

**DEVELOPING A GUIDE FOR BASELINE SALMONELLA AGGLUTININ
TITRES ACCORDING TO AGE, GENDER AND HIV STATUS IN
PATIENTS ATTENDING AT HOSPITALS IN NORTHERN NAMIBIA**

**A THESIS SUBMITTED IN FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF**

DOCTOR OF PHILOSOPHY (PUBLIC HEALTH)

OF

THE UNIVERSITY OF NAMIBIA

BY

SYDNEY CHIKUKWA

SEPTEMBER 2012

**SUPERVISORS: Dr S IIPINGE (University of Namibia) &
Dr PM. CHIMWAMUROMBE (University of Namibia)**

ABSTRACT

In Namibia, the Widal test (a blood test that detects level of *Salmonella* antibodies) is widely used in the diagnosis of typhoid fever. There are no available normal population baseline *Salmonella* agglutinins titres in Namibia which can be used to come up with country specific diagnostic titres for typhoid fever. The normal population baseline *Salmonella* agglutinin titre is used as the basis for the interpretation of the Widal test. Since there is no guide, it means that standardization of patient care in Namibia is not possible. The aim of the study was to develop a guide for the interpretation of diagnostic *Salmonella* agglutinin titres for typhoid fever after performing a Widal test. The objectives of the study were to; determine the prevalence of typhoid fever for age, gender and HIV status among patients attending five hospitals in northern Namibia; establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever; develop a conceptual framework based on the outcome of the situational analysis; develop a guide for baseline *Salmonella* agglutinin titres for age, gender and HIV status in patients attending hospitals in northern Namibia and finally to implement, monitor and evaluate the efficacy of the guide on baseline. Blood samples were collected from 400 subjects; 200 males and 200 females, of the 200 males 100 were children (<16years) and of the 200 females 100 were also children (<16years), half of each gender and age group were HIV positive and the other half HIV negative; the blood samples were examined for the presence and levels of *Salmonella* antibodies by Widal agglutination technique. Standard *S. typhi* O and H and *Salmonella paratyphi* AH and BH suspension (FORTRESS) were used as antigens. This study reports that Typhi O and Typhi H titres greater than 80 are

diagnostic of typhoid fever in the studied population regardless of age, gender and HIV status. Typhi O titres greater than 40 and Typhi H titres greater than 80 are diagnostic of typhoid fever in HIV positive patients whilst typhi O titres greater than 80 and typhi H titres greater than 40 are diagnostic of typhoid fever in HIV negative patients. The current diagnostic titre for typhoid fever of 160 for both typhi O and typhi H is too high and many typhoid cases are being missed and must be changed urgently, by the adoption of the guide developed by this study. The current Widal procedure should include a 1 in 120 dilution, to give a titre 120 between titre 80 and titre 160, so as to minimise the risk of missing cases of typhoid fever with titres greater than 80 but less than 160. This study has revealed that the current typhoid diagnostic titre of 160 for both typhi O and H is too high. A guide for the interpretation of Widal test has been developed specifically for northern Namibia. The guide can only be used in patients who are older than 2 years as these are capable of forming antibodies. This study has shown that there is a significant association between HIV and Salmonella agglutinin titres. This revelation could be used to lobby for policy change as in revision of treatment and management of HAART (Highly Active Antiretroviral Therapy) and typhoid vaccination policies. This study has shown that typhoid fever is highly prevalent in Northern Namibia and it calls for drastic public health intervention by all stake holders under the leadership of Ministry of Health and Social Services. Laboratories should implement a comprehensive quality assurance program to enhance the validity and reliability of the Widal test. This will improve accurate diagnosis and give the surest way to reverse the deteriorating health status of Namibians.

TABLE OF CONTENTS

ABSTRACT	i
TABLE OF CONTENTS	iii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
ABBREVIATIONS	xv
ACKNOWLEDGEMENTS	xvii
DEDICATION	xviii
DECLARATION	xix
CHAPTER 1: ORIENTATION AND BACKGROUND INFORMATION	
OF THE STUDY	1
1.1 INTRODUCTION	1
1.2 STATEMENT OF PROBLEM	8
1.3 DEFINITION OF CONCEPTS	9
1.4 THEORETICAL CONCEPTUALISATION OF THE STUDY	10
1.5 PURPOSE AND OBJECTIVES OF THE STUDY	12
1.5.1 Purpose of the study	12
1.5.2 Objectives	12
1.6 HYPOTHESES	12
1.7 SIGNIFICANCE OF THE STUDY	13
1.8 LIMITATIONS OF THE STUDY	14
1.9 SUMMARY	15
CHAPTER 2: LITERATURE REVIEW	16

2.1	INTRODUCTION	16
2.2	TRANSMISSION AND RISK FACTORS	19
2.3	GLOBAL BURDEN OF DISEASE	22
2.3.1	Asia and the Indian sub-continent	25
2.3.2	Typhoid fever in the African continent	27
2.4	BASELINE SALMONELLA AGGLUTININ TITRES IN POPULATIONS	29
2.5	THE ORGANISM <i>SALMONELLA</i>	33
2.6	PATHOGENESIS	34
2.6.1	Enteric fever (typhoid fever)	34
2.6.2	The disease pathology	34
2.7	DIAGNOSIS OF TYPHOID FEVER	35
2.7.1	Signs and symptoms	36
2.7.2	Complications of typhoid fever	37
2.7.3	Investigations	37
2.8	MANAGEMENT AND TREATMENT	38
2.8.1	Primary intervention	38
2.8.2	Secondary intervention	38
2.8.3	Clearance of chronic carriers	39
2.8.4	Health education	39
2.9	WIDAL AGGLUTINATION	39
2.9.1	Performance technique	42
2.9.2	Interpretation of the test results	44
2.9.3	Causes of negative Widal agglutination tests	46

2.9.4	Causes of positive Widal agglutination tests	46
2.9.5	Limitations of the Widal test	48
2.10	ANTIGEN DETECTION AS AN ALTERNATIVE TO WIDAL TEST	50
2.11	THE BIOMEDICAL MODEL	51
2.12	SUMMARY	56
	CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY	57
3.1	INTRODUCTION	57
3.2	RESEARCH DESIGN	57
3.2.1	Exploratory design	59
3.2.2	Descriptive design	60
3.3	RESEARCH METHOD	61
3.3.1	Phase one: needs assessment	61
3.3.2	Study population	62
3.3.2.1	Inclusion criteria	63
3.3.2.2	Exclusion criteria	64
3.4	SAMPLING	64
3.4.1	Sample size criteria	66
3.4.1.1	The level of precision	66
3.4.1.2	The confidence level	66
3.4.1.3	Degree of variability	67
3.4.2	Sample Collection	69
3.5	TESTING PROCEDURES	70
3.5.1	Rapid slide agglutination	71

3.5.2	Reading and interpretation	71
3.5.3	Tube agglutination	72
3.6	DATA COLLECTION	73
3.7	DATA ANALYSIS	74
3.8	PHASE TWO: CONCEPTUAL FRAMEWORK	74
3.9	PHASE THREE: DEVELOPMENT OF THE GUIDE FOR INTERPRETATION OF THE WIDAL TEST	74
3.10	PHASE FOUR: IMPLEMENTATION, MONITORING AND EVALUATION OF THE GUIDE	75
3.11	ETHICAL ISSUES	76
3.11.1	Permission	77
3.11.2	Confidentiality and anonymity	78
3.12	SUMMARY	78
	CHAPTER 4: RESULTS OF THE STUDY	79
4.1	INTRODUCTION	79
4.1.1	Purpose of the study	79
4.1.2	Overview of the Widal test	80
4.2	TESTING PROCESS	80
4.3	END TITRE DISTRIBUTION AMONG 400 SUBJECTS	81
4.4	END TITRE DISTRIBUTION BY AGE GROUP AMONG THE 400 SUBJECTS	84
4.4.1	<i>Typhi O</i> end titre distribution by age	85
4.4.2	<i>Typhi H</i> end titre distribution by age	87
4.4.3	<i>Typhi O</i> end titre distribution by HIV status	90

4.4.4	<i>Typhi H</i> end titre distribution by HIV status	92
4.4.5	<i>Typhi O</i> end titre distribution by gender	98
4.4.6	<i>Typhi H</i> end titre distribution by gender	100
4.5	SUMMARY	103
CHAPTER 5: CONCEPTUALISATION OF THE STUDY		104
5.1	PHILOSOPHICAL BASIS OF THE STUDY	104
5.2	CONCEPTUAL FRAMEWORK	105
5.2.1	The agent	108
5.2.1.1	Conclusion	109
5.2.2	The recipient	110
5.2.2.1	Conclusion	112
5.2.3	Dynamics, problems and challenges	112
5.2.3.1	Attitude	113
5.2.3.2	Knowledge and skills	113
5.2.3.3	Roles and functions	114
5.2.3.3.1	Conclusion	114
5.2.4	The procedure	115
5.2.5	Terminus/end point	115
5.3	SUMMARY	116
CHAPTER 6: DEVELOPMENT OF THE GUIDE FOR SALMONELLA AGGLUTININ TITRES ACCORDING TO AGE, GENDER AND HIV STATUS		117
6.1	INTRODUCTION	117

6.2	RATIONALE FOR DEVELOPMENT OF THE GUIDE ON THE INTERPRETATION OF THE WIDAL TEST	118
6.3	GUIDE DEVELOPMENT PROCESS	118
6.3.1	Key stakeholders	119
6.3.1.1	Meeting with stakeholders	120
6.3.1.2	Discussion points with stakeholders	121
6.3.1.3	Outcomes from stakeholders (feedback sessions)	121
6.3.1.3.1	Feedback from medical practitioners	121
6.3.1.3.2	Feedback from MoHSS	122
6.3.1.3.3	Feedback from NIP laboratory staff	122
6.3.1.3.4	Feedback from medical seminar	123
6.3.2	Amended draft guide	124
6.4	IMPLEMENTATION, MONITORING AND EVALUATION OF THE GUIDE	124
6.4.1	Implementation, Monitoring and Evaluation Stakeholders	124
6.4.2	Feedback from stakeholders	125
6.4.2.1	Feedback from medical practitioners	125
6.4.2.2	Feedback from NIP laboratory staff	126
6.4.3	Final guide on interpretation of Widal test	127
6.5	THE GUIDE FOR INTERPRETATION OF THE WIDAL TEST ACCORDING TO HIV STATUS IN NORTHERN NAMIBIA	127
6.5.1	Guideline 1: Specimen collection	128

6.5.1.1	Construct 1: All illness and symptoms and signs within the body arise from underlying disease	128
6.5.1.2	Objective	128
6.5.2	Guideline 2: Interpretation of Widal test	129
6.5.2.1	Construct 2: All disease give rise to symptoms	129
6.5.2.2	Objective	130
6.5.3	Guideline 3: Deciding if it is a current or past infection	132
6.5.3.1	Construct 3: Health is the absence of disease	132
6.5.3.2	Objective	132
6.5.4	Guideline 4: Screening of food handlers by a Widal test	133
6.5.4.1	Construct 2: All disease give rise to symptoms	133
6.5.4.2	Objective	133
6.5.5	Guideline 5: Performance of Widal test in the laboratory	135
6.5.5.1	Objective	135
6.6	SUMMARY	136
	CHAPTER 7: CONCLUSION AND RECOMMENDATIONS	137
7.1	INTRODUCTION	137
7.2	PURPOSE AND OBJECTIVES OF THE STUDY	137
7.3	CONCLUSION	138
7.3.1	Objective 1: To determine the prevalence of typhoid fever among patients attending hospitals in northern Namibia	138
7.3.2	Objective 2: To establish age, gender and HIV status presumptively diagnostic <i>Salmonella</i> agglutinin titres for the diagnosis of typhoid fever	139

7.3.2.1	General presumptive diagnostic <i>Salmonella</i> agglutinin titres	139
7.3.2.2	Age dependent presumptive diagnostic <i>Salmonella</i> agglutinin titres	141
7.3.2.3	Gender dependent presumptive diagnostic <i>Salmonella</i> agglutinin titres	142
7.3.2.4	HIV status dependent presumptive diagnostic <i>Salmonella</i> agglutinin titres	143
7.3.3	Objective 4: To develop a guide for baseline <i>Salmonella</i> agglutinin titres for age, gender and HIV status among patients attending hospitals in northern Namibia	145
7.3.4	Objective 5: To implement, monitor and evaluate the efficacy of the guide on baseline <i>Salmonella</i> agglutinin titres	145
7.4	RECOMMENDATIONS	146
7.4.1	Recommendations for objective 1	146
7.4.2	Recommendations for objective 2	147
7.5	UNIQUE CONTRIBUTIONS OF THIS STUDY	152
7.6	RECOMMENDATIONS FOR PRACTICE	153
7.7	RECOMMENDATION FOR EDUCATION	154
7.8	RECOMMENDATION FOR RESEARCH	154
7.9	SUMMARY	155
	REFERENCES	156
	ANNEXURE A: UNAM Letter of Permission 1	174
	ANNEXURE B: MoHSS Ethical Clearance 1	175
	ANNEXURE C: NIP Letter of Permission 1	176

ANNEXURE D: A guide for interpretation of Widal test in the diagnosis of typhoid fever	178
ANNEXURE E: Amended guide for interpretation of Widal test in the diagnosis of typhoid fever	182

LIST OF TABLES

Table 2.1	Summary of the integration of biomedical model of illness: <i>Salmonella</i> and HIV status of persons	55
Table 3.1	Total number of sample stratified according to age, gender and HIV status	70
Table 3.2	Preparation of double dilution for tube dilution method	73
Table 4.1	End titre distribution among 400 subjects	81
Table 4.2	<i>Typhi O</i> end titres distribution by age group among the 400 subjects	85
Table 4.3	<i>Typhi H</i> end titres distribution by age group among the 400 subjects	89
Table 4.4	<i>Typhi O</i> end titres distribution by HIV status among the 400 subjects	91
Table 4.5	<i>Typhi H</i> end titres distribution by HIV status among the 400 subjects	93
Table 4.6	Baseline and diagnostic <i>Salmonella</i> agglutinin titres by HIV status	97
Table 4.7	<i>Typhi O</i> end titres distribution by gender among the 400 subjects	98
Table 4.8	<i>Typhi H</i> end titres distribution by gender among the 400 subjects	101
Table 6.1	Widal test interpretation chart	131

**Table 7.4 Presumptively diagnostic titres for typhoid fever according
to HIV status**

149

LIST OF FIGURES

Figure 2.1	The application of the biomedical model in disease diagnosis	52
Figure 4.1	Percent end titre distribution among the 400 subjects	82
Figure 4.2	<i>Typhi O</i> percent end titre distribution by age among the 400 subjects	86
Figure 4.3	<i>Typhi H</i> end titre distribution by age among 400 subjects	90
Figure 4.4	<i>Typhi O</i> end titre percent distribution by HIV status	91
Figure 4.5	<i>Typhi H</i> end titre percent distribution by HIV status among 400 subjects	94
Figure 4.6	<i>Typhi O</i> end titre percent distribution by gender among the 400 subjects	99
Figure 4.7	<i>Typhi H</i> end titre percent distribution by gender among the 400 subjects	102
Figure 5.1	Reasoning map	107

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Clinic
ART	Anti Retroviral Therapy
ARVs	Anti Retro Virals
CDC	Centre for Disease Control
CD4	Cluster Designate 4
CMO	Chief Medical Officer
DOMI	Disease of Most Impoverished
DNA	Deoxy Ribonucleic Acid
DRC	Democratic Republic of Congo
FBC	Full Blood Count
GDP	Gross Domestic Product
HLA	Human Leukocyte Antigen
HIV	Human Immune Virus
IHO	Intermediate Hospital Oshakati
IL	Inter Leukin
IR	Incidence Rate
LAC	Legal Assistance Centre
LIS	Laboratory Information System
MCS	Microscopy Culture and Sensitivity
MoHSS	Ministry of Health and Social Services
NIP	Namibia Institute of Pathology

NTS	Non-Typhoid Salmonella serovars
PHC	Primary Health Care
SADC	Southern African Development Community
SPSS	Statistical Program for Social Sciences
TB	Tuberculosis
TNF	Tumour Necrosis Factor
UNAIDS	Joint United Nations Program on HIV/AIDS
USA	United States of America
WHO	World Health Organisation

ACKNOWLEDGEMENTS

Glory is to God, Ebenezer; you have taken me this far through your strength.

I wish to express my sincere gratitude to the following people:

I greatly appreciate the generosity of Namibia Institute of Pathology (NIP) for funding this thesis; Berlin Dermatology Foundation for paying my tuition fees for the first two years of my study, Dr. S. Ipinge, my supervisor, for guiding me meticulously throughout my work. I would like to thank Dr. P. Chimwamurombe my co-supervisor, for his patient guidance, advice and encouragement throughout the course of this work. I humbly thank the administrators at University of Namibia for allowing me to study at their institution.

I would also like to thank Mr. M.L. Munro for editing my proposal and all my colleagues, relatives and friends who assisted and wished me well during this endeavour. To all medical practitioners and laboratory staff that worked hard in the finalisation of the Guide on the interpretation of Widal test, I thank you all and this project will not have succeeded without your effort.

May almighty God bless all of you.

DEDICATION

This thesis is dedicated to my wife, Portia, for her continual encouragement, support and love and to my beautiful daughters, Lisa, Lisy and Lisian. Let this accomplishment be a source of inspiration. To my father Maxwell Chikukwa, thank you for believing in me and this is your reward for investing in education. I dedicate this document to all my best friends I lost in touch during the years I was working on this document.

DECLARATION

I hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree from any other institution of higher education. No part of this thesis may be reproduced, stored in a retrieval system, or transmitted in any form, or by any means (e.g. electronic, mechanical, photocopying, recording, or otherwise) without the prior permission of the author, or the University of Namibia on his behalf. I grant the University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which the University of Namibia may deem fit, for any person or institution requiring it for study and research; providing that the University of Namibia shall waive this right if the whole thesis has been or is being published in a manner satisfactory to the University.

.....

Date.....

SYDNEY CHIKUKWA

CHAPTER 1

ORIENTATION AND BACKGROUND INFORMATION OF THE STUDY

1.1 INTRODUCTION

Salmonella infections in humans are divided into typhoid fever caused by *S. typhi* and *S. paratyphi*, and a range of diarrhoeal disease caused by a large number of non-typhoid *Salmonella* serovars (NTS). These NTS, usually have a broad vertebrate host range, and show dramatically more severe and invasive presentation in immune compromised individuals; especially HIV carriers. Also included are patients with severe and progressive disease, such as chronic granulomatosis disease, blockading of IL-12/ IL-23/IL-17 and TNF suppurative foci and bacteraemia which may be recurrent (WHO, 2010). Invasive recurrent NTS bacteraemia associated with HIV disease is becoming a huge problem worldwide (Gordon, 2008).

Typhoid fever is a systemic infection which is caused by the typhoid bacillus *Salmonella enteritica serovar typhi* (commonly referred to as *S. typhi*). It is the most common cause of enteric fever; this also includes paratyphoid fever caused by *S. paratyphi* A, B and C (WHO, 2010). These pathogens only infect humans and the disease is transmitted by ingestion of food, including dairy products, or water contaminated by excreta from patients or chronic carriers or handled by infected persons (Levine, 2008).

Typhoid fever is spread by the faecal oral route and is closely associated with poor hygiene, lack of clean drinking water and inadequate sanitation. The disease is almost exclusively transmitted by water and food contaminated by the faeces and urine of patients and carriers. Polluted water is the most common source of transmission (WHO/CDC, 2003). However, shellfish taken from sewage-contaminated beds, uncooked vegetables that are fertilised with night-soil, contaminated milk, and milk products, are known sources of infection (WHO, 2010).

Although typhoid fever has practically disappeared from industrialised countries, it remains a serious public health problem in several Asian regions of the former USSR (Union of Soviet Socialist Republics) in parts of South and Southeast Asia, in Africa and in South America. In the last typhoid outbreak in the Democratic Republic of Congo (DRC), between 27 September 2004 and early January 2005, no less than 42 564 cases were reported, including 214 death and 696 cases of peritonitis and intestinal perforations (WHO, 2010). Typhoid is an important cause of morbidity in many regions across the world, with an estimated 13 million cases occurring in Asia alone (House, Wain, Ho, Diep, Chinh, & Bay 2001).

According to the World Health Organisation (WHO, 2010) the burden of typhoid fever in developing countries is difficult to estimate. However, it is estimated that 22 million (range 16 to 33 million) cases occur annually causing 216 000 deaths, predominantly in school aged children and young adults (Crump, Luby & Mintz, 2004). Asia with 274 cases per 100 000 persons has the highest incidence of typhoid fever worldwide, especially in Southeast Asian countries and on the Indian sub-

continent. These regions are followed by sub-Saharan Africa, and Latin America, with 50 cases per 100 000 persons (WHO, 2009). On the African continent, East and Central Africa have the highest incidence of typhoid fever; countries such as Nigeria, Senegal, DRC and Kenya are the hardest hit (Onunkwo, Nwonkwo & Umolu, 2001). In an urban slum in Dhaka the incidence of bacteraemic typhoid fever was found to be 390 cases per 100 000 persons. This is a nine fold higher risk for pre-school children than older persons (Brooks, Hossain, Goswami, Nahar, Alam & Ahmed, 2005). A prospective population-based disease-surveillance study conducted by the Disease of Most Impoverished (DOMI) programme, which was supported by the Bill and Melinda Gates Foundation, focused on five sites: China, India, Indonesia, Pakistan and Vietnam. The findings revealed high rates of typhoid fever among children in urban slums, including children below five years of age. In three urban slums of Karachi, Kolkata, and North Jakarta, the incidence of blood-confirmed typhoid fever cases among five to 15 year olds ranged from 180 cases to 494 cases per 100 000 (DeRoeck, Jodar & Clemens, 2007).

Typhoid fever is prevalent in developing countries due to many interrelated factors; these include amongst others, variable efficacies of currently available vaccine preparations. Unplanned urbanisation causes growth of peri-urban slums which lack safe water supply and sanitation facilities. Furthermore, increased movements of large numbers of migrant workers make a developing country the hub of typhoid fever (Mishra, Gupta, Ali, Nath & Chandral, 1994). The extra-intestinal infections caused by *Salmonella* are very fatal. The incidence of typhoid fever remains very high in impoverished areas and the emergence of multidrug resistance have made the

situation worse. To combat and to reduce the morbidity and mortality caused by typhoid fever, many preventive measures and strategies have been employed, the most important being vaccination. In recent years, many *Salmonella* vaccines have been developed including live attenuated as well as DNA vaccines and their clinical trials have shown encouraging results. But with the increasing antibiotic resistance, the development of potent vaccine candidate for typhoid fever is a need of the hour (Sanndhya, Amit, Vidya and Dipshikha, 2012).

In 2000 and 2008, WHO has explained the importance of vaccine against typhoid fever. There have been many efforts done by different groups of scientists to develop an effective vaccine against *Salmonella*. But at present, only two licensed vaccine for typhoid fever - a subunit (Vi PS) and a live attenuated *S. Typhi* strain (Ty21a) are commercially available. Continuous efforts are being undertaken to develop typhoid vaccine with the advancement of Vi polysaccharide conjugate vaccine and live attenuated *Salmonella* strain to attain higher antibody titres and increased immunogenicity (Garmory, Griffin, Leary, Perkins, Brown & Titball, 2002). Thus vaccination turns to interfere with interpretation of the Widal test results. In Namibia, there is no vaccination initiatives for the general population. This is mainly because Namibia is not considered a typhoid endemic area.

In many typhoid endemic areas the human immune deficiency virus (HIV) infection is a serious public health concern (UNAIDS, 2006, UNAIDS, 2007). Although not reported from other endemic areas, a study done in Peru indicated that typhoid fever was 60 times more frequent in HIV infected individuals as compared to the general

population. This could presumably be due to HIV-induced impairment of the host's natural antibacterial activity against *S. typhi* and direct faecal oral route transmission of *Salmonella* within the homosexual population (Khan, 2004).

Although there is limited data on the prevalence of typhoid fever in Southern Africa, it is most likely that the burden is immense in view of lack of safe drinking water and sanitation facilities in all the countries; especially in rural communities which comprise more than 50% of the population. The other factor that tends to augment the burden of typhoid fever is the HIV/AIDS (Acquired Immunodeficiency Syndrome) pandemic in Southern Africa which is the hardest hit region in the world. It is estimated that 22.4 million people are living with the HIV in Southern Africa (UNAIDS, 2009). In the SADC (Southern African Development Community) region Swaziland has a national prevalence estimated at 42%, Botswana 25%, Namibia 17.8% and Zimbabwe 15.8% (UNAIDS, 2009).

Namibia has an area of 825214 square kilometres and is in Southern Africa. Its borders are Zimbabwe, Botswana, Zambia, Angola, South Africa, and the South Atlantic Ocean. The country has a good road network and communication infrastructure. The 2001 census indicated that 67.6% of the population lives in communal areas and 32.4% in commercial urban areas. The Namibian economy is heavily dependent on the extraction and processing of minerals for export. Mining accounts for 20% of the GDP (gross domestic product); the rich alluvial diamond deposits make Namibia a primary source for quality gems. The mining sector employs about 3% of the population whereas about half of the population depend on

subsistence agriculture for their livelihoods. Agriculture accounts for 11.5% of GDP, industry 29.8%, and services 58.7% (countryfacts.com, 2003).

According to an assessment report on the Millennium Development Goals (MDGs), countries around the world agreed to a number of factors to improve their countries MDGs. One of the Namibian goals was to improve access to safe drinking water in urban areas to 100% of the population and in rural areas to 87% of the population. Additionally, Namibia pledged to provide 98% of urban households and 65% of rural households, respectively, with basic sanitation (Legal Assistance Centre (LAC), 2009).

It seems that Namibia will likely meet its target to provide safe drinking water to 100 % of urban households by 2015 because according to the 2006 statistics 97% already had access to safe drinking water. Eighty percent (80%) of rural households have access to safe drinking water hence the country is on track to meet its 87% goal. However, in terms of the right to adequate sanitation Namibia has failed to meet its goals. In 2006, 58% of urban households and 14% of rural households had access to basic sanitation services (Legal Assistance Centre (LAC), 2009).

In the previous year, it has been noted by the Namibia Institute of Pathology (NIP), Laboratory Information System (LIS) that the requests for the Widal test at Oshakati increased from 1000 in 2007-2008 to 3000 in 2008-2009. Most of the specimens tested had O titres greater or equal to 1:160. The current cut-off titre for diagnosis of typhoid fever is 1:160 and above for O titre. It remains unclear whether northern

Namibia is an endemic area for typhoid fever. According to NIP laboratory information system (2009) from 1 July 2008 to 15 June 2009 the Oshakati state hospital laboratory culture confirmed 68 cases of typhoid fever due to *Salmonella*. The majority of cases (n=54) occurred between November 2008 to April 2009. It also remains uncertain whether the borrowed diagnostic titre of 1:160 is applicable to northern Namibia or whether some cases are being over or under diagnosed. Prevalence of typhoid fever and population baseline *Salmonella* agglutinin titres in Namibia, specifically in the northern part, is unknown. This makes interpretation of the Widal test virtually impossible.

Most Namibian hospitals do not have laboratory facilities to culture bone marrow, urine, stool, or blood for the diagnosis of typhoid fever; the Widal test therefore becomes the only option. It is a cheap easy test that offers paramount diagnostic cues. It provides results within a short time frame which allows for timeous treatment of a patient thereby averting irreversible complications of typhoid fever. In order for the test to be of clinical relevance it is very important to establish a population baseline of *Salmonella* agglutinin titres. This data could then be used as a base for interpretation of the Widal test.

HIV/AIDS is a disease that is known to compromise the host's immune system. Therefore the rate of antibody production in HIV/AIDS patients is diminished and baseline *Salmonella* agglutinin titres may be affected. In Namibia, including the northern part, interpretation of *Salmonella* agglutinin titres is similar regardless of the HIV status of a patient since there are no official guidelines for the interpretation

of Widal tests. This is not only peculiar to Namibia as it occurs worldwide: under or over diagnosis of typhoid fever in an HIV/AIDS positive patient is inevitable and serious medical consequences may result. To summarise, there is currently no guide in Namibia for the interpretation of *Salmonella* agglutinin titres for age, gender and HIV status. Standardization of patient care is therefore impossible to implement.

1.2 STATEMENT OF PROBLEM

In Namibia the Widal test (a blood test that detects level of *Salmonella* antibodies) is widely used in the diagnosis of typhoid fever. The test is readily available and the turnaround time for results is very short. Although the test is widely used in Namibia its interpretation leaves more questions than answers. Interpretation of the test is based on borrowed/exotic diagnostic *Salmonella* agglutinin levels and as such there might be under or over diagnosis of cases of typhoid fever. There is no available population baseline *Salmonella* agglutinins titre in Namibia and as such is of relevance to this study which focuses on northern Namibia in particular. The normal population baseline *Salmonella* agglutinin titre is used as the basis for the interpretation of the Widal test. However, since there is no available baseline to refer to, it means that interpretation of the Widal test is not scientifically based. In other words there is no country specific guide for the interpretation of *Salmonella* agglutinin titres following a Widal test.

This argument can be taken further in terms of the need for a guide for the interpretation of *Salmonella* agglutinin titres for age, gender and HIV status. Since

there is no guide, it means that standardization of patient care in Namibia is not possible. Within this context there is scarce information, as well as a lack of a guide, that takes into consideration the HIV status of individuals when interpreting the Widal test. It is against this background that a quantitative descriptive, explorative serological study was conducted to determine the baseline *Salmonella* agglutinin titres according to age, gender and HIV status among patients attending hospitals in northern Namibia. The aim of the study was to develop a guide for the interpretation of diagnostic *Salmonella* agglutinin titres for typhoid fever.

The research aimed to answer three questions, namely:

1. What is the age and gender prevalence of typhoid fever in patients with or without HIV?
2. Is there an association between, age, gender, HIV/AIDS and baseline *Salmonella* agglutinin titres?

1.3 DEFINITION OF CONCEPTS

- A guide is a concise reference book providing specific information about a subject. In this study a guide refers to a concise reference document providing information on normal and abnormal *Salmonella* agglutinin titers for age, gender and HIV status with a Namibian context.
- Baseline is a measurement, calculation or location used as a basis for comparison. In this study a baseline refers to population normal levels of

Salmonella agglutinin titers.

- Agglutinin is an antibody that causes agglutination of a specific antigen and in this study it refers to antibodies produced against *Salmonella* bacteria.
- Titre is the concentration of a solution as determined by titration and in this study it refers to the *Salmonella* antibody level in patient serum.

1.4 THEORETICAL CONCEPTUALISATION OF THE STUDY

Nearly all research studies in the social and behavioural sciences, regardless of programmes, require a rationale or basis for conducting research. This rationale is often called the theoretical framework (Radhakrishna, Yoder & Ewing, 2007). Sekaran (2000) defines a theoretical concept as a conceptual model of how one makes logical sense of the relationships among several factors identified to be important. In essence the conceptual model attempts to integrate key pieces of information, especially variables, in a logical manner, thereby conceptualising a problem that can be tested. The conceptual model usually frames the bigger picture of a study, identifies categories for literature review, and directs research objectives. A typical conceptual model provides a schematic description of relationships among independent, dependent, moderator, control and extraneous variables so that a reader can easily comprehend the theorised relationships (Radhakrishna *et al.*, 2007). The theoretical conceptualisation of the current study is based on the biomedical model of illness which combines several closely related sets of beliefs (Wade & Halligan, 2004). The biomedical model of illness has been applied in both a medical and

psychological context and rests on three constructs (Wade & Halligan, 2004), namely:

- All signs and symptoms within the body arise from an underlying disease
- All disease give rise to symptoms
- Health is the absence of the disease

Dickoff, James and Wiedenbach's (1968) practice oriented theory was applied to conceptualise the findings. In the practice oriented theory the following questions are asked.

- Who performs activity?
- Who or what is the recipient?
- In what context is the activity performed?
- What is the guiding procedure?
- What are the challenges?
- What is the end point?

The biomedical model is described in detail in Chapter 2 of this study and a summary of the integration of biomedical model of illness is presented in Table 2.1. A thorough description of the conceptualisation of the study using practice oriented theory is detailed in Chapter 5 of this study.

1.5 PURPOSE AND OBJECTIVES OF THE STUDY

1.5.1 Purpose of the study

The purpose of the study was to develop a guide on baseline *Salmonella* agglutinin titres according age, gender and HIV status among patients attending hospitals in northern Namibia.

1.5.2 Objectives

- i. To determine the prevalence of typhoid fever for age, gender and HIV status among patients attending five hospitals in northern Namibia.
- ii. To establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever.
- iii. To develop a conceptual framework based on the outcome of the situational analysis.
- iv. To develop a guide for baseline *Salmonella* agglutinin titres for age, gender and HIV status in patients attending hospitals in northern Namibia.
- v. To implement, monitor and evaluate the efficacy of the guide on baseline *Salmonella* agglutinin titres.

1.6 HYPOTHESES

Three hypotheses were formulated to test the research questions.

- ***Hypothesis one***

H_O: Typhoid fever is not prevalent in HIV/AIDS patients attending hospitals in northern Namibia.

H_A: Typhoid fever is prevalent in HIV/AIDS patients attending hospitals in northern Namibia.

- ***Hypothesis two***

H_O: There is no relationship between age, gender, HIV/AIDS and baseline *Salmonella* agglutinin titres.

H_A: There is a relationship between age, gender, HIV/AIDS and baseline *Salmonella* agglutinin titres..

1.7 SIGNIFICANCE OF THE STUDY

This study focuses on the prevalence of typhoid fever in order to establish presumptively diagnostic cut-off values for *Salmonella* agglutinin titres according to age, gender and HIV status in northern Namibia. The guide will help clinicians and other healthcare workers to effectively diagnose manage and treat patients with typhoid fever thereby improving patient outcomes. The results of the study could be significant to policy makers, to initiate policy changes especially in management of HIV positive patients with typhoid fever. The results could be significant to health

care and laboratory managers, in resource mobilization, allocation and prioritizing for management of HIV positive patients with typhoid fever. Findings on the prevalence of typhoid fever should also help in resource mobilization, prioritization and channelling to needy areas for the prevention of typhoid fever. This information could be used to review current management and treatment guidelines for HIV positive patients.

1.8 LIMITATIONS OF THE STUDY

Limitations of a study are normally in the design, population, sample or data collection instrument. In this study there were five main limitations. Firstly, the normal subjects who were not suspected of having typhoid fever could even be suffering from typhoid fever even if they visited the hospital for routine medical examinations. Therefore the sample cannot be ascertained with 100% confidence that all were normal population without any typhoid related illness, even though they did not have signs and symptoms of typhoid fever. They could have been in the prodromal or incubatory phase of illness. Secondly, cross reaction of the Widal test with other diseases may cause false positives. A positive Widal test does not only indicate presence of typhoid fever because patients suffering from other illnesses, such as malaria may also show a positive Widal test, since malaria and *Salmonella* share common immunogens. Use of positive and negative controls minimised this limitation and thereby improving the sensitivity of the Widal test. Third, staging of AIDS may affect the Widal test result even though the patients were selected in

terms of them being either HIV positive or negative but not according to HIV/AIDS WHO disease staging.

Fourthly, the study subjects were more than two years old and as such the *Salmonella* agglutinin guide is not applicable to subjects less than two years. Fifthly, although one of the objectives of the study was to have diagnostic *Salmonella* agglutinin titres according to age, gender and HIV status, it was not possible to come up with age and gender specific diagnostic titres. This was because the outcome of the statistical analysis of significance association between age and gender with *Salmonella* typhi O and H agglutinin titres. The Chi-square test showed that there was no significant association between gender and age with *Salmonella* agglutinin titres, and as such it was no longer necessary to differentiate the diagnostic titres according to age and gender.

1.9 SUMMARY

This chapter covers the global picture of typhoid fever and the burden associated with it. It also addresses the continental as well as regional distribution and burden of typhoid fever. An analysis is presented of Namibia's infrastructure and shortfalls that may predispose the country to typhoid fever. The analysis includes HIV/AIDS and water and sanitation as factors that may contribute to the prevalence of typhoid fever. The research objectives and hypothesis were also discussed in this chapter. The significance of the study and five limitations of the study were also presented in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Typhoid fever was a major cause of morbidity and mortality in the United States of America (USA) and Europe in the 19th century (Osler, 1912). After the provision of clean water and good sewage systems there was a dramatic decrease in the incidence of typhoid in these regions. The disease, however, remains a serious public health problem in developing countries (Crump, Luby & Mintz, 2004). For example, it is endemic in India and causes significant morbidity and mortality in both paediatric and adult populations. It is difficult to obtain reliable data to estimate the burden of disease in these areas. Many hospitals in Asia and Africa lack facilities for blood culture and up to 90% of patients with fever are treated as outpatients (Kothari, Pruthi & Chugh, 2008).

Typhoid fever is a systemic infection caused by *Salmonella enterica* serotype *typhi* (*S. typhi*). As stated above typhoid remains an important public health problem in developing countries with an estimated annual incidence of 540 per 100 000. In 2008 it was estimated that over 17 million episodes of typhoid occurred worldwide resulting in 216 000 deaths; more than 90% of this morbidity and mortality occurred in Asia (WHO, 2008). Although improved water quality and sanitation constitute ultimate solutions to this problem, WHO recommends vaccination in high-risk areas as a potential short-to-intermediate term control strategy.

Despite the current limitations of available epidemiologic data, a number of recent trends in enteric disease epidemiology have emerged in the African, Asian, and Latin American regions (Alnwich, 2001). In Asia disease burden estimates have normally relied on clinically diagnosed cases of typhoid fever compiled by governments and hospitals, usually with uncertain denominators (Ochiai, Acosta & Donovaro-Holiday, 2008). Population-based estimates of blood culture confirmed typhoid cases are scarce. In sub-Saharan Africa, where the burden of enteric fever is the least well characterized, hospital-based studies indicate that non-*typhi* serotypes of *Salmonella*, particularly *S. enterica serotype enteritidis* and *S. enterica serotype typhimurium*, greatly outnumber *S. typhi* and *S. paratyphi* as causes of bloodstream infection (Shaw, Reddy & Crump, 2008). Nonetheless, outbreaks of typhoid fever are frequently reported from sub-Saharan Africa with large numbers of patients often presenting with intestinal perforations. Important questions regarding epidemiology of enteric fever in the region are however not explored (Muyembe-Tamfum, Veyi, Kaswa, Lunguya, Verhaegen & Boelaert, 2009). In Asia a large population-based prospective study that used standardized surveillance methods estimated typhoid fever incidence in China, India, Indonesia, Pakistan, and Vietnam. This was done to consider a typhoid fever vaccine policy. Not only did the study confirm the high incidence of typhoid fever in the region, particularly among children and adolescents, but it also demonstrated that substantial variation in incidence occurs between surveillance sites in the same region (Ochiai *et al.*, 2008). *S. paratyphi A* was responsible for a growing proportion of enteric fever in a number of Asian countries, sometimes accounting for 50% of *Salmonella* bloodstream isolates among patients with enteric fever. This trend raises important concerns

about the impact of typhoid fever vaccine on enteric fever rates (Woods, Murdoch & Zimmerman, 2006). There is evidence in Latin America that typhoid fever incidence has decreased in parallel with economic transition and water and sanitation measures that were introduced to control cholera during the last pandemic (Crump *et al.*, 2004).

So what tests are available since the disease has dire consequences? The Widal test is easy, inexpensive and relatively non-invasive (Malik & Malik, 2001). The test can be of diagnostic value when blood cultures are not available or practically impossible (Otegbayo, Daramola, Onyegbutulem, Balogun & Oguntoye, 2002). Widal test results must be interpreted cautiously because of the low sensitivity of the test (Malik & Malik, 2001). A Widal test done on a convalescent serum gives more reliable results with high specificity and sensitivity than that of a single serum sample. In a situation where a second sample collection is not feasible, knowledge of baseline *Salmonella* agglutinin titres in normal population can form the basis to formulate diagnostic titres using a single Widal test (Ibekwe, Okonko, Onunkwo, Onunkwo, Donbraye & Babalola, 2008).

Although effective treatment with chloramphenicol was introduced in 1948 (Woodward, Smadel, Ley, Green & Mankikar, 1948), the emergence of resistance to this drug, as well as ampicillin and cotrimoxazole, is of concern (Mirza, Beeching & Hart, 1996). Currently the drugs of choice for treatment of typhoid fever are fluoroquinolones and third generation cephalosporins, respectively. However, recent reports of decreased susceptibility to these drugs have led to the prospect of re-

emergence of untreatable typhoid fever and an increasing global burden (Zenilman, 1997). Transmission of typhoid fever is based on availability of carriers and sick patients as well as the level of water and sanitation provision (Aftab & Khurshid, 2009).

2.2 TRANSMISSION AND RISK FACTORS

Typhoid fever is caused by *Salmonella typhi* a gram negative short bacillus which is motile due to the peritrichous flagella but non-flagellated variants do occur. They are non-capsulated intestinal pathogens which comprise *S. typhi* that cause an enteric fever known as typhoid fever (Philip, 2000). *S. typhi* has somatic antigens and glycolipid microcapsule the Vi or virulence antigen. Phage typing can be used to distinguish different strains of the organism.

Typhoid fever is transmitted via the faecal oral route through food or water, contaminated by faeces of patients or chronic carriers (CDC, 2008). Patients with typhoid fever carry the bacteria in their bloodstream and intestinal tract hence can spread the infection directly to other people by contaminating food or water (Utar, 2005). Risk is greatest among travellers to South Asia, Africa, the Caribbean, and central and South America. For example, travellers to South Asia are at a higher risk of infections with *Salmonella* that is nalidixic acid or multidrug (cotrimoxazole, chloramphenicol and ampicillin) resistant (Ibekwe *et al.*, 2008).

S. typhi is entirely parasitic and so too is *Salmonella paratyphi*. It differs from others parasitic diseases because humans are its only natural host and in laboratory experiments it is of low virulence in mice and other animals. Although epidemics are usually spread through water or food the source of infection is usually a human patient or carrier. Typhoid fever is usually characterized by a sustained fever as high as 40°C, profuse sweating, gastroenteritis and non bloody diarrhoea. Less commonly a flat, rose-coloured spots rash may appear (CDC, 2002).

Humans are the natural host and reservoir for *Salmonella enterica serovar typhi*. *Salmonella* bacteria can survive for days in ground or seawater, and for months in contaminated eggs and frozen oysters (Wait & Sobsey, 2001). The infectious dose varies between 10³-10⁶ organisms given orally (Kothari, Pruthi & Chugh, 2008). Transmission of infection occurs by ingestion of food or water contaminated with faeces. Other established risk factors include recent contact with a typhoid patient or carrier; eating ice cream, flavoured iced drinks or food from street vendors; and raw fruit and vegetables grown in fields fertilised with sewage (Bhan, Bahl & Bhatnagar, 2005).

A study undertaken in the slums in Bangladesh implicated eating raw papaya as being associated with the disease (Ram, Naheed, Brooks, Hossain, Mintz, Breiman, 2007). Papaya has a neutral pH and its cut surface can support the growth of various microorganisms. Hosoglu, Celen, Geyik, Akalin, Ayaz and Acemoglu (2006) concluded that eating lettuce salad and cig kofte (a traditional raw food) was significantly associated with the development of typhoid fever in Turkey. While

living in a crowded household was independently associated with typhoid fever (Hosoglu *et al.*, 2006). Preventive measures include routine washing of vegetables (Srikantiah, Vafokulov, Luby, Ishmail, Earhart and Khodjaev, 2007) and use of a latrine for defecation (Ram *et al.*, 2007). Overcrowding probably represents a greater opportunity for person-to-person transmission within households. In a case-control study in Indonesia, paratyphoid fever was found to be associated with flooding and with consumption of food from street vendors (Vollaard, Ali, Hagh, Suhariah, Widjaja & Visser, 2004).

Previous antimicrobial use has been shown in multiple epidemiological studies to increase the risk of infection with both antibiotic-resistant and drug-sensitive serotypes of *S. typhi* (Glynn, Reddy, Hutwagner, Rabatsky-Ehr, Shiferaw & Vugia, 2004). However, two case-control studies in Turkey and Bangladesh failed to show such a link (Hosoglu *et al.*, 2006). It is postulated that in addition to providing a selective advantage to resistant *S. typhi* strains, antimicrobial exposure can lead to prolonged alterations in gastro-intestinal flora and a decreased barrier to bacterial colonisation thereby lowering the dose of *Salmonella* necessary to cause infection (Barza & Travers, 2002).

Bhan, Bahl, and Sazawal (2002) found a significant association between the presence of serum anti-*Helicobacter pylori* IgG antibodies and typhoid fever. This provided empirical evidence that *Helicobacter pylori* (*H. pylori*) causes reduced gastric acidity and is associated with an increased risk of typhoid fever. Involvement of host genetic factors is implicated in susceptibility or resistance to infection with typhoid.

In Vietnam nucleotide polymorphisms in specific HLA alleles and the TNF-alpha promoter were associated with lower risk (Dunstan, Stephens, Blackwell, Duc, Lanh & Dudbridge, 2001). HLA-DRB1 was associated with protection against complicated typhoid fever (Dharmana, Joosten, Tijssen, Gasem, Indarwidayati & Keuter, 2002). These genetic factors may be partly responsible for the wide variation in the incidence of typhoid fever among the developing countries with similar standards of public health and hygiene.

2.3 GLOBAL BURDEN OF DISEASE

Salmonella typhi is a bacterium that causes typhoid fever (enteric fever). Typhoid fever is an acute, life-threatening febrile illness caused by bacterium *Salmonella enterica serotype typhi* (CDC 2008). Typhoid fever is a global health problem but its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections (WHO, 2003). WHO (2003) notes that the disease is underestimated because there are no bacteriological laboratories in most areas of developing countries. These factors are believed to result in many cases not being diagnosed.

Ibekwe *et al.* (2008) state that typhoid fever is a public health problem that is responsible for 10% fatality rate. They state that poor sanitary conditions and lack of or inadequate potable water is a concern in terms of transmission of disease. It is therefore a major public health problem in developing countries, such as Asia and Africa which includes Namibia. The WHO (2008) estimated an annual infection rate

of 21, 6 million and an approximate mortality rate of 600 000 with the highest percentage in Africa and Asia. Typhoid fever is endemic in the tropics and subtropics and has become a major public health problem in developing countries with an estimated annual incidence of 540 per 100 000. It constitutes serious sources of morbidities and mortalities within these regions.

Earlier estimates of the global burden of typhoid fever indicated there are at least 16 million new cases every year with 600 000 deaths (WHO, 1996). This data was first presented in 1984 and similar estimates were published around the same time (Edelman & Levine, 1986). This data, however, excluded China and did not account for the age distribution of typhoid fever (Crump *et al.*, 2004).

A 2004 retrospective study of 22 blood culture positive population-based Asian and African studies estimated that typhoid caused 21,650,974 illnesses and 216,510 deaths during the year 2000, and that paratyphoid fever caused 5,412,744 illnesses (Crump *et al.*, 2004). However, this estimate was based on data from only a few countries, with only three studies providing data for the entire continent of Africa. Since there is scant reliable population-based data on the incidence of paratyphoid fever, an estimate of this incidence was extrapolated from the 1997 global survey of *Salmonella* serotyping practices and results, which was conducted by the WHO Collaborating Centre for Food Borne Disease Surveillance and the United States Centres for Disease Control and Prevention (2003). A conservative case-fatality rate of 1% was chosen on the basis of estimates from hospital-based typhoid fever

studies, the mortality data from countries with reliable national typhoid surveillance systems, and expert opinion.

Crump *et al.* (2004) studies relied also on typhoid vaccine studies and as such this raises questions over data quality. To achieve favourable sample sizes typhoid vaccine studies are usually conducted at sites with known high incidence of typhoid fever, of which the high incidence introduces a bias towards the final estimation of disease burden. The incidence of typhoid was high (>100 cases per 100 000 population per year) in South-Central Asia, South East Asia and Southern Africa, and medium (10-100 cases per 100 000 population per year) in the rest of Asia, Africa, Latin America, the Caribbean islands and Oceania (Crump *et al.*, 2004). The incidence of typhoid fever was estimated to be low in Europe, North America, Australia and New Zealand (<10 cases per 100 000 population per year) (Crump *et al.*, 2004).

Typhoid fever incidence rates (IR) during various vaccine trials in Egypt varied from 209/100 000 in 1972-73 (Wahdan, Sippel, Makhail, Rahka, Anderson & Sparks, 1975) to 48/100 000 persons in 1978-81 (Wahdan, Serie, Germanier, Lackany, Cerisier, Guerin 1980). However, Crump *et al.* (2003) reported a lower IR, namely 13/100 000 persons in their Egyptian study. Most cases in developed countries arise in travellers and domestically acquired disease is infrequently reported (Reller, Olsen, Kressel, Moon, Kubota & Adcock, 2003). A total of 1,393 typhoid cases were reported between 1994 and 1999 in the USA and 74% of these cases were related to travel. The rest were acquired domestically; 7% of these cases were part of

recognised outbreaks (Olsen, Bleasdale, Magnano, Landrigan, Holland & Tauxe, 2003).

As evident from the above studies, typhoid is an endemic disease in developing countries; it is sporadic in more developed, industrialised nations with universal provision of safe drinking water and sanitation. In such a scenario it may be relevant to estimate region-specific burden of disease. In this context, Crump *et al.* (2004) reported the crude incidence of typhoid fever cases as 50/100 000 persons in Africa and 274/100 000 persons in Asia. Their reported incidence of the disease is remarkable given that most socio-economic indices, including provision of safe drinking water and sanitation, are much lower in most parts of Africa compared to South-East and South-Central Asia. This anomaly may be due to the lack of available data on typhoid fever incidence for Eastern, Central and Western Africa, as regional incidences were derived from extrapolation from three African incidence studies (Crump *et al.*, 2004). These statistics may also reflect the different environmental conditions in different parts of Asia and Africa, and the adaptability of *S. typhi* to survive and thrive in these varying conditions. This data highlight the need for more population-based studies of typhoid fever incidence from different parts of Africa to clarify the typhoid fever situation for this continent.

2.3.1 Asia and the Indian sub-continent

Attempts to measure the incidence of febrile illness are hampered by problems associated with surveillance sensitivity and specificity. Although conducting

surveillance at a tertiary hospital level is attractive such an approach tends to underestimate disease incidence. While a routine door-to-door visit to identify febrile persons is a highly sensitive technique, it has cost and time constraints. Similarly, although a syndrome based classification requires no laboratory capacity it lacks specificity. Only a few developing countries have national typhoid fever surveillance systems, leading to an over-reliance on vaccine studies for estimates of typhoid fever incidence in these countries. Data from Vietnam report an IR of typhoid fever ranging from 11.3 per 100 000 in 1991 to 12.2/100 000 in 2001 (Kelly-Hope, Alonso & Thiem, 2008). However, between 1994 and 1997, the IR shot up to 33.8 /100 000, indicating the presence of both seasonal and temporal trends in typhoid fever (Kelly-Hope, Alonso & Thiem, 2007). The mean IR for typhoid in this period was 23.3/100 000 (Kelly-Hope *et al.*, 2007). However, the highest incidence of the disease worldwide is found in the Indian subcontinent which in a study conducted in 2006 in Pakistan revealed an IR of 170/100 000 (using blood culture) whereas a serology based IR using Typhidot was 710/100 000 (Siddiqui, Rabbani, Hasan, Nizam & Bhutta, 2006). Brooks *et al.* (2005) reported an overall IR of 3.9/1000 person-years in an urban slum in Bangladesh.

The results of these studies highlight the wide variation in the incidence of disease even within a country. This could be due to various factors, including methodological differences, differences in standards of sanitation and hygiene, different geographical locations, lack of standardisation among the study populations, and the impact of availability of an effective vaccine in the recent studies, to name a few. Therefore, it is important to point out that a reported

incidence in a study may not necessarily be generalized to the entire country (Kothari, Pruthi & Chugh, 2008). This caveat was noted in Namibia. To avoid generalisation of the entire population meant that guidelines for typhoid fever were compiled for northern Namibia.

Hospital-based data in typhoid endemic areas have reported that most cases occurred in children aged 5-19 years and young adults (Mahle & Levine, 1993). However, more recent population-based studies from India, Indonesia, and Vietnam suggest that in some settings typhoid fever is also common in 1-5 year old children (Lin, Vo, Phan, Nguyen, Bryla & Tran, 2000). Ochiai *et al.*(2008) reported in their multi-centric trial that the mean age of typhoid was significantly lower in the South Asian sites (Pakistan and India) compared to the South East and North East Asian sites and they suggested that there was an inverse correlation between typhoid incidence and mean age of cases.

2.3.2 Typhoid fever in the African continent

Typhoid fever caused by the bacterium *Salmonella enterica serovar typhi* has become rare in industrialised countries but remains a major cause of enteric disease in children in developing countries (Kariuki, 2008) since it results in an estimated annual incidence of 50 cases per 100 000 persons predominantly in school-aged children. However, this statistic is a conservative estimate because not many studies have been done in most African countries. Kariuki (2008) acknowledges that the burden of typhoid fever in Africa is not fully known because credible measures of

disease incidence, which inherently require confirmed diagnosis of typhoid based on blood or bone marrow culture, are almost non-existent in many endemic countries where laboratory capacity is frequently limited.

So far only one study on population based incidence of typhoid has been done in Egypt with a reported estimated incidence of typhoid fever of 59 cases per 100 000 persons per year. However, a number of hospital based surveillance and case reports from several African countries suggest that typhoid is indeed a major public health concern, especially among school aged children (Kariuki. 2008).

An earlier incidence study in Mali by Ferreccio, Levine, Manterola, Rodriquez, Rivara and Panzel (1984) confirmed that *S. typhi* and *S. paratyphi* A were major causes of bacteraemia among children younger than two years of age. In Nigeria cases of ileum perforation due to typhoid were common in children younger than five years of age (Uba, Chirdan, Ituen & Mohammed, 2007). Several studies indicate that the burden of typhoid fever is high in Senegal, Nigeria, Uganda, the DRC and Burundi (Oyeyinka & Salimonu, 2002).

A breakdown in the potable water system in a South African suburb caused an outbreak of typhoid in nearly 4 000 people as well as several deaths (Sidley, 2005). In Kenya multidrug-resistant *S. typhi* strains in adult and school-aged children were previously associated with sporadic outbreaks in resource poor settings (Kariuki *et al.*, 2004). Outbreaks of typhoid fever have been reported in Addis Ababa and Ethiopia (Worku, 2000).

2.4 BASELINE SALMONELLA AGGLUTININ TITRES IN POPULATIONS

A number of studies have been carried out on baseline titres in the world. Ibekwe *et al.* (2008) determined the prevalence of typhoid fever between age and gender. They then established *Salmonella* agglutinin titres that are not diagnostically significant but that are normal in study population and titres that could be used as presumptively diagnostic of typhoid fever in eastern Nigeria. The researcher of this study explored similar objectives as well as furthering the research by assessing baseline *Salmonella* agglutinin titres according to HIV status, age, and gender. The study concluded by developing a guide for the interpretation of the Widal test. The study sample in the work of Ibekwe *et al.* (2008) was healthy fishermen in Awka. However, the sample used by the researcher in this study was of patients, attending hospitals in northern Namibia, who were not suspected of having typhoid fever, and also presumably healthy patients who underwent medical examinations.

A study by Patil, Kulkarni and Kulkarni (2007) determined the baseline agglutinin titres in apparently healthy children in Davangere, India. They found out that Out of 250 children, 64.2% had a titre of less than 1:20, 22.4% had a titre equal to 1:20, 9.6% had a titre of 1:40 and 3.6% had a titre of 1:80 to 'O' antigen and 67.2% had a titre of less than 1:20, 21.2% had a titre equal to 1:20, 8% had a titre of 1:40 and 3.6% had a titre of 1:80 to 'H' antigen of *S. enterica* subsp. *enterica* ser. Typhi. No children in age group 6 months-2 years had a titre of 1:80 to either antigen. All children in this age group had a titre of less than 1:20 to AH antigen and older children had a titre up to

1:40 dilution. However, the HIV status of the children was not known; this disease plays a major role in determining immune response which the Widal test heavily depends on. The cited study primarily focused on children from six months and above, whereas the current study focused on both children two years and above, as well as adults to obtain extensive information across ages.

Ibadin and Ogbimi (2004) determined the anti-typhoid agglutinins in African school children with malaria. They concluded that enhanced agglutinin to O and H antigens may be a phenomenon associated with childhood malaria.

A study in India compared Typhidot and Widal tests in patients with typhoid fever and the findings were that the Widal test has a sensitivity of 74% and specificity of 83% for typhoid fever (Sherwal, Dhamja & Randhawa, 2004). Sherwal *et al.* (2004) concluded that typhidot is a new, inexpensive, and reliable serodiagnostic test available commercially and studied in many endemic areas with reports of higher sensitivity and specificity. They studied typhidot test for its usefulness in patients of typhoid fever presenting at their hospital and observed that it has a sensitivity of 92% and specificity of 87.5%, which was higher than that of Widal test and comparable to the studies done elsewhere in India and outside. A similar study carried out in the southern part of India reported typhidot of having a sensitivity of 100% and a specificity of 80% and was recommended for its utility in conjunction with Widal test for an early diagnosis of typhoid fever (Jsudasson, Esther and Mathai, 2002). In another study group of typhoid patients in Pakistan, typhidot test had a comparable sensitivity of 94% and specificity of 77%, while Widal test had sensitivity and

specificity of 63% and 83% only (Butter & Mansurali, 1999). The effectiveness of typhidot test in early diagnosis of typhoid fever patients was also studied in two different studies in Malaysia. Its sensitivity and specificity was reported as 90.3% and 91.9% respectively in the first study, and was significantly higher, while the second study also showed sensitivity and specificity of 98% and 76.6% respectively (Choo, Davis and Ismail 1999), (Gopalakrishan, Sekher and Soo, 2002). Both the Malaysian studies showed it to be a better test in contrast to Widal test for rapid diagnosis as well as for its simplicity of ease in use. Results of all the studies done to evaluate typhidot test in developing countries have consistently shown similar and comparable results.

It has been reported that a single Widal test, in an unvaccinated individual showing elevated O and H titres, is strongly suggestive of typhoid fever if the person comes from a non-endemic area or is a child less than 10 years of age in an endemic area (Myron, Oscar, Robert, William, Rene & William, 1978). According to Lateef and Aprileona (2000) there is great deal of controversy surrounding Widal test. It has become increasingly obvious that the Widal test, while offering a simple methodology, often results in misleading information because of the polyvalent nature of the antigens involved. On the other hand Ayse, Onder and Banu (2002) concluded in their study on the use of the Widal test in the diagnosis of typhoid fever in Turkey, that it is easy to do as well as being inexpensive and relatively non-invasive. They further concluded that it can be of diagnostic value when blood cultures are not available or impractical. The results must be interpreted cautiously because of low sensitivity of the test, however. Kulkarni and Rego (1994) concluded

in their study on the value of a single Widal test in the diagnosis of typhoid fever that the frequency and concentration of O and H agglutinins vary much less in different parts of the world. Baseline surveys of zero-prevalence of *S. typhi* O and H antibodies in normal population must therefore be carried out as a guideline for the interpretation of the Widal test. They stated that numerous studies have produced data that cast serious doubt on the value of the Widal test in the diagnosis of typhoid fever. Their study revealed that a single Widal test is still a useful diagnostic tool in typhoid fever. An O titre in isolation, an H titre in isolation, and an O and H titre considered together equal or more than 1:160, with relevant clinical findings are highly suggestive of typhoid fever. At a titre more than or equal to 1:160 the sensitivity of O titre (70%) was greater than that of the H titre (30%), and the overall accuracy of the O and H titre was greater (90.8% compared to 83.1%). Hence the O titre was considered to be of greater diagnostic significance.

In their South African study on typhoid fever and asymptomatic HIV infections, Khan, Yacoob and Sturm (1997) reported that HIV is a serious public health concern in many developing countries where typhoid fever is endemic. It is biologically plausible that HIV infection can influence the clinical course of typhoid fever; a previous study found that circulating peripheral mononuclear cells in HIV infected people decreased natural antibacterial activity against *S. typhi* and *S. paratyphi C*. In addition it may have decreased antibody activity against *S. typhi* lipopolysaccharide antigens.

It is against this background that the current study explored prevalence as well as baseline *Salmonella* agglutinin titres according age, gender and HIV status in order to develop a guide for the correct interpretation of the Widal test. This study covered some of the shortfalls of previous studies in areas of HIV status in relation *Salmonella* agglutinin titre levels.

2.5 THE ORGANISM SALMONELLA

Salmonella are the most complex of all the *Enterobacteriaceae*, with more than 2400 serotypes described in the current Kauffmann-White scheme (Cheesbrough, 2006). The first isolate of *Salmonella* group was reported in 1884 by Gaffky (*Bacterium typhosa*); in 1886 Salmon and Smith reported the *Salmonella choleraesuis*. The development of *Salmonella* nomenclature has been very complex and a matter of dispute (Washington *et al.*, 2006). *Salmonella* was formally classified as separate species but DNA hybridization studies have now shown that all pathogenic *Salmonella* belong to one species *S. enterica* which is subdivided into seven subspecies (Cheesbrough, 2006).

Prior to July 1983 there were three species of *Salmonella* that were used to report positive results: *S. choleraesuis*, *S. typhi* and *S. enteritidis*. Presently all former species and subgroups of *Salmonella* and *Arizona* are globally considered to be the same species but can be separated into seven taxa representing six distinct subgroups. The only exception is *S. bongori*, previously known as *sub genera V*, thus there are

two species and six sub-species of *S. enterica* in the current system used by CDC (Washington *et al.*, 2006).

2.6 PATHOGENESIS

Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. Each year 1.4 million cases of salmonellosis occur among humans in the USA, resulting in 16000 hospitalizations and nearly 600 deaths (Washington *et al.*, 2006).

2.6.1 Enteric fever (typhoid fever)

The serious form of this fever is caused by *S. typhi* and the milder form by *S. paratyphi* A, B and C and is usually accompanied by bacteraemia. These *Salmonella* are usually found only in humans and are excreted in urine and faeces of infected patients and carriers. Infection is by ingestion of the organisms residing on contaminated hands. *S. typhi* is mainly water-borne and *S. paratyphi* is mainly food-borne (Cheesborough, 2006).

2.6.2 The disease pathology

During an acute infection *S. typhi* multiplies in mononuclear phagocytic cells before being released into the blood stream. After ingestion of contaminated water or food, the typhoid organisms enter the intestines where, through rapid penetration of the

mucosal epithelium, they reach the lamina propria, thereby rapidly eliciting an influx of macrophages that ingest the bacilli but do not generally kill them. Some of the bacilli remain within macrophages of the small intestines lymphoid tissue; the others are drained into the mesenteric lymph nodes where there is further multiplication and ingestion by macrophages.

Typhoid bacilli reach the blood stream principally by lymph drainage from the mesenteric nodes, after which they enter the thoracic duct and the general circulation. As a result of this silent primary bacteraemia, the pathogens reach intracellular haven within 24 hours of ingestion throughout the reticulo-endothelium organs (spleen, liver and bone marrow). The size of the inoculum, and immune status of hosts, influence the incubation period which usually decreases with increasing inoculum and diminishing immune system (WHO, 2003).

2.7 DIAGNOSIS OF TYPHOID FEVER

The gold standard for diagnosis of typhoid fever rests on recovery and identification of the causal organism from blood samples during the first few days of illness and from faeces during the second and third weeks of illness and from urine in the third and fourth week (Mgbor & Osuafor, 1990; Easman, 2002). A blood or stool sample is needed for the diagnosis of typhoid fever and these are examined for the presence of *S. typhi* (Utah, 2005). A serological test, namely the Widal test, is used as an indirect test to detect the “shadows”, “footprints” of *S. typhi* groups. The possibility

of a quick sero-diagnostic test for typhoid fever has engaged scientists over the last few years (Onunkwo, Nwankwo & Umolu, 2001).

Towo, Fadiora, Oparinde and Olowe (2007) concluded in their study on the use of Widal agglutination titres in the diagnosis of typhoid fever that because of the difficulties in isolating *S. typhi* from blood, stool or other body fluids in developing countries, Widal agglutination titre is a valuable diagnostic tool for typhoid fever when used with population baseline titres of the particular locality. Epidemiology of cross-reacting antigens determines the baseline titre of Widal test as the antibody produced in these diseases may cross-react with *Salmonella* antigens. Therefore, a fourfold rise in antibody titres between acute and convalescent phases is considered as a significant change in a given person. Since this type of comparison is not practically helpful in establishing diagnosis of an acute illness, a single cut-off value is widely used. In a given population, interpretation of a single Widal test result needs to be based on average baseline titre among healthy individuals. Antibody titres beyond a cut-off value should be regarded as significantly elevated titres. These titres may be used for diagnosis in an appropriate clinical setting (Kariuki, 2008).

2.7.1 Signs and symptoms

Typhoid fever is characterized by a number of signs and symptoms, namely:

- High grade fever $>38^{\circ}\text{C}$ with profuse sweating

- Headache, malaise and anorexia
- Exanthema (rose spots) on chest, abdomen and back
- Gastroenteritis and constipation
- Non-bloody diarrhea

2.7.2 Complications of typhoid fever

Untreated cases of typhoid fever may result in a complicated fever characterized as follows:

- Intestinal perforation with all signs of peritonitis accompanied by sudden rise in pulse rate and hypotension
- Marked abdominal tenderness, rebound tenderness and guarding and subsequent abdominal rigidity
- Altered mental status

2.7.3 Investigations

A diagnostic workout for typhoid fever includes the following:

- Stool occult blood
- Rectal swab/ stool microscopy culture and sensitivity (MCS)
- Bone marrow/blood culture
- Urine MCS

- Full blood count (FBC) (left shift)
- Widal test
- Abdominal radiographs (free air)

Note: Blood, urine and stool specimen should be submitted at different time intervals to facilitate isolation of *Salmonella* as it is shed intermittently.

2.8 MANAGEMENT AND TREATMENT

2.8.1 Primary intervention

The focus of primary intervention should be on oral or intravenous hydration and nutrition. Treatment should include antipyretics and antibiotics such as ceftriaxone 1g twice daily for 10 to 14 days. Primary intervention is normally done at a health center or primary health care (PHC) level.

2.8.2 Secondary intervention

This is normally done at a referral center and involves antibiotic treatment with chloramphenicol 500mg four times a day for 14 to 21 days. Chloramphenicol may be substituted with ciprofloxacin 250mg to 500mg daily dose for one week.

2.8.3 Clearance of chronic carriers

Ciprofloxacin 750 mg twice a day for 28 days is the best therapy used to rid chronic carriers of typhoid fever.

2.8.4 Health education

Key issues to emphasize include, but are not limited to:

- avoid faecal contamination of water and food
- personal hygiene, for example, washing hands with soap after using the bathroom and before food preparation
- screening of food handlers biannually for *Salmonella*
- avoiding raw food, shell fish and ice
- isolation measures for the patient
- disinfection measures

The above information was adopted from the Namibian standard treatment guidelines (MoHSS, 2011).

2.9 WIDAL AGGLUTINATION

Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody which is directed against a specific antigen. Such

tests have been widely used for a long time for the detection of antibodies against various disease-producing micro-organisms in serum. The Widal agglutination test was developed by F. Widal in 1896 in Britain (Widal, 1896) to aid in the diagnosis of typhoid fever. It utilises a suspension of killed *Salmonella typhi* as antigen to detect typhoid fever in serum from suspected *S typhi*-infected patients who present with febrile illness.

The value and clinical application of this test in developed countries has diminished considerably in recent years and a large number of antigenically related determinants, of both typhoid and non-typhoid *Salmonella* organisms, are now recognised. This test measures agglutinating antibody levels (agglutinins) against *S. typhi* and *paratyphi* O and H antigens. *Salmonellae* can be characterized by their somatic (O) and flagella (H) antigens, the latter existing in some serotypes in phases 1 and 2. Some *Salmonellae* have an envelope antigen called Vi (virulence). Usually O antibodies appear on days 6 to 8 and H antibodies appear on days 10 to 12 after onset of the disease. The test is usually performed on an acute serum (at first contact with patient). A convalescent serum should preferably also be collected so that paired titrations can be performed. In practice, however, it is often difficult (Olopoenia & King, 2000). The test has only moderate sensitivity and specificity as it can be negative in culture proven cases of typhoid fever. This may be because of prior antibiotic therapy that blunted the antibody response. On the other hand *S typhi* share O and H antigens with other *Salmonella* serotypes and cross reacting epitopes with other *Enterobacteriaceae*, and this can lead to false positive results. Such results may also occur in other clinical conditions, such as malaria, typhus, bacteraemia

caused by other organisms, and cirrhosis. In endemic areas there is often a high background level of antibodies in normal population. Determining an appropriate cut-off for positive result can be difficult since it varies between areas and between times in given areas (Clegg, Passey & Thong, 1996).

It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which the antibody titre is considered clinically significant. This is particularly important if, as usually happens, a single acute sample is available for testing. If paired sera are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic. Quality control of the test is achieved by running a standard serum with known antibody titre in parallel with each batch of assays. The variations in the standard serum should not exceed one tube, namely double dilution.

Despite its limitations the test may be useful, particularly in areas that cannot afford the more expensive diagnostic methods (Pang, 1989). This is acceptable as long as the results are interpreted with care in accordance with appropriate local cut-off values for the determination of positivity (WHO, 2003). In developed countries, the use of Widal agglutination as a laboratory tool to aid in the diagnosis of typhoid fever during the acute phase of the illness has largely been abandoned (Washington, 1991), because the need for such a test is minimal, especially in view of the low prevalence of typhoid fever. In addition, adequate and improved sanitation, sewage systems, proper hygiene and better means of isolating the organism from culture are available. Unfortunately, in some developing countries the situation is quite different

and the Widal test appears to be the only laboratory method employed in the diagnosis of typhoid fever among suspected patients. As the test suffers from serious cross-reactivity with other infectious agents, it may produce false-positive results, leading to an over-diagnosis of typhoid fever. Olopoenia and King (2000) concluded that diagnosis of typhoid fever based on serology (Widal agglutination) alone is frequently inaccurate. Concomitant with this increase in diagnosis is the abuse of the first-line drug of choice (chloramphenicol), which has led to the selection of resistant strains of *S. typhi*.

2.9.1 Performance technique

The Widal test reaction involves the use of bacterial suspensions of *S. typhi* and *S. paratyphi* A and B. These bacterial suspensions are treated to retain only the O and H antigens. These antigens are employed to detect corresponding antibodies in the serum of a patient suspected of having typhoid fever. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persists for longer (Olopoenia & King, 2000).

Two types of agglutination techniques are available: the slide test and the tube test. The slide test is rapid and is used as a screening procedure. Using commercially available antigens of *S. typhi*, a drop of the suspended antigen is added to an equal amount of previously prepared serum. An initial positive screening test requires the determination of the strength of the antibody. This is done by adding together equal

amounts of antigen suspension to the serially diluted serum from the suspected patient. Agglutinations are visualised as clumps. Weakly reactive agglutinations may require an adequate light source for proper visualisation, while strongly reactive agglutinations are easily seen. The result of the tests are scored from 0 to 4+ (0 = no agglutination; 1+ = 25% agglutination; 2+ = 50% agglutination; 3+ = 75% agglutination; 4+ = 100% agglutination). The smallest quantity of serum that exhibits 2+ (50%) agglutination is considered the end-point of serum activity or titre. The tube agglutination test requires much more technical work than the rapid slide test, and it is a macroscopic test (Chart, Cheesbrough & Waghorn, 2000). It also serves as a means of confirming the results of the slide test. A mixture of suspended antigen and antibody is incubated for up to 20 hours at 37°C in a water bath. Agglutinations are visualised in the form of pellets that are clumped together at the bottom of the test tube. Results are also scored from 0 to 4 + positive agglutination as described above for the slide test.

The tube test is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test. Since the ultimate goal of the test is antigen–antibody complex reaction, cross-reactions are encountered when an antibody produced by non-typhoid antigens reacts with typhoid-specific antigens. Several other diseases caused by non-*Salmonella* organisms (malaria, dengue, miliary tuberculosis, endocarditis, chronic liver disease and brucellosis) have been shown to exhibit this cross-reactivity in typhoid endemic regions. These cross-reactions increase the error rate of the result of the Widal test. Lack of standardisation of antigens also compromises the technique, as shown by Devillier (cited in King & Olopoenia,

2000). The value of the Widal test depends upon the standardisation and maintenance of the antigens to produce consistent results. It has become evident from work done in recent years on standardisation of the Widal test and interpretation of the results that both the O and H antigens are necessary for proper serologic analysis of the suspected serum. However, according to Welch (1936), no Widal test, regardless of the composition and standardisation of the antigens used, is infallible, and thus it is unlikely that any will be developed that will lower the validity of the isolation of the etiologic agent. Unfortunately, more than 60 years after Welch published his paper, the problems of ambiguity, insensitivity and non-specificity of Widal antigens continue. The widespread use of typhoid–paratyphoid vaccine, as well as the large number of cases of repeated exposure to *Salmonella* species, tends to lower the specificity of the Widal test.

2.9.2 Interpretation of the test results

While performance of the test may require some detailed technical work, interpreting the test result is the most arduous task. *Salmonella* are divided into distinct serologic groups (A through E) on the basis of their somatic O antigens. While all group D organisms, such as *S. typhi*, possess O antigen 9, about 60 of the 78 group D serotypes, including *S. typhi*, also have O antigen 12 (King & Olopoenia, 2000). Infection by any of the group D serotypes can therefore produce antibodies that can react with the O antigen used in the Widal reaction. Also, since all groups A and B organisms possess O antigen 12, cross-reactions with O antibody of group D is possible. The relative quality and quantity of antigenicity of the O antigens 9 and 12

contained in other common non-typhoid *Salmonella* serotypes, argument cross-reaction to occur frequently to lessen considerably the diagnostic specificity of the Widal reaction. A comparative study of *S. typhi* O antigen obtained from different manufacturers tested against the same serum, which had previously been shown to be positive by the slide agglutination test, revealed marked variability associated with the Widal agglutination titre (King & Olopoenia, 2000). A negative agglutination test may be due to one of several reasons. A negative Widal test result does not therefore necessarily rule out the presence of infection. Such results are best kept as a reference for subsequent comparative analysis (Mintz, 2003).

A positive agglutination tests (on two successive occasions) on the other hand, may also be open to several different interpretations. Although there are controversies surrounding the increase in titre beyond the first week of illness in some endemic areas, it is generally accepted by clinicians that toward the end of the first week of illness, titres of either O or H antibody may rise to as high as 1:160. However, the lack of paired sera may lead to an erroneous interpretation of test results (Olopoenia, Oyewole & Nafowokan, 1995). In endemic typhoid regions, a single testing of a serum specimen for Widal agglutinin cannot provide a reliable diagnosis due to repeated exposure to small inoculum of *S. typhi* or to other *Salmonella* species that contain type 9 or 12 antigens.

2.9.3 Causes of negative Widal agglutination tests

These include:

- absence of infection by *S. Typhi*;
- the carrier state;
- an inadequate inoculum of bacterial antigen in the host to induce antibody production;
- technical difficulty or errors in the performance of the test;
- previous antibiotic treatment; and
- variability in the preparation of commercial antigens.

2.9.4 Causes of positive Widal agglutination tests

These include:

- The patient being tested has typhoid fever
- Previous immunisation with *Salmonella* antigen.
- Cross-reaction with non-typhoid *Salmonella*.
- Variability and poorly standardised commercial antigen preparation
- Infection with malaria or other *Enterobacteriaceae*
- Other diseases, such as dengue
- Previous typhoid fever immunisation

- Other infectious agents, such as malaria.

Although a number of reports from some developing countries have suggested that a single Widal test is sufficient to make the diagnosis of typhoid fever (Choo, Razif, Openheimer, Ariffin, Lau & Abrahan, 1993) others have disputed the usefulness of such a single test result. In some developing countries, where the use of a single Widal test appears to be the norm, there has been an increase in the rate of false-positive results. Olopoenia and King (2000) studied Widal agglutinin in malaria infection in a Nigerian population and found that 85% of patients with a negative *S. typhi* culture but positive malaria smear had Widal titres of 1:40, 12% had titres of 1:80, and 3% had titres of 1:160. In contrast, 45% of patients with both *S. typhi* cultures and malaria smears negative had Widal titres of 1:40, 15% had titres of 1:80, and 10% had titres of 1:160.

When interpreting Widal test results, it is important that there should be close communication between the physician requesting the test and the laboratory, since modifications of technique in individual laboratories may affect the Widal titres and some patients with bacteriologically confirmed typhoid fever may fail to develop the usual rise of antibody titres. The results of the tests should be reported as either 'no agglutination' or, if agglutination is present, in titres (1:20, 1:40 or 1:80) rather than in descriptive (negative or positive) terms, as the latter may be misleading and contribute to the false interpretation of the test result by the physician. The function of the laboratory is to perform and report the test result to the requesting physician, who in turn will use the data to help make the proper diagnosis. Unfortunately, in

several areas of developing countries, the laboratory performs the test, makes the diagnosis, and prescribes the antibiotics.

It should be stressed that a single Widal agglutination test has no diagnostic significance. According to Hoffman (cited in Olopoenia & King, 2000) the results of a single Widal test, tube dilution, and micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known, as well as predictive values. Even in the extreme case of a high titre in a single Widal agglutination test, the causative organism may often be due to other species of *Salmonella*, rather than *S. typhi*. In an individual with no prior exposure to *S. typhi* infection (either lack of active infection or absence of passive immunisation), a higher than 1:50 or 1:100 titre on an initial single test, usually correlates fairly well with exposure to typhoid fever. However, even these single high value titres in an endemic area where repeated exposures to *S. typhi* may have occurred, do not have any clinical relevance in the absence of a positive isolate of the causative organism or its antigen (Olopoenia & King, 2000).

2.9.5 Limitations of the Widal test

While the Widal test has played a major role in the diagnosis of typhoid fever in the past, recent technical developments have revealed several pitfalls in its use and interpretation of its result. Clinically, it is obvious that a single Widal test in an unvaccinated or unexposed patient may have some diagnostic relevance. However,

the result of such a single test has no diagnostic significance in an endemic region; in part due to difficulty in establishing a steady-state or baseline titre of Widal agglutination, which limits the usefulness of the test as a reliable diagnostic indicator of the disease process.

One would not therefore expect any patient to have any specific Widal agglutinin in his/her serum, unless there are related, undetected, antigenic determinants of *S. typhi* present in the cells of other organisms. The presence of Widal agglutinin under conditions of positive malaria smear, negative *S. typhi* culture, and negative prior typhoid immunisation, would suggest that malaria parasite may have some undefined antigenic determinants similar to *S. typhi* which can induce antibody production. This could explain the febrile condition seen in some of these patients. On the other hand, the presence of Widal agglutinin, under conditions of negative malaria smear, negative *S. typhi* culture and negative prior immunisation against typhoid fever, suggests that other infectious agents, in addition to *Salmonella* and malaria parasite, may also share common antigenic determinants with *S. typhi*. These findings are in agreement with other reports from India with similar environmental and disease (malaria, typhoid) conditions (Olopoenia *et al.*, 1995).

The use of the Widal test to diagnose typhoid fever should therefore be limited to situations in which there is no other confirmatory supportive test, such as positive culture, available. Similarities between typhoid and non-typhoid *Salmonella* antigens mean that a serological method of diagnosis is the least accurate for typhoid fever. Due to the inexperience of some clinicians in typhoid endemic countries,

many cases of pyrexia of unknown origin receive the diagnosis of typhoid fever, based upon a false-positive Widal test result rather than a positive culture of *S. typhi*.

2.10 ANTIGEN DETECTION AS AN ALTERNATIVE TO WIDAL TEST

While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. In an acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a rapid, accurate, specific and sensitive test should be used to differentiate typhoid from non-typhoid febrile illnesses.

Clinicians usually elect to treat according to patient's symptoms, rather than wait for blood or stool culture results, which may take 3 to 5 days. While there might be some merit in this approach, particularly in areas where culture facilities are either poor or not available. Empirical treatment should be substituted by the use of rapid antigen screening directly from the stool of the suspected patient would be more useful.

Olopoenia and King (2000) described a new rapid immuno-enzymatic dipstick test for detection of *Salmonella* directly from the stool. It is a non-invasive test which involves homogenisation of stool sample in a buffer solution and immersion of a dipstick (previously coated with antibodies) in a tube containing the supernatant from the homogenised stool samples. The contents of the tube (dipstick and supernatant) are incubated at room temperature for 15 minutes; a second tube is incubated for an

additional 5 minutes for full development of colour. The dipstick is air dried and the result is visualised as a horizontal mark on the dipstick.

2.11 THE BIOMEDICAL MODEL

A model is a conceptual framework that unifies a topic and provides a way of thinking about it and investigating its usefulness. Once accepted, a model guides the research of scientists and directs the diagnosis and treatments of practitioners (Nettleton, 1995).

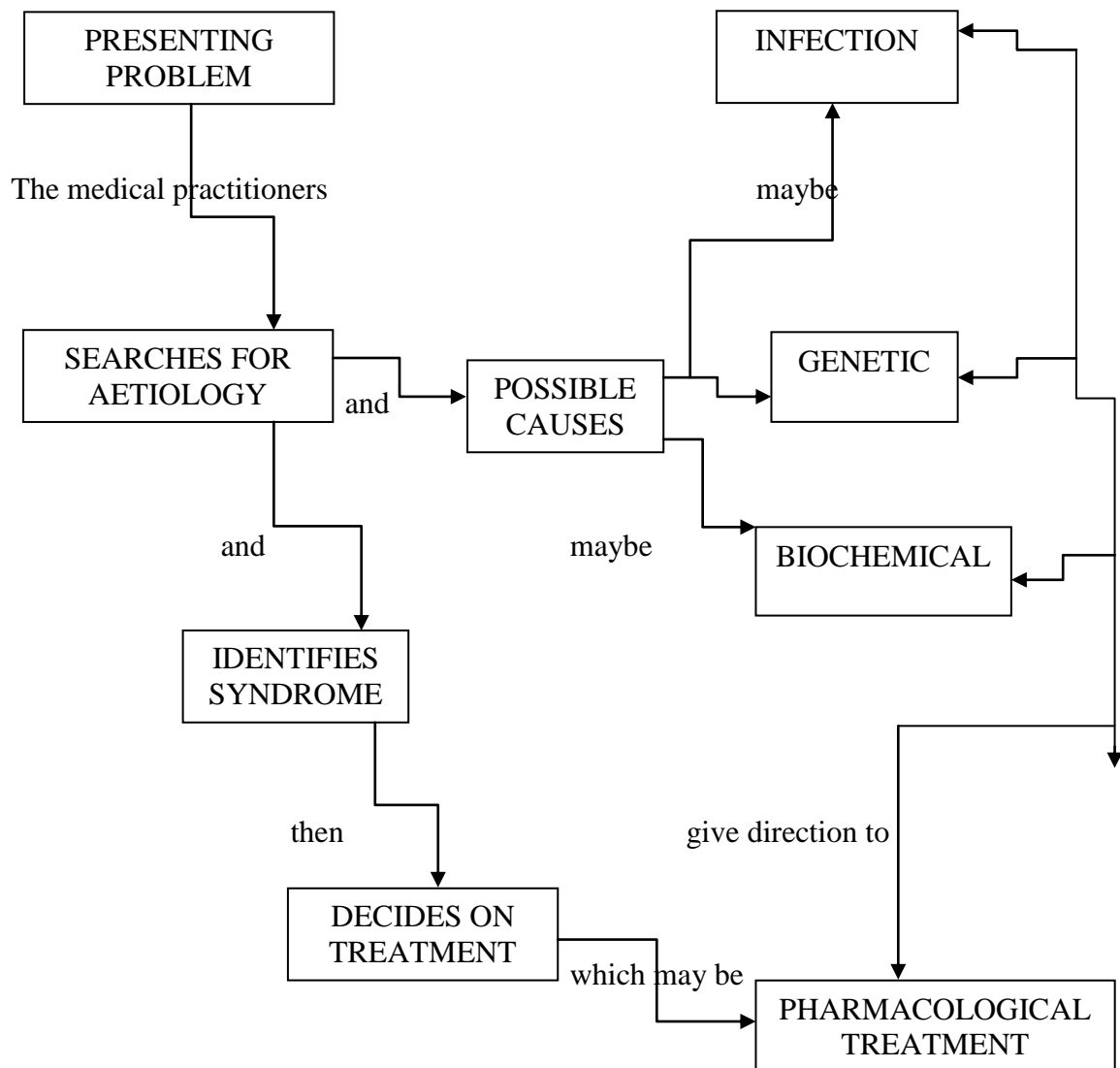


Fig 2.1: THE APPLICATION OF THE BIOMEDICAL MODEL IN DISEASE

DIAGNOSIS

Since the 19th century the leading model of health and illness has been the biomedical model. It gained strength in research areas such as physiology and medicine and led to the identification of infectious agents that cause diseases (Nettleton, 1995). The biomedical model originates from Galen's (Greek physician, 200AD) concept of pathogens (germ theory), namely, the disease is produced when a

pathogen enters the body. The model is reductionist in character as it looks at underlying organic factors, and the belief that the cause of a disorder, including a psychological disorder, is physical problem.

The biomedical model of illness is based on the following assumptions:

- Disease is an organic condition: non-organic factors associated with the human mind are considered unimportant or are ignored altogether in search for biological causes of pathological symptoms.
- Disease is a temporal organic state that can be eradicated or cured by medical intervention.
- Disease is experienced by a sick individual, who then becomes the object of treatment.
- Disease is treated after symptoms appear: the application of medicine is reactive healing process.
- Disease is treated in medical environment, such as a surgery or hospital, away from the site where the symptoms first appeared (Evans & Lee, 2002).

This model is based on a technically powerful science that has made a massive contribution to key areas of health (for example, vaccination). The biomedical model also underlies the official definition of health and disease adopted by states and international authorities (Doyal, 1995). The shortfalls of the biomedical model include the fact that people's perception of health and illness is culturally variable, highly context-specific, and dynamic and subject to change. Crucially there is no

clear-cut relationship between existence of a physical or emotional feelings and the judgement that this indicates illness requiring consultation with a doctor and becoming a patient. The fact that disease is due to a physical pathological problem is short sightedness as there are a lot of psychological conditions not associated with pathogens (Nettleton, 1995). Table 2.1 below summaries the integration of the biomedical model of illness in HIV and typhoid fever infection.

Table 2.1 Summary of the integration of biomedical model of illness:***Salmonella* and HIV status of persons***

Biomedical model of illness	HIV positive adults	HIV negative adults	HIV positive children	HIV negative children
All illness and symptoms and signs within the body arise from an underlining disease	Severely diminished immune system due to HIV and infection by <i>Salmonella</i> causes severely diminished <i>Salmonella</i> agglutinin titres	Infection with <i>Salmonella</i> results in normal immune response in the absence of HIV and normal production of salmonella agglutinin titres.	Severely diminished immune system due to underdeveloped immune system and HIV and infection by <i>Salmonella</i> causes severely diminished <i>Salmonella</i> agglutinin titres	Infection with <i>Salmonella</i> and underdeveloped immune system results in normal immune response in the absence of HIV and almost normal production of <i>Salmonella</i> agglutinin titres.
All disease give rise to symptoms	Severe symptoms due to co infection	Symptoms may or may not be as severe in the absence of HIV	Confection plus underdeveloped immune system results in severe form of disease	Underdeveloped immune system and infection with <i>Salmonella</i> moderate symptoms in the absence of HIV
Health is the absence of disease	In the absence of typhoid fever the HIV positive is regarded as healthy in HIV context	In the absence of typhoid fever the patient becomes healthy	In the absence of typhoid fever the HIV positive is regarded as healthy in HIV context	In the absence of typhoid fever the patient becomes healthy

*Adopted from Wade & Halligan, 2004, pp. 1398-1401.

2.12 SUMMARY

This chapter covered an extensive review of literature in the areas of global and regional burden of typhoid fever, as well comparison of studies done on baseline *Salmonella* agglutinin titres, the genera *Salmonella* and its pathogenesis. The chapter also looked at signs, symptoms, treatment, and complications of typhoid fever. The issue of diagnosis of typhoid fever, using in particular the Widal test, was extensively covered. A thorough description of the biomedical model of illness was also presented in this chapter including its integration in HIV and typhoid infection. In the following chapter the methodology is presented in detail and the research design is explicitly covered.

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1 INTRODUCTION

This chapter presents an in-depth analysis of both the design and methodology of the research. The main purpose of the study is to develop a guide on baseline *Salmonella* agglutinin titres according to age, gender and HIV status among patients attending hospitals in northern Namibia.

Four hundred (n=400) blood samples were collected from patients attending hospitals in northern Namibia. The samples were analysed for the presence of *Salmonella* agglutinins using slide and tube Widal agglutination tests.

3.2 RESEARCH DESIGN

A research design seeks answers to the research question (Polit & Hungler, 1997). The design is a general plan or blueprint that describes how the research will be conducted. It focuses on the kind of study proposed and its desired result. It begins with a problem, or question, and in the context of the logic of the research, determines what kind of evidence will address the research question adequately (Mouton, 2002).

It is a plan according to which the researcher obtains research participants (subjects)

and collects information from them. In it the researcher describes what activities were done in the field with a view to reaching conclusions about the research problem (Welman, Kruger and Mitchell, 2009). McGivern (2006) views a research design as a two tier process. In the first level, research design is about the logic of the research, its framework and structure. In view of what is known about the problem to be researched and the sort of research enquiry (exploratory, descriptive or explanatory) which that demands, decisions about structure of the research are made at the first level. The cited author further stresses that the structure may comprise a cross sectional, a longitudinal or an experimental design, or a case study. Decisions on units of analysis are also made in the first level of research design and include the 'who' or 'what' to question or to observe.

The secondary level of research design is about the research process: what type of data (primary or secondary, qualitative or quantitative or a combination), what method of data collection, what sampling strategy, and so on (McGivern 2006). In conclusion McGivern (2006) eludes that the first level is about designing the overall structure of the research so that it can deliver the sort of evidence one needs to answer the research problem and the second level concerns decisions about how to collect that evidence. Welman, Kruger *et al.* (2009) state that in research design researchers have to specify:

- i. The number of groups that should be used (necessary to decide which statistical technique to use).
- ii. Whether these groups are to be drawn randomly from the populations involved

and whether they should be assigned randomly to groups.

iii. What exactly should be done with them in case of experimental research?

In view of the above a quantitative descriptive, explorative serological study was utilised in this study.

3.2.1 Exploratory design

According to McGivern (2006) explorative research is undertaken to explore an issue or topic. It is particularly useful in helping to identify a problem, clarify the nature of the problem or define the issues involved. It can be used to develop propositions and hypotheses for further research, or to look for new insights or to research a greater understanding of an issue. For example, one can conduct exploratory research in order to understand what is meant by “elder abuse” (McGivern 2006). An explorative research study is conducted when limited information is available regarding the phenomenon under investigation (Brink, 1996). So to contextualise this there is limited understanding about the prevalence of typhoid fever in northern Namibia and there are no baseline titres for *Salmonella* agglutinins in Namibia. Interpretation of the Widal test is not standardized in Namibia as there is no guide for the interpretation of Widal test. The relationship between HIV and *Salmonella* baseline agglutinin titres within populations has not been established for Namibia. Consequently, this study sought to produce in-depth information about the prevalence of typhoid fever, as well as establish *Salmonella* baseline agglutinin titres according to age, gender and HIV status for populations attending hospitals in

northern Namibia.

Although studies about *Salmonella* baseline titres have produced valuable information on the phenomenon, it has not been investigated in the Namibian context, where scarce information is available. In addition no study has produced a guide that takes into consideration the HIV status of individuals when interpreting the Widal test.

3.2.2 Descriptive design

A great deal of market and social research is about description, as well as exploration, used to find answers to questions: Who?; What?; Where?; When?; How?; and how many? The purpose of descriptive research is to answer research questions. Descriptive research aims to build a picture of the market, a set of experiences, for example. It aims to identify, describe and in some cases count things (Babbie & Monton, 2001). It can be used to examine some of the key issues facing marketers and policy-makers (McGivern, 2006). The researcher defined the descriptive components of a quantitative study of baseline *Salmonella* agglutinin titres according to age, gender and HIV status for patients attending hospitals in northern Namibia.

3.3 RESEARCH METHOD

Research methodology focuses on the research process and the tools and procedures utilised. Beginning with the tasks it must accomplish, namely, data collection and sampling, it focuses on individual steps in the research process, trying to employ objective (unbiased) procedures (Mouton, 2002). Quantitative data is information that can be numerically measured and analysed. This involves computer analysis of statistical data to test their significance. Quantitative research methodology facilitates easy comparison of data and reproduction of results (Brink, 1996). In this study quantitative descriptive statistical methods were used. Graphs and tables were used to present the data and the Statistical Programme for Social Studies (SPSS) ANOVA was used to analyse the data.

The study was undertaken in four phases: needs assessment; developing a guide on baseline *Salmonella* agglutinin titres according age, gender and HIV status for Namibia; implementation of the guide; monitoring and evaluation of the effectiveness of the guide and each phase is described below.

3.3.1 Phase one: needs assessment

The specific objectives covered by this phase were:

1. To determine the prevalence of typhoid fever for age, gender and HIV status among patients attending five hospitals in northern Namibia.

2. To establish age, gender and HIV status presumptively diagnostic salmonella agglutinin titres for the diagnosis of typhoid fever.

3.3.2 Study population

A population is any defined group that is selected as a subject for research. If a population can be defined, from oxygen molecules in the universe to supercomputers in the world, then it can be subjected to study and analysis (Melville & Goddard, 1996). A study population includes all the members, or units, of a group that can be clearly defined in terms of its distinguishing criteria, whether they are people, objects or events (Uys & Basson, 1991).

In a research context, population refers to the universe of enquiry or, put another way, to the people, organisations, events or items that are relevant to the research problem (McGivern, 2006). It is important to define the population of interest as precisely as possible. Any flaws in definition of the population will mean flaws in the sample drawn from it (McGivern, 2006). On the other hand Welman, Kruger *et al.* (2009) define population as the study object that consists of individuals, groups, organizations, human products and events, or conditions to which they are exposed. A research problem relates to a specific population and the population encompasses the total collection of all units of analyses about which the researcher wishes to make specific conclusions (Welman, Kruger *et al.*, 2009).

The way in which a study population is defined depends on the issues the research aims to address. For example, if a study of the health and social welfare needs of older people has been commissioned to help develop policy in relation to community health activities the researcher may decide that those in residential care, nursing homes or hospitals are not part of the relevant population (McGivern, 2006).

McGivern (2006) further categorizes population as target and survey population, respectively. Moser and Kalton (cited in McGivern 2006) make a distinction between these two populations. The target population is the one from which the results are required and the survey population is that actually covered by the research. The two populations should ideally be the same but for practical reasons they may not be.

The population of this study is defined as all patients whose clotted blood samples are sent from hospitals in northern Namibia to the Namibia Institute of Pathology (NIP) for analyses other than typhoid screen (Widal test).

3.3.2.1 Inclusion criteria

The hospitals that were included in this study included Oshakati Intermediate hospital, Onandjokwe hospital, Engela hospital, Eenahana hospital, Oshikuku hospital, Tsandi hospital, Okahao hospital and Outapi hospital. Some of the samples originated from Katima and Rundu army and police hospitals send for medical examinations of new recruits. To be included in this study, the patients were divided

into two major groups: Group 1 was HIV positive patients; Group 2 was HIV negative patients. General inclusion criteria for all groups included the following:

- All patients whose clotted blood specimen was submitted to NIP laboratories in the northern Namibia.
- The patients were not suspected to be having or diagnosed of typhoid fever.
- The patients whose blood samples were not lipaemic or haemolysed.
- Group 1 were HIV positive patients' blood.
- Group 2 were HIV negative patients' blood (all samples were screened for HIV).

3.3.2.2 Exclusion criteria

This study excluded:

- all patients whose blood samples were not clotted;
- all patients whose blood samples were submitted to NIP laboratories by hospitals that were not participating in this study; and
- all patients suspected or diagnosed with typhoid fever.

3.4 SAMPLING

A sample is a group of people or elements that form part of a study population. Results from a study sample allow general observations to be made about the entire

population (Melville & Goddard, 1996). De Vos (2002) defines a sample as a small portion of the total set of the population; together they comprise the subject of the study. Sampling is the most feasible way of studying large populations when there are resource, time and financial limitations.

Once the population is clearly defined a researcher must decide whether to collect data from every member or element of that population (usually defined as a census) or from a representative subset or sample of it (McGivern, 2006). In most health and social research the population of interest is often too large for census to be practicable either in terms of time it would involve or cost. The argument for using a well designed sample rather than a census rests on two issues: on the practical issue of the time and cost involved in administering it, and on the methodological issue of the ability of a sample to be representative of the population (to deliver external validity).

Welman, Kruger *et al.* (2009) define a sample as a miniature image or likeness of the population. The aspect of generalising is extremely important. It is only when the results can be generalised from a sample to a population that the results of research have meaning beyond the limited setting in which they were originally obtained. A sample must therefore be a true representative of the population: the sample must have the exact properties in the exact same proportions as the population from which it was drawn but in smaller numbers (Welman, Kruger *et al.*, 2009). The sample for this study included 400 clotted blood samples from equal numbers of HIV positive and negative, adult and children, females and males.

3.4.1 Sample size criteria

In addition to the purpose of the study and population size, three criteria usually need to be specified to determine the appropriate sample size. The level of precision; the level of confidence or risk; and the degree of variability in the attributes being measured should be clearly specified (Miaoulis & Michener, 1976). The level of precision is also known as sampling error and is discussed in the following section.

3.4.1.1 The level of precision

This level, which is sometimes called, sampling error, is the range in which the true value of the population is estimated to be. This range is often expressed in percentage points (for example, ± 5 percent), in the same way that results for political campaign polls are reported by the media. Thus, if a researcher finds that 60% of farmers in the sample have adopted a recommended practice with a precision rate of $\pm 5\%$, then the researcher can conclude that between 55% and 65% of farmers in the population have adopted the practice. In this study the level of precision was set at 5% which is the maximum precision point (Miaoulis & Michener, 1976).

3.4.1.2 The confidence level

The confidence or risk level is based on ideas encompassed under the central limit theorem. The key idea encompassed in this theorem is that when a population is repeatedly sampled, the average value of the attribute obtained by those samples is

equal to the true population value. Furthermore, the values obtained by these samples are distributed normally about the true value, with some samples having a higher value and some obtaining a lower score than the true population value. In a normal distribution, approximately 95% of the sample values are within two standard deviations of the true population value (the mean value). In other words if a 95% confidence level is selected, 95 out of 100 samples will have the true population value within the range of precision specified earlier. There is always a chance that the obtained sample does not represent the true population value. Such samples with extreme values are represented by the 5% (2.5% on each side) area under the normal distribution curve. This risk is reduced for 99% confidence levels and increased for 90% (or lower) confidence levels (Miaoulis & Michener, 1976).

3.4.1.3 Degree of variability

The degree of variability in the attributes being measured refers to the distribution of attributes in the population. The more heterogeneous a population, the larger the sample size required to obtain a given level of precision. The smaller the sample size the less variable the population is. Note that a proportion of 50% indicates a greater level of variability than either 20% or 80%. This is because 20% and 80% indicate that a large majority do not or do, respectively, have the attribute of interest. Since a proportion of 0.5 indicates the maximum variability in a population it is often used in determining a more conservative sample size: the sample size may be larger than if the true variability of the population attribute were used (Miaoulis & Michener, 1976).

Since in this study there is a large population, and little is known about the variability of *Salmonella* agglutinins titers, an assumption was made for a maximum variability, $p=.5$ (maximum variability). Furthermore, a 95% confidence level and $\pm 5\%$ precision was desired in this study. The resulting sample size is demonstrated in the equation below:

$$n_0 = \frac{Z^2 pq}{e^2} = \frac{(1.96)^2 (.5)(.5)}{(.05)^2} = 385 \text{ blood samples}$$

Which is valid where n_0 is the sample size, Z^2 is the abscissa of the normal curve that cuts off an area at the tails ($1 -$ equals the desired confidence level, for example, 95%), e is the desired level of precision, p is the estimated proportion of an attribute that is present in the population, and q is $1-p$. The value for Z is found in statistical tables which contain the area under the normal curve. From the above equation it follows then that at least 385 blood samples were needed in this study and the researcher rounded off the figure to 400 blood samples. This sample size accorded the researcher the ability to out rightly reject outliers and also make inferences for the population (Miaoulis & Michener, 1976).

All blood samples submitted to NIP laboratories are always accompanied by a specifically designed laboratory request form. This form always indicates the name of the ward or department that submitted the specimen. This was used for collecting samples from the HAART department. The form also includes a portion where the

clinical data of the patient are recorded. This therefore enabled the researcher to exclude samples of patients suspected of having or diagnosed with typhoid fever.

3.4.2 Sample Collection

Four hundred (n=400) clotted blood samples were collected by purposive sampling over eighteen months, namely September 2009 to April 2011. The samples were stratified according to patients' ages, gender and HIV status. Table 3.1 presents the distribution of these samples among the different strata. There was an equal number of samples of HIV positive subjects (n=200) and HIV negative subjects (n=200). The sample comprised equal numbers of male (n=200) and female (n=200) subjects. Fifty percent of the sample were adults (n=200) and fifty percent were children (n=200). Half of each sample comprised HIV positive adults and children: n=100 adults and n=100 children. The HIV negative sample was also equally divided: n=100 adults and n=100 children. This division also applied to the HIV positive subjects see Table 3.1 below.

Table 3.1 Total number of sample stratified according to age, gender and HIV status

	MALE ADULTS	FEMALE ADULTS	MALE CHILDREN (2-15yrs)	FEMALE CHILDREN (2-15yrs)	TOTAL
HIV POSITIVE	50	50	50	50	200
HIV NEGATIVE	50	50	50	50	200
GRAND TOTAL	100	100	100	100	400

3.5 TESTING PROCEDURES

Clotted blood samples were spun at 3000 r.p.m. for 10 minutes in an automatic centrifuge. The serum was used to perform the Widal test using FEBSTHO5 febrile antigen kit (United Kingdom) from Fortress Diagnostic.

A rapid slide screening test (qualitative) was carried out first, followed by tube agglutination test according to the manufacturer's specifications. The FEBSTHO5 is suitable for both the rapid and tube agglutination tests against human sera for the detection of *Salmonella* agglutinins. The stained antigen suspensions are killed bacteria, stained to enhance the reading of agglutination tests. The blue stained

antigens are specific to the somatic O antigens whilst the red stained antigens are specific to the flagella H antigens.

3.5.1 Rapid slide agglutination

The rapid slide agglutination technique was used to screen samples for typhoid fever and entailed a specific procedure, namely:

- The reagents and samples were brought to room temperature.
- 50ul or one drop of the sample and 1 drop of each control were placed into separate circles on the card.
- The antigen was gently re-suspended.
- A drop of the latex reagent was added to each circle next to the sample to be tested.
- The two drops were mixed together with disposable pipette/stirrer and spread over the entire area enclosed by the ring and a new stirrer was used for each sample.
- The cards were rotated at 1000 r.p.m for 2 minutes on a rotator.

3.5.2 Reading and interpretation

Reading and interpretation of the Widal test depend on visual interpretation of the agglutination patterns. The procedure was as follows:

- The cards were examined macroscopically for the presence or absence of clumps or agglutination within one (1) minute of removing the card from the rotator, comparing with the controls.
- Negative results showed no signs of agglutination.
- Positive results showed visible clumps a clear sign of agglutination.

3.5.3 Tube agglutination

Positive (2+) results from slide agglutination were confirmed and titrated by the tube agglutination test using the following technique:

- Eight (8) tubes were labelled as set out in the Table 3.1 below.
- A 1/20 dilution of serum and saline was made in the first tube.
- 1ml of the 1/20 dilution in tube 1, was transferred to tube 2 and proceeded by serial dilutions as shown below until tube 7.
- Tube 8 served as the blank or negative control as it contained only saline.
- Positive and negative controls were diluted 1/10 with 0.85% saline.
- A drop of the appropriate antigen suspension was added to each tube and mixed well.
- The tubes were incubated as follows:- Antigens at 36 degrees Celsius for 24hrs or accelerated incubation by: Somatic (O) antigens 48-50 degrees Celsius for 4hrs; Flagella (H) antigens 48-50 degrees Celsius for 2hrs.

- The tubes were examined for signs of agglutination. The titre was taken as the last tube that showed agglutination.

Table 3.2 Preparation of double dilution for tube dilution method

Tube No.	Saline ml	Serum ml	Dilution Titre
1	1.9ml	0.1ml	1/20
2	1.0ml		1/40
3	1.0ml	1ml	1/80
4	1.0ml	serial	1/160
5	1.0ml	dilution	1/320
6	1.0ml		1/640
7	1.0ml		1/1280
8	1.0ml	blank	

Titres <20 are negative for typhoid fever, while any titre >20 is positive for typhoid fever.

3.6 DATA COLLECTION

Demographic data were generated from the laboratory request form and the LIS (Laboratory Information System). Data on *Salmonella* agglutinin titre were generated from laboratory Widal tests done on blood samples collected for the study.

3.7 DATA ANALYSIS

Data, presented as descriptive statistics and tables, graphs and pie charts, were used for comparative analysis of data according to age, gender and HIV status. The SPSS ANOVA computer program was used for statistical analysis of data.

3.8 PHASE TWO: CONCEPTUAL FRAMEWORK

A conceptual framework was formulated using the biomedical of illness model which culminated in the development of a guide on the baseline *Salmonella* agglutinin titres according to age, gender and HIV status for patients attending hospitals in northern Namibia. The conceptual framework is discussed in detail in Chapter 5 of the study.

3.9 PHASE THREE: DEVELOPMENT OF THE GUIDE FOR INTERPRETATION OF THE WIDAL TEST

The guide was developed using the findings of phase one: needs assessment. Development of the guide for the interpretation of *Salmonella* agglutinin titres was based on the results of this study of baseline *Salmonella* agglutinin titres according to age, gender and HIV status. The process for developing the guide is fully described in Chapter 6.

3.10 PHASE FOUR: IMPLEMENTATION, MONITORING AND EVALUATION OF THE GUIDE

After the inputs from all stakeholders the draft guide was submitted to medical practitioners who handle cases of typhoid fever at the Oshakati Hospital in the in-patient and out-patients departments including HIV clinics and the laboratory. The period for this phase was one full calendar month from the first of October 2011 to end of October 2011. The clinicians were advised to check the Widal test results of the patients and compare them with the clinical picture of the respective patient. The clinicians were also asked to monitor prognosis of patients after commencing treatment as directed by the guide. The Oshakati Hospital was selected for the implementation of the study as it was convenient for the researcher and also because it handles the highest number of patients in the northern region. The draft guide was implemented for a full calendar month by two medical officers and two specialist physicians working in the respective internal medicine in-patient/out-patient departments. One medical officer at the CDC clinic was part of the implementation team as well as the immunochemistry technologists at NIP Oshakati laboratory. The clinicians used the guide as an aid for clinical diagnosis and management of typhoid fever; the laboratory used it to establish normal or critical ranges for the Widal test and to compile the necessary comments that usually accompany laboratory test results.

The draft guide was presented to the medical seminar where about 200 medical personnel gather to share experiences and discuss cases. Most doctors who were

present expressed satisfaction and said the guide was long overdue and they really welcomed the development. There was not much criticism except clarification on methodology and criteria for sampling.

A small caucus meeting between the researcher, two physicians, a principle medical officer in internal medicine, and a dietician, was held in the NIP lecture room. The main agenda was to discuss laboratory diagnosis of typhoid fever and the role of the draft guide. The meeting also discussed on the application of the guide to food handlers when they are being screened for typhoid fever. Extensive professional input was given by these specialists including recommendations on proper screening of food handlers. This information was co-opted in the final guide that has a section on screening of food handlers for typhoid fever. Copies of the revised guide were given to doctors responsible for seeing patients with or suspected to be having typhoid fever. Some copies were also distributed to clinicians working with HIV positive patients on HAART, so as to use them during screening and management of patients with, or suspected of having, typhoid fever and then to give feedback to the researcher after one month.

3.11 ETHICAL ISSUES

Conducting research implies the acceptance of responsibilities. A researcher is responsible to fellow researchers, to respondents, to society as a whole and, most importantly, to himself (Melville & Goddard, 1996). A high professional standard regarding confidentiality was strictly maintained. De Vos (2002) identifies ethical

issues that are of utmost importance for the researcher, as follows: For permission to analyse and test samples submitted to NIP, the researcher engaged management and research committee of NIP. A copy of the proposal was send to the general manager, human resource manager, and chairperson of NIP Research committee. The NIP research committee approved the research (see attached copy annexure C.). For ethical clearance the researcher applied to the MoHSS and the research was also approved (see annex B).

3.11.1 Permission

Permission to conduct the study was sought and obtained from the University of Namibia Post-Graduate Committee (see annexure A). The written proposal was reviewed by the committee to ensure that it conformed to ethical standards of scientific research. In order to obtain permission to analyse and test samples submitted to NIP, the researcher engaged and got approval from management and research committee of NIP. A copy of the proposal was sent to the general manager, human resource manager, and chairperson of NIP Research committee. Prior to using blood specimens in this study written authorization from NIP top level management was sought and obtained.

3.11.2 Confidentiality and anonymity

All patient information on specimens and request forms was treated with the strictest confidentiality. The data obtained were used for the stated purpose of the research and no other person had access to the patients' specimen details.

This condition is reflected by Le Beau (1998) who states that confidentiality entails that information shared by someone is not divulged to others. Specimens meeting the inclusion criteria were assigned a survey code and poured into a new tube that did not include patient name and laboratory or hospital numbers. Study samples were not traceable to the submitting patient.

3.12 SUMMARY

This chapter discussed the research design and research methods concentrating on descriptive and explorative designs. It also looked at the population and sampling methods. Data collection and analysis methods were also discussed in this chapter. Ethical issues of confidentiality, anonymity and permission were also discussed.

CHAPTER 4

RESULTS OF THE STUDY

4.1 INTRODUCTION

This chapter presents the findings on baseline *Salmonella* agglutinin titres according to age, gender and HIV status among patients attending hospitals in northern Namibia. The results are presented as descriptive statistics in tables and graphs in terms of the objectives of the study.

4.1.1 Purpose of the study

The purpose of the study was to develop a guide on baseline *Salmonella* agglutinin titres according to age, gender, and HIV status, among patients attending hospitals in northern Namibia. The objectives of the study were, namely:

1. To determine the prevalence of typhoid fever among patients attending hospitals in northern Namibia.
2. To establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever. To determine the prevalence of typhoid fever for age, gender and HIV status among patients attending five hospitals in northern Namibia.
3. To develop a conceptual framework based on the outcome of the situational analysis

4. To develop a guide for baseline *Salmonella* agglutinin titres for age, gender and HIV status in patients attending hospitals in northern Namibia.
5. To implement, monitor and evaluate the efficacy of the guide on baseline *Salmonella* agglutinin titres.

4.1.2 Overview of the Widal test

The Widal test, when used in conjunction with baseline *Salmonella* agglutinin titres normal to the population, may suffice. Clinicians usually elect to treat a patient rather than wait for culture results. The latter can take between three to eight days to be available. Although there may be some merit in the said approach, particularly in areas where cultures facilities are either poor or non-existent, the Widal test can be used to raise the suspicion index and help minimize costs and resistance to antibiotics.

4.2 TESTING PROCESS

The *Salmonella* agglutinins (end titres), for *typhi O* and *H* as well as *Salmonella paratyphi AH* and *BH* were determined on separated serum by standard Widal slide and tube agglutination tests; appropriate controls were included in each procedure. The end titre distribution was analysed according to age, gender and HIV status and

these results are presented in the subsequent sections below. The samples were collected as seen in Table 3.1.

4.3 END TITRE DISTRIBUTION AMONG 400 SUBJECTS

The distribution of *Salmonella* agglutinin titres obtained from the 400 (100%) subjects is shown in Table 4.1 below. *Salmonella* titres <20 indicate negative for typhoid fever, whilst any titre >20 indicate positive for typhoid fever.

Table 4.1 End titre distribution among 400 subjects

<i>Salmonella</i>	End Titre Frequencies							Grand Total	>20	Percent >20
	<20	20	40	80	160	320				
<i>Typhi H</i>	318 79.5%	14 3.5%	26 6.5%	33 8.3%	2 0.5%	7 1.8%	400	82	20.5%	
<i>Typhi O</i>	301 75.3%	24 6%	50 12.5%	23 5.8%	2 0.5%	0 0%	400	99	24.8%	
<i>Paratyphi</i> <i>AH</i>	400 0%	0 0%	0 0%	0 0%	0 0%	0 0%	400	0	0.0%	
<i>Paratyphi</i> <i>BH</i>	382 95.5%	8 2%	7 1.8%	3 0.8%	0 0%	0 0%	400	18	4.5%	

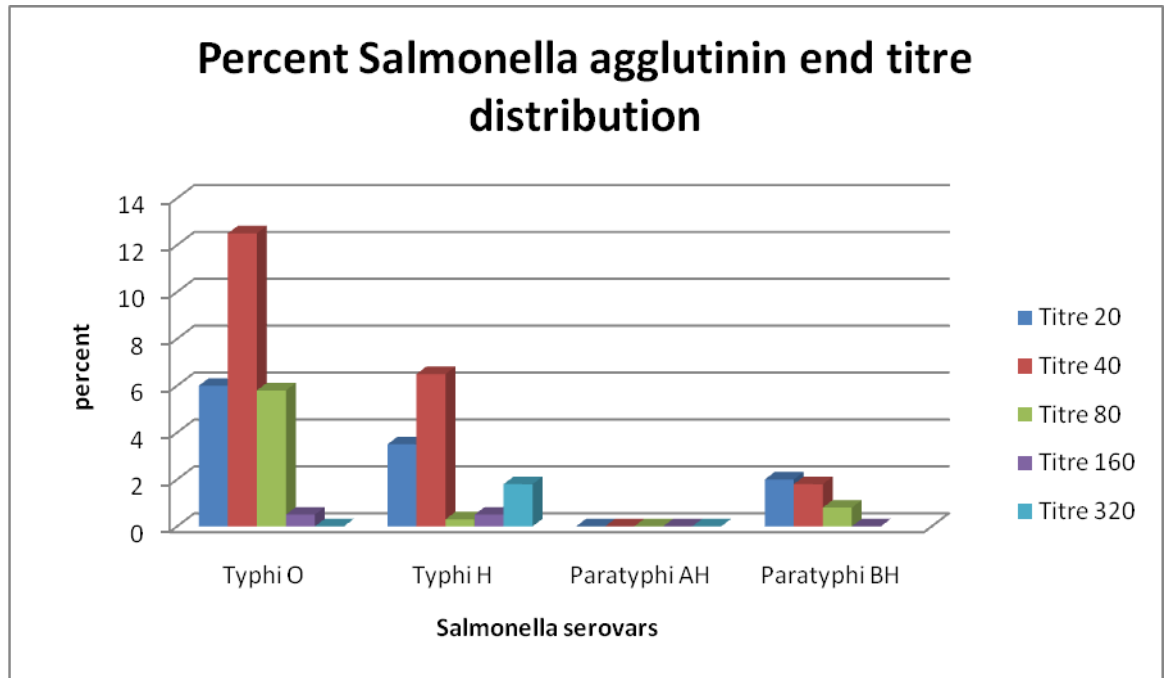


Figure 4.1 Percent end titre distribution among the 400 subjects

About 82 (20.5%) of the all the subjects (n=400) had positive end titres (≥ 20) for *S. typhi O*, 99 (24.8%) had *typhi H* end titres ≥ 20 , and 0% of the subjects had *paratyphi AH* end titres ≥ 20 , and 4.5% of the subjects had *paratyphi BH* end titres ≥ 20 as shown in table 4.1 and figure 4.1. None of the subjects had *typhi O* and *paratyphi BH* end titres of 320. For *typhi O* end titres, 24 (6%) subjects had titre 20, 50 (12.5%) had titre 40, 23 (5.8%) had titre 80 and only 2 (0.5%) had titre 160. Three hundred and one (301) (75.2%) subjects had negative titres < 20 for *typhi O* end titres. The highest significant ($p < 0.05$) *typhi O* end titres occurring in $> 5\%$ of the subjects was titre 80.

For *typhi H* end titres, 318 (79.5%) had titres <20; 24 (3.5%) had *typhi H* end titre of 20; 26 (6.5%) had *typhi H* end titres of 40; 33 (8.3%) had titre 80, and 2 (0.5%) had titre 160, and 7 (1.8%) had titre 320. The highest significant ($p<0.05$) *typhi H* end titre occurring in > 5% of the subjects was titre 80. *Paratyphi BH* had significantly ($p<0.05$) the lowest titres among all the subjects ($n=400$) with an average of 4.5%. Eight (2%) subjects had *paratyphi BH* end titre of 20, and 7 (1.8%) had titres of 40, and 3 (0.8%) had titre of 80. None of the subjects had *paratyphi BH* titre of 160 and 320.

In their study on plasma titres of antibodies to *Salmonella* O and H antigens in Ibadan, Nigeria, Oyeyinka and Salimonu (2002) found that *S. paratyphi BH* represented the least prevalence at 1.1%. This study does agree with the fact that *paratyphi BH* is of the least prevalence but it has also shown a slightly higher prevalence of *paratyphi BH* of 4.5% compared to 1.1% finding of Oyeyinka and Salimonu. On the other hand Ibekwe *et al.* (2008) found that the highest *typhi O* end titres occurring in > 5% of the sample in their study was titre 40, and 80 for *typhi H* end titres. On contrary this study showed that the highest *typhi O* end titre occurring in > 5% of the subjects was titre 80, and for *typhi H* end titre occurring in > 5% of the subjects was titre 80. So titre 40 found by Ibekwe *et al.* (2008) was slightly lower than the findings of this study.

Bharat, Rajendra, Shyam and Janak (2009) in their study on distribution of antibody titre against *Salmonella enterica* among healthy individuals in Nepal, found that 62 % of their 100 samples showed agglutination in titre ≥ 20 for antibodies against *typhi*

O or *H* and *paratyphi* AH and BH. However, this study revealed that 49.8% of the subjects had *Salmonella* agglutinin titres ≥ 20 for O or H antibodies against *S. typhi* and *paratyphi* B. Bharat *et al.* (2009) found that titre 80 for *typhi O* was the highest titre occurring in $> 5\%$ of the subjects and titre 160 for *typhi H*. These findings are in accord with this study on *typhi O* titre of 80 but differ substantially on the *typhi H* end titre of 160 as this study had titre 80.

In this study there were no end titres ≥ 20 for *S. paratyphi* AH since none of the subjects had positive titres for *paratyphi* AH. The study by Bharat *et al.* (2009) revealed 12 (12%) of the subjects had *paratyphi* AH end titres ≥ 20 . Of particular interest was the distribution of *S. paratyphi* BH positive titre of 3% which is almost close to this study's positive end titre of 4.5 % for *paratyphi* BH.

4.4 END TITRE DISTRIBUTION BY AGE GROUP AMONG THE 400 SUBJECTS

The end titre distribution for *Salmonella typhi O* and *H* was analysed according to age for both *S. typhi O* and *H* and the results are presented in table 4.2 and figure 4.4 below.

4.4.1 *Typhi O* end titre distribution by age

The age group of the subjects ranged from 2 years to >55yrs, the < 16years and the 16-25 years age groups had the highest number of subjects.

Table 4.2 *Typhi O* end titres distribution by age group among the 400 subjects

End Titres <i>Typhi O</i>								
Age groups	<20	20	40	80	160	Grand Total	>20	Percent >20
<16	159 79.5%	8 4%	20 10%	12 6%	1 0.5%	200	41	20.5%
16-25	51 63.8%	4 5%	16 20%	8 10%	1 1.3%	80	29	36.3%
26-35	41 78.8%	6 11.5%	4 7.7%	1 1.9%	0 0%	52	11	21.2%
36-45	27 79.4%	2 5.9%	5 14.7%	0 0%	0 0%	34	7	20.6%
46-55	14 66.7%	3 14.3%	3 14.3%	1 4.8%	0 0%	21	7	33.3%
>55	9 69.2%	1 7.7%	2 15.4%	1 7.7%	0 0%	13	4	30.8%
Grand Total	301	24	50	23	2	400	99	24.8%

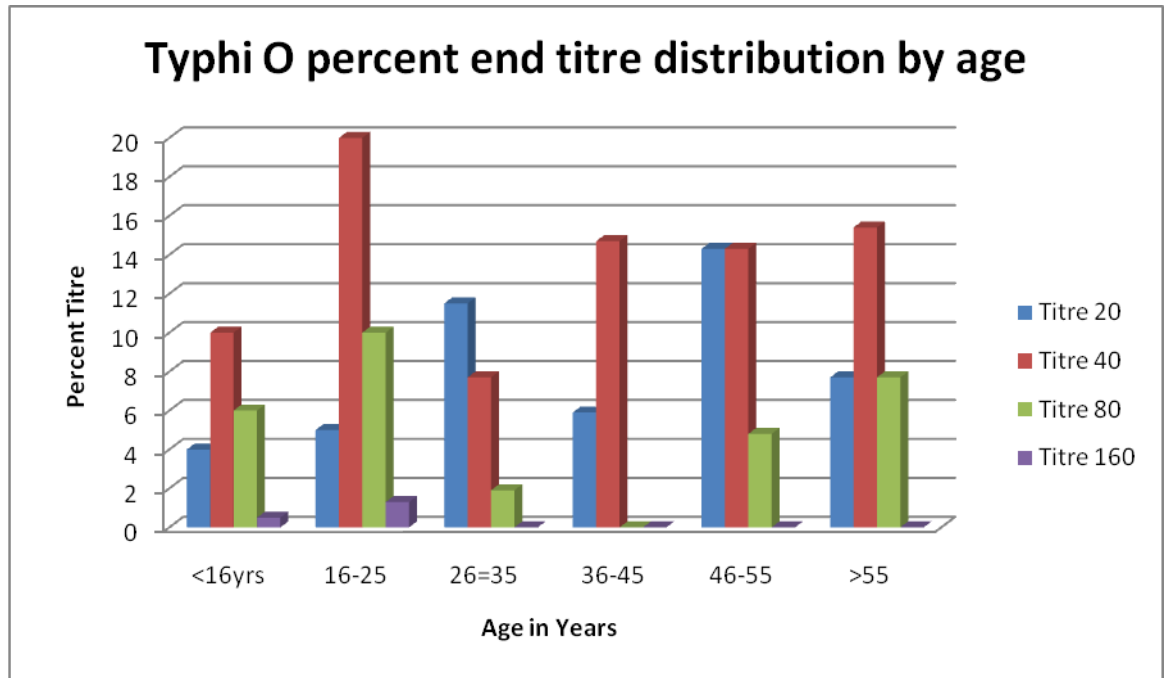


Figure 4.2 *Typhi O* percent end titre distribution by age among the 400 subjects

In this study the Pearson Chi-Square statistic revealed that there is no significant ($p > 0.05$) association between age and *Salmonella* agglutinin titres. A significant ($p < 0.05$) proportion of the children (< 16 years) showed end titres ≥ 20 for *typhi O* accounting for 20.5% of the subjects, whilst 36.3% of young adults (16 to 25 years) had titres ≥ 20 for *typhi O*. On the other hand 21.2% of the 26-35 year age group had end titres ≥ 20 for *typhi O* and 20.6% of the 36-45 year age group had *typhi O* end titres ≥ 20 . A third (33.3%) of the 46-55 year age group had *typhi O* end titres ≥ 20 , whilst 30.8% of the >55 year age group had *typhi O* end titres ≥ 20 . The highest *typhi O* end titre occurring in > 5% of the subjects aged <16 years, 16-25 years, and > 55 years, was titre 80; the highest *typhi O* end titre occurring in subjects aged 26-35 years, 36-45 years and 46-55 years was titre 40.

In their study, on plasma titres of antibodies to *Salmonella* O and H antigens, Oyeyinka and Salimonu (2002) showed that significantly higher titres ($p < 0.05$) of antibody to *S. typhi* were observed in the 6-25 year age group.

This study also showed a significant ($p < 0.05$) higher titres of antibody to *S. typhi* O among the < 16 year and 16-25 years aged groups. These findings concur with those of Oyeyinka and Salimonu (2002). The latter showed that the mean titre for *S. typhi* O ranged from 15 ± 23.0 in 26 to 45 year olds to 19 ± 9.8 in subjects 46 to 65 years old in their study. In this study the mean *Salmonella typhi* O agglutinin titres ranged from 50.7 ± 28.3 for the < 16 year; 50.4 ± 30.0 for the 16 to 25 year age group; 32.7 ± 17.6 for the 26 to 35 year age group; 34.3 ± 9.8 for the 36 to 45 year age group; 37.1 ± 21.4 for the 46 to 55 year age group; and up to 13.8 ± 43.9 for the > 55 year age group.

4.4.2 *Typhi* H end titre distribution by age

In this study the Pearson Chi-Square statistic revealed that there is no significant association ($p > 0.05$) between age and *typhi* H agglutinin titres. Table 4.3 and Figure 4.3 present the *typhi* H end titre distribution by age. Children (< 16 year) and young adults had the least *typhi* H end titres ≥ 20 of 17.0% and 8.8%, respectively, whilst the 46 to 55, and > 55 year age groups, respectively, had the highest *typhi* H end titres ≥ 20 of 61.5% and 57.1%.

For the < 16year age group 4.5% had *typhi H* end titre of 20; 5.5% had *typhi H* end titre of 40; 4% had *typhi H* end titre of 80 whilst 3% had *typhi H* titre of 320. Age group 16 to 25 years had *typhi* end titres of 20 and 40 at 3.8% and 5%, respectively; the 26 to 35, 36 to 45, 46 to 55, and the >55year old groups had titre 80 as the highest titre occurring > 5 % of the subjects. The highest *typhi H* end titre occurring in > 5% of the < 16years and 16-25years age group was titre 40.

In a study on the Widal test in the diagnosis of typhoid fever in Turkey, Onder and Banu (2002) observed that the majority of their subjects had titres for *S. typhi H* of 200. These figures differ substantially from the findings of this study where the majority ($p < 0.05$) of the subjects had titre 80 for *typhi H*.

Table 4.3 *Typhi H* end titres distribution by age group among the 400 subjects

Age Group	End Titres <i>Typhi H</i>								
	<20	20	40	80	160	320	Grand Total	>20	Percent >20
<16	166 83%	9 4.5%	11 5.5%	8 4%	0 0%	6 3%	200	34	17.0%
16-25	73 91.3%	3 3.8%	4 5%	0 0%	0 0%	0 0%	80	7	8.8%
26-35	41 78.8%	0 0%	5 9.6%	6 11.5%	0 0%	0 0%	52	11	21.2%
36-45	24 70.6%	1 2.9%	3 8.8%	5 14.7%	0 0%	1 2.9%	34	10	29.4%
46-55	9 4.3%	1 4.8%	1 4.8%	8 38.1%	2 9.5%	0 0%	21	12	57.1%
>55	5 38.5%	0 0%	2 15.3%	6 46.2%	0 0%	0 0%	13	8	61.5%
Grand Total	318	14	26	33	2	7	400	82	20.5%

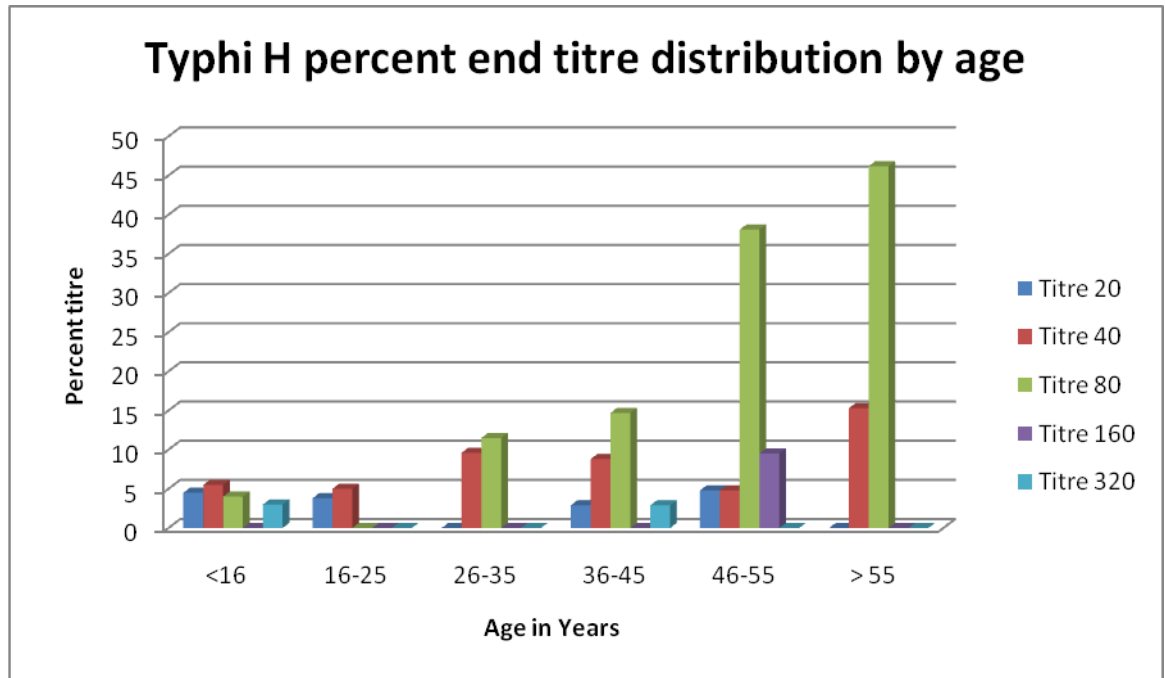


Figure 4.3 *Typhi H* end titre distribution by age among 400 subjects

4.4.3 *Typhi O* end titre distribution by HIV status

The *typhi O* end titres were analysed for their distribution among all the subjects (n=400) by HIV status. The findings are presented in Table 4.4 and Figure 4.4 below.

Table 4.4 *Typhi O* end titres distribution by HIV status among the 400 subjects

HIV STATUS	End Titres <i>Typhi O</i>					Grand Total	>20	Percent >20
	<20	20	40	80	160			
NEGATIVE	140 70%	12 6%	30 15%	16 8%	2 1%	200	60	30.0%
POSITIVE	161 80.5%	12 6%	20 10%	7 3.5%	0 0%	200	39	19.5%
Grand Total	301	24	50	23	2	400	99	24.8%

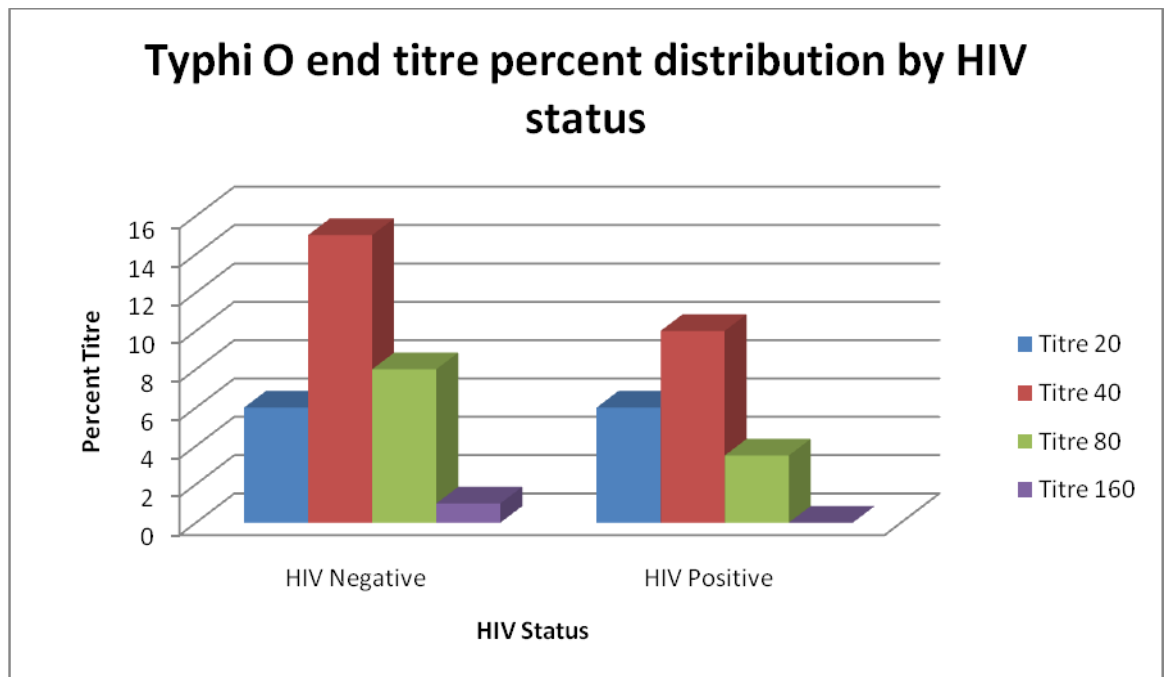


Figure 4.4 *Typhi O* end titre percent distribution by HIV status

In this study the Pearson Chi-Square statistic showed that there is significant ($p < 0.05$) association between HIV status and *typhi O* agglutinin titres. At least 30% of the 200 HIV negative subjects had *typhi O* end titres ≥ 20 while 19.5% of the 200 HIV positive subjects had *typhi O* end titres ≥ 20 . HIV negative subjects produced significantly ($p < 0.05$) higher *typhi O* titres compared to the HIV positive subjects. Seventy percent (70%) of the HIV negative subjects had *typhi O* end titres < 20 (negative) while 80.5 % of the HIV positive subjects had *typhi O* end titre < 20 . Of the 200 HIV negative subjects 6% had *typhi O* end titres of 20; 15% had *typhi O* end titres of 40; 8% had *typhi O* end titres of 80; and 1% had *typhi O* end titres of 160. None of the HIV negative subjects ($n=200$) had *typhi O* end titres of 320.

On the other hand significantly ($p < 0.05$) low *typhi O* end titres ≥ 20 were observed for all of HIV positive subjects ($n=200$) which ranged from 0 to 10 %. Of the HIV positive subjects ($n=200$) 6% had *typhi O* end titre of 20; 10% had *typhi O* end titre of 40; and 3.5% had *typhi O* end titre of 80. None of the HIV positive subjects had *typhi O* end titre ≥ 160 . The highest *typhi O* end titre occurring in $> 5\%$ of the HIV negative subjects was titre 80, whilst the highest *typhi O* end titre occurring in $> 5\%$ of the HIV positive subjects was titre 40.

4.4.4 *Typhi H* end titre distribution by HIV status

Typhi H end titres were analysed according to HIV status of all the subjects ($n=400$). The findings are presented in Table 4.5 and Figure 4.5 below.

Table 4.5 *Typhi H* end titres distribution by HIV status among the 400 subjects

HIV Status	End titre <i>Typhi H</i>								
	<20	20	40	80	160	320	Grand Total	>20	Percent >20
NEGATIVE	177 89%	4 2%	9 5%	6 3%	0 0	4 2%	200	23	12%
POSITIVE	141 71%	10 5%	17 9%	27 14%	2 1%	3 2%	200	59	30%
Grand Total	318	14	26	33	2	7	400	82	21%

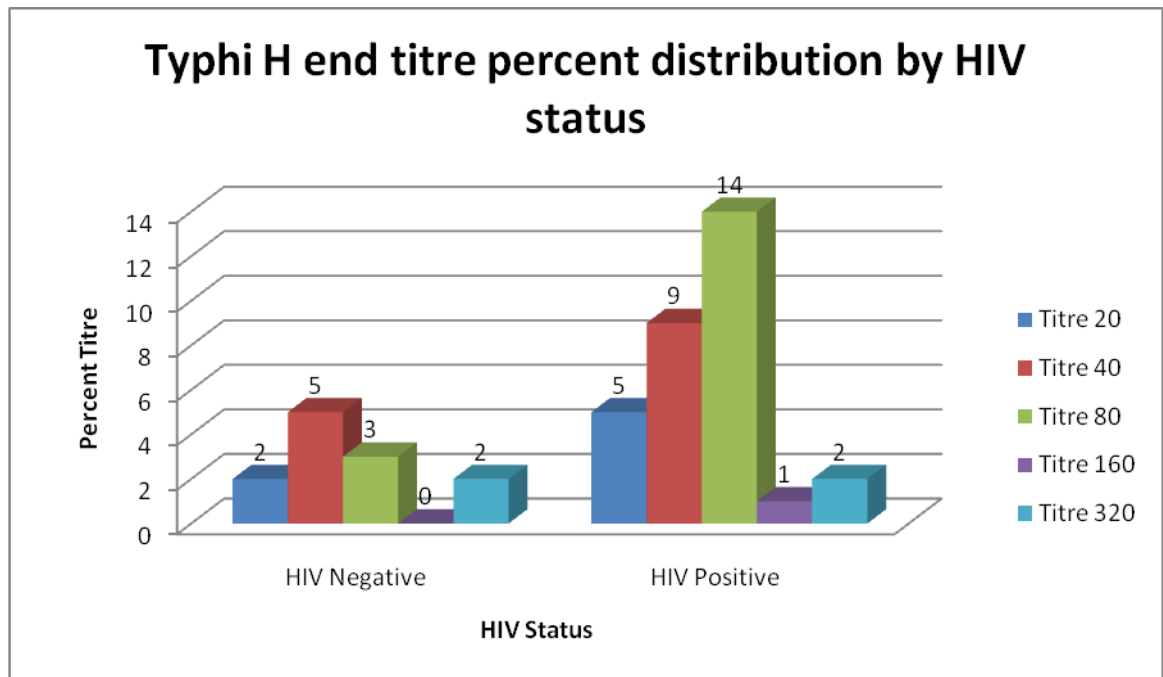


Figure 4.5 *Typhi H* end titre percent distribution by HIV status among 400 subjects

In this study the Pearson Chi-Square statistic revealed a significant ($p < 0.05$) association between HIV status and *typhi H* agglutinin titres. About 12% of the 200 HIV negative subjects had *typhi H* end titres ≥ 20 whilst 30 % of the 200 HIV positive subjects had *typhi H* end titres ≥ 20 . Significantly higher ($p < 0.05$) *typhi H* end titres were found among the HIV positive compared to the HIV negative subjects. In terms of the HIV negative subjects ($n=200$) 89 % had *typhi H* end titres < 20 ; 2% had *typhi H* end titre of 40; 3% had *typhi H* end titre of 80; and 2% had *typhi H* end titre of 320. None of the HIV negative subjects had *typhi H* end titre of 160.

In terms of the HIV positive subjects ($n=200$), 71% had *typhi H* end titre < 20 ; at least 5% had *typhi H* end titre of 20; 9% had *typhi H* end titre of 40; 14% had *typhi H*

end titre of 80; 1% had *typhi H* end titre of 160; and 2% had *typhi H* end titre of 320. The highest *typhi H* end titre occurring in > 5% of the HIV negative subjects was 40 and for HIV positive subjects it was 80. There could be two explanations for these observations. Firstly, degradation of *typhi H* antibodies is faster in immune competent HIV negative hosts than in immune compromised HIV positive hosts. Thus a *typhi H* antibody tends to persist even much longer and at higher titres in HIV positive hosts than in HIV negative hosts. Secondly, persistency of *typhi H* titres is a function of the switching off of secondary immune response after clearance of an infection. This switching off, or termination, of an immune response is a function of the immune competency of the hosts. Immune competent hosts tend to terminate secondary immune responses faster than immune compromised hosts. Increased persistency of *typhi H* agglutinins in HIV positive subjects can therefore be attributed to failure, or delayed termination of secondary immune response in immune compromised HIV positive subjects.

HIV co-infection with other diseases is known to cause complications in the diagnosis, management and treatment of these diseases. Typhoid fever, tuberculosis (TB) and malaria have not been spared from the complications associated with HIV co-infection among HIV positive patients. According to the WHO (2010), in their country specific report on TB, antiretroviral treatment (ARV) is known to interfere with TB treatment and *vice versa*. Most of the HIV positive patients used in this study was receiving ARVs. In normal circumstances an HIV negative patient has a 10% chance of developing the TB disease in a life time whereas co-infection with HIV increases the risk of developing TB disease to 10% per year. The progression

of TB in HIV patients is faster because as the CD4 cell tries to fight the TB bacilli it is exposed to the HIV. This then quickens the depletion of CD4 cells leading to enhanced progression from HIV infection to AIDS and/or TB.

The treatment of malaria and typhoid fever co-infection is a common phenomenon in Africa. Malaria and typhoid fever remain a treatment problem for many people in sub-Saharan Africa for several reasons. For example, increasing poverty, deterioration in public health services, a high prevalence of HIV/AIDS, an increasing resistance of malaria parasites to antimalarial medicines, a lack of potable water, and a wide spread misuse of Widal test for diagnosing typhoid fever, all contribute to problems when treating these diseases (Alnwich, 2001). Malaria and typhoid fever often present with mimicking signs and symptoms especially in the early stages of typhoid fever (Nsutebu & Ndumbe, 2001). It is very common to see patients undergoing both typhoid and malaria treatment even if diagnosis of these diseases has not been confirmed. There are more typhoid cases in areas with drug resistant malaria thus cross reaction between malaria parasites and *Salmonella* antigens may cause a false positive Widal test. As HIV/AIDS weakens the immune system it is anticipated that immune response (both primary and secondary) to typhoid fever by HIV positive patients will be severely affected. In their study on the rate of co-infection with malaria parasites and *Salmonella typhi* in Zaria, Kaduna state, Nigeria, Mbuh, Galadima and Ogbadu (2003) found a co-infection rate with malaria of 5% in culture diagnosis of typhoid fever compared to a 10% rate in a Widal test.

Diagnostic titres for *typhi O* in HIV positive subjects are greater than 40 where as for *typhi H* it is greater than 80. On the other hand diagnostic *typhi O* titres in HIV negative subjects are greater than 80 and *typhi H* titres are greater than 40. Table 4.6 presents a summary of these findings.

Table 4.6 Baseline and diagnostic *Salmonella* agglutinin titres by HIV status

SUBJECTS	NORMAL TITRES (Found in normal population)		DIAGNOSTIC TITRES (Found in Typhoid patients)	
	<i>TYPHI O</i>	<i>TYPHI H</i>	<i>TYPHI O</i>	<i>TYPHI H</i>
	HIV POSITIVE	40	80	>40
HIV NEGATIVE	80	40	>80	>40

Currently the diagnostic titre for both *typhi O* and *typhi H* titre is pegged at 160 which seem to be very high and most cases of typhoid fever are being missed. Unfortunately there is no published and/or unpublished data on the distribution of *typhi O* and *H* end titre by HIV status. The researcher is therefore of the opinion the current study is the first ever undertaken in Namibia, and in the whole world.

4.4.5 *Typhi O* end titre distribution by gender

Typhi O end titre were analysed for their distribution according to gender. The results for gender related *typhi O* end titre distribution are presented in Table 4.7 and Figure 4.6 present *typhi O* below.

Table 4.7 *Typhi O* end titres distribution by gender among the 400 subjects

<i>TYPHI O</i>			
END TITRE	FEMALE	MALE	Grand Total
<20	149 74.5%	152 76%	301
20	18 9%	6 3%	24
40	24 12%	26 13%	50
80	8 4%	15 7.5%	23
160	1 0.5%	1 0.5%	2
>20	51	48	99
Grand Total	200	200	400
PERCENT			
>20	25.5%	24.0%	24.8%

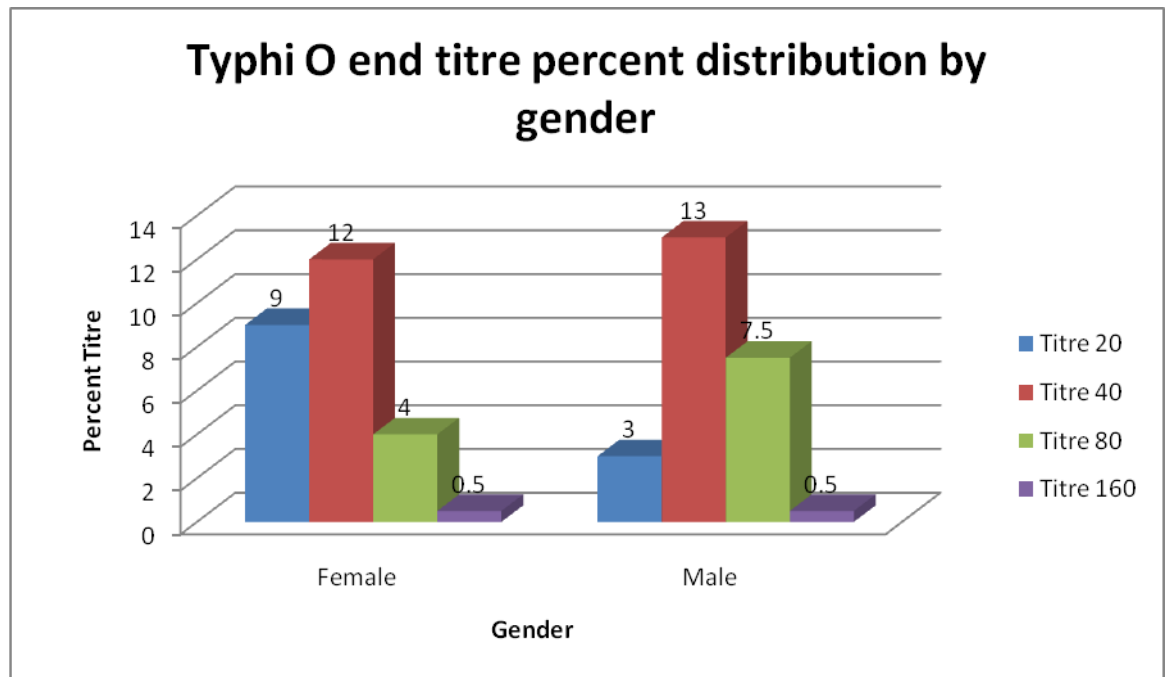


Figure 4.6 *Typhi O* end titre percent distribution by gender among the 400 subjects

In this study the Pearson Chi-Square statistics revealed that there is no significant ($p > 0.05$) association between gender and *typhi O* agglutinin titres. The distribution of positive *typhi O* end titres between male and female subjects was almost similar at 24.0% and 25.5%, respectively. In terms of the 200 females 74.5% had *typhi O* end titre of < 20 ; 9 % had *typhi O* end titre of 20; 12% had *typhi O* end titre of 40; 4 % had *typhi O* end titre of 80; and 0.5% had *typhi O* end titre of 160. About 76% of the 200 male subjects had *typhi O* end titres < 20 ; 3% had *typhi O* end titre of 20; 13% had *typhi O* end titre of 40; 7.5% had *typhi O* end titre of 80; and 0.5% had *typhi O* end titre of 160.

The highest *typhi O* end titre occurring in $> 5\%$ of the female subjects was titre 40 whereas for the male subjects it was titre 80. It was found that more female subjects

(n=18) had *typhi O* titre 40 compared to the male subjects (n=6). On the other hand more male subjects (n=15) had *typhi O* end titre of 80 compared to female subjects (n=8). Ibekwe et al. (2008) found in their study, on baseline *Salmonella* agglutinin titres in apparently healthy Nigerian fishermen, that 32% of male subjects had *typhi O* titre ≥ 20 . However, in this current study 24% of the male subjects (n=200) had *typhi O* titre ≥ 20 . This current study obtained significant ($p < 0.05$) high *typhi O* titre ≥ 20 for females (25.5%) compared to the results of Ibekwe et al. (2008) since they found *typhi O* positive end titre for females to be 12% in their study.

In a study by Oyeyinka et al. (2002) it was found that in general there was no significant differences in *typhi O* end titres between males and female subjects. This does not concur well with this current study which reveals that the highest *typhi O* end titre occurring in $>5\%$ of the female subjects is titre 40 and for males it is titre 80.

4.4.6 *Typhi H* end titre distribution by gender

Table 4.8 and Figure 4.7 below present *typhi H* end titres according to gender among the 400 subjects. About 20.5% of the all the subjects (n=400) had *typhi H* end titres ≥ 20 whilst 22% of the 200 females had *typhi H* end titres ≥ 20 and 19.0% of the 200 male subjects had *typhi H* titres ≥ 20 . None of the male subjects had titre 160 for *typhi H* while 1% of the female subjects had *typhi H* titre 160. About 1.5% of the females, and 2% of the male subjects, had *typhi H* titre of 320.

Table 4.8 *Typhi H* end titres distribution by gender among the 400 subjects

<i>TYPHI H</i>			
END TITRE	FEMALE	MALE	Grand Total
<20	156 78%	162 81%	318
20	5 2.5%	9 4.5%	14
40	18 9%	8 4%	26
80	16 8%	17 8.5%	33
160	2 1%	0 0%	2
320	3 1.5%	4 2%	7
>20	44	38	82
Grand Total	200	200	400
PERCENT			
>20	22.0%	19.0%	20.5%

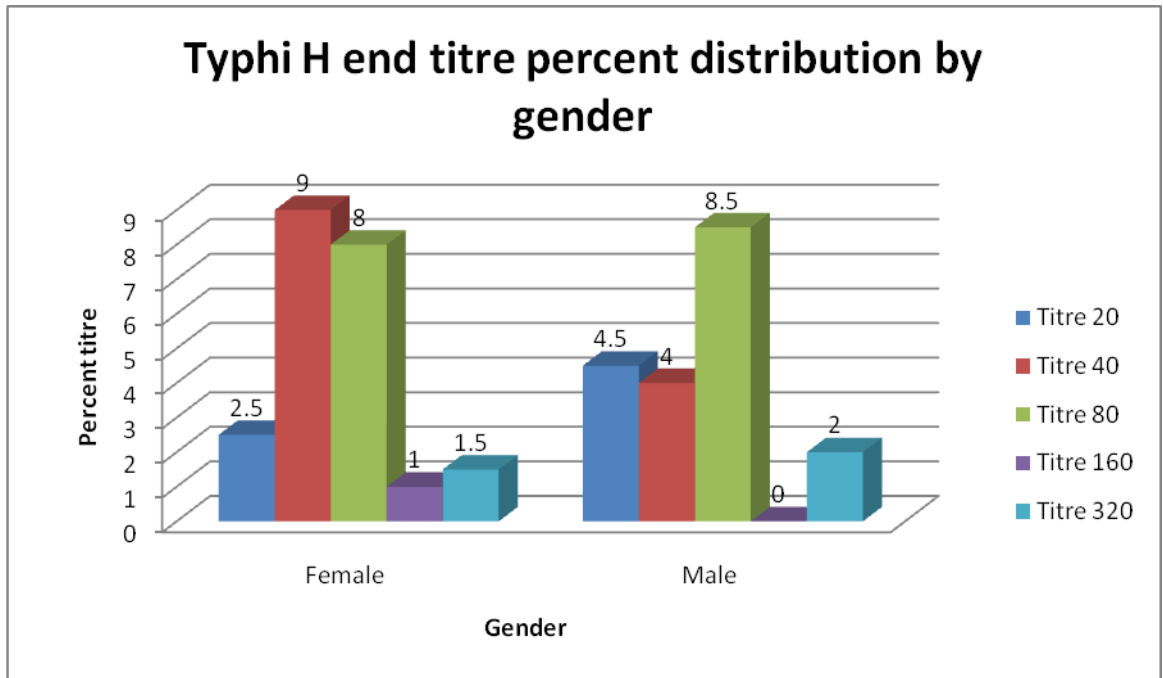


Figure 4.7 *Typhi H* end titre percent distribution by gender among the 400 subjects

The Pearson Chi-Square statistics revealed that in this study there was no significant ($p > 0.05$) association between gender and *typhi H* agglutinin titres. The general distribution of *typhi H* end titre among male subjects and female subjects was almost similar. Of the 200 female subjects 2.5% had *typhi H* end titres of 20; 9% had *typhi H* end titre of 40; 8% had *typhi H* end titre of 80; 1% had *typhi H* end titre of 160; and 1.5% had *typhi H* end titre of 320. Unlike the female subjects 4.5% of the male subjects had *typhi H* end titre of 20; 4% had *typhi H* end titre of 40; 8.5% had *typhi H* end titre of 80; none had *typhi H* end titre of 160; and only 2% had *typhi H* end titre of 320.

In terms of the *typhi O* end titres none of the subjects had titre 320 but for *typhi H* end titres of 320 were present in both the female and male subjects. The highest *typhi H* end titre occurring in > 5% of both male and female subjects was titre 80. Ibekwe et al. (2008) found that 41.5% of the male subjects in their study had *typhi H* end titre ≥ 20 and 29.5% of their female subjects had *typhi H* end titre ≥ 20 . Ibekwe et al. (2008) also found out that all agglutinin titres tested were present in the sera of all females up to titre 160, and that *typhi H* end titres in male subjects were higher than those of *typhi O* end titres. According to them the female subjects in their study had *typhi O* end titres higher than those of the *typhi H* end titres. The same was found to be true in this study: *typhi O* end titres in female subjects are higher than *typhi H* end titres. On the other hand in this study *typhi H* end titres in female subjects range from 1 to 9% and were present in sera of all females up to titre 320 whereas *typhi H* end titres in males are lower than *typhi O* end titres.

4.5 SUMMARY

The findings of the study are presented in this chapter. The presentation places particular emphasis on the overall distribution of *S. typhi O*, *H* and *paratyphi AH* and *BH* among all the subjects (n=400). Also presented was the distribution of *typhi O* and *H* end titres according to age, gender and HIV status. Discussions on *S. paratyphi AH* and *BH* were excluded as the results showed they were insignificant in the subjects studied. A discussion was presented on the relationship between this current study and other published studies.

CHAPTER 5

CONCEPTUALISATION OF THE STUDY

5.1 PHILOSOPHICAL BASIS OF THE STUDY

The philosophical basis of the study was positivism for the following reasons. The researcher moved in an orderly and systematic fashion through the: identification of the problem; selection of concepts on which to focus; the design of the study and collection of data to the conclusion of the study. Deductive reasoning was used to generate hunches that are tested in the real world. In this study there was significant ($p < 0.05$) association between HIV status and *Salmonella* agglutinin titres, whilst there was no significant ($p > 0.05$) association between age, gender and *Salmonella* agglutinin titres. Deductive reasoning seeks to extract specific propositions from general account of reality. In addressing the research questions/objectives, the researcher used empirical evidence. In this study a quantitative descriptive and explorative serological study was conducted.

An important goal of a scientific study is to understand the phenomenon, not in isolated circumstances alone, but in broad general sense {(generalisation) (Burns & Groove, 2005)}. In this study a representative sample from the entire population of the study was used to allow generalisation of the findings to the specific population under study.

5.2 CONCEPTUAL FRAMEWORK

A conceptual framework can be defined as a set of highly abstract terms related to constructs that broadly explain phenomenon of interest, express assumptions and reflect a philosophical basis (Burns & Groove, 2005). It can be stated then that a conceptual framework classifies, or categorises, the phenomenon and enables a researcher to link findings to the body of knowledge and field of practice. Objective 3 of the study was to develop a conceptual framework based on the outcome of situation analysis. It is clear from the findings of this study that HIV status influences the diagnostic *Salmonella* agglutinin titres for people living with HIV/AIDS. It is also clear that gender and age have no significant effect on *Salmonella* agglutinin titres. In the absence of HIV/AIDS a patient with typhoid infection will mount a normal primary and secondary response resulting in the normal production of *typhi O* and *typhi H* agglutinins. When the infection or disease is cleared in these HIV negative hosts, the immune response is switched off/terminated timeously and degradation of *typhi H* agglutinins is initiated and executed efficiently. Thus in HIV negative hosts infected with typhoid fever there are higher titres of *typhi O* agglutinins and lower titres of *typhi H* agglutinins.

In the presence of HIV/AIDS, subjects infected with typhoid fever will mount an inferior primary and secondary response resulting in below normal production of *typhi O* agglutinins and slightly raised *typhi H* agglutinins. Since the immune system of HIV positive subjects is compromised, CD4 cells are few and activation of B lymphocytes into plasma cells is compromised in number and time of onset resulting

in the production of low *typhi O* titres. Termination of immune response is severely hampered in the presence of HIV/AIDS as the negative feedback mechanisms are affected and these result in delayed degradation of *typhi H* agglutinins therefore the persistency of *typhi H* agglutinins at higher titres observed in HIV positive subjects.

The conceptual framework of this study derived from the practice theory of Dickoff, James and Wiedenbach (1968). The checklist that Dickoff et al. (1968) proposed consists of the several questions that are presented below. The responses to each question in terms of this study follow each question (see reasoning map in Figure 5.1).

- Who or what performs the activity i.e. the agent? In this study the researcher performed the activity, namely the development of the guide.
- Who is the recipient of the activity? In this study the recipient of the activity includes clinicians, nurses, dieticians, health inspectors, laboratory technologists, and scientists.
- In what context is the activity performed? In this study the activity is performed in a clinical and laboratory medicine set-up.
- What are the challenges? The challenges that may hinder the activity to realise the terminus point include: endogenous limitations of the activity itself (i.e. implementation of the guide) could be the attitude and level of appreciation of the activity by the recipients which include health care workers in general.

- What is the procedure activity? The procedure activity is the development and implementation of the guide for *Salmonella* agglutinin titres according to age, gender and HIV status among patients attending hospitals in northern Namibia.
- What is the terminus or end point of the activity? The terminus of the activity is adoption of the guide and its use in clinical and laboratory medicine in Namibia.

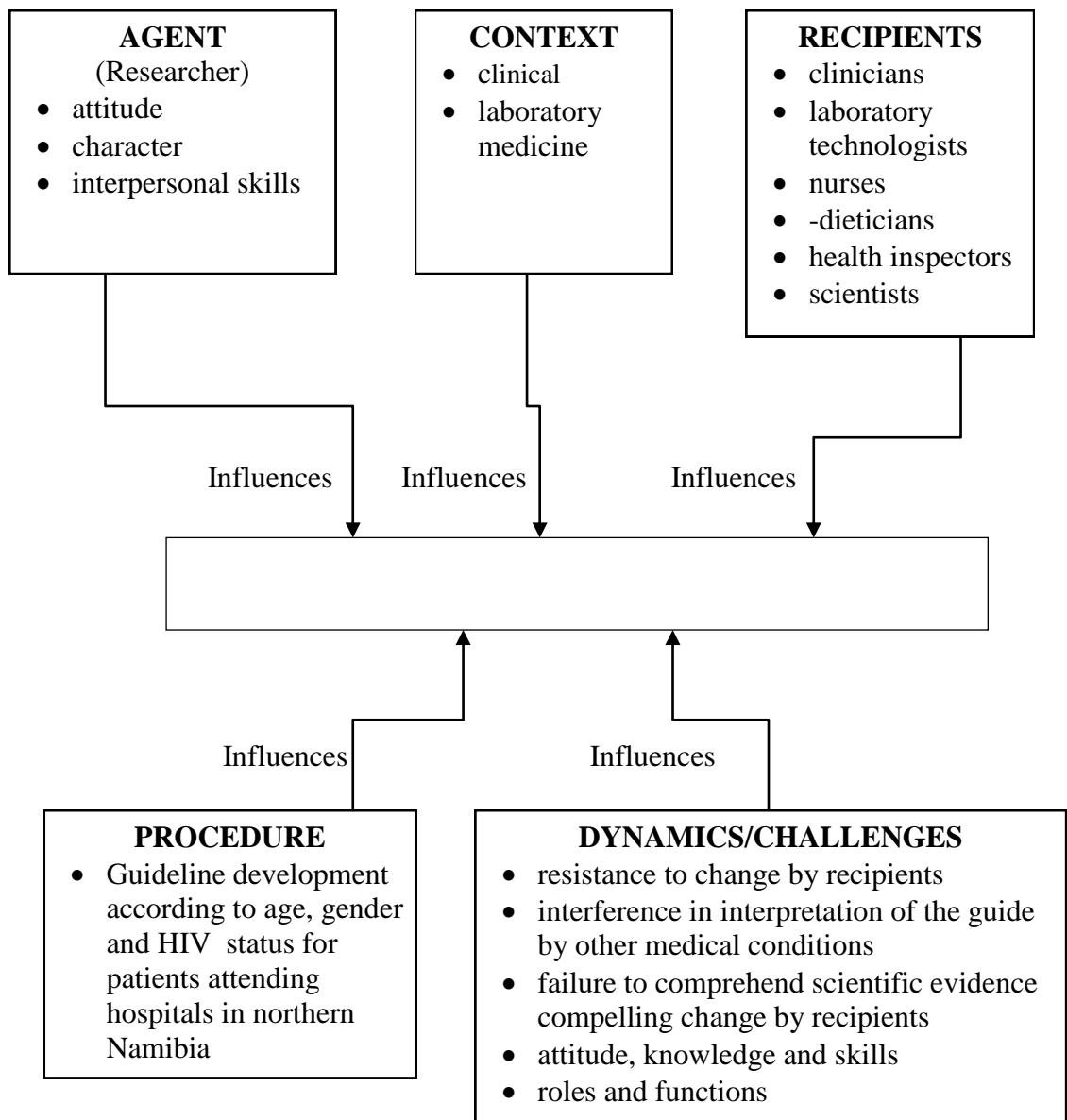


Figure 5.1 Reasoning map

In order to achieve the terminus of the activity, specific characteristics of the agent and recipient must be fulfilled. What follows is a description of the attributes of the agent and recipient, as well as elaborations of the other factors that may hinder the realisation of the terminus of the activity.

5.2.1 The agent

The role of the agent or, in particular the researcher, is to sell the guide to recipients without prejudice or coercion. According to CMO Council, (2008) the researcher must have the following characteristics, attributes, knowledge and skills to sell the guide to recipients.

- Recipient centric: puts the recipients' needs, wants and desires first. The researcher must own the recipients and work to activate them to be loyal and must practice word of mouth advocacy.
- Competitive strategy: the agent must be a big picture thinker by anticipating recipient behaviour and championing ethical strategies to modify recipients' behaviours. The researcher must know the recipients inside and out.
- Strong activity driver: the agent must have a sound grasp of fundamentals of typhoid fever and HIV. The agent must map routes to winning the minds and hearts of recipients, not through coercion, but through use of factual and scientific means.
- Guide advocate and champion: the researcher must demonstrate personal passion for the guide and its adoption and use in Namibia and must ensure

consistency and accuracy of message to all recipients (adopted from CMO Council, 2008).

- Ability to secure MoHSS and NIP executive management support and foster cross-functional relationships during the selling of the guide to the recipients. The agent must be an inspirational leader who is competent in offering support to recipient teams and valuable targeted initiatives for them to understand and implement the guide accordingly. The agent must by all means legitimize the change from the old (existing) guide to the newly developed guide. The agent must not try to misrepresent the benefits of the guide so as to induce recipients' participation, or to look good or to avoid losing face and looking bad (De Paulo, Kashy, Kirkendol & Wyer, 1996).
- A visionary and thoughtful leader: the agent must be a master strategist who embraces multi-channel and multi-level strategies to reach and involve the recipients, ensuring the future of the guide as well as realising the terminus of the activity.

5.2.1.1 Conclusion

The agent must therefore have good communication and interpersonal skills and sound knowledge of the subject in order to ensure adoption and use of the guide by the recipients. The agent must be able to create a clear, focused concept of the guide and communicate it clearly to the recipients in order to realise the terminus of the activity.

5.2.2 The recipient

The recipient (Healthcare workers) must be committed to adopt and use the new guidelines. Recipients therefore have roles and responsibilities to play in order to realise the terminus of the activity. To a large extent it is through the meanings that recipients form regarding a change that leads to the impacts of change initiatives (George & Jones, 2001). The recipients must have good interpersonal skills and be able to view the value of the new guidelines as profitable to the profession and management of a patient. The recipients must be able to make sense out of the activity, namely the new guidelines as well as making sense of the change which involves an array of information. This information may include recipients' understandings of the nature of change that the agents expose, appraisal of whether implementation deviates from the articulated plan and personal impacts of change. In general such effects of participation on sense-making are expected in view of the information that actively involves recipients as well as what they derive from their experiences with the change itself and their greater exposure to the influence of change agents (Weber & Manning, 2001).

Some of the sense-making mediating variables likely to affect the adoption of the guide include: vision consistent with participation of recipients; contradictions and inconsistencies; personal impact of change on the recipient. If the recipients are able to share the same vision with the agent and, have less contradictions and inconsistencies, then the chances of adoption of the guidelines will be high. If the

guide is going to have a high positive personal impact on recipients then its adoption will be easy whilst the opposite is true.

The perceived outcome variables by the recipients will influence the adoption of the guide. According to Weber & Manning, (2001), the successful adoption of the guide is usually underscored by perceived losses or gains as presented below.

- Quality of care: if the recipients perceive the guide will bring gains in the quality of care then the adoption of the guidelines will be easy. On the other hand if the recipients do not foresee improvements in quality of care then they will be hesitant to adopt and use the guidelines.
- Professional development: the recipients will adopt and use the guide if they perceive training on the guide as well as its adoption and use as a platform to develop their profession.
- Work relationship: such a relationship between the agent and the recipient will also have an effect on the adoption of the guide. Poor working relationship between the agent and the recipients will result in resistance in adoption of the guide, whereas good professional work relationship will guarantee adoption and use of the guide all things being equal.
- The affect: the affect of the guide on the recipient will also influence its adoption and usage. If the affect is positive the guide will be adopted and used by the recipients.

5.2.2.1 Conclusion

Recipient participation, sense-making and affect of change have direct influence on the adoption and use of the new guidelines. The agent and recipient must not be prescriptive in nature or view each other as an antagonist; they should work as a team to implement the new guide in a manner conducive to both parties. It must be acknowledged that recipients' impressions of an intervention may be both consistent with, and diverge, from what the change agent intends. Change agents must actively solicit support and then work to understand and address misunderstandings of a change initiative held by its recipients as the initiative progresses. These are sometimes both positive and negative; sometimes contradictory perceptions may contain valuable information that can allow change agents to work together in devising midcourse corrections (George & Jones, 2001).

5.2.3 Dynamics, problems and challenges

The dynamics of selling, adopting and use of the guidelines are not immune to challenges and problems. The most likely challenges and problems that will bring about dynamism are the attitudes, knowledge, skills, roles and function of both the agent and the recipients. The following section discusses these problems and challenges.

5.2.3.1 Attitude

The respective attitudes of the agent and the recipient may result in failure in the adoption and use of the guide. If it is perceived that the recipients are resistant to change the agent may fail to positively see constructive criticisms from recipients and in so doing personal shortcomings may be overlooked. The agent must adopt a professional non-prescriptive attitude, open to constructive criticisms in order to gain understanding of the recipients concerns and fears and to capitalise on them: to turn perceived resistant to opportunity to sell the guide. The recipients on the other hand must also demonstrate professional and ethical commitment to adopt and use the guide in terms of its merit and clinical relevance for the betterment of patient management. Personal egos and grudges should never be allowed to take precedence of patient care; objectivity and subjectivity should be applied accordingly in judgement.

5.2.3.2 Knowledge and skills

The researcher must have exceptional knowledge of typhoid fever and HIV/AIDS relevant to the guide for easy and clear transfer of information to the recipients. The agent must be well versed in adult education and facilitation. In the absence of such knowledge and skills the adoption and use of the guide may be threatened. The researcher/agent must be a communicator who is very sensitive and respectful of the recipients. On the other hand the recipients must have sound knowledge of old guidelines in order to appreciate their shortfalls as well as the benefits of using the

new locally developed guidelines for the diagnosis of typhoid fever according to age, gender and HIV status. The recipients must be very inquisitive and at the same time possess good listening skills.

5.2.3.3 Roles and functions

The recipients and agent must have well delineated roles and responsibilities which they must accept and practice accordingly. A thorough knowledge of roles and function should limit incidences of role ambiguity. The primary role of the agent is to educate, train and provide skills to the recipients on how to use the guide. The agent must present as much accurate and reliable information as possible on the guide in order for the recipients to activate their sense-making process. The agent must also create conducive and enabling environments that allow recipients to air their concerns and raise constructive criticisms. The recipients must be committed to adopting and using the guidelines in clinical and laboratory medicine. They must also give feedback on areas that need improvement or change in relation to the application of guidelines in daily practice.

5.2.3.3.1 Conclusion

The successful adoption and use of the guide depends on well defined roles and functions of both the agent and recipients of the activity. Role ambiguity should be avoided through the agent's ability to give as much information as possible to

recipients. The recipients must be active and give feedback of challenges and areas for improvement to the agent.

5.2.4 The procedure

In this study the procedure is the guide on *Salmonella* agglutinin titres which is developed according to age, gender and HIV status among patients attending hospitals in northern Namibia. The guidelines include sections on patient preparation, specimen collection and preparation, normal and abnormal *Salmonella* agglutinins for diagnosis. It has a section on the interpretation of infection either as current or past and guidelines on screening of food handlers for typhoid fever.

5.2.5 Terminus/end point

In this study the end point is the adoption and use of the guidelines in Namibia. To ensure competency in the use of the guidelines, the recipients should be thoroughly trained by the researcher in small workgroup workshops at all hospitals in northern Namibia. Once training on the new guide is concluded the guidelines will be available and easily accessible for use in clinical and laboratory medicine. The agent in collaboration with NIP and MoHSS will be responsible for the training and availability of the guide.

5.3 SUMMARY

This chapter looked at the philosophical basis of the study, biomedical model and the conceptual frame-work. The philosophical basis of the study was positivism and deductive reasoning was used to generate hunches that are tested in the real world. The biomedical model is a reductionist one as it looks at underlying organic factors and the belief that the cause of a disorder, including a psychological disorder, is physical problem and has three crucial constructs. The conceptual framework of this study was derived from the practice theory of Dickoff, James and Wiedenbach (1968). Various issues, such as the agent, recipient, challenges, procedure and the terminus were discussed in terms of their respective impact on realising the terminus of the study.

CHAPTER 6

DEVELOPMENT OF THE GUIDE FOR *SALMONELLA* AGGLUTININ TITRES ACCORDING TO AGE, GENDER AND HIV STATUS

6.1 INTRODUCTION

The guidelines are based on the results of the study and tied to the biomedical model framework. The diagnostic significance of serological test depends, among other factors, on the positivity rate in the general population. Antibody build-up against specific antigens in a given community depends on endemicity of the specific infecting agents therefore their plasma antibody titres differ widely from place to place. In view of this it is necessary to determine the titres in a patient and general health population in order to know what titres indicate on-going or active infection (Oyeyinka & Salimonu, 2009). Indeed for reasons of cross reaction and anamnesis reaction, baseline level of antibodies in normal healthy individuals of any community should be known (Nsutebu & Ndumbe, 2001). Therefore it is a requirement to establish *Salmonella* agglutinin titres in the normal population of northern Namibia in order to establish titres that are diagnostic of typhoid fever. Since these *Salmonella* agglutinin titres differ from place to place, the current practice of using diagnostic titres which are not derived from the Namibian population constitute mismanagement of typhoid fever. It is against this background that this study established Namibian baseline *Salmonella* agglutinin titres according to age, gender and HIV status among patients attending hospitals in northern Namibia.

6.2 RATIONALE FOR DEVELOPMENT OF THE GUIDE ON THE INTERPRETATION OF THE WIDAL TEST

Before a patient can be diagnosed with typhoid fever by means of a Widal test, it is important to know the level of *Salmonella* agglutinins titres in normal populations. When the normal levels are known anything above the normal levels of *Salmonella* agglutinin titres will be regarded as diagnostic of typhoid fever. The first stage in the development of the guide was to do a serological study to determine the normal (baseline) *Salmonella* agglutinin titres for the population of northern Namibia. This was established from the study results in chapter 4. Before this guide was established HIV negative and positive patients, respectively, underwent similar screening for typhoid fever and the diagnostic titre for *typhi O* was 160. Therefore any patient who was found with titre <160 was regarded as negative for typhoid fever. The ramification of this was that some HIV patients were regarded as healthy but in actual fact were suffering from typhoid fever as the diagnostic titre used in the old guidelines was too high. In a nutshell the use of this guide for diagnosis of typhoid fever means that HIV positive and negative patients can accurately be diagnosed and regarded as truly healthy if the Widal test is negative.

6.3 GUIDE DEVELOPMENT PROCESS

The researcher developed a draft guide based on the results of the study (see Annexure D). The draft guide was then presented to the study supervisors to assess the content and face value of the document. It was the role of the researcher to

identify key stakeholders who are directly working with patients suspecting of typhoid fever or those testing specimens from patients suspected of having typhoid fever. The following steps were followed in the guide development process, starting with the identification of multidisciplinary guide development stakeholders.

6.3.1 Key stakeholders

The following people were identified because certain skills and expertise are required:

- The medical practioners at Oshakati hospital who handle cases of typhoid fever in both the out and in patient departments as well as HIV clinics.
- The laboratory staff at Oshakati hospital.
- The staff of the MoHSS Directorate of Policy and Planning.
- The guide was also presented at the Medical Seminar where about 200 medical officers in the region were present including other key note speakers from other regions in Namibia.

The medical practioners at Oshakati hospital's in and out patient departments were included as they are the one screening and treating patients suspected of typhoid fever. The medical practioners will be the ones using the guidelines in their day to day activities, and as such their involvement from the initial phase guarantees ownership and uptake of the guidelines.

On the other hand the laboratory staff was included in the stakeholder group as they are the ones performing the test and as such their involvement gave valuable information on the laboratory testing part of the guide.

The MoHSS directorate of policy and planning were included to give guidance on policy development in tandem with the Ministry of Health's policy development framework. The participants at the medical seminar were also used as stakeholders because it gave a multidisciplinary platform for assessment of the draft guide as it was constituted by various medical professionals from different regions in northern part of Namibia. The participants at the medical seminar came from different parts of Namibia and this helped to advocate for the use and adoption of the guide across the regions and countrywide.

6.3.1.1 Meeting with stakeholders

The researcher called for separate meetings with each of the stakeholder group and acted as the chairperson in all the meetings. At each meeting the researcher started by welcoming the participants and then explained the purpose of the meeting. The researcher also explained the commitment requested from each and every stakeholder. The draft guidelines were provided to the stakeholders, and they were asked to go and read and prepare discussion in puts at the feedback session after a week. These meetings were held from middle of August to begging of September 2011.

6.3.1.2 Discussion points with stakeholders

During discussion meetings with stakeholders, the researcher encouraged them at each occasion to scrutinize the document and to give an input into the document. In summary, the mandate of the stakeholders was to assess the following issues about the guide:

- Layout of the guide, including how easily accessible is the information given pressure associated with consultation rooms.
- Adequacy of information provided, including the clarity of instructions.
- Relevance of information in the guide.
- Any other changes or omissions noted and additional comments.

6.3.1.3 Outcomes from stakeholders (feedback sessions)

The stakeholders were able to contribute to and influence the form and final guidelines and this gave the much needed sense of ownership of the guidelines. The feedback was given on one on one basis with each stakeholder group. The following are the outcome of the discussions.

6.3.1.3.1 Feedback from medical practitioners

The medical practitioners felt the layout of the guide was too cramped as some of the information was not in point form. They felt the information was not easily

accessible, and some places in the guide instructions were not clearly stated including the finer details on specimen handling. The guide was found lacking on the screening of food handlers for typhoid fever and the medical practitioners felt that a section on screening of food handlers should be included in the guide. On the relevance of information in the guide, the medical practitioners felt the information was good enough. The issues raised by the medical practitioners were modified and incorporated in the final guide to be piloted for one month.

6.3.1.3.2 Feedback from MoHSS

The ministry of health officials did not raise any issues on the guide. This could have been the fact that they were pleased with the guide or they are waiting for the final version.

6.3.1.3.3 Feedback from NIP laboratory staff

The laboratory staff wanted clarity on the issue of modification of the Widal test procedure which was included in the guidelines. This included adding a 1 in 120 dilution between the 1 in 80 and 1 in 160 dilutions, as a measure to minimise missing of positive cases. This issue was explained thoroughly by the researcher and some practical training on the use of the guidelines was also given to medical laboratory technologist and technicians.

6.3.1.3.4 Feedback from medical seminar

The medical seminar presented a good opportunity for the guide to be scrutinized by a lot of professionals from different backgrounds. The majority of the medical practitioners present felt the guide was long overdue and welcomed the tool. Most of the practitioners wanted to know if the guide could be used across the whole country, and the researcher pointed out that the guide was specifically for northern Namibia, but, however, in the absence of other region specific guides, it is much better to use this guide as it reflects the Namibian situation better than using other guidelines from other countries. The other issues raised at the seminar were the sampling technique used, and whether healthy subjects were used and or patients suffering from typhoid fever. The researcher referred the participants to chapter 3 of the study on exclusion and inclusion criteria, which clearly stated that patients suspected or diagnosed with typhoid fever were excluded from the study. The other concern raised during the medical seminar pertained to the issue of specificity and sensitivity of the Widal test. The researcher clarified these issues by explaining the incorporation of internal quality controls in the Widal test as well as that screening for *Brucellosis* and *Proteus* infections was run parallel with the test to exclude false positives. The researcher also explained that the Widal test is not immune to these false positives or negatives results. He highlighted that it is an easy, cheap test whose results are comparable to the gold standard and that results are available on the same day and can be used in resource limited facilities.

6.3.2 Amended draft guide

During the feedback sessions with the stakeholders, the researcher noted all the concerns and suggestions of the stakeholders and used the information to amend the first draft guide into amended draft guide (see Annexure E). The amended draft guide was then piloted for a month by stakeholders working with cases of typhoid fever or samples from patients suspected of typhoid fever.

6.4 IMPLEMENTATION, MONITORING AND EVALUATION OF THE GUIDE

The guide was given to stakeholders who implemented it at Oshakati hospital for one full month from the first of October to the end of October 2011. The researcher had group meeting with the stakeholders and asked to critically assess the correlation between the clinical picture of the patient and the Widal test result.

6.4.1 Implementation, Monitoring and Evaluation Stakeholders

The following were identified and utilised as implementation, monitoring and evaluation stakeholders, for the reason that they are the ones involved in either screening and treatment of both in and out patients suspected of typhoid fever, or they are the ones involved in the testing of blood samples from patients suspected of typhoid fever.

- Medical practitioners working at in and out patients departments including HIV clinic, this team included specialist physicians and general practitioners.
- Medical Laboratory technicians and technologists working in immunochemistry at NIP Oshakati regional laboratory.

6.4.2 Feedback from stakeholders

After one month of implementation of the guidelines on interpretation of the Widal test, feedback meetings between the researcher and stakeholders were held. The key question was about the correlation of the clinical picture of the patient and the Widal test result. The stakeholders presented their recommendations and amendments to the researcher. Each stakeholder group gave their feedback to the researcher.

6.4.2.1 Feedback from medical practitioners

The following issues were raised by medical practitioners working with cases of typhoid fever. One of the most interesting case that raised doubts on correlation between clinical picture and Widal test results, was that of a medical doctor who presented with signs and symptoms highly suggestive of typhoid fever and a Widal test was done and the results were positive 1 in 160 for both typhi O and H and treatment was started. Two days after initiation of treatment, the doctor developed chicken pox and then everyone wondered whether the Widal test is really sensitive. The researcher was presented with the case and initiated an investigation including a

repeat of the test in the laboratory as well as a repeat test on the doctor two weeks later and all the results were positive.

The conclusion was that the doctor was not suffering from typhoid fever as indicated by the Widal test result but in fact she had a high background titre of *salmonella* agglutinin titres as she was from India. In India *salmonella* agglutinin titres of 1 in 180 for both typhi O and H are considered normal since it's a typhoid endemic area. This further reinforced the fact that diagnosis of typhoid fever by Widal test is only possible if normal background *salmonella* agglutinin titres are established for regions and countries, and also that this guide is specific for Namibians and not other nationalities.

The medical practioners also raised concerns on stability of *salmonella* antibodies in the serum when refrigerated as they felt false negatives may result from degeneration of the antibodies in an improperly stored serum sample. The researcher clarified that from past studies results, salmonella antibodies are stable up to 8 hours.

Over all the impression of the guide to the practioners was good and they promised to continuously monitor and give feedback on the guide.

6.4.2.2 Feedback from NIP laboratory staff

The laboratory staff managed to use the guide in their day to day performance of the Widal test and really appreciated the inclusion of the 1 in 120 dilution between the 1

in 80 and 1 in 160 dilutions during the performance of Widal test since a lot of positive patients were being missed. They had concerns on the lack of positive and negative controls for *Brucellosis* and *Proteus* but this was resolved by the procurement department of NIP who managed to buy the controls.

6.4.3 Final guide on interpretation of Widal test

After the implementation, monitoring and evaluation of the guidelines on interpretation of the Widal test, feedback was given by stakeholders to the researcher and a final document of the guidelines was developed. The literature was also consulted to ensure the final guideline document also concur with other documents developed internationally. The final guidelines developed are presented in the following section below.

6.5 THE GUIDE FOR INTERPRETATION OF THE WIDAL TEST ACCORDING TO HIV STATUS IN NORTHERN NAMIBIA

After wide consultation with multidisciplinary stakeholders, as well as results of this study and literature review, a final guideline document on the interpretation of Widal test according to HIV status in northern Namibia was developed.

6.5.1 Guideline 1: Specimen collection

6.5.1.1 Construct 1: All illness and symptoms and signs within the body arise from underlying disease

In this study it was found that there is a highly significant association between HIV status and *Salmonella* agglutinin titres. Furthermore, HIV negative subjects had higher titres for *Salmonella typhi O* agglutinin titres as compared to HIV positive subjects (patients) since they had very low titres of *typhi O* agglutinins. Therefore, the signs of low *Salmonella* agglutinin titres in HIV positive patients were as a result of an underlying disease which, in this case, was HIV. This underlying disease weakened the immune system and led to development of low titres of *Salmonella* agglutinins. The HIV negative subjects do not have an underlying disease their immune system is very efficient and produce high titres of *Salmonella* agglutinins.

6.5.1.2 Objective

The objective is to explain the important aspects of specimen collection from the patient suspected of having typhoid fever.

1. Blood specimen should be collected at the acute phase of illness within five to six days of onset of illness. At least 3ml (milliliters) of clotted blood should be submitted to the laboratory.

2. Samples should be collected from patients who are at least two years old as the immune systems in <2 year old children are not yet fully developed.
3. Gel tubes (yellow top) or plain tube (red top) may be used to collect the blood.
4. The blood specimen should be refrigerated if immediate delivery to the laboratory is not possible. Blood specimens may be refrigerated for a maximum of eight hours
5. No lipaemic or haemolysed blood specimens should be used.

6.5.2 Guideline 2: Interpretation of Widal test

6.5.2.1 Construct 2: All disease give rise to symptoms

In view of a compromised immune system in HIV patients they are not able to produce enough agglutinins to effectively defend themselves against HIV and typhoid fever. Thus symptoms of HIV are more pronounced in patients who are co-infected with typhoid fever compared to those patients with HIV only. Equally the symptoms of typhoid fever are masked in HIV positive patients compared to HIV negative patients because the former have a weakened immune system.

6.5.2.2 Objective

The objective of this guideline is to explain the interpretation of the Widal test. The following interpretations are based on a single Widal test done in northern Namibia where baseline *Salmonella* agglutinin titres have been established.

- A Widal test result in children <2 years is irrelevant and typhoid fever diagnosis in this age group should be done clinically because their immune system is immature hence they may fail to produce antibodies to *S. typhi* even in the presence of disease.
- Co-infection of HIV and typhoid fever is very common and if not accurately diagnosed may result in complications.
- There is significant ($p < 0.05$) association between HIV status and *Salmonella* agglutinin titres.
- Age and gender had no influence in the interpretation of the Widal test as there is not any significant association between age, gender and *Salmonella* agglutinin titres.

Table 6.1 Widal test interpretation chart

PATIENT	BASELINE TITRES		DIAGNOSTIC TITRES	
	(NORMAL TITRES)		(ABNORMAL TITRES)	
	<i>TYPHI O</i>	<i>TYPHI H</i>	<i>TYPHI O</i>	<i>TYPHI H</i>
GENERAL POPULATION HIV UNKNOWN	80	80	>80	>80
HIV POSITIVE PATIENT	40	80	>40	>80
UNKNOWN HIV STATUS BUT CLINICALLY SUGGESTIVE OF HIV#	40	80	>40	>80
HIV NEGATIVE PATIENT	80	40	>80	>40

Seek patient consent to do an HIV test. If consent is declined then proceed as if the patient is clinically suggestive of HIV.

NB. Use of the chart applies to patients who are above 2years and from northern Namibia.

6.5.3 Guideline 3: Deciding if it is a current or past infection

6.5.3.1 Construct 3: Health is the absence of disease

In an HIV context, in the absence of typhoid fever an HIV positive patient is regarded as healthy. It stands to reason therefore that for HIV negative patients the absence of typhoid fever makes them healthy. This study showed that clinically diagnostic *Salmonella* agglutinins for HIV positive patients are titre >80 for *typhi H* and titre > 40 for *typhi O*. This means that an HIV positive patient with *typhi O* > 40 is regarded as not healthy because of the typhoid infection. Treatment of this patient will reduce the titre to <40 and the patient will then be regarded as healthy. Therefore this study addresses an opportunity for HIV positive patients, who are diagnosed as not healthy, to be able to seek treatment and become healthy again.

6.5.3.2 Objective

The objective of this guideline is to explain how to decide if the patient positive for typhoid fever has current or past infection.

- *Salmonella typhi O* agglutinins are of the IgM type and as such their presence indicates current active infection.
- On the other hand *typhi H* agglutinins are of the IgG type and their presence indicates past or late stages of infection.

- A patient with a normal *typhi O* titre and an abnormal *typhi H* titre may indicate past typhoid fever infection, whilst a patient with abnormal *typhi O* and a normal *typhi H* may indicate current acute active typhoid fever infection.
- A patient with both abnormal *typhi O* and *typhi H* may indicate current persistent typhoid fever infection.

6.5.4 Guideline 4: Screening of food handlers by a Widal test

A food handler is any person who handles and prepares food for both commercial and public consumption. Food handlers are found in restaurants, hospitals and supermarkets, meat processing factories and butcheries.

6.5.4.1 Construct 2: All disease give rise to symptoms

Typhoid fever is a communicable disease and as such food handlers need to be screened every six months for typhoid fever. Recommendations are presented for screening of food handlers by a Widal test.

6.5.4.2 Objective

The objective of this guideline is to elaborate the screening process for food handlers who come for medical examination.

1. For feasibility purpose, every six month a single serum sample will be collected from the patient and tested for *Salmonella* using Widal test.
2. Those with titres >80 and above (with or without symptoms of typhoid fever) should be treated for typhoid fever or treated as carriers of typhoid fever.
3. After completion of treatment three stool specimens 24 hours apart should be collected for culture and no follow-up Widal test will be needed if the stool results are negative.
6. At least 3ml (milliliters) of clotted blood should be submitted to the laboratory.
7. Gel tubes (yellow top) or plain tube (red top) may be used to collect the blood.
8. The blood specimen should be refrigerated if immediate delivery to the laboratory is not possible. Blood specimens may be refrigerated for a maximum of eight hours.
9. No lipaemic or haemolysed blood specimens should be used.
10. Interpretation of the Widal test is based on the same premise as of patients (see Table 6.1 above).

6.5.5 Guideline 5: Performance of Widal test in the laboratory

Good laboratory practice is accomplished through the adoption and implementation quality assurance in the laboratory. Widal test results' reliability and accuracy depends mainly on the quality assurance of the testing process.

6.5.5.1 Objective

To give guidance to laboratory technicians and technologist on quality assurance when performing the Widal test.

- Therefore, to make the Widal test more sensitive and specific, it is recommended that laboratories that are performing the test should use the best standardised commercial bacterial antigens from reputable suppliers with proven quality control data.
- Every effort should be made to use the bacterial antigens in accordance with the manufacturer's instructions.
- Quality assurance (QA) should be instituted in laboratories performing the Widal test.
- QA should include running known commercial positive and negative controls as well as external QA through proficiency testing.
- Laboratories should include controls to rule out non-specific agglutination, such as cross reaction with other bacterial or parasitic antigens.

- The following controls should always be included when performing each batch of Widal tests to enhance reliability and validity of results: *Brucella abortus*, *E. coli*, *Proteus* and a saline suspension to act as a control for non-specific auto-agglutination of the bacterial antigen suspensions.
- This study recommends the inclusion of titre 1:120 dilution between 1:80 and 1:160 in order to minimise the risk of missing some cases of typhoid fever.

6.6 SUMMARY

This chapter discussed the applicability of the biomedical model of illness constructs relative to the objectives of the guide. This chapter further presented the guide for baseline *Salmonella* agglutinin titres according to HIV status. The guide contains several guidelines that pertain to: specimen collection; interpretation of the Widal test; deciding whether the infection is current or past; and screening of food handlers. This guide, together with findings of this study, enabled the researcher to draw conclusions and make necessary recommendations.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 INTRODUCTION

In this chapter the main research findings are summarised and conclusions are drawn in the context of the purpose and stated objectives of the study. From these conclusions recommendations are formulated and presented with acknowledgement of the limitations of the study. These limitations are highlighted and contextualised.

7.2 PURPOSE AND OBJECTIVES OF THE STUDY

The purpose of the study was to develop a guide on baseline *Salmonella* agglutinin titres according age, gender and HIV status among patients attending hospitals in northern Namibia.

The specific objectives of the study were:

1. To determine the prevalence of typhoid fever for age, gender and HIV status among patients attending five hospitals in northern Namibia.
2. To establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever.

3. To develop a conceptual framework based on the outcome of the situational analysis.
4. To develop a guide for baseline *Salmonella* agglutinin titres for age, gender and HIV status in patients attending five hospitals in northern Namibia.
5. To implement, monitor and evaluate the efficacy of the guide on baseline *Salmonella* agglutinin titres.

7.3 CONCLUSION

The conclusions are presented in relation to the specific objectives.

7.3.1 Objective 1: To determine the prevalence of typhoid fever among patients attending hospitals in northern Namibia

It is worth noting that in Namibia there is no vaccination against typhoid fever done by the MoHSS and as such established prevalence of typhoid fever found in this current study are a true reflection of typhoid fever prevalence in Northern Namibia. About half (49.8%) of all the research subjects (n=400) tested positive for the Widal test (Table 4.2). There is a high prevalence of typhoid fever in northern Namibia. It was seen from this study that typhoid fever due to *S. paratyphi A* is non-existent in the population under study whilst *S. paratyphi B* prevalence in the study subjects was very low at 4.5% (Table 4.2). These findings are in accord with the observations of Okonko *et al.* (2010) in their study on the prevalence of *Salmonella typhi* among

patients in Abeokuta, South-Western Nigeria; 673(80.1%) of the 840(100%) blood samples gave positive Widal reactions.

7.3.2 Objective 2: To establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever

Based on this study there is no significant association ($p>0.05$) between age and *Salmonella* agglutinin titres. There is no significant association ($p>0.05$) between gender and *Salmonella* agglutinin titres among the subjects. This conclusion is drawn from the Pearson Chi-square statistic as well as established serological prevalence premise from past research. A premise that was first coined by Collard (1959) (cited by Ibekwe *et al.*, 2008) states that for practical purposes titres occurring in more than or equal to 5% of the subjects under study are not diagnostically significant and should be regarded as normal in that population (baseline *Salmonella* agglutinin titres). Therefore, any titres occurring above those normal to the population will be regarded as diagnostic titres for typhoid fever. In this study the Pearson Chi-Square test showed that there is a highly significant association ($p<0.05$) between HIV status and *Salmonella typhi O* and *typhi H* titres.

7.3.2.1 General presumptive diagnostic *Salmonella* agglutinin titres

From Table 4.2 it is concluded that for *typhi O*, titre 80 is the highest to occur in more than 5% of the study subjects and as such it is a normal titre in the study population and is diagnostically insignificant. Therefore, for *typhi O* in the general

(age, gender and HIV status independent) study subjects, titre 80 is normal. Thus *typhi O* titre greater than 80 is diagnostically significant for typhoid fever.

From Table 4.2 it is concluded that for *typhi H* titre, 80 is the highest titre to occur in more than 5% of the study subjects and as such it is a normal titre in the study population and is diagnostically insignificant. Therefore, for *typhi H* in the general study subjects its titre 80 is normal. *Typhi H* titre greater than 80 is diagnostically significant for typhoid fever in northern Namibia.

These findings are in agreement with those of Ibekwe *et al.* (2008) in their study on baseline *Salmonella* agglutinin titres in apparently healthy freshman in Akwa, South Eastern Nigeria. They found that *typhi O* and *typhi H* titre of 80 occurred in greater than 5% of the subjects and was hence normal to their study population. They concluded that titres above 80 for both *typhi O* and *typhi H* were diagnostic of typhoid fever in their study population. However, Okonko *et al.* (2010) in their study on the prevalence of *Salmonella typhi* among patients in Abeokuta, South-West Nigeria, reported that *typhi O* and *typhi H* titre of 160 occurred in greater than 5% of their study population and as such were normal; titres above 160 for both *typhi O* and *typhi H* were diagnostic of typhoid fever in their study population.

Therefore, *typhi O* titres above 80 are diagnostic of typhoid fever in the general study population whilst *typhi H* titres above 80 are also diagnostic of typhoid fever in the study population (northern Namibia). The current diagnostic cut-off titre for

Namibia of 160 for both *typhi O* and *typhi H* is very high and some cases of typhoid fever are being missed.

7.3.2.2 Age dependent presumptive diagnostic *Salmonella* agglutinin titres

From Tables 4.3 and 4.4 comparison between adults (>16years) and children (<16 years) shows that there is a negligible difference between children and adults on the prevalence of typhoid fever and titre distribution. From the total study sample (n =400) 41(20.5%) children and 58(29%) adults were positive for *Salmonella* agglutinins.

The Pearson Chi-Square statistics showed that there is no significant association ($p < 0.05$) between age and *Salmonella* agglutinin titres. Therefore, there is no observable risk difference of getting typhoid fever between the age groups. Children and adults were almost equally affected by typhoid fever. Titre 80 for both *typhi O* and *typhi H* occurred in greater than 5% of the subjects: both adults and children. Thus titre 80, in children and adults, for both *typhi O* and *typhi H* is normal. Titres above 80 are therefore diagnostically significant for typhoid fever across age groups. Oyeyinka and Salimonu (2002), in their study, in Ibadan, Nigeria, on plasma titres of antibodies to *Salmonella O* and *H* antigens, found significantly higher titres in children than in adults for both *typhi O* and *typhi H*. In this study there is no significant association between age and *Salmonella* agglutinin titres.

7.3.2.3 Gender dependent presumptive diagnostic *Salmonella* agglutinin titres

The Pearson Chi-Square statistic showed that there is no significant association ($p>0.05$) between gender and *Salmonella* agglutinin titres. This study shows that males and females are equally affected by typhoid fever. About 25.5% of the females, and 24.0% males, were positive for *Salmonella typhi O* agglutinin titre; on the other hand 22.0% of the females and 19.0% of males were positive for *Salmonella typhi H* agglutinin titres (Tables 4.8 and 4.9 and Figures 4.6 and 4.7).

In this study since titre 80 occurred in greater than 5% of males and females for both *typhi O* and *typhi H* it is normal among the study population; it is therefore clinically insignificant. Hence titres above 80 for both *typhi O* and *typhi H* are clinically diagnostic across gender among the study population of northern Namibia.

In their study on prevalence of *Salmonella typhi* among patients in Abeokuta South-Western Nigeria, Okonko *et al.* (2010) found that, of the 840 sera tested, the majority of the positive ones were females. They found a 2:1 female to male preponderance. In their study in Ibadan, Nigeria, on plasma titres of antibodies to *Salmonella O* and *H* antigens, Oyeyinka and Salimonu (2002) concluded that in general there were significant titre differences between males and females except for two comparable age groups. In the six to 25 year and >65 year age groups males had lower titres compared to females. In this study there was no significant association between gender and *Salmonella* agglutinin titres; titres greater than 80 for both *typhi O* and *typhi H* are diagnostic of typhoid fever across males and females.

7.3.2.4 HIV status dependent presumptive diagnostic *Salmonella* agglutinin titres

In this study the Pearson Chi-Square statistic showed that there is a high significant association ($p < 0.05$) between HIV status and *Salmonella* agglutinin titres. The study revealed that HIV negative (30%) subjects were more positive for *typhi O* agglutinin titres as compared to HIV positive (19.5%) subjects (see Table 4.5 and Figure 4.4). This is due to the fact that HIV positive subjects have a weakened immune system so if they are infected by typhoid fever they are not capable of producing antibodies at the same level and quantity as their HIV negative counterparts. The study further showed that *typhi O* titre of 80 occurred in greater than 5% of the HIV negative subjects and as such is normal to the study area among HIV negative subjects. *Typhi O* titres greater than 80 are therefore diagnostic of typhoid fever in HIV negative subjects from northern Namibia.

On the other hand in this study *typhi O* titres of 40 occurred in more than 5% of the HIV positive subjects under study and as such are normal to the study area among HIV positive subjects. *Typhi O* titres above 40 are therefore diagnostically significant for HIV positive patients from the study area.

Immunological response to typhoid infection may be compromised in two ways: the quantity of *typhi O* antibodies is severely reduced and/or the time of onset of an immune response may be prolonged. Thus the primary immune response mediated by the IgM *typhi O* antibodies is lower in HIV positive subjects than in HIV negative

subjects. Clinically diagnostic titre for *typhi O* is greater than 40 in HIV positive subjects whereas for HIV negative subjects, *typhi O* titre should be greater than 80. This applies to the population in northern Namibia: the study area of this research.

In this study 30% of HIV positive subjects were positive for *typhi H* agglutinin titres whilst 12% of the HIV negative subjects were positive for *typhi H* agglutinin titres (Table 4.6 and Figure 4.5). Secondary immune response to typhoid infection is mediated by the IgG *typhi H* antibodies. This antibody class switching is a function of immune competency and determines the persistency, as well as quantity, of *typhi H* antibodies. *Typhi H* agglutinins tend to persist longer than *typhi O* agglutinins.

This study showed that *typhi H* titre 80 occurred in greater than 5% of HIV positive subjects and thus it is normal among these subjects in the population of the study area. Therefore, diagnostic *typhi H* titres should be greater than 80 for HIV positive subjects in the population of northern Namibia. On the other hand in this study *typhi H* titre 40 occurred in greater than 5% of the HIV negative subjects and as such is normal to these subjects among the population of the study area. Therefore, *typhi H* titres greater than 40 are diagnostic of typhoid fever in HIV negative subjects in the study area population.

Diagnostic titres for *typhi O* in HIV positive subjects is greater than 40, whereas for *typhi H* it is greater than 80. Diagnostic *typhi O* titres in HIV negative subjects is greater than 80 and *typhi H* titres is greater than 40 (Table 4.7). Currently the

diagnostic titre for both *typhi O* and *typhi H* titres is pegged at 160 which are very high and most cases of typhoid fever are being missed.

7.3.3 Objective 4: To develop a guide for baseline *Salmonella* agglutinin titres for age, gender and HIV status among patients attending hospitals in northern Namibia

This study showed that there is no significant association ($p > 0.05$) between gender, age and *Salmonella* agglutinin titres. However, there was significant ($p < 0.05$) association between HIV status and *Salmonella* agglutinin titres. This applies to all age groups above two years and is applicable to both males and females.

7.3.4 Objective 5: To implement, monitor and evaluate the efficacy of the guide on baseline *Salmonella* agglutinin titres

The implementation of the guide revealed that it is a valuable tool in the management and treatment of typhoid fever. This study also showed that the use of the guide will facilitate good clinical outcomes in patients with co-infection of HIV and typhoid fever. Overall, from the feedback from all stake holders involved in the development, monitoring and evaluation of the guide, the guide will definitely make a difference in management of typhoid fever and HIV in northern Namibia. The whole process of implementation, monitoring and evaluation of the guide is an ongoing process as feedback and amendments will continuously be done as new developments arise.

7.4 RECOMMENDATIONS

From the above presented conclusions in section 7.3 various recommendations are presented according to the above objectives.

7.4.1 Recommendations for objective 1

This objective was to determine the prevalence of typhoid fever among patients attending hospitals in northern Namibia. In this study 199 (49.8%) of the research subjects (n= 400) tested positive for the Widal test (Table 4.2). This indicates a high prevalence of typhoid fever in northern Namibia. It was seen from this study that typhoid fever due to *S. paratyphi A* is non-existent in the population under study; *S. paratyphi B* prevalence in the study subjects was very low at 4.5% (Table 4.2). This prevalence reveals that typhoid fever is a public health problem and warrants public health interventions. Two recommendations are made in terms of this high seroprevalence of typhoid fever in northern Namibia.

1. There is a need to do a blood culture based prevalence study on the typhoid fever in northern Namibia. Blood culture is the gold standard for the diagnosis of typhoid fever and as such a more accurate prevalence rate would be established. The Widal test is a cheap, simple and reliable test which is not immune to some shortfalls, such as cross reaction with other bacterial and parasitic antigens resulting in false positives. These false

positives are due to the sharing of common antigens between *salmonella* and some bacteria and parasites.

2. Serological prevalence of typhoid fever in northern Namibia is very high and as such it calls for drastic public health interventions spearheaded by the Ministry of Health and Social Services (MoHSS). It is recommended that the MoHSS should take a leading role in educating the public on typhoid fever. The public should be informed about the disease in terms of: what it is: its signs, symptoms and modes of transmission; treatment and preventative measures; for example. Public education of the disease could involve, for example, mass media campaigns, community mass mobilisations and use of IEC material tailor made for the target population. It is anticipated that education of the community about typhoid fever should lessen the burden of the disease on an already strained health care delivery system. Such community education would improve the lives of the Namibian people. As the general public becomes aware of the typhoid disease, the incidence of complications, such as perforating ulcers, would be reduced. Furthermore there should be a reduction in new cases of typhoid because the Namibian people would be empowered to take preventive measures to protect themselves.

7.4.2 Recommendations for objective 2

This objective was to establish age, gender and HIV status presumptively diagnostic *Salmonella* agglutinin titres for the diagnosis of typhoid fever. This study showed

that there is no significant ($p>0.05$) association between age, gender and *Salmonella* agglutinin titres. Therefore, interpretation of the Widal test should not be based on age or gender. In this study there was a highly significant ($p<0.05$) association between HIV status and *Salmonella* agglutinin titres. So whenever the HIV status of a patient is known, the Widal test should be interpreted using the presumptively diagnostic titres established by this study. The following are the recommended presumptive diagnostic *Salmonella* agglutinin titres for typhoid fever in northern Namibia.

1. Where the HIV status of a patient is unknown a *typhi O* titre greater than 80 is presumptively diagnostic of typhoid fever and a *typhi H* titre greater than 80 is presumptively diagnostic of typhoid fever. It is worth noting that the current diagnostic titre in Namibia for both *typhi O* and *typhi H* is greater than 160. These diagnostic titres are too high and many cases of typhoid fever could have been missed.
2. If a patient is HIV positive a *typhi O* titre greater than 40 is presumptively diagnostic of typhoid fever; a *typhi H* titre greater than 80 is presumptively diagnostic of typhoid fever. It can also be noted from these findings that most of the cases of typhoid fever in HIV positive patients could have been missed and could have resulted in death of these patients. Such patients could also have had complications associated with untreated typhoid fever and could also have spread the infection to the community.

3. If a patient is HIV negative a *typhi O* titre greater than 80 is presumptively diagnostic of typhoid fever; a *typhi H* titre greater than 40 is presumptively diagnostic of typhoid fever.
4. If a patient's HIV status is unknown but clinically is suspiciously HIV positive then the patient should be offered HIV counselling and testing. However, if a patient opts out then presumptive diagnostic titres of HIV positive subjects should be applied.

The above are summarised in Table 7.4. Note that the values listed in Table 7.4 are only applicable to northern Namibian persons older than 2 years.

Table 7.4 Presumptively diagnostic titres for typhoid fever according to HIV status

PATIENT	DIAGNOSTIC TITRES	
	<i>Typhi O</i>	<i>Typhi H</i>
General population HIV unknown	>80	>80
HIV positive patient	>40	>80
Unknown HIV status but clinically suggestive of HIV#	>40	>80
HIV negative patient	>80	>40

Seek patient consent and do HIV test. If consent is withheld the patient should be taken as being clinically suggestive of HIV.

It is also recommended that paired sera be used where possible in the diagnosis of typhoid fever. Paired sera are two serum samples collected one week apart for the Widal test. Demonstration of a two to fourfold rise in *Salmonella* agglutinin titres is highly suggestive of typhoid fever. However, paired sera are not always feasible in most northern Namibian health facilities because most patients are treated on the same day since they have limited access transport. In other words many patients usually travel long distance to come to a health facility and may not have ready access to transport on a specific day to return for a second sera sample to be taken.

Where blood culture facilities are available then typhoid fever should be diagnosed as such. However, the unavailability of such facilities and the urgent need for a result in acute typhoid fever limit the use of blood culture. Blood culture results are available between three to eight days and this further restricts the use of blood culture in routine diagnosis of typhoid fever. Where possible clinicians could hospitalise patients with complicated cases of typhoid fever; this would enable taking blood cultures in order to implement treatment in terms of antimicrobial susceptibility patterns. This would help to limit the emergence of drug resistant strains of *Salmonella* species which is currently on the increase globally. When the gold standard diagnosis of typhoid fever is not feasible for various reasons then it is necessary to resort to Widal test usage. The test can be done in most resource limited health facilities, and it offers relatively comparable results. Therefore, to make the Widal test more sensitive and specific, it is recommended that laboratories that are performing the test should use the best standardised commercial bacterial antigens

from reputable suppliers with proven quality control data. Every effort should be made to use the bacterial antigens in accordance with the manufacturer's instructions. Quality assurance (QA) should be instituted in laboratories performing the Widal test. QA should include running known commercial positive and negative controls as well as external QA through proficiency testing. Laboratories should include controls to rule out non-specific agglutination, such as cross reaction with other bacterial or parasitic antigens. The following controls should always be included when performing each batch of Widal tests to enhance reliability and validity of results: *Brucella abortus*, *E. coli*, *Proteus* and a saline suspension to act as a control for non-specific auto-agglutination of the bacterial antigen suspensions.

Another laboratory recommendation pertains to the Widal test procedure. The Widal test is meant to test for double dilutions of sera in a geometric progression: titrations start at 1:20, 1:40, 1:80, 1:160 and 1:320 and so on. If critically analysed the gap between say 1:80 and 1:160 may lead to false negatives in the diagnosis of typhoid fever. If diagnostic titre for *Salmonella* agglutinins is set at titre greater than 80, then a positive patient will only be detected from a positive titre of 160 and above. A patient who has a titre of 100, for example, is clinically positive for typhoid fever. However, the procedure does not make provisions for such patients to be detected as it only has titrations of 1:80 and then jumps to 1:160 creating a wide gap in between. All patients whose titres fall in this gap will be regarded as negative since the first point of detection of a positive result, as per the current Widal test procedure, is titre 160. A patient with a titre above 80 generally will be regarded as having typhoid fever under the new protocol established by this study. If a patient is

seen as having titres above 80, the titre 160 should be positive as it is the only titre after 80 in the current Widal test. So a patient with titre above 80 but less than 160 would still be considered negative for typhoid fever. It is against this critical analysis of the Widal test procedures that this study recommends the inclusion of titre 1:120 dilution between 1:80 and 1:160 in order to minimise the risk of missing some cases of typhoid fever. Since the Widal test is a serological test it should preferably be performed ONLY on subjects older than two years since by this age the immune system is fully developed.

7.5 UNIQUE CONTRIBUTIONS OF THIS STUDY

1. The findings of this study reveal that the current cut off titre of 160 is far too high, such that a number of cases of typhoid fever are being missed. The diagnostic titre should be as indicated in Table 7.4 above on presumptively diagnostic titres for typhoid fever according to HIV status.
2. The laboratory test procedure for Widal test has wide gaps between the double dilutions: patients with titres above 80 but less than 160 are being regarded as negative. This study found out that it is necessary to include titre 1:120 in order to minimise the risk of missing some cases of typhoid fever.
3. This study recommends that since the Widal test is a serological test, it follows then that the immune status of children less than two years old is underdeveloped and not able to secrete antibodies effectively. Therefore the Widal test should be done on patients more than two years old.

4. The findings should contribute immensely to the national and international knowledge bank.
5. A guide on the interpretation of the Widal test is developed to act as an important tool for proper laboratory diagnosis and interpretation of the Widal test to improve case detection and management of typhoid fever.
6. Valuable information on the relationship between HIV status and baseline *Salmonella* agglutinin titres is determined from this study.
7. A policy on vaccination against typhoid fever may be promulgated from these valuable findings especially since the study revealed that northern Namibia is has a high prevalence of typhoid fever. An identified association of HIV and typhoid fever, as well as differences in *Salmonella* agglutinin titres across both HIV positive negative persons, could be used to lobby for policy change and revision of treatment and management of HAART (highly active antiretroviral therapy) programme in Namibia.

7.6 RECOMMENDATION FOR PRACTICE

This study recommends that this guide on interpretation of the Widal test be implemented in all clinical and laboratory practice in northern Namibia. Before the implementation of this guide, it is important to give training on the new guidelines to all the implementers. In the absence of a Namibian national guideline on interpretation of the Widal test, these guidelines may be adopted by other regions in

Namibia whilst effort is being made by these regions to develop their region specific guidelines.

7.7 RECOMMENDATION FOR EDUCATION

This study recommends that the new guidelines on the interpretation of the Widal test according to HIV status among patients in northern Namibia, be included as part of training in medical laboratory science, medicine and nursing programmes to raise awareness as well as give strong base in typhoid fever diagnosis and management. All new and current staff members working with typhoid fever cases in hospitals and laboratories should be given continuous in-service training on these guidelines.

7.8 RECOMMENDATION FOR RESEARCH

This study recommends that this study be duplicated in other regions of Namibia, in an endeavour to come up with region specific guidelines on the interpretation of the Widal tests. These region specific guidelines may be consolidated to come up with a national guideline on the interpretation of the Widal test.

This study recommends further study on the prevalence of typhoid fever, using bone marrow or blood culture prevalence studies which are more specific and more sensitive.

Further research may also be done to establish the unique complications of untreated typhoid fever in HIV positive patients compared to HIV negative patients.

Furthermore an impact study could be done to evaluate the trends and prevalence of typhoid in different regions over the years after the adoption and use of these guidelines.

7.9 SUMMARY

This chapter presented conclusions and recommendations based on the objectives of the study. The high prevalence of typhoid fever in northern Namibia calls for drastic public health interventions. HIV/AIDS has a high significant association with *typhi* agglutinin titres and as such interpretation of the Widal test should be based on HIV status dependent presumptive *Salmonella* diagnostic titres set by this study. This study showed that there is no significant association between age, gender and *Salmonella* agglutinin titres therefore the Widal test should be interpreted similarly between gender and age. In order to enhance sensitivity and specificity of the Widal test the laboratories that are performing the test should implement a comprehensive quality assurance (QA) programme. This chapter further discussed recommendations for education, practice and research.

REFERENCES

Aftab, R. & Khurshid, R. (2009). Widal agglutination titre: A rapid serological diagnosis of typhoid fever in developing countries. *Pakistan Journal of Physiology*, 5(1), 65-69.

Alnwich, D. (2001). Meeting the malaria challenge. *Africa Health*, 23, 18-19.

Ayşe, W. Onder, E. & Banu, B. (2002). Widal test in the diagnosis of typhoid fever in Turkey. *Journal of Clinical Diagnostic Laboratory Immunology*, 9(4), 938-941.

Babbie, E. & Mouton, J. (2001). *The practice of social research*. Oxford: Oxford University Press.

Barza, M. & Travers, K. (2002). Excess infections due to antimicrobial resistance: the “Attributable Fraction”. *Clinical Infectious Disease*, 34: 126- S136.

Bhan, M. K., Bahl, R. & Bhatnagar, S. (2005). Typhoid and paratyphoid fever. *Lancet*, 366: 749-762.

Bhan, M. K., Bahl, R. & Sazawal, S. (2002). Association between *Helicobacter pylori* infection and increased risk of typhoid fever. *Journal of Infectious Disease*, 186: 1857-1860.

Brink, H. I. (1996). *Fundamentals of research methodology for health care professionals*. Cape Town: Creda Press.

Brooks, WA., Hossain, A., Goswami, D., Nahar, K., Alam, K. & Ahmed, N. (2005). Bacteraemic typhoid fever in children in an urban slum, Bangladesh. *Emerging Infectious Disease*, 11:326-329.

Butta, ZA.& Mansurali, N. Rapid serological diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot – enzyme immunoassays and the Widal test. *American Journal of Tropical Medicine Hygiene* 1999; 61: 645-7.

Burns, N. & Groove, S. K. (2005) *The Practice of Nursing Research* (5th.ed.). St. Louis: Elsevier Saunders

CDC. (2002). *Typhoid fever general information*. Atlanta, GA: CDC

CDC. (2008). *Health information for international travel*. Retrieved February 20, 2009 from: <http://www.cdc.gov/travel/index.htm>

Chart, H., Cheesbrough, J. S. & Waghorn, D. J. (2000). The serodiagnostic of infection with *Salmonella typhi*. *Journal of Clinical Pathology*, 53:851–853.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries*. (2nd edition). Norfolk: Cambridge University Press.

Choo, K. E., Razif, A. R., Openheimer, S. J., Ariffin, W. A., Lau, J. & Abrahan, T.(1993). Usefulness of the Widal test in diagnosing childhood typhoid fever. *Journal of Paediatrics Child Health*, 29 (1): 36-39.

Choo, KE., Davis, T M. & Ismail, A. (1999). Rapid and reliable serological diagnosis of enteric fever, comparative sensitivity and specificity of typhi dot and typhi dot M in febrile Malaysian children. *Acta Trop* 72: 175-83.

Clegg, A., Passey, M. & Thong, K.L. (1996). Molecular analysis of isolates of *salmonella typhi* from patients with fatal and nonfatal typhoid fever. *Journal of Clinical Microbiology*, 34: 1029.

CMO council (2008). *Leadership qualities*. Retrieved March 14, 2012 from <http://www.cmocouncil.org>

Countryfacts.com. (2003). Namibian Economy GDP currency Exports, Namibian Economics. Retrieved September 5, 2009 from <http://www.countryfacts.com/Namibia/economy>

Crump, J. A., Luby, S. P. & Mintz, E. D. (2004). The global burden of typhoid fever. *Bull World Health Organ*, 82:346-353.

Crump, J. A., Youssef, F. G., Luby, S. P., Wasfy, M. D., Rangel, J. M., & Talaat, M. (2003). Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerging Infectious Disease*, 9(5): 539-544.

DePaulo, B.M., Kashy, D.A., Kirkendol, S.E. & Wyer, M.M. (1996). Lying in everyday life. *Journal of Personality and Social Psychology*, 70: 979-995.

DeRoeck, D., Jodar, L. & Clemens, J. (2007). Putting typhoid vaccination on the global health agenda. *New England Journal of Medicine*, 357: 1069-1071.

De Vos, A. S. (2002). *Research at grassroots*. (2nd edition.). Pretoria: J L van Schaik.

Dharmana, E., Joosten, I., Tijssen, H. J., Gasem, M. H., Indarwidayati, R., Keuter, M. et al. (2002). HLA-DRB1 is associated with protection against complicated typhoid fever, independent of tumour necrosis factor alpha. *European Journal of Immunogenetics*, 29: 297-300 .

Dickoff, J., James, P. & Wiedenbach, E. (1968). Theory in a practice discipline. *American Journal of Nursing Company*, 17 (5): 415-444.

Doyal, L. (1995). *What makes women sick: Gender and Political Economy of Health*, Palgrave: Polity press.

Dunstan, S. J., Stephens, H.A., Blackwell J.M., Duc, C. M., Lanh, M. N., Dudbridge, F. et al. (2001). Current trends in typhoid fever. *Journal of Infectious Disease*, 183: 261-268.

Evans, M. & Lee, E. (2002). *Real bodies: A sociological introduction*. Palgrave: Polite Press.

Ferreccio, C., Levine, M. M., Manterola, A., Rodriguez, G., Rivara, I., Panzel, I. et al. (1984). Benign bacteremia caused by *Salmonella typhi* and paratyphi in children younger than 2 years. *Journal of Pediatrics*, 104: 899-901.

Garmory HS, Griffin KF, Leary SE, Perkins SD, Brown KA, Titball RW. (2002). The effect of recombinant plasmids on *in vivo* colonisation of *Salmonella enterica* serovar Typhimurium strains is not reflected by *in vitro* cellular invasion assays. *Vaccine*; 20: 3239-43.

George, J.M. & Jones, G.R. (2001). Towards a process model of individual change in organisations. *Human Relations*, 54: 419-444.

Glynn, M. K., Reddy, V., Hutwagner, L., Rabatsky-Ehr, T., Shiferaw, B. & Vugia, D. J. (2004). Prior antimicrobial agent use increases the risk of sporadic infections with multi-drug resistant *Salmonella enterica* serotype Typhimurium: a Foodnet case-control study. *Journal of Clinical Infectious Disease*, 38 (3): S227-S236.

Gopalakrishnan, V., Sekher, WY. , & Soo, EH. (2002). Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercially available diagnostic kits for detection of antibodies to *S. typhi* 2002. *Singapore Med J.* 43 (10): 495.

Gordon, M. A. (2008). *Salmonella* infections in immunocompromised adults. *Journal of Infectious Diseases*, 56: 413-22.

Ibadin, M. O. & Ogbim, A. (2004). *Antityphoid agglutinins in African school aged with Malaria*. Benin: University of Benin Teaching Hospital.

Ibekwe, A. C., Okonko, I. O., Onunkwo, A. U., Donbraye, E., Babalola, E. T. (2008). *Baseline Salmonella agglutinin titres in apparently healthy freshmen in Awka, South Eastern Nigeria*. Retrieved January 15, 2009 from <http://www.academicjournals.org/SRE>

Hosoglu, S., Celen, M., Geyik, M. F., Akalin, S., Ayaz, C., Acemoglu, H. et al. (2006). Risks factors for typhoid fever among adult patients in Diyarbakir, Turkey. *Journal of Epidemiology of Infectious Disease*, 134: 612-616.

House, D., Wain, J., Ho, V. A., Diep, T. S., Chinh, N. T., Bay, P. V. et al. (2001). Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. *Journal of Clinical Microbiology*. 39(3): 1002 – 1007.

Khan, M. E. H. (2004). *Typhoid fever in South African in-patient population*.

Retrieved January 15, 2009 from:

<http://www.dissertations.ub.rug.nl/files/medicine/2004/m.e.h.khan/summary.pdf>.

Kariuki, S. (2008). Typhoid fever in sub-Saharan Africa: Challenges of diagnosis and management of infection. *Journal of Infectious Diseases in Developing Countries*, 2(6): 443-447.

Kariuki, S., Revathi, G., Muyodi, J., Mwituria, J., Munyalo, A., Mirza, A. et al. (2004). Characterization of multidrug resistant typhoid outbreaks in Kenya. *Journal of Clinical Microbiology*, 42: 1477-1482.

Kelly-Hope, L. A., Alonso, W. J. & Thiem, V. D. (2007). Geographical distribution and risk factors associated with enteric diseases in Vietnam. *American Journal of Tropical Med Hygiene*, 76(4): 706-712.

Kelly-Hope, L. A., Alonso, W. J. & Thiem, V. D. (2008). Temporal trends and climatic factors associated with bacterial enteric disease in Vietnam, 1991-2001. *Environmental Health Perspective* 116(1): 7-12.

Khan, M., Yacoob, M. C., Connolly, C. & Sturm, A. W. (1997). The early diagnosis of typhoid fever prior to the Widal test and bacteriological culture results. *Acta Tropica*, 69(2): 165-173.

Kothari, A., Pruthi, A. & Chugh, T.D. (2009). The burden of enteric fever. *Journal of Infectious Disease in Developing Countries*, 2(4): 253-259.

Kulkarni, M. L. & Rego, S. J. (1994). Value of a single Widal test in the diagnosis of typhoid fever. *Journal of Indian Paediatrics*, 31: 1373-1377.

Lateef, A. O, & Aprileona, L. K. (2000). Widal agglutination test-100 years later: still plagued by controversy. *Postgraduate Medicine Journal* 76: 80-84.

Legal Assistance Centre, (2009). *LAC to fight for rights to adequate sanitation, water.*

Retrieved March 14, 2010 from http://www.lac.org.na/news/published_30/11/2009

LeBeau, D. (1998). *Sociological research methods workbook (Part 1) for module 1 (Soc 3201)*. Windhoek: University of Namibia, Centre for External Studies.

Levine, M. M. (2008). *Typhoid fever vaccines*. (5th edition.). Plotkin SA: Offit PA editors.

Lin, F.Y., Vo, A. H., Phan, V.B., Nguyen, T.T., Bryla, D., Tran, C. T. et al. (2000). The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *American Journal of Tropical Medicine Hygiene*, 62: 644-648.

Mahle, W. T. & Levine, M. M. (1993). *Salmonella typhi* infection in children younger than five years of age. *Paediatrics Infectious Disease Journal*, 12: 627-631.

Malik, A.S. & Malik, A.H. (2001). Typhoid fever in Malaysian children. *Medical Journal Malaysia*, 56(4): 478-490.

Mbuh, F. A., Galadima, M. & Ogbadu, L. (2003). Rate of co-infection with malaria parasites and *salmonella typhi* in Zaria, Kaduna state, Nigeria. *Annals of African Medicine*, 2: 64-67.

McGivern, Y. (2006). *The practice of market and social research, an introduction*. (2nd edition.). Dublin: Pearson Education Ltd.

Melville, S. & Goddard, W. (1996). *Research methodology, an introduction for science & engineering students*. Cape Town: Creda Press.

Mgbor, S. O. & Osuafor, T. O. K. (1990). Are your Widal test requests rational? *Orient Journal of Medicine* 2:3-5.

MoHSS, (2011). *Namibia standard treatment guidelines*. Windhoek: Namibia government press.

Miaoulis, G. & Michener, R. D. (1976). *An introduction to sampling*. Dubuque, Iowa: Kendall & Hunt.

Mintz, E. D. (2003). Sexual transmission of typhoid fever: a multistate outbreak among men who have sex with men. *Clinical Infectious Disease*, 37(1): 141 – 144.

Mirza, S. H., Beeching, N. J. & Hart, C. A. (1996). Multidrug resistant typhoid: a global problem. *Journal of Medical Microbiology*, 44: 317-319.

Mishra, O. P., Gupta, B. L., Ali, Z., Nath, G. & Chandra, L. (1994). Adenosine deaminase activity in typhoid fever. *Journal of Indian Pediatrics*, 31: 1379-1384.

Mouton, J. (2002). *How to succeed in your Master's and Doctoral studies*. Pretoria: J. L. van Schaik.

Muyembe-Tamfum, J.J., Veyi, J., Kaswa, M., Lunguya, O., Verhaegen, J. & Boelaert, M. (2009). An outbreak of peritonitis caused by multidrug-resistant *Salmonella typhi* in Kinshasa, Democratic Republic of Congo. *Travel Med Infectious Disease*, 7: 40-43.

Myron, M. L., Oscar, G., Robert, H.G., William, E.W., Rene, S.P. & William, W. (1978). Diagnostic value of Widal test in areas endemic for typhoid fever. *American Journal of Tropical Medicine Hygiene*, 27(4): 795-800.

Nettleton, S. (1995). *The sociology of health and illness*. Palgrave: Polity press

Nsutebu, E. & Ndumbe, P. (2001). The Widal test for typhoid fever: is it useful?
Africa Health, 23: 5-9.

Ochiai, R.L., Acosta, C.J. & Donovaro-Holiday, M.C. (2008). A study of typhoid fever in five Asian countries: disease burden and implications for control. *Bull WHO*, 86 (4): 260-268.

Okonko, I.O., Soley, F.A., Eyarefe, O.D., Amusan, T.A., Abubakar, M.J., Adeyi, A.O. et al. (2010). Prevalence of *Salmonella typhi* among patients in Abeokuta, South-Western Nigeria. *British Journal of Pharmacology and Toxicology*, 1(1): 6-14.

Olopoenia, L.A. & King, A.L. (2000). Widal agglutination test still plagued by controversy 100 years later. *Postgraduate Medical Journal*, 76: 80–84.

Olopoenia, L., Oyewole, F. & Nafowokan, R. I. (1995). Widal agglutination in malaria infection in Nigeria (abstract). *Clinical Immunology and Immunopathology*, 75: 314.

Olsen, S.J., Bleasdale, S.C., Magnano, A.R., Landrigan, C., Holland, B.H., Tauxe, R.V. et al. (2003). Outbreaks of typhoid fever in the United States, 1960-99. *Epidemiology Infectious Disease*, 130: 13-21.

Onunkwo, A.U., Nwonkwo, C.H. & Umolu, D.N. (2001). Scholastic appraisal of the routine serodiagnostic method for enteric fever in Nigeria. *Journal of Scientific Engineering Technology*, 8 (1): 2964-2973

Osler, W. (1912). *The principles and practice of medicine designed for the practitioners and students of medicine*, (8th ed.). New York: D Appleton.

Otegbayo, J.A., Daramola, O.O., Onyegbutulem, H.C., Balogun, W.F. & Oguntoye, O.O. (2002). Retrospective analysis of typhoid fever in a tropical tertiary health facility. *Tropical Gastroenterology*, 23(1): 9-12.

Oyeyinka, G.O. & Salimonu, L.S. (2002). Plasma titres of antibodies to *Salmonella* O and H antigens in Ibadan, Nigeria. *Tropical Journal Of Health Sciences*, 9: 221-223.

Pang, T. & Puthuchear, S. D. (1989). False positive Widal test in none typhoid *salmonella* infections. *Journal of Tropical Medicine and Public Health*, 20:163-164.

Patil, A.M., Kulkarni, M.L., Kulkarni, A.M. (2007). *Baseline Widal titres in healthy children*. Karnataka: Springer India.

Philip, Y.K. (2000). *A brief review of typhoid fever in Nigeria*. *Nigeria Medical Practice*, 38: 4-6.

Polit, D.F. & Hungler, B.P. (1997). *Nursing research, principles and methods*. (5th edition). London: JB Lippincott Company.

Radhakrishna, R. B., Yoder, E.P. & Ewing, J. C. (2007). *Strategies for linking theoretical framework and research types*. Pennsylvania: Pennsylvania State University.

Ram, P.K., Naheed, A., Brooks, W.A., Hossain, M.A., Mintz, E.D., Breiman, R.F. et al. (2007). Risks factors for typhoid fever in a slum in Dhaka, Bangladesh. *Epidemiology of Infectious Disease*, 135: 458-465.

Reller, M.E., Olsen, S.J., Kressel, A.B., Moon, T.D., Kubota, K.A., Adcock, M.P. et al. (2000). *Research methods for business*. New York: John Wiley & Sons.

Sandhya, AM., Amit, L., Vidya, DN. & Dipshikha, C. (2012). Typhoid fever & vaccine development: a partially answered question. *Indian Journal Med. Res*, 135: 161-169.

Sekaran, U. (2000). *Research methods for business: A skill building approach*. Dublin: Pearson Education Ltd.

Shaw, A.V., Reddy, E.A. & Crump, J.A. (2008). *Etiology of community-acquired bloodstream infections in Africa [abstract L-620]*. Program and abstracts of the 46th

Annual Meeting of the Infectious Diseases Society of America. Washington, DC: Infectious Diseases Society of America.

Sherwal, B.L., Dhamja, R.K. & Randhawa, V.S. (2004). A comparative study of Typhidot and Widal test in patients with typhoid fever. *Journal of Indian Academy of Clinical Medicine*, 5(3), 244-246.

Sidley, H. (2005). Typhoid outbreak prompts protests over inadequate water system. *BMJ.*, 331: 665.

Siddiqui, F.J., Rabbani, F., Hasan, R., Nizam, S.Q. & Bhutta, Z.A. (2006). Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. *International Journal of Infectious Disease*, 10 (3): 215-222.

Srikantiah, P., Vafokulov, S., Luby, S.P., Ishmail, T., Earhart, K., Khodjaev, N. et al. (2007). Epidemiology and risks factors for endemic typhoid fever in Uzbekistan. *Tropical Med International Health*, 12 (7): 838-847.

Taiwo, S.S., Fadiora, S.O., Oparindo, D.P. & Olowe, O.A. (2007). Widal agglutination titres in the diagnosis of typhoid fever. *West African, Journal of Medicine*, 26: 97-101.

Utah Department of Health. (2005). *Immunization and epidemiology program working document*. Utah: Utah Department of Health.

Uba, A.F., Chirdan, L.B., Ituen, A.M. & Mohammed, A.M. (2007). Typhoid intestinal perforation in children: a continuing scourge in a developing country. *Pediatric Surgical International Journal* 23: 33-39.

UNAIDS. (2006). *Report on Global HIV/AIDS epidemics, June 2006*. Geneva

UNAIDS. (2007). *Report on Global HIV/AIDS epidemics. December 2007*. Geneva

UNAIDS. (2009). *Report on Global HIV/AIDS epidemics. December 2009*. Geneva.

Uys, H.H.M. & Basson, A.A. (1991). *Research methodology in nursing*. Pretoria: Kagiso Tertiary.

Vollaard, A.M., Ali, S., Hagh, V.A., Suhariah, I., Widjaja, S., Visser, L.G. et al. (2004). Risk factors for transmission of foodborne illness in restaurants and street vendors in Jakarta, Indonesia. *JAMA*, 291: 2607-2615.

Wade, D.T. & Halligan, P.W. (2004). Do biomedical models of illness make good healthcare systems? *BMJ*, 329: 1398-1401.

Wahdan, M.H., Serie, C., Germanier, R., Lackany, A., Cerisier, Y., Guerin, N. et al. (1980). A controlled field trial of live oral typhoid vaccine Ty 21a. *Bull WHO*, 58: 469-474.

Wahdan, M.H., Sippel, J.E., Makhail, I.A., Rahka, A.E., Anderson, E.S. & Sparks, H.A. (1975). Controlled field trial of a typhoid vaccine prepared with a non motile mutant of *Salmonella typhi* Ty2. *Bull WHO*, 52: 69-73.

Wait, D.A., & Sobsey, M.D. (2001). Comparative survival of enteric viruses and bacteria in Atlantic Ocean seawater. *Water Science Technology*, 43: 139-142.

Washington, J.A. (1991). *Enterobactireaece of medical importance In JB Henry JB (Ed.), Medical microbiology in clinical diagnosis and management by laboratory methods* (18th ed.). Philadelphia, PA: WB Saunders.

Washington, C. W. , Stephen, D. A., William, M. J., Elmer, W. K., Paul, C. S., Gary, W. P., et al. (2006). *Koneman's colour atlas and textbook of diagnostic microbiology*. Philadelphia: Lippincott Williams & Wilkins

Weber, P.S. & Manning, M.R. (2001). Everyday Sense giving: A Closer Look at Successful Plant Managers. *The Journal of Applied Behavioral Science*, 46: 220-244

Welch, H. & Mickle, F.L. (1936). A rapid slide test for the serological diagnosis of typhoid and paratyphoid fevers. *American Journal of Public Health*, 26: 248–55.

Welman, C., Kruger, F. & Mitchell, B. (2009). *Research methodology* (3rd ed.). Cape Town: Oxford Southern Africa Press.

WHO. (1996). *Fighting disease, fostering development*. Geneva: WHO

WHO. (2003). *Background document: Diagnosis, treatment and prevention of typhoid of typhoid fever*. Retrieved January 14, 2009 from <http://www.who.int/vaccines-document>

WHO/CDC. (2003). *Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world*. Geneva: WHO.

WHO. (2008). *The HIV/AIDS programme highlights 2008-2009*. Geneva: WHO.

WHO. (2009). *Diarrhoeal diseases (Update February 2009)*. Retrieved March 03, 2009 from <http://www.who.int/vaccines-research/diseases/diarrhoeal/en/print.html>

WHO. (2010), *Universal access to HIV/AIDS prevention, treatment and care*. Geneva: WHO

Widal, F.M. (1896). Serodiagnostic de la fièvre typhoïde a-propos d'une modification par MMC. *Bull Soc Med Hop Paris*, 13:561–566.

Woods, C.W., Murdoch, D.R. & Zimmerman, M.D. (2006). Emergences of *Salmonella enterica* serotype paratyphi A as a major cause of enteric fever in Kathmandu, Nepal. *Trans R Soc Trop Med Hyg*, 100: 1063-1067.

Woodward, T.E., Smadel, J.E., Ley, H.L., Green, R. & Mankikar, D.S. (1948). Preliminary report on the beneficial effect of chloromycetin in treatment of typhoid fever. *Annals of International Medicine*, 29: 131-34.

Worku, B. (2000). Typhoid fever in an Ethiopian children's hospital: 1984-1995. *Ethiopian Journal of Health Development*, 14: 311-315.

Zenilman, J.M. (1997). Typhoid fever (clinical conference). *JAMA*, 278: 847-50.

Annexure A: UNAM Letter of Permission 1**UNIVERSITY OF NAMIBIA**

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pionierspark, Windhoek, Namibia

**FACULTY OF MEDICAL AND HEALTH SCIENCES**

**Letter of permission:
Post graduate students**

Date: 14 Aug 2009

Dear Student: Mr S Cikukwa

The post graduate studies committee has approved your research proposal.

Title: A GUIDE FOR BASELINE SALMONELLA AGGLUTININ TITRES
ACCORDING TO AGE, GENDER AND HIV STATUS OF PATIENTS
ATTENDING AT HOSPITALS IN NORTHERN NAMIBIA

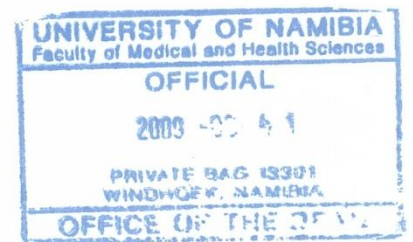
You may now proceed with your study and data collection and formal registration for the degree.

It may be required that you need to apply for additional permission to utilize your target population. If so, please submit this letter to the relevant organizations involved. It is stressed that you should not proceed with data collection and fieldwork before you have received this letter and got permission from the other institutions to conduct the study. It may also be expected that these organizations may require additional information from you.

Please contact your supervisors on a regular basis.

Faculty representative Post graduate committee

[Handwritten Signature]
14 Aug 2009



Annexure B: MoHSS Ethical Clearance 1



REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198
Windhoek
Namibia

Ministerial Building
Harvey Street
Windhoek

Tel: (061) 2032125

Fax: (061) 272286

E-mail: amuheua@mhss.gov.na

Enquiries: A. Muheua

Ref.: 17/1/1

Date: 13 November 2009

OFFICE OF THE PERMANENT SECRETARY

Mr. S. Chikukwa
P.O. Box 15709
Oshakati

Dear Mr. Chikukwa

Re: A guide for baseline salmonella agglutinin titres according to age, gender and HIV status of patients attending at hospitals in Northern Namibia

1. Reference is made to your application to conduct the above-mentioned study.
2. The proposal has been evaluated and found to have merit.
3. Kindly be informed that approval has been granted under the following conditions:
 - 3.1 The data collected is only to be used for academic purposes;
 - 3.2 A quarterly progress report is to be submitted to the Ministry's Research Unit;
 - 3.3 Preliminary findings are to be submitted to the Ministry before the final report;
 - 3.4 Final report to be submitted upon completion of the study;
 - 3.5 Separate permission to be sought from the Ministry for the publication of the findings.

Wishing you success with the project.

Yours sincerely,


MR. K. KAHURE
PERMANENT SECRETARY



Annexure C: NIP Letter of Permission 1



NAMIBIA INSTITUTE OF PATHOLOGY (NIP) LTD.

Tel: +264-61-295 4200, Fax: +264-61-255566, P.O. Box 277, Windhoek, Namibia

Reg. No. 200

OFFICE OF THE CHIEF EXECUTIVE OFFICER

Enquiries: Ms. M Pendukeni

29 September 2009

Mr. S. Chikukwa
Via: Mr H T Kaura
Chairperson: NIP Research Committee

RESEARCH PROPOSAL TO BE UNDERTAKEN AT NIP LABORATORIES

Dear Mr. Chikukwa

I refer to your email message dated 14 September 2009, regarding the above subject.

Kindly be informed that it was found out that your research proposal including the research design is sound and would add value to the diagnosis of typhoid fever in Namibia, more especially in the northern regions. This is a benefit to NIP and as a result, a decision was taken as part of NIP contribution to assist your research by covering the cost breakdown as follows:

- | | |
|-------|---------------------------------------|
| (i) | 400 Rapid HIV test = N\$2,463.40 |
| (ii) | 400 Wild tests slides = N\$3,584.00 |
| (iii) | 400 Tube wild test = <u>N\$296.00</u> |
| | Total = N\$6,343.40 |

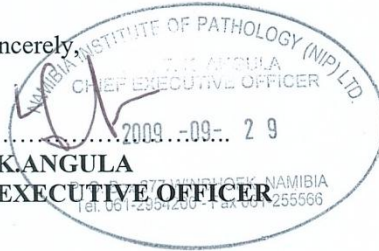
However, NIP will not be able to cover for subsistence and travelling expenses to be incurred by you when travelling to collect data. This is your own responsibility thus you need to find other alternatives. With regard to the study leave, you are advised to take your accumulated one day leave per month as stipulated in the Human Resources Policy page 38 clause 6.8.11.2. You are further advised to combine your one day accumulated study leave days with your annual leave, if they are not enough to cover for the one month study leave requested.

Undertaking of this research is therefore hereby approved on the following conditions:

1. That you will recognise/acknowledge the Namibia Institute of Pathology (NIP) in this regard.
2. Such undertaking is further subject to approval by the Permanent Secretary of the Ministry of Health and Social Services (MOHSS). You are advised to approach him for further approval.

Finally, I would like to wish you luck on this big step you have taken to undertake a PhD in Public Health.

Yours Sincerely,



MRS T.K. ANGULA
CHIEF EXECUTIVE OFFICER

Directors: A. September (Chairperson); K. Kahuure; K. Von Wenzel - Obelholzer; A. Biwa; U. Maamberua; M. Shivute;
H. Sepiso
T.K. Angula (CEO)

ANNEXURE D: A guide for interpretation of Widal test in the diagnosis of typhoid fever

1.0 Introduction

Antibody build-up against specific antigens in a given community depends on endemicity of the specific infecting agents therefore their plasma antibody titres differ widely from place to place. It is therefore necessary to determine the titres in the patient and general health population in order to know what titres indicate on-going or active infection (Oyeyinka& Salimonu, 2009, p.1). Indeed for reasons of cross reaction and anamnestic reaction, baseline level of antibodies in normal healthy individuals of any community should be known (Nsutebu& Ndumbe, 2001, pp.5-9). It is therefore a prerogative to establish the *Salmonella* agglutinin titres in the normal population of northern Namibia, so as to be able to establish what titres are diagnostic of typhoid fever. Since these *Salmonella* agglutinin titres differ from place to place, the current practice of using diagnostic titres which are not derived from Namibian population constitute mismanagement of typhoid fever. It is against this background that this study established Namibian baseline *Salmonella* agglutinin titres according to age, gender and HIV status.

The guide contains four main sections as presented below.

1.1 Guide on specimen collection

Blood should be collected at the acute phase of illness within five to six days of onset of illness. At least 3ml (milliliters) of clotted blood should be submitted to the laboratory. Samples should be collected from patients who are at least two years old as the immune systems in < 2 year old children are not yet fully developed. Gel tubes (yellow top) or plain tube (red top) may be used to collect the blood. The blood

specimen should be refrigerated. Lipaemic and haemolysed specimens must not be used.

1.2 Guide on interpretation of the Widal test (*Salmonella* agglutinin titres)

The following interpretations are based on a single Widal test done in northern Namibia where baseline *Salmonella* agglutinin titres have been established (Chikukwa, 2012). It is worth noting that interpretation of a Widal test result in children <2 years should be more clinically based because their immune system is immature hence they may fail to produce antibodies to *S. typhi* even in the presence of disease. In view of the burden of HIV/AIDS it is important that clinicians realize that co-infection of HIV and typhoid fever is very common and if not accurately diagnosed may result in complications. There is significant ($p<0.05$) association between HIV status and *Salmonella* agglutinin titres. Age and gender had no influence in the interpretation of the Widal test as it was found that there was not any significant association between age, gender and *Salmonella* agglutinin titres (Chikukwa 2012). Table 5.1 presents data for interpretation of the Widal test.

Table 1.1.Widal test interpretation chart.

PATIENT	BASELINE TITRES (NORMAL TITRES)		DIAGNOSTIC TITRES (ABNORMAL TITRES)	
	<i>TYPHI O</i>	<i>TYPHI H</i>	<i>TYPHI O</i>	<i>TYPHI H</i>
GENERAL POPULATION HIV UNKNOWN	80	80	>80	>80
HIV POSITIVE PATIENT	40	80	>40	>80
UNKNOWN HIV STATUS BUT CLINICALLY SUGGESTIVE OF HIV#	40	80	>40	>80
HIV NEGATIVE PATIENT	80	40	>80	>40

Seek patient consent to do an HIV test. If consent is declined then proceed as if the patient is clinically suggestive of HIV.

NB. Use of the chart applies to patients who are above 2years and from northern Namibia.

1.3 Guide on deciding if it is a current or past infection

Salmonella typhi O agglutinins are of the IgM type and as such their presence indicates current active infection. On the other hand *typhi H* agglutinins are of the IgG type and their presence indicates past or late stages of infection. A patient with a normal *typhi O* titre and an abnormal *typhi H* titre may indicate past typhoid fever infection, whilst a patient with abnormal *typhi O* and a normal *typhi H* may indicate current acute active typhoid fever infection. A patient with both abnormal *typhi O* and *typhi H* may indicate current persistent typhoid fever infection.

1.4 Guide on Performance of Widal test in the Laboratory

1. Therefore, to make the Widal test more sensitive and specific, it is recommended that laboratories that are performing the test should use the best standardised commercial bacterial antigens from reputable suppliers with proven quality control data.
2. Every effort should be made to use the bacterial antigens in accordance with the manufacturer's instructions.
3. Quality assurance (QA) should be instituted in laboratories performing the Widal test.
4. QA should include running known commercial positive and negative controls as well as external QA through proficiency testing.
5. Laboratories should include controls to rule out non-specific agglutination, such as cross reaction with other bacterial or parasitic antigens.
6. The following controls should always be included when performing each batch of Widal tests to enhance reliability and validity of results: *Brucella abortus*, *E. coli*, *Proteus* and a saline suspension to act as a control for non-specific auto-agglutination of the bacterial antigen suspensions.
7. This study recommends the inclusion of titre 1:120 dilution between 1:80 and 1:160 in order to minimise the risk of missing some cases of typhoid fever

ANNEXURE E: Amended guide for interpretation of Widal test in the diagnosis of typhoid fever

The guide contains four main sections as presented below.

1.1 Guide on specimen collection

Blood should be collected at the acute phase of illness within five to six days of onset of illness. At least 3ml (milliliters) of clotted blood should be submitted to the laboratory. Samples should be collected from patients who are at least two years old as the immune systems in < 2 year old children are not yet fully developed. Gel tubes (yellow top) or plain tube (red top) may be used to collect the blood. The blood specimen should be refrigerated. Lipaemic and haemolysed specimens must not be used.

1.2 Guide on interpretation of the Widal test (*Salmonella* agglutinin titres)

The following interpretations are based on a single Widal test done in northern Namibia where baseline *Salmonella* agglutinin titres have been established (Chikukwa, 2012). It is worth noting that interpretation of a Widal test result in children <2 years should be more clinically based because their immune system is immature hence they may fail to produce antibodies to *S. typhi* even in the presence of disease. In view of the burden of HIV/AIDS it is important that clinicians realize that co-infection of HIV and typhoid fever is very common and if not accurately diagnosed may result in complications. There is significant ($p < 0.05$) association between HIV status and *Salmonella* agglutinin titres. Age and gender had no influence in the interpretation of the Widal test as it was found that there was not any

significant association between age, gender and *Salmonella* agglutinin titres (Chikukwa 2012).

Table 5.1. Widal test interpretation chart.

PATIENT	BASELINE TITRES (NORMAL TITRES)		DIAGNOSTIC TITRES (ABNORMAL TITRES)	
	<i>TYPHI O</i>	<i>TYPHI H</i>	<i>TYPHI O</i>	<i>TYPHI H</i>
GENERAL POPULATION HIV UNKNOWN	80	80	>80	>80
HIV POSITIVE PATIENT	40	80	>40	>80
UNKNOWN HIV STATUS BUT CLINICALLY SUGGESTIVE OF HIV#	40	80	>40	>80
HIV NEGATIVE PATIENT	80	40	>80	>40

Seek patient consent to do an HIV test. If consent is declined then proceed as if the patient is clinically suggestive of HIV.

NB. Use of the chart applies to patients who are above 2years and from northern Namibia.

1.3 Guide on deciding if it is a current or past infection

Salmonella typhi O agglutinins are of the IgM type and as such their presence indicates current active infection. On the other hand *typhi H* agglutinins are of the IgG type and their presence indicates past or late stages of infection. A patient with a normal *typhi O* titre and an abnormal *typhi H* titre may indicate past typhoid fever

infection, whilst a patient with abnormal *typhi O* and a normal *typhi H* may indicate current acute active typhoid fever infection. A patient with both abnormal *typhi O* and *typhi H* may indicate current persistent typhoid fever infection.

1.4 Guide on screening of food handlers by a Widal test

Typhoid fever is a communicable disease and as such food handlers need to be screened every six months for typhoid fever. Four recommendations are presented for screening of food handlers by a Widal test.

1. For feasibility purpose only a single serum sample will be collected from the patient and tested for *Salmonella* using Widal test
2. Interpretation of the Widal test is based on the same premise as of patients (see Figure 5.1 above).
3. Those with titres of >80 (with or without symptoms of typhoid fever) should be treated for typhoid fever or cleared as carriers of typhoid fever
4. After treatment three stool specimens 24 hours apart must be collected for culture and no follow-up Widal test will be needed.

1.5 Guideline on Performance of Widal test in the Laboratory

7. Therefore, to make the Widal test more sensitive and specific, it is recommended that laboratories that are performing the test should use the best standardised commercial bacterial antigens from reputable suppliers with proven quality control data.
8. Every effort should be made to use the bacterial antigens in accordance with the manufacturer's instructions.

9. Quality assurance (QA) should be instituted in laboratories performing the Widal test.
10. QA should include running known commercial positive and negative controls as well as external QA through proficiency testing.
11. Laboratories should include controls to rule out non-specific agglutination, such as cross reaction with other bacterial or parasitic antigens.
12. The following controls should always be included when performing each batch of Widal tests to enhance reliability and validity of results: *Brucella abortus*, *E. coli*, *Proteus* and a saline suspension to act as a control for non-specific auto-agglutination of the bacterial antigen suspensions.
13. This study recommends the inclusion of titre 1:120 dilution between 1:80 and 1:160 in order to minimise the risk of missing some cases of typhoid fever.