

OCCURENCES OF *Salmonella typhi* IN DRINKING WATER SOURCES AS A RISK FACTOR
OF TYPHOID FEVER OUTBREAK IN THE COMMUNITY OF PAYA SUBCOUNTY, TORORO-
UGANDA

SSENKIMA BENSON

BU/UP/2018/3545

A RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF BIOLOGY IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
BACHELOR OF SCIENCE WITH EDUCATION (BIOLOGICAL) OF BUSITEMA UNIVERSITY

JUNE, 2022

DECLARATION

This thesis is my original work and has not been submitted for a degree or any other award elsewhere.

Signature.....

Date.....

Benson Ssenkima

BU/UP/2018/3545

Department of Biology

APPROVAL BY THE SUPERVISORS

This thesis has been submitted to the Department of Biology, Faculty of Science and Education with approval of the following supervisors:

Signature.....

Date.....

Dr Hannington Ochieng
Department of Biology
Busitema University

Signature.....Date.....

Ms Flavia Natukunda
Department of Biology
Busitema University

ACKNOWLEDGEMENT

A project report like this cannot come to a conclusion in isolation, owing to the complexity and time demanding that comes with it. I want to say **THANK YOU**, to the highly trusted **Almighty GOD**, who really hears me every time as I turn to Him for knowledge, guidance, protection, support and good health. I prayed for his facilitation throughout the whole process of this project. I remember my parents, lecturers and colleagues at the university who believed that I

could do this, even when my instincts told me something different. Cheers to my supervisors, **Dr. H. Ochieng** and **Ms F. Natukunda** for the concerted efforts who made sure this is possible. The supervisors have really guided me with the positive energy that has kept me moving to the right direction. Finally, it's worth mentioning the help rendered by the Laboratory Technician of Biology laboratory especially in regard to the microbiological work, at some point, I didn't know the reagents and apparatus to use but he came in for my rescue!

ABSTRACT

Typhoid fever is a major cause of human mortality throughout large areas of the world. Human typhoid occurs following the ingestion of the *Salmonella typhi* bacteria, mostly found in contaminated water, animal products or close association with an infected individual. This study investigated the bacterial load of the water samples, and identified of *Salmonella typhi* bacteria isolated from different drinking water sources in Paya Sub County. Sampling and collection of the water samples took three weeks with one sampling per week. Aseptically, 100 ml of each of the samples of river and borehole, well, and tap water were collected from different sources and sites in Paya sub county. 5 petri dishes of XLD (xylose lysine deoxycholate) Agar were inoculated with each water sample by streak method at 37°C for 24hrs. Colonies on nutrient agar were counted using colony counter technique and the plates having colonies were recorded. Pure cultures of salmonella were made on TSA (tryptic soy agar) which were then gram stained before microscopy. Clear red rod-shaped colonies with black centers, gram -negative rods clustered together (staphylobaccilli) were observed in the microscope. Analysis of variance (ANOVA) at 5% confidence level was used to compare all numeric analysis for the total plate count were made using the F-parametric test where statistical significance existed between the observed two data sets . The null hypothesis was therefore rejected; and alternative hypothesis accepted. Therefore, the stream and pond water sources contained significant bacterial loads that were responsible for the persistent typhoid fever outbreaks in Paya community. Conclusively, the natives were advised to improve on their personal and environmental hygiene, boil water for drinking, emphasize regular hand washing, eating hot food and ensure proper human waste disposal. However, more research needs to be done to explore further on the water quality in relation to typhoid fever, multi-drug resistance of salmonella in many typhoid patients and the cause of this drug resistance.

TABLE OF CONTENTS	
DECLARATION	i
APPROVAL BY THE SUPERVISORS	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	v
LIST OF TABLES	vi
LIST OF GRAPHS	vi
LISTS OF ABBREVIATIONS	vi
CHAPTER ONE:	1
INTRODUCTION	1
1.1 Background.	1
1.2 Problem statement.	2
1.3. AIM of the Study:	2
1.4 Justification of the study.	2
1.5 Research Questions	3
1.6 Study Hypotheses	3
1.7 OBJECTIVE.	4
1.7.1 Specific Objectives:	4
CHAPTER TWO:	5
LITERATURE REVIEW	5
2.1 History of Typhoid Fever.....	5
2.2 The etiology of typhoid fever.....	5
2.3 Clinical Manifestations of Typhoid Fever	5
2.4 Major Risk Factors Leading to the Spread of Typhoid Fever in Paya sub county.....	6
2.4.1 Used Water and Sewage Discharge.....	6
2.4.2 Water Sources.....	7
2.5 Prevention of typhoid fever.....	7
CHAPTER THREE:	9
METHODS AND MATERIALS	9
3.0 Sampling locations:	9
3.1 Drinking water sources:.....	9
3.2 Sample collection.....	9

3.3 Inoculation and Incubation of the Prepared Samples.....	9
3.4 Data collection.....	10
3.5 Data processing and analysis.....	10
CHAPTER FOUR.....	11
RESULTS.....	11
4.1 COLONY CHARACTERISATION.....	11
4.2 ANALYSIS OF THE RESULTS.....	16
4.3 Analysis of variance (ANOVA).....	18
CHAPTER FIVE.....	21
5.1 DISCUSSIONS.....	21
5.2 CONCLUSIONS.....	22
5.3 RECOMMENDATIONS.....	22
REFERENCES.....	24

LIST OF FIGURES

Figure 1 : shows the slopes being prepared for making a pure culture of Salmonella typhi in McCartney bottles.....	12
Figure 2: shows some plates of stream water and well water whereas borehole and tap water plates were clear with no growth of the Petri plates of the cultured salmonella on XLD agar.....	12
Figure 3: represents Petri plates of stream water and tap water . There is some evident bacterial growth on the former whereas the latter are clear with no growth of salmonella.....	12
Figure 4: represents the images of the gram stained bacteria of the first sampling after being viewed under microscope. The bacteria were seen to be clustered around each other in very many clusters.....	13
Figure 5: represents images of the gram stained bacteria of the second sampling as seen under microscope.....	14
Figure 6: represent images of the gram stained bacteria of the second sampling as seen under microscope.....	15
Figure 7: shows Pictures which were taken from the site of collection in the field and they are of the well and stream respectively.....	16

LIST OF TABLES

Table 1: showing results of the first sampling. Date: 12/02/2022.....	13
Table 2: showing results of the second sampling. Date: 18/02/2022.....	14
Table 3: showing results of the Third sampling. Date: 25/02/2022.....	15
Table 4: Average number of colonies per sampling.....	16
Table 5: summary of statistics for the ANOVA Test.....	17
Table 6: ANOVA Test calculations.....	17

LIST OF GRAPHS

Graph 1: showing the number of colonies on each petriplate for each water sample of the first sampling.....	18
Graph 2: showing the number of colonies on each petriplate for each water sample of the second sampling.....	19
Graph 3: showing the number of colonies on each petriplate for each water sample of the third sampling.....	19
Graph 4: showing the number of colonies on each petriplate for each water sample of all the three samples.....	20
Graph 5: relating the number of colonies on each petriplate for the stream water and well water for all the three samples.....	21

LISTS OF ABBREVIATIONS

ANOVA: Analysis of variance

TSA: Tryptic soy agar

XLD: Xylose lysine deoxycholate

CFU: Colony forming units

S.typhi: *Salmonella typhi*

WHO: World Health Organization

MoH: Ministry of Health

DF: Degrees of Freedom

Ho: Null hypothesis

Ha: Alternative hypothesis

UNICEF: United Nations International Children Emergency Fund

CHAPTER ONE:

INTRODUCTION

1.1 Background.

Typhoid fever is caused by ingestion of food or contaminated water with *Salmonella typhi*. Symptoms include high fever, malaise, headache, abdominal pain, and constipation or diarrhea, and lead to sensitive intestinal destruction and death if untreated. Symptoms begin 1-6 weeks after exposure. Lack of improved sanitation and access to safe food and water among populations living in low and middle income countries is related to an elevated risk of typhoid fever.

Nearly 12 million cases and 130,000 deaths were estimated to have occurred worldwide in 2010. After adjusting for water-related risk factors (i.e. access to improved water), high incidence of typhoid fever has been reported in 45 African countries, and there has been an increase in the number of reported outbreaks throughout the African continent over the last 10 years. (Salaria, 2018)

Paya sub-county is in West Budama central constituency Tororo district found in Eastern Uganda is among the areas where typhoid fever has been endemic. It is somewhat densely populated with over 5,000 people. In early 2019, the typhoid fever outbreak began in the town council slums of Paya town council and subsequently spread throughout the sub-county and adjacent sub-counties in the district. The attack was higher in males (110/1000) than in females (84/1000) residents and higher in adults 15-59 than in children under 15 (20/1000) residents.

In response to water borne disease outbreak, public health officials often conduct an investigation to define the scope and potential causes of outbreak and inform implementation of effective control measures to reduce the disease burden. Despite the high incidence of typhoid fever globally, relatively few outbreak investigations incorporate drinking water testing and routine water testing for typhi is rarely done in regions where *S. typhi* is endemic. While these studies have provided useful information, more comprehensive sampling and testing strategies are needed to identify disease transmission risks and areas of focus for public health interventions.

The objective of this study is to determine the salmonella typhi in different drinking water sources in Paya Sub County in order to identify water sources that may have been contributing to the outbreak.

REFERENCES

- (Muyembe, B. R. (2009). Spatial Distribution of diarrhoea and microbial quality of domestic water. *Health, Population and Nutrition* , 652-659.
- Amoah E.D, B. R. (2005). Surveillance for for water borne disease outbreaks associated with drinking water. *MMWR morbidity and mortality weekly report* , 23-45.
- Anderson, K., & Davidson, P. (2010). Water microbiology, bacterial pathogens and water. *International journal of environmental research and public health* , 3657-36703.
- B., J., & Shively, D. F. (2015). Multistate outbreaks of salmonella serotype infections associated with drinking water. *Centres for disease Control and prevention* , 713-720.
- Bhan. (2015). Environmental analysis of water for bacteria. *Department of environment and primary prevention hygiene of water* , 299-304.
- Block, R. a. (2011). Distribution and genetic diversity of salmonella enterica in the upper swanee river. *International journal of microbiology* , 307-313.
- Butler . W. (2013). Threats to sources of drinking water and aquatic ecosystems health in canada. *National Water Research institute* , 39-50.
- Crump, e. (2004). Typhoid and other invasive salmonellosis. *vaccine-preventable disease surveillance standards* , 458-465.
- Farouqui. (2009). Microbial analysis of household wells and streams. *journal of water and health* , 56-69.
- G., C., Adingira, A., & Kouadio, A. (2012). Bacteriological analysis of surface water collected from the Grand-Lahou lagoon. *African journal of microbiological Research* , 77-82.
- Graham A, R. P. (2000). Typhoid fever in a slum in Dhaka. *Epidemiology & infection* , 458-465.
- Karuk, G., Zeng, B., Yang, Z., & Wang, C. (2012). Abundance of clinical enteric bacterial pathogens in coastal waters and shellfish. *water research* , 51-56.
- Mints, C. a. (2010). *WATER RESEARCH*. Alberta, Canada: water research institute.
- MOH. (2009). distribution, diversity and sesonality of water borne salmonellae in a rural watershed. *applied and environmental microbiology* , 75-81.
- N, A. (2009). Vulneribility of drinking water wells to enteric-virus contamination from surface water contributions. *Applied and environmental MICROBIOLOGY* , 1011-1019.
- Polo, F. A. (2012). Water microbiology, bacterial pathogens in water. *international journal of environmental research and public health* , 453-456.
- Pruss T, B. R. (2006). Serotyping, PCR, Phage typing and antibiotic sensitivity testing of

salmonella serovars isolated from urban drinking water sources. *BMC Public Health* , 899-904.

ROSA. (2013). microbial pollutants in our nation's water. *environmenal and public health issues* , 45-56.

Salaria. (2018). Analysis of microbial properties of water. *water research institute, CNR* , 315-318.

UNICEF. (2012). Salmonella contamination in various watersheds and microbiological diversity. *food borne pathogens and disease* , 666-675.

WHO. (2018). TYPHOID AND OTHER INVASIVE SALMONELLOSIS. *Vaccine-preventable diseases* , 15-19.

WHO. (2012). Analysis of drinking water for salmonellosis. *Progress in drinking water and sanitation* , 299-304.

WHO, U. (. (2012). Analysis of drinking water for salmonelosis. *progress on drinking water and sanitation* , 299-304.