# Characterization of enteric and blood pathogens and associeted factors among patients presenting with typhoid fever like symptoms in Buhigwe district Kigoma Tanzania

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# CHARACTERIZATION OF ENTERIC AND BLOOD PATHOGENS AND ASSOCIETED FACTORS AMONG PATIENTS PRESENTING WITH TYPHOID FEVER LIKE SYMPTOMS IN BUHIGWE DISTRICT KIGOMA TANZANIA

**By**

**Pius Raphael Tarimo**

**A Dissertation/Thesis Submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Sciences (Epidemiology and Laboratory Management) of**

**Muhimbili University of Health and Allied Sciences**

**October, 2017**

# CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Health Sciences a dissertation entitled **"*Characterization of enteric and blood pathogens and associated factors among patients presenting with typhoid fever like symptoms in Buhigwe District Kigoma Tanzania 2017* "** in fulfillment of the requirements for the degree of Master of Science (Epidemiology and Laboratory Sciences) of Muhimbili University of Health and Allied Sciences.

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Date: _____

# DECLARATION AND COPYRIGHT

I, **Pius Raphael Tarimo**, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature…… Date…….....

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## DEDICATION

I would like to dedicate this work to my entire family for their great encouragement and prayers and all resident of Buhigwe district who for couples of years back they have been experiencing outbreak of unknown illness and in one way or another they have been affected either socially, economically or psychologically.

## ACKNOLEDGEMENT

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## LIST OF ABREVIATIONS

| | |
|---|---|
| AOR | Adjusted Odds Ratio |
| CI | Confidence Interval |
| DAEC | Diffusely adherent Escherichia coli |
| DMO | District Medical officer |
| DNA | deoxyribonucleic acid |
| dNTP | Deoxynucleotide Triphosphates |
| EAEC | Enteroagrigative Escherichia Coli |
| EDTA | Ethylene Diamine Tetraacetic Acid |
| EHEC | Enteroinvasive E. coli |
| EIEC | Enteroinvasive Escherichia *coli* |
| EPEC | Enteropathogenic Escherichia coli |
| ETEC | Enterotoxigenic Escherichia coli |
| KCRI | Kilimanjaro Clinical Research Institute |
| LLIN | Long Lasting Insecticide Net |
| MOHCDGEC | Ministry of Health, Community Development, Gender, Elderly and Children |

| | |
|---|---|
| MUHAS | Muhimbili University of Health and Allied Sciences |
| PCR | Polymerase Chain Reaction |
| RMO | Regional Medical Officer |
| RNA | Ribonucleic acid |
| PhHV | Phocine Herpesvirus |

## DEFINITIONS OF TERMS

1. **Fever:** Having a body temperature above the normal range due to an increase in the body's temperature set-point.($\geq 37.5^0$C)
2. **Outbreak:** Occurrence of cases of diseases in excess of what would normally be expected in a defined community geographical area or season.
3. **Primer:** A short strand of RNA or DNA (generally about 18-22 bases) that serves as a starting point for DNA synthesis.
4. **Probe:** Fragment of DNA or RNA of variable length (usually 100–1000 bases long) which can be radioactively labeled.
5. **TaqMan® Array Card:** is a 384-well micro fluidic card designed to perform 384 simultaneous real-time PCR (qPCR) reactions.
6. **Treated water:** Water which is free from disease causing organism after boiling or chlorination
7. **Typhoid fever:** An illness caused by Salmonella typhi that is often characterized by insidious onset of sustained fever, headache, malaise, anorexia, relative bradycardia, constipation or diarrhea, and nonproductive cough(CDC)

## ABSTRACT

**Background:** Typhoid fever is a life threatening waterborne and food borne illness caused by bacterium *Salmonella typhi*. Approximately 21 million cases and 222 000 typhoid-related deaths occur annually worldwide. People living in Buhigwe district for couple of years have been experiencing seasonal outbreak of fever and gastrointestinal illness of unknown origin similarly to that of typhoid fever.

**Broad objective:** The aim of this study was to characterize enteric and blood pathogens among patients presenting with typhoid fever like symptoms in Buhigwe district.

**Material and Methods:** This was cross-sectional designed health facilities based study conducted in Buhigwe district. Four health facilities was selected randomly form Kajana, Muyama, and Muzenze and Songambele wards. After systematic sampling of study participants blood and stool sample were collected and analyze using Real Time Polymerase Chain Reaction (PCR).Questionnaires were used to collect information on factors associated with enteric and blood pathogens. Collected data was analyzed using Epi info version 3.5.1, Stata and excel statistical software

**Results:** Total of 246 participants were enrolled into the study. Their age range from 1to 82 years with 72.8% female**.** Majority of them were farmers (63.8%). Thirty five percent aged less than 16 years old. Participants presenting with typhoid fever like symptoms were having multiple pathogens. Blastocystis, Enteroagrigative Escherichia Coli (EAEC), Giardia and malaria were the most prevalent enteric and blood pathogens. Drinking untreated water was significantly associated with enteric infections AOR (95%CI) =2.8(1-7.8). Not having insecticide treated bed net was associated with getting malaria

infection AOR (95%CI) =3.5(1.4-8.8).Living in Kajana ward was significant associated with both getting malaria and enteric infections AOR (95%CI) = 2.3(1-4), AOR (95%CI) =8(2.7-23.9) respectively. Getting schistosomiasis infection was significant associated with washing clothes in open water bodies AOR (95%CI) =2.2(1.2-4.0).

**Conclusions.** Malaria, **s**chistosomiasis, giardiasis and to a lesser extent other enteric and blood pathogens may commonly be present in patients presented with typhoid fever like symptoms or in patients suspected of typhoid fever.

Poor personal hygiene practices, not having mosquito bed net and taking shower or washing clothes in open water bodies was significant associated with getting enteric infections, malaria and schistosomiasis infections respectively.

# CHAPTER ONE

# INTRODUCTION.

## 1.1 BACKGROUND.

Typhoid fever is a life threatening waterborne and food borne illness caused by bacterium *Salmonella typhi*(1).According to the most recent estimates (published in 2014), approximately 21 million cases and 222 000 typhoid-related deaths occur annually worldwide(2). Estimates on non epidemic incidences suggest that south central Asia, south east Asia and southern Africa are region with high *Salmonella* typhi infection(3).

In Africa more than 400,000 typhoid fever cases are reported annually with East Africa contributing 98,000 of these cases while sub Saharan Africa studies shows that incidence rate of typhoid fever is 724.6/100,000 cases/year(4).*Salmonella typhi* are gram negative rod shaped bacteria belonging to the family Enterobacteriaceae. This organism was identified as human pathogen in the late 19$^{th}$ century and as health monitoring and tracking continue to be improved it was identified that these organisms causes significant issues in food born disease outbreak (5).

There are approximately 2400 serotypes (common regarded as species of *Salmonella*) which are divided into two main groups typhoidal and nontyphoidal *Salmonella*. The most common serovars are nontyphoidal serovars which cause self-limiting gastrointestinal illness inhuman. Typhoidal serovars consists of group of *Salmonella* which are adapted to humans and do not occur in other animals. These include *Salmonella typhi* and *Salmonella paratyphi A,B*.Paratyphi A is a very similar bacteria to *Salmonella* typhi but often cause less severe disease (6).

Organisms responsible for typhoid fever occurs predominantly in association with poor sanitation and lack of clean drinking water, they are trans-mitted faeco-orally and shed acutely during an infection(3,7).Once *Salmonella typhi* bacteria are eaten or drunk, they multiply within the gastrointestinal truck (GIT) and spread into the bloodstream. The body reacts with fever and other signs and symptoms including headache**,** fatigue, abdominal pain, diarrhoea and vomiting. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract.

Other individuals, may progress to become long-term asymptomatic carriers, as both *Salmonella typhi* and *Salmonellaparatyphi A* can colonize and survive for prolonged periods within the gall bladder both ill persons and carriers shed *Salmonellatyphi* in their feces(1).

Group of people who are at higher risks of *Salmonella* infections are older adult people with weakened immune system and children who are under five years of age. *Salmonella* infection in this group can be more severe resulting in long term consequences or death. Typhoid fever can be prevented by using vaccines which can be administered orally and parenterally and are safe(8).

Fever is one of the signs and symptoms presented during *Salmonella* infection. It is also one of the most common presenting symptoms in clinical practice(9). Most of the time fever is either self-limiting or can persist for a certain period of time especially when definite underlying etiology is not yet identified(9).

Several studies conducted to assess prevalence or incidence of *Salmonella* typhi results reveals presence of other microbiological pathogens other than S.typhi in blood or stool of febrile patients presenting with typhoid fever like symptoms. The identified groups of pathogens consist of other bacteria apart from *Salmonella* species, viruses and parasites and this supports the evidence that patients presenting with typhoid fever like symptoms are usually inflicted with other pathogenic microorganisms. These pathogens can be divided into those that are easy to identify using microscope or culture and those which require serological or molecular technique(10,11).

Many pathogenic microorganisms can cause non specific chronic or low grade fever, making the specific causative agent difficult to predict. But with improvement in diagnostic technology most of pathogenic microorganisms now days are easy to identify when correct diagnostic techniques are performed on correct patient specimen(10).

Globally, infectious diseases which are caused by these pathogenic microorganisms remain the leading cause of morbidity and mortality. New diseases are arising worldwide and old diseases are re-emerging as infectious agents evolve and spread as changes occur in ecology, socioeconomic conditions, and population patterns (12). These diseases are becoming health problems for a significant proportion of the population especially in developing countries.

Despite all these there is both good news and bad news concerning infectious diseases control globally. The good news is that some of infectious diseases have already been eradicated e.g small pox, other infectious disease like poliomyelitis eradication is on track, and others are under effective control in much of the world(12).

The bad news is that propagation and transmission of pathogenic microorganisms which cause infectious diseases are favored in today's world resulting in increase threat to human health and welfare to all countries(11–13).

Infectious diseases are caused by pathogenic microorganisms such as viruses, bacteria, parasites or fungi(14).Some of these microorganisms are transmitted by bites from insects or animals and others are acquired by ingestion of contaminated food or drinks or being exposed to the organism in the environment(15).Transmission of these microorganisms can broadly be grouped into direct or indirect transmission.

Direct transmission occurs when microorganisms pass from an infected person to a health person via direct physical contact with blood or body fluid e.g person to person contact or through droplet spread. Indirect transmission of infectious agents occurs when there is no direct human-to human contact e.g airborne transmission, contaminated object, food and drinking water, insect bite or animal to person transmissions(15).

After infectious agents get in the body of susceptible host they may be categorized as gastrointestinal infectious agent when they are confined to the gastrointestinal tract or initiated to the gut before spreading to the other part of the body (15).

Gastrointestinal illness has a significant impact on health of community and carries a substantial economic burden. The burden of gastrointestinal illness in developing countries remains significantly high(16).Significant proportion of vulnerable people in sub-Saharan Africa remain at risk for contracting gastrointestinal illness due to the presence of many risk factors facilitating transmission(16).

Organism responsible for gastrointestinal illness are transmitted by the faecal-oral route and food ingested are major vehicle of this transmission(17)**.**Always before taking any action to reduce the impact of gastroenteritis the organism responsible for the disease must first be identified(18)**.**

## 1.2 STATEMENTS OF THE PROBLEM

People living in Buhigwe district in Kigoma region Tanzania for couple of years have been experiencing seasonal outbreak of fever and gastrointestinal illness most of them termed as outbreak of unknown illness. In 2013 a total of 690 suspected cases of Hepatitis E virus with detection rate of 7% (19). In 2015 another outbreak of unknown illness occurred in the same area where approximately 1462 suspected cases were reported. Only 5% of the suspected cases revealed presence of infectious agent**s** with Salmonellosis being the main cause (unpublished data). Both outbreaks presented with the similar clinical picture although the etiological agent were totally different.

In 2016 in the same district 1704 suspected cases of salmonellosis were reported , 84 cases (5%) were taken for laboratory confirmation, among confirmed cases 11(13%) were positive for salmonellosis and 8(10%) were positive for other viral**,** parasitic and bacterial infections. Clinical presentations of all these cases were similar to those of typhoid fever and they were treated as typhoid fever cases(20)**.**

Disease caused by these pathogens have important socio-economic impacts in the affected communities and the government in general as several days are required for a patient to recover and resume duties as well as the costs incurred during investigation and treatment. As current global estimates of typhoid infection are based on very limited data, especially from Africa(21).

Despite the fact that studies have established that some patients presenting with typhoid like symptoms are usually inflicted by other pathogens, characterization of enteric and blood pathogens among patients presenting with typhoid fever like symptoms in Buhigwe district has not been done. Predominantly factors associated with acquiring these blood and enteric pathogens are not clearly identified. This may result in failure to plan preventive measures for these yearly outbreak**s** termed as outbreak of unknown illness.

## 1.3 RATIONALE

Results obtained from this study provide a clear picture on characterization of enteric and blood pathogens together with associated risk factors among study participants. Study finding also provide a picture on possible enteric and blood pathogens which may be present in patients presenting with sign and symptoms of typhoid fever in Buhigwe district Kigoma.

## 1.4 RESEARCH QUESTIONS

1. What are the most prevalent enteric and blood pathogens in patients presenting with typhoid fever like symptoms in Buhigwe?

2. What are the factors associated with enteric and blood pathogens among patients presenting with typhoid fever like symptoms?

## 1.5 OBJECTIVES

### 1.5.1 Broad objective.

To characterize enteric and blood pathogens and risk factors associated with them among patients presenting with typhoid fever like symptoms in Buhigwe district.

### 1.5.2 Specific objectives

**1.** To characterize enteric and blood pathogens among patients presenting with typhoid fever like symptoms in Buhigwe district.

**2.** To identify risk factors associated with blood pathogens among patients presenting with typhoid fever like symptoms in Buhigwe district.

**3.** To identify risk factors associated with enteric pathogens among patients presenting with typhoid fever like symptoms in Buhigwe district.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Typhoid fever epidemiology

Typhoid fever (enteric fever) is a systemic prolonged febrile illness caused by certain salmonella serotype. *Salmonella enterica serovars typhi*(*S.typhi*) and *Salmonella enterica serotype paratyphi A, B and C* (*S.paratyphi* A, B and C) are the major species responsible for causing salmonella infection which is a common bacterial disease that affect intestinal tract.

Among these mentioned species *Salmonella typhi* is the most common species responsible for the disease(22).In 2000, typhoid fever caused approximately 2.16 million episodes with 21600 deaths globally(23) but recently WHO data shows that approximately 21 million cases and 222 000 typhoid-related deaths occur annually worldwide(2).In developing countries burden of gastrointestinal illness remain significantly high despite a marked decrease in mortality rate from 4.6 million in 1980 to about 1.5 million in 1999(17), however the disease still affect 25.1 millions of people each year(24).

In most cases gastrointestinal illness are caused by enteric pathogens which are transmitted by faecal-oral root**.** Globally, typhoid fever is most prevalent in low income countries especially in areas that are overcrowded and with poor access to sanitation. This influence less frequently hand washing behavior and sources of water are more likely to be contaminated(25). In south central and southeastern Asia infant**s**, children and adolescents experience the greatest burden of the illness (26).

In other endemic areas, the incidences of typhoid fever are considered to be low in the first few years of life, peaking in school-aged children and young adults and then falling in the middle aged(27).The observable peak in school aged children and young adults may be due to unsafe feeding habit which may result in an increase in consumption of contaminated food and water. Many diseases unlike typhoid exhibit marked seasonal variations in their incidence; however typhoid fever usually occurs at any season of the year. In some places around the world typhoid fever is more likely to be contracted during the late summer or autumn.

## 2.2 Challenges in diagnosis of typhoid fever

For several decades it is clearly known that definitive diagnosis of typhoid fever is isolation of *Salmonella typhi* from blood or bone marrow culture, however inadequate infrastructure to perform culture technique has been regarded as one of the challenges which contribute to many health facilities and hospitals to rely on cheaper serological diagnostic techniques which results in incorrect estimation of the disease burden(28,29).

The accurate and rapid clinical diagnosis of enteric fever is obfuscated by the range of other common fever-causing infections including malaria, dengue fever, leptospirosis, shigellosis and the rickettsioses. Accurate diagnosis to differentiate typhoid fever from these other infections is often difficult, both for clinicians and laboratorians, and is imperative for effective treatment selection. Even in highly-resourced western countries, physicians often start typhoid treatment empirically whilst awaiting confirmation of the diagnosis. All these also have an impact in correct estimation of the burden caused by salmonellosis(30).

Due to non specific nature of the symptoms and lack of reliable diagnostic tests remarkable delay in presentation of cases to appropriate health facilities has been observed. Despite all these challenges prompt treatment with appropriate antibiotics minimizes illness severity. In most of developing countries in spite of availability of several specific drugs for typhoid fever treatment morbidity and mortality due to typhoid fever continue to be recorded(29).

Antimicrobial resistance can be explained as resistance to the three first line agents used to treat typhoid fever. Treatment decisions are further complicated by the increasing prevalence of antibiotics resistance amongst clinical isolates due to plasmid-mediated multidrug resistance. The observed resistance of the agents to common known drugs of choice is fast becoming a major public health problem in management of typhoid fever(31).

## 2.3 Transmission and risk factors of *Salmonella typhi*

Humans are the only natural host and reservoir of *Salmonella typhi*(32).*Salmonella typhi* can survive for prolonged periods in water, ice, dust and dried sewage which become sources of infection(27).*Salmonellatyphi* is mainly transmitted by ingestion of food or water contaminated with faeces from an infected person(33).The highest incidence occurs where water supplies serving large populations are contaminated with faeces. Flavored iced drinks or food from street vendors, shellfish taken from contaminated water and raw fruit and vegetables fertilized with sewage are significant risk factors for transmission of typhoid fever(27).All these mentioned sources can also harbor other bacteria associated with poor hygiene thus serve as transmission routes(8)

## 2.4 Laboratory diagnosis of typhoid fever

For many years clinical signs and symptoms of typhoid fever have been described many times and are helpful in diagnosis of salmonellosis(34).In recent years due to development in science and technology definitive diagnosis of typhoid fever depends on isolation of *Salmonella typhi* from clinical specimen. Blood and bone marrow aspirate culture is the gold standard diagnosis technique for typhoid fever (32).

Specific diagnosis of typhoid fever requires access to a competent laboratory that can process blood and stool through culture technique. Culture technique is the one recommended for best results. Even under the best culture conditions, the organisms may not be isolated from blood, especially after antimicrobial treatment is commenced(35). Emerging drug resistances among circulating serotype of *Salmonella* species has complicate the treatment of *Salmonella* and increase awareness on the requirement of accurate diagnostic and appropriate selection of antimicrobial agents to which pathogens has remain susceptible(36).

Due to the fact that Salmonella species have extremely limited genetic diversity within their population and having unique genome, all these facts provide room for effective molecular diagnostics techniques. Detecting the presence of *Salmonella typhi* in clinical samples using highly sensitive molecular techniques is not a recent development(30).

After the early studies using DNA probes and hybridization techniques attention was turned to the use of polymerase chain reaction (PCR) methods for the detection of both *Salmonella typhi* and *Salmonella paratyphi A* for diagnosis of enteric fever(37).

The use of PCR technique in diagnosis of Salmonella infection has been observed as a good approach over the other methods as it has the ability to detect Salmonella typhi in patients who have been on antibiotic prior to diagnosis. PCR technique reinforce the clinical diagnosis in culture negative cases thus avoid other unnecessary treatment options (37)**.**

## 2.5 Features of diseases caused by bacteria isolated along side with *Salmonella typhi*

Apart from Salmonella species other pathogenic microorganisms can be isolated or detected in stool or blood samples .The magnitude of bacteremia (presence of bacteria in blood) dependent on age, immune status and virulence of the strains. Signs and symptoms presented as results of bacteremia include fever over (38.3$^{o}$C), chills, malaise, abdominal pain, nausea vomiting, shortness of breath it is important to note that presentation of these symptoms depends on type of bacteria present in blood.

## 2.6 Common pathogens associated with poor hygiene

Sanitation and hygiene are critical to health, survival, and development, as it helps prevent typhoid, Cholera, Shigella and many other common gastro-enteric infections. Human faeces when comes into contact with food and drinking water are the main sources of diarrheal infections which claim the lives of 1.87 million children under the age of 5 each year according to the World Health Organization(38).

## 2.7 Malaria and typhoid fever

Although, malaria and typhoid fever have different etiologies, they are seems to be a physiological convergence in the symptomatic patterns of onset. People living in endemic areas are at risk of contracting both infections concurrently(39).These two diseases are associated with poverty and underdevelopment. It is known that diagnostic laboratories are often poorly resourced in developing countries, and sparsely distributed(40).

All the mentioned challenges may contribute much on clinical diagnosis rather than laboratory confirmation in an effort to provide treatments for a certain disease.

The similarities of clinical features of both disease which is preceded by fever leads to misdiagnosis and mistreatment of patients(41).Reliable diagnostic method is important for effective management of cases to prevent unexpected drug resistance, misuse and wastage of drugs.

# CHAPTER THREE

# METHODOLOGY

## 3:1 Study area

Study was conducted in four randomly selected wards (Kajana, Muyama, Muzenze and Songambele) out of twenty wards in Buhigwe district in Kigoma region. Buhigwe District is one of the eight districts within the Kigoma Region in Tanzania. The District has 2 Hospital one owned by Faith Based Organization and one under government control; 4 Health Centre whereby 2 are owned by private, 2 by government and 30 Dispensaries. Buhigwe District is bordered to the North by Burundi, to the East by Kasulu Rural District and Kasulu Urban District and to the west by Kigoma Rural District According to the 2012 Tanzania National Census; the population of Buhigwe District was 254,342.

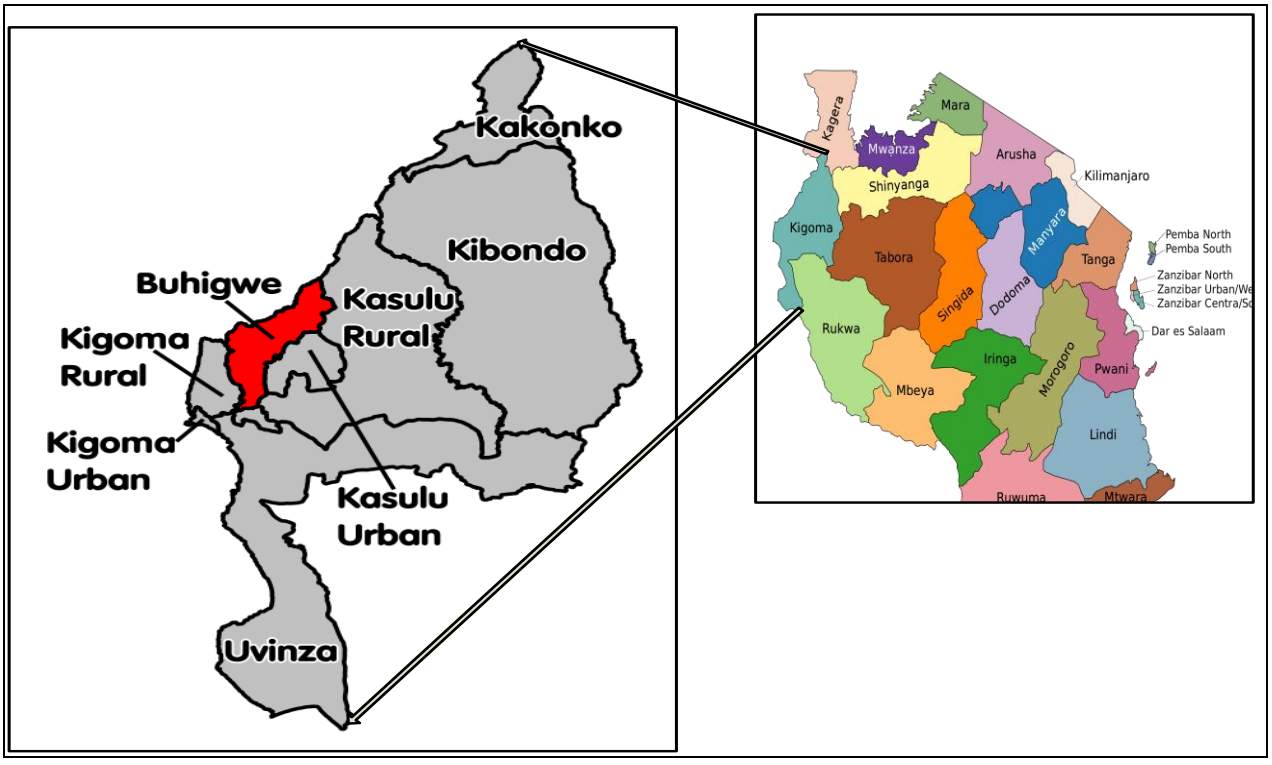



**Figure 1: Map of Tanzania showing Kigoma region and Buhigwe district**

### 3.2 Study design:

This study was facilities based cross sectional study design conducted in four selected health facilities in Buhigwe district.

### 3.3 Study population:

All patients presenting with typhoid fever like symptoms in Buhigwe District

### 3.4 Inclusion criteria

Buhigwe residence of any sex and any age group, who presented with typhoid fever symptoms, which were fever with or without headache general body malaise, diarrhoea, joint pains, abdominal pain, vomiting and or dizziness.

### 3.5 Exclusion criteria

Patient**s** who met inclusion criteria but who were too sick to communicate or failure to provide stool or blood sample.

### 3.6 Study variables

Independent variables were exposure factors that were known to be associated with common enteric or blood infections.

i) Socio economic factors e.g. occupation, age, sex, marital status

ii) Behavioral factors e.g hand washing habit before meal or after visiting a toilet, habit of eating food prepared by street vendors ,swimming,washingclothes,or taking bath in ponds, mosquito use behavior,

Dependent variable for this study was testing positive for selected group of common enteric and blood pathogens

### 3.7 Sample size estimations:

The following formula for estimating sample size of the proportion will be used

$$n = \frac{z^2PQ}{d^2}$$

Where:

n=Minimum sample size expected

Z=Confidence interval at 95% =1.96

d=4% margin of error

P=Prevalence obtained from previous study 4.2% prevalence of typhoid in Egypt(43).

Q=1-P

$$n=\frac{1.96^2\times 0.0423(1-0.0423)}{0.04^2}=\frac{0.1618}{0.04^2}=101$$

N=101

As study participants will be selected from three different health facilities the selected health facilities provide services to community members who will be coming from different places within Buhigwe district. Due to this expected differences in study participants designing effect will be considered and sample size will be multiplied by 2.

101×2=202 Total of 202 study participants will be enrolled.

### 3.8 Sampling procedure.

Four health facilities within Buhigwe district was selected randomly from 4 health centres and 30 dispensaries. Monthly uptake was determined so as to allocate number of patients to be enrolled from each health facilities. Patients attending any of four randomly selected health facilities (Kajana dispensary, Muyama health centre, Muzenze health centre and Songambele health centre) in Buhigwe district was examined clinically for signs and symptoms of typhoid fever by a clinician, Systematic random sampling was used to select study participants who meet inclusion criteria. The formula below indicates how number of study participants from each facility was calculated.

$F=X/X_0\times N$

F=Number of study participants enrolled from particular health facility

X=Average No. of patients seen at single facility per monthly

$X_0$=Average total number of patients seen in all three health facilities per monthly

N=Minimum study sample size

For facility No 1,2,3,4

F1= $X_1/X_0 \times N$ F2= $X_2/X_0 \times N$ F3= $X_3/x_0 \times N$ F4=$X_4/X_0 \times N$

### 3.9 Data collection

Data was collected by Principal Investigator together with trained research assistants after obtaining informed concent from study participants. Structured questionnaires were used to collect information on socio demographic characteristics of study participants together with associated factors for enteric and blood pathogens through interview. Structured questionnaires were given ID numbers which was then labeled on participants stool and blood containers.

Small amount of stool sample was collected in a clean container together with 3mls of blood samples in EDTA blood collection tubes. All questionnaires and participants samples were cross checked for completeness and proper labeling. At Kigoma hospital 2-3grms of stool samples was aliquoted into cryovial tubes and stored at 2-8$^0$C before transport them to KCRI laboratory. Cold chain environment was maintained during specimen transportation.

### 3.10 Laboratory investigation:

**TaqMan RT PCR**

All blood and stool samples were analyzed using real time PCR technique. TaqMan Real Time PCR card were used during analysis.

Extraction of total nucleic from blood samples was done in which 2.5 mls of blood sample undergo lysate preparation process using high pure viral nucleic acid large volume kit. Purification and elution of nucleic acid using spin column was done including a high pure extender assembly for initial large volume .Extrinsic control PhHV and MS2 were added to each sample during lysate preparation to evaluate extraction and amplification efficiency. At the end extracted nucleic acid was stored at -80 ready for testing.

Extraction of total nucleic acid was done using modified extraction procedure to isolate DNA and RNA from fecal specimen. Fecal sample undergoes lysate preparation process among them were mechanical disruption (bead beating), removal of inhibitors, purification and elution of DNA and RNA was done using spin column. Extrinsic control PhHV and MS2 were added to each sample during lysate preparation to evaluate extraction and amplification efficiency. At the end extracted nucleic acid was stored at -80 ready for testing. To rule out contamination during extraction process a blank was also processed through the complete protocol.

### 3.11 Data management

After laboratory investigation of all specimens results were obtained in excels format, data cleaning was done using Epi info version 3.5.1 and excel software followed by univariate analysis of socio demographic and behavioral characteristic. Figures and tables were used to present results.

At bivariate analysis level three outcome variables were defined to simplify analysis of risk factors (bacterial infection, malaria infections and **s**chistosomiasis infection)**.**Bacterial infections were group of enteric pathogens which are associated with daily unhygienic practices which include those transmitted via fecal oral route. Independent variables which were found to be significantly associated with dependent variable at 95%CI with P value <0.05 were subjected to multivariate analysis.

Forward addition logistic regression for variables which showed association at bivariate level with p value <0.2 was done to look for independent risk factors.

## 3.11 Ethical considerations.

Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences ethical review board. Permission to conduct the study was requested from Kigoma Region Medical Officer (RMO) and Buhigwe District Medical Officer (DMO). Final permission was obtained from respective hospital in charge in selected health facilities in Buhigwe district. From study participants informed consent was requested. Participants with age below 18 years were required to give verbal consent so as to be enrolled and written consent was signed by their parents or

guidance. Protection of study participants and confidentiality of collected data was ensured during and after the study. For those study participants whose enteric or blood pathogens was detected in their samples results was communicated with medical officer in charge of their nearby health facilities for medication. Results were shared with Kigoma RHMT and Buhigwe CHMT for farther medical attention in which they started by distributing ant worms for primary school pupils and supply more ant malaria to different health facilities in Buhigwe district.

# CHAPTER FOUR

# RESULTS

## 4.1 Socio demographic characteristics of study participants (N=246)

Of the 246 study participants enrolled into the study during the study period, 118 (47.9%),54(22%), 45(18.3%) and 29(10.6%) were from Kajana, Muyama, Muzenze and Songambele wards respectively. The median age was 21 year with a range of 1-82 years. Female constituted 179 (72.8%) with majority (35%) of study participants being less than 16 years Table 1.

**Table 1: Socio demographic characteristics of study participants**

| Variable | | Number (n) | Percentage (%) |
|---|---|---|---|
| Sex | | | |
| | Female | 179 | 72.76 |
| | Male | 67 | 27.24 |
| Age group | | | |
| | <16 | 86 | 34.96 |
| | 16-22 | 20 | 8.13 |
| | 23-31 | 30 | 12.19 |
| | 32-50 | 72 | 29.27 |
| | >50 | 38 | 15.44 |
| No. of family members | | | |
| | <6 | 102 | 41.46 |
| | ≥6 | 144 | 58.54 |
| Ward | | | |
| | Kajana | 118 | 47.97 |
| | Muyama | 54 | 21.95 |
| | Muzenze | 45 | 18.29 |
| | Songambele | 29 | 11.79 |
| Marital status | | | |
| | Married | 146 | 59.3 |
| | Single | 100 | 40.7 |
| Occupation | | | |
| | Child | 5 | 2 |
| | Peasant | 157 | 63.8 |
| | Student | 84 | 34.1 |
| Level of education | | | |
| | None | 69 | 28.05 |
| | Primary | 173 | 70.32 |
| | Secondary | 4 | 1.63 |

All study participants were presented with fever (≥37.5).Other signs and symptoms presented were abdominal pain 89%, headache 88.2%, joint pain 58.5%, general body weakness 57.7% and others as shown in table 2 below.

**Table 2: Clinical presentation of study participants**

| Signs and symptoms | Number | % |
|---|---|---|
| Headache | 217 | 88.2 |
| Abdominal pain | 219 | 89 |
| Joint pain | 144 | 58.5 |
| Fever | 246 | 100 |
| Nausea | 92 | 37.4 |
| Blood stain stool | 24 | 9.8 |
| Vomiting | 35 | 14.2 |
| Diarrhoea | 77 | 31.3 |
| General body weakness | 142 | 57.7 |
| Hematuria | 15 | 6.1 |

## 4.2 Detection rate of enteric pathogens

Stool samples were collected from 246 study participants and subjected to Real Time Multiplex PCR which has the capacity to detect 40 different pathogens. From stool samples 25 different pathogens ranging from bacterial, parasites to viruses were identified. Of the pathogens identified bacterial pathogens were the most prevalent accounting for 16(64%) with parasites and viruses accounting for 6/25(24%), 3/25(12%) respectively Figure 1.

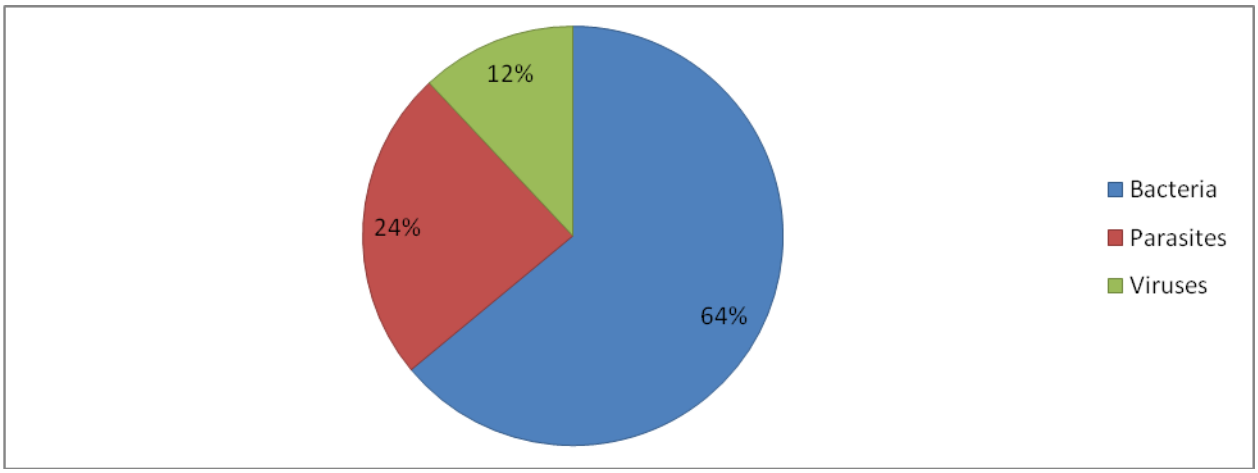
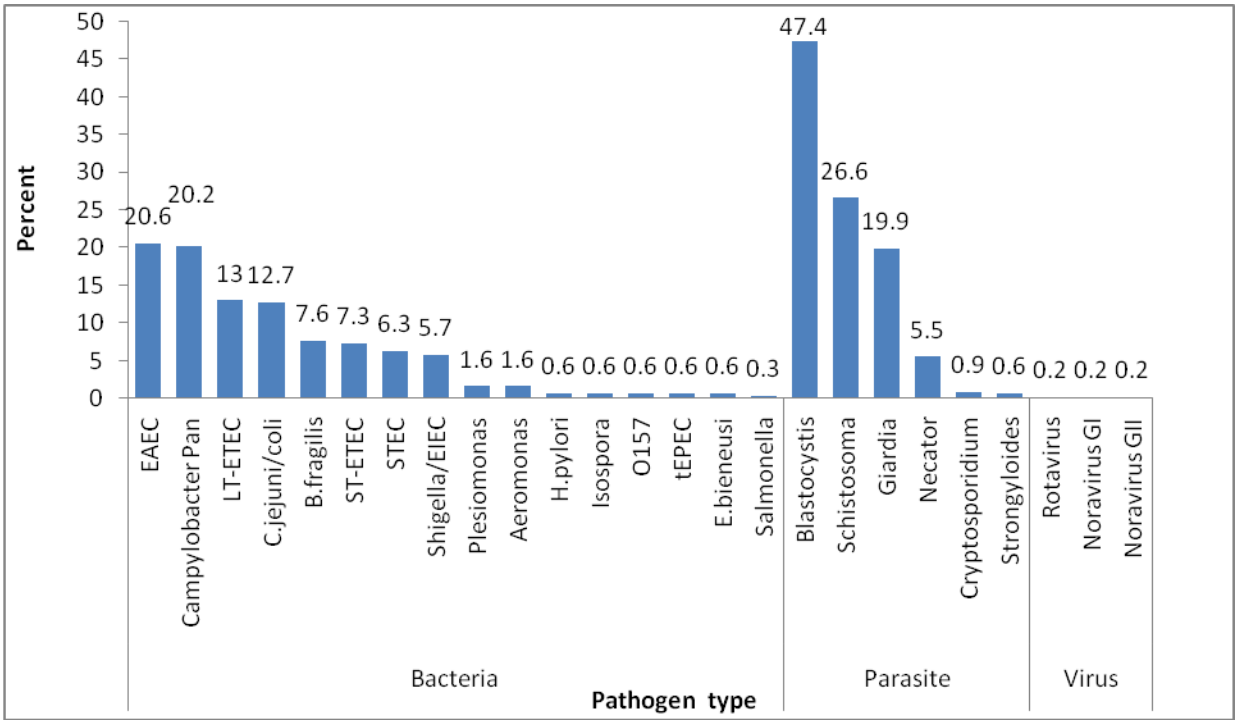



**Figure 2:.Group of Pathogens detected in stool sample**

Of the 246 stool samples analyzed parasites including *Blastocystis hominis,Schistosoma mansoni* and *Giardia lamblia* were identified in 164(47.4%), 92/246(26.6%),69(19.9%),of the samples respectively. Several bacterial pathogens were identified among them include Enteroagrigative E.coli 65(20.6%) Campylobacter spp 64(20.2%).Only Norovirus GI and GII were identified in 0.2% of the samples Figure 3.




**.Figure 3: Detected pathogens in stool samples**

### 4.3 Detection rate of blood pathogens

From blood samples 8 different pathogens including bacteria, parasites and to a lesser extent viruses were identified. Based on type or group of pathogen detected bacterial pathogens were the most prevalent accounting for 5 types of bacteria (62.5%) of the 8 different pathogens identified. Parasites and viruses accounting for 1(12.5%), 2(25%) respectively. Table three below identifies number of individual pathogens among each group (bacteria, parasites and viruses) and their percentages.

**Table 3: Distributions of pathogens detected in blood**

| | **Blood pathogens** | **n** | **%** |
|---|---|---|---|
| **Bacteria** | C.burnetii | 4 | 2.20 |
| | Bartonella | 2 | 1.10 |
| | S.aureus | 1 | 0.55 |
| | Rickettsia | 1 | 0.55 |
| | Brucella | 1 | 0.55 |
| **Parasites** | Plasmodium spp | 161 | 88.46 |
| **viruses** | Dengue | 11 | 6.04 |
| | Chikungunya | 1 | 0.55 |

## 4.4 Factors associated with blood pathogens (Plasmodium spp)

Table four below shows analysis of factors associated with malaria infections at bivariate level

**Table 4: Bivariate analysis of factors associated with malaria infection:**

| Variable | n | Yes | % | COR(95%CI) | P-Value |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| Female | 179 | 115 | 64.2 | 0.8(0.5-1.5) | 0.5 |
| Male | 67 | 46 | 68.7 | Ref | |
| **Age group(Years)** | | | | | |
| 16-22 | 20 | 12 | 60 | 0.5(0.2-1.4) | 0.2 |
| 23-31 | 30 | 18 | 60 | 0.5(0.2-1.2) | 0.1 |
| 32-50 | 72 | 45 | 62.5 | 0.6(0.3-1.1) | 0.1 |
| >50 | 38 | 22 | 57.9 | 0.4(0.2-1) | 0.07 |
| <16 | 86 | 64 | 74.4 | Ref | |
| **Occupation** | | | | | |
| Student | 89 | 68 | 76.4 | 2(1.2-4) | 0.007 |
| Peasant | 157 | 93 | 59.2 | Ref | |
| **Marital status** | | | | | |
| Single | 99 | 74 | 74.7 | 2(1.2-3.6) | 0.01 |
| Married | 147 | 87 | 59.2 | Ref | |
| **Ward** | | | | | |
| Kajana | 118 | 92 | 78 | 3(1.7-5.3) | <0.001 |
| Other wards | 128 | 69 | 53.9 | Ref | |
| **Ownership of bed net** | | | | | |
| No | 50 | 44 | 88 | 4.95(2-12) | <0.001 |
| Yes | 196 | 117 | 59.7 | Ref | |
| **Frequency use of bed net** | | | | | |
| Sometimes | 26 | 21 | 80.8 | 2.4(0.9-6.6) | 0.06 |
| Always | 170 | 96 | 56.5 | Ref | |
| **Mosquito net status** | | | | | |
| **Old with some holes** | 96 | 61 | 63.5 | 0.9(0.5-1.5) | 0.6 |
| **New** | 100 | 56 | 56 | Ref | |
| **Level of education** | | | | | |
| Primary | 173 | 118 | 68.2 | 1.6(0.9-2.8) | 0.1 |
| Secondary | 4 | 3 | 75 | 2.2(0.2-22.5) | 0.5 |
| None | 69 | 40 | 58 | Ref | |
| **Family members** | | | | | |
| ≥6 | 144 | 101 | 70.1 | 1.6(1-2.8) | 0.07 |
| <6 | 102 | 60 | 58.8 | Ref | |

Ref=References group

## 4.5 Bivariate analysis of risk factors associated with enteric infections.

Table five below shows factors which were associated with enteric infections among study participants.

**Table 5: Bivariate analysis of factors associated with enteric infections**

| Variable | n | Yes | % | COR(95%CI) | P-Value |
|---|---|---|---|---|---|
| **Age group** | | | | | |
| 16-22 | 20 | 18 | 90 | 0.8(0.1-4) | 0.8 |
| 23-31 | 30 | 25 | 83.3 | 0.4(0.1-1.5) | 0.2 |
| 32-50 | 72 | 63 | 87.5 | 0.6(0.2-1.8) | 0.4 |
| >50 | 38 | 30 | 78.9 | 0.3(0.1-1) | 0.05 |
| <16 | 86 | 79 | 91.9 | Ref | |
| **Sex** | | | | | |
| Female | 179 | 156 | 87.2 | 0.92(0.4-2) | 0.8 |
| Male | 67 | 59 | 88.1 | Ref | |
| **No. Family members** | | | | | |
| ≥6 | 144 | 131 | 91 | 2(1-4.6) | 0.04 |
| **<6** | 102 | 84 | 82.4 | Ref | |
| **Occupation** | | | | | |
| Students | 89 | 82 | 92.1 | 2(0.9-5.1) | 0.09 |
| Peasants | 157 | 133 | 84.7 | Ref | |
| **Ward** | | | | | |
| Kajana | 118 | 114 | 96.6 | 7.6(2.6-22) | <0.001 |
| Other Places | 128 | 101 | 78.9 | Ref | |
| **Hand washing style** | | | | | |
| Dipping hands in a basin with water | 173 | 159 | 91.9 | 3.4(1.6-7.4) | 0.001 |
| Running water | 71 | 55 | 77.5 | Ref | |
| **Source of drinking water** | | | | | |
| Unclean source | 38 | 33 | 86.8 | 0.9(0.3-2.6) | 0.5 |
| Clean source | 208 | 182 | 87.5 | Ref | |
| **Drinking untreated water** | | | | | |
| Yes | 216 | 192 | 88.9 | 2.4(0.9-6) | 0.06 |
| No | 30 | 23 | 76.7 | Ref | |
| **Hand washing after toilet** | | | | | |
| No | 78 | 70 | 89.7 | 1.4(0.6-3.3) | 0.5 |
| Yes | 167 | 145 | 86.8 | Ref | |
| **Habit of eating out of home** | | | | | |
| Yes | 35 | 33 | 94.3 | 2.6(0.6-11.6) | 0.1 |
| No | 211 | 182 | 86.3 | Ref | |

Ref=References group

Table 6 below shows results of analysis of factors associated with schistosomiasis infections among study participants

**Table 6: Bivariate analysis of factors associated with schistosomiasis**

| Variable | n | Yes | % | COR(95%CI) | P-Value |
|---|---|---|---|---|---|
| Male | 67 | 26 | 38.8 | 0.9(0.5-1.6) | 0.8 |
| Female | 179 | 66 | 36.9 | Ref | |
| **Occupation** | | | | | |
| Students | 89 | 53 | 59.6 | 4.5(2.6-7.8) | <0.001 |
| Peasant | 157 | 39 | 24.8 | Ref | |
| **Level of education** | | | | | |
| Primary | 177 | 78 | 44.1 | 3.1(1.6-6) | <0.001 |
| None | 69 | 14 | 20.3 | Ref | |
| **Age group** | | | | | |
| 16-22 | 20 | 5 | 25 | 0.2(0.1-0.7) | 0.004 |
| 23-31 | 30 | 9 | 30 | 0.3(0.1-0.7) | 0.004 |
| 32-50 | 72 | 16 | 22.2 | 0.2(0.1-0.4) | <0.001 |
| >50 | 38 | 10 | 26.3 | 0.2(0.1-0.6) | <0.001 |
| <16 | 86 | 52 | 60.5 | Ref | |
| **Washing clothes in river/dam** | | | | | |
| Yes | 98 | 50 | 51.0 | 3(1.5-4.5) | <0.001 |
| No | 148 | 42 | 28.4 | Ref | |
| **Showering out of home** | | | | | |
| Yes | 72 | 37 | 51.4 | 2.3(1.3-4) | 0.004 |
| No | 173 | 55 | 31.8 | Ref | |

Ref=References group

## 4.6 Stratification analysis

During analysis stratification was done to assess the effect measure of modification and effect of potential confounder factors associated with enteric and blood pathogens. No any confounding variables or interaction was observed among the variable associated with enteric or blood pathogens.

## 4.7 Multivariate analysis of risk factors

All factors which were found to show association at bivariate lever and having P value less than 0.2 were subjected to multivariate logistic model.

**Malaria infections.**

The odds of getting malaria infections were 4 times higher among those who were not having bed net compared to those who were having bed net. The second risk factor which was independently associated with malaria infection was living in Kajana ward in which the odds of getting malaria infection were 2.3 times higher among Kajana resident compared to those who were not. Other factors were assessed but were not statistically significant associated with malaria infection in Buhigwe as shown in table **7** below.

**Table 7: Multivariate analysis of factors associated with malaria infection.**

| Variable | Yes | % | COR(95%CI) | P-Value | AOR(95%CI) | P-Value |
|---|---|---|---|---|---|---|
| **Age group(Years)** | | | | | | |
| 16-22 | 12 | 60 | 0.5(0.2-1.4) | 0.2 | 0.5(0.2-1.5) | 0.2 |
| 23-31 | 18 | 60 | 0.5(0.2-1.2) | 0.1 | 0.5(0.2-1.3) | 0.2 |
| 32-50 | 45 | 62.5 | 0.6(0.3-1.1) | 0.1 | 0.6(0.3-1.1) | 0.1 |
| >50 | 22 | 57.9 | 0.4(0.2-1) | 0.07 | 0.5(0.2-1.0) | 0.06 |
| <16 | 64 | 74.4 | Ref | | | |
| **Occupation** | | | | | | |
| Student | 68 | 76.4 | 2(1.2-4) | 0.007 | 1(0.2-3.4) | 1 |
| Peasant | 93 | 59.2 | Ref | | | |
| **Marital status** | | | | | | |
| Single | 74 | 74.7 | 2(1.2-3.6) | 0.01 | 2(0.6-6.8) | 0.2 |
| Married | 87 | 59.2 | Ref | | | |
| **Ward** | | | | | | |
| Kajana | 92 | 78 | 3(1.7-5.3) | <0.001 | 2.3(1.3-4) | 0.007 |
| Other wards | 69 | 53.9 | Ref | | | |
| **Bed net ownership** | | | | | | |
| No | 44 | 88 | 4.95(2-12) | <0.001 | 4(1.7-10) | 0.002 |
| Yes | 117 | 59.7 | Ref | | | |
| **Frequency of bed net use** | | | | | | |
| Sometimes | 21 | 80.8 | 2.4(0.9-6.6) | 0.06 | 2.7(1-7.8) | 0.06 |
| Always | 96 | 56.5 | Ref | | | |
| **Family members** | | | | | | |
| ≥6 | 101 | 70.1 | 1.6(1-2.8) | 0.07 | 1.1(0.6-2) | 0.8 |
| <6 | 60 | 58.8 | Ref | | | |

Ref=References group

**Bacterial infections**

The odds of getting enteric infection were 8 times higher among individual who were living in Kajana ward compared to those who were living in other three wards. The second factor was drinking untreated water in which the odds of getting enteric infections were 2.8 times higher among those who drinking untreated water compared to those who drink treated water. The odds of getting enteric infections were 2 times higher among those who wash hands by dipping hands in a basin containing water compared to those who use running water. Other factors believed to be associated with enteric infections were assessed but the association were not statistically significant e.g. living in a family with more than six members or being a students. However having age above 50 years was observed as protective against enteric infection AOR (95%CI) =0.4(0.1-1.1) P-Value 0.007.

**Table 8: Multivariate analysis of factors associated with bacterial infections**.

| Variable | Yes | % | COR(95%CI) | P-Value | AOR(95%CI) | P-Value |
|---|---|---|---|---|---|---|
| **Age group** | | | | | | |
| 16-22 | 18 | 90 | 0.8(0.1-4) | 0.8 | 0.9(0.2-4.5) | 0.9 |
| 23-31 | 25 | 83.3 | 0.4(0.1-1.5) | 0.2 | 0.5(0.1-1.7) | 0.3 |
| 32-50 | 63 | 87.5 | 0.6(0.2-1.8) | 0.4 | 0.6(0.2-1.8) | 0.4 |
| >50 | 30 | 78.9 | 0.3(0.1-1) | 0.05 | 0.4(0.1-1.1) | 0.007 |
| <16 | 79 | 91.9 | Ref | | | |
| **No. Family members** | | | | | | |
| ≥6 | 131 | 91 | 2(1-4.6) | 0.04 | 1.3(0.3-0.6) | 0.6 |
| <6 | 84 | 82.4 | Ref | | | |
| **Occupation** | | | | | | |
| Students | 82 | 92.1 | 2(0.9-5.1) | 0.09 | 0.6(0.1-2) | 0.5 |
| Peasants | 133 | 84.7 | Ref | | | |
| **Ward** | | | | | | |
| Kajana | 114 | 96.6 | 7.6(2.6-22) | <0.001 | 8(2.7-23.9) | <0.001 |
| Other Places | 101 | 78.9 | Ref | | | |
| **Hand washing style** | | | | | | |
| Dipping hands in a basin with water | 159 | 91.9 | 3.4(1.6-7.4) | 0.001 | 2(0.9-4.6) | 0.009 |
| running water | 55 | 77.5 | Ref | | | |
| **Drinking untreated water** | | | | | | |
| Yes | 192 | 88.9 | 2.4(0.9-6) | 0.06 | 2.8(1-7.8) | 0.05 |
| No | 23 | 76.7 | Ref | | | |

Ref=References group

## Schistosomiasis infection

Factors which were found to be associated with **s**chistosomiasis infection were assessed and study finding revealed that the odds of getting **s**chistosomiasis infections were 2.2 times higher among those who were washing their clothes in rivers or dams than those who were not. By considering occupation students were 4.4 times more likely to get **s**chistosomiasis infections compared to peasant.

**Table 9: Multivariate analysis of factors associated with schistosomiasis infections**.

| Variable | Yes | % | COR(95%CI) | P-Value | AOR(95%CI) | P-Value |
|---|---|---|---|---|---|---|
| **Occupation** | | | | | | |
| Students | 53 | 59.6 | 4.5(2.6-7.8) | <0.001 | 4.4(2.3-8.2) | <0.001 |
| Peasant | 39 | 24.8 | Ref | | | |
| **Age group** | | | | | | |
| 16-22 | 5 | 25 | 0.2(0.1-0.7) | 0.004 | 0.8(0.2-3.5) | 0.7 |
| 23-31 | 9 | 30 | 0.3(0.1-0.7) | 0.004 | 1.6(0.3-8.8) | 0.6 |
| 32-50 | 16 | 22.2 | 0.2(0.1-0.4) | <0.001 | 1.1(0.2-5.3) | 0.9 |
| >50 | 10 | 26.3 | 0.2(0.1-0.6) | <0.001 | 1.3(0.2-7.1) | 0.7 |
| <16 | 52 | 60.5 | Ref | | | |
| **Level of education** | | | | | | |
| Primary | 78 | 44.1 | 3.1(1.6-6) | <0.001 | 1.7(0.8-3.6) | 0.1 |
| None | 14 | 20.3 | Ref | | | |
| **Showering out of home** | | | | | | |
| Yes | 37 | 51.4 | 2.3(1.3-4) | 0.004 | 1.6(0.9-3.0) | 0.1 |
| No | 55 | 31.8 | Ref | | | |
| **Washing clothes in river/dam** | | | | | | |
| Yes | 50 | 51.0 | 3(1.5-5.5) | <0.001 | 2.2(1.2-4) | 0.009 |
| No | 42 | 28.4 | Ref | | | |

Ref=References group

# CHAPTER FIVE

## DISCUSSIONS

In this health facility based cross sectional study of 246 participants presenting with typhoid fever like symptoms we demonstrated different etiologies including bacteria, parasites and to lesser proportion viruses. It is clearly known that typhoid fever continue to be burden in most developing countries where diagnostic capacities are not well developed. Several factors including emerging and re-emerging diseases have been associated with increase in the number of patients presenting with typhoid fever.

Lack of proper clinical cases definition and inability to identify etiology of fever due to lack of laboratory diagnostic capacity has led to the observed increase in the number of typhoid cases. In this study we use real time PCR diagnostic technique to analyze participants stool and blood sample aimed at characterizing enteric and blood etiologies among patients presenting with typhoid like fever in Buhigwe District, Kigoma region.

Our study results shows that parasites, viruses and bacteria (gram negative and gram positive) were detected in participant blood samples. Prevalence of bacterial infection in blood was (3.7%) the observed prevalence is in agreement with study done in Kenya by Mureithi M (27) in which prevalence of bacteria detected in blood was 4%. The other similarities were the detection of *S.aureus* in blood sample which was observed in both studies. Different bacteria were also detected in blood sample of study participants in which80% of detected bacteria were gram negative bacteria. This finding not in agreement with a five years study done in Dar es Salaam in which more gram positive bacteria were isolated in blood compared to gram negative. The observed different were possibly due to location of the study, duration and detection technique used between the two studies(37).

Eight different types of pathogens were detected in blood of study participants. These finding are not in agreement with several studies which comes out with high level of isolated or detected bacterial more than what we observe in our study. This can be explained by the fact that their

study populations were in patients who were admitted with significant level of diseases compared to ours which we enroll outpatients (21).

In most of hospitals and health facilities when patients presenting with typhoid fever like symptoms there is a great possibility clinician will end up requesting laboratory investigation to confirm the diagnosis. In our results we found that among all cases presenting with typhoid fever like symptoms only one participant was found to have salmonella infection. Our observed results are not in agreement with study done to detect salmonella typhi in patients with febrile illness in which more than 60% were found to have salmonella infections(44).

Both malaria and typhoid are known as major diseases of public health important and cause of morbidity and mortality in tropical and subtropical countries in Africa(22). It is clearly known that there is a considerable overlapping of sign and symptoms between the two infections(45). Our study found that out of all study participants who were presenting with typhoid fever like symptoms 65.4% were having malaria parasite. The observed high prevalence of malaria is in contrast with study conducted in Abia state in Nigeria (37).Despite high prevalence of malaria infection the rate of co-infection between malaria and salmonella among study participants were too low (0.4%).The observed high malaria prevalence can be explained by several factors but we assume collection of data near the end of rain season may be among the contributing factors as is the conducive period for high mosquito breeding.

The most prevalent detected enteric bacteria were Enteroagrigative E.coli (EAEC).Others were *Shigella spp* and O157. The observed results differ from study done in Nigeria(46) in which they observed1.8% prevalence of *Salmonella* which is slightly higher compare to the one observed in Buhigwe(0.4%). In our study the prevalence of pathogenic form of E.coli was 0.8% these finding are not in agreement with study done in Nigeria in which they didn't isolate pathogenic form of *E.coli* (O157). Among other factors sensitivity of Real time PCR compared to culture technique can be considered as contributing factors.

Another result observed were the detection of other group of E.coli (EAEC, EPET, ETEC and others).These group of *E.coli* are not commonly isolate in routine culture diagnostic technique in different hospitals in Tanzania. Detection of these *E.coli* subtypes in this study was different from study done in southwest Nigeria(46) in which prevalence of isolated E.coli subtype was

62%.This is higher compared to 38.2%. The observed difference was possibly due to difference in study designs used and diagnostic approach, the study design used in southern western Nigeria was case control study and they use culture together with PCR while this was cross section study and only PCR technique was used.

Other pathogenic microorganisms apart from bacteria were screen in participants blood sample among them were blood parasites. Results show that 65.4% were having malaria infection. The observed prevalence is higher compare to study done in Kilosa Morogoro Tanzania, Kilosa district is an area with holoendemic malaria transmission with seasonal peak following long and short rainy seasons. Prevalence of Malaria in this district was found to be 23.0% (47).

As we were asses some factors which were associated with blood pathogens and Plasmodium spp being the most prevalence blood pathogens found, the following factors found to be associated with being infected by plasmodium species.

Living in Kajana ward was significant found to be associated with malaria infection among Buhigwe community members. Those who live in Kajana were 2.3 more likely to be infected with malaria parasites compared to those who live in Muyama, Songambele and Muzenze. The second significantly associated factor was not having mosquito net in which the odds of having malaria infections were 3.5 times higher among those who were not having mosquito net than to those who own mosquito net.

This observation were different from those observed in Tubu village in northern Botswana(48) in their study they found no significant association between not possessing mosquito net and getting malaria infections. The observed difference possibly may be due to different study site and study design used.

In our study we found that living in Kajana and drinking untreated water was statistically significant associated with acquiring enteric infection. The associated factors of drinking untreated water was in agreement with study done in Kenya(43). As most of developing countries facing challenges in obtaining clean and safe drinking water, preparation of drinking water is among the encouraged hygiene practices emphasized in communities so as to protect community members against different water born disease.

Other non adherence to hygiene practices was also found to be associated with acquiring enteric infections, practice like hand washing style by dipping hands in a container with water, not washing hands after visiting toilet, not washing hands by using soap even though these factors was not statistically significant .

The odds of washing clothes in rivers or dams were 2.6 higher among individuals who were having schistosomiasis infection than those who were not having schistosomiasis. Being student was also found to be associated with schistosomiasis infections but the association was not statistically significant. Our observed risk of getting schistosomiasis infection was higher compared to study done in Yemen which they found the odds were 1.3 times higher among those who bath in open water source than those who don't(49).The observed different possibly was due to the fact that study done in Yemen was cross section community based and only children were involved.

## CONCLUSIONS

From our study results we concluded that Plasmodium species, Schistosoma species, Giardia species and to a lesser extent other enteric and blood pathogens may commonly be present in patients presented with typhoid fever like symptoms or in patients suspected of typhoid fever.

Poor personal hygiene practices like drinking untreated water contribute much in acquiring enteric infections among community members. Malaria infections was significant associated with not having mosquito bed net among community members especially those who reside in Kajana ward, lastly taking shower or washing clothes in open water bodies in Kajana wards like rivers and dams is associated with schistosomiasis infestation.

## RECOMMENDATIONS

We recommend the following:-

There should be periodic investigations or studies in Buhigwe district following any interventions which will be done specifically in area of malaria, **s**chistosomiasis and **g**iardiasis. This will assist in in-depth speculation of individual factors associated with these pathogens as our study has already provide broad pictures of circulating enteric and blood pathogens. Gold standard diagnostic methods should be available in all health facilities to facilitate differential diagnostic as signs and symptoms presented overlap each other among different diseases.

There should be mass campaign on preventions procedures for malaria, giardiasis and schistosomiasis together with emphasis on personal hygiene improvement among Buhigwe community members so as to protect themselves from mentioned diseases and discourage multiplications of different causative agents of enteric infections that thrive in poor hygiene environment.

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# ANNEXES

## Annex 1 Questionnaire English version

Questionnaire No.……Name of health facility……

1. Participant age (Years)…….....Gender Male ☐ Ke ☐

2. Participants Village…….Wad…….Hamlet..……

3. Marital status: A) Married ☐ B) Single ☐ C) Widow ☐ D) Divorce ☐
E) Cohabiting ☐

4. Participants occupation. A) Farmer ☐ B) Businessman ☐ C) Pastoralist ☐

D) Formal employee ☐ E) Mwanafunzi ☐

F)……

5. Number of family members ……..

6. Level of education A) Primary ☐ B) Secondary form four ☐ C) Secondary form six ☐

D) College after form four ☐ E) Colleges after six ☐

F) University ☐ G) None ☐

7. Do you have any of the following signs and symptoms?

| | | | | | | |
|---|---|---|---|---|---|---|
| Headache | Yes | No | | Vomiting | Y | N |
| Abdominal pain | Y | N | | Diarrhoea | Y | N |
| Fever | Y | N | | General body weakness | Y | N |
| Joint pain | Y | N | | Hematuria | Y | N |
| Nausea | Y | N | | | | |
| Having blood stained stool | Y | N | | | | |

8. Where do you get your daily drinking water? A) Community tape ☐

B) Dam ☐ C) River ☐ D) From deep well ☐ E) Rain water ☐
F)……

9. How do you prepare your drinking water at home? A) Boiling ☐ B) Chlorination C) No any preparation ☐

10. Do you have mosquito net? A) Yes ☐ B) No ☐

11. How many times do you use your mosquito net? A) Every day when I go to sleep ☐

B) Sometimes ☐

12. What is the status of your mosquito net A) New ☐ B) Old with some holes ☐

13. Do you use mosquito insect side at your home A) Yes ☐ B) No ☐

14. Do you wash your hands before meal? A) Yes everyday ☐ B) No ☐

15. How do you wash your hands before meal? A) By dipping hands in a container with water ☐ B) Using running water poured by someone else ☐ C) By using running water from the tape ☐

16. Do you use soap? A) Yes ☐ B) No ☐

17. Do you have a habit of eating outdoor? A) Yes once per week ☐ B) Yes 2 to 4 times per week ☐ C) Yes all of the week ☐ D) No I don't have habit of eating outdoor ☐

18. Do you have a toilet? A) Ndiyo ☐ B) Hapana ☐

19. If yes what kind of a toilet do you have? A) Pit latrine build with bric ☐ B)Pit latrine covered with branches of trees ☐ C) Modern sink latrine ☐

20. If question 18 is yes is there water inside the toilet? A) Yes tape water ☐

B) Yes water in a bucket ☐ C) No there is no water inside the toilet ☐

21. Do you have a habit of washing hands after visiting toilet? A) Yes every time ☐ B) Yes sometimes ☐ C) No ☐

22. If yes do you use soap? A) Yes ☐ C) No ☐

23. Where do you usually take shower? A) Home bathroo ☐ B) In the riv ☐ C) In ☐ dams

24. Do you have a habit of washing clothes in rivers or dams? A) Yes ☐ B) No ☐

25. If yes mention the name of the river or dam …….....

26.Do you keep animals A)Yes ☐ B)No ☐

27. Mention what types of animal do you keep …….

THANKS FOR PARTICIPARTING.

**Annex 2 Dodoso la kushiriki kwenye utafiti**

Namba ya dodoso……Jina la hospital/Kituo cha afya……

1. Umri wa mshiriki (Miaka)……...Jinsia ya mshiriki Me ☐ Ke ☐

2. Kijiji atokako mshiriki…….Kata…….Mtaa……

3. Hali ya ndoa: A) Nimeoa/Nimeolewa ☐ B) Sijaoa/sijaolewa ☐ C) Mjane ☐ D) Tumetengana ☐ E) Ninaishi na mpenzi ☐

4. Kazi ya mshiriki. A) Mkulima ☐ B)Mfanyabiashara ☐ C)Mfugaji ☐

D) Mwajiriwa ☐ E) Mwanafunzi ☐ F)……

5. Idadi ya watu katika familia ……..

6. Kiwango cha elimu A) Elimu ya msingi ☐ B) Kidato cha nne ☐ C) Kidato cha sita ☐

D) Mafunzo baada ya kidato cha nne ☐ E) Mafunzo baada ya kidato cha sita ☐

F) Elimu ya chuo kikuu ☐ G) Sijasoma ☐

7. Je una dalili yeyote kati ya hizi zifuatazo?

| Maumivu ya kichwa | Ndiyo | Hapana | | Kutapika | N | H |
|---|---|---|---|---|---|---|
| Maumivu ya tumbo | N | H | | Kuharisha | N | H |
| Homa | N | H | | Mwili kukosa nguvu | N | H |
| Kukosa nguvu kwenye viungo | N | H | | Kujisaidia haja ndogo yenye damu | N | H |
| Kichefuchefu | N | H | | | | |
| Kujisaidia haja kubwa yenye damu | N | H | | | | |

8. Maji ya namna gani huwa mara nyingi unatumia kwa kunywa? A) Maji ya bomba ☐

B) Maji ya bwawa ☐ C) Maji ya mto ☐ D) Maji ya kisima cha kuchimbwa ☐

E) Maji ya mvua kutoka kwenye bati la nyumba ☐ F)……..

9.Ni njia gani unatumia kuandaa maji yako ya kunywa nyumbani? A) Nayachemsha ☐

B) Nayatibu kwa dawa kama water guard ☐ C) Hakuna maandalizi yeyote ninayoyafanya ☐

10. Una chandarua nyumbani? A) Ndiyo ☐ B) Hapana ☐

11. Mara ngapi unakitumia chandarua chako? A) Kila siku nikilala ☐ B) Mara moja moja ☐

12. Chandarua unachokitumia wewe kina hali gani A)Kipya ☐ B)Kimechakaa/kina matobo ☐

13.Huwa unatumia dawa ya kupulizia kuuwa mbu nyumbani A) Ndiyo ☐ B) Hapana ☐

☐ ☐

14. Huwa unanawa mikono kabla ya kula? A) Ndiyo kila mara B) Hapana

15.Ni namna gani unanawa mikono kabla ya kula? A) Kwa kutumbukiza mikono kwenye chombo chenye maji ☐ B) Kwa kumiminiwa na mtu mwingine ☐ C) Kunawia kwenye bomba inayotiririsha maji ☐

16. Huwa unatumia sabuni? A) Ndio ☐ B) Hapana ☐

17. Je huwa una mazoea ya kula vyakula nje ya nyumbani kwako mfano magengeni au mama ntilie/lishe?

A) Ndio mara moja kwa wiki ☐ B) Ndiyo mara 2 hadi 4 kwa wiki ☐ C) Ndiyo wiki nzima ☐

D) Hapana ☐

18. Una choo nyumbani kwako?

A) Ndiyo ☐ B) Hapana ☐

19. Kama jibu ni ndio choo ulicho nacho ni cha namna gani A) Cha shimo kilichojengwa kwa matofali ☐ Cha shimo chenye uzio wa nyasi/miti C☐na sinki ☐

20. Kama jibu la swali la 11 ni ndio je ndani ya choo chako kuna maji? A) Ndiyo maji ya bomba ☐ B) Ndiyo maji kwenye chombo kama ndoo ☐ C) Hapana hakuna maji ☐

21Je huwa una nawa mikono baada ya kutoka kujisaidia? A) Ndio kila mara ☐ B) Ndio mara ☐ moja moja ☐ C) Hapana ☐

22. Kama jibu ni ndio je huwa unatumia sabuni A) Ndio ☐ C) Hapana ☐

23. Mara nyingi huwa unaoga wapi? A) Bafu la nyumbani ☐ B) Mtoni ☐ C) Kwenye bwawa ☐

24. Huwa una mazoea ya kufulia nguo mtoni au kwenye maji yaliyotwama kama bwawa

A) Ndiyo ☐ B) Hapana ☐

25. Kama ni ndio ni mto upi taja jina la mto /bwawa …….....

26.Una mifugo nyumbani kwako A)Ndio ☐ B) Hapana ☐

27.Taja aina ya mifugo …….

ASANTE KWA KUSHIRIKI.