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YELLOW FEVER AND MALARIA AMONG FEBRILE PATIENTS IN AND AROUND THE EPICENTRE OF A YELLOW FEVER OUTBREAK IN GHANA.

BY

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, UNIVERSITY OF GHANA IN PARTIAL FULFILLMENT OF THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY IN MEDICAL MICROBIOLOGY.

DEPARTMENT OF MEDICAL MICROBIOLOGY.

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DECLARATION

I, Lidiwan Mensah, do hereby declare that the work presented in this thesis with title, "Yellow fever and malaria among febrile patients in and around the epicentre of a yellow fever outbreak in Ghana" is my original work and that this thesis has neither in part nor whole been presented to the University or elsewhere for any degree. I declare that the thesis is the result of the research work I undertook towards the award of Master of Philosophy in the Department of Medical Microbiology, Medical School, College of Health Sciences, University of Ghana. I further declare that the authors whose works were referred to have been duly acknowledged.

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DEDICATION

I dedicate this thesis to the Almighty God, and then to my father, Mr. Mustapha Mensah and mother, Mrs. Georgina Gaisie, my entire family, my abled supervisors and colleagues at the Department of Medical Microbiology of the University of Ghana Medical School. This research would not have been a successful one without their constant supervision, motivation, prayers, constructive critics and financial assistance.

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TABLE OF CONTENTS

DECLARATION .... **ii**

DEDICATION .... **iii**

ACKNOWLEDGEMENT .... **iv**

TABLE OF CONTENTS .... **v**

LIST OF TABLES .... **x**

LIST OF FIGURES .... **xi**

LIST OF ABBREVIATIONS .... **xii**

ABSTRACT .... **xiv**

CHAPTER ONE

1.0 INTRODUCTION **1**

1.1 Background **1**

1.2 Problem Statement **3**

1.3 Justification **4**

1.4 Aim **5**

1.5 Specific Objectives **5**

CHAPTER TWO

2.0 LITERATURE REVIEW **6**

University of Ghana http://ugspace.ug.edu.gh

2.1 Worldwide outbreak of Yellow Fever ………………………………………………. 6

2.2 Yellow Fever Outbreaks in Africa ……………………………………………. 6

2.3 Burden of Yellow Fever in Ghana ……………………………………………. 7

2.4 Transmission of Yellow Fever and other Arboviral Viruses ………………... 8

2.5 Molecular Detection of Yellow Fever Virus …………………………………. 9

2.6 Control, Prevention and Treatment of Yellow Fever ……………………….. 9

2.7 Yellow Fever Vaccination ……………………………………………………… 10

2.8 Burden of Yellow Fever Surveillance in Africa ……………………………… 10

2.9 Burden of malaria in Ghana ………………………………………………... 12

2.10 Transmission of malaria …………………………………………………….. 12

2.11 Detection of malaria ………………………………………………………… 13

CHAPTER THREE

3.0 METHODOLOGY ……………………………………………………………. 14

3.1 Study Design and Sites ……………………………………………………… 14

3.2 Study Population …………………………………………………………….... 15

3.3 Sample Size Calculation ……………………………………………………….. 15

3.4 Inclusion Criteria ……………………………………………………………. 16

3.5 Exclusion Criteria …………………………………………………………… 16

University of Ghana http://ugspace.ug.edu.gh

3.6 Participant Sampling and Data Collection ……………………………….... 16

3.7 Sample Collection ………………………………..………………….. 17

3.8 Molecular Detection of Yellow Fever Virus …………………………….. 18

3.8.1 RNA Extraction of Yellow Fever Virus ………………………………... 18

3.9 Detection of *Plasmodium falciparum* by Microscopy …………………. 21

3.10 Molecular Detection of *Plasmodium falciparum* by Polymerase Chain Reaction ……………………………………………………………………… 22

3.10.1 DNA Extraction of *Plasmodium falciparum* from Blood Paper Blot using Chelex DNA Extraction Protocol ……………………………………… 22

3.10.2 Plasmodium detection: Conventional Nested PCR …………………... 22

3.11 Data Management Plan ……………………………………………… 26

3.12 Data Analysis ……………………………………………………….. 26

3.13 Ethical considerations ……………………………………………….. 27

CHAPTER FOUR

4.0 RESULTS …………………………………………………………… 28

4.1 Socio-demographics of Participants …………………..……………….. 28

4.2 Carriage of yellow fever virus in febrile participants ………………… 30

4.3 Study site Malaria Percentage Prevalence by Microscopy ..………….. 31

University of Ghana http://ugspace.ug.edu.gh

4.4 Age-specific Malaria Percentage Prevalence by Microscopy ………… 32

4.5 Study site Malaria Percentage Prevalence by Conventional Nested PCR …… 33

4.6 Age-specific Malaria Percentage Prevalence by Conventional Nested PCR …. 35

4.7 Carriage of Plasmodium parasites in febrile participants …………………….. 36

4.8 Malaria Prevalence by Different Technique of Plasmodium Parasite Detection ……………………………………………………………………… 37

4.9 Association between Malaria Infections and Socio-demographic factors Using Microscopy ……………………………………………………………… 37

4.10 Association between Malaria Infections and Socio-demographic factors Using Conventional Nested PCR …………………………………………………… 38

CHAPTER FIVE

5.0 DISCUSSION ……………………………………………………………………... 39

CHAPTER SIX

6.0 CONCLUSION ……………………………………………………………………… 42

6.1 RECOMMENDATIONS ………………………………………………………… 42

REFERENCES ……………………………………………………………………........... 43

APPENDIXES

APPENDIX 1: Information Sheet ……………………………………………………. 52

INTEGRI PROCEDAMUS

University of Ghana http://ugspace.ug.edu.gh

APPENDIX 2: All Data Collection Instruments …………………..……………… 64

APPENDIX 3: Principal/Co-Investigators and Collaborators Curriculum Vitae… 66

APPENDIX 4: College of Health Science Ethical Clearance Form ………………. 78

APPENDIX 5: Ghana Health Service Ethical Clearance Form ………………….. 79

University of Ghana http://ugspace.ug.edu.gh

LIST OF TABLES

Table 3.1 Pipetting scheme for Yellow fever virus detection by RT- PCR analysis …….. 19

Table 3.2 Detection of Yellow Fever Virus using the YFA, YFAs primers and YFP probe with the AgPath ID OneStep RT-PCR Kit ………………………………………………………. 20

Table 3.3 Primer sequences for Plasmodium parasite identification ………………….. 24

Table 3.4 Primer sequences for the first nested PCR analysis ………………………… 24

Table 3.5 Reaction conditions for first Nested PCR analysis …………………………. 25

Table 3.6 Primer sequences for the second nested PCR analysis ……………………… 25

Table 3.7 Reaction Conditions for first Nested PCR analysis …………………………. 26

Table 4.1 Socio-demographics of Participants ………………………………………… 29

Table 4.2 Clinical presentations of participants ……………………………………… 30

Table 4.3 Malaria prevalence by microscopy and mean temperatures of participants in all study sites ……………………………………………………………………………… 31

Table 4.4 Malaria prevalence according to yellow fever outbreak and non-outbreak zones by microscopy …………………………………………………………………………… 31

Table 4.5 Malaria Prevalence by Different Technique of Plasmodium Parasite Detection .. 37

Table 4.6 Association between Malaria Infections and Socio-demographic factors Using Microscopy ……………………………………………………………………………… 38

University of Ghana http://ugspace.ug.edu.gh

LIST OF FIGURES

Figure 3.1: A map of Ghana showing the various study sites 14

Figure 4.1 PCR amplification curve showing results of yellow fever detection output 30

Figure 4.2 Study site Malaria Percentage Prevalence by Microscopy 32

Figure 4.3 Age-specific Malaria Percentage Prevalence by Microscopy 33

Figure 4.4 Study site Malaria Percentage Prevalence by Conventional nested PCR 34

Figure 4.5 Age-specific Malaria Percentage Prevalence by Conventional Nested PCR 35

Figure 4.6 Agarose gel picture of PCR products for the detection of *Plasmodium falciparum* ... 36

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LIST OF ABBREVIATIONS

RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
GHS	Ghana Health Service
CRF	Case Fatality Rate
W.H.O	World Health Organization
CDC	Centre for Disease Control
HIV	Human Immunodeficiency Virus
°C	Degree Celsius
EDTA	Ethylenediaminetetraacetic Acid
ml	Milliitre
µl	Microlitre
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
rpm	Revolutions Per Minute
™	Trademark Symbol
p-value	Probability value
CI	Confidence interval

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ct	Cycle threshold
V	Volts

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ABSTRACT

Background

Yellow fever is endemic in Ghana and the country has been faced with outbreaks of the disease in the past years. From October, 2021 to February, 2022, yellow fever cases and deaths were recorded in the Savannah, Upper West, Bono and Oti regions of Ghana. Individuals infected with yellow fever have clinical presentations like fever, headache, body pains and vomiting which are similar to that of malaria. Malaria is endemic in Ghana, and is known to be the commonest microbial etiology of fever in febrile patients in Ghana. Infections with yellow fever virus and malaria parasites among febrile inhabitants in and around the epicenter of the yellow fever outbreak zones are unknown after the outbreak. Thus, there is the need to investigate yellow fever infections and malaria parasites in the febrile patients in order to put in the appropriate prophylactic measures in controlling the etiology of fever.

Aim

The aim of this study was to determine the carriage of yellow fever virus and malaria parasites among febrile patients in and around the epicenter of a yellow fever outbreak in Ghana.

Methodology

This was a cross-sectional study that was conducted in four districts in Ghana. Two of these districts (Wenchi and Damongo) experienced a yellow fever outbreak in 2021 and the other districts (Tamale and Kumbungu) are in close proximity to the outbreak foci. A total of 498 blood samples was collected during both dry and rainy seasons in Ghana from febrile patients in the study sites. The blood samples were processed for the detection of yellow fever virus and malaria parasites. One-step RT-PCR was performed on serum samples to determine the carriage of yellow

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fever virus in the participants while conventional nested PCR and microscopy were performed to determine the carriage of malaria parasites in the participants.

Results

Out of a total of 498 febrile human participants, none of them had the yellow fever virus as detected by real-time PCR. The non-outbreak zones (Tamale or Kumbungu) had the highest prevalence of malaria irrespective of the season of sample collection or technique in disease diagnosis. During the dry season, Tamale and Kumbungu had malaria prevalence of 35% and 21.6% respectively using microscopy. During the rainy season, Tamale and Kumbungu had recorded prevalence of 23% and 30.1% respectively using microscopy. During the rainy season, Kumbungu recorded prevalence of 47.6% using conventional nested PCR. Tamale recorded the highest proportions of malaria infections (35%) during the dry season with the use of microscopy while Kumbungu recorded the highest proportions of malaria infections (30.1%) during the rainy season with the use of microscopy. Also, Kumbungu recorded the highest proportions of malaria infections (47.6%) during the rainy season with the use of conventional nested PCR The highest proportions of malaria infections (12.2%) were observed among the older group (above 15 years) during the rainy season with the use of microscopy while children less than 5 years recorded the highest proportions of malaria infections (9.1%) during the dry season with the use of microscopy. Also, the older age group (above 15 years) recorded the highest proportions of malaria infections (15%) during the rainy season with the use of conventional nested PCR.

Conclusion

There was no detectable yellow fever virus in the febrile patients. The overall prevalence of malaria infections in the febrile patients was high in this study. High vaccination coverage against yellow

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fever should be encouraged in yellow fever endemic countries like Ghana and malaria control interventions should target all age groups to completely tackle reservoir infections.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Yellow fever is endemic in Ghana and there have been several major outbreaks in the country in past years which resulted in numerous cases and deaths (Gardner *et al* 2010; GHS, 2021). It has been reported that yellow fever outbreaks occur in a ten-year to twelve-year duration of cycle in the Southern part of Ghana (Agadzi *et* al., 1986). However, the recurring reported cases and deaths caused by outbreak of the disease in these parts of Ghana recently in 2011, 2016 and 2021 suggest otherwise.

From October, 2021 to February, 2022, an outbreak of yellow fever took place to which numerous cases and deaths were recorded in several districts in the Savannah, Upper West, Bono and Oti regions in Ghana. The *Aedes aegypti* mosquito, the primary vector of yellow fever transmits the yellow fever virus from one place to another easily without any borders which makes all neighbouring districts around the epicentre of the yellow fever outbreak zones at risk of being infected. As of November 27 2021, the yellow fever outbreak in Ghana had recorded 70 cases and 35 deaths (with a CFR of 17%) as confirmed by the Ghana Health Service (GHS, 2021).

Yellow fever is a viral hemorrhagic fever caused by a mosquito-borne flavivirus that belongs to the family *Flaviviridae* and the virus is transmitted primarily by the *Aedes aegypti* mosquito in Ghana (Kwallah *et al*., 2015). The virus is transmitted through the bite of an infected *Aedes aegypti* mosquito and infects both humans and non-human primates like monkeys (Monath *et al* 2015; Garkse *et al*., 2014). Some infected individuals with yellow fever present with clinical symptoms like fever, chills, fatigue, dehydration, myalgia, nausea, vomiting, headache, hepatitis with

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jaundice, multisystem organ failure and hemorrhage (Domingo *et al*., 2018). There is no specific antiviral treatment of the disease, however, treatment of underlying clinical manifestations like fever, dehydration and antibiotics for associated bacterial infections in an infected individual could enhance a better treatment outcome (Beasley *et al*., 2015). The disease can be prevented by a live attenuated yellow fever vaccine, from a virus which was isolated from a Ghanaian patient called Asibi in 1927, and the vaccine is known to provide a lifelong immunity (Barrett *et al*., 1997; Barrett *et al*., 2017). Mass yellow fever vaccination was deployed after yellow fever suspected cases were confirmed through laboratory findings. Since 6th November, 2021, a mass vaccination campaign which targeted 54 964 people from the ages of 6 months to 60 years in about 80 communities within the affected regions took place (WHO, 2021).

Clinical presentations of the yellow fever disease overlap with other febrile infections, thus in Africa, the most frequently occurring disease is assumed (without any clinical laboratory confirmation) to affect a person with febrile illness which makes yellow fever cases go underdiagnosed (Adams & Jassoy, 2021). Yellow fever is not considered as part of routine clinical investigations of febrile illness in most clinical settings in Ghana, thus lack of appropriate laboratory diagnosis hampers epidemiological surveillance systems leading to the making of an inaccurate information on the true incidence of the disease (Adams & Jassoy, 2021).

Febrile illness is a major cause of morbidity and mortality in Africa (Maze *et al*., 2018). As part of a list of common etiologies of acute febrile illness reported in patients who present with fever, malaria has been known to be the most prevalent in Ghana. (Chrispal *et al*., 2010; Sunden *et al*., 2017). Malaria is endemic in Ghana and it is responsible for about 40% of outpatient attendances

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and 23% of inpatient admissions in the country (Malm, Sackey, & Wurapa, 2014). Malaria is a differential diagnosis of yellow fever.

There is the need for a more specific, sensitive and differential laboratory detection of yellow fever infections. Also, since the virus can spread from one place to another by the vector there is the need to investigate if the virus has been among people especially the unvaccinated population in the non-outbreak zones.

It is crucial to investigate yellow fever and malarial infections responsible for causing fever in patients the selected sites in order to implement the appropriate preventive measures in containing the etiology of fever.

1.2 Problem Statement

The mortality rate of the yellow fever disease is high, causing deaths in about 30% to 60% in affected individuals, especially those with high severity form of the disease (Johansson *et al*., 2014). Assessing the burden of yellow fever infections has been problematic in the past years (W.H.O. 1990). There is rare diagnosis of arboviral infections (including yellow fever) by the healthcare facilities in Ghana as a result of lack of diagnostic capacity (resources, expertise, etc) (Adams & Jassoy, 2021).

Yellow fever is not considered as part of routine clinical investigations of febrile illness in most clinical settings in Ghana, thus lack of appropriate laboratory diagnosis of yellow fever hampers epidemiological surveillance systems leading to the making of an inaccurate information on the true incidence of the yellow fever disease (Adams & Jassoy, 2021).

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Febrile illness is a major cause of morbidity and mortality in Africa (Maze et al., 2018). As part of a list of common etiologies of acute febrile illness reported in patients who present with fever, malaria has been known to be the most prevalent in Ghana. (Chrispal et al., 2010; Sunden et al.,

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2017). It is therefore crucial to investigate malaria infections responsible for causing fever in the patients in order to implement the appropriate preventive measures.

Yellow fever is sometimes misdiagnosed as malaria due to similar clinical presentations such as fever, headache and general body pains. Yellow fever is most often misdiagnosed as malaria sine malaria is the comment disease in Ghana, and malaria-infected individuals have similar symptoms to individuals infected with yellow fever. As a result of the similar clinical presentations between these two groups of people, and malaria been the most prevalence febrile disease in Africa, yellow fever is misdiagnosed as malaria in most cases (Adams & Jassoy, 2021).

1.3 Justification

Epidemiological knowledge about the incidence and prevalence of a particular disease helps in the clinical assessment of the disease.

Malaria is the most prevalent febrile illness in Ghana. In yellow fever endemic areas, it is important to determine the carriage of both yellow fever virus and malaria parasites in febrile patients.

There is the need to determine the carriage of malaria parasites and yellow fever virus in the febrile patients, which are the likely leading pathogens that cause fever in the community. This will help to implement the appropriate preventive measures in containing the etiology of fever.

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1.4 Aim

To determine the carriage of yellow fever virus and malaria parasites in febrile patients in the selected districts.

1.5 Specific Objectives

i. To determine the carriage of yellow fever virus in febrile patients in the selected districts.

ii. To determine the carriage of malaria parasites in febrile patients in the selected districts.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Worldwide outbreak of Yellow Fever

Worldwide, around 1 billion people from 49 endemic countries are considered at risk of the yellow fever disease (Gardner & Ryman, 2015). Yellow fever remains a global public health problem that leads to high mortality and morbidity rates in Africa and South America. In South America, countries like Brazil, Peru, Bolivia and Colombia have experienced yellow fever outbreaks in past years. In recent years, African countries like Angola and Democratic Republic of Africa have experienced yellow fever outbreaks (Paules & Fauci, 2017). An outbreak which started in Angola in December, 2015 reported a massive widespread of the yellow fever disease, which even affected many unvaccinated Chinese workers who had returned to China from Angola (Vasconcelos & Monath, 2016). Ghana has also recorded a number of yellow fever cases in recent years.

2.2 Yellow Fever Outbreaks in Africa

Yellow fever is endemic in Africa (Monath *et al.,* 2015). As of 2021, yellow fever outbreaks have been recorded in Ghana, Ethiopia, Nigeria, Guinea, Angola, and Democratic Republic of Congo over the past decades (Chen *et al*., 2020). A modeling study based on African data sources estimated the burden of yellow fever during 2013 to be 84,000–170,000 severe cases and 29,000–60,000 (Garske *et al*., 2014). As of 2014, according to the WHO, an estimated 180,000 yellow fever cases and 25,000 deaths are reported every year in Africa (WHO, 2014). Between 1960 and

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01962, Ethiopia was faced with yellow fever outbreak recording 100,000 cases and 30,000 deaths. In the 1966 yellow fever outbreak, Arba-Minch district recorded 2200 cases and 451 deaths (Ardoin *et al*., 1976). Thereafter, between 1984 and 1990, Nigeria was faced with yellow fever outbreak recording 21,299 deaths (Adam & Jassoy, 2021). In Guinea, between September 4, 2000 and January 7, 2001 the country was faced with yellow fever outbreak recording 688 cases and 225 deaths (Nathan *et al*., 2001). Another yellow fever struck in Africa from December 5, 2015, to November, 2016, where 7334 suspected cases with 962 confirmed cases of yellow fever, accounting for 393 deaths were recorded in Angola and the Democratic Republic of the Congo (WHO, 2016).

2.3 Burden of Yellow Fever in Ghana

Yellow fever is endemic in Ghana and there have been major outbreaks of the disease in the country in 1950, 1969-1970, 1977-1980 and 1982-1983, which resulted into more than 400 deaths; for which many deaths may have gone unreported (Addy et al., 1986). In 1963, there were cases reported from Kumasi in the Ashanti region and Damongo in the Northern region. In the 1969-1970 yellow fever outbreak in Ghana, the country recorded 319 cases and 79 deaths. The outbreak started in 1969 at Pong-Tamale in the Northern region, recording 5 cases and 3 deaths. In the same year (1969) 303 cases and 72 deaths were reported from Bolgatanga, Navrongo, Nandom and Jirapa in the northern part of Ghana.

Few years after, the 1971-1975 yellow fever outbreak that occured in Dormaa Ahenkro, Berekum and Hwidiem in the Brong Ahafo region recorded 12 cases. Thereafter, the 1977-1980 yellow fever outbreak in Ghana recorded 827 cases and 189 deaths (Addy, 1984). In 2011-2012, a number of cases and deaths of yellow fever were reported in the Upper East, Upper West and Greater Accra regions of Ghana (DREF, 2012). Thereafter, in 2016, the Ghana Health Service confirmed cases

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in the Brong Ahafo region and Volta region. From October, 2021 to February, 2022, an outbreak of yellow fever took place to which numerous cases and deaths were recorded in several districts in the Savannah, Upper West, Bono and Oti regions in Ghana.

2.4 Transmission of Yellow fever and other Arboviral Viruses

Arboviruses stands for "Arthropod-borne viruses", which represents any viral pathogen transmitted by an arthropod vector (CDC, 2007). Availability and distribution of arthropods like mosquitoes, sandflies and ticks are essential for infections with arboviruses (Adam & Jassoy, 2021). Yellow fever belongs to the family *Flaviviridae* and genus Flavivirus. The genus consists of human and veterinary viruses such as Dengue virus, Zika virus, West Nile virus, Japanese encephalitis virus and Tick-borne encephalitis virus (Gould *et al*., 2008). Yellow fever is a vector-borne disease that is transmitted primarily by the bite of an infected *Aedes aegypti* mosquito (Shearer *et al*., 2017). The disease affects humans and non-human primates in tropical areas of Africa. Eradication of yellow fever is almost impossible as a result of sylvatic wild life reservoir (Garske *et al*., 2014). Humans are the main vertebrate hosts of yellow fever virus (Garske *et al*., 2014). The virus was first isolated from a Ghanaian patient called Asibi in 1927 (WHO 2018). Transmission of yellow fever virus to humans takes place in three cycles, namely; sylvatic, intermediate and urban transmission (Shearer *et al*., 2017). With the sylvatic transmission, non-human primates (monkeys) serve as forest reservoirs, and after getting infected through the bite of infected mosquitoes, humans then get infected when they work or visit the jungle where the virus is transmitted to the humans by infected mosquitoes. With intermediate (Savannah) transmission, humans who live in the border areas of the jungle get infected by the bite of infected mosquitoes that feed on both humans and non-human primates. Lastly, with urban transmission, humans who live in areas of high population get infected when urban infected *Aedes aegypti* mosquitoes

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transmit the virus from one person to another (Gardner *et al*., 2010). Yellow fever outbreaks are often triggered by the sylvatic and intermediate forms of transmission, where humans become infected when the virus is transmitted by the mosquitoes to humans as humans come into close contact with monkeys (Kleinert *et al*., 2019).

2.5 Detection of Yellow Fever Virus

Detection of yellow fever by polymerase chain reaction (PCR) is most reliable 5–7 days after symptom onset, during the viremic phase (Fischer *et al*., 2017). A more rapid and accurate for quantifying load of the yellow fever virus is made possible with the recent development of quantitative Real-Time PCR (Mackay *et al*., 2002). Real-time reverse transcription polymerase chain reaction (RT-PCR) can detect viral RNA in serum samples during the first 10 days since the onset of symptoms.

2.6 Control, Prevention and Treatment of Yellow Fever

From research findings conducted on the spread of yellow fever virus outbreak in Angola, human mobility and vector sustainability are important predictors for the spatial spread of yellow fever virus (Kraemer *et al*., 2017). The yellow fever spreads from one place to the other through transportation and urbanization of new areas as these factors also ensure the spread of the mosquito vector (Suzuki *et al*., 2016). The widespread pyrethroid resistance in the *Aedes aegypti* mosquito-vector also has helped in the transmission and perpetuation of the yellow fever virus (Suzuki *et al*., 2016). The eradication of the disease is almost impossible as a result of sylvatic wild life reservoir of the yellow fever virus (Garske et al., 2014).

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The yellow fever disease can be prevented by a single dose of a live-attenuated yellow fever vaccine (17D yellow fever vaccine) which provides lifelong immunity (Mokaya *et al*., 2021). Currently, in 2021, there is no specific antiviral treatment for yellow fever. However, treatment of clinical manifestations such as dehydration, fever, organ failure and antibiotics for associated bacterial infections could help better treatment outcome (Mokaya *et al*., 2021).

2.7 Yellow Fever Vaccination

Vaccination is the most important means of preventing the yellow fever infection (WHO, 2018). Yellow fever can be prevented using a live-attenuated yellow fever vaccine which was first developed in 1937. All current yellow fever vaccines are derived from the 17D strain, which was obtained following the attenuation of the Asibi strain by serial passage through chicken tissue or embryonic mouse. Anti-yellow fever virus neutralizing antibodies have been detected for as long as 35 years (Waggoner, Rojas, & Pinsky, 2018). The vaccine is administered intramuscularly or subcutaneously to adults travelling to endemic areas or at times when there is an outbreak, and to children aged 9 months or above through native childhood immunization (Domingo, 2018). There is evidence that a single dose of the vaccine of the yellow fever vaccine can provide lifelong immunity (WHO, 2019). The vaccine is contraindicated in children below 9 months, pregnant women and people with severe immunodeficiency (Verma, Khanna, & Chawla, 2014).

2.8 Burden of Yellow Fever Surveillance in Africa

Laboratory investigations are not done. And because is non-specific and only a syndromic approach is used, it leads to inappropriate diagnosis.

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Informed knowledge on the spread of yellow fever can be useful in the disease control, as this can be obtained through laboratory investigations of the disease (Basile *et al*., 2016). However, the existence of natural reservoirs (monkeys) of the yellow fever virus and the widespread of vector populations make elimination of the disease impossible even in the presence of the vaccine (Waggoner *et al*., 2018).

The main problem in Africa is distinguishing between the causative agents of febrile illnesses with similar clinical presentations including yellow fever, malaria, measles, acute HIV infection and chikungunya fever. Most often than not, arboviral infections are frequently undiagnosed (Adam & Jassoy, 2021). Laboratory investigations methods of arboviral infections include detection of the viral genome, viral antigen and viral antibodies (Adam & Jassoy, 2021).

Rural areas lack the required laboratory facilities to diagnose arboviral infections. For such reason, in the event of arboviral infections, a team of experts must call upon to investigate the outbreak and samples must be collected and sent to the appropriate lab facility for laboratory investigations (Adam & Jassoy, 2021).

In many African countries, strategies for prevention of yellow fever are protection from mosquito bites, community vector control, routine immunization for children aged 9 months or above, mass vaccination campaigns to wider age ranges and early investigation and detection of the disease outbreak (Ahmed *et al*., 2017).

Vaccinated individuals are deemed to be protected against a natural infection after approximately 10 days post-vaccination. The efficiency of yellow fever vaccination in a given population is dependent on factors such as the availability of the 17D vaccine, the percentages of the population that take the vaccine and the spread of the disease (Basile et al., 2016).

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2.9 Burden of malaria in Ghana

Malaria is endemic in Ghana (W.H.O. 2016). In Ghana, malaria affects people of all ages and throughout the year. However, there is a shift in the number of malaria cases by locations and seasons. Generally, there is a higher prevalence of malaria around irrigated farming areas compared to neighbouring areas without irrigation agriculture (Baeze *et al*., 2011). The increased prevalence rate of malaria in irrigated farming areas is due to the availability of water from the irrigation system that enhances breeding sites for the mosquito vectors, thus resulting into high density of the anopheles mosquito vector (Afrane *et al*., 2012). Children below the age of 5 years are the most affected age group and *Plasmodium falciparum* is the mosquito that causes most malaria infections in Ghana (Basu *et al*., 2017; Nyarko *et al*., 2014). Clinical presentations of uncomplicated malaria include fever, headache, chills, joint pains and diarrhoea (Caraballo & King 2014). Individuals infected with malaria presents with fever and between the years of 2000 to 2013, febrile illness has accounted for more than half of all clinic visits in Ghana (Stoler & Awandare,2106). Some measures taken to control malaria in Ghana are the use of long-lasting insecticides nets, indoor residual spraying, and intermittent preventive treatment (Ndong *et al*., 2019).

2.10 Transmission of malaria in Ghana

Female Anopheles mosquito is responsible for the spread of malaria parasites.

For the Anopheles mosquito to become infected, they must bite, or take a blood meal, from a person with the malaria parasites. The mosquito will then inject the parasites through her saliva into the next person she bites. And the cycle of infection perpetuates (Castro, 2017).

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2.11 Detection of malaria

Malaria diagnosis among suspected infected individuals includes the detection of malaria parasites or antigens in the patient blood. The initial clinical presentations of malaria like fever, headache, body pains, nausea and chills are often verifying and non-specific (Looareesuman *et al*., 1999). Laboratory diagnosis of malaria is achieved by techniques such as the conventional microscopic diagnosis by staining thin and thick peripheral blood smears, rapid diagnostic tests, quantitative buffy coat (QBC), and polymerase chain reaction (Vo *et al*. 2007).

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design and Sites

This was a cross-sectional study. The study was conducted in three regions in Ghana. The districts were Tamale, Kumbungu, Wenchi and Damongo; Wenchi (7.7420 ˚ N, 2.1008 ˚ W), Kumbungu (9.5605 ˚ N, 0.9486 ˚ W), Damongo (9.3634˚ N, 1.6761 ˚ W) and Tamale (9.4034 ˚ N, 0.8424 ˚ W). Whereas Damongo and Wenchi are part of the yellow fever outbreak zones, Kumbungu and Tamale have not yet experienced yellow fever. **Selection of Wenchi and Damongo districts was based on the fact that they had an experienced a yellow fever outbreak recently; whilst Tamale and Kumbungu were selected as non-outbreak areas, due to their close proximity to the outbreak foci. Also, the sites and health facilities were selected by convenient sampling as there were ongoing studies at these sites.**

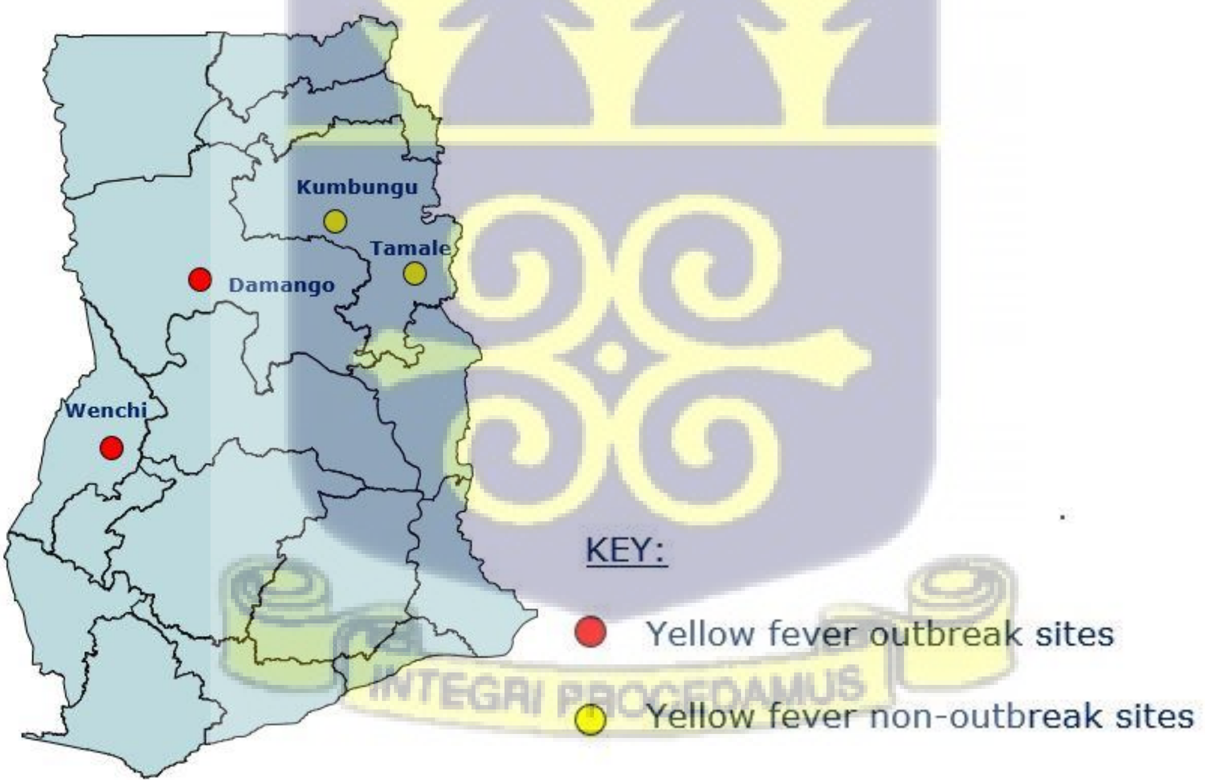

Figure 3.1: A map of Ghana showing the various districts.

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3.2 Study Population

The study population was made up of febrile patients drawn from health facilities in the study districts. The health facilities included one hospital from each district; Tamale Central Hospital from Tamale, Kumbungu's Kings Medical Centre from Kumbungu, Wenchi Methodist Hospital from Wenchi and West Gonja District Hospital from West Gonja. These hospitals were settled on due to convenience sampling. Febrile patients are individuals who have fever, and have a body temperature of at least 37.5 ℃.

3.3 Sample Size Calculation

Based on a previous study by Nwaiwu *et al*., (2021) in Ghana, the expected proportion of yellow fever cases was 10000 per 100000. The margin of error or level of precision (e) to be used was 0.05 (5%). The formula used for the determination of sample size is:

$$n = \frac{z^2 \times p\ (1-p)}{e^2}$$

With confidence level of 95%, z= 1.96, e =5% (0.05), p= 10000/100000 (0.1),

$$n = \frac{1.96^2 \times 0.1\ (1-0.1)}{0.05^2}$$

n = 138 (minimum sample size)

The study involved 498 febrile participants.

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3.4 Inclusion Criteria

Febrile patients of any age in the health facilities of the selected study sites who provided informed consent or child assent to participate in the study. Febrile participants are individuals with a body temperature of 37.5 ˚C or above.

3.5 Exclusion Criteria

Individuals with a body temperature less than 37.5 ˚C were excluded from this study. Also, febrile patients who did not provide informed consent or child assent to participate in the study or were too sick or weak at the time of data or sample collection were excluded from the study.

'Too sick' or 'too weak' patients are febrile patients who have overwhelming clinical presentations at the time of sample collection which makes it uncomfortable to include them in the study due to ethical issues.

3.6 Participant Sampling and Data Collection

Letters having the requisite information seeking permission to conduct the research study were sent to the Medical Directorate of the health facilities of the study sites captured.

The febrile participants were sampled by random sampling at the specified health facilities at the study sites. Random sampling was a probability sampling method where each febrile participant had an equal chance of being selected. Febrile patients were picked at random for the study. The febrile participants were recruited from health facilities at the study sites captured. Febrile participants were considered as individuals with a body temperature equal to or greater than 37.5 ˚C. The background and purpose of the study were properly read and explained to the participants. Informed consent or assent were sought before the participants were involved in the study.

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After consent or assent was obtained from the participants, participants were asked to provide answers to a previously developed questionnaire containing information about age, sex, address, occupation, vaccination status, travel history, current clinical presentations, presence of primitive primates near residency and residency proximity to native vegetation and plantation. For each participant, venous blood of about 3 ml was taken by venipuncture into serum separator tubes and EDTA tubes.

3.7 Sample Collection

About 3 ml of venous blood samples were collected by venipuncture from each participant. A portion of the blood samples was collected into serum separator tubes, which was processed into serum for the detection of yellow fever virus and the remaining portion was collected into EDTA tubes used for the diagnosis of malaria. Conventional Nested PCR was performed and microscopy were performed. Microscopy was used to determine malaria parasite density and parasite species.

RT-PCR was performed at the Virology Laboratory of Noguchi Memorial Institute for Medical Research. RT-PCR was used to amplify yellow fever viral nucleic acid (RNA) to detect the presence of yellow fever virus.

DNA extraction from blood paper blot was done after sample collection. After blood was taken from patients, a portion of it was collected into ethylenediaminetetraacetic acid (EDTA) tubes and then drops of blood were put on the blood paper blot. DNA extraction was performed using Chelex extraction method in the laboratory after samples were sent to the laboratory.

Serum samples were kept at 4 ˚C on ice packs in the field immediately after collection before it was transported at optimal temperature to the Virology Laboratory of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon in the Accra Metropolis. In the laboratory, the serum samples were stored at a temperature of -80 ˚C.

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3.8 Molecular Detection of Yellow Fever Virus

3.8.1 RNA Extraction of Yellow Fever Virus

A total of 140µl of each patient's serum sample was submitted for RNA extraction using the QIAmp Viral RNA Mini Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instruction.

A volume of 560µL prepared Buffer AVL containing carrier RNA was pipetted into a 1.5ml microcentrifuge tube. 140µL of patient's serum was added to the Buffer AVL- carrier in the microcentrifuge tube. It was mixed by pulse-vortexing for 15 seconds and incubated at room temperature for 10 minutes. The tube was centrifuged for a short time to remove drops from the inside of the lid. 560 ethanol (96-100%) was added to the sample, and mix by pulse-vortexing for 15 seconds. After mixing, the tube was centrifuged to remove drops from the lid. 630 µl of the solution was carefully applied from the previous step to the QIAamp Mini column (in a 2 ml collection tube) without the rim. The cap was closed, and centrifuged at 6000 ×g (8000 rpm) for 1 minute. The QIAamp Mini column was placed into a clean 2 ml collection tube and then tube containing the filtrate discarded. The QIAamp Mini column was carefully opened and the previous step was repeated. The QIAamp Mini column was carefully opened, and the previous step was repeated. The QIAamp Mini column was opened carefully and 500 µl Buffer AW1 added. The cap was closed, and then centrifuged at 6000 ×g (8000 rpm) for 1 minute. The QIAamp Mini column was placed in a clean 2 ml collection and then tube containing the filtrate discarded. The QIAamp Mini column was opened carefully, and 500 µl Buffer AW2. The cap was closed and centrifuged at full speed (20, 000 × g, 14, 000 rpm) for 3 minutes. Any possible Buffer AW2 carryover was centrifuged until there was no visible traces of droplets at the base of the tube. The QIAamp Mini column was placed in a new 2 ml collection tube, and the old collection tube with the filtrate

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discarded. It is centrifuged at full speed for 1 minute. The QIAamp Mini column is placed in a clean 1.5 ml microcentrifuge tube. The collection tube containing the filtrate was discarded. The QIAamp Mini column was opened carefully and 60 µl AVE was added to room temperature. The cap is closed, and then incubated at room temperature for 1 minute. It is centrifuged at 6000 × g (8000 rpm) for 1 minute. A single elution with 60 µl Buffer AVE was done to elute the viral RNA.

Pipetting scheme for Yellow fever virus detection by RT- PCR analysis has been shown in the table below.

Table 3.1 Pipetting scheme for Yellow fever virus detection by RT- PCR analysis

Reagent	×1 (µl)
Luna one-step reaction mix (2×)	10
Enzyme mix (×20)	1
Yellow fever probe (YFP)	0.4
Yellow fever forward primer (YFS)	0.8
Yellow fever reverse primer (YFAs)	0.8
Nuclease-free water	5

Real time Reverse Transcription polymerase chain reaction (RT-PCR) was performed using the Applied Biosystems (ABI) 7500 Fasc machine.

A known yellow fever positive control was used for the set-up.

A known yellow fever negative control was used for the set-up.

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Detection of Yellow Fever Virus using the YFA, YFAs primers and YFP probe with the AgPath ID OneStep RT-PCR Kit has been shown in the table below.

Table 3.2 A table showing detection of Yellow Fever Virus using the YFA, YFAs primers and YFP probe with the AgPath ID OneStep RT-PCR Kit.

Reagent	Volume per reaction (µl)
Nuclease free water	5
2× Reaction buffer	10
YFS	1
YFAs	1
YFP	0.5
Enzyme Mix	0.5
Template DNA	2
Total	20µl

Steps	Stage	Number of cycles	Temperature [°C]	Time [min:sec]
Reverse transcription	Hold	1	45	20:00
Denaturation	Hold	1	95	05:00
Amplification	Cycling	45	95	00:05
			60	00:35

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3.9 Detection of *Plasmodium falciparum* by Microscopy

A drop of whole blood, about 6 µl was dispensed at the center of a pre-cleaned labelled glass slide and about 2 µl dispensed at a corner of the same glass side. Thin and thick blood films were prepared. The thin blood smear was fixed in absolute methanol, and then allowed to airdry at room temperature. 10% Giemsa stain (1 ml of Giemsa to 9ml of distilled water) was added to the blood smears and allowed to stand for 10 minutes. The stain was washed in water and the blood smears allowed to air dry. A drop of immersion oil was then added on the blood smears and observed under the light microscope using oil immersion magnification in detecting the Plasmodium parasites.

The smears were observed or screened at the least objective lens magnification (×10, ×40 objective lenses) to determine appropriate fields of the smear. The smears were then examined using ×100 oil immersion. Fields of the blood smears were observed at least 100 high power before a thick film was known as positive or negative. For every malaria positive slide, Plasmodium parasites and total number of white blood cells (lymphocytes, basophils, eosinophils and neutrophils) in each field of the blood smears were counted, and with the help of a tally counter until a total number of 200 white blood cells were counted and the corresponding Plasmodium parasites counted and recorded respectively. Parasite density was estimated by counting the number of Plasmodium parasites per 200 white blood cells.

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3.10 Molecular Detection of *Plasmodium falciparum* by Conventional Nested Polymerase Chain Reaction

3.10.1 DNA Extraction of *Plasmodium falciparum* from Blood Paper Blot using Chelex DNA Extraction Protocol

The hole puncher was sterilized with the use of 10% bleach, 80% ethanol and distilled water. A punch of each labelled blot filter paper to a size of about 5mm made into a sterile 1.5 ml Eppendorf tube. The hole puncher was sterilized before the next sample was processed with the use of 10% bleach, 80% ethanol and distilled water. 50µl of 10% Chelex and 1ml of phosphate buffer saline was added to each labelled Eppendorf tube. It then vortexed for a short time and then incubated overnight at 4°C whilst shaking to enhance erythrocytes lysis to release the plasmodium parasites. The Eppendorf tube was vortexed and spinned at 10,0000rpm for 1 minute. The reddish phosphate buffer solution/ saponin supernatant was decanted. Ice ml of ice-cold PBS was added to the tube containing the filter and then vortexed for a short time and then incubated at 4°C for 30 minutes. The tubes were spinned at 10,000rpm for 1 minute, and the supernatant aspirated. 100µl of distilled water 50µl of 20% Chelex was added and incubated at 95°C for 10 minutes and then vortexed at a 2 minutes interval. The Chelex reagents prevents degradation of the DNA and then traps the contaminants. The tubes were spinned at 13,000rpm for 6 minutes. The supernatant (DNA) was transferred into a sterile labelled 0.5 ml microfuge tube and stored at -20°C.

3.10.2 Plasmodium detection: Nested Conventional Polymerase Chain Reaction

Extracted DNA samples were tested for Plasmodium species using nested conventional PCR (thermocycler: Applied Biosytems™ SimpliAmp™). Nested PCR involves two PCR analyses. Amplicons from the first PCR analysis was served as a template for the second PCR. The first

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PCR analysis was done to detect 18S rRNA genes. The second PCR analysis was done to detect Merozoite surface protein (msp2), specific for *Plasmodium falciparum* After the reaction, the PCR products were resolved on 1.5% agarose gel stained with ethidium bromide and then visualized under UV light.

Nested Conventional Polymerase Chain Reaction was done using a two-primer set and successive PCR with the use of a thermocycler to detect *Plasmodium falciparum*. In the DNA extract. Nested Dream Taq Master mix which is made up of Taq polymerase, buffer, Magnesium chloride, deoxynucleotide triphosphates and primers. The first PCR produced amplicons which was used as a template for the second PCR. A volume of 0.4µl rPLU5, 0.4µl rPLU6, 7.5µl of Dream Taq master mix, 3.7µl of double distilled water and 3µlof the template was used in Nest 1 to detect 18S rRNA genes. Polymerase chain reaction was then performed with a thermocycler (Applied Biosystems™ SimpliAmp™) with reaction conditions made up of cyclical conditions of 94°C for 2 minutes, followed by 94°C for 30 seconds, 54°C for 1 minute, 68°C for 1 minute and the reaction subjected to 35 cycles. The final step is a 5-minute extension at a temperature of 68°C.

Nest 2 reaction was made up of 0.4µl ofrFAL2, 0.4µl rFAL3, 7.5µl of Dream Taq master mix, 6.20µl of double distilled water and 2µl of Nest 1 PCR product was used in Nest 2 to detect merozoite surface protein msp2, a specific target sequence for *Plasmodium falciparum*. It has a band size of 200 base pairs. The second cycling conditions for Nest 2 was 94°C for 2 minutes, followed by 94°C for 30 seconds, 59°C for 1 minute, 68°C for 1 minute and the reaction subjected to 35 cycles. The final step is a 5-minute extension at a temperature of 68°C. Size-fraction of the PCR products was done on 2% agarose gel stained with Ethidium bromide (Biotium, Hayward,

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California, USA). The gel was then run at 130V for 45 minutes and then visualized under the ultraviolet light.

Primer sequences for Plasmodium parasite identification has been shown in the table below.

Table 3.3 A table showing primer sequences for Plasmodium parasite identification

Primer	Sequence: 5' – 3'
rFAL2	ACACAATGAACTCAATCATGACTACCCGTC
rFAL3	TTAAACTGGTTTGGGAAAACCAAATATATT
rPLU5	CCTGTTGTTGCCTTAAACTTC
rPLU6	TTAAAATTGTTGCAGTTAAAACG

Primer Sequences for the first nested PCR analysis has been shown in the table below.

Table 3.4 A table showing primer sequences for the first nested PCR analysis

Primer	Sequence
rPLU5	5'-CCTGTTGTTGCCTTAAACTTC-3'
rPLU6	5'-TTAAAATTGTTGCAGTTAAAACG-3'

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Reaction Conditions for first Nested PCR analysis has been shown in the table below.

Table 3.5 A table showing reaction conditions for first Nested PCR analysis

Reagent	**×1 (µl)**
Double distilled water	3.7
DNA template	3
Nested Dream Taq Master mix	7.5
rPLU5	0.4
rPLU6	0.4

Primer Sequences for the second nested PCR analysis has been shown in the table below.

Table 3.6 A table showing primer sequences for the second nested PCR analysis

Target gene	**Sequence**	**Base pair**
rPLU5	**5'-CCTGTTGTTGCCTTAAACTTC-3'**	200
rPLU6	**5'-TTAAAATTGTTGCAGTTAAAACG-3'**	200

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Reaction Conditions for first Nested PCR analysis has been shown in the table below.

Table 3.7 Reaction Conditions for first Nested PCR analysis

Reagent	**×1 (µl)**
Double distilled water	6.2
Nest 1 PCR product	2
Nested Dream Taq Master mix	7.5
rPLU5	0.4
rPLU6	0.4

3.11 Data Management Plan

There was high confidentiality in the keeping of data. Data was saved in both hardcopies and softcopies for analysis. Softcopies of data collected was kept confidential in an encrypted personal computer for analysis. Hardcopies of data collected was kept under lock and key. Data was stored on an external drive for backup which was made inaccessible to non-researchers.

3.12 Data Analysis

Laboratory results for each participant was put in Microsoft Excel Version 2019. Data was analyzed using Statistical Package for Social Sciences (SPSS) Version 25.0 and presented as frequencies and percentages in the form of tables and graphs.

Significance level for all statistical analysis was set at p-value less than 0.05.

Chi-square analysis was used to analyse categorical variables, which was used to compare malaria prevalence across different study sites and age groups.

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Prevalence of malaria was calculated by dividing the number of malaria positives by total number of patients multiplied by a percent.

3.13 Ethical Considerations

Ethical approval was sought from the Ghana Health Service Ethical Review Committee and College of Health Sciences, University of Ghana.

Approval number is GHS-ERC:031/03/22.

Permission was sought from the Medical Directorate of the various health facilities of the selected study sites.

An informed written consent or assent was obtained from each participant who was 18 years and above and below 18 years respectively. Background and purpose of study was properly explained to the participants in their native language.

Participants benefitted from the study by knowing they have been infected by the yellow fever virus or not. Infected individuals were then asked to seek medical care and treatment. Extreme respect was adhered to for the rights and confidentiality of the participants during data collection and handling.

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CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographics of Participants

A total of 498 febrile participants was sampled during dry and rainy seasons. All participants had a body temperature of 37.5°C or higher at the time of participants recruitment and human blood sample collection. The mean age of the participants was 26.09 years. A higher proportion of females (313, 62.9%) than males (185, 37.1%) was recorded in this study as seen in **Table 4.1**. Higher proportions of the participants were observed in the older age group (321, 64%). Almost an equal proportion of the participants were observed in all study sites, with Tamale having 120 participants, Kumbungu having 140 participants, Wenchi having 101 participants and Damong having 137 participants, and a higher proportion of human blood samples was collected during the rainy season (410, 82.3%) than the dry season (88, 17.7%) as seen in **Table 4.1**. A higher proportion of the febrile patients (293, 58.8 %) in this study were vaccinated against yellow fever as seen in **Table 4.1**.

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Table 4.1 A table showing socio-demographics of Participants

Characteristic		Frequency	Percentage (%)
Sex	Male	185	37.1
	Female	313	62.9
Age group	< 5	97	19.5
	5-10	46	9.2
	11-15	34	6.8
	> 15	321	64.5
Study site	Tamale	120	24.1
	Kumbungu	140	28.1
	Wenchi	101	20.3
	Damongo	137	27.5
Season of sample collection	Dry	88	17.7
	Rainy	410	82.3
Vaccination	Yes	293	58.8
	No	205	41.2

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Table 4.2 A table showing clinical presentations of participants

Clinical presentation	Number of participants (%)
Fever	498 (100)
Headache	498 (100)
Body/joint pains	498 (100)
Nausea/vomiting	223 (44.8)
Jaundice	106 (21.3)

4.2 Carriage of yellow fever virus in febrile participants

Out of a total of 498 febrile participants, all tested negative to yellow fever virus using one-step real-time reverse transcription polymerase chain reaction as seen in **Figure 4.1.** The positive control used for the PCR had a CT value of 34.9938 with a sigmoid curve as shown in **Figure 4.1.** Any sample with value greater than 40 was negative.

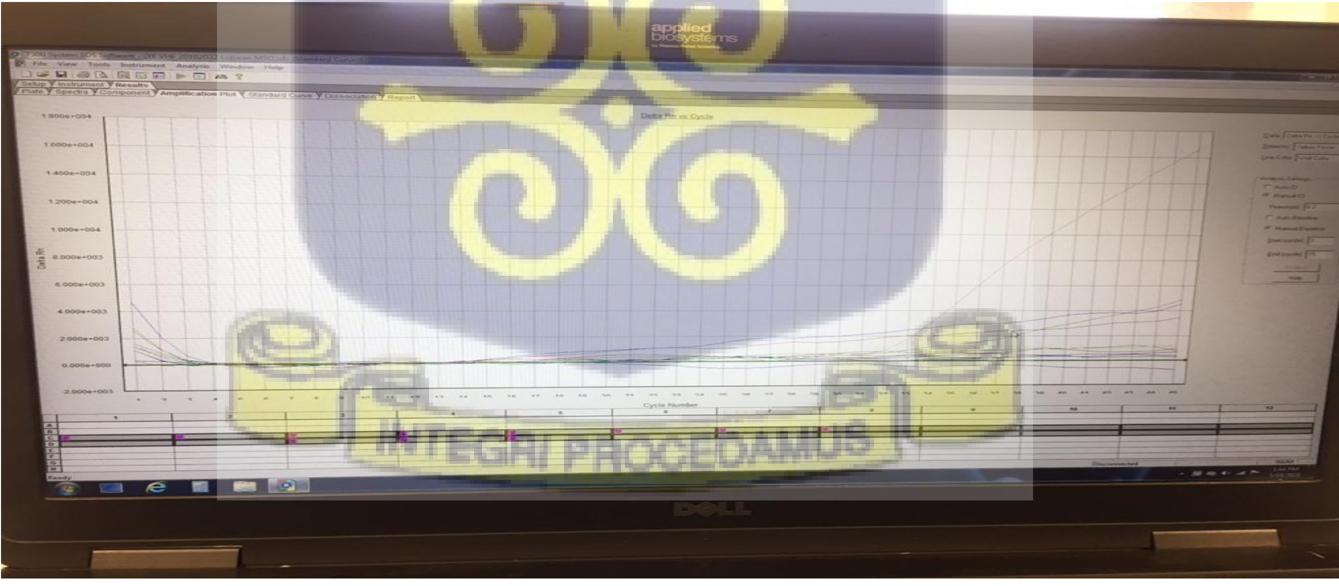

Figure 4.1 RT-PCR amplification curve showing results of yellow fever detection output

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Table 4.3 A table showing malaria prevalence by microscopy and mean temperatures of participants in all study sites

Study site	Number of samples (dry season)	Number of samples (rainy season)	Number of malaria positives (dry season) (%)	Number of malaria positives (rainy season) (%)	Mean temperature of participants (°C)
Tamale	20	100	7(35)	23(23)	38.2
Wenchi	37	103	8(21.6)	31(30.1)	38.8
Kumbungu	31	70	6(19.4)	2(2.9)	37.8
Damongo	-	137	-	21(15.3)	38

Table 4.4 A table showing malaria prevalence according to yellow fever outbreak and non-outbreak zones by microscopy

Zones	Number of participants	Number of positives (%)
Outbreak zones	238	29(12.18)
Non-outbreak zones	260	69(26.54)

4.3 Study site Malaria Percentage Prevalence by Microscopy

During the dry season, the highest proportions of malaria infections using microscopy were observed among participants in Tamale (35%) while the highest proportions of malaria infections were observed among participants in Kumbungu (30.1%) during the rainy season as seen in **Figure 4.2**. However, Wenchi (2.9%) recorded the least proportions of malaria infections during both the

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dry and rainy seasons. There was no sample collection at Damongo during the dry season as seen in **Figure 4.2**.

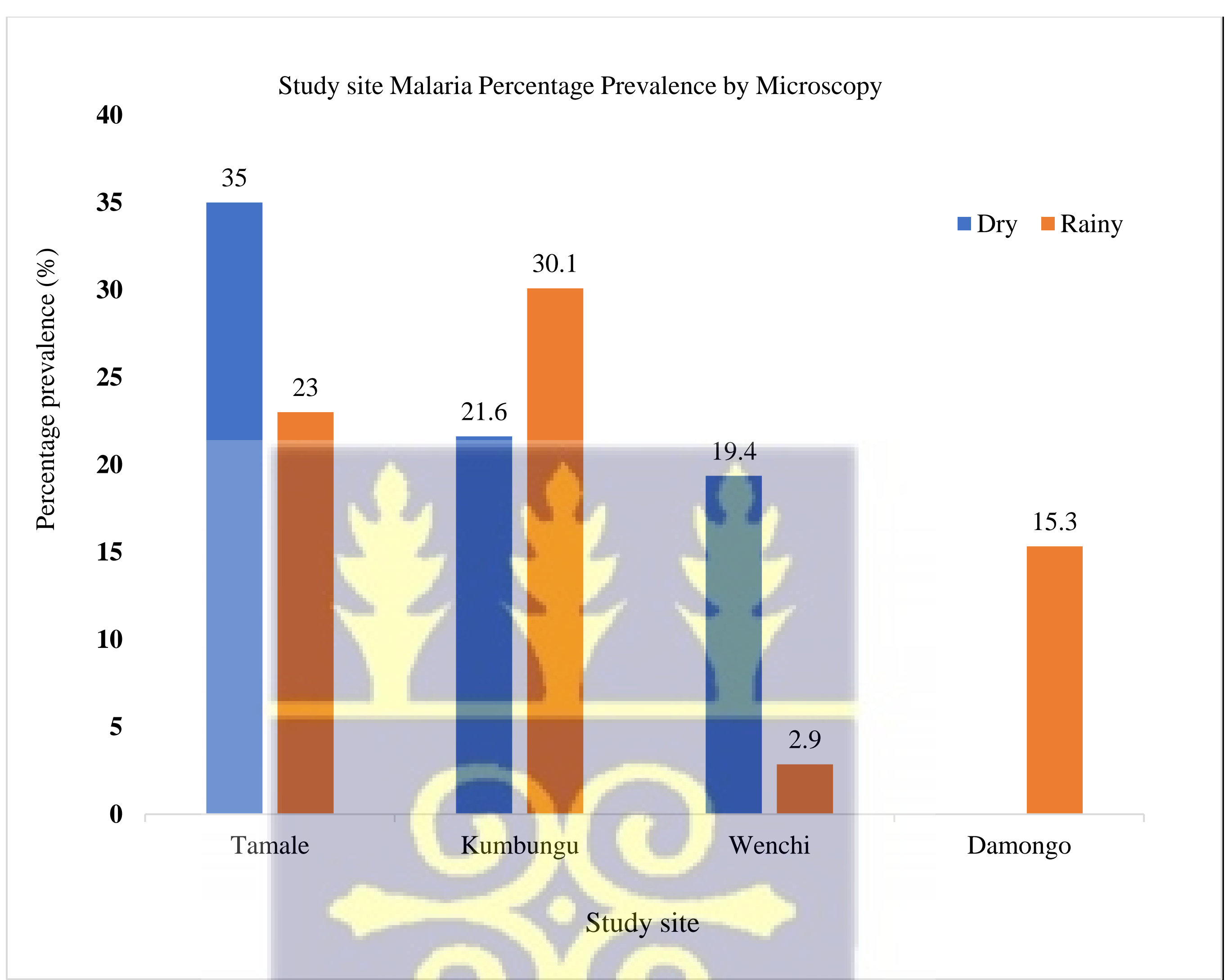

Figure 4.2 Study site Malaria Percentage Prevalence by Microscopy

4.4 Age-specific Malaria Percentage Prevalence by Microscopy

During the dry season, the highest proportions of malaria infections using microscopy were observed among participants less than 5 years (9.1%) as seen in **Figure 4.3**. However, the highest proportions of malaria infections were observed among participants in the older age group (12.2%) during the rainy season as seen in **Figure 4.3**.

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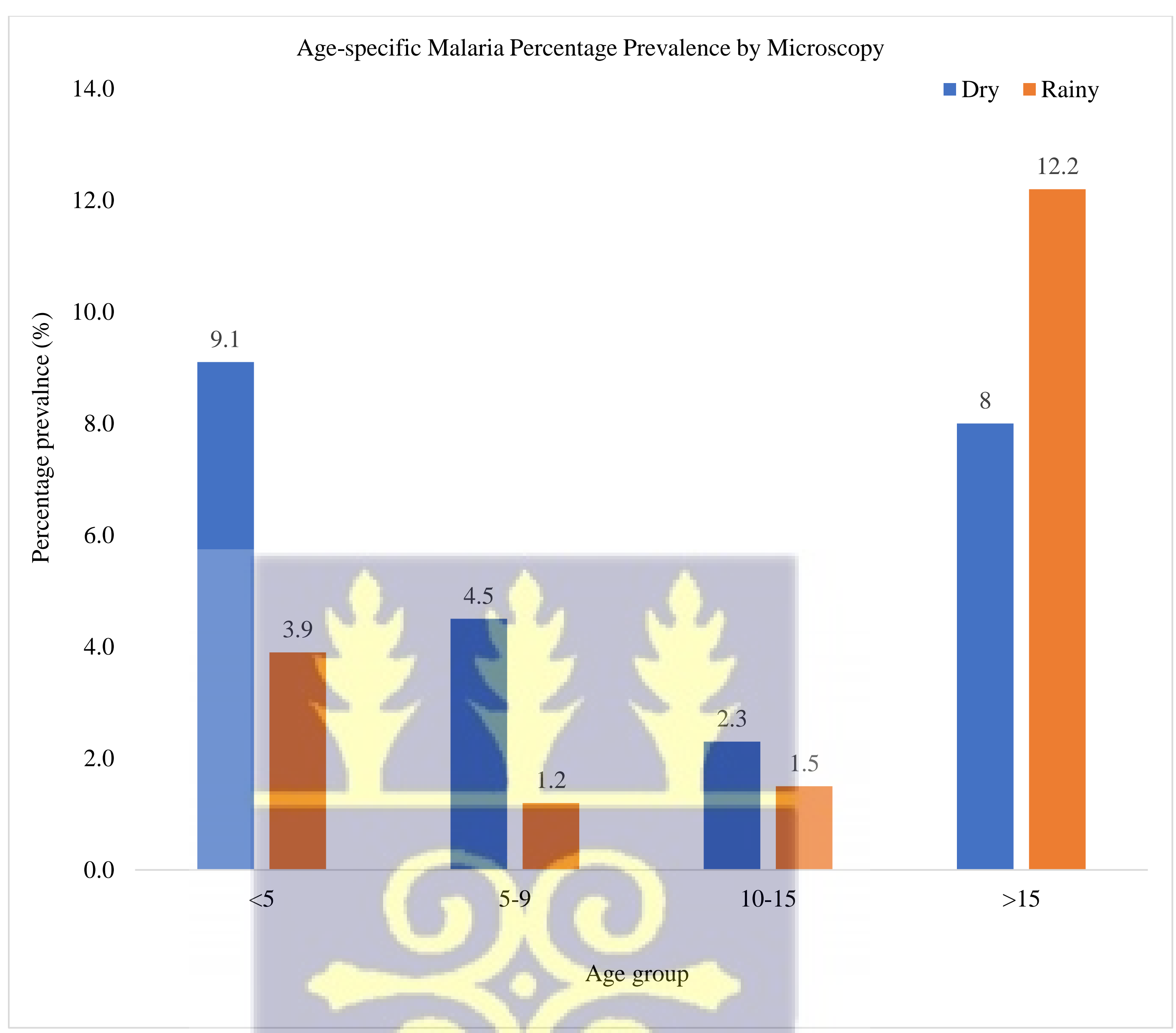

Figure 4.3 Age-specific Malaria Percentage Prevalence by Microscopy

4.5 Study site Malaria Percentage Prevalence by Conventional Nested PCR

During the rainy season, the highest proportions of malaria infections using conventional nested PCR were observed among participants from Kumbungu (47.6%). There was no PCR done on samples from Tamale as seen in **Figure 4.4**.

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Study site Malaria Percentage Prevalencce by Conventional Nested PCR

Percentage prevalence (%)

50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0

47.6
15.7
23.4

Kumbungu
Wenchi
Damongo

Study site

Figure 4.4 Study site Malaria Percentage Prevalence by Conventional nested PCR

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4.6 Age-specific Malaria prevalence by Conventional Nested PCR

During the rainy season, the highest proportions of malaria infections using conventional nested PCR were observed among the older age groups (15%) as seen in **Figure 4.5.**

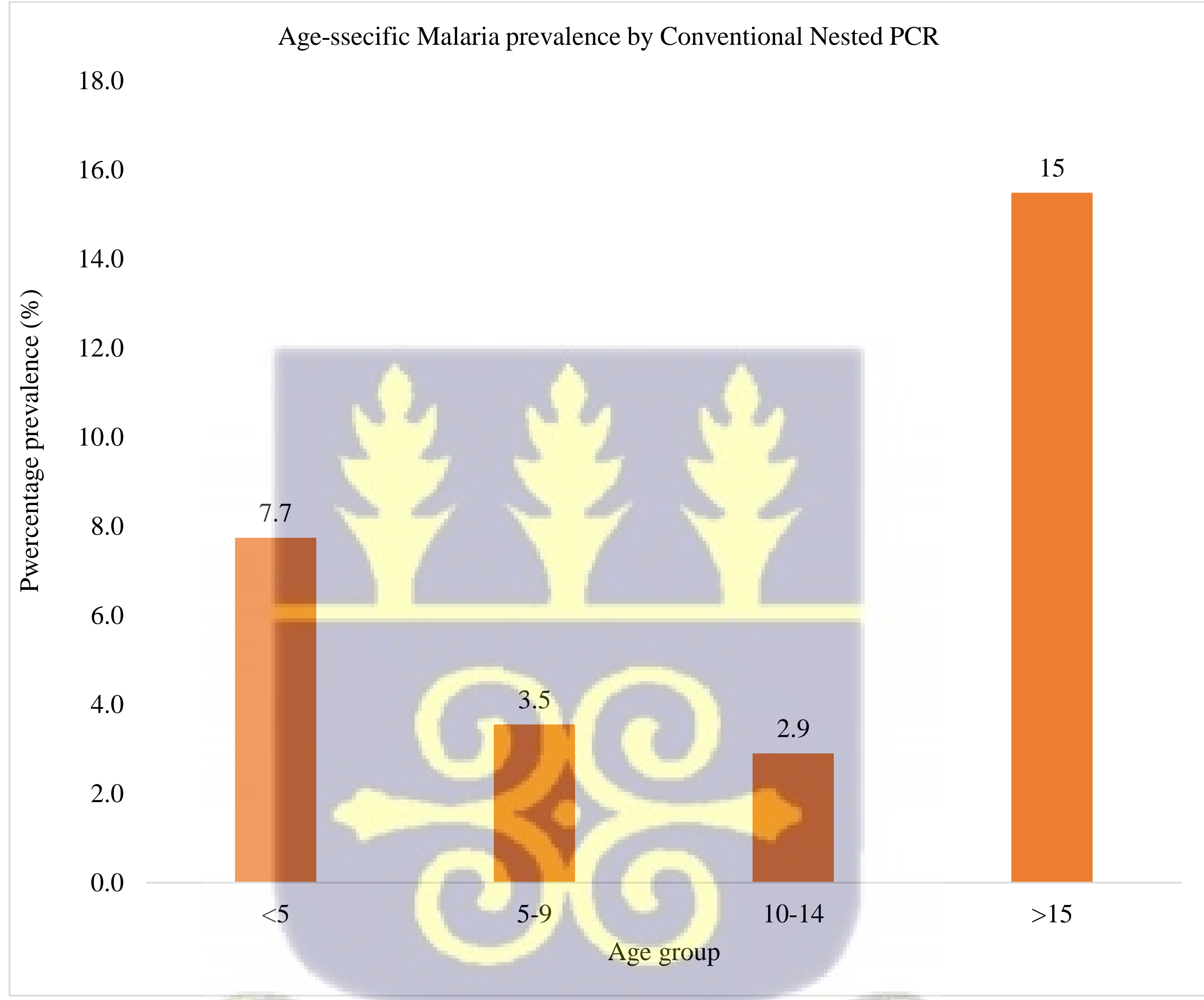

Figure 4.5 Age-specific Malaria Percentage Prevalence by Conventional Nested PCR

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4.7 Carriage of Plasmodium parasites in febrile participants

Out of a total of 498 febrile participants, all tested negative to yellow fever virus using one-step real-time reverse transcription polymerase chain reaction in Figure 4.1 and Figure 4.2. The positive control used for this RT-PCR had a value of 34.9938 with a red sigmoid curve as shown in Figure 4.1. Any sample with value greater than 40 was negative.

Figure 4.6 Agarose gel picture of Conventional Nested PCR products for the detection of *Plasmodium falciparum*

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4.8 Malaria Prevalence by Different Technique of Plasmodium Parasite Detection

310 human blood samples were collected for conventional nested PCR, of which 92 (29.7%) while out of 498 human blood samples collected for microscopy, 98 (19.7%) were positive as seen in **Table 4.2.**

Table 4.5 A table showing Malaria Prevalence by Different Technique of Plasmodium Parasite Detection

Technique Of Parasite Detection	Conventional Nested PCR	Microscopy
Total number of samples	310	498
Number of samples that tested positive	92	98
Percentage prevalence (%)	29.7	19.7

4.9 Association between Malaria Infections and Socio-demographic factors Using Microscopy

Using the microscopy results, there was no association between sex ($p = 0.743$) and malaria infection as shown in **Table 4.3**. There was no association between age group ($p = 0.450$) and malaria infection in **Table 4.3**. Also, there was no association between season of sample collection ($p = 0.276$) and malaria infection as shown in **Table 4.3**. However, there was an association between study site ($p = 0.000$) and malaria infection as shown in **Table 4.3**.

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Table 4.6 A table showing association between Malaria Infections and Socio-demographic factors Using Microscopy

Socio-demographic factor	Positive	p-value
Sex	98	0.743
Age group	98	0.450
Study site	98	0.000
Season of sample collection	98	0.276

4.10 Association between Malaria Infections and Socio-demographic factors Using Conventional Nested PCR

With the use of conventional nested PCR, there was no association between sex (p = 0.465) and malaria infection as shown in **Table 4.4**. However, there was an association between age group (p = 0.003) and malaria infection as shown in **Table 4.4**. Also, there was an association between season of study site (p = 0.000) and malaria infection as shown in **Table 4.4**.

Table 4.7 A table showing association between Malaria Infections and Socio-demographic factors Using Conventional Nested PCR

Socio-demographic factor	Positive	p-value
Sex	92	0.465
Age group	92	0.003
Study site	92	0.000

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CHAPTER FIVE

5.0 DISCUSSION

Yellow fever (YF) is a viral hemorrhagic fever that presents with clinical symptoms similar to malaria. In endemic areas, it is important to determine the carriage of both yellow fever virus and malaria parasites in inhabitants in these areas. Yellow fever is endemic in Ghana and the country was faced with an outbreak of the yellow fever disease in 2021 which affected four regions in the country (Gardner *et al* 2010; GHS, 2021). Malaria is a differential diagnosis of yellow fever and the most predominant cause of fever in febrile patients in Ghana (Simon, Hashmi & Torp 2022; Chrispal *et al*., 2010; Sunden *et al*., 2017). In this study, yellow fever virus and malaria parasites were investigated in febrile patients in and around areas in Ghana that experienced a yellow fever outbreak in 2021. From the study, out of total of 498 febrile participants, all the participants tested negative to the yellow fever virus using one-step real-time reverse-transcription polymerase chain reaction. Also, out of a total of 498 febrile participants, 98 (19.7%) of them were found to have the *Plasmodium falciparum* parasite using microscopy while out of a total of 310 febrile participants, 92 (29.7%) of them were found were found to have the *Plasmodium falciparum* parasite by conventional nested polymerase chain reaction. The commonest clinical presentations of the febrile participants included headache, generalized body pains, fatigue, joint pains and nausea.

None of the febrile participants tested positive to the yellow fever virus. These findings are similar to a study conducted in Central African Republic that reported only one female to have the yellow fever virus (the East-Central African genotype) using real-time RT PCR (Tchetgna *et al*., 2022). However, in a study conducted in Ghana in 188 febrile patients, 70 (37.2%) tested positive to the yellow fever virus using real-time RT PCR. The study was conducted from October, 2021 to

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February, 2022 and during the time of the yellow fever outbreak (Bonney *et al*., 2022). Yellow fever has a short period of viremic phase for the detection of the yellow fever virus by polymerase chain reaction (Fischer *et al*., 2017). Thus, the use of real-time PCR is difficult to detect the virus after the viremic phase. Selection of the non-outbreak zones (Tamale and Kumbungu) is based on the fact that these districts are in close proximity to the outbreak foci. The Northern region of Ghana, especially Tamale has been known to recorded yellow fever cases in past yellow fever outbreaks in the country (Agadzi *et al* 1984). 98/498(19.7%) of the febrile patients were found to have the *Plasmodium falciparum* parasite using microscopy while 92/498 (29.7%) of them were found were found to have the *Plasmodium falciparum* parasite by conventional nested polymerase chain reaction.

Malaria has been known to be the most prevaledisease in Ghana. (Chrispal *et al*., 2010; Sunden *et al*., 2017). Malaria is endemic in Ghana and it is responsible for about 40% of outpatient attendances and 23% of inpatient admissions in the country (Malm, Sackey, & Wurapa, 2014). Malaria was diagnosed across all age groups; however, the highest proportions of infections (12.2%) were observed among the older age group (above 15 years) during the rainy season with the use of microscopy. Also, the highest proportions of malaria infections (15%) were observed among the older age group during the rainy season with the use of conventional nested PCR. This suggests a possible shift of the burden of malaria from children under 5 years to the older age groups (Yaro *et al*., 2022; Kigozi *et al*., 2020). However, during the dry season, the highest proportions of malaria infections (9.1%) were observed among children under 5 years with the use of microscopy. This is similar to a study conducted in South-west Burkina Faso and Uganda (Yaro *et al*., 2022; Kigozi *et al*., 2020).

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There is the need for a more specific, sensitive and differential laboratory detection of yellow fever infections. Also, since the virus can spread from one place to another by the *Aedes aegypti* mosquito there is the need to investigate if the virus has been shared among people especially the unvaccinated population in the non-outbreak zones. It is crucial to investigate malarial infections responsible for causing fever in the patients in order to implement the appropriate preventive measures in containing the etiology of fever.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

There was no detectable yellow fever virus in febrile patients in and around the epicentre of a yellow fever outbreak in Ghana in this study. The non-outbreak zones (Tamale or Kumbungu) had the highest proportions of malaria infections irrespective of the season of sample collection (dry or rainy) or method of malaria parasites detection (microscopy or conventional nested polymerase chain reaction). The overall prevalence of malaria infections in the febrile patients was high in this study. The highest proportions of malaria infections were observed among the older age group (above 15 years) during the rainy season and children under 5 years during the dry season. Malaria control interventions should target all age groups to completely tackle reservoir infections.

6.2 RECOMMENDATIONS

Based on the results of this research study, the following recommendations are given;

1. The use of both serum and plasma samples for each participant for the detection of yellow fever virus should be considered.
2. High vaccination coverage of the population against yellow fever in yellow fever endemic countries like Ghana should be encouraged.
3. Also, a further look out for other etiologies of febrile illness (apart from yellow fever and malaria) would be contributory.

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REFERENCES

1. Addy, P. A., Minami, K., & Agadzi, V. K (1986). Recent yellow fever epidemics in Ghana (1969-1983). Eas African medical journal 63(6) 422-434.

2. Afrane Y. A., Lawson B. W. Brenya R., Kruppa T. & Yan G (2012). The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. *Parasites & Vectors*. 2012; 5:233.

3. Agadzi,, V. K., Boatin, B. A, Appawu, M. A. Mingle, J. A., & Addy. P. A. (1984).

Yellow fever in Ghana, 1977-1980. *Bulletin of the World Health Organization,*62(4), 577-583.

4. Ahmed, Q. A. & Memish, Z. A. Yellow fever from Angola and Congo: a storm gathers.

Trop Doct. 2017;47(2): 92-96.

5. Appawu, M., Dadzie S, Abdul, H., Asmah, H., Boakye, D., Wilson., & Ofori-Adjei, D. (2006). Surveillance of viral hemorrhagic fevers in Ghana: entomological assessment of the risk of transmission in the northern regions. *Ghana medical journal,* 40(4) 137-141.

6. Ardoin, P., Rodhain, F., & Hannoun, C. (1976). Epidemiologic study of arboviruses in the Arba-Minch district of Ethiopia. *Tropical and geographical medicine*, 28(4), 309315.

7.Baba M. M, Yahaya K. M., Ezra E. M., Adamu M., Kulloma B. M., Ikusemoran M.,

Momoh J. P., … Oderinde B. S. (2021). Assessment of immunity against Yellow Fever virus infections in northeastern Nigeria using three serological assays 93(8), 4856-4864.

University of Ghana http://ugspace.ug.edu.gh

8. Baeza A., Bouma M. J., Dobson A. P., Dhiman R., Srivastava H. C & Pascual M. (2011). Climate forcing and desert malaria: the effect of irrigation. *Malaria journal*. 2011; 10:190.

9. Barrett A. D. (2017). Yellow fever live attenuated vaccine: A very successful live attenuated vaccine but still we have problems controlling the disease. *Vaccine* 2007;35:5951-5.

10. Barrett A. D. (1997). Yellow fever vaccines. *Biologicals*: *journal of the International Association of Biological Standardization*, 25(1), 17-25.

11. Basile, A. J., Goodman C., Horiuchi, K., Laven, J., Panella, J., Kosoy, O., Lanciotti R. S., & Johnson, B. W. (2015). Development and validation of an ELISA kit (YF MAC-HD) to detect IgM to yellow fever virus. *Journal of virological methods*, 225, 41-48.

12. Bonney, J. H. K., Osei-Kwasi, M., Adiku, T. K., Barnor, J. S. … Gunther, S. (2013). Hospital-Based Surveillance for Viral Hemorrhagic Fevers and Hepatitides in Ghana, *7*(9), 1-10.

13.Cammack N, Gould E. A. Antigenic analysis of yellow fever virus glycoproteins: use of monoclonal antibodies in enzyme-linked immunosorbent assays. *Journal Virology Method.* 1986(13), 135-142.

14. CDC information on arboviral encephalitides. Retrieved from wwwn.cdc.gov/nndss/conditions/arboviral-encephalitis.

15. Chen, L. H., & Wilson, M. E. (2020). Yellow fever control: current epidemiological and vaccination strategies. *Tropical diseases, travel medicine and vaccines* 6, 1.

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16. De Rezende I. M., Alves P. A., Arruda M. S., Goncalves A. P., Oliveira G. F. G., Pereira L. S., … Drumond B. P. (2019). Yellow fever Virus Genotyping Tool and Investigation of Suspected Adverse Events Following Yellow fever Vaccination, 7 (206), 1-10.

University of Ghana http://ugspace.ug.edu.gh

17. Domingo, C., Charrel R. N., Schmidt-Chanasit J. & Reusken, C. (2018). Yellow fever in the diagnostics laboratory. *Emerging Microbes & Infections*, 7(1), 129.

18. Fabiyi, A. (2017). Yellow Fever at Tema, Ghana, 1959 : A Serological Survey by Complement Fixation, *Annals of tropical medicine and parasitology,* 55, 235-241

19. Fernandes-Monteiro, A. G., Trindade, G. F., Yamamura, A. M. Y., Moreira, O. C., de Paula, V. S., Duarte, A. C., Britto, C., & Lima, S. M. (2015). New approaches for the standardization and validation of a real-time qPCR assay using TaqMan probes for quantification of yellow fever virus on clinical samples with high quality parameters.
Human Vaccines and Immunotherapeutics, *11*(7), 1865–1871.

20. Fischer, C., Torres, M. C., Patel, P., Moreira-soto, A., Gould, E. A., … Drexler, J. F. (2017). Lineage-Specific Real-Time Reverse Transcription PCR for Yellow Fever Virus Outbreak Surveillance, Brazil, *Emerging infectious diseases, 23*(11), 1867– 1871.

21. Gardner, C. L., & Ryman, K. D. (2010). Yellow fever: a reemerging threat. *Clinics in laboratory medicine*, 30(1), 237-260.

22. Garske, T., Kerkhove, M. D. Van, Yactayo, S., Ronveaux, O., Lewis, R. F., Staples, J. E., Perea, W., Ferguson, N. M., & Yellow fever Expert Committee (2014). Yellow
Fever in Africa: Estimating the Burden of Disease and Impact of Mass Vaccination from Outbreak and Serological Data. *PLoS medicine,* 111(5), e1001638.

23. GHS: The Health Sector in Ghana Facts And Figures in 2015. Ghana Health Service 2015.

24. Gotuzzo E., Yactayo S. & Cordova E. (2013). Efficacy and Duration of Immunity after Yellow fever Vaccination: Systematic Review on the Need for a Booster Every 10 Years, 89(3), 434-444.

University of Ghana http://ugspace.ug.edu.gh

27. Gould E. A., & Solomon, T. (2008). Pathogenic flaviviruses. *Lancet (London, Enland)*, 371(9611), 500-509.

28. Henriette, M., Ndione, D., Gaye, A., & Barry, M. A. ... Faye O. (2021). Yellow Fever Outbreak in Eastern Senegal, 13(1475), 2-12.

29. Johansson, M. A., Vasconcelos, P. F., & Staples, J. E. (2014). The whole iceberg: estimating the incidence of yellow fever virus infection from the number of severe cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 108(8), 482-487.

30. Johnson, B. W., Demanou, M., Fall, G., Betoulle, J., Obiekea, C., Basile, A. J., … Kayiwa, J. (2021). Laboratory capacity assessments in 25 African countries at high risk of yellow fever, August- December 2018, 38(402) 1-18.

31. Kleinert, R. D. V, Montoya-diaz, E., Khera, T., Welsch, K., Tegtmeyer, B., Hoehl, S.,

Ciesek, S., & Brown, R. (2019.). Yellow Fever: Integrating Current Knowledge with Technological Innovations to Identify Strategies for Controlling a Re-Emerging Virus.
Viruses, 11(10), 960.

32. Kraemer, M. U. G., Faria, N. R., Jr, R. C. R., Golding, N., Nikolay, B., Stasse, S., … Cauchemez, S. (2017). Spread of yellow fever virus outbreak in Angola and the
Democratic Republic of the Congo 2015 – 16 : a modelling study. *The Lancet Infectious Diseases*, *17*(3), 330–338.

33. Kwagonza L., Masiira B, Kyobe-Bosa H., Kadobera D., Auheire E. B., Lubwama B … Bao-Ping Zhu. (2018). Outbreak of yellow fever in central and southwestern Uganda, February-May 2016, 18 (548), 1-9.

University of Ghana http://ugspace.ug.edu.gh

34. Kwallah, A.o, Inoue, S., Thairu-Muigai, A.W., Kuttoh, Morita, K., & Mwau, M. (2015). Seroprevalence of yellow fever virus in selected health facilities in Western Kenya from 2010 to 2012. *Japanese journal of infectious diseases,* 68(3), 230-234.

35. Looareesuwan S., Malaria. Looareesuwan S, Wilairatana P., editors. Clinical Tropical Medicine. 1st ed. Bangkok, Thailand: Medical Media; 1999. Pp. 5-10.

36. Mackay, I. M., Arden, K. E., & Nitsche, A. (2002). Real-time PCR in virology. *Nucleic acids research*, 30(6), 1292-1305.

37. Manu, S. K., Bonney, J. H. K., K., Pratt, D., Abdulai, F. N., Agbosu, E. E., … Adiku, T. K. (2019). Arbovirus circulation among febrile patients at the greater Accra Regional Hospital, Ghana. *BMC Research Notes*, 12(332) 1–5.

38. Mascheretti M. Tengan C. H., Suzuki A., Maeda M., Brasil R., Pereira M. ... Ribeiro A. F. (2013). Yellow fever: reemerging in the state of Sao Paulo, Brazil, 2009. 47 (5), 1-9.

39. Maze M. J., Bassat Q., Feasy N.A., Mandomando I., Musicha O., Crump J. A. (2018). The epidemiology of febrile illness in sub-Saharan Africa: implications for diagnosis and management. *Clin Microbiol infect*. 2018;24(8):808-814.

40. Mokaya, J., Kimathi, D., Lambe, T., & Warimwe, G. M. (2021). What Constitutes Protective Immunity Following Yellow Fever Vaccination? *Vaccines,* 9(6), 671.

41. Monath, T. P., & Vasconcelos, P. F. (2005). Yellow fever. *Journal of clinical viroloy: the official publication of the Pan American Society for Clinical Virology,* 64, 160173.

42. PAHO/WHO. *Laboratory Diagnosis of Yellow Fever Virus Infection* (PAHO/WHO, 2018).

University of Ghana http://ugspace.ug.edu.gh

43. Pulendran B. (2009). Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. *Nature reviews. Immonulogy*, 9(10), 741-747.

44. Sory E. (2009). The health sector in Ghana. Facts and figures. Accra (Ghana): Ghana Health Service; 2009. P. 31.

45. Staples, J. E., & Monath T. P. (2008). Yellow fever: 100 years of discovery. *JAMA,* 300(8), 960-962.

46.Staples, J. E., Barrett, A, Wilder-Smith A., & Hombach (2020). Review of data and knowledge gaps regarding yellow fever vacine-induced immunity and duration of protection. NP*J vaccines*, 5(1), 54.

47.Sunden-Cullberg J., Rylance R. S., Norrby-Teglund A., Bjork J. Inghammar M. (2017). Fever in the emergency department predicts survival of patients with severe sepsis and septic shock admitted to the ICU. *Crit Care Med*. 2017;45(4):591-599.

48.Suzuki, T., Osei, J. H., Sasaki, A., Adimazoya, M., Appawu, M., Ohta, N., & Dadzie, S. (2016). Risk of transmission of viral haemorrhagic fevers and the insecticide susceptibility status of *Aedes aegypti* (linnaeus) in some sites in Accra, Ghana, *Ghana medical journal 50*(3), 136–14.

49. Staples, J. E., & Monath T. P. (2008). Yellow fever: 100 years of discovery. *JAMA,* 300(8), 960-962.

50. Staples, J. E., Barrett, A, Wilder-Smith A., & Hombach (2020). Review of data and knowledge gaps regarding yellow fever vacine-induced immunity and duration of protection. NP*J vaccines*, 5(1), 54.

University of Ghana http://ugspace.ug.edu.gh

51. Sunden-Cullberg J., Rylance R. S., Norrby-Teglund A., Bjork J. Inghammar M. (2017). Fever in the emergency department predicts survival of patients with severe sepsis and septic shock admitted to the ICU. *Crit Care Med*. 2017;45(4):591-599.

52. Suzuki, T., Osei, J. H., Sasaki, A., Adimazoya, M., Appawu, M., Ohta, N., & Dadzie, S. (2016). Risk of transmission of viral haemorrhagic fevers and the insecticide susceptibility status of *Aedes aegypti* (linnaeus) in some sites in Accra, Ghana, *Ghana medical journal 50*(3), 136–141.

53. Tomori O. (2004). Yellow fever: the recurrin plague. *Critical reviews in clinical laboratory sciences,* 41(4), 391-427

54. Tsai, T. F., Bolin, R. A., Montaya, M., Bailey R. E., Francy, D. B., Jozan, M. & Roehrig , J. T., 1987. Detection of St. Louis encephalitis virus antigen in mosquitoes by enzyme immunoassay. *Journal of clinical microbiology* 25, 370-376.

55. Tsegaye, M. M., Beyene, B., Ayele, W., Abebe, A., Tareke, I., Sall, A., … Tayachew, A. (2018). Sero-prevalence of yellow fever and related Flavi viruses in Ethiopia: a public health perspective, *BMC Public Health* 18(1011), 1–10.

56. Tuboi S. H., Costa Z. G., da Costa Vasconcelos P. F. Hatch D. (2007). Clinical nd epidemiological characteristics of yellow fever in Brazil: analysis of reported cases 1998-2002. *Transactions of the Royal Society of Tropical Medicine and hygiene.*101(2) 169-175.

57. 57. Verma, R., Khanna, P., & Chawla, S. (2014). Yellow fever vaccine: An effective vaccine for travelers. *Human Vaccines and Immunotherapeutics*, *10*(1), 126–128.

58. Waggoner, J. J., Rojas, A., & Pinsky, B. A. (2018). Yellow fever virus: Diagnostics for a persistent arboviral threat. *Journal of Clinical Microbiology*, *56*(10), 1–13.

University of Ghana http://ugspace.ug.edu.gh

59. Wamala J. F., Malimbo M. Okot C. L., Atai-Omoruto A. D., Tenywa J. R. M. Balinandi S. ...Mbonye A. K. (2012). Epidemiological and laboratory characterization of a yellow fever outbreak in northern Uganda, October 2010-January 2011, e536-e542.

60. Wichmann O, Muhlberger N & Jelinek T. (2003). Dengue-the underestimated risk in travelers. *Dengue Bull*. 2003; 27:126-137.

61. World Health Organization (2016). World Malaria Report. Geneva: World Health Organization. 2016. Retrieved from http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1.

62. World Health Organization (2020). Retrieved from http://www.who.int/csr/disease/yellowfev/case-definition/en/.

63. World Health Organization. Situation report. Yellow fever. 28 October 2016. World Health Organization; Geneva: 2016. Retrieved from http://apps.who.int/iris/bistream/10665/250661/1/yellowfeversitrep28Octeng.pdf?u=1

64. Vo, T. K., Bigot, P., Gazin, P., Sinou, V., De Pina, J. J., Huynh, D. C., Fumoux, F., & Parzy, D. (2007). Evaluation of a real-time PCR assay for malaria diagnosis in patients from Vietnam and in returned travelers. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101(5), 422-428.

65. https://www.who.int/emergencies/disease-outbreak-news/item/yellow-fever---ghana.

66. https://www.cdc.gov/yellowfever/vaccine/index.html.

67. Drosten, C., Göttig, S., Schilling, S., Asper, M., Panning, M., Schmitz, H., & Günther, S. (2002). Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus,

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Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *Journal of clinical microbiology*, *40*(7), 2323–2330. https://doi.org/10.1128/JCM.40.7.2323-2330.2002

68. Catherine I. Paules and Anthony S. Fauci, 2017 Yellow Fever-One Again on the Radar Screen in the Americas.

69. Lindsey, N. P., Horton, J., Barrett, A. D. T., Demanou, M., Monath, T. P., Tomori, O., Van Herp, M., Zeller, H., Fall, I. S., Cibrelus, L., & Erin Staples, J. (2022). Yellow fever resurgence: An avoidable crisis?. *NPJ vaccines*, *7*(1), 137. https://doi.org/10.1038/s41541-022-00552-3

70. Gardner, C. L., & Ryman, K. D. (2010). Yellow fever: a reemerging threat. *Clinics in laboratory medicine*, *30*(1), 237–260. https://doi.org/10.1016/j.cll.2010.01.001

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APPENDIXES

APPENDIX 1-Information Sheet

Informed Consent Form

Title of Research Study: Assessment of immunity against yellow fever virus infections among people following a yellow fever outbreak in Ghana

Principal Investigator: Lidiwan Mensah

Address: Department of Medical Microbiology, University of Ghana, Legon

Telephone: 0545165145 **Email:** lidiwan19.mensah@gmail.com

Background and Purpose of Study

The aim of this study is to check whether people in and around the epicentre of a yellow fever outbreak in Ghana have adequate protection against yellow fever virus infection. There is the need for a more specific, sensitive and differential laboratory diagnosis of yellow fever infections to make appropriate interventions and recommendations on yellow fever prevention and control in Ghana. Study will be conducted from May to July, 2022.

About 2 tablespoons of whole blood will be collected from all study participants and used to assess immunity against yellow fever.

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Possible Risks and Discomforts

During the collection of blood sample, you may experience a bit of pain. Blood samples will be taken by laboratory technicians with the expertise to minimize any risk or discomforts.

Possible Benefits

There are no immediate benefits to you for participating in this study. No money or gift will be offered in exchange but the data generated. This will help decision makers in the Ministry of Health to improve upon yellow fever surveillance and introduce effective yellow fever diagnostic capacity.

Confidentiality

Information about the study population will be protected from the populace reach to the best of our ability. Their names will not be presented in any reports. The samples taken from you will be coded and cannot be accessed by anyone who is not a member of the research team.

Voluntary participation

Your participation in this research study is completely voluntary and you have the right to withdraw from the study at any time you want. Withdrawal from the study will not affect any benefit (like treatment) you are having

Questions, Concerns or Complains

INTEGRI PROCEDAMUS

If you have any issues relating to this study, please direct them to Prof. Yaw A. Afrane

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(0542286113), Rev. Prof. Kwamena Sagoe (0277408528) of the Department of Medical Microbiology, University of Ghana Medical School, University Ghana, Legon and Dr. Joseph Humphrey Kofi Bonney (0243568041) of the Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.

Questions about your rights as a participant and ethical issues can be directed to the EPRC

Administrator. Mr. Daniel Nana Yaw Abankwah (+233 [030] 2665103/4, +233 [030] 2940528; eprc@chs.edu.gh).

Statement of Consent

I acknowledge that I have read or have had the purpose and contents of the study and all questions have been explained to me to my satisfaction. I consent voluntarily to partake in this study and I have the rights to withdraw from the study at any point in time.

I understand that a copy of the information sheet and the informed consent forms will be offered to me to take home after it has been signed.

I …………………………………….. have read the consent form and agree voluntarily to partake in this study.

_______________________   _______________________

Signature/Thumb print of Participant   Date

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_________________________________ _____________________________

Signature of Researcher Obtaining Consent Date

Statement of Witness

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research voluntarily.

_________________________________ _____________________________

Signature/Thumb print of Witness Date

_________________________________ _____________________________

Signature of Researcher Obtaining Consent Date

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Parental Consent Form

Title of Research Study: Assessment of immunity against yellow fever virus infections among people following a yellow fever outbreak in Ghana

Principal Investigator: Lidiwan Mensah

Address: Department of Medical Microbiology, University of Ghana, Legon

Telephone: 0545165145 **Email:** lidiwan19.mensah@gmail.com

Background and Purpose of Study

The aim of this study is to check whether people in and around the epicentre of a yellow fever outbreak in Ghana have adequate protection against yellow fever virus infection. There is the need for a more specific, sensitive and differential laboratory diagnosis of yellow fever infections to make appropriate interventions and recommendations on yellow fever prevention and control in Ghana. Study will be conducted from May to July, 2022.

Possible Risks and Discomforts

During the collection of blood sample, you may experience a bit of pain. Blood samples will be taken by laboratory technicians with the expertise to minimize any risk or discomforts.

INTEGRI PROCEDAMUS

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Possible Benefits

There are no immediate benefits to you for participating in this study. No money or gift will be offered in exchange but the data generated. This will help decision makers in the Ministry of Health to improve upon yellow fever surveillance and introduce effective yellow fever diagnostic capacity.

Confidentiality

Information about the study population will be protected from the populace reach to the best of our ability. Their names will not be presented in any reports. The samples taken from you will be coded and cannot be accessed by anyone who is not a member of the research team.

Voluntary participation

Your child's participation in this research study is completely voluntary and your child has the right to withdraw from the study at any time.

Questions, Concerns or Complains

If you have any issues relating to this study, please direct them to Prof. Yaw A. Afrane

(0542286113), Rev. Prof. Kwamena Sagoe (0277408528) of the Department of Medical Microbiology, University of Ghana Medical School, University Ghana, Legon and Dr. Joseph Humphrey Kofi Bonney (0243568041) of the Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.

Questions about your rights as a participant and ethical issues can be directed to the EPRC

University of Ghana http://ugspace.ug.edu.gh

Administrator. Mr. Daniel Nana Yaw Abankwah (+233 [030] 2665103/4, +233 [030] 2940528; eprc@chs.edu.gh).

Statement of Consent

I acknowledge that I have read or have had the purpose and contents of the study and all questions have been explained to me to my satisfaction.

I …………………………………. have read the parental consent form and agree for my child to partake voluntarily in this study.

_______________________ _______________________

Signature/Thumb print of Parent Date

_______________________ _______________________

Signature of Researcher Obtaining Consent Date

Statement of Witness

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research voluntarily.

_______________________ _______________________

Signature/Thumb print of Witness Date

University of Ghana http://ugspace.ug.edu.gh

_______________________________ _______________________

Signature of Researcher Obtaining Consent Date

University of Ghana http://ugspace.ug.edu.gh

Child Assent Form

Title of Research Study: Assessment of immunity against yellow fever virus infections among people following a yellow fever outbreak in Ghana

Principal Investigator: Lidiwan Mensah

Address: Department of Medical Microbiology, University of Ghana, Legon

Telephone: 0545165145 **Email:** lidiwan19.mensah@gmail.com

Background and Purpose of Study

The aim of this study is to check whether people in and around the epicentre of a yellow fever outbreak in Ghana have adequate protection against yellow fever virus infection

About 2 tablespoons of whole blood will be collected from all study participants and used to assess immunity against yellow fever.

Possible Risks and Discomforts

During the collection of blood sample, you may experience a bit of pain. Blood samples will be taken by laboratory technicians with the expertise to minimize any risk or discomforts.

Possible Benefits

There are no immediate benefits to you for participating in this study. No money or gift will be offered in exchange but the data generated. This will help decision makers in the Ministry of Health to improve upon yellow fever surveillance and introduce effective yellow fever diagnostic capacity.

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Confidentiality

Information about the study population will be protected from the populace reach to the best of our ability. Their names will not be presented in any reports. The samples taken from you will be coded and cannot be accessed by anyone who is not a member of the research team.

Voluntary participation

Your participation in this research study is completely voluntary and you have the right to withdraw from the study at any time you want.

Questions, Concerns or Complains

If you have any issues relating to this study, please direct them to Prof. Yaw A. Afrane

(0542286113), Rev. Prof. Kwamena Sagoe (0277408528) of the Department of Medical Microbiology, University of Ghana Medical School, University Ghana, Legon and Dr. Joseph Humphrey Kofi Bonney (0243568041) of the Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.

Questions about your rights as a participant and ethical issues can be directed to the EPRC

Administrator. Mr. Daniel Nana Yaw Abankwah (+233 [030] 2665103/4, +233 [030] 2940528; eprc@chs.edu.gh).

INTEGRI PROCEDAMUS

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Statement of Consent

I acknowledge that I have read or have had the purpose and contents of the study and all questions have been explained to me to my satisfaction. I consent voluntarily to partake in this study and I have the rights to withdraw from the study at any point in time.

I understand that a copy of the information sheet and the informed consent forms will be offered to me to take home after it has been signed.

I **…………………………………..** have read the consent form and agree voluntarily to partake in this study.

________________________ ________________________

Signature/Thumb print of Child Date

______________________________ __________________________

Signature of Researcher Obtaining Consent Date

Statement of Witness

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research voluntarily.

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______________________________ ______________________

Signature/Thumb print of Witness Date

______________________________ ______________________

Signature of Researcher Obtaining Consent Date

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APPENDIX 2 – All Data Collection Instruments

Assessment of Immunity Against Yellow Fever Virus Infections Among People Following A Yellow Fever Outbreak In Ghana

1. Study ID Number: …………………………….   Date ……………………
2. Hospital ID number: …………………………..
3. Admitting department: Out patients[   ]   In patients[   ]
4. Admission date: ……………………………….
5. Current temperature: …………………………..
6. Sex: Male[   ]   Female[   ]
7. Age: ………………………………………….
8. Occupation :……………………………….
9. Residence: ……………………………………..
10. Telephone number: …………..........................

11. Current Medical history:

a) Hypertension [   ]   b) Diabetes [   ]   c) Asthma [   ]

d) Malaria [   ]   e) Sickle cell disease [   ]   f) Other ………………..

12. Symptoms (main) that the patient presents with ……………………………………….

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13. Do you have any immunodeficient condition (like HIV/AIDS, viral hepatitis, cancer)?

Yes [ ] No [ ]

14. If yes, name of condition ……………………………

15. Have you been vaccinated for yellow fever? Yes [ ] No [ ]

16. If yes, date of vaccination ……………………………….

18. Do you have a proof of vaccination (yellow vaccination card)? Yes [ ] No [ ]

19. Have you travelled recently? Yes [ ] No [ ]

20. If yes, recent travel history …………………………….

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APPENDIX 3 – Principal/Co-Investigators and Collaborators

Curriculum Vitae

First Collaborator: Prof. Yaw A. Afrane

Department of Medical Microbiology

University of Ghana Medical School

College of Health Sciences

University of Ghana

Personal Profile

Email: yafrane@ug.edu.gh**;** yawafrane@gmail.com;

Tel: +233 542 286113

Nationality: Ghanaian

Education and Qualifications

PhD – June 2006. Kwame Nkrumah University of Science and Technology Ghana.

MPhil – June 2003. Kwame Nkrumah University of Science & Technology, Ghana

BSc. – June 2000 Kwame Nkrumah University of Science & Technology, Ghana

Career Summary

March 2015 – present

Associate Professor

Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana.

August 2013 – February 2015

Associate Professor

School of Health Sciences, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya.

April 2012 – March 2014

Dean

School of Health Sciences, Jaramogi Oginga Odinga University of Science and Technology, Kenya

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July 2010 – July 2013

Senior Lecturer

School of Health Sciences, Jaramogi Oginga Odinga University of Science and Technology, Kenya.

September 2007 – July 2010: Lecturer
School of Public Health, Maseno University, Maseno, Kenya

GRADUATE STUDENT THESIS SUPERVISOR

Name	Year enrolled	Degree	Thesis Title	Role	Status
Majidah Ahmed	2016	PhD	Intra-Species Indoor And Outdoor-Resting Behaviour in Malaria Vectors	Main Supervisor	Completed
Isaiah Debrah	2018	PhD	Investigating Insecticide Resistance Status, Mitogenome Diversity and Behavioral Changes of *An. funestus* in Different Ecological Landscapes of Western Kenya And Ghana	Main Supervisor	Ongoing
Christopher OwusuAsenso	2020	PhD	Effect of microbial diversity of larval habitats on mosquito productivity	Main Supervisor	Ongoing
Isaac Amankona Hinne	2018	MPhil	Ecology of *Anopheles* Mosquito Larvae in Different Ecological Zones in Ghana	Co-Supervisor	Completed

Selected Publications in Peer Reviewed Journals (From over 70 publications)

2021

1. Crava CM, Varghese FS, Pischedda E, Halbach R, Palatini U, Marconcini M, Gasmi L, Redmond S, **Afrane Y**, Ayala D, Paupy C, Carballar-Lejarazu R, Miesen P, van Rij RP, Bonizzoni M. Population genomics in the arboviral vector Aedes aegypti reveals the genomic architecture and evolution of endogenous viral elements. *Mol Ecol*. 2021;30(7):1594-1611. doi:10.1111/mec.15798

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2020

2. Hamid-Adiamoh M, Amambua-Ngwa A, Nwakanma D, D'Alessandro U, Awandare GA, Afrane YA. Insecticide resistance in indoor and outdoor-resting Anopheles gambiae in Northern Ghana. Malar J. 2020 Aug 31;19(1):314. doi: 10.1186/s12936-020-03388-1. PMID: 32867769; PMCID: PMC7460795.

2019

3. Machani MG, Ochomo E, Sang D, Bonizzoni M, Zhou G, Githeko AK, Yan G, Afrane YA. Influence of blood meal and age of mosquitoes on susceptibility to pyrethroids in Anopheles gambiae from Western Kenya. Malar J. 2019 Apr 2;18(1):112. doi: 10.1186/s12936-019-2746-6.

2018

4. Jeffries CL, Lawrence GG, Golovko G, Kristan M, Orsborne J, Tantely LM, Raharimalala FN, Keita K, Camara D, Barry Y, Manzambi EZ, Afrane YA, Hughes GL, Walker T et al.. Novel Wolbachia strains in Anopheles malaria vectors from Sub-Saharan Africa. Wellcome Open Res. 2018 Nov 27 [revised 2018 Jan 1];3:113. doi: 10.12688/wellcomeopenres.14765.2. eCollection 2018. PMID:30483601

Research Grants

1. Forgaty International Centre / National Institute of Health (NIH) D43TW011513
2021-2026
"Regional Centre for Vector Borne Diseases in West Africa (RCVBD)" Role: Program Director
2. National Institute of Health (NIH) R01 AI123074 2016 – 2021
"Impact of insecticide resistance on the behavior and fitness of malaria vectors"
Role: Principal Investigator
3. WHO/TDR (2015)
"Predicting and validating the consequences of insecticide resistance on malaria transmission"
Role: Principal Investigator
4. National Institute of Health (NIH) R01 AI094580 2011- 2016
"Epidemiology of Clinical Malaria in the western Kenya Highlands" Role: Principal Investigator

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Second Collaborator: Rev. Prof. Kwamena Sagoe

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

NAME: Kwamena W Sagoe eRA COMMONS USER NAME (credential, e.g., agency login):KWSAGOE

POSITION TITLE: Associate Professor

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Ghana, Accra, Ghana	BSc	10/89	Zoology/Biochemistry
Karolinska Institute, Stockholm, Sweden	MSc	06/99	Virology
University of Ghana, Accra, Ghana	PhD	02/10	Microbiology/Virology

EDUCATION/TRAINING

B. Positions and Honors

10th Jun 2016 to date — Associate Professor, Department of Medical Microbiology, School of Biomedical a Allied Health Sciences, Accra, Ghana.

Aug 2015 to July 2019
Associate Professor, Department of Medical Microbiology, School of Biomedical a Allied Health Sciences, Accra , Ghana.

1st Feb. 2012-31st Dec. 2013 — Senior Lecturer, Department of Physician Assistantship, Central University College, Miotso, Ghana [Two-year sabbatical leave].

31st Oct. 2007 - 9th Jun 2016 — Senior Lecturer, Department of Medical Microbiology, University of Ghana Medical School, Accra, Ghana.

1st May 2000-30th Oct. 2007 Lecturer, Department of Microbiology, University of Ghana Medical School, Accra, Ghana.

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Jan. 1997-July 1999 Postgraduate Research in HIV at Clinical Virology Division, Department of Immunology, Microbiology, Pathology and Infectious Diseases, Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden.

Mar 1991-Apr 2000

Snr. Research Assist., Virology Unit, Department of Microbiology, University of Ghana Medical School, Accra, Ghana.

National Committees

1. Member, Expert Committee on HIV Resistance Monitoring (Ghana), National AIDS Control Programme, July 2006 to date.

2. Member, National Polio Expert Committee, 2005 to date.

Membership of Associations

1. Ghana Science Association (GSA)
2. Ghana Association of Biomedical Scientists (GABS)
3. International AIDS Society (IAS)
4. American Society for Microbiology (ASM)

C. Contribution to Science 1. Below are some publications:

a. **Sagoe, K. W. C.**, Lartey, M., Agyei, A. A., Boamah, I., Mingle, J. A. A., & Arens, M. (2005). Implications for antiretroviral therapy of dual HIV-1/HIV-2 serologic profiles in Accra, Ghana, West Africa. *HIV AIDS Rev, 4(2),* 24-27

b. **Sagoe, K. W.**, Britton, S., Mingle, J. A., Affram, K., Djokoto, A., & Sonnerborg, A. (1999). Similar HIV-1 subtype A V3 loop sequences are found in dual HIV-1/HIV-2 and HIV-1-only seropositives in Ghana. *AIDS Res Hum Retroviruses, 15*(8), 775-779. doi:10.1089/088922299310863

c. Martin-Odoom A, Brown CA, Odoom JK, Bonney EY, Ntim NAA, Delgado E, Lartey M, **Sagoe KW**, Adiku T, Ampofo WK. Emergence of HIV-1 drug resistance mutations inmothers on treatment with a history of prophylaxis in Ghana. Virol J. 2018 Sep17;15(1):143. doi: 10.1186/s12985-018-1051-2. PubMed PMID: 30223845; PubMedCentral PMCID: PMC6142311.

d. **Sagoe, K. W.**, Duedu, K. O., Ziga, F., Agyei, A. A., Adiku, T. K., Lartey, M., Mingle, J. A. & Arens, M. (2016). Short-term treatment outcomes in human immunodeficiency virus type-1 and hepatitis B virus co-infections. *Ann Clin Microbiol Antimicrob, 15*(1), 38. doi:10.1186/s12941-016-0152-2

e. **Sagoe, K. W.**, Dwidar, M., Lartey, M., Boamah, I., Agyei, A. A., Hayford, A. A., Mingle, J. A. A. & Arens, M. Q. (2007). Variability of the human immunodeficiency virus type 1 polymerase gene from treatment naive patients in Accra, Ghana. *J Clin Virol, 40*(2), 163-167. doi:10.1016/j.jcv.2007.07.016

The complete list of my published work in PubMed is

University of Ghana http://ugspace.ug.edu.gh

https://www.ncbi.nlm.nih.gov/sites/myncbi/1V_8gjzPOlK5K/bibliography/48330049/public/? sort=date&d irection=ascending

D. **Research Support**

Projects

1. ***Funding Agency/Institution*:** Ghana AIDS Commission

***Total Amount*:** $40,000.00

***Role*:** Consultant Virologist

***Period*:** May to November 2013

2. ***Funding Agency/Institution*:** NIH - NINDS/NHGRI

(Grant Award Number: 1U54HG007479-01)

***Total Amount*:** $600,000

***Role*:** Co - Investigator (Biostatistics and Bioinformatics Core) ***Period*:**

20th September, 2013 - 31st July, 2017 (4years).

***Primary Grantee*:** University of Ibadan

Title: Stroke Investigative Research & Educational Network (SIREN)

3. ***Funding Agency/Institution*:** World Health Organization

***Total Amount*:** $5,000.00 to $6,000.00 annually

***Role*:** Co-Investigator

***Period*:** From 2006 to date

Title: Surveillance for rotavirus gastroenteritis in children <5 years hospitalised with acute diarrhoea

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Third Collaborator: Dr. Joseph Humphrey Kofi Bonney

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Bonney, Joseph Humphrey Kofi	Senior Research Fellow

eRACOMMONS USER NAME: JHKBONNEY

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Ghana, Legon	BSc.	1997	Botany and Biochemistry
University of East London, London, UK	MSc.	2002	Molecular medical Microbiology
University of Ghana Medical School, Legon	PhD	2013	Microbiology

A. Personal Statement

Skilled professional, who welcomes the opportunity to discuss and/or share results and observations with work mates and colleagues, very well abreast with good laboratory practice gained from school internships, work placements and years of working in a reputable bio-medical research institute, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra Ghana. I believe I have a lot to contribute to this project in terms of laboratory investigations and diagnosis of the microbial agents of infection we seek to evaluate. Started my formal education in Ghana and went through basic to obtaining my first degree in Botany and Biochemistry from the University of Ghana. I had the opportunity to complete my master's in medical microbiology at the University of East London, UK and started a PhD in Bacterial Genomics and Pathogenesis at the Leicester University in the UK. Due to financial limitations, I could not complete my doctorate at Leicester University, but had the opportunity to do a 'sandwich' doctorate between the University of Ghana Medical School and the Bernhard Nocht Institute for Tropical Medicine in Hamburg, on Viral Hemorrhagic fevers in Ghana. I won an award for a 6-month post-doctoral fellowship at the Diseases Dynamics Unit of the Department of Veterinary Medicine, University of Cambridge, UK to work on "the use of

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advanced genomic analysis in identifying viral pathogens". Currently a Senior Research Fellow at the NMIMR and have a research interest in Molecular and Serological investigation of viral agents of emerging and dangerous pathogens: Viral Hemorrhagic Fevers such as Ebola, Lassa Fever, Crimean-Congo, Rift Valley, Yellow Fever, Dengue Fever and Laboratory investigations and Surveillance of viral agents of infectious diseases such as viral hepatitis and respiratory tract infections of viral origin. My role in this funding opportunity, 'Does the intensification of pig farming in West Africa increase the likelihood of the next pandemic', is to offer my experience and expertise to bear as a co-investigator.

B. **Positions and Honors**

Positions and Employment

2016- Senior Research Fellow, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana

2013-2016

Research Fellow, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana

2007-2013

Chief Research Assistant, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon.

2003

Student Research Assistant University of East London, School Health Sciences, Stratford, London, UK

2005-2009

Senior Research Assistant, Noguchi Memorial Institute for Medical Research (NMIMR)

University of Ghana, Legon, Ghana

<u>Honors</u>

2018

An award to participate in the CDC China maiden training program on **ModPad** by China CDC from 29th October – 8th November 2018 in Beijing, Wuhan and Shenzhen, China.

2017

A 6-month Post-Doctoral Fellowship won under the Cambridge Africa Partnership for Research Excellence (CAPREx) Cambridge University, UK, School of Veterinary Medicine. Won in June 2017 and was undertaken from January to June 2018.

2016-2018 University of Ghana Research Grant for Multi-disciplinary Grant Category for the project,

"Arboviral infections of Public Health importance in Ghana"

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2016-2017

The Royal Society International Exchanges Award.

C. Contributions to science

1. My area of interest has been to use molecular and serological tools in investigating viral agents of emerging and dangerous pathogens including the viral hemorrhagic fevers such as Ebola, Lassa Fever, Crimean-Congo, Rift Valley and Arboviruses including Yellow Fever, Dengue Fever, Chikungunya, West Nile and Zika.

a. Ngoi JM, Quashie PK, Morang'a CM, **Bonney JH**, Amuzu DS, Kumordjie S, Asante IA, Bonney EY, et. al. Genomic analysis of SARS-CoV-2 reveals local viral evolution in Ghana. Exp Biol Med (Maywood). 2020 Dec 16;1535370220975351. doi: 10.1177/1535370220975351. [Epub ahead of print] PubMed PMID: 33325750; PubMed Central PMCID: PMC7746953.

b. **Bonney JHK,** Hayashi T, Dadzie S, Agbosu E, Pratt D, Nyarko S, et al. Molecular detection of dengue virus in patients suspected of Ebola virus disease in Ghana. PLOS ONE (To be published in December 19, 2018).

c. Gabriel M, Adomeh D, Ehimuan J, Ovakhilome J, Omomoh M, IghodaloY, Olokor T, **Bonney K,** et al. Development and evaluation of antibody-capture immunoassays for detection of Lassa virus nucleoprotein-specific immunoglobulin M and G. PLOS Neglected Trop. Diseases. March 2018

d. **Bonney JH**, Nyarko EO, Ohene S, Amankwa J, Ametepi RK, et al. Molecular confirmation of Lassa fever imported into Ghana. African Journal of Laboratory Medicine. 2016 April 25; 5(No 1):6 pages.

e. **Bonney JH,** Osei-Kwasi M, Adiku TK, Barnor JS, Amesiya R, Kubio C, Ahadzie L, Olschläger S, Lelke M, Becker-Ziaja B, Pahlmann M, Günther S. Hospital-based surveillance for viral hemorrhagic fevers and hepatitides in Ghana. PLoS Negl Trop Dis. 2013 Sep 19;7(9):e2435. doi: 10.1371/journal.pntd.0002435. eCollection 2013. PubMed PMID: 24069490.

2. My other research interest is in the laboratory investigations and surveillance of viral agents of infectious diseases such as viral hepatitis and respiratory tract infections.

a. Attiku K, **Bonney J**, Agbosu E, Bonney E, Puplampu P, Ganu V, et al. (2021) Circulation of hepatitis delta virus and occult hepatitis B virus infection amongst HIV/HBV co-infected patients in Korle-Bu, Ghana. PLoS ONE 16(1): e0244507. https://doi.org/10.1371/journal.pone.0244507

b. Owusu M, **Bonney JHK**, Annan AA, et al. Aetiology of viral hepatitis among jaundiced patients presenting to a tertiary hospital in Ghana. PLoS ONE, September 2018; 13(9): e0203699.

c. E, Ruf MT, Aboagye S, Kpeli G, Akuoku V, Pereko J, Paintsil A, Bonney K, Ampofo W, Pluschke G, Yeboah-Manu D. Challenges Associated with Management of Buruli Ulcer/Human Immunodeficiency Virus Coinfection in a Treatment Center in Ghana: A Case Series Study. Am J Trop Med Hyg. 2015 Aug;93(2):216-23. doi: 10.4269/ajtmh.14-0571. Epub 2015 Jun 8. PubMed PMID: 26055745; PubMed Central PMCID: PMC4530737.

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d. **Bonney JHK**, Kronmann KC, Lindan CP, Asante IA, Parbie P, Aboagye J, Amankwah J, Odoom JK, Adjabeng M, Nzussouo NT, Ahadzie L, Barthel RV, Cornelius C, Amofah G, Oyofo B, Ampofo WK. Virological surveillance of influenza-like illness among children in Ghana, 2008-2010. J Infect Dis. 2012 Dec 15;206 Suppl 1:S108-13. doi: 10.1093/infdis/jis577. PubMed PMID: 23169955.

D. Research Support

1. **Title:** Preventing emergence and spillover of bat viruses in high-risk global hotspots
Role: Co-investigator

Funding: Montana University, USA and US DoD

Period: 2019 – 2021

2. **Title**: Arboviral Viral Infections of Public Health Importance
Role: Principal Investigator

Funding: Royal Society UK and University of Ghana Research grant

Period: 2016 – 2018

3. **Title**: Molecular Epidemiology of Dengue Fever and Chikungunya Viruses in Ghana

Role: Principal Investigator

Funding: AMED, Japan

Period: 2015 – 2020

4. **Title**: Preparedness against Ebola and other emerging infectious diseases in Sierra Leone and Guinea
Role: Coordinator on Work Package 2

Funding: The Netherlands Government

Period: 2015 – 2017

5. **Title**: Assessing the effectiveness of the revised International Health Regulations (2005) at mitigating the emergence and spread of newly emerging infectious diseases in African settings **Role**: Co-Principal Investigator

Funding: University of Cambridge, UK

Period: 2014 – 2016

6. **Title**: Hospital-based surveillance for viral hemorrhagic fevers and hepatitides in Ghana. **Role**: Principal Investigator

Funding: VW foundation

Period: 2010 – 2013

INTEGRI PROCEDAMUS

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Principal Investigator Curriculum vitae

Name: Lidiwan Mensah

Nationality: Ghanaian

Telephone: (+233)545165145

Address: lidiwan19.mensah@gmail.com

Education & Certificates

2014-2018: BSc. Biomedical Sciences; University of Cape Coast, Second Class Honours (Upper Division).

2009-2013: West African Secondary School Certificate Examination; General Science, Prempeh College.

Working Experience

2019: Volunteer worker, Bomso Specialist Hospital Laboratory, Bomso, Kumasi

2018-2019: National Service Personnel (Teaching/Research Assistant), Pathology Department, University of Cape Coast School of Medical Sciences.

2017: Intern, Department of Infectious Diseases Epidemiology; project on transmission of malaria in an area of co-endemicity with schistosomiasis (TRANSMAL), Kumasi Centre for Collaborative Research into Tropical Medicine.

2016: Intern, Department of Microbiology; project on Severe Typhoid in Africa (SETA); Kumasi Centre for Collaborative Research into Tropical Medicine.

2013/2014: Integrated Science teacher, Dunkwa St. Mark International Junior High School.

Personal Skills

- Computing Skills; IBM SPSS, Excel

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- Event planning and organizing
- Data collection and analysis
- Able to work as a team member

Referees

- Prof. Yaw A. Afrane

  Associate Professor, University of Ghana Medical School.

  0542286113.

- Rev. Prof. Kwamena Sagoe

  Associate Professor, University of Ghana Medical School,

  0277408528

- Dr. J. H. Kofi Bonney

  Senior Research Fellow, Noguchi Memorial Institute for Medical Research,

  0243568041

- Dr. Patrick Kafui Akakpo,

  Senior Lecturer, University of Cape Coast School of Medical Sciences, 0206301058

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APPENDIX 4: College of Health Science Ethical Clearance Form

COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

EPRC/SEP/2022

September 27, 2022.

Mr. Lidwan Mensah
Department of Medical Microbiology,
University of Ghana Medical School
Accra.

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.2 – P5.11 /2022-2023***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on September 22, 2022 reviewed and approved your research protocol.

Title of Protocol: "Assessment of immunity against Yellow Fever Virus infections among people following a Yellow Fever Outbreak in Ghana"

Principal Investigator Mr. Lidwan Mensah

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till, September, 27 2023.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: ....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, UGMS
Head, Medical Microbiology,

P. O. Box LG 52, Legon, Accra, Ghana | Tel: +233 (0) 303 665 103/4 | Fax: +233 (0) 302 660 762

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APPENDIX 5: Ghana Health Service Ethical Clearance Form

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Email: ethics.research@ghsmail.org

My Ref. GHS/RDD/ERC/Admin/App /22/195
Your Ref. No.

10th May, 2022

Lidiwan Mensah
Department of Medical Microbiology
College of Health Sciences
University of Ghana

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC: 031/03/22**
Study Title	Assessment of Immunity against Yellow Fever Virus Infections among People Following a Yellow Fever Outbreak in Ghana
Approval Date	10th May, 2022
Expiry Date	9th May, 2023
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED...

Mr. Kofi Wellington
(GHS ERC Vice Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra