFM1 was detected in 99.1 % of all urine samples at a range of 25 to 2375 pg/mL (Table 1). This gives a typical picture of what happens when aflatoxins are ingested in contaminated food and animal products. In the liver, the ingested aflatoxin (AFB1) is converted to aflatoxin M1 (AFM1), a metabolite that we have used in our study as a biomarker of AFB1 exposure, as it is excreted in the urine, and therefore suitable for our study.

TABLE 1. The urinary concentration of Aflatoxin in residents of Makueni County

Characteristics	The concentration of AFs in pg/ml									
	Below 100	101-300	301-500	501-700	701-900	901-1100	1101-1300	1301-1500	1501-1700	Above 1701
Age groups										
15-25	7 (28.0%)	2 (8.0%)	1 (4.0%)	3 (12.0%)	3 (12.0%)	4 (16.0%)	1 (4.0%)	2 (8.0%)	0 (0.0%)	2 (8.0%)
26-35	2 (8.0%)	9 (36.0%)	1 (4.0%)	10 (40.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	2 (8.0%)	0 (0.0%)	0 (0.0%)
36-45	2 (11.8%)	2 (11.8%)	3 (17.6%)	2 (11.8%)	4 (23.5%)	1 (5.9%)	1 (5.9%)	1 (5.9%)	0 (0.0%)	1 (5.9%)
46-55	4 (30.8%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	3 (23.1%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
56-65	2 (14.3%)	2 (14.3%)	1 (7.1%)	0 (0.0%)	3 (21.4%)	2 (14.3%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	0 (0.0%)
above 66	2 (16.7%)	4 (33.3%)	2 (16.7%)	1 (8.3%)	2 (16.7%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gender										
Male	7 (23.3%)	3 (10.0%)	3 (10.0%)	6 (20.0%)	3 (10.0%)	3 (10.0%)	1 (3.3%)	2 (6.7%)	0 (0.0%)	2 (6.7%)
Female	12 (15.8%)	17 (22.4%)	6 (7.9%)	11 (14.5%)	9 (11.8%)	8 (10.5%)	4 (5.3%)	6 (7.9%)	1 (1.3%)	2 (2.6%)

DISCUSSION

Aflatoxins metabolites are common in groundnuts, cereals, and spices and herbs, which are widely used as the main food commodities for human and animal consumptions globally. [17-27] All food supplies are plant-related products, animal products such as meat and dairy products are in danger of being contaminated with aflatoxin. Animal products get tainted when such animals are on plant diet that is tainted with aflatoxin. The level of contamination in both cereal and animal products varies from country to country or even at a local level. For example, the Kenya National Bureau of Standards, a state organ with the mandate to ensure the safety of consumer products in the country found that 19 out of 53 samples of dairy products were tainted with AFM1, ranging from 3.5 to 100.5 ng/L. [28-34] The Kenyan population is among some of the African people who consume cereals products daily as this forms one of their staple food supply. [35]

The current prevalence rates of Aflatoxicosis

In our study, the prevalence rate was at 99.1% from the 106 urine samples analyzed. This prevalence of urinary AFM1 was relatively higher than the 79% and 83% reported by Kang'ethe et al 2017 [36, 37] in their previous studies in children below the age of five years in Makueni and Nandi counties, respectively. The high prevalence found in the present study could be attributed to continuous exposure to aflatoxins tainted food even at the time of adulthood. These results were also similar to the 86% prevalence reported by Polychronaki et al. 2008 among children in Guinea [38] The prevalence rate was also higher than those reported in other countries such as 30 % in Egypt [38], Sanchez et al. 2019 reported a 41.7% in Colombia children [39], Ali et al also found a 40 % prevalence rate among a rural population in Bangladesh [40] and Ayelign et al 2017 reported a prevalence rate of 17 % from Ethiopian children. [41] The presence of AFM1 in urine as seen in this study gives a picture of continuous exposure to aflatoxin-tainted food commodities in the entire lives of individuals from this County. This high number can be explained by the high level of aflatoxins contamination and continuous ingestion of AFB1 in food, which is eventually degraded to AFM1 by the liver and easily excreted by kidneys. Aflatoxins metabolized by the liver results into end products such as AFB1-lysine adduct [42-44], AFB1-N7-guanine adduct [45], and urinary AFM1 commonly found in urine. Aflatoxin contamination of animal products has been reported in various studies across the world. [46-57] The first Aflatoxicosis case related to aflatoxin contamination was reported in turkeys and ducklings in the 1960s, where millions died as a result of consuming AFB1-tainted feeds. [58-60]

All these cases could be related to the higher consumption of aflatoxin-tainted staple food, an aspect that contributed to the largest outbreak of human Aflatoxicosis and food contamination in Makueni and subsequent attacks as reported by various studies from different regions of the country.[4, 7, 10, 36, 48, 61-85] The outbreaks reported as high as 8000 µg/kg of aflatoxin contamination in maize in which 125 of people who consumed it never survived the poisoning. Most mycotoxins are stable at the time of food processing [17, 86-90] so it can even show up in food products such as peanut butter and many processed products. However, certain food preparation techniques can minimize the level of toxicity. [91]

CONCLUSION

The results from this study provided the current situation on aflatoxin exposure to the people of Makueni and at the same time offered feedback that served to monitor what is happening and if the initiated preventive strategies for Aflatoxicosis are working. From what is evident from our study, the findings suggest higher aflatoxin concentration among the resident of Makueni County, where there is an urgent need to mitigate the long-term effect of this high exposure. Therefore, the study is recommending to the concerned ministry to have a broader focus on the extent of aflatoxin food contamination from this region plus other regions across the country. This will be so important to protect vulnerable communities and the general population of the country as a whole from this carcinogenic exposure. Some of the recommendations we propose include; regular training on good agricultural practices, routine food sampling for aflatoxin levels, the introduction of a modern method to control aflatoxin food contamination and more research to be done to understand the impact of this high exposure to the health of the vulnerable population.

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Conflict of Interest

The authors declare no conflict of interest related to this study

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REVIEW ARTICLE

The effect of coexistence between larvae of *Anopheles gambiae* and *Culex quinquefasciatus* on larvicidal efficacy of *Bacillus thuringiensis* var. *israelensis*

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ABSTRACT

Background: The efficacy of *Bacillus thuringiensis* var. *israelensis* (*Bti*) is affected by several factors including the species of the mosquito. Mosquito larvae of different species are found to coexist in larval breeding habitats. This study evaluated whether the coexistence between *Anopheles gambiae* and *Culex quinquefasciatus* affect the larvicidal activity of *Bti*.

Methods: Two parallel larval bioassay experiments were conducted to test *A. gambiae* sensu stricto (s.s) and *C. quinquefasciatus* larvae susceptibility to *Bti*. They were followed by three parallel bioassays in which *A. gambiae* s.s and *C. quinquefasciatus* larvae were mixed in different proportions such that the earlier species contributed three quarters, half and a quarter of the larvae in each testing cup respectively. In each bioassay, six *Bti* concentrations were tested in four replicates and repeated on three different days. Larvae mortality was scored 24 hours after application of *Bti* and subjected to Probit analysis.

Results: *C. quinquefasciatus* was significantly more susceptible to *Bti* than *A. gambiae* s.s at both lethal concentration values (LC_{50} and LC_{95}). In coexisting scenario, LC_{50} of *Bti* was significantly lower when the proportion of *C. quinquefasciatus* exceeded 50%. No significant variation in susceptibility to *Bti* was observed at LC_{95} in any proportion of coexistence between the two species.

Conclusion: The findings show that larvae of *C. quinquefasciatus* were significantly more susceptible to *Bti* than those of *A. gambiae* s.s. Moreover, when larvae of the two species coexisted, there was a general trend of increase in sensitivity to *Bti* with higher proportion of *C. quinquefasciatus*. Although this increase in sensitivity of coexisting larvae to *Bti* is worth noting, our findings suggest that it will not impact larval control where *A. gambiae* s.s and *C. quinquefasciatus* coexist.

Keywords: *Anopheles gambiae* sensu stricto, *Culex quinquefasciatus, Bacillus thuringiensis* var. *israelensis,* Larval bioassays.

INTRODUCTION

Insecticide-based malaria vector control interventions have contributed significantly to the reduction of global malaria transmission and consecutively renewed interest in global malaria elimination.[1] However, new novel tools are urgently needed not only to complement the core malaria vector control interventions (insecticide treated nets and indoor residual spraying) but also with the potential to tackle threats posed by insecticide resistance and behavioral adoptions by malaria vectors.[2] Application of bacterial larvicide products based on *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) has been found to be effective and with potential to control both indoor and outdoor biting malaria vectors and possibly delay the evolution of insecticide resistance.[2] Furthermore, the potential role of larviciding for malaria vector control increases as malaria continues in a declining trend, creating a more appropriate condition for the interventions as summarized elsewhere.[3]

Bacterial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) (Bacillales: Bacillaceae) is gram-positive, spore-forming aerobic bacteria isolated from a multitude of aquatic larval habitats.[4] It has been used extensively as a larvicide for mosquito (Diptera: Culicidae) and black fly (Diptera: Simuliidae) control globally.[4,5] The larvicidal activity of *Bti* is based on delta-endotoxins produced by this bacterium at the time of sporulation.[5] When ingested by susceptible mosquito larvae, these toxins bind to the surface membranes of the epithelial cells of the larval midgut disrupting osmotic balance and resulting in the death of the larvae.[5] Based on review of bacterial larvicides, 13 *Bti* based products have been evaluated and found to be effective for malaria vector control in sub-Saharan Africa (SSA).[3] However, only the *Bti* strain AM65-52 (Vectobac® granules (GR) and VectoBac® water dispersible granules (WG)) has been prequalified by the World Health Organization (WHO) to be used for malaria vector control.[6] In addition to its proven effectiveness in mosquito control, *Bti* is generally safe to other non-target organisms coexisting with mosquito larvae in aquatic habitats.[7] The reported efficacy and safety of *Bti* when used for malaria vector control make it ideal for inclusion in Integrated Vector Management (IVM) operations to supplement other vector control strategies.[3] A study conducted in western Kenya demonstrated the potential role of integrating larviciding into adult malaria mosquito vector control interventions in reducing malaria transmission.[8]

Efficacy of *Bti* in mosquito control has been reported to vary greatly, mainly due to factors related to target mosquitoes (species of mosquito, their respective feeding strategies, age and density of larvae), larval habitat conditions (temperature, solar radiation, depth of water, turbidity, organic contents and presence of vegetation) and larvicide properties (application rates, toxin contents, type of carrier, how effective the material reach the target, settling rate, means of application and frequency of treatment).[4,5] In this regard, control efficacy and persistence of *Bti* based products have been shown to vary greatly in different ecological settings in SSA. [3] Understanding factors that cause variation of the effectiveness of *Bti* is important, particularly when planning larviciding interventions in different geographical settings. Like any other larviciding intervention, the *Bti* application must be guided by adequate knowledge of the prevailing mosquito vectors species, including their ecology, and the properties of the larvicide used.[5]

Studies have reported contradictory results on the level of susceptibility of larvae of *Anopheles gambiae* complex (Diptera: Culicidae) and *Culex* species (Diptera: Culicidae) to *Bti*. In controlled conditions, studies have shown that these two-mosquito species are equally susceptible to *Bti*.[9–11] On the other hand, in laboratory settings, *Culex quinquefasciatus* (Diptera: Culicidae) larvae were reported to be up to 4 times more susceptible to *Bti* than *A. gambiae* complex.[12] A field study conducted on the Kenyan coast showed that *A. gambiae* complex were more susceptible to *Bti* than *C. quinquefasciatus*.[13] From an ecological perspectives, it has been documented that the larvae of *A. gambiae* complex spend much more time feeding on the water surface whereas *C. quinquefasciatus* larvae feed under the surface of the water.[14] The nature and properties of a particular larvicide (including its settling rate) is likely to affect the larvicidal exposure rate between surface and bottom feeding larvae.[15]

Immature stages (larvae and pupae) of *A. gambiae* complex and *C. quinquefasciatus* have been found to coexist freely in the natural larval habitats.[16,17] In some instances, the coexistence of *A. gambiae* complex and *C. quinquefasciatus* has been detected in all potential anopheline larval habitats surveyed.[16] This coexistence has shown to harm the fitness of resulting adult mosquitoes due to competition for resources.[18] The coexistence may lead to competition for available larvicide toxins in a treated larval habitat which may subsequently impact on the effectiveness of *Bti*. It has been shown that *C. quinquefasciatus* has a relatively

higher particulate filtration rate than *A. gambiae*.[15] In the coexistence scenario, it is assumed that *C. quinquefasciatus* may filter more *Bti* toxins due to its inherent effective feeding behaviour and will succumb more to the lethal effect of the larvicide than *A. gambiae* complex. The current study was designed to evaluate whether co-existence between *A. gambiae* sensu stricto (s.s) and *C. quinquefasciatus* in the same larval habitat could influence the larvicidal activity of *Bti*.

MATERIALS AND METHODS

Study area and test mosquitoes

The study was conducted at the insecticide testing facility of the National Institute for Medical Research, Amani Medical Research Centre in Muheza, Tanga, Tanzania. Larvae of *A. gambiae* s.s (Kisumu strain) and *C. quinquefasciatus* (Tropical Pesticide Research Institute stain), both of which were fully susceptible to insecticides were used for the laboratory bioassays. The tested colony of *A. gambiae* s.s has been maintained for 30 generations at the insectary of the Amani Medical Research Centre whereas *C. quinquefasciatus* (at their 40th generation) were obtained from the Tropical Pesticide Research Institute, Arusha, Tanzania. *A. gambiae* s.s (Kisumu strain) is a reference strain, considered susceptible to insecticides and has been used extensively in bioassay experiments across Africa.[19]

Adult *A. gambiae* s.s and *C. quinquefasciatus* were maintained at an average room temperature of 26.5 °C (24–29°C) and an average relative humidity of 77.5% (65–90%) whereas larvae were kept at an average room temperature of 32 °C (29–35 °C). Before larval bioassays, first and second stage (early instar) larvae of *A. gambiae* s.s were fed on instant yeast (Istanbul, Turkey) whereas third and fourth stage (late instars) were fed Aquafin® fish food (Quanzhou, China). Larvae of *C. quinquefasciatus* were fed Whiskas® cat food (Mars Africa, South Africa). The feeding strategy was such that, 0.25–0.5 g of instant yeast was reconstituted in 4 mL of water and then 1 mL solution was added to 1500 mL chlorine free tap water, which was sufficient to feed around 500 respective first and second stage larvae of *A. gambiae* s.s for one day. For third and fourth stage larvae of this species, 0.1g Aquafin® was added to 1500 mL chlorine free tap water to feed around 500 larvae per day. For *C. quinquefasciatus*, 1g of Whiskas® cat food was added in 1500 mL of chlorine free tap water to feed approximately 500 larvae per day.

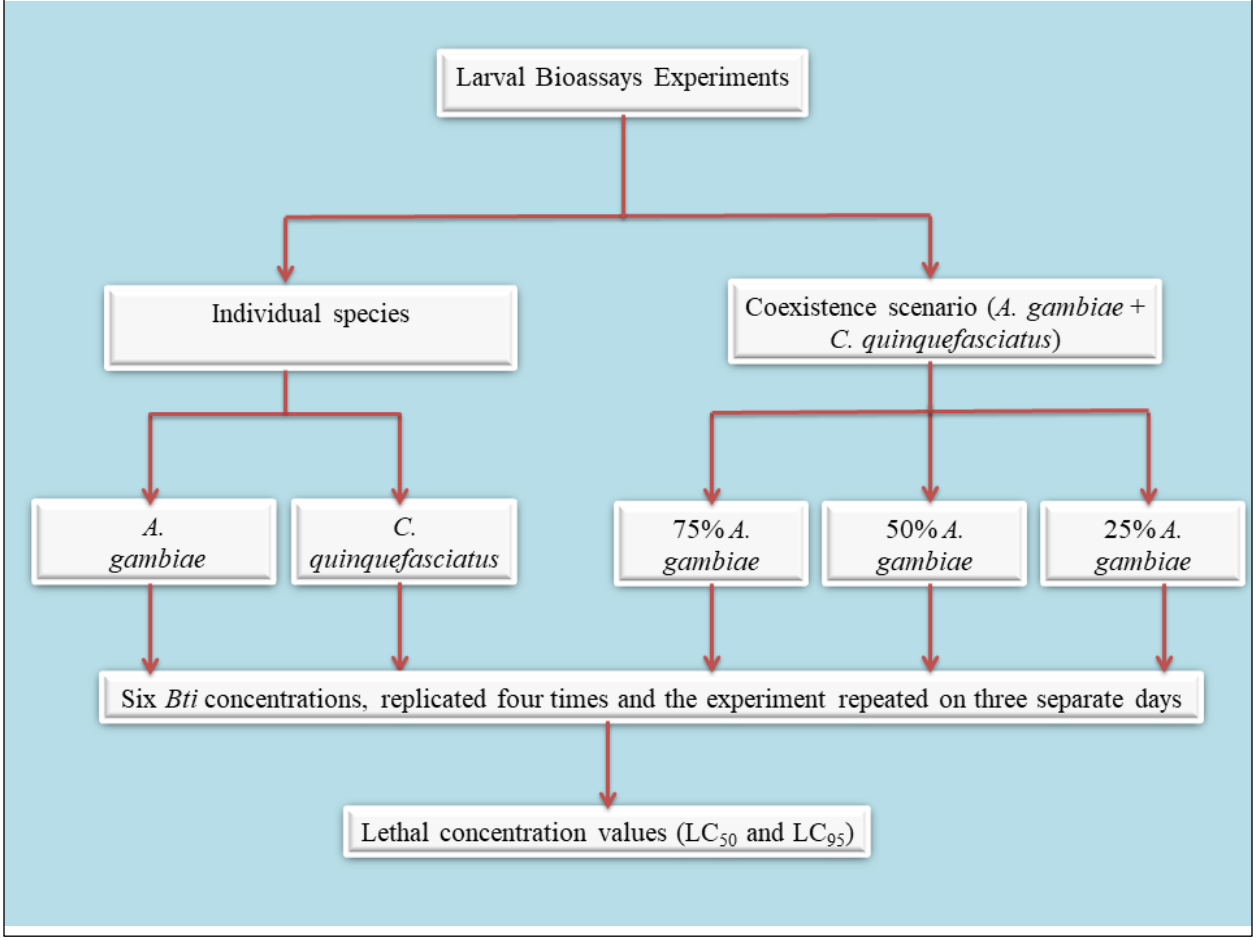
Preparation of Test Larvicides

Larval bioassays experiments were conducted with *Bti* strain Becker Microbial Products (BMP) 144 (potency 7000 ITU [International Toxic Units]/mg) form Becker Microbial Products, Inc, (11146 NW 69th Place, Parkland, FL 33076, USA). Testing solution of *Bti* larvicide was prepared and serially diluted as per recommended practice.[20] In brief, a stock solution was made by dissolving 200 mg of *Bti* powder in 20 mL distilled water. The resultant 10 mg/mL stock solution was kept frozen in 2 mL aliquots until use. On the day of the experiment, one aliquot of the stock solution of *Bti* was thawed and serially diluted in distilled water as recommended. Ten-fold dilution series was prepared by first transferring 2 mL of stock solution to 18 mL of chlorine-free tap water to make 1.0 mg/mL concentration, and then by subsequently repeating this procedure by transferring 2 mL of the latest solution to 18 mL of chlorine-free tap water to make 0.1, 0.01 and 0.001 mg/mL concentrations of *Bti*. The last three concentrations (0.1, 0.01 and 0.001 mg/mL) were used in the subsequent larvicide bioassays.

Study design

Larval bioassay experiments with *A. gambiae* s.s and *C. quinquefasciatus* were run from August to October 2019. At the beginning, two parallel larval bioassay experiments were conducted to separately test the susceptibility of *A. gambiae* s.s and *C. quinquefasciatus* larvae to *Bti*. They were then followed by three parallel larval bioassay experiments in which *A. gambiae* s.s and *C. quinquefasciatus* larvae were mixed (coexistence scenario) such that *A. gambiae* s.s contributed three quarters (15 out of 20), half (10 out of 20) and a quarter (5 out of 20) of the larvae in each testing cup (Figure 1).

FIGURE 1: Larval bioassay experiments



Larval bioassay experiments

At the start of each experiment, 20 third stage larvae were transferred from the larval rearing pans to the labelled disposable paper cups with 100 mL of chlorine-free tap water by use of disposable Pasteur pipettes. Using a pipette with disposable tips, and starting with the lowest concentration, appropriate volumes established in range finding bioassays (0.5 to 0.158 mL) of each of the three last dilutions of *Bti* were then added to the experimental cups (with mosquito larvae in 100 mL of chlorine-free tap water). In each larval bioassay, six concentrations of *Bti* (including a negative control) were tested in four replicates and repeated on three different days. In negative control test cups, 0.5 mL of chlorine free tap water was used. The test cups were held at an average ambient temperature of 29.0 °C and photoperiods of 12 hours light followed by 12 hours of darkness. Larval mortality was recorded at 24 hours after treatment with *Bti*. Only experiments with control larval mortality below 5% were included for further analysis.

Data analysis

Data on larval mortality were entered in Microsoft (MS) Excel (Microsoft Corporation, 2007) spread sheets and subsequently analysed to establish lethal concentrations (LC) of *Bti* that caused 50 and 95% mortality of test larvae (LC_{50} and LC_{95}), lethal concentration ratios (LCR) including their 95% confidence limits by using Probit/Logit analysis software Polo Plus (2002-2003 LeOra Software, Petaluma CA, USA)[21]. Polo Plus has been shown to be robust enough for analysis of mortality-concentration regression and its output compares fairly well with other analysis softwares.[22] The variation in LCs and

LCRs among the tested mosquitoes were compared by examining their 95% confidence limits, a common way to compare lethal concentrations or other point estimates. If the confidence limits overlap, then the LCs or LCRs do not differ significantly.[21] Experiments were considered valid if control mortality did not exceed 5%.

RESULTS

Overall, for the five larval bioassay experiment conducted, the lethal concentration of *Bti* that caused 50% and 95% mortality of the tested larvae (LC_{50} and LC_{95}) ranged from 0.021 mg/L to 0.065 mg/L and 0.105 mg/L to 0.423 mg/L, respectively (Table 1). *A. gambiae* s.s and *C. quinquefasciatus* displayed a different level of sensitivity to *Bti*, with the latter species being significantly more susceptible at both LC_{50} and LC_{95} (Table 1). In the coexisting scenario, LC_{50} of *Bti* was found to be significantly lower when the proportion of *C. quinquefasciatus* exceeded 50%. At LC_{95}, there was no significant variation in susceptibility between the tested larvae at any level of coexistence, although a small trend of increased sensitivity to *Bti* was observed with increasing proportion of *C. quinquefasciatus*. (Table 1). These findings were confirmed by examining lethal concentration ratios of *Bti* calculated by comparing LC values of the tested larvae with that of *A. gambiae* s.s (Table 2). At LC_{50}, the lethal concentration ratio (LCR) of coexisting larvae was significantly different when the proportion of *C. quinquefasciatus* exceeded 50% and no variation in susceptibility was observed in LCR at LC_{95} (Table 2).

TABLE 1: Laboratory bioassay results of *Bacillus thuringiensis* var. *israelensis* against larvae of *Anopheles gambiae* s.s and *Culex quinquefasciatus* in different proportions of coexistence

Mosquito species (proportion in %)	No. tested‡	LC_{50}† (95% CI)	LC_{95}† (95% CI)	Slope ±SE	χ^2 (df)	Heterogeneity
A. gambiae s.s (100)	1440	0.065 (0.056–0.075)	0.359 (0.283–0.484)	2.211±0.113	101.34 (58)	1.747
A. gambiae s.s (75) + *C. quinquefasciatus* (25)	1440	0.059 (0.053–0.067)	0.423 (0.345–0.540)	1.925±0.097	53.923 (58)	0.930
A. gambiae s.s (50) + *C. quinquefasciatus* (50)	1440	0.039 (0.035–0.045)	0.387 (0.303–0.519)	1.659±0.083	63.867 (58)	1.100
A. gambiae s.s (25) + *C. quinquefasciatus* (75)	1440	0.038 (0.033–0.042)	0.300 (0.241–0.391)	1.821±0.090	62.916 (58)	1.085
C. quinquefasciatus (100)	1440	0.021 (0.019–0.023)	0.105 (0.088–0.130)	2.358±0.135	52.042 (58)	0.897

Note: ‡1200 subjects and 240 controls in all tests (control mortality did not exceed 1.3% in any experiment); †mg/litre at 24 hours

Abbreviations: CI, confidence interval, SE, standard error; df, degrees of freedom, s.s, sensu stricto

TABLE 2: Lethal dose ratios for *Bacillus thuringiensis* var. *israelensis* against larvae of *Anopheles gambiae* s.s and *Culex quinquefasciatus* in different proportions of coexistence

Mosquito species (proportion in %)	Bti-Lethal Concentration Ratios† at	
	LC_{50} (95% CI)	LC_{95} (95% CI)
A. gambiae s.s. (75) + *C. quinquefasciatus* (25)	1.093 (0.927–1.289) a	0.848 (0.626–1.147) a
A. gambiae s.s. (50) + *C. quinquefasciatus* (50)	1.639 (1.391–1.933) b	0.928 (0.672–1.281) a
A. gambiae s.s. (25) + *C. quinquefasciatus* (75)	1.723 (1.466–2.026) b	1.195 (0.881–1.621) a
C. quinquefasciatus (100)	3.101 (2.674-3.597) c	3.436 (2.593–4.554) b

†Compared to *An. gambiae* sensu stricto (s.s)

Values in the columns followed by the same letter are not statistically significant (overlapping confidence interval)

DISCUSSION

Bti has been used extensively for the control of mosquitoes and black flies.[4,5] However, the activity of *Bti* based products is affected by a multitude of factors related to the target mosquitoes, their ecology, and inherent properties of the larvicide formulations.[5] Of relevancy to the current study, the control efficacy of *Bti* is known to vary with the species of the mosquito, mainly due to variation in larval feeding strategies.[23,24] In larvae ecology, the coexistence of different species of mosquito larvae in aquatic habitats, particularly anopheline and culicine species is not uncommon. The current study was designed to establish whether this coexistence could affect the larvicidal activity of *Bti* under laboratory settings. Understanding factors that affect the activity of larvicide has both epidemiological and economic advantages in the control of mosquito-borne diseases using *Bti* based products.

The findings have shown that larvae of *A. gambiae* s.s and *C. quinquefasciatus* tested were readily susceptible to *Bti* at relatively low application rates, which corroborates well with the results of other studies as summarized elsewhere[4]. A comparison between the two species revealed that *C. quinquefasciatus* were up to three times more susceptible to *Bti* than *A. gambiae* s.s. The high sensitivity of *C. quinquefasciatus* observed in the current study has also been reported in other studies,[12,23,24] and has been linked to the inherent high particulate filtration rate of this mosquito species.[25] In addition to particulate filtration rate, the larvicide settling rate has been identified to impact the activity of bacterial larvicides.[5] In this regard, the rapid settling rate of *Bti* toxins has been shown to lower larvicidal activity of surface feeding *Anopheles* mosquitoes.[25,26] Although the settling rate of *Bti* was not measured in this study, the test solutions were found to settle to the bottom of storage tubes and required gentle shaking before dispensing to the testing cups. Vigorous to gentle shaking or stirring of tubes containing re-suspended bacterial larvicide preparations before application to the target larvae has been emphasized in many testing protocols.[20] Although higher particulate filtration rate is known to increase the sensitivity of *C. quinquefasciatus* to *Bti*, it also appears likely that this species is considerably more exposed due to its bottom-feeding habit when tested with products with relatively high settling rate. The findings suggest that, if other factors that affect larval susceptibility to *Bti* remained constant, *C. quinquefasciatus* may respond better to *Bti* intervention than *A. gambiae* s.s.

In larval bioassay experiments in which larvae of *A. gambiae* and *C. quinquefasciatus* were mixed to represent various proportions of coexistence, an increased in sensitivity to *Bti* was recorded when compared to the LC values of *A. gambiae* s.s. At LC_{50}, the increase in larval sensitivity to *Bti* was significantly higher when the proportion of *C. quinquefasciatus* in test cups exceeded 50%. Likewise, relatively lower *Bti* concentrations were required to cause 95% mortality (LC_{95}) of test larvae with increased proportion of *C. quinquefasciatus* but this did not reach statistical significance in any of the three coexistence experiments. Our findings suggest that the increase in sensitivity of coexisting larvae to *Bti* was possibly due to relatively high susceptibility of *C. quinquefasciatus* with an overall effect of increasing sensitivity of coexisting larvae. This assumption is supported by previous findings showing rapid onset of toxic manifestation and reduced feeding in *C. quinquefasciatus* after an initial period of feeding on *Bti* toxins ranging from 15-20 minutes.[26] Provided that LC_{95} represents the minimum effective dose by which field application rates are based,[20] lack of significant variation in susceptibility of coexisting larvae at LC_{95} has important practical implications relevant to larviciding. In this regard, it can be safely generalized that larval control interventions using *Bti* can be scaled-up with little consideration to the level of coexistence between *A. gambiae* complex and *C. quinquefasciatus*.

In practical field applications, when anophelines and culicines coexist, treating the two species as a single unit in terms of susceptibility to *Bti* is appropriate and it has been previously documented.[9–11] This approach reduces logistical challenges pertaining to resources, time and efforts that would have been required in the larvicide intervention and monitoring if coexistence had an influence on activity of *Bti*. Thus, this assumption allows for rapid scale-up of larviciding interventions in different ecological settings where association between anopheline and culicine species is common. Although the efficacy of *Bti* is affected by a multitude of factors, when these factors are considered, *Bti* is effective and can be successfully incorporated in integrated vector management programs.

CONCLUSIONS

The findings of the current study have shown that larvae of *C. quinquefasciatus* were more susceptible to *Bti* than those of *A. gambiae* s.s and when the two species coexisted, there was a general increase in sensitivity to *Bti, which* increases proportionally with *C. quinquefasciatus*. Although this increase in

sensitivity of coexisting larvae to *Bti* is worth noting, our findings suggest that it will not impact larval control where *A. gambiae* and *C. quinquefasciatus* coexist.

Authors' contributions

YAD, EJK, WNK, GY, AKG and FWM conceived and designed the study. YAD conducted laboratory experiments and performed data analysis. YAD drafted the manuscript with contributions from EJK, WNK, GY, AKG and FWM. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical statement

Not applicable.

Availability of data and materials

All relevant data supporting the conclusions of this article are included in the article.

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REVIEW ARTICLE

The Role of Pig Production and Market Value Chain in the Occurrence of African Swine Fever in Songwe and Ruvuma Regions, Tanzania

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ABSTRACT

Background: In recent years, the pig industry in Tanzania has faced frequent occurrence of outbreaks of African swine fever (ASF). However, there is inadequate information on the pig value chain operation in relation to occurrence of ASF. This study aimed at mapping pig value chain and assess its contribution to the occurrence and spread of ASF in Tanzania.

Methods: A cross sectional study was carried out in Songwe, Momba, Songea and Mbinga districts of Tanzania. Study districts were purposively selected based on the density of pig population, differences in production systems and history of ASF outbreaks. A total number of 484 pig producers and 28 traders were involved in the study. Random sampling was used to select pig producers. Pig traders were selected using snowball technique. Structured questionnaires were used to collect data on pig management and production practices, veterinary services, pig marketing practices and biosecurity measures using Open Data Kit (ODK) software. Semi structured interviews were conducted with key informants on perceived risk practices that are related to ASF outbreaks, challenges and recommendations on ASF prevention and control measures. Observation method was used to assess structure, facilities and practices within the pig production chain.

Results: The main actors in the pig production chain were pig producers, assemblers, wholesalers and retailers. Unknown stock source (30%), poor husbandry practice such as free ranging (5%), poor management of waste products (73%) and poor handling of feed (73%) were risk practices in the production node. Transportation nodes operated under high risk due to frequent movements and pick-ups of ≥ 30 pigs per trip.

Conclusion: The results demonstrated that different actors operate in the pig production, distribution and marketing chain. Each node operated under low biosecurity measures, and poor infrastructures that are likely to contribute to occurrence of ASF. There is need to improve good husbandry practices, marketing and infrastructures to increase production while ensuring pork safety.

Keywords: African swine fever; animal protein; pig value chain; Tanzania

INTRODUCTION

Globally, pig production is increasingly driven by the demand for animal protein in the market. The pig industry accounts for 118.7 million tonnes of pork meat of which a large amount is produced in China, European Union countries, the United States of America, Brazil and Russia.[1] The world pork meat market has increased by 1.4% from 8.1 million tonnes in 2017 to 8.3 million tonnes in 2018.[2] In Sub-Saharan Africa (SSA), South Africa accounts for 26% of the pig

production followed by Nigeria (19%) and Uganda (12%).[3] Available statistics indicate that there are approximately 2 million pigs in Tanzania.[4] The consumption of pork meat is increasing due to the price of substitute meat products, increasing wealth, and growing population in both rural and urban centers.[5] Parallel to this, Tanzania's pig industry has the capacity to grow and reduce a production-consumption deficit for pork from 8000 to 1350 tonnes in 2025.[4] Despite this rapid growth of the sector its economic contribution is threated by outbreaks of African swine fever (ASF).[6]

ASF is an infectious disease and number one killer of pig populations.[4] The disease is caused by Deoxyribonucleic acid (DNA) virus that belongs to the genus *Asfivirus* of the family *Asfarviridae*.[7] It is a major constraint in development of pig industry in Europe, Asia and Africa due to a mortality rate of up to 100% in domestic pigs.[8] In sub Saharan Africa, over 22 countries have reported ASF epidemics.[6] The disease was first reported in Eastern African countries in 1921.[9]In the absence of a vaccine, ASF control has relied on culling/ slaughtering of pigs, proper disposal of carcasses, quarantine, sanitation and hygiene measures and education interventions.[10]

The prevention and control of ASF depends on available information on chains operation and performance using value chain analysis.[11] The analysis describes the system dynamic, classifying interaction and linkages as well as assess the behavioral risk practices such feeding swill, farm visitors, sharing boars and free ranging which contribute to disease transmission.[11] Understanding of the pig value chain, drivers and factors contributing to transboundary animal disease spread can contribute to designing of effective control strategies. Value chain refers to a full range of activities which are required to bring products or services from production, marketing channels to end consumers and final disposal after use.[12] It can be classified based on two approaches: The first approach is based on the role of chain governance and the second approach on coordination and operation of activities within chains. The former determines the rule of trade and who decides what to produce (producers' or buyers' chain) while the latter defines how people or actors in value chain perform and the decisions they make. [13, 14]

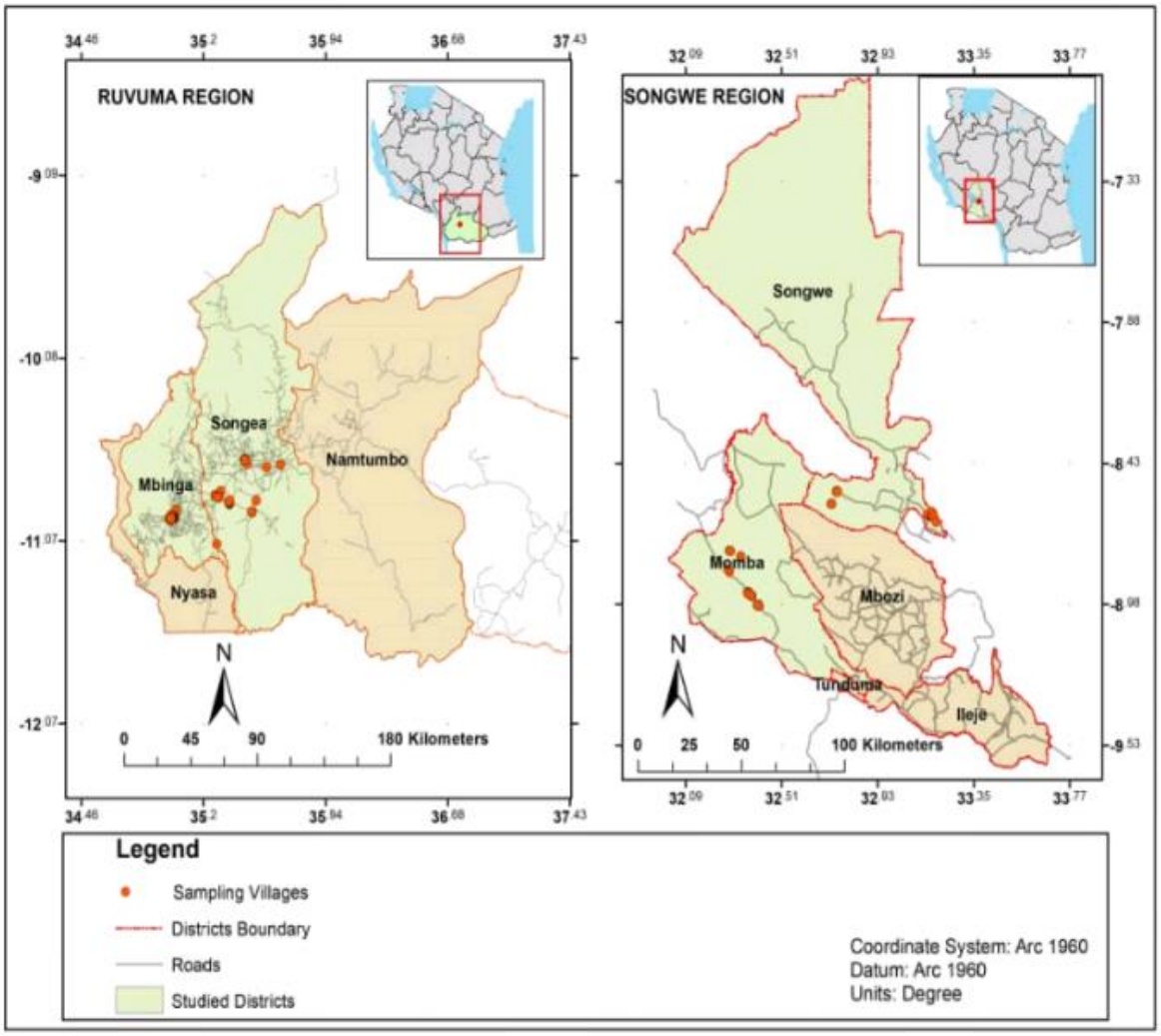
While previous studies in Tanzania have reported on ASF epidemiology and molecular identification of African swine fever virus (ASFV) genotypes circulating in different parts of the country,[15, 16, 17]there is dearth of information on the role played by pig value chain operation in disease epidemiology. Such information could contribute in designing effective ASF disease management within the country. Therefore, the objective of this study was to map pig value chain and assess its role in the occurrence and spread of ASF in selected districts of Tanzania.

METHODS

Study Area

The study was conducted in Songwe and Ruvuma regions of southern Tanzania. In Songwe two districts, Momba and Songwe were involved; while in Ruvuma, Mbinga district and Songea district were involved. The populations of Songwe, Momba, Songea and Mbinga districts are 133,692, 196,818, 173, 821 and 353, 683 respectively.[18] Crop and livestock production are the most important economic activities in these study areas. Additionally, the areas were purposively chosen based on different ecosystems of animal production, history of ASF outbreaks and high density of pig population; whereby Songwe region has approximately 79,513 pigs, [19] and Ruvuma region has a population of 183,276 pigs.[18]There are five veterinary health services (consultation and drug outlets) within study areas. In Songwe district selected wards and villages were Ifwenkenya ward (Ifwenkenya and Iyela villages) and Namkukwe ward (Namkukwe and Mheza villages); Momba district, Ikana ward (Ikana and Nyenjele villages) and Myunga ward (Namshinde and Mfuto villages); Songea district, Peramiho ward (Peramiho A village), Ngogosi ward (Namatuhi village), Kizuka ward (Ngahokora village), and Magagura ward (Magagura village); Mbinga district, Mbinga Mjini B ward (Misheni street), Luhuwiko ward (Luhuwiko A and Luhuwiko B streets), Bethelehemu ward (Mahela and Bethelehemu streets) and Lusonga ward (Ruvuma and Kihaha streets) (Fig 1).

FIGURE 1: Map of the Study Areas



Study Design and Sample Collection

A cross sectional study was carried out in which data were collected from October to November 2018 in Songwe and Momba districts, and from June to July 2019 in Songea and Mbinga districts. The sample size for pig producers was determined by the formula developed by Kothari, [20] i.e. $n = z2pq/d^2$ where by n is the sample size, z is 1.96 corresponding to the 95% confidence interval level and significant level of 5%, p is the prevalence of disease which is estimated at 50% [21], q is 1- p, and d is the permissible error of estimation (0.05). Therefore $1.96^2 \times 0.5 \times 0.5/0.05^2 = 384$. A contingency of 26% was added to account for non-responses and incomplete data was added resulting to a final sample size of 484 households keeping pigs. Since there were no physical markets for pigs in study districts, sampling of traders was based on known information, accessibility and availability of them during data collection. Thus, a total number of 28 pig traders were obtained from all surveyed districts.

Prior information on ASF outbreaks distribution in the study areas was obtained from respective district veterinary offices. Livestock Field officers provided lists of pig producers within study areas that served as sampling frames for pig producers. In this study we

interviewed pig producers with and without pigs at the time of data collection. Snowball sampling technique was used to obtain traders within study areas. The information about the first trader came from field officers in villages, thereafter, the same trader was used to identify other fellow traders one after another for interviews and the process was repeated until the point of thematic saturation was reached. The targeted pig trader populations were assemblers, wholesalers and retailers.

The value chain in this study was based on coordination and operation of activities within the pig production system. An assumption was made that operations and activities in pig value chain are independent and absence of command within chains.[22] In addition, this approach could enable assessment of risk practices in relation to transmission of disease within chains.

Data Collection

A semi-structured questionnaire was used to collect data from pig producers and pig traders. The questionnaire for pig producers focused on household characteristics, source of stocks, production system, production constraints, breeding type, herd size, feed materials, premises structures, management of waste products, training and veterinary services, and biosecurity measures. While the questionnaire for pig traders included questions on characteristics of pig traders, type of business, initial capital, source of stock, marketing prices, marketing distance, marketing constraints, management of waste products and biosecurity measures. Three research assistants were recruited and trained on study objectives and protocol. Software was used to collect data. The English version of a semi-structured questionnaire was translated to Kiswahili. The questionnaire was installed in the android mobile device with Open Data Kit software. It was pre-tested in an area in Songwe district that was not involved in the actual study. Modifications were made to the questionnaire before been used for actual data collection. A single interview took approximately 30 minutes to complete.

Moreover, we interviewed district livestock officers (n=2) and field officers (n=8) as key informants. Furthermore, we directly observed the presence of veterinary services, water supply, means of animal transport, slaughter facilities, animal inspection, pig premises, presence of fence, ticks, warthogs, disposal of waste products and behavioral risk practices such as feeding swills, free ranging, farm visitors and sharing boars and farm equipment without disinfectant that could contribute to introduction and spread of ASF. These parameters were marked good practices as 1, poor practices as 2 and not applicable as 3.

Statistical Data Analysis

Data analysis was done using Statistical Package for Social Sciences (IBM SPSS) version 25® (IBM Corp, Armonk New York, USA). Data were summarized using descriptive statistics including percentage, median and frequency tables to describe general characteristic and graphical representation of key actors in pig value chain. For qualitative data thematic analysis was used whereby specific themes were deduced from qualitative research questions. Thematic areas addressed in this study include knowledge on risk practices that related to ASF outbreaks, opinion on factors limiting the control of ASF and recommendations on ASF prevention and control measures.

Ethical Approval

The study was approved by the Sokoine University of Agriculture (reference number SUA/CVMBS//R.1/2018/2019/2). Permission to conduct the study was obtained from the respective district and village authorities. Informed consent was obtained from pig owners.

RESULTS

A total of 484 respondents were involved in the study. Of these, 99 were from Songwe, 97 from Momba, 119 from Songea and 169 were from Mbinga. Of the total respondents, 45% were females and 55% males between minimum age 13 to maximum 85 years. Over 50% of pig producers had attained primary education as shown in Table 1.

TABLE 1: Socio-demographic characteristics of pig producers in Songwe and Ruvuma regions

Category	Variables	Songwe Region			Ruvuma Region		
		Songwe (n = 99)	Momba (n =97)	Total (N = 196)	Songea (n =119)	Mbinga (n= 169)	Total (N = 288)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Sex	Male	58 (58.6)	88 (90.7)	146 (74 .5)	62 (52.1)	58 (34.3)	120 (41.7)
	Female	41 (41.4)	9 (9.3)	50 (25.5)	57 (47.9)	111 (65.7)	168 (58.3)
Age	Mean	40.2	38.1	39.2	44.1	46.2	45.3
	Median	37	35	38	41	44	40.5
	Std. Deviation	10	13	11.81	14.7	14.1	14.4
	Minimum	21	15	15	13	16	13
	Maximum	68	89	89	95	83	95
Marital Status	Single	2 (2.0)	4 (4.1)	6 (3.1)	16 (13.4)	18 (10.7)	34 (11.8)
	Married	90 (90.9)	89 (91.8)	179 (91.3)	92 (77.3)	116 (68.6)	208 (72.2)
	Widowed	5 (5 .1)	3 (3.1)	8 (4.1)	10(8.4)	31 (18.3)	41 (14.2)
	Divorced	2 (2.0)	1 (1.0)	3 (1.5)	1 (0.8)	4 (2.4)	5 (1.7)
Education status	Never attend school	16 (16.2)	12 (12.4)	28 (14.3)	1 (0.8)	9 (5.3)	10 (3.5)
	Informal school	0 (0.0)	1 (1.0)	1 (0.5)	2 (1.7)	0 (0.0)	2 (0.7)
	Primary school	74 (74.7)	71 (73.2)	145 (74.0)	101 (84.9)	118 (69.8)	219 (76.0)
	Secondary school	8 (8.1)	13.4	21(10.7)	11 (9.2)	34 (20.1)	45 (15.6)
	Tertiary education	1 (1.0)	0 (0.0)	1 (0.5)	4 (3.4)	8 (4.7)	12 (4.2)
Household size	1 - 5	33 (33.3)	40 (41.2)	73 (37.2)	74 (62.2)	110 (65.1)	184 (63.9)
	6 - 10	46 (46.5)	52 (53.6)	98 (50.0)	45 (37.8)	59 (34.9)	104 (36.1)
	11 - 15	14 (14.1)	3 (3.1)	17 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)
	16 - 20	5 (5.1)	0 (0.0)	5 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
	21 - 25	0 (0)	2 (2.1)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
	>25	1 (1.0)	0 (0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Reason for keeping pigs	Income generation	99 (67.3)	97 (93.0)	196 (78)	117 (92.13)	167 (81.5)	284 (85.5)
	Reproduction	28 (19.0)	1 (1.0)	29 (12)	3 (2.36)	0 (0.0)	3 (0.9)
	Manure	2 (1.4)	1 (1.0)	3 (1)	7 (5.51)	34 (16.6)	41 (12.3)
	Domestic consumption	14 (9.5)	3 (3)	17 (7)	0 (0.0)	4 (2.0)	4 (1.2)
	Cultural	4 (2.7)	2 (2)	6 (2)	0 (0.0)	0 (0.0)	0 (0.0)

Socio-Demographic Characteristics of Pig Traders

A total of 28 pig traders were obtained during data collection. Out of them, seven were from Songwe district, five from Momba district, 11 were from Songea and five were from Mbinga districts (Table 2). All five assembler traders were married males with minimum age 27 and maximum 44 years, and four had acquired primary education. On the other hand, 21 retailer traders were males and two were females with age minimum of 17 years and maximum of 65 years". Over 50% attended primary education and only two were not married. In order to start pig business, the minimum initial capital investment for selling pork meat was between USD 22 and USD 87 (Table 2).

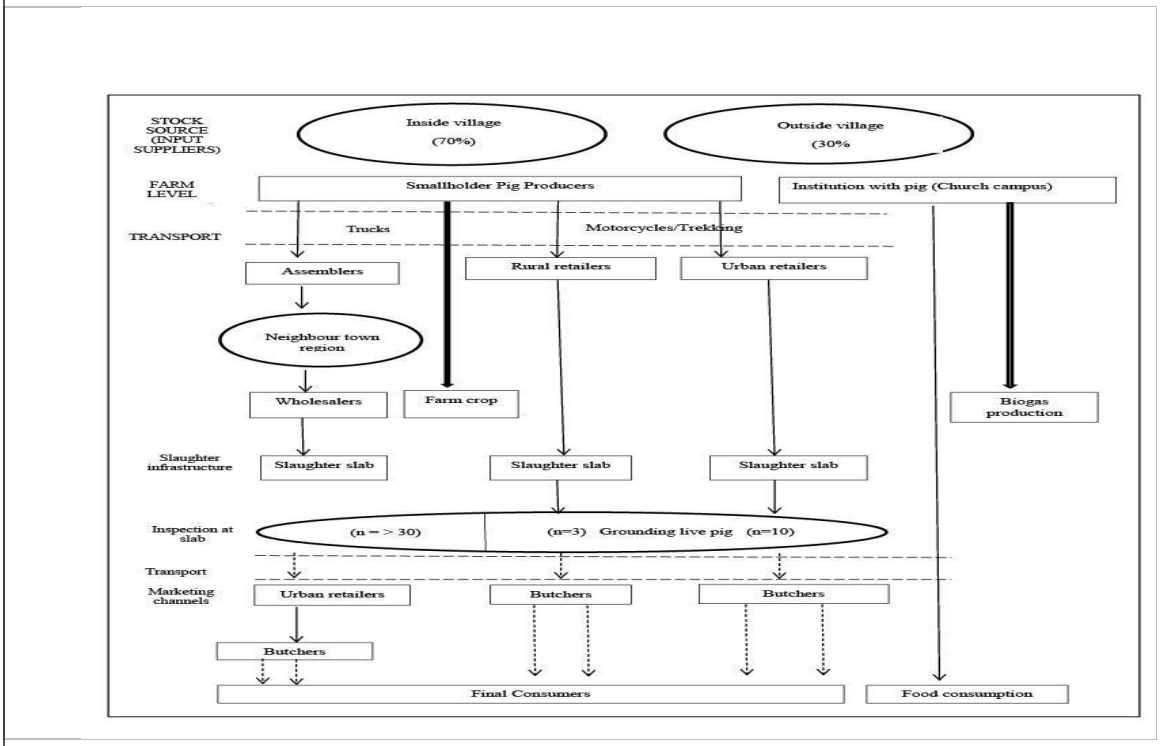
TABLE 2: Socio-demographic characteristics of pig traders in Songwe and Ruvuma regions, Tanzania

Category	Variable	Songwe (n=7)		Momba (n=5)		Total (N=12)	Songea (n=11)	Mbinga (n=5)	Total (N =16)
		No	No	No	No	No	No	No	No
		Assemblers (n= 2)	Retailers (n=5	Assemblers (n= 3)	Retailers (n=2)		Retailers (n=11)	Retailers (n=5)	
Age	Mean	35.5	30.6	35.7	30.5	32.7	37.5	50	41.4
	Median	35.5	30	35	30.5	31	39	48	40
	Std. Deviation	8.5	9	4.9	1.5	7.64	7.53	10.5	10.3
	Minimum	27	17	30	29	17	24	38	24
	Maximum	44	45	42	32	45	52	65	65
Sex	Male	2	4	3	2	11	11	4	15
	Female	0	1	0	0	1	0	1	1
Marital status	Single	0	1	0	0	1	1	0	1
	Married	2	4	3	2	11	10	5	15
Education status	Never attended school	1	1	0	0	2	0	0	0
	Primary school	1	4	3	2	10	10	5	15
	Secondary school	0	0	0	0	0	1	0	1
Household size	1 - 5	1	4	2	1	8	7	4	11
	6 - 10	1	1	1	1	4	4	1	5
Initial capital investment	22 - 87 USD	0	4	0	2	6	3	0	3
	109 – 217 USD	0	1	0	0	1	8	4	12
	> 217 - 435USD	1	0	0	0	1	0	1	1
	> 435 USD	1	0	3	0	4	0	0	0

Main Actors in Pig Production Value Chain

Through the mapping process, it was found that the main actors involved in production, distribution and marketing were pig producers, assemblers, wholesalers, rural and urban retailers. The flow of products involved live pigs, raw pork, offal, heads, legs and pig's oil. Pig producers were involved in rearing pigs and selling directly to traders. Assembler traders were engaged in transportation of live pigs within and outside districts. The wholesale traders were involved in buying and selling pigs in large quantity from assemblers while retail traders were involved in buying live pigs in small quantity from pig producers, slaughtering and selling raw pork or roasted pork to end consumers (Fig 2).

FIGURE 2. Mapping of actors and flow of products in pig value chain in Songwe and Ruvuma regions, Tanzania.



Straight arrow shows the flow of live animals for selling and food consumption.

Interrupted arrow shows the flow of pork meat and offal heads, legs and pig oil.

Bold arrow shows the flow of manure of which used for crop farming and biogas generation.

The Production Node

Pig Management and Production Practices

Over 50% of pig producers reported to have acquired pig stocks from neighboring farms within study areas. However, it was found that producers in Songwe (7; 7.1%), Momba (29; 29.9%), Songea (6; 5%) and Mbinga (21; 12.4%) obtained pigs from outside villages and neighboring regions. Majority of pig producers in Songwe (93; 93.9%), Momba (89; 91.8%), Songea (94; 79.0%) and Mbinga (138; 81.7%) preferred to keep indigenous breeds compared to pure breeds, cross breeds and exotic breeds. Most of pig producers in Songwe (42; 42.4%), Momba (33;

34%), Songea (81; 68.1%) and Mbinga (158; 94.1%) owned about 1.00 acres to 2.00 acres for pig production. A few producers practiced free ranging in Songwe (4; 4%), Momba (5; 5.2%), Songea (1; 0.8); while Semi confinement production system was observed in Songwe (44; 44.4%) and Momba (66; 68%) (Table 3).

TABLE 3. Pig Producers' management and production practices in Songwe and Ruvuma Regions, Tanzania

Category	Variables	Songwe Region			Ruvuma Region		
		Songwe (n = 99)	Momba (n =97)	Total (N = 196)	Songea (n =119)	Mbinga (n= 169)	Total (N = 288)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Source of stock	Neighbouring farm	85 (85.9)	58 (59.8)	143 (72.9)	96 (80.7)	124 (73.4)	220(76.4)
	Outside village	7 (7.1)	29 (29.9)	36 (18.4)	6 (5.0)	21(12.4)	27 (9.4)
	Mission camp	0 (0.0)	0 (0.0)	0 (0)	14 (11.8)	19 (11.2)	33(11.5)
	Family/relative	6 (6.0)	7 (7.2)	13 (6.6)	1 (0.8)	4 (2.4)	5 (1.7)
	Others	1 (1.0)	3 (2.0)	4 (3.0)	2(1.7)	1 (0.6)	3 (1.1)
Breed type	Indegenous breed	93 (93.9)	89 (91.8)	182(92.9)	94 (79.0)	138 (81.7)	232 (80.6)
	Exotic pure breed	2 (2.0)	3 (3.1)	5 (2.6)	9 (7.6)	4 (2.4)	13 (4.5)
	Cross breed	4 (4.0)	5 (5.2)	9 (4.6)	16 (13.4)	27 (16.0)	43 (14.9)
Herd size	0	14 (14.1)	17 (17.5)	31 (15.8)	32(26.9)	30 (17.8)	62 (21.5)
	1-4	44 (44.4)	31 (32.0)	75 (38.3)	58 (48.7)	106 (62.7)	164 (56.9)
	5-9	20 (20.2)	27 (27.8)	47(24.0)	17 (14.3)	17 (10.1)	34 (11.8)
	10 - 14	11 (11.1)	12 (12.4)	23 (11.7)	5 (4.2)	12 (7.1)	17 (5.9)
	15-19	2 (2.0)	3 (3.1)	5 (2.6)	3 (2.5)	1 (0.6)	4 (1.4)
	>20	8 (8.1)	7(7.2)	15 (7.7)	4 (3.4)	3 (1.8)	7 (2.4)
Land Size	Don't own land	10 (10.1)	11 (11.3)	21 (10.7)	9 (7.6)	9 (5.3)	18(6.3)
	1.00 - 2.00 acres	42 (42.4)	33 (34.0)	75 (38.3)	81 (68.1)	159 (94.1)	240 (83.3)
	> 2.00 - 4.00 acres	21 (21.2)	31 (32.0)	52 (26.5)	22 (18.5)	0 (0.0)	22 (7.6)
	> 4.00 _ 10.00 acres	14 (14.1)	17 (17.5)	31 (15.8)	6 (5.0)	1 (0.6)	6 (2.1)
	>10 acres	12 (12.1)	5(5.2)	17 (8.7)	1 (0.8)	1 (0.6)	2 (0.7)
Production system	Confinement	41 (41.4)	26 (26.8)	67 (34.2)	113 (95.0)	169 (100)	282 (97.9)
	Semi confinement	44 (44.4)	66 (68.0)	110 (56.1)	4 (3.4)	0 (0.0)	4 (1.4)
	Free range	4 (4.0)	5(5.2)	9 (4.6)	1 (0.8)	0 (0.0)	1 (0.3)
	Tethering	10 (10.1)	0 (0.0)	10 (5.1)	1 (0.8)	0 (0.0)	1 (0.3)
Who take care of pigs	Husband	15 (15.2)	20 (20.6)	35 (17.9)	13 (10.9)	16 (9.5)	29 (10.1)
	Wife	31 (31.3)	27(27.8)	58 (29.6)	27 (22.7)	87 (51.5)	114 (39.6)
	Children	2 (2.0)	3(3.1)	5 (2.6)	6 (5.0)	9 (5.3)	15 (5.2)
	Both 1& 2	23 (23.2)	24(24.1)	47 (24.0)	40 (33.6)	26 (15.4)	66 (22.9)
	All 1, 2 & 3	27 (27.3)	22 (22.6)	49 (25.0)	22 (18.5)	18 (10.7)	40 (13.9)
	Hired labour	1 (1.0)	0(0)	1 (0.5)	3 (2.5)	5 (3.0)	8 (2.8)
	Himself/herself	0(0)	1 (1.0)	1 (0.5)	4 (3.4)	1 (0.6)	5 (1.7)

	Others	0(0)	0(0)	0(0)	4 (3.4)	7 (4.1)	11 (3.8)

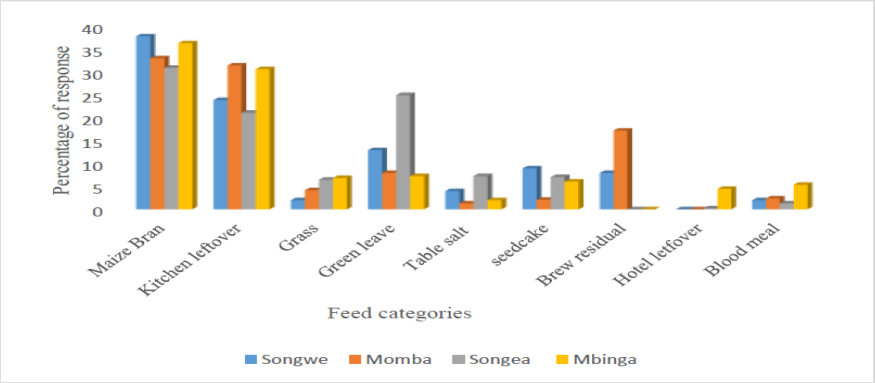
Continued

TABLE 3. Continued

Category	Variables	Songwe Region			Ruvuma Region		
		Songwe	Momba	Total (N = 196)	Songea	Mbinga (n= 169)	Total
		(n = 99)	(n =97)		(n =119)		(N = 288)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Disposal of waste product	Proper (Deep burial collecting waste, burning)	44 (44.4)	57 (58.8)	101(51.5)	32(26.9)	79(46.7)	111(38.5)
	Improper (throwing away, feed to pigs, disposal of dead pigs)	55(55.6)	40 (41.2)	95(48.5)	87(73.1)	90 (53.3)	177 (61.5)
Boiling feed/ leftovers	Yes	27(27.3)	26(26.8)	53(27)	42 (35.3)	81 (47.9)	123(42.7)
	No	72(72.7)	71 (73.2)	143(73	77 (64.7)	88(52.1)	165(57.3)
Wash/Use of protective gears	Yes	25(25.3)	17(17.5)	42(21.4)	71(59.7)	91(53.8)	162(53.3)
	No	74(74.7)	80(82.5)	154(78.6)	48(40.3)	78 (46.2)	126(43.8)
Animal treatment services	Yes	64 (64.6)	37 (38.1)	101 (51.5)	115(96.6)	146 (86.4)	261 (90.6)
	No	35 (35.4)	60 (61.9)	95 (48.5)	4 (3.4)	23 (13.6)	27(9.4)

It was found that wives were source of family labour in Songwe (31; 31.3%), Momba (27; 27.8%), Songea (27; 22.7%) and Mbinga (87; 51.5%); while very few producers in Songwe (2; 2%), Momba (3; 3.1%), Songea rural (6; 5%) and Mbinga (9; 5.3%) reported that children were the ones taking care of pigs. Poor implementation of biosecurity measures such as non-use of protective gears and washing equipment without use of disinfectants were reported in Songwe (74; 74.7%), Momba (80; 82.5%), Songea (48; 40.3) and Mbinga (78; 46.2%). Moreover, it was found that, producers in Songwe (55; 55.6%), Momba (40; 41.2%), Songea 87 (73.1%) and Mbinga (90; 53.3%) managed waste products inappropriately by throwing them away. Different proportions of pig producers in Songwe (35; 35.4%), Momba (60; 61.9%), Songea (4; 3.4%), and Mbinga (23; 13.6%) had not treated sick animals due to lack of veterinary services (Table 3). Different feeds were used to feed pigs in the study areas. Maize ban was highly used in Songwe (95; 38%), brew residuals in Momba (41; 17%), green leaves in Songea (96; 25%), and hotel leftovers in Mbinga (20; 5%) (Fig 3).

FIGURE 3: Type of feeds used to feed pigs in Songwe and Ruvuma regions, Tanzania



Cooking of pig feeds was not common in Songwe (72; 72.7%), Momba (71; 73.2%), Songea (77; 64.7%) and Mbinga (88; 52.1%) (Table 3). The common materials used to construct pig premises were grass thatched on roof, timber on walls and soil/timber on floor (Fig. 4a, 4b, 4c, 4d). Some pig premises (18%) within the study areas were left open on the roof while 5.0% to (44; 44.4%) had mud floor (Fig 5). The analysis showed that in all districts less than 50% of pig producers used to clean pig premises on daily or weekly basis (Fig 6).

FIGURE 4a: *Peramiho village (Songea District)*

FIGURE 4b: *Magagura village (Songea District)*

FIGURE 4c: ***Nyenjele Village (Momba District)***

FIGURE 4d: ***Misheni Street (Mbinga District)***

FIGURE 5: Type of materials used for pig premises

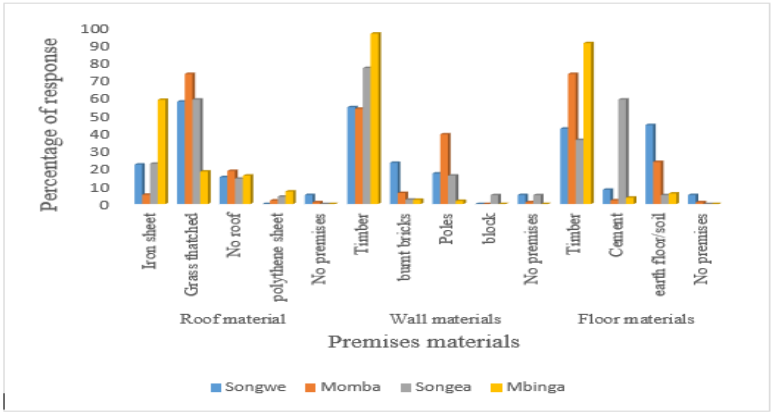
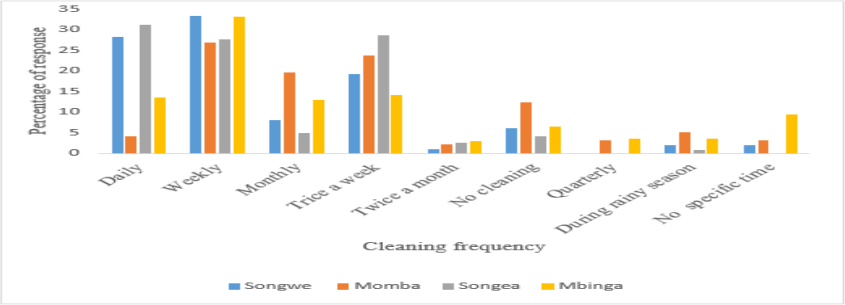


FIGURE 6: Routine cleaning of premises in Songwe and Ruvuma regions, Tanzania.



Transportation Node

Assemblers Marketing Practices

All 5 surveyed assemblers adopted sole proprietorship form of business, they reported not to have business licenses. Two (2) assemblers lived within study areas and while the rest three (3) came from neighbouring districts and towns in Mbeya and Njombe regions. Assemblers (n=5) purchased live pigs from more than 5 producers and transported them to neighbouring towns/regions. Depending on pig weight, the price ranged between US$ 22 and 174. The residential assemblers reported to transport between 6 and 10 live pigs per trip while those from outside the districts transported large numbers of live pigs (≥30) per trip. Both residential and non-residential assemblers hired trucks to transport purchased live pigs from study areas to pig slaughter slabs in the neighbouring region/town/city. The assemblers reported to transport live pigs during night and spent an average of 2 hours in short distances of 70 to 100 kilometres and more than 5 hours in long distances of 396.3 kilometres or more. All assemblers reported not to observe bio security measures such as disinfecting the vehicles/trucks before loading and after unloading of animals and during transportation (Table 4).

TABLE 4: Pig traders' marketing activities and practices in Songwe and Ruvuma Regions, Tanzania

Category	Variable	Songwe		Momba		Total (n=12)	Songea	Mbinga	Total (N =16)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
		Assembler (n= 2)	Retailers (n=5	Assembler (n= 3)	Retailers (n=2)		Retailers (n=11)	Retailers (n=5)	
Business Ownership	Sole proprietorship	2(100)	4(80.0)	3(100)	2(100)	11(91.7)	11 (68.7)	5 (31.3)	16(100)
	Partnership	-	1 (20.0)	-	-	1(8.3)	-	-	-
License	Yes	-	-	-	-	-	7(63.6)	5(100)	12(75.0)
	No	2(100)	5(100)	3(100)	2(100)	12(100)	4(36.4)	-	4(25.0)
Residential Status	Within district/urban	1(100)	5(100)	-	2(100)	8(66.7)	11(100)	5(100)	16(100)
	Outside district/urban	1(100)	-	3(100)	-	4(33.3)	-	-	-

Source of stock	Both within and outside	2(100)	5(100)	3 (100)	2(100)		11(100)	5(100)	16(100)
Number of producers	>5	2(100)	5(100)	3(100)	2(100)	12(100)	11(100)	5(100)	16(100)
Number of live pigs purchase	1	0(0)	3(60.0)	0(0)	2(100)	5(41.7)	7(63.6)	1(20.0)	8(50.0)
	>1 - 5	0(0)	2(40.0)	-	-	2(16.6)	4(36.3)	4(80.0)	8(50.0)
	6 - 10	2(100)	-	-	-	2(16.7)	-	-	-
	>10 - 30	-	-	-	-	-	-	-	-
	>30	-	-	3(100)	-	3(25.0)	-	-	-
Means of Transport	Trekking	-	2(40.0)	-	1(50.0)	3 (25.0)	-	-	-
	Hired truck	2(100)	-	3(100)	-	5(41.7)	-	-	-
	Motorcycle	-	3(60.0)	-	1(50.0)	3(33.3)	11(100)	(5(100)	16 (100)
Number of hours on road	30 – 45 mins	-	5(100)	-	2(100)	7(58.3)	7(63.6)	3(60.0)	10(62.5)
	1 – 2 hours	2(100.0)	-	-	-	2(16.7)	4 (36.4))	2(40.0)	6(37.5)
	>5 hours	-	-	3 (100)	-	3(25.0)	-	-	
Frequency of purchase	4-10 days/month	2(100)	-	3(100)	-	5(41.7)	-	1(20.0)	1(6.3)
	30days/months	-	3(60.0)	-	-	3(25.0)	6(54.5)	4(80.0)	10(62.5)
	Once/week	-	2(40.0)	-	2(100)	4(33.3)	5(45.5)	-	5(31.2)
Protective gears	Yes	-	-	-	-	1(8.3)	3(27.3)	1(20.1)	4(25.0)
	No	2(100)	5(100.0)	3(100)	2(100)	11(91.7)	8(72.7)	4(80.0)	12(75.0)

Marketing Node

Wholesalers and Retailers Marketing Practices

The distribution and marketing of live pigs and pig products involving wholesaler traders were not common in all study districts. However, it was mentioned by assemblers that wholesalers could be found at pig slaughter slabs in neighbouring town/city/region, that is to say,in Mbeya and Njombe regions. In all districts, the retailer traders shared common practices. Majority of pig retailers (n=22) operated in sole proprietorship while a few operated in partnership. Retailer traders (n=7) had no business licenses, while 12 had business licenses. Traders reported to purchase live pigs from pig producers both within and outside their villages of residence. Retailers (n=13), purchased live pigs daily, slaughtered and sold raw pork or roasted pork. Depending on the distance, several means of transport including trekking/ motorcycle/bicycle and trucks were used to transport live pigs from farm to different destinations within the study districts. In all districts, retailers were purchasing at least one live pig. Besides, 2 retailers in Songwe, 4 in Songea and 4 in Mbinga reported to purchase more than one live pigs and kept them at home before slaughtering. Slaughtering practices were conducted at slaughter slabs. It was observed that the slabs were open structures, without water supply and other relevant equipment and had poor waste products management. It was observed that retailer traders (n=4) were in gumboots and white overalls at slaughter slabs. The infrastructures and equipment used in butcheries and pork roast shops were of poor hygiene (unclean environment) as they could not protect pork meat from external contamination (Table 4).

Challenges in Prevention and Control of ASF

The respondents (Livestock Field Officers) reported on poor husbandry practices such as free ranging as well as low bio security measures at farm level and marketing channels. In order to improve production

and preventing disease outbreaks, application of livestock policy and bylaws within the chain is a necessity. The respondents mentioned presence of animal movement policy, bylaws against free ranging practices, inspection checkpoints, and penalty for violation of by laws. However, it was claimed that monitoring and implementation of the bylaws and regulations was the major challenge. One respondent had this to say: '*During ASF outbreak in 2018, we caught a retailer trader selling pork that was not inspected by livestock in charge officer.* We reported the case to the Police Station. However, the suspect won the case due to lack of strong evidence against him' (FLO, Songea district).

Moreover, the respondents reported that there were backyard-slaughtering practices especially during ASF outbreaks. For instance, one respondent reported that *'I caught a trader who slaughtered at backyard and sold out pork unsuitable for human consumption but due to bureaucracy with long chain of procedures, that trader was able to run away'* (FLO, Mbinga district).

In addition, it was reported that the sources of disease outbreaks included farm visitors, animal movements and products from infected villages. One of the respondent had this to say: *'In order to ensure implementation of animal movement regulations; the Ministry of Livestock and Fisheries should consider to delegate issuing of animal permit to Village Government Authority in order to control illegal movement of animals'* (Extension officer, Songwe district).

FINDINGS

The findings from this study have shown that there are many actors in pig industry from production, distribution, marketing to consumers. Basing on pig value chain performance within districts, the production was dominated by pig producers keeping 1 to 4 pigs. This finding is consistent with census report on pig production in Tanzania.[18] Many pig producers owned small pieces of land ranging from 1 to 2 acres, this is considered to be enough space to keep 1 to 4 pigs for small scale production. An interesting finding was the fact that only one type of breed was kept by pig producers in all the 4 districts. Many preferred to keep indigenous breeds with a reason that they are less susceptible to diseases including ASF.

The production chain involving pig producers highlighted key information that could contribute to transmission of ASF. Outsourcing pig stocks from various places without considering the health status of pigs could have been the source of ASF introduction and spread in the 4 study districts. ASF transmission through unknown sources has also been reported in previous studies in Uganda[23,24] and Nigeria.[25] It was found that practices such as free ranging system during dry season were common in rural areas compared to urban areas. Probably this observation could partly explain the spread of previous outbreaks by free roaming pigs in rural areas. This practice is similar with the characteristic of smallholder producers in Uganda.[26]

Other practices such as low bio security level in pig premises could contribute to introduction of ASF into un-infected pig herds. Pig premises were observed to be very dirty even those in which pig producers claimed to perform daily cleanliness, waste products were maintained nearby the farm. The open roof structures and those with mud floor have a high chance of exposing pigs to a number of infections. These findings are in agreement with those of Dione[26] in Uganda who reported that open pig premises exposed animals to predators and stray animals. Different feed types were used to feed pigs but yet there was poor handling of the feeds, use of dirty equipment, and feeding pigs on leftovers could also be a source of farm outbreaks. It seems that majority of pig producers have inadequate knowledge on implementation of bio security measures suggesting that purposive education and awareness campaigns should be conducted. Similar findings were reported in Tororo and Busia districts in Uganda and Busia and Teso district in Kenya.[24]

In our study districts, pig marketing was dominated by male traders, implying that there is high disease risk in marketing channels since males are less likely to implement bio security measures. This finding is supported by a study in Uganda that reported a belief by males that bio security measures such as cleanliness are women responsibilities.[27]

Small capital investment to purchase live pigs was observed to be the main concern of pig traders in marketing activities. Along with this, lack of license among pig traders was common in all the study districts. The absence of business licensing especially in rural areas result in lack of data on pig marketing channels, making it nearly impossible for industry planning and development as well as in intervention against ASF.

Transportation of live pigs during night hours could be the source of disease transmission from infected areas to uninfected areas. This finding is consistent with what was found in a previous study in Uganda which reported on behaviour of traders to avoid inspection as

a loophole for transporting infected pigs.[26] Moreover, the practice of collecting live animals from more than one farms/producers, loading and unloading of animals without cleaning and disinfection of vehicles could be sources of disease transmission within and outside the districts. Similar observations have been reported in Kenya and Uganda.[28] Meanwhile, long transportation periods carried high disease risks of infected pigs to affect health pigs as previously reported in Uganda.[29]

Marketing activities of pork meat and other products were more common in urban areas compared to rural areas meaning that marketing activities are economically driven by urbanisation and income growth. On average, the urban retailers slaughtered 10 pigs daily compared to 3 pigs in rural areas. This is an indication that traders in urban areas practice more farm visits due to consumers demand. Additionally, this observation entail that the emerging towns and cities require strict measures to fight against ASF. This is because most towns and cities are targeted market for pork meat.

Poor hygiene was observed in slaughter slabs. Waste products were discarded in open areas and this could be the source of disease outbreaks. Apart from facilitating disease spread, unhygienic environment in slaughter slab, as well as roast shops and butchers, indicate that there is a high chance of consumers to end up with unsafe pork meat.

husbandry system, sharing boars, feeding pork leftovers, and poor disposal of dead pigs to all actors involved in pig production and marketing. The formation of pig business associations should be encouraged to provide an opportunity to improve pig value chain operations and ensure access to market information. Moreover, improved availability and accessibility to veterinary services are likely to play major roles in reducing disease outbreaks through

i) It was conducted when there were no ASF outbreak cases. Although its findings could inform chains operation and sources of ASF outbreaks, more attention should be given to pig chain performance during ASF outbreaks,

ii) The time interval is unlikely to have had influence on practices and there was no interventions implemented in the study areas during the period. However, we cannot account on unknown sources of changes in the practices and their impacts such as exchanges of information among the actors/pig owners between the study sites,

iii) Poor record keeping practices by pig producers and pig traders. Despite being able to capture production practices within the pig value chain; it was not possible

The key informants highlighted that behaviour of traders to avoid animal inspection and slaughtering practice in the backyard played great role in the spread of ASF. This finding entailed the need to improve good animal husbandry practices in order to enhance productivity and meat safety. Meanwhile, field officers pointed out that among the factors that hinder control of animal movements could be the limited involvement of livestock officers and extension officers regarding the issuance of animal movement permits. This weakened enforcement of animal movement control has been attributed to spread of diseases in many countries of Africa and in the Middle East.[30] Recommendation on decentralised power in issuing the animal movement permits was given as a way forward in solving animal control movements. Parallel to this, quarantine of animals that are being moved between regions and animals introduced within regions should be emphasised in order to prevent the introduction and spread of diseases.

Recommendations to Future Interventions

Since there is no effective treatment available against ASF, prevention would be much achieved by enforcement of strict bio security measures. It is important to increase awareness on risk practices such as free-ranging

routine disease surveillance, training and extension services. Training should provide knowledge transfer on good husbandry such as confinement of pigs to avoid free-ranging practices associated with outbreaks. Lastly, it is important to improve infrastructures such as slaughter facilities not only to control diseases but also to ensure meat safety.

Study Limitations

to establish relationship between pig management and factors influencing pig productivity due to absence of records on different variables including the number of piglets born, number of births and age of sow.

CONCLUSIONS

The findings demonstrated that pig producers, assemblers, and retailer traders were operating in pig production, distribution and marketing chains in Tanzania. Each node performed under poor bio security measures, poor infrastructure and facilities which could contribute to introduction and spread of ASF. The transportation node carried a high disease risk due to many movements and pick-ups. There is need to improve good husbandry practices and marketing

infrastructures to increase productivity and ensure pork safety. It is anticipated that an overview information on chains operation and possible disease risk practices from this study will be used as inputs in the design of future ASF prevention and control measures in Tanzania.

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EAHRC
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ORIGINAL ARTICLE

Etiologies of bloodstream infection and antimicrobial resistance: A cross sectional study among patients in a tertiary hospital, Northern Tanzania

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ABSTRACT

Background: Bloodstream infections are important causes of morbidity and mortality in people of all age groups, especially in sub-Saharan Africa. In Tanzania, a recent report indicates that case fatality rate of 37% is attributed to bloodstream infections. The aim of this study was to determine the prevalence and factors associated with bloodstream infections as well as to determine resistance pattern of bacterial isolates among patients visiting Kilimanjaro Christian Medical Centre (KCMC).
Methods: A cross-sectional study was conducted from April to June 2019 at KCMC. A total of 200 patients were included in the study. Blood samples were collected for culture, malaria rapid test, typhoid and brucella tests. Clinical features, co-morbid conditions and patients' hospitalization data were recorded in the questionnaire. Logistic regression was used to examine the factors associated with bloodstream infections. Predictors of the outcome were considered significant at $p<0.05$.
Results: The prevalence of bloodstream infections was 52(26%). Participants with stomachache had less odds of having bloodstream infections as compared to other patients with symptoms (AOR=0.22, 5.33, 95%CI=0.05-0.97; p=0.04). Of the XX identified isolates Staphylococcus aureus showed the highest rates of resistance for Meropenem 8(88.8%), Cefotaxime 6(66.6%, Amikacin 6(66.6%), Gentamicin 6(66.6%) and Imipenem 6(66.6%). The lowest level of resistance was observed in Ceftriaxone 1(11.1%).
Conclusion: Bloodstream infections were highly prevalent in this sample (26%). Staphylococcus *spp* was the most commonly isolated organism and exhibited a high resistance rate to most antibiotics. This calls for increased and coordinated efforts to improve the identification, treatment and management of bloodstream infections and antimicrobial resistance, thereby improving clinical practice.

INTRODUCTION

Bloodstream infections (BSIs) are important causes of morbidity and mortality among children and adults in most African Countries[1–3]. In a meta-analysis on community-acquired BSI in Africa, the mean mortality rate was reported to be 18.1%[2]. In 2007, a study done at Muhimbili National Hospital, Dar es Salaam among a pediatric population, reported mortality rates of 20.2%, 43.5% and 16.7% for malaria, gram-negative BSIs and gram-positive BSIs respectively[4]. More recently findings from the same hospital indicated a case mortality rate of 37% among patients of all age groups[5]. The prevalence of BSIs ranges from 13% to 14.5% in different parts of the Tanzania[4,6,7].

There are increased reports of antimicrobial resistance (AMR) among patients with BSI[7–10]. More importantly, rates of BSI are increasing owing to multidrug-resistant extended-spectrum beta-lactamase (ESBL)[8,11,12], methicillin-resistant Staphylococcus aureus (MRSA)[13], and Vancomycin resistance[14].
In Tanzanian, most of BSIs are misdiagnosed as malaria infections[1]. This is due to the lack of capacity to properly identify the cause of febrile illness[15]. Consequently, Clinicians often rely on clinical features

to guide the treatment of patient who present with febrile illness[16] which is less accurate in identifying BSIs, hence risking poor clinical outcomes and promotion of AMR[17].

Various factors have been found to be associated with BSI, including; socio-demographics, comorbid conditions, prior hospitalization and recent exposures[7]. Comorbid conditions found to be associated with BSI include: acute and chronic renal failure, hepatic disease, diabetes, hypertension, congestive heart failure, and intravenous drug abuse[18].

However, there is limited information about BSIs in Northern Tanzania. Specifically, the prevalence, level of resistance and factors associated have not been documented. Notably, the extent of AMR in most BSIs has not been studied. Understanding BSI at KCMC, resistance patterns, and the factors associated with BSIs will guide clinical management and appropriate antibiotic use. Therefore, the present study was designed to document prevalence, factors associated with BSI and resistance patterns of bacteria isolated among patients with BSI.

METHODS

Study design and area

This was a hospital-based cross-sectional study. The study was conducted at KCMC referral hospital, located in the foothills of the snowcapped Mount Kilimanjaro in Tanzania (http://www.kcmc.ac.tz/). KCMC has a 650-bed capacity and the second largest consultant referral university teaching hospital in the country serving over 15 million people from northern and central regions of Tanzania, attending more than 800 – 1000 outpatients daily.

Study population and inclusion criteria

The study included all out- and inpatients suspected with BSI. The study included patients of all age suspected of having bacterial or malarial infections. All patients who were critical ill, mentally unfit, and not able to communicate were excluded from the study.

Sample Size Estimation

Samples was calculated based on the following formula:

$$N = \frac{Z_P^2 \,(1-P)}{\varepsilon^2}$$

Where: N = Sample size, Z = Level of confidence (1.96) , P =proportion previous prevalence (13.4%) 6 and ε = Margin error (5%), plus 10% non-response. Sample size = 1.962*0.134*(1-0.134)/(0.05)2. The minimum sample size was 178+10%=196, we rounded up to give a sample size approximation of 200 patients.

Data collection

Interview

Data was collected by three research assistants who were experienced in data collection. All information was collected during face-to-face interviews using questionnaire in Swahili language. The interview lasted between 30 and 45 minutes. The interviews for Out-patients were conducted in a private room where no one other than the research assistant and the patients and/or guardian were allowed to be present. For inpatient, bedside interviews were conducted. The children's information's were given by their parents/ guardians.

The questionnaire had three sections. Section 1: recorded patient's socio-demographic characteristics such as age, sex, occupation, level of education, income per month and residence. Section 2: assessed patient's clinical features and conditions. Other patients' information such as clinical outcomes were retrieved from the patient's medical records. Section 3: collected details on antibiotic usage, and lastly section 4 collated the laboratory investigations

Pre-testing of the questionnaire

To maximize its validity, the questionnaire was pretested on appropriate respondents before distribution. Interviews were conducted in five (5) patients to examine how patients understood and responded to the questions. In addition to the pilot, two experts in the field of survey design approved the quality of the questionnaire. After the pretest, adjustments in phrasings were made as necessary so that the questionnaire was simple and easily understood.

Blood sample collection and Blood culture

Venous blood was drawn aseptically from each patient. A total of 2-5ml of blood was collected in BD BACTEC bottles from pediatrics patients (BD BACTEC Peds PlusTM/F Culture Vials, Becton Dickinson and Company) and 8-10ml from adults (BD BACTEC Plus Aerobic/F Culture Vials, Becton Dickinson and Company). Blood samples were immediately transported at room temperature to the KCMC clinical laboratory and incubated in the BACTEC machine for further investigation. Blood samples were incubated in BD BACTEC machine for a maximum of 5 days. Positive blood cultures were inoculated on Blood agar, Chocolate agar (both from HI Media Laboratories, Mumbai, India), and MacConkey agar (Becton Dickinson and Company, Cockeysville, MD, USA) and incubated for 18–24 h at 37°C. Standard microbiological technique were conducted to identify bacteria including colony morphology, Gram stain, and biochemical tests (Oxoid). Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results. Gram-negative rods were identified by performing a series of biochemical tests such as Kligler Iron Agar (KIA), Simon's citrate agar, Indole, urea, and motility. Blood collected for malaria and serology were analyzed within 30 minutes in their

respective sections after collection. Samples were collected and processed by qualified laboratory technologist.

Susceptibility Test

Antimicrobial susceptibility testing was performed using disc diffusion on Müller–Hinton Agar according to Clinical Laboratory Standards Institute guidelines[19]. Bacterial isolates were tested against amoxicillin-clavulanic acid (30 µg), Amikacin (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Imipenem (30 µg), Trimethoprim-sulfamethoxazole (23.75 µg/1.25 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Meropenem (10 µg), Vancomycin (30 µg), Tetracycline (30 µg), Cefotaxime (30 µg), Penicillin (10 µg) and Chloramphenicol (30 µg). All antibiotic discs were from Oxoid, ThermoFisher, and Scientific, USA. Antimicrobial sensitivity was reported as resistant, intermediate, and sensitive according to the Clinical Laboratory Standard Institute[19]. The choice of antibiotic agents varied depending on the range of antibiotics available to the laboratory.

Case definition

BSI was defined as having either positive blood culture, malaria parasites, a positive serological result or any co-infections.

Data analysis plan

Data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY, USA). Cross-tabulation of categorical variables was calculated using Chi-square test ($\chi 2$) while Fisher's exact test was used in cases when expected counts were ≤ 5. The association between categorical predictors and the BSI was presented as odds ratio (OR) with 95% confidence intervals (95% CI) using logistic regression. Predictors significantly associated with BSI in the bivariate analysis were selected for multivariable analysis in the final model. A significance level ≤ 0.05 was used throughout.

Ethical Consideration

Ethical approval to conduct this study was obtained from the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (CRERC) with ethics certificate number 2472. Written consent was signed before filling the questionnaire. Permission to conduct the study was obtained from KCMC administration. Though all measures to protect the privacy and confidentiality was considered that neither name nor registration number was mentioned during data collection.

RESULTS

Demographic characteristics of the study population

A total of 200 patients were enrolled in the study, giving a response rate of 100%. The modal age group was 0-5 years with a frequency of 72 (36%). More than half of the participants were female 110 (55%). The majority of the participants lived in urban 119 (59.5%). Most participants were self-employed 65 (32.5%). Lastly, 58 (29%) and 62 (31%) had primary and secondary education respectively (***Table 1***).

Prevalence and common etiologies of BSI infections

Overall, 52/200 (26%) of the participants were evident of having BSI infections. A total of 123 blood cultures were performed, 76/123 (61.8%) from children (0-17 years) and 47/123 (38.2%) from adults >17 years. Positive bacterial growth was observed in 41/123 (33.3%) isolates with 18/123 (14.6%) being significant for antimicrobial susceptibility testing. The number of pathogens recovered in the study period is presented in ***Figure 1***. A total of 67 samples were subjected to serological tests. Out of these, 27(40.3%) were tested for Widal, 20(29.9%) tested for Brucella and the remaining were tested for syphilis. The decisions for specific tests on samples were based on the clinical symptoms presented by the patient and the tests ordered by the attending clinician. A total of 10(37.0%) were positive for Widal while 2(10.5%) were positive for Brucella. Two of the participants had co-infection (typhoid and Brucellosis), ***Figure 2***. A total of 51 participants were tested for malaria, only 1(2.0%) was found positive.

Antimicrobial susceptibility patterns of bacterial isolates

Antibiotic-resistance patterns of bacteria against the antimicrobial agents is shown in ***Table 2***. Coagulase Negative Staphylococcus (CoNS) showed highest resistance for Gentamicin 24 (96%), Trimethoprim/sulfamethoxazole 24 (96%), Imipenem 24 (96%), Chloramphenicol 24 (96%), Ciprofloxacin 23 (92%), Clindamycin 23 (92%) and Gentamycin 22 (88%). Staphylococcus aureus showed the highest resistance for Meropenem 8 (88.8%), Chloramphenicol 8 (88.8%), Cefotaxime 6 (66.6%), Tetracycline 6 (66.6%), Amikacin 6 (66.6%), Gentamicin 5 (66.6%) and Imipenem 6 (66.6%). The lowest level of resistance was observed in Ceftriaxone 1 (11.1%).

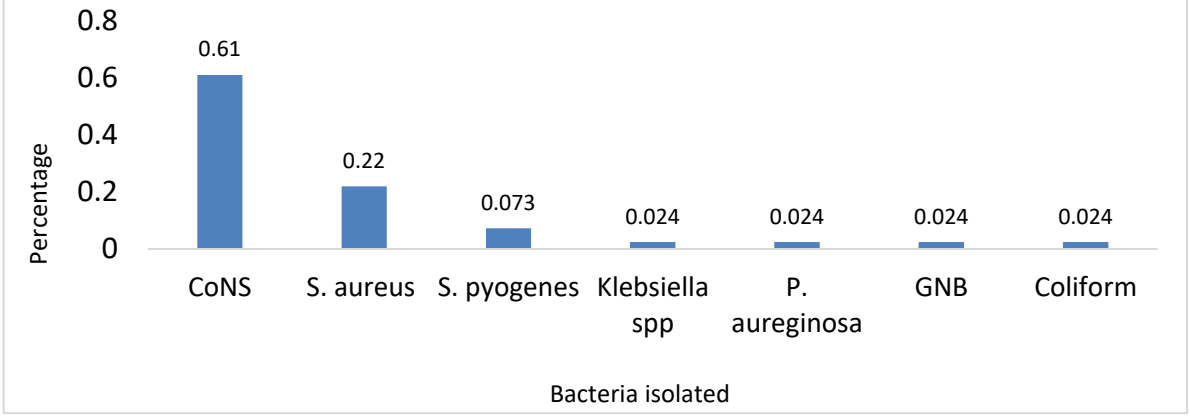
Risk factors associated with BSI

The results of the bi- and multivariate analyses of the association between selected predictors and BSI are shown in ***Table 3***. In the bivariate analysis: age of the participants, education level, infection risk, admission, length of admission and hospitalization showed an association with the outcome and were then selected to be included in the multivariate model. Only two factors remained independently associated with the occurrence of BSI after adjusting for confounding. Participants who reported having a diploma as their highest level of education had a higher odds of having BSIs compared to those with a degree (AOR= 5.33, 95%CI=1.39-20.38; P=0.01). Participants with a stomachache had lower odds of having BSI compared to other patients with symptoms (AOR=0.22, 5.33, 95%CI=0.05-0.97; P=0.04).

TABLE 1: Socio-demographic characteristics of the respondents (N =200)

Variable	n (%)	BSI infection	
		Positive n (%)	Negative n (%)
Age group (years)			
0-5	72 (36.0)	26 (49.1)	46 (31.3)
6-17	7 (3.5)	1 (1.9)	6 (4.1)
18-45	69 (34.5)	17 (32.1)	52 (35.4)
More than 45	52 (26.0)	9 (17.0)	43 (29.3)
Locality			
Urban	119 (59.5)	31 (58.5)	88 (59.9)
Rural	81 (40.5)	22 (41.5)	59 (40.1)
Sex			
Male	90 (45.0)	26 (49.1)	64 (43.5)
Female	110 (55.0)	27 (50.9)	83 (56.5)
Education level			
Illiterate	4 (2.0)	0 (0.0)	4 (2.7)
Primary level	58 (29.0)	19 (35.8)	39 (29.5)
Secondary level	62 (31.0)	17 (32.1)	45 (30.6)
Diploma level	32 (16.0)	2 (3.8)	30 (20.4)
Degree level	32 (16.0)	11 (20.8)	21 (14.3)
Others	12 (6.0)	4 (7.5)	8 (5.4)
Occupation status			
Employed	61 (30.5)	13 (24.5)	48 (32.7)
Unemployed	43 (21.5)	12 (22.6)	31 (21.1)
Student	31 (15.5)	7 (13.2)	24 (16.3)
Self-employed	65 (32.5)	21 (39.6)	44 (29.9)
Income			
Less than 300,000/=	62 (31.0)	18 (34.0)	44 (29.9)
300,000/= to 1,000,000/=	57 (28.5)	14 (26.4)	43 (29.3)
More than 1,000,000/=	4 (2.0)	1 (1.9)	3 (2.0)
No income	77 (38.5)	20 (37.7)	57 (38.8)

FIGURE 1: Isolated Bacteria



CoNS- Coagulase negative Staphylococcus
GNB- Gram negative bacilli

FIGURE 2: Other Laboratory Tests Performed

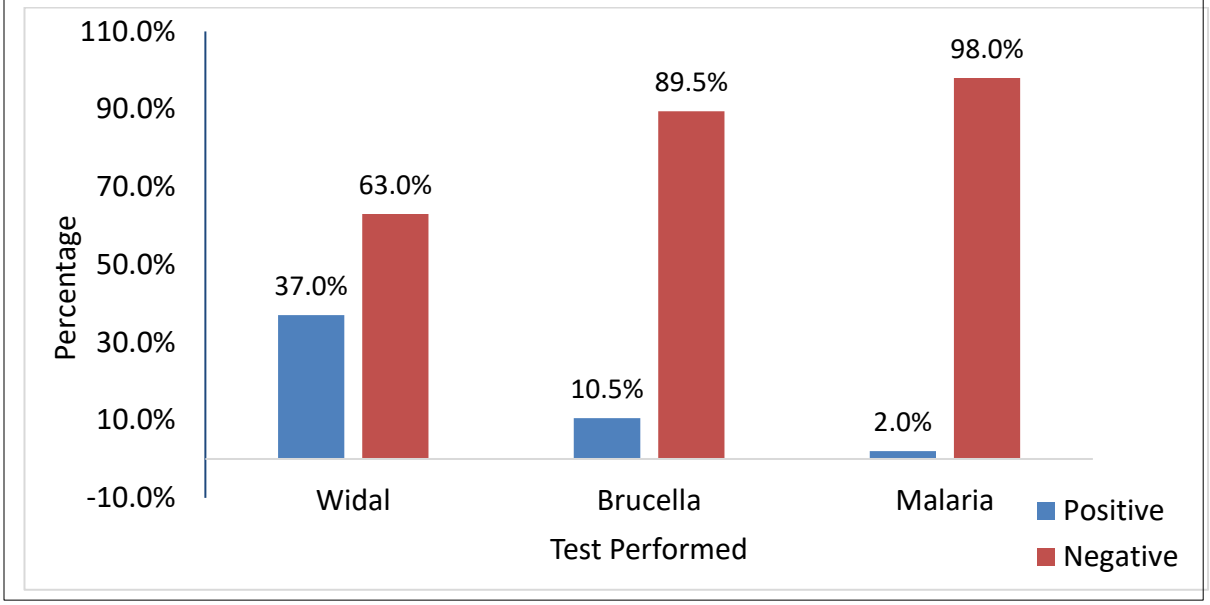


TABLE 3: Factors associated with BSI

Variable		COR	P-value	AOR	P-value
Age group (years)					
	0-5	0.37 (0.15-0.87)	0.02	1.29 (0.17-9.41)	0.7
	6-17	1.25 (0.13-11.74)	0.8	3.77 (0.25-55.05)	0.3
	18-45	0.69 (0.27-1.72)	0.4	0.61 (0.20-1.89)	0.3
	More than 45	Reference		Reference	
Locality					
	Urban	1.10 (0.58-2.10)	0.7		
	Rural	Reference			
Sex					
	Male	0.84 (0.44-1.59)	0.6		
	Female	Reference			
Education level					
	Primary level	1.12 (0.45-2.73)	0.8	2.37 (0.69-8.07)	0.1
	Secondary level	1.47 (0.60-3.61)	0.3	2.75 (0.89-8.53)	0.07
	Diploma level	5.04 (1.44-17.58)	0.01	5.33 (1.39-20.38)	0.01
	Degree level and above	Reference		Reference	
Occupation					
	Employed	1.93 (0.86-4.32)	0.1	1.69 (0.59-4.79)	0.3
	Unemployed	1.65 (0.78-3.47)	0.1	1.44 (0.59-3.49)	0.4
	Self-employed	Reference			
Income					
	Less than 300,000/=	0.91 (0.09-9.11)	0.9		
	300,000/= to 1,000,000/=	1.02 (0.09-10.65)	0.9		
	More than 1,000,000/=	Reference			
Symptoms					
	Fever	0.78 (0.35-1.75)	0.5	0.67 (0.27-1.69)	0.4

Headache	1.56 (0.44-5.52)	0.4	1.14 (0.27-4.84)	0.8
Stomachache	0.44 (0.13-1.53)	0.1	0.22 (0.05-0.97)	0.04
Diarrhea	0.44 (0.06-3.01)	0.4	0.22 (0.02-2.13)	0.1
Others*	Reference			
Co-morbidities				
Diabetes	1.78 (0.20-16.67)	0.6		
Hypertension	0.89 (0.26-2.98)	0.8		
Cancer	0.35 (0.49-2.60)	0.3		
Others	1.06 (0.27-4.13)	0.9		
None	Reference			
Infection risks (N=87)				
Urinary catheter	0.59 (0.14-2.45)	0.4	0.80 (0.15-4.13)	0.7
Intravascular catheter	0.43 (0.22-0.84)	0.01	0.74 (0.29-1.85)	0.5
None	Reference		Reference	
Admission				
Yes	0.32 (0.16-0.65)	0.02	0.12 (0.005-3.10)	0.2
No	Reference		Reference	
Duration of hospitalization				
Less than 7 days	0.41 (0.19-0.87)	0.02	1.35 (0.02-68.09)	0.8
7 to 14 days	0.29 (0.11-0.73)	0.009	0.71 (0.01-39.24)	0.8
More than 14 days	0.18 (0.04-0.82)	0.02	0.46 (0.006-34.71)	0.7
Not admitted	Reference		Reference	
Ward				
Medical	0.75 (0.26-2.15)	0.5	4.70 (0.35-62.17)	0.2
Surgical	0.34 (0.07-1.57)	0.1	3.22 (0.17-59.01)	0.4
Pediatric	0.32 (0.15-0.67)	0.02	1.31 (0.07-22.70)	0.8
Others	Reference		Reference	

COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio

* Other symptom such as Rashes and vomit/nausea

DISCUSSION

This study was conducted among patients who visited KCMC. The study aimed at determining the prevalence of BSIs, associated factors, and antibiotic susceptibility. The prevalence of positive blood culture in this study was found to be 14.6% which is comparable to other studies conducted in the same region (Zanzibar (14.0%)[20], Muhimbili-Tanzania (13.4%).[6] A higher prevalence was observed in a study done in Malawi which reported a prevalence of 30%, also difference may be due to different study population whereby it was specific to adults only who presented with fever[22]. Coagulase Negative Staphylococcus (61.0%) was the most predominant bacteria isolated among participants with bacterial infections, followed by Staphylococcus aureus (22.0%). This pattern is similar to a study conducted in Dar Es Salaam Tanzania which also reported that Staphylococcus aureus as the most common cause of BSIs[6]. CoNS was also reported to be the most isolated bacteria in a study conducted in Ghana [23], while Staphylococcus aureus was the second most isolated bacteria among patients with BSI in Ghana[23] and Malawi[24]. Salmonella typhi was the most common organism isolated among the positive participants. Different studies have also reported Salmonella being a major cause of infection in Bangladesh (36.9%)[21], Nepal (71%)[25] and Malawi[24].

Coagulase Negative Staphylococcus showed the highest rates of drug resistance to Trimethoprim/sulfamethoxazole, Imipenem, Chloramphenicol, Ciprofloxacin, Clindamycin and Gentamycin. Similarly, a study in India reported that Ciprofloxacin and Clindamycin was resistance to CoNS[26]. Staphylococcus aureus showed the highest rates of resistance for gentamicin (66.6%). This finding is comparable to similar reports from Brazil[27], India[26] and Senegal[28]. In contrary, a study conducted in Zanzibar reported high susceptibility of Staphylococcus aureus to Cefotaxime and Tetracycline[20]. In general, our findings suggest that the that isolates found on the Tanzanian mainland are quite similar to those found elsewhere[26–28]. However, we noted small differences with a study conducted in Zanzibar[20]. It is possible that the smaller population and more limited circulation of people reduces the transmission of some strains of the pathogen. Based on the observed results in this study, treatment should follow microbiology investigations and not be solely based on clinical symptoms. This will improve the accuracy of diagnosis and support the reduction in the occurrence of AMR. Further efforts are needed to raise awareness amongst the community and healthcare workers as to the rapid increase in drug

resistance and advocate on the antimicrobial stewardship program in these settings.

The education of individuals can help tackling the spread of disease, given that educated people may be more aware how diseases circulate in their community. The probability of having or dying from infections such as malaria is inversely related to income and education[29]. However, this was not the case in this study, our results suggest that those with a higher level of education (diploma) were more likely to have a BSI. It is possible that this result is caused by a selection bias, the study was hospital based, so it is possible that those who are more educated may have better health seeking behavior and therefore more likely to attend hospital and then be included in this study. A study conducted in America showed that education level was a significant factor associated with BSIs for more than 30% of participants[18]. Though lacking statistical significance our findings suggest that participants with diabetes were 78% more likely to have BSIs compared to others. Our sample size was small, and this may have reduced our power to detect an association. Further studies should be conducted to investigate this association. Finally, our study suggests that increased efforts should be taken to reduce opportunities for infection.

The findings of this study should be considered with the following limitations in mind. Firstly, a single blood culture specimen was collected from each patient, therefore it was not possible to determine if the patients with CoNS isolation had true bacteremia or the finding was due to skin contamination. The data for this study were obtained for three months between April and June 2019, and therefore seasonal variations in the frequency of BSI and causative microorganisms could not be assessed.

CONCLUSION

We identified a high prevalence of BSIs across all age groups and more commonly associated with education level. Staphylococcus spp was the most commonly isolated organism among patients with BSIs and exhibited high levels of AMR. We recommend that laboratory investigations and susceptibility tests of isolated bacteria should guide treatment protocols, and these should not be solely based on clinical symptoms.
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Conflict of interest
There was no conflict of interest

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EAHRC
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ORIGINAL ARTICLE

Detection of mixed infection of *Plasmodium* species in the Southern province of Rwanda. Case study: Ruhango, Bunyogombe and Kibilizi Health centres

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ABSTRACT

Background: Malaria is a leading cause of mortality and morbidity in sub-Saharan Africa including Rwanda. Though the prevalence of malaria has been reduced due to the use of indoor residual sprayings and insecticide-treated bed nets, it is still a disease that kills many people annually. Many studies conducted revealed that in sub-Saharan Africa including Rwanda there is a high prevalence of Plasmodium falciparum. However, there is still a gap in the identification of the presence of mixed Plasmodium infection. This study was conducted to determine the overall prevalence of Plasmodium species as well as that of mixed plasmodium infection in Ruhango and Kibilizi Health centres.

Methods: Descriptive cross-sectional study was conducted on 126 participants in Ruhango, Bunyogombe and Kibilizi health centres located in the southern province of Rwanda. The conventional sampling strategy was used for the selection of individuals who consented to participate in the study. Blood samples were used to detect Plasmodium species and the obtained data were analyzed using Microsoft excel and IBM SPSS version 21.

Results: Among 126 participants presenting with signs and symptoms of malaria, the overall positive cases of Plasmodium species were 61(48.4%) and among the total positive cases 56 (44.5%) were infected with single Plasmodium species while 5 (4%) were infected with mixed Plasmodium species. Plasmodium falciparum was the most prevalent species infecting 49 (39%) participants while Plasmodium vivax was the least prevalent infection, detected in only 1(0.8%) participant.

Conclusion: The study identified the significant prevalence of mixed-species of Plasmodium infection as well as the high prevalence of Plasmodium falciparum infection in the study population. These findings suggest that there is a need for continued monitoring of non-falciparum infection in this population and the introduction of species-specific RDTs that can be used for diagnostic purposes.

Keywords: Malaria, Plasmodium species, Mixed Plasmodium infection, Infection, Prevalence, Descriptive Cross-sectional study, Samples, Purposive sampling, WHO.

INTRODUCTION

Malaria is a human disease caused by five Plasmodium parasites: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*. Plasmodium species are differently distributed in the world.[1] The prevalence of malaria cases and deaths differs during different seasons.[2] In 2017, there were an estimated 219 million cases of malaria in 90 countries. Malaria deaths reached 435 000 in 2017.[3] The World Health Organisation (WHO) African Region carries a disproportionately high share of the global malaria burden in 2017 as well as in 2018. In 2017, the region was home to 92% of malaria cases and 93% of malaria deaths[3] while In 2018, malaria cases were 93% and 94% of malaria deaths.[4]

In Africa, including Rwanda, many epidemiological studies demonstrate that P. falciparum is the most prevalent malarial species.[5,6] Some population groups are at considerably higher risk of contracting malaria and developing severe disease than others.[7] These include infants, children under five years of age, pregnant women and patient with HIV AIDS as well as non-immune migrants, mobile populations and

travelers.[8] In Rwanda, the Malaria indicator survey (MIS) indicated that in 2017 the prevalence of Malaria in the general population was 7% where Eastern province presented 12%, Southern province 9%, Western province 2%, Northern 1%, and Kigali city 3%.[9a] The Rwandan National Malaria control programmes have been at the forefront of scaling up malaria interventions over the past decade, including the distribution of insecticide-treated bed nets, indoor residual spraying in high malaria transmission foci and epidemic-prone areas, early adoption of artemisinin-based combined therapy and use of rapid diagnostic test (RDTs) for clinical management of malaria.[9b]

Rapid and accurate diagnosis of Plasmodium infections is crucial for morbidity and mortality reduction in tropical areas, especially in regions where mixed infections are prevalent such as sub-Saharan Africa including Rwanda, where all four parasites infecting humans coexist and mixed-species infections are common and not well characterized but may play roles in disease progression and outcomes.[10] For improving accuracy in large epidemiological studies, molecular diagnostic tools permitting high throughput analysis for the identification and quantification of malaria parasites would be of great benefit.[11] Traditionally, light microscopy (LM) examination of blood smears has been considered the gold standard for the diagnosis of malaria.[8] Light Microscopy has clear advantages over molecular typing since it incurs only low costs, does neither need complex sample preparation nor advanced technology and permits species identification and quantification.[8] In Rwanda, the use of Rapid diagnostic tests (RDT) by community health workers has greatly facilitated the diagnosis of malaria disease in remote areas where Plasmodium parasites are known to be endemic.[12] However, their use is limited due to the lack of sensitivity to differentiate between existing Plasmodium species.[8]

For improving accuracy in diagnosis and treatment of malaria, identifying malaria species and or mixed species of Plasmodium infections are reliable methods to move towards the elimination of Plasmodium parasite.[5] It follows that further understanding of the distribution of local malaria species and co-infections of plasmodium parasites is important for developing appropriate preventive as well as diagnostic and treatment options. This study aimed to determine the prevalence of Plasmodium species co-infection in Ruhango, Bunyogombe Kibilizi health centres. This information is necessary to guide and inform management and control strategies as wells as provide evidence for strategic malaria diagnostics service choices and treatment options.

METHODOLOGY

Fisher's exact test for proportions, were used to test for independence.

Design and Study area

This was a descriptive cross-sectional study conducted during the dry season for a period of three months from May to September 2019 at Ruhango Health Center of Ruhango district and Kibilizi Health Center of Gisagara district located in the southern province of Rwanda. The above districts are known to be the hot spot zone of malaria disease in Rwanda.

Sample size determination and Sampling

A purposive sampling strategy was used to select the participants attending Ruhango and Kibilizi health centres presenting signs and symptoms of malaria. Sample size calculation used the prevalence of malaria in the southern province in 2017 which was 9% as indicated by Rwanda Malaria indicator survey.[9] The minimum sample size was calculated using Daniel's formula which is $n = \frac{z^2 * p(1-p)}{d^2}$,

where n=sample size, p= expected prevalence, d=allowed error which is 5%, and z which is statistical of confidence 95%.[13]

The sample size was: $n = \frac{z^2 * p(1-p)}{d^2} = \frac{1.96^2 * 0.09(1-0.09)}{0.05^2} = 126$

Data collection and Diagnostic procedures

Finger prick was performed to prepare thick and thin smears of 1 to 1cm and allow the prepared smears to air dry. The well-dried smears were stained with 10% Giemsa solution for 20 minutes and examined under a light microscope using a 100x objective (immersion oil objective) for detecting the presence of the Plasmodium parasites. Once the parasites were detected on a thick smear using a light microscope, the thin smear was performed for differentiation of Plasmodium species after fixing the smear in methanol and staining it using Giemsa solution diluted 10% for 20 min and examine under the light microscope using 100x objective for species identification. Slides were considered as positive only after being confirmed by two more technicians. Parasite density was determined as the number of parasites per 200 leukocytes count of 8000/µl of blood. A negative result was considered if no parasites were detected per 200 leukocytes. The four technicians for microscopy detection were well trained on malaria diagnosis, blood collection, thick and thin blood smear preparation, Giemsa staining and using slide reader.

Data record and analysis

The data were recorded in Microsoft excel and were analyzed using IBM SPSS version 21 (Chicago- USA). The Mantel Haenszel chi-square test, and in some cases

Ethical Approval

Ethical clearance for the study was obtained from the Institutional Review Board of the College of Medicine

and Health Sciences at the University of Rwanda (CMHS/IRB/216/2019). The ethical clearance was presented to study sites administration at Ruhango and Kibilizi Health Centres and an approval letter for the data collection was provided. The community at the study sites was sensitized and appreciated the objectives of the study. Informed consent forms for data collection were signed and the participant's information was kept confidential. Included in the study were the residents of the study site for 6 months preceding the study with clinical signs and symptoms of malaria. Those excluded were nonresidents, those on anti-malarial treatment and those who declined to give informed consent.

RESULTS

This study was carried out in the southern province, at Ruhango and Kibilizi Health Centers for a period of 3 months from May to September 2019. The study aimed to detect the mixed infection of Plasmodium species using microscopy technique.

Socio-Demographic Characteristics of the study population

One hundred and twenty-six participants aged between 3 and 67 years old from Ruhango, Bunyogombe and Kibilizi health centres have participated in the study (***Table 1***).

TABLE 1: Socio Demographic Characteristics of the Study Population

		Health Facility			
Characteristics		Ruhango	Bunyogombe	Kibilizi	Total (%)
Sex	Male	37	8	6	51(40.5)
	Female	58	11	6	75 (59.5)
	Total	**95**	**19**	**12**	**126**
Age	<10	28	3	2	33 (26.2)
	10-20	16	6	3	25(19.8)
	21-30	26	4	1	31(24.6)
	31-40	11	1	1	13(10.3)
	41-50	8	1	3	12(9.5)
	>50	6	4	2	12(9.5)
	Total	**95**	**19**	**12**	**126**

The most affected population aged <10 years old and represent 26.2%(33/126) of the total participants. In this study, 75 (59.5%) were females and 51(40.5%) were males. All the participants were from Southern province especially at Ruhango Health Center, Buntogombe Health Post and Kibirizi Health Center.

Prevalence of Plasmodium species infection in Ruhango, Kibilizi, and Bunyogombe health centres. As summarized in ***Figure 1***, among the 126 persons who participated in the study, 61(48.4%) were positive for plasmodium infection and 65(51.6%) were negative for Plasmodium infection. Among the total positive cases, 56(44.5%) were infected by single species of plasmodium while 5(4%) were mixed plasmodium species infections. The most prevalent Plasmodium species was Plasmodium falciparum with 49(39%) and the least prevalent species was Plasmodium vivax with 1(0.8%). The prevalence of Plasmodium species per health facility are detailed in the ***Table 2*** below.

Prevalence of mixed infections of plasmodium species As shown in ***Table 2***, out of 126 participants who participated in the study, 5(4%) had mixed Plasmodium infection. 3(2.4 %) were infected with both Plasmodium falciparum and Plasmodium ovale while 2(1.6 %) were infected with both Plasmodium falciparum and Plasmodium malariae.

FIGURE 1: Prevalence of Plasmodium species Infection in Ruhango, Kibilizi, and Bunyogombe Health Centers

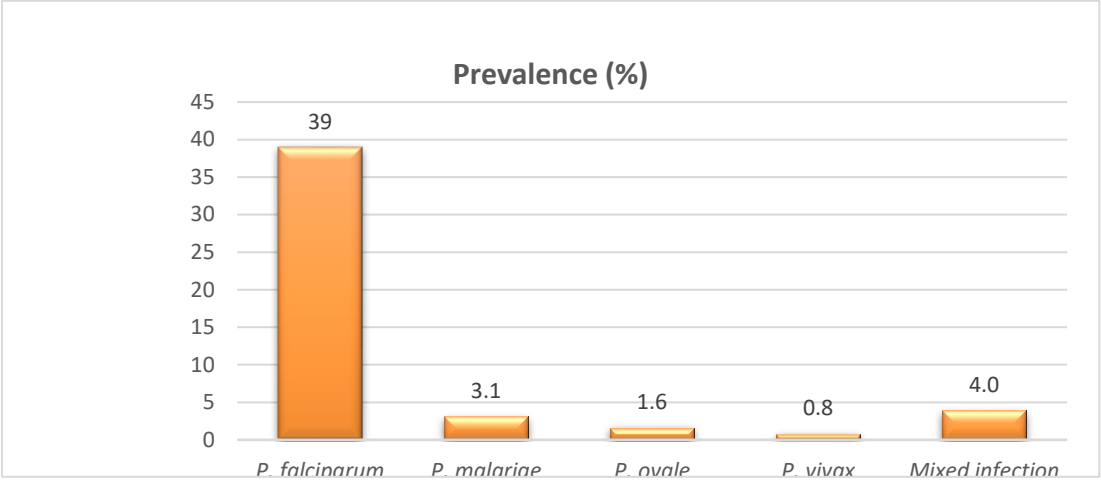

TABLE 2: Socio Demographic Characteristics of the Study Population

Health facilities	Number of samples	Prevalence of mixed infections of plasmodium species			
		P. Falciparum & P. ovale	P. Falciparum & P. Malariae	Total	P Value
Ruhango	95	2(1.6%)	1(0.8%)	3(2.4%)	.004
Bunyogombe	19	1(0.8%)	1(0.8%)	2(1.6%)	<.001
Kibilizi	12	0(0%)	0(0%)	0(0%)	
Total	**126**	**3(2.4%)**	**2(1.6%)**	**5(4%)**	

DISCUSSIONS

This study showed a significantly high prevalence of Plasmodium species in Ruhango, Bunyogombe and Kibilizi health centres located in the southern province. The overall prevalence was 48.4%. Plasmodium falciparum presented a high prevalence of 39%. Other Plasmodium species such as Plasmodium malariae had 3.1%, Plasmodium ovale 1.6% and Plasmodium vivax 0.8%. The study demonstrated that mixed infections account for 5% of the prevalence of the total infections. The prevalence of mixed Plasmodium infection in this study is not different from a study conducted in South Korea which indicated a 2.1% prevalence of mixed Plasmodium falciparum and Plasmodium ovale co-infection among Korean individuals who had returned from the Democratic Republic of Congo suggesting that in the region of sub-Saharan Africa there is a significant prevalence of mixed Plasmodium infection which are often unrecognized.[16] Ruhango health centre had a high percentage of positives cases (37.3%) and had a higher prevalence of Plasmodium species compared to Kibilizi health center located in the Gisagara District. These findings suggest that there is a need for continued monitoring of non-falciparum infection in this population to decide when the species-specific RDTs can be introduced for diagnostic purposes as the country moves towards malaria elimination. In addition, ACTs, the first-line treatment does not clear plasmodium hypnozoites and radical treatment with primaquine, effective against hypnozoites may play an important role in the control and eventual elimination of P. vivax, P ovale and P. malariae.[15] The high prevalence may be attributed to the fact there was no use of indoor residual sprayings at the time of data collection which could reduce malaria vectors and the transmission rate. This prevalence of Plasmodium species in Ruhango and Kibilizi health centres is significantly higher compared to other studies conducted by Gahutu et al, 2011 and

Nyirakanani et al, 2018 with a prevalence of 16.7% and 12.2 %, respectively.[14] The patterns of the burden of infection observed for the different species were not surprising as similar observations have been reported elsewhere.[17,18] However, the fact that the non-falciparum malaria cases were present in the study population necessitates the need to have tools at the national level that facilitate the detections of non-falciparum parasites. Also there is a need to conduct refresher courses for laboratory staff and microscopists to strengthen non-falciparum malaria diagnosis. Therefore, future effective malaria control requires an elaborate understanding of the interactions among different plasmodium species.

LIMITATIONS

This study was conducted in Ruhango and Kibilizi Health centres located in Ruhango and Gisagara districts, respectively and the outcome of the two Health centres cannot be applied to the whole country. Further research is needed to establish the exact prevalence of mixed Plasmodium infection in the whole country.

CONCLUSION

This study revealed the presence of mixed infections in the Southern province of Rwanda with a prevalence of 5%. The overall prevalence of Plasmodium *species* was 48.4% where *P. falciparum* is the most prevalent species with 39% prevalence. This observation calls for improvement of existing malaria control strategies at the National level. The presence of non-falciparum infections varies with transmission patterns, demographic trends and geographical contrasts. There is a need to monitor those factors through a functional surveillance system to understand the epidemiological profile. Given that most of the mixed infections cannot be detected by HRP2- based RDTs there is a need to strengthen the detection of malaria infection with microscopy examination in Health care facilities to facilitate the detection of non-falciparum infections. Nonetheless, there is a need for further studies to generate a detailed profile and epidemiology of plasmodium species in the country.

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EAHRC
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ORIGINAL ARTICLE

The rate of sample rejection and pre-analytical errors at KCMC Clinical Laboratory in Moshi, Kilimanjaro

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ABSTRACT

Background: Over the years, modern medicine has provided better quality services to patients. However, medical error is still prevalent and has a lot of negative consequences on patients' outcomes. These may include delayed treatment, longer hospital stays, or even worsening of the patient's condition. This study aimed to determine the sample rejection rate at KCMC Clinical Laboratory and characterize pre-analytical errors that contribute to it.

Methods: Data extraction sheet was used to collect information from rejection forms from January to December 2016. The data collection form gathered information on where samples were collected and send for laboratory analysis, and the types of rejected sample. Information on the reasons for sample rejection were also collected.

Results: The proportion of rejected samples stand at 0.19% (out of 117181 samples received from January to December 2016, 234 were rejected). The highest rates of rejection were from haematology section 78 (33.3%). The major type of rejected sample was blood (86.3%) and 20% of the rejected samples came from internal medicine department, mainly from its inpatient department (13.6%). The commonest reason for rejection was unpaid specimens 84 (35.9%).

Conclusion: Study results indicate the need for improving quality control system which will enhance sample management and prompt identification of deficiencies. The high rate of sample rejection due to lack of payment signify the need for improved government policies that address health expenses coverage. Provision of trainings on phlebotomy and blood samples transportation can reduce the rate of sample rejection due to clotting.

Keywords: Clinical laboratory, Pre-analytical errors, Rejected samples, Rejection rate.

INTRODUCTION

Clinical laboratory services play a significant role in the management of patients. Clinicians rely on laboratory investigations for confirmation of diagnosis and ensuring quality service to patients. The proper management of patients is mainly influenced by accurate[1] and timely released results from the laboratory[2]. Laboratory errors stand as barriers to achieving this. These errors can occur during the pre-analytical, analytical and post-analytical phases of sample analysis, but pre-analytical errors account for around 70% of all the errors.[2-6] The pre-analytical phase includes procedures that start from the clinician requesting a laboratory test on a patient, identification of the patient and sample, patient preparation, collection of the samples, sample handling, transportation, processing and storage[7].

Clinical laboratories worldwide have established protocols for sample collection and analysis and several criteria to judge the quality of a sample for analysis. The protocols are essential in guiding appropriate testing and quality results. In the pre-analytical phase, a sample is deemed inappropriate for analysis in the following case scenarios: unlabelled specimen, insufficient patient information, haemolysed specimen, wrong container, the inadequate volume of sample, wrong specimen, prolonged transport, clotted sample, no specimen submitted, leaking specimen containers, contaminated request forms, and unpaid specimens[1,8].

The occurrence of laboratory errors cause delays in sample analysis and prevents the clinicians from providing timely management to their patients which negatively impacts patients' outcome[9], especially for the critically ill. A longer stay in the hospital due to delay in sample analysis and treatment also means wasted economic resources on the patient and insurance companies. Sample rejection causes the need to repeat withdrawing more samples and lead to much discomfort, especially for painful procedures like withdrawing blood or cerebrospinal fluid.

Identifying quality indicators is a critical step in the laboratory services that enables the user to quantify, document, monitor, and improve quality[2]. Quality indicators such as patient safety, effectiveness, equity, patient-centeredness, timeliness and efficiency are objective measures to evaluate critical healthcare domains[10]. Determining the suitable quality indicators for the pre-analytical phase is critical as most of the errors occur in this phase of the total testing process (TTP). The initial stages of TTP are neither done in the clinical laboratory nor solely carried out by laboratory personnel. Evaluation, monitoring and improving all the initial process of the TTP is crucial for appropriate patient management[11,12].

To ensure the quality of results in the laboratory, external quality control is done to measure laboratory performance in pre-analytical, analytical and post-analytical phases. The external quality control is a performance indicator for our quality system and positively contributes to reducing errors in all phases. Sample handling guidelines are available in KCMC clinical laboratory and other hospital departments in order to minimise errors in pre-analytical phases because these errors occur in different places and not only laboratory personnel are involved but also clinicians, nurses and patients[13]. Errors in the pre-analytical phase affect both analytical and post-analytical phases[14]. Information on what goes wrong during the pre-analytical phase is vital in deciding where to focus interventions and what improvements can be brought to the currently available guidelines.
There is also a laboratory information system (LIS) which is used to record the required details of all samples received in the laboratory, entering test results and authorising their release. Information of

In spite of guidelines on sample handling being available at KCMC clinical laboratory and other hospital departments, there is still an obvious need to improve on the quality of samples provided to the laboratory for analysis. With a better understanding of the sample rejection rate and pre-analytical errors, it will be possible to develop strategies that can help improve the quality of samples submitted to the clinical laboratory department and provide timely management to the patients. This study aimed to assess the overall rate of sample rejection, the reasons and type of sample rejected, the hospital departments they come from, and the clinical laboratory sections they go to.

METHODS

Study design

This was a hospital based cross-sectional study. The KCMC Clinical Laboratory Department systematically records every rejected sample using a sample rejection form. The rejection forms are kept in the laboratory's archives and are arranged chronologically.

Setting

This study was conducted in the Clinical Laboratory department at KCMC Referral Hospital Moshi, Kilimanjaro Region in the Northern zone of Tanzania. This clinical laboratory is accredited as Zonal Reference Laboratory for the Arusha, Dodoma, Kilimanjaro, Singida and Tanga regions. The clinical laboratory is divided into different sections, namely Haematology, Clinical biochemistry, Microbiology, Serology, Blood transfusion, Parasitology and Molecular diagnostics. It receives specimens from outpatient department (OPD) clinics, Casualty, and inpatient departments (Internal Medicine, Obstetrics and Gynaecology, Surgical departments, namely General surgery, Orthopaedics, Urology, ENT, Ophthalmology, and Paediatrics). The laboratory is also a teaching area for students from different programs (Doctor of Medicine, Bachelor of Science in Health Laboratory Sciences, and Diploma in Health Laboratory Sciences).

Data Collection

Basing on the study's objectives, a data extraction sheet was created and used to manually record the relevant information from the sample rejection forms from the 1st of January to the 31st of December 2016. This will include the patient's hospital number, the hospital department, the time of sample collection and reception, the test requested, the clinical laboratory section, the type of sample and the reason for rejection.

the total number of samples received in the laboratory in 2016 was obtained from LIS.

Data Analysis

Data were entered, cleaned and analysed using Statistical Package for Social Science (SPSS); IBM Corporation, New York, USA released in 2012, IBM SPSS Statistics for window, version 20. After extracting information from LIS using data extraction sheets, data were entered in SPSS. Our outcome of interest, number of samples rejected was defined as number of samples rejected during the study period. Rejected samples were coded 1 as samples rejected at the laboratory and 0 as samples that were received and tested in the laboratory. Rejected samples were categorised based on reasons for rejection which included; incomplete patient information (unlabelled samples), samples missing payment details, samples with wrong test, samples with prolonged transport time, haemolysed and clotted samples. Categorical data were summarised by using frequencies and percentages. Graphs and tables were used for data presentation.

Ethical Approval

Ethical approval was sought from the Kilimanjaro Christian Medical University College (KCMUCo) Ethical Review Committee (Ethical approval reference number: 2398). Permission to access rejected samples data was requested from the Executive Director and Director of Clinical Laboratory Department of KCMC referral hospital. Patients' rejected samples were identified by hospital numbers and no names were mentioned to ensure confidentiality.

RESULTS

Rate of Sample Rejection and Reasons

A total number of 117181 samples were registered by the KCMC clinical laboratory from the 1st of January to the 31st of December 2016. Out of these, 234 were rejected over this time period for not fulfilling the requirements of the laboratory's collection manual. This means the proportion of sample rejection was 0.2% and rejection rate was 200 per 100,000 samples. The rates of sample rejection varied in different sections, the highest rates being recorded in the haematology and biochemistry sections. (***Table 1***). Analysis of sample rejection rate in different steps of the pre-analytical phase revealed that most of the sample were rejected at the stage of sample identification and data entry (***Table 2***). In most cases, the reason for sample rejection included unpaid request, clotted and haemolysed samples, and wrong sample collection container (***Table 3***). Analysis by types of samples (not shown in table) revealed that blood was the most frequently rejected sample 202 (86.3%) followed by urine 28 (12%) and stool 4 (1.7%).

TABLE 1: Number, Frequency and Rate of Sample Rejection in Different Laboratory Sections

Laboratory type	Rejected samples	Frequency of rejection	Rate of rejection (per 100,000 samples)
Haematology	78	33.3	67
Biochemistry	69	29.5	59
Serology	34	14.5	29
Parasitology	24	10.3	20
Microbiology	12	5.1	10
Blood transfusion	10	4.3	9
Molecular biology	7	3.0	6
All	234	100.0	200

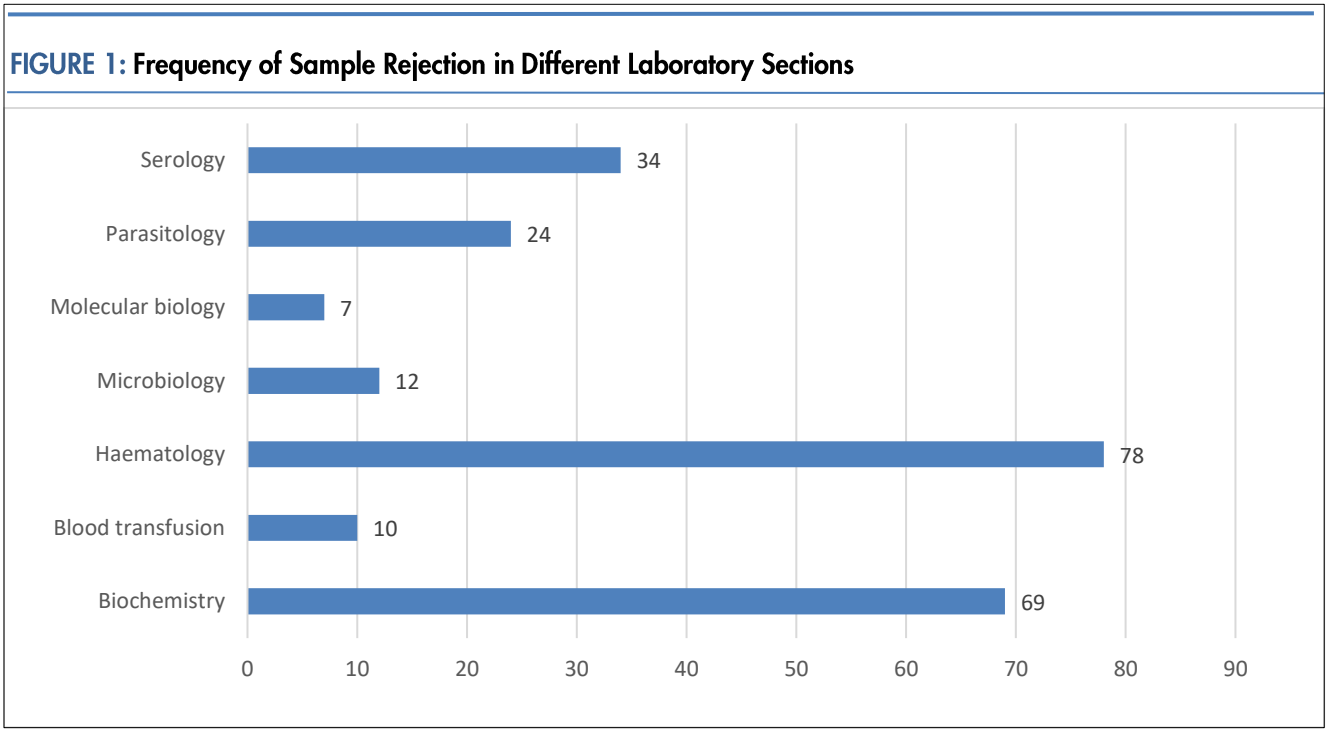
TABLE 2: Rate of Sample Rejection at Each Pre-Analytical Stage (N=234)

Pre-analytical stage	Rejected samples	Rejection frequency (%)	Rejection rate (per 100,000 samples)
Patient identification	5	2.14	4.3
Data entry of the request	85	36.32	73
Sample identification	11	4.70	94
Sample collection	53	22.65	46
Transport of sample	14	5.98	12
Suitability of sample	66	28.21	56

TABLE 3: Reasons for Sample Rejection

Description	Rejected samples	Rejection frequency	Rejection rate (per 100,000 samples)
Clotted sample	33	14.10	28
Haemolyzed specimen	33	14.10	28
Inadequate sample volume	9	3.85	8
Missing patient information	5	2.14	4
No specimen submitted	10	4.27	9
Prolonged sample transportation	4	1.71	3
Unlabeled specimen	11	4.70	9
Unpaid sample	84	35.90	72
Wrong sample collection container	36	15.38	31
Wrong specimen	8	3.42	7
Wrong test ordered	1	0.43	1
	234	100.00	200

FIGURE 1: Frequency of Sample Rejection in Different Laboratory Sections



The highest frequency of sample rejection was observed for samples collected in the internal medicine department 47 (20%), with the frequency of rejection from the inpatient medical wards being 32 (13.6%) which was more than double that of the outpatient clinic 15 (6.4%). The ophthalmology department had the lowest rejection frequency of 5 (2.1%) among all departments. The frequency of sample rejection from different hospital departments are shown in ***Table 4***. Haematology and biochemistry section of clinical laboratory had the highest number of rejected samples (***Figure 1***).

TABLE 4: Frequency of Sample Rejection by Hospital Departments (N=234)

Department	n (%)
Internal medicine total	47 (20)
OPD	15 (6.4)
IPD	32 (13.6)
Obstetrics and Gynecology total	33 (14.1)
OPD	27 (11.5)
IPD	6 (2.6)
Surgery total	16 (6.8)
OPD	9 (3.8)
IPD	7 (3)
Pediatrics total	9 (3.8)
OPD	4 (1.7)
IPD	5 (2.1)
Dermatology	15 (6.4)
ENT	8 (3.4)
Ophthalmology	5 (2.1)
Urology	32 (13.7)
Casualty	40 (17.1)
CCFCC	6 (2.6)
Diabetic clinic	6 (2.6)
IDC	6 (2.6)
SSHS	11 (4.7)

DISCUSSION

The proportion of sample rejection was 0.19% for the year 2016 in KCMC clinical laboratory. This finding is consistent with the findings from China[15], Q-Probe analysis of 78 clinical laboratories[9] and close to that conducted in Texas, USA[8]. The sample rejection frequency in this study is within (0.1% to 5.3%) the reported range from different laboratory settings.[2,6,8,9,15-18]

This study also looked at different causes of sample rejection, and unpaid specimen 84 (35.9%) was the most commonly mentioned reason for sample rejection. This can be possibly explained by a lack of careful inspection of sample collection forms to ensure that the requested sample has been paid for or patients could not afford to pay for laboratory tests. Laboratory personnel at the sample collection area or in phlebotomy should verify a payment stamp on the sample collection forms before collecting a specimen. The limited number of personnel compared to the number of patients that require laboratory services can be another contributing factor to this overlook. An introduction of digital health technology could be the best option to significantly minimise this problem both in the outpatient and inpatient set up. The patients' socioeconomic status is a factor that cannot be overlooked in explaining the lack of payment for received samples. In addition to this, it is important to realise that medical insurance coverage is not available to all patients, limiting their ability to afford laboratory services. A lack of understanding of payment procedures and a readily available tariff of the cost of collection and analysis of different laboratory samples to the patients is another contributing factor to this problem. However, there is a contrast between this study and others where sample clotting was mentioned as the commonest reason for rejection, with a 55.8% in a Turkish university hospital laboratory[2] 43.8% in Porto Alegre, Brazil[5] and 51.2 % in Indian[1]. If the laboratory test needs whole blood or plasma, blood sample should be drawn into anticoagulant tubes. Clotting in laboratory blood samples usually occurs when blood is left too long in a tube without an anticoagulant or when it is not adequately mixed in the anticoagulant containing tube[19]. In our study, clotting was ranked second among the reasons for rejection with a frequency of 14.1%, which is also a bit lower than the study done in Ethiopia, which had 16.4% and higher than the study done in Kenya reported 1.4%[20,21]. The high frequency of clotted samples might be explained by the lack of in-depth training in phlebotomy, and blood samples transportation for nurses and physicians, as most of clotted samples came from inpatient departments. The high workload for phlebotomists, nurses and physicians is also a contributing factor to these findings.

Blood was the most commonly rejected type of sample with a frequency of 86.3%. This correlates with previous studies conducted in Turkey, China and India.[2,15,18] The high frequency of rejection of blood samples might be due to different factors which include the difficulty of collecting these samples, the frequency of orders made for these samples by clinicians, and the special requirements for their

containment and transportation. Among other factors investigated was the origin of the rejected samples. The frequency of sample rejection from different hospital departments was calculated and compared. The internal medicine department had the highest frequency of sample rejection 47(20%), with most of these coming from its inpatient division. This finding is different from a study conducted in Brazil, which showed that the paediatrics department had a higher sample rejection frequency than other departments[5]. However, concerning inpatient departments, our findings were in line with a study conducted in Turkey that found that adult inpatient departments had a higher frequency of sample rejection than outpatient departments[2].

The highest sample rejection frequency being from the haematology section 78(33.3%) and followed by biochemistry 69 (29.5%) can be explained by the fact that these clinical laboratory sections receive a higher load of samples compared to others. It also correlates to a study conducted in Brazil where the haematology section was responsible for most recollection requests 43.6%, followed by the biochemistry section 29%[16]. Clotted and haemolysed samples are the main reasons for high rejections for haematology and biochemistry sections.

CONCLUSION

This study allows for a better understanding of the factors that may contribute to laboratory sample rejection in KCMC hospital. Keeping in mind the negative consequences of a delayed sample analysis on a patient's management plan, addressing these factors is invaluable in improving patient care at the pre-analytical phase of the laboratory process.
This can be done through an improved quality control system of laboratory processes that will allow for prompt identification of deficiencies in sample management. Therefore, corrective measures can be put in place in a timely manner to allow for improved quality of care.

The high rate of sample rejection due to lack of payment observed in this study can indicate the need for improved government policies that address health expenses coverage.
Identifying vulnerable patients and the availability of support services that help in the payment of their hospital bills can be an initial step in addressing this issue. The introduction of an electronic medical record system that allows easy access to patient information and financial status with regards to hospital expenses can be a possible solution.

Additionally, further training in phlebotomy and blood samples transportation can reduce the high number of samples rejected due to clotting. This can be done through regular, continuous medical education (CME) sessions and joint ward rounds, including clinicians and laboratory personnel. The information gathered from this study can serve as a basis for more research and guide policy makers in implementing these interventions.

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