EPIDEMIOLOGICAL STUDY AND POTENTIAL OF LATE PCR FOR DIAGNOSIS OF SCHISTOSOMIASIS IN NORTH WESTERN REGION OF NIGERIA

KABIRU MOHAMMED

UNIVERSITI SAINS MALAYSIA

2015

EPIDEMIOLOGICAL STUDY AND POTENTIAL OF LATE PCR FOR DIAGNOSIS OF SCHISTOSOMIASIS IN NORTH WESTERN REGION OF NIGERIA

by

KABIRU MOHAMMED

Thesis submitted in fulfillment of the requirements
For the degree of
Doctor Philosophy

JULY 2015

ACKNOWLEGEMENTS

In the name of Allah, the most Gracious and the most merciful

All praise be to Allah for the strength and blessing in completing this thesis. I have been privilege to be supervised by Professor Dr. Mohamed Rusli bin Abdullah whose broad expertise, innovative ideas and exceptional leadership have been the spirit of this thesis. I would like to expressed my special gratitude to him for his patience in seeing me through this project that I am able to complete and also for his invaluable help, constructive comments and suggestions throughout the period of my study in Universiti Sains Malaysia (USM). He had contributed immensely to the success of this research.

I would like to express my appreciation to my co-supervisors Dr Aziah Ismail, the Director of Institute for Research in Molecular Medicine (INFORMM) for the expert advice, support and innovative ideas regarding the molecular part of this research work and for reviewing the thesis and manuscript as well as providing valuable feedback throughout my study. I would like to express my special gratitude to her for this novel contribution.

I would like to also express my gratitude and appreciation to Dr Julia Omar, a co-supervisor and Head, Chemical Pathology Department, school of Medical Sciences, U S M for her expert advice, support, and encouragement.

I would like to also express my humble gratitude and appreciation to my field supervisors Professor Ikeh Eugene Ifeanyi and Professor Fabiyi joseph Popoola for their constructive criticism, support and advice at all times. I would like to extend my regards to Associate Professor Dr Kamarul Imran Musa, Associate Professor Dr Nor Azwany Yaacob of the department of Community Medicine, school of Medical

Science Universiti Sains Malaysia for their statistical advice and questionnaire design.

My special thanks goes to my research friends who have helped me in their own special ways in motivating me into the completion of my studies, Faizul S. Jaftri, Nooramalina Zulkiply, Noorain, Nuramin Ahmad. I would like to extend my gratitude to all the staff of INFORMM for their continuous help and support during part of my research study period especially Zafri, Badrul Sham Zainuddin, for their technical advice.

To my clients, I whole heartedly wish to express my special thanks to them for their patience, kindness, and consent to participate in the study and to the emirs, district heads, School teachers, Headmasters and parents/guardians of the children for their consent.

My special thanks and appreciation also goes to Directors state Ministry of Health Sokoto, Zamfara and Kebbi states, Nigeria for giving me permission to carry out this study.

I am indebted to my wife Aishah Mohammed Isa, my daughter Zainab, and my sons Al- Amin, Khalifah Omar, and Ahmad who have shown patience, understanding and lost my endure love due to my study in Malaysia. Without their support and encouragement as well as understanding it would not have been possible for me to the complete my study. My special thanks and gratitude's goes to my brothers, sisters and their families for their love and support.

Lastly, I remain grateful to my friends in the School of Medical Science Usmanu Danfodiyo University, Sokoto, Mohammad Kabiru Garba, Nataala S. Usman, Hussaini ladan and Usmanu Danfodiyo University Teaching Hospital, Sokoto, Kebbi and Zamfara states, Bala Mohammed Bashir, Ibrahim Umar Sanda. for their unrelenting support and encouragement.

My special thanks to my friends in USM who advise me in one way or the other Dr Badr, Dr. Mustapha and Dr Amir. Thank you and God bless.

TABLE OF CONTENTS

ACKNOWLEGEMENTS ii
TABLE OF CONTENTS v
ABSTRAK xxvii
ABSTRACT xxxi
Chapter 1 : INTRODUCTION 1
1.1 Background: What is Schistosomiasis 1
1.2 Schistosomiasis in Nigeria 8
1.3 Statement of the Problems 10
1.3.1 High Disease Burden 10
1.3.2 Socio-economic Impact of Schistosomiasis 11
1.3.3 High Infection Rate Results in Serious Complications 11
1.3.4 Lack of Effective Prevention and Control Methods 12
1.3.5 Lack of Sensitivity and Specificity of Some Methods in Low Endemic Areas 12
1.3.6 LATE-PCR Dipstick Provide Alternative Solutions to Current problems 12
1.4 Rationale of the Study/Justification of the Study 13
1.5 Approach to the Study of Schistosomiasis 15
1.6 Control of Schistosomiasis 15
1.6.1 Snail Control 17
1.6.2 Chemical Control 18
1.6.3 Biological Control 20
1.6.4 Environmental Modification 20
1.6.5 Improved Water and Sanitation 21
1.6.6 Health Education 22
1.6.7 Praziquentel (PZQ) Drug 26
1.6.8 Chemotherapy of Schistosomiasis 27
1.6.9 Development of a Schistosome Vaccine 29
1.7 Strategies for Prevention and Control 30
1.8 OBJECTIVES OF THE STUDY 32
1.8.1 Aims of the Study 32
1.8.2 Objectives of the Study 32
1. To determine the proportion of Schistosomiasis among all parasitic diarrhoel and urinary tract diseases in the study area 32
Chapter 2 : LITERATURE REVIEW 35
2.1 Schistosomiasis: Disease Burden 35
2.2 Prevalence and Incidence of Schistosomiasis 38
2.2.1 Mortality and Morbidity of Schistosomiasis 41
2.2.2 Post Intervention Assessment using Praziquentel 43
2.2.3 Eosinophil Cationic Protein (ECP) Detection 43
2.3 General Morphological characteristic of Schistosomiasis 44
2.3.1 Structural Characteristic 44
2.3.2 Biological Characteristics 46

2.3.3 Genetics Characteristics....46
2.3.4 Biochemical Characteristic....47
2.4 Epidemiology of Schistosomiasis....48
2.4.1 Epidemiology and Situation of Schistosomiasis in Nigeria....49
2.5 Distribution of Schistosomiasis....51
2.6 Transmission of Schistosomiasis....53
2.6.1 Life cycle of Schistosomiasis....54
2.7 Risk Factors for Schistosomiasis....57
2.7.1 Water Development Projects....58
2.7.2 Water Contact and Exposure....60
2.7.3 Irrigation Scheme....61
2.7.4 Man-made Lakes....61
2.7.5 Immigrants (Refugees)....63
2.7.6 Species Specific....63
2.7.7 Genetic Factors....64
2.7.8 Intrinsic Factor....65
2.7.9 Environmental Factor....67
2.8 Diagnosis of Schistosomiasis Infection....69
2.8.1 Parasitological Methods of Schistosomiasis.....70
2.9 Immunological Methods....74
2.9.1 Rapid Diagnostic Test....75
2.9.2 Detection of CCA Methods....76
2.9.3 POC-CCA Methods....76
2.10 Molecular Diagnosis of Schistosomiasis....77
2.10.1 PCR Amplification....78
2.10.2 Gene Targets of PCR Based Assay....79
2.11 Management of Schistosomiasis....81
2.11.1 Pathogenesis, and Pathology of Schistosomiasis....82
2.12 Immune Response to Schistosomiasis....91
2.13 Surveillance/Data Base/ Registry....92
2.13.1 Active Case Detection Surveillance....92
2.13.2 Passive Surveillance....93
2.13.3 Snail Surveillance....93
Chapter 3 : EPIDEMIOLOGICAL STUDY (PART ONE)....94
3.1 Introduction....94
3.2 Study Design....94
3.3 Selection of the Study Areas....99
3.3.1 Selection of Study Population....99
3.3.2 Study Location (North-western Nigeria)....99
3.3.3 Sokoto State, North-western Nigeria....102
3.3.4 Kebbi State in North-western Nigeria....105
3.3.5 Zamfara State in North-western Nigeria....107
3.4 SAMPLING AND SAMPLING METHOD....109
3.4.1 Study Population....109
3.4.2 Reference Population....109
3.4.3 Source Population....109
3.4.4 Target Population....110
3.4.5 Sampling Frame....110
3.4.6 Inclusion and Exclusion Criteria....110
3.4.7 Sampling Techniques....111

3.4.8 Sampling Method....112
3.4.9 Sample Size Determination....113
3.4.9.1 Objective One....113
3.4.9.2 Objective Two (A)....115
3.4.9.3 Objective Three....117
3.5 Parasitological Methods of Sample Examinations....120
3.5.1 Filtration Concentration Techniques for *Schistosoma haematobium*....120
3.5.2 Stool Sample Collection....121
3.5.3 Stool Sample Analysis....121
3.5.4 Formol Ether Concentration Techniques....122
3.6 Cohort Study....123
3.6.1 Introduction....123
3.6.2 Follow-up and Study Procedures....124
3.6.3 Sampling and Sample Size Calculation....124
3.7 Research Tools....126
3.7.1 Questionnaire....127
3.7.2 Validation Process....127
3.7.3 Domains of the questionnaire....128
3.8 Data Management....129
3.9 Statistical Analysis....129
3.9.1 Descriptive Statistics....129
3.9.2 Data exploration and cleaning....130
3.9.3 Simple Logistic Regression....131
3.9.4 Multiple Logistic Regression for *S. haematobium and S. mansoni*....132
3.9.5 Checking Multicolinearity and Interaction for *S. haematobium and S. mansoni*....132
3.9.6 Checking Linearity of Continuous numerical variables *S. haematobium and S. mansoni*....133
3.9.7 Checking Assumptions (goodness of fit model *for S. haematobium and S. mansoni*....134
3.9.8 Hosmer Lemeshow test) *for S. haematobium and S. mansoni*....134
3.9.9 Study Variable....140
3.10 Expected Outcome....140
3.11 Ethical Approval MOH Nigeria and Ethics Review Committee, USM....141
3.12 Results....142
3.12.1 Part one (A) : Epidemiological study of schistosomiasis....142
3.12.2 Comparison of Socio-Demographic Characteristic of the Participants in Three States of North-western Nigeria....142
3.12.3 Prevalence rate of *Schistosoma haematobium* Infection....145
3.12.4 Comparism of Socio-demographic characteristic of S. haematobium between Sokoto and Zamfara and Kebbi state, Nigeria....150
3.12.5 Comparism of Socio-demographic characteristic and prevalence of *S. mansoni* between Sokoto and Zamfara and Kebbi state, Nigeria....153
3.12.6 Factors Associated with Urinary Schistosomiasis Infection....156
3.12.8 Preliminary model variable selection....161
3.12.9 Multicolinearity and Interaction....164
3.12.10 Correlation Matrix for the Model for *S.haematobium*....164
3.12.11 Interaction Terms between Variables in the Model....165
3.12.12 Checking Assumptions for *S. haematobium*....166
3.12.13 Hosmer Lemeshow Test for *S.haematobium*....166

3.12.14 Classifications Table for *S. haematobium* 167
3.12.15 Area under the (ROC) curve for *S. haematobium* 168
3.13 Regression diagnostic for *S. haematobium* 169
3.13.1 Remedial Measures 172
3.14 Multiple Logistic Regression for *S. haematobium* 173
3.15 Interpretation of the Results for *S. haematobium* final model 176
3.15.1 Established Final Model For *S. haematobium* Infection 176
3.16 Prevalence of *Schistosoma mansoni* Infection 178
3.16.1 Factors Associated with *Schistosomiasis mansoni* Infection 183
3.16.2 Multicolinearity and interaction for *S. mansoni* 191
3.16.3 Checking assumptions for *S. mansoni* 192
3.16.4 Hosmer Lemeshow test for *S. mansoni* 193
3.16.5 Classification table for *S. mansoni* 193
3.16.6 Area under the receiver operating curve for *S. mansoni* 194
3.17 Regression diagnostic for *Schistosoma mansoni* 195
3.17.1 Remedial measures for S. mansoni 198
3.17.2 Interpretation 200
3.17.3 Established Final Model For *S. mansoni* Infection 201
3.17.4 Intensity and prevalence of schistosomiasis haematobium infection in North-western Nigeria 202
3.18 Standardized Incidence Rate of Schistosomiasis 206
3.18.1 Direct Method of Standardization (Sokoto State) 208
3.18.2 Interpretation of the results for incidence of *S. haematobium* 211
3.18.3 Age- sex standardized incidence rate for Sokoto state 212
3.18.4 Interpretation of the results for age-sex incidence of *S. haematobium and S.mansoni* 215
3.18.5 Age standardized incidence rate in Sokoto state using standard world population 216
3.18.6 Interpretation of the results using world standard population 218
3.18.7 Interpretation of the result for *S. haematobium and S. mansoni* 221
3.19 Direct standardization (Zamfara state) 222
3.19.1 Interpretation of the results 225
3.19.2 Interpretation of the results for Zamfara state 228
3.20 Direct standardized incidence rate for Kebbi state, Nigeria 229
3.20.1 Interpretation of the results for *S. haematobium and S. mansoni* 232
3.20.2 Interpretation of the results for *S. haematobium and S. mansoni* 234
Chapter 4 : Part 2: ENHANCED DETECTION TOOLS USING MLECULAR METHODS 235
4.1 Background 235
4.1.1 Study Design 242
4.1.2 Study Population 244
4.1.3 Sampling Methods 244
4.2 Collection of Samples 244
4.2.1 Study Samples 244
4.3 Method of Sample Collection 245
4.4 Human Ethical Committee Approval from USM Malaysia 245
4.4.1 Material and Method 246
4.4.2 Preparation of buffers 251
4.5 Preparation of Reagents for Agarose Gel Electrophoresis 254
4.5.4 Preparation of Primer Stock Solution (100 µM) 255

4.5.5 Preparation of Working Primers Solution 255
4.5.6 Reagents and Materials used for LATE-PCR LFA 256
4.6 Preparation of Reagents for Lateral Flow Assay 258
4.6.4 Preparation of Lateral Flow Strip 259
4.6.5 Lining Method of the capture with Nitrocellulose.....260
4.7 Preparation of genomic DNA 261
4.7.1 Genomic DNA Extraction 261
4.8 Determination of DNA Concentration Using ND-100 Nanodrop Machine 263
4.9 Primer Design 264
4.9.1 Primer Sequences Used in this Study 265
4.10 PCR Product Detection and Analysis Using Agarose Gel Electrophoresis 269
4.10.4 Optimization of LATE-PCR.....271
4.10.5 LATE-PCR Lateral Flow Amplification 273
Results.....294
4.14.1 Optimization of annealing temperature by gradient PCR.....295
4.14.2 Optimization of Primer Ratio 295
4.14.3 Optimization of primer concentration.....296
4.14.4 Optimization of dNTPs.....296
4.14.5 Optimization of Magnesium chloride concentration 296
4.14.6 Optimization of dNTPs.....298
4.15 Optimization of LATE-PCR Conditions 299
4.15.1 Optimization of DMSO of LATE-PCR dipstick for *S. mansoni* 299
4.15.2 Optimization $MgCl_2$ of LATE-PCR dipstick for *Schistosoma mansoni*……..300
4.15.3 Optimization $_{dNTPs}$ of LATE-PCR dipstick for *S. mansoni*.....301
4.15.4 Optimization of Taq polymerase LATE-PCR dipstick for *S. mansoni* 302
4.15.5 Optimization $MgCl_2$ of LATE-PCR for *S. haematobium*.....303
4.15.6 Optimization dNTPs of LATE-PCR dipstick for *S. haematobium*.....304
4.15.7 Optimization of Taq polymerase LATE-PCR dipstick for *S. haematobium*.....305
4.16 Confirmation of LATE-PCR LFA product by DNA Sequencing 306
4.16.1 Analytical sensitivity of LATE-PCR LFA for *S. mansoni* Using diluted DNA.....307
4.16.2 Analytical sensitivity of LATE-PCR LFA for *S. haematobium* using diluted DNA.....308
4.16.3 Gel profile showing evaluation of sensitivity and specificity of *S. mansoni* LATE-PCR Assay.....309
4.16.4 Gel profile showing evaluation of sensitivity and specificity of *S. mansoni* LATE-PCR Assay.....310
4.16.5 Gel profile showing evaluation of sensitivity and specificity using DNA from positive stool samples with *S. mansoni*.....311
4.16.6 Gel profile showing evaluation of specificity using DNA from negative stool samples with *S. mansoni*.....312
4.16.7 Gel profile showing evaluation of sensitivity and specificity using DNA from urine samples with *S.haematobium*.....314
4.16.8 Evaluation of sensitivity and specificity using DNA from urine samples with S. haematobium positive.....315

4.16.9 Evaluation of specificity using DNA from urine samples with *S.haematobium Negative* 316
4.16.10 Evaluation of specificity using DNA from urine samples with *S.haematobium Negative* 317
4.16.11 LATE-PCR LFA using confirmed positive stool samples for S. mansoni....319
4.16.12 LATE-PCR LFA using confirmed positive stool samples for *S. mansoni*....320
4.16.13 LATE-PCR LFA using confirmed negative stool samples for *S. mansoni*....321
4.16.14 LATE-PCR LFA using confirmed negative stool samples for *S. mansoni*....322
4.16.15 LATE-PCR LFA using confirmed negative stool samples for *S. mansoni*....323
4.16.16 LATE-PCR LFA using confirmed positive urine samples for *S. haematobium*....324
4.16.17 LATE-PCR LFA using confirmed positive urine samples for *S. haematobium*....325
4.16.18 LATE-PCR LFA using confirmed positive urine samples for *S. haematobium*....326
4.16.19 LATE-PCR LFA using confirmed positive urine samples for *S. haematobium*....**Error! Bookmark not defined.**
4.16.20 LATE-PCR LFA using confirmed negative urine samples for *S. haematobium*....327
4.16.21 LATE-PCR LFA using confirmed negative urine samples for *S. haematobium*....328
4.16.22 Evaluation of analytical specificity of 30 positive and 20 negative stool sample for *Schistosoma mansoni*....329
4.16.23 Evaluation of analytical specificity of 30 positive and 20 negative urine sample for *Schistosoma haematobium*....330
Chapter 5 : GENERAL DISCUSSION....331
5.1 Epidemiological Study (Part one)....331
5.1.1 High Disease Burden of Schistosomiasis 332
5.1.2 Socio-economic Impact of Schistosomiasis 334
5.1.3 High Infection Rate Results in Serious Complications 339
5.1.4 Diagnosis, Prevention and Control of Schistosomiasis 340
5.1.5 Comparism of Socio-demographic characteristic and prevalence of *S. haematobium* infection between Sokoto, Zamfara and Kebbi state, Nigeria 341
5.1.6 Comparism of Socio-demographic characteristic and prevalence of *S. mansoni* infection between Sokoto, Zamfara and Kebbi state, Nigeria… 343
5.1.7 Associated Risk Factors for Schistosomiasis Infection 344
5.1.8 Intensity of Schistosomiasis Infection in North-western Nigeria....346
5.1.9 Standardized Incidence and Prevalence Rate of Schistosomiasis 348
5.2 Laboratory/ Molecular Study (Part two)....349
Chapter 6 : GENERAL CONCLUSION 357
6.1 Epidemiological Study (part one) 357
6.2 Laboratory Based Molecular Study (Part two) 358
6.3 Recommendations....360

6.4 Study Limitations 362

LIST OF TABLES

Table 3.1 Socio-Demographic characteristics of the participants in three states of North-western Nigeria (n=2451).....143

Table 3.2 Socio-Demographic characteristics of the participants in three states of North-western Nigeria (n=2451).....144

Table 3.3 Prevalence and Distribution of *S. haematobium* infection with respect to socio- demographic among the participants in North-western Nigeria (n=2451) ...147

Table 3.4 Prevalence and Distribution of S. haematobium infection 148

Table 3.5 Distribution of *S. haematobium* infection with respect to socio-geographical location among the participants in North-western Nigeria (n=2451)149

Table 3.6 Comparism of Socio-demographic characteristic and prevalence of *S.*...152

Table 3.7 Comparison of Socio-demographic characteristic of *S. mansoni* between Sokoto, Zamfara and Kebbi state, Nigeria 155

Table 3.8 *Schistosoma haematobium* infection with respect to socio-economic and demographic factors among the participants in North-western Nigeria from Simple Logistic Regressions (n=2451) 158

Table 3.9 *Schistosoma haematobium* infection in relation to water contact activities among the participants in North-western Nigeria from Simple Logistic Regressions (n=2451).....160

Table 3.10 Preliminary main effect model for variable selection into the final model for *S. haematobium* infection (n=2451 162

Table 3.11 Multivariate analysis for potential factors associated with *S. haematobium* infection (n=2451).....163

Table 3.12 *Schistosoma haematobium* infection with respect to Standard error of the model selection for each variable.....164

Table 3.13 Correlation Matrix for the model for *S. haematobium*.....165

Table 3.14 Interaction Terms between Variables in the Model for *S.haematobium* 165

Table 3.15 Hosmer lame-show test and Pearson Chisquare test for *S. haematobium* 166

Table 3.16 Classifications table for *S. haematobium* 167

Table 3.17 Result of area under receiver operation characteristic (ROC) curve ..168

Table 3.18 *Schistosoma haematobium* infection with respect to socio- economic and demographic factors among the participants in North-western Nigeria from Multiple Logistic Regressions (n=2451) 174

Table 3.19 Established final model and Summary of Univariable and Multivariable analysis for potential risk factors associated with *S. haematobium* infection (n=2451).....175

Table 3.20 Prevalence and Distribution of *S. mansoni* infection in relation to socio-demographic characteristics among the participants in North-western Nigeria (n= 2451) 180

Table 3.21 Prevalence and Distribution of *S. mansoni* infection in relation to water contact activities among the participants in, North-western Nigeria (n=2451) 181

Table 3.22 Prevalence and Distribution of S. mansoni infection in relation to socio-geographical location among the participants in North-western Nigeria (n=2451).182

Table3.23 *Schistosoma mansoni* infection in relation to socio-demographic factors among the participants in North-western Nigeriafrom Simple Logistic Regression (n=2451).....185

Table 3.24 Simple Logistic Regressions.....186

Table 3.25 Factors associated withS.mansoni infection with respect to Water contact activitiesamong the participants in North-western Nigeria from Simple Logistic Regressions (n=2451) 187

Table 3.26 Factors associated withSchistosoma mansoni infection with respect to geographical location namong the participants in North-western Nigeria from Simple Logistic Regressions.....188

Table 3.27 Preliminary main effect model for variable selection into the final model for *S. haematobium* infection (n=2451 190

Table 3.28 Multivariate analysis for potential factors associated with *S. haematobium* infection (n=2451).....191

Table 3.29 Shows Standard error of the model selection for each variable in the model which are relatively small, therefore multicolinearity does not exist in the model.....191

Table 3.30 Correlation matrix for the model 192

Table 3.31 Correlation matrix for the model for *S. mansoni* 192

Table 3.32 Interaction terms between variables in the model for *S. mansoni* 192

Table 3.33 Hosmer lame-show test and Pearson Chi-square test for *S. mansoni* 193

Table 3.34 Classification table for *S. mansoni*.....193

Table 3.35 Result of area under receiver operation characteristic (ROC) curve for *S.mansoni* 195

Table 3.36 Scatter plot of leverage (h) versus estimated predicted probability from the fitted model for *S. mansoni* 196

Table 3.37 Scatter plot of Delta deviance versus estimated predicted probability from the fitted model for *S. mansoni* 196

Table 3.38 Scatter plot of Delta Chi-square versus estimated predicted probability versus from the fitted model for *S. mansoni* 197

Table 3.39 Scatter plot of Pregibon's dbeta versus estimated predicted probability from the fitted model for *S. mansoni* 197

Table 3.40 Final established model of multiple logistic regression for risk Factors associated with *S. mansoni* infection 200

Table 3.41 Intensity Distribution of infection in relation to age, gender and monthly income of the participants (n=2451) 204

Table 3.42 Intensity Distribution of infection in relation to Occupation and educational status of the participants (n=2451) 205

Table 3.43 Age specific incidence rate of *schistosomiasis haematobium* in Sokoto state (n=635) 209

Table 3.44 Age standardized Incidence rate with S.haematobium *and S.mansoni* infection among participants resident in Sokoto state, Nigeria (n=635).....210

Table 3.45 Age standardized Incidence rate with *S.haematobium and S.mansoni* infection among participants resident in Sokoto state, Nigeria (n=635).....211

Table 3.46 Age-Sex standardized Incidence rate with *S.haematobium and S.mansoni infection* in Sokoto state, Nigeria (n=635) 212

Table 3.47 Age-Sex standardized Incidence rate with *S.haematobium and S.mansoni*(n=635) 213

Table 3.48 Age-Sex standardized Incidence rate with *S.haematobium and S.mansoni infection* among participants resident in Sokoto state, Nigeria (n=635).....214

Table 3.49Age specific incidence rate of *S. haematobium* in Sokoto state, Nigeria Using World standard population (n=635) 216

Table 3.50 Age standardized Incidence rate with S.haematobium *and S.mansoni* infection among participants resident in Sokoto state, Nigeria (n=635).....217

Table 3.51 Age-Sex standardized Incidence rate among participants resident in Sokoto state, Nigeria using standard population (n=635).....219

Table 3.52 Age-sex standardized Incidence rate with *S.haematobium and S.mansoni infection in Sokoto state using standard world population(n=635)* 220

Table 3.53 Age-specific standardized Incidence rate with *S. haematobium and S. mansoni infection* among participants resident in Zamfara state, Nigeria 222

Table 3.54 Age-specific standardized Incidence rate with *S.haematobium and S.mansoni infection* among participants resident in Zamfara state, Nigeria(n=635) 223

Table 3.55 Age specific incidence rate Continued.....224

Table 3.56 Age-Sex standardized Incidence rate with *S. haematobium* and *S.mansoni infection* t in Zanfara state, Nigeria (n=635) 226

Table 3.57 Age-Sex standardized Incidence rate with *S. haematobium and S.mansoni infection using standard population (n=635).*.....227

Table 3.58 Age-Sex standardized Incidence rate for *S. haematobium* and *S.mansoni infection in Kebbi state.(n=635).* 229

Table 3.59 Age-Sex standardized Incidence rate with Schistosomiasis infection t in Kebbi state,.....230

Table 3.60 Age-Sex standardized Incidence rate with Schistosomiasis infection t in Kebbi state,.....231

Table 3.61 Age standardized Incidence rate *with S.haematobium and S.mansoni* infection Cohort study among participants resident in Kebbi state, Nigeria 233

Table 3.62 Age standardized Incidence rate with S.haematobium *and S.mansoni infection* among participants resident in Kebbi state, Nigeria 234

Table 4.1 List of Chemicals and Reagents used for LATE PCR 246

Table 4.2 List of Chemicals and Reagents used for LFA 247

Table 4.3 List of equipment's used for LATE PCR LFA 248

Table 4.4 List of equipment's used for agarose gel electrophoresis & extraction 249

Table 4.5 List of equipment's used in the Study.....250

Table 4.6 List of labeled primers, probes sequences used for *S. haematobium*...257

Table 4.7 List of labeled primers, probes sequences used for *S. .mansoni* LATE-PCR-Lateral flow. 257

Table 4.8 DRA1_F and DRA1_R for detection of *S. haematobium*, and SM1-7_F and SM1-7_R for detection of *S. mansoni* 265

Table 4.9 Compositions of the Standard PCR Mixture for *S. haematobium* 272

Table 4.10 Master mix reagents Preparation for *S. mansoni* 274

Table 4.11 Primer design of LATE-PCR dipstick for *Schistosoma mansoni* 277

Table 4.12 Primer design of LATE-PCR dipstick for *Schistosoma haematobium* 278

Table 4.13 List of regents and the concentrations used 284

Table 4-.15 Evaluation study on 50 stool samples showed 100% sensitivity and specificity suggesting the primers and designed probe is specific without any cross-detection with other microorganisms 329

List of Figures

Figure 1.1 Types of human Schistosoma species.....4

Figure 1.2 Water contact activities of the people in the community 5

Figure2.1Schistosoma adult flukes adopted from Source:http://cc.shsmu.edu.cn/G2S/Template/View.aspx?courseId=5240&topMenuId=27449&action.....45

Figure 2.2 Map of Global epidemiology and geographical distribution of schistosomiasis adopted from (Source CDC) 51

Figure 2.3 Life Cycle of Schistosomiasis 55

Figure 3.1 Flowchart of Epidemiological Study of Schistosomiasis in North-Western Region of Nigeria.....97

Figure 3.2 Layout conceptual frame work of associated risk factor for Schistosomiasis 98

Figure 3.3 Map of Nigeria showing North-western Nigeria adopted from101Figure 3.4 Sultan Palace adopted fromSource: http://www.upcinn.com/state_details.php?id=sokoto 103

Figure 3.5 Map of Sokoto state. Adopted from Source: http://www.upcinn.com/state_details.php?id=sokoto 104

Figure 3.6 Map of Kebbi state, Nigeria adopted from Source: http://www.nigerianstat.gov.ng/information/details/Kebbi National Bereu on Statistic 106

Figure 3.7 Map of Zamfara state Nigeria adopted from Source: http://www.ncocusa.com/constituencies_zamfara.html Nigerian Chamber of Commerce USA (NICOCUSA). 108

Figure 3.8 Statistical flow chart of Multiple Logistic Regression Analysis 139

Figure 3.9 Receiver Operating Characteristics Curve.....168

Figure3.10 Scatter plot of predicted probability versus leverage for *S. haematobium* 169

Figure3.11 Scatter plot of predicted probability versus DDelta influence for *S. haematobium*.....170

Figure3.12 Scatter plot of D Pearson Chi-square versus estimated predicted probability for *S.haematobium* from the fitted model.....170

Figure 3.13 Scatter plot of Delta Chisquare versus estimated predicted probability for *S. haematobium* from the fitted model.....171

Figure 3.14 Scatter plot of Standardized Pearson residual versus estimated predicted probability for *S. haematobium*.....171

Figure 3.15 Scatter plot of predicted probability Pregibon's DBeta versus estimated predicted probability for *S. haematobium from the fitted model.* 172

Figure 4.1 Conceptual frame work of Lab. Molecular study (Part two).....243

Figure 4.2 Shows typical configuration of a Lateral flow immunoassay test strips adopted from Source(http://www.chamicabioanaliticaunito.it/lateral%20flow.png). 276

Figure 4.3 Showing positive and negative control and positive for *S.haematobium* 282

Figure 4.4 Showing positive and negative control and positive for *S.mansoni* 282

Figure 4.5 Optimization of DMSO of LATE PCR LFA for S.mansoni…….298

Figure 4.6 Optimization of MgCl2 of LATE PCR for S. mansoni……..299

Figure 4.7 Optimization of dNTP for LATE PCR for S.mansoni……..300

Figure 4.8 Optimization of Polymerase enzymes for LATE PCR for S. mansoni……301

Figure 4.9 Optimization of MgCl2 for LATE PCR for S. haematobium……302

Figure 4.10 Optimization of dNTP for LATE PCR for S. haematobium…….303

Figure 4.11 Optimization of polymerase enzymes for S.haematobium…….. …304

Figure 4.12 Optimization of DNA Sequencing result for *S. mansoni*..……..305

Figure 4.13 DNA sequencing result for *S. haematobium* 306

Figure 4.14 Analytical Sensitivity of LATE PCR LFA for *S. mansoni*……..306

Figure 4.15 Analytical Sensitivity of LATE PCR for *S. haematobium*..……307

Figure 4.16 Evaluation of Sensitivity and Specificity using DNA from positive stool 308

Figure 4.17 Evaluation of Sensitivity and Specificity using DNA from positive stool…309

Figure 4.18 Evaluation of Sensitivity and Specificity using DNA from positive stool..310

Figure 4.19 Evaluation of Specificity using DNA from Negative stool Samples…..311

Figure 4.20 Evaluation of Specificity using DNA from Negative stool……..312

Figure 4.21 Evaluation of Sensitivity and Specificity using DNA from Positive urine…313

Figure 4.22 Evaluation of Sensitivity and Specificity using DNA from Positive urine..314

Figure 4.23 Evaluation of Specificity using DNA from Negative urine……....315

Figure 4.24 Evaluation of Specificity using DNA from Negative urine…….316

figure 4.25 Evaluation of Specificity using DNA from negative urine with *S. haematobium* ……..318

Figure 4.26 LATE PCR LFA using confirmed positive stool sample for S. mansoni..319.

Figure 4.27 LATE PCR LFA using confirmed positive stool sample for *S. mansoni*..320

Figure 4.28 LATE PCR LFA using confirmed negative stool sample for *S. mansoni*.321.

Figure 4.29 LATE PCR LFA using confirmed negative stool sample for *S. manson*i.322.

Figure 4.30 LATE PCR LFA using confirmed negative stool sample for *S. mansoni*..323

Figure 4.31 LATE PCR LFA using confirmed positive urine for *S. haematobium*……324

Figure 4.32 LATE PCR LFA using confirmed positive urine for *S. haematobium*…..325

Figure 4.33 LATE PCR LFA using confirmed negative urine for *S.haematobium*…..326

Figure 4.34 LATE PCR LFA using confirmed negative urine for *S. haematobium*….327

Figure 4.35 LATE PCR LFA using confirmed negative urine for *S. haematobium*…328

LIST OF ABBREVIATIONS

A	Adenine
AOR	Adjusted Odds Ratio
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
BSA	Bovine Serum Albumin
^{0}C	Degree Celsius
CCA	Circulating Cathodic Antigen
C.I	Confidence interval
CDC	Centre for Disease Control
%	Percentage
DATP	Deoxyadenosine triphosphate
DNA	Deoxyribonucleic Acid
DNTPs	Deoxyribonucleoxide triphosphate
dsDNA	Double Stranded DNA
ECP	Eosinophil Cationic Protein
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzymes Linked Immunosorbent assay
epg 10 ml	Eggs per 10 ml of urine
epg	Eggs per gram of faeces
AE	Elusion buffer
FAM	Carboxyfluorescein

FITC	Fluorescein isothiocyanate
g	Gram
GOF	Goodness of Fit
GM	Geometric mean
H_2O	Water
HCl	Hydrogen chloride
HH	Household
IAC	Internal amplification control
Kb	Kilo base
KCl	Potassium chloride
LATE	Linear -After - The- Exponential
LoD	Limit of detection
M d	Mean difference
mg	Milligram
mg/ml	Milligram per millimetre
Mm	MilliMolar
MLgR	Multiple Logistic Regression
MOH	Ministry of health
mmol/l	Millimole per litre
$MgCL_2$	Magnesium chloride
Ml	Millilitre
mM	Millimolar
MDA	Mass Drug Administration
NaCl	Sodium, chloride
NaOH	Sodium hydroxide

NALF	Nucleic acid lateral flow
NCBI	National Centre for Biotechnology Information
NIPD	National Institute for Prevention of Disease
NA	Not Applicable
ng	Nano gram
ng/ul	Nanogram per microliter
Nm	Nanometre
Nmole	Nano mole
NPV	Negative predictive value
OD	Optical density
OR	Odds Ratio
PBS	Phosphate Buffer Saline
PCR	Polymerase chain reaction
Pmol	Pico mole
LFA	Lateral flow Assay
LOD	Limit of Detection
MGS	Multiple Cloning site
POC-CCA	Point Of Contact Circulating Cathodic Antigen
PPV	Positive predictive value
RNA	Ribonucleic acid
ROC	Receiving Operating Characteristics
RH	Relative Humidity
SGC	Streptavidin-colloidal gold conjugate
SCP	Schistosomiasis Control Program
SLgR	Simple Logistic Regression

Sh	Schistosoma haematobium
Sm	Schistosoma mansoni
Sp	Species
STH	Soil Transmitted Disease
Ss DNA	Single stranded DNA
SIR	Standardized Incidence Rate
T	Temperature
T	Time
Ta	Annealing temperature
TE	Tris- EDTA
TAE	Tris-acetate EDTA
Th	T-helper
USA	United States of America
USD	United States Dollar
V	Volts
USM	Universiti Sains Malaysia
PZQ	Praziquentel drug
PI	Principal Investigator
WHO	World Health Organization

Operational definitions

Terms	Definition
Incidence	Number of new cases of disease occurring per period
Prevalence	Number of both old and new cases to the total population at the point in time
Intensity of *S.haematobium* infection	Number of eggs detected per 10 ml of urine
Burden of disease	Measures gap between current health status & ideal situation
Schistosomiasis	Is a disease caused by Schistosoma specie
Urinary schistosomiasis	Is a disease caused by *Schistosoma haematobium*
Intestinal schistosomiasis	Is a disease caused by *S. mansoni*
Cercariae	Parasite in its infective stage
Snail host	Intermediate host of schistosoma specie
Sample	Selected elements choosen for participation in study
Sampling	Process of selecting a group of people for a study
Sampling frame	List of all the elements in the population from which sample is withdrawn
Population study	Study of a group with a common characteristic
Risk factors	Characteristic associated with increase risk of disease
ROC Curve	Graph plotted to discriminate between sensitivity and specificity
Children	Any one under the age of 18

Adult	Any one at or above the age of 18
Primers	Short artificial DNA strands
Sensitivity	Proportion of people without disease

EPIDEMIOLOGI KAJIAN DAN POTENSI LAWAT PCR UNTUK DIAGNOSIS OF SCHISTOSOMIASIS DALAM UTARA-WESTERN DI BARAT LAUT DI NIGERIA

ABSTRAK

Latarbelakang:. Schistosomiasis dianggap jangkitan yang paling biasa dan meluas parasit dengan ketara kebimbangan kesihatan sosio -ekonomi dan orang ramai di seluruh dunia dan terus menjadi penyebab utama kecacatan dan kematian dalam membangun dan negara-negara mundur , termasuk Nigeria

Objektif: Kajian ini bertujuan untuk menentukan kekerapan dan risiko faktor-faktor yang dikaitkan dengan jangkitan oleh Schistosoma haematobium dan Schistosoma mansoni di tiga negeri Utara - Barat Nigeria, dan untuk membangunkan dan menilai Linear - Selepas -The- Exponential- Polymerase Chain Reaction (PCR - LEWAT) mencelup stik untuk mengesan *S. haematobium* dan *S. mansoni* dalam air kencing dan najis sampel. Yang terakhir ini telah dilakukan untuk mewujudkan dan meningkatkan diagnosis tepat jangkitan Schistosomiasis

Keputusan : Hasil: Berdasarkan kepada 2451 peserta , kajian menunjukkan kelaziman keseluruhan jangkitan *S. haematobium* menjadi 61.2 % manakala kelaziman dengan Schistosoma parasit mansoni adalah 54.7 %. Majoriti responden adalah lelaki (85.5 %). Kalangan kanak-kanak sekolah kelaziman tertinggi jangkitan telah dikesan dalam 10-14 tahun (68.4 %). Dalam analisis Univariable , kanak-kanak yang dipunyai oleh isi rumah berpendapatan rendah ialah 1.43 kali kemungkinan tertinggi dijangkiti S. haematobium [COR : 95 % CI, 1.02 , 2.00) p-nilai 0,042) berbanding dengan yang dimiliki oleh keluarga isi rumah dengan pendapatan bulanan daripada > USD 500. S. mansoni menunjukkan tiada hubungan

yang signifikan antara pendapatan dan risiko jangkitan. Faktor-faktor lain yang didapati berkaitan dengan Schistosoma jangkitan haematobium dalam analisis univariat termasuk status perkahwinan di mana yang satu mempunyai lebih banyak kemungkinan mendapat jangkitan berbanding responden berkahwin dengan (COR 1.97 (95 % CI, 1.41 : 2.76 ; *P*= 0.001).

Dalam perlawanan akhir analisis model multivariat ditubuhkan, faktor-faktor risiko yang masih ketara yang dikaitkan dengan jangkitan Schistosomiasis termasuk kumpulan umur, hubungan siput, rumah disewa, sistem tandas lubang, Open tandas ruang dan tangan terkena air dari sungai. Untuk *S. haematobium* faktor risiko jangkitan adalah kumpulan umur 5-17 (AOR, 2.10: 95% CI, 1.29,2.06, *P* = 0.001), 18-29 tahun (AOR, 1.37; 95% CI: 1.08,1.74, *P* = 0.007), siput kenalan (AOR: 1.34,95% CI: 1.07,1.67, *P* = 0.009), rumah sewa (AOR, 1.61, 95% CI: 1.17,2.19: *P* = 0.001), manakala bagi S. mansoni utama faktor risiko dalam model analisis terakhir ditubuhkan pembolehubah ialah siput kenalan (AOR, 1.44,95% CI: 1.09,1.70, *P* = 0.006), sistem Pit (AOR, 0.68,95% CI: 0.56,0.84, *P* = 0.001) dan akhir sekali terbuka defaecation ruang (AOR: 1.26, 95% CI: 1.06,1.51, P = 0.009). Dapatan kajian menunjukkan bahawa kumpulan umur, hubungan siput dan defeacating di kawasan lapang adalah antara faktor risiko penentu utama bagi kedua-dua spesies schistosomiasis, ia itu oleh itu disyorkan bahawa pembuat keputusan dasar perlu memperhebatkan usaha ke arah mewujudkan kesedaran

Yang LATE PCR mencelup stik dikesan schistosoma DNA serendah 1 fg / µl parasit DNA dalam air kencing dan 1 ng / µl DNA dalam sampel najis .. Reaksi penguatan menunjukkan untuk menjadi tertentu tanpa sebarang reaksi balas dengan DNA daripada mikro usus lain organisma

Metodologi: Dijalankan dalam dua fasa , fasa pertama kajian ini adalah kajian keratan rentas yang dijalankan antara September 2011 hingga September 2012. Para peserta telah dipilih berdasarkan persampelan rawak mudah. Soal selidik berstruktur digunakan untuk menilai maklumat sosio - demografi, dan lain-lain faktor penentu berkaitan peserta. Sampel najis telah diperiksa menggunakan mikroskop dan formol teknik penumpuan eter manakala sampel air kencing telah diperiksa menggunakan teknik penumpuan Penapisan . Data telah dimasukkan dan dianalisis menggunakan SPSS Versi 22.0 perisian statistik. Terurus logistik Mudah dan pelbagai ujian dan chisquare digunakan untuk meneroka hubungan antara faktor-faktor risiko yang berkaitan . Statistik yang signifikan telah diambil pada tahap 5 % tersebut.

Bahagian kedua kajian ini melibatkan pembangunan LATE PCR kaedah mencelup stik untuk mewujudkan dan meningkatkan diagnosis tepat Schistosoma haematobium dan Schistosoma jangkitan mansoni .Primers dan kuar mensasarkan , telah direka untuk dua spesies amplifikasi yang tertentu. Yang LATE PCR parameter mencelup stik telah dioptimumkan dan pengesanan produk PCR telah dilakukan ke atas 2 % gel agarose elektroforesis , membran nitroselulosa itu disalut dengan biotinylated anti - tetikus IgG (line kawalan) , anti - FITC (garis sasaran) dan dipasang sebagai aliran sisi jalur .

Kesimpulan: Kesimpulan: Prevalens schistosomiasis direkodkan dalam kajian ini adalah sangat tinggi dan memerlukan kawalan dan pengurusan strategi yang berkesan . Kumpulan umur dewasa terdedah kepada nelayan dan pekerjaan pertanian , menyeberangi sungai telanjang kaki , hubungan siput , dan rumah yang disewa adalah beberapa faktor yang dikenal pasti untuk S. haematobium dan S. jangkitan mansoni di Utara- Barat , Nigeria predisposing . Mencelup stik LEWAT - PCR

dibangunkan dalam kajian ini menyediakan alternatif yang berharga untuk mengesan *Schistosoma haematobium* dan *Schistosoma jangkitan mansoni* di kawasan kajian untuk mempercepatkan diagnosis sebagai tambahan kepada kaedah konvensional yang kini digunakan

EPIDEMIOLOGICAL STUDY AND POTENTIAL OF LATE PCR FOR DIAGNOSIS OF SCHISTOSOMIASIS IN NORTH-WESTERN REGION OF NIGERIA

ABSTRACT

Background: *Schistosomiasis* is considered the most common and widespread parasitic infection with significant socio-economic and public health concern worldwide and continues to be a significant cause of morbidity and mortality in developing and underdeveloped countries, including Nigeria.

Objectives: This study aimed to determine the prevalence and risk factors associated with infections by *S. haematobium and S. mansoni* in three states of North-western Nigeria, and to develop and evaluate Linear-After-The-Exponential- Polymerase Chain Reaction (LATE-PCR) LFA for the detection of *S. haematobium and S. mansoni* in urine and stool samples. The latter was performed to establish and enhance accurate diagnosis of *Schistosomiasis infection.*

Materials and Methods: Conducted in two phases. The first phase of the study was a cross-sectional survey, conducted between September, 2011 to September, 2012. Participants were selected based on simple random sampling. Structured questionnaire was used to assess socio-demographic information, and other associated determinant factors of the participants. Stool samples were examined using microscopy and formol ether concentration techniques while urine samples were examined using Filtration concentration techniques. Data were entered and analyzed using SPSS version 22.0 statistical software. Simple and multiple logistic regressions and chisquare test were used to explore the relationships between associated risk factors. Statistical significant was taken at the 5% level.

The second part of the study involved development of LATE-PCR dipstick method to establish and enhance accurate diagnosis of *S. haematobium and S. mansoni* infection .Primers and probes targeting for, were designed for the two species specific amplification. The LATE-PCR LFA parameters were optimized and detection of PCR products was performed on 2% agarose gel electrophoresis, the nitrocellulose membrane was coated with biotinylated anti-mouse IgG (control line), anti-FITC (target line) and assembled as lateral flow strips.

Results: Based on the 2451 participants, the study showed an overall prevalence of infection with *S. haematobium* to be 61.2% while the prevalence with *Schistosoma mansoni* parasites was 54.7%. The majority of the respondents were males (85.5%). Among the school children the highest prevalence of infection was detected in the 10-14 years (68.4%). In Univariable analysis, children who belonged to low income household were 1.43 times highest odds of being infected with *S. haematobium* [COR: 95% CI, 1.02, 2.00) p-value 0.042) as compared to those belonging to household families with monthly income of >USD 500. *S. mansoni* showed no significant association between income and risk of infection. Other factors that were found to be associated with *S. haematobium* infection in univariate analysis includes marital status whereby being single had more odds of getting infection compared to married respondents with (COR 1.97 (95% CI, 1.41:2.76*;* *P*=0.001).

In final established multivariate analysis model, risk factors that remain significantly associated with Schistosomiasis infection include age group, snail contact, rented house, pit latrine system, Open space toilet and hands contact with water from the river. For *S.haematobium* infection risk factors were age group 5-17 (AOR,2.10 :95% CI,1.29,2.06,*P*=0.001), 18-29 years (AOR,1.37;95% CI: 1.08,1.74, *P*=0.007),

Snail contact (AOR: 1.34,95% CI: 1.07,1.67,*P*=0.009), Rental house (AOR,1.61, 95% CI: 1.17,2.19: *P*=0.001), while for *S. mansoni* the main risk factors in the final established multivariable analysis model were Snail contact (AOR,1.44,95% CI:1.09,1.70, *P*=0.006), Pit system (AOR,0.68,95% CI:0.56,0.84,*P*=0.001) and lastly open space defaecation (AOR: 1.26, 95% CI: 1.06,1.51,*P*=0.009). The study findings revealed that age group, snail contact and defeacating in an open space was some of the major determinant risk factors for both species of schistosomiasis, it was thus therefore recommended that policy decision makers should intensify effort towards creating awareness through health education for prevention and control.

The LATE-PCR LFA detected schistosoma DNA as low as 1 fg/µl of parasite DNA in urine and 1 ng/µl of DNA in stool samples.. The amplification reaction showed to be specific without any cross reaction with DNA from other intestinal micro-organism

Conclusion: The prevalence of schistosomiasis recorded in this study was extremely high and requires effective control and management strategies. Adult age group exposed to fishing and farming occupation, crossing river bare footed, snail contact, and rented house are some of predisposing factors identified for *S. haematobium and S. mansoni* infection in North-western, Nigeria. LATE-PCR LFA developed in this study provides a valuable alternative for the detection of *S. haematobium and S. mansoni* infection in the study area to speed up diagnosis in addition to the conventional method currently used.

Chapter 1: INTRODUCTION

1.1 Background: What is Schistosomiasis

Schistosomiasis otherwise called ‘’Bilharziasis’’ or snail fever”’ is a parasitic waterborne intravascular infections named after Bilharz, who discovered the parasites in autopsy in Egypt (Wu and Halim, 2000). Bilharzia also known to many local people as red water fever, Katayama disease or even big belly due to swollen abdomen as a result of the disease. World Health Organization (WHO) estimate that the disease is spread world-wide with 235 million cases of schistosomiasis, in which 732 million people are probably at risk of infection. Another 200,000 people died each year mainly in sub-Saharan African regions where the disease is said to be endemic (Alebie *et al.*, 2014). In 2007, WHO reported between 391 and 587 million people have active cases of schistosomiasis worldwide and that 1.7 to 4.5 million loss per annum of disability adjusted life years are due to schistosomiasis (W H O, 2004; Charles, 2010; Alebie *et al.*, 2014).

Schistosomiasis is currently the second and leading parasitic challenge to public health after malaria (Chitsulo *et al.*, 2000; Saleh, and Samir, 2011; Elbaz and Gamal, 2013). One of the characteristics of schistosomiasis is the involvement of male and female living in copulation within mesenteric vein of the host vascular system: each gravid female capable of producing several hundreds and even thousands of eggs per day (Saleh, and Samir, 2011).

Schistosomiasis is a serious public health problem among school age children which are the target and probably the most affected group in the society (Saathot *et al.,* 2004 , Risikat and Ayoade, 2012). The infection with cercariae which is an infective stage occurs following contact with contaminated water during their daily activities such as swimming, bathing, fishing as well as irrigation purpose.

.

Schistosoma is trematode fluke (worm) belonging to the phylum Platyhelminthes. Schistosoma causes significant morbidity and mortality worldwide with the highest peak prevalence and intensity of infection among the 10-20 years age group (Rambu *et al.,* 2013). Depending on the causative agents, the disease involves either gastrointestinal or urinary tract of the definitive host (Gray *et al.*, 2011). It is regarded to be one of the major health related challenges among neglected tropical diseases in Africa. WHO in its annual report estimated that, thousands are expected to die of the disease each year with several billions at risk of being infected where even brief exposure to contaminated water may result in infection by schistosoma specie (W H O, 2004). Adult worm of the parasite causes chronic granulomatous inflammation as result of the eggs released and plexus invasion around the urinary bladder, as well as inflammation in the mucosal and sub mucosal of the urinary bladder.

Chronic granulomatous inflammation may often lead to the development of squamous metaphasis of the transitional epithelium. It can also lead to bladder fibrosis due to bacterial conversion of nitrites into nitrosamines which later cause stasis and bacterial super-infection (Rambau *et al.,* 2013).

Increase in schistosomiasis distribution and prevalence in some parts of endemic countries were attributed to climatic, human migration, as well as ecological changes. Snails intermediate host, environmental risk factors as well as human were attributed in the transmission of this disease which is highly focal (Koukounari *et al.*, 2011). Urinary and intestinal schistosomiasis remains one of the serious and most prevalent parasitic diseases in Nigeria. Schistosomiasis among school age-children found to have several impact on nutritional deficiency, physical, and intellectual growth as well as cognitive (Partnershipforchild Development, 1997).

The main form of human schistosomiasis is caused by six main species of trematodes or blood flukes belonging to the genus Schistosoma. The genus schistosoma contains 19 species (World Health Organization, 1993), six of which are (*Schistosoma. mansoni, Schistosoma haematobium, Schistosoma. japonicum, Sschistosoma.mekongi, Schistosoma.intercalatum* and *Schistosoma malayensis*) which are of major pathological significance especially to humans, while others affect only non-human mammals, even- though some zoonotic transmission to humans do occur.

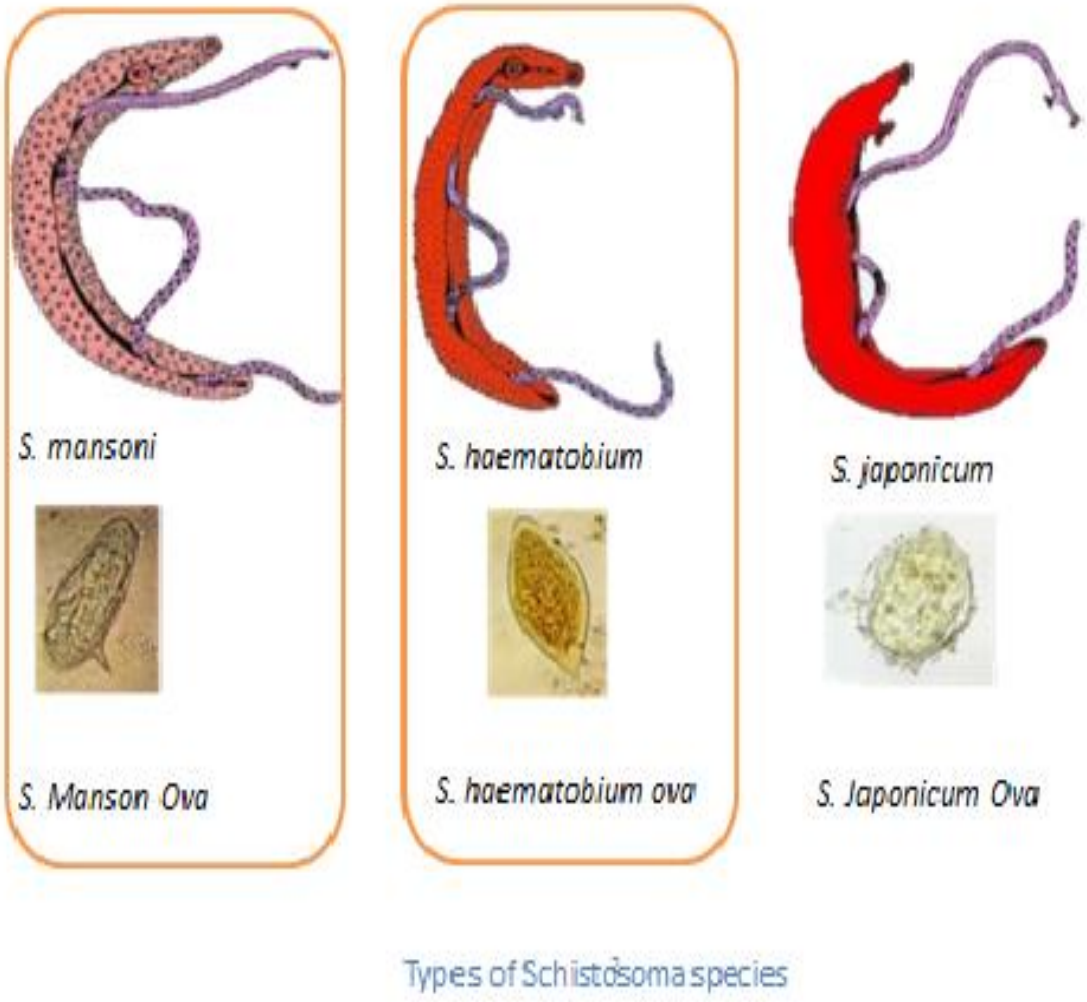

Figure 1.1 Types of human Schistosoma species

All of these flukes (flatworms) require a freshwater snail as an intermediate host and do not replicate in their definitive hosts, humans. The disease is due to the eggs of small, thread-like parasitic worms living inside the blood vessels of the liver, gut and even bladder. These eggs induce an immunological response after being trapped in some part of the body organs such as urogenital tract, liver, and gut wall and thus the disease become established as a result of this immunological

response (Farley, 1991). The prevalence of infection and its clinical consequences depend on interactions between the distribution of the intermediate hosts, and the social and cultural behaviour of human (Conlon, 2005).

WHO estimates 95% or more of human infection are due to S. *haematobium and S. mansoni* especially in the North-western Nigeria (World Health Organization, 1993).

The disease is closely related to human water contact activities around rivers, streams, ponds, dams and so on, especially in rural and agricultural areas of developing countries. Man acquires the infection as he goes about his daily activities that are related to his occupation, recreation, domestic and personal hygiene as a result of his poor sanitary habits (WHO, 1991).

Figure 1.2 Water contact activities of the people in the community

Studies conducted among children have documented increased risk of repeated and prolonged exposure because of greater opportunities for group swimming, bathing, and washing (Pugh *et al.*, 1980; Patwani and Aneja., 1988); Pelwani and Aneja,1988). These children harbour the heaviest infection accounting for more than 75% of the infected communities, forming a source of infection (Akogun and Akogun, 1996,). Several factors influence the transmission of schistosomiasis which include amongst others are ignorance of mode of transmission, false cultural beliefs, low hygienic practices, lack of functional social amenities and primary health care (PHC) services including school health clinics (WHO, 1991).

Schistosomes have approximately about 100 species and 13 genera. Some of these species belong to family schistosomatidae which contain the parasites of mammals, birds and crocodiles. Some 18,000 species belong to phylum Platyhelminthes of the digenetic trematodes with class trematoda and Digenea as the subclass (Luker and Mkeji, 2005). But for the scope of our study we dealt with only two species, *S. haematobium* and *S. mansoni* that affect only human (Luker and Mkeji, 2005).

The gold standards for diagnosis in population based studies rely on detection of eggs in urine or stool of the infected patients (Hamburger *et al.*, 2010). One of the limitations of detection of eggs in urine or stool is that low or light infection may be missed due to insensitivity of the methods (Hamburger *et al.*, 2010).

Developments of molecular techniques such as PCR in the identification of *S. haematobium and S. mansoni* infections have been established in previous studies (Hamburger *et al.,* 2001b; Pontes *et al.,* 2002a; ten Hove *et al.,* 2008.). Studies have shown that PCR based assay for detection of specific DNA for *S. haematobium* and *S. mansoni* parasites is sensitive, specific, and reliable. Most of these PCR techniques used agarose gel electrophoresis for the detection of amplified product. Agarose gel electrophoresis on the other hand has many limitations such as equipment dependent, time consuming and tedious. The nucleic acid can be stained using either ethidium bromide or Syber green vouaer, in which UV-transiluminator is require for analysing the PCR-products.

1.2 Schistosomiasis in Nigeria

Schistosomiasis was reported to be endemic in Nigeria with over 30 million infected. Its prevalence among children remains high especially those between the age of 6-12 years, who acquire the greater burden of the disease. Among 162 million projected populations of Nigerians over 18.5% have schistosomiasis (Useh , 2013).

S. haematobium infection has been reported among children in all regions of Nigeria with 60-75% prevalence in some communities. *S. mansoni* endemicity have also been reported but the prevalence is not as widespread as the former. The high prevalence reported prompted the Nigerian government to initiate a National schistosomiasis control committee in which the fund to carry out research on prevalence in order to delineate endemic communities were not made available. Regrettably, the committee only existed on paper as there was no fund to works with (Useh, 2013). A national control programme for schistosomiasis was established with the sole aimed of surveying prevalence among children aged 5-14 years, treating infected children with praziquentel once every two years and lastly planning for operational research (World Health Organization, 1993).

Schistosomiasis main public health impact is on socio-demographic and economic development of the people. This was due to premature death and incapacity to work leading to decrease productivity, and economic loss to the community (van der Werf *et al.*, 2003).

One important factor that influence severity of schistosomiasis associated morbidity is the geographical variation, in which many external factors play a role in determining the severity of schistosomiasis. Inflammatory marker for the assessment of schistosomiasis associated morbidity was also of significant importance in determining post-treatment assessment of organ enlargement (Vernnervald, 2005).

In Nigeria, the National Schistosomiasis Control Program (NSCP) estimated that the disease affects 29 million people farming across all parts of the Federation States. United Tanzania Republic and Nigeria with 19 million each and lastly 15 million each from Democratic Republic of Congo and Ghana (Steinmann *et al.,* 2006).

Schistosomiasis were reported to have caused more than 200,000 thousand deaths in Sub-Saharan Africa and millions more are said to be at risk of infection Women and their children are vulnerable to infection due to their activities like washing clothes and for children playing in contaminated mud water that make them at risk of infection in Africa (Kabatereine *et al.*, 2004; WHO, 2010:). The risk of infection with schistosomiasis is highest among those who lived close to dam, rivers or any water bodies and prevalence and intensity of infection is expected to increase with age which sometimes reaches highest peak within 15 to 20 years of age but intensity decreases in adult with no change in prevalence (Leder and Weller, 2009; WHO, 2010).

1.3 Statement of the Problems

1.3.1 High Disease Burden

Schistosomiasis is an important public health problem associated with high disease burden in tropical and subtropical African countries and their effects results in high morbidity and mortality especially in the North-western Nigeria. Irrigation and dams construction are said to be essential but are associated with health problems that resulted in reduction of productivity and welfare of the people engaged in irrigation agriculture. Schistosomiasis infection may result in disability and even death to vast number of people resulting in economic loss to the affected areas.

The annual mortality rate due to urinary schistosomiasis could be as high as 150,000 while number of patient dying due to *S. mansoni* infection could be as low as that of the *S. haematobium* (Fenwick, and Savioli , 2003). One major factor that promotes higher worm burdens is poverty and can also result in lower income among those infected due to their poor health conditions induced by schistosomiasis. Poverty may result in many consequences due to schistosomiasis which may include among others disability, long term illness, reduced productive capacity, impaired growth and cognitive development among children (Ross *et al.*, 2002; Hotez *et al.*, 2006).

1.3.2 Socio-economic Impact of Schistosomiasis

Socio-economic impact of schistosomiasis can be looked in terms of declining well- being and productivity of the infected irrigation farmers such as reduction in crop production, unable to pay school fees for their children and unable to build or construct houses for their living. In children the disease may result low cognitive impairment, stunted growth, poor educational achievement, delay in learning and development and poor school attendance (King, 2009).

1.3.3 High Infection Rate Results in Serious Complications

Human schistosomiasis is often linked to socio-economic activities of the people which may include occupational, recreational, domestic and socio-cultural factors on human water contact patterns especially among the vulnerable school children where the infection rate were high and resulted in serious complications (Okpala, *et al.*, 2004).A complication which may result due to high infection rate includes bladder cancer and infertility due to *S. haematobium*, neurological leision due to spinal cord schistosomiasis, or liver cancer as a result of infection with *S. mansoni*.

1.3.4 Lack of Effective Prevention and Control Methods

Another challenge is to prevent and control the spread of the disease using various diagnostic techniques available. Some of which include parasitological techniques, molecular base methods, antigen-antibody techniques and the most recent ones called lateral flow assay (LFA). Each of the methods mentioned above have its limitation in prevention and control of schistosomiasis.

1.3.5 Lack of Sensitivity and Specificity of Some Methods in Low Endemic Areas

Current parasitological methods such as urine filtration concentration and Formol ether concentration techniques used for the diagnosis of human schistosomiasis often lack sensitivity and specificity especially in areas of low endemicity. It was also observed that the techniques are time consuming, labour intensive and require skilled personnel. Immunodiagnostic techniques cannot differentiate between current and past infections as well as the issue of cross reaction which is more obvious.

1.3.6 LATE-PCR Dipstick Provide Alternative Solutions to Current problems

The ultimate challenge for all researchers is aim in improving current diagnostic methods and management of the disease by responding to threat of praziquentel resistance as well as environmental changes. As already mentioned above establishment of new or evaluation of the current diagnostic methods to a simple ,rapid and reliable to diagnose light infection are much needed in this direction(such as simple dipstick, PCR base assays). In this present research study,

we put the effort to establish LATE-PCR Dipstick which is one of the DNA–base PCR assay to detect both *Schistosoma haematobium and Schistosoma mansoni* infection in human urine and stool samples respectively.

1.4 Rationale of the Study/Justification of the Study

Water development projects such as dams and canals are associated with increase in the incidence, prevalence and transmission of urinary Schistosomiasis. And yet these are closely related to the culture, habits and routines of the population. Moreover, because of the changes in flora and fauna following these water projects there could be a shift in the epidemiology of urinary and intestinal schistosomiasis in endemic areas from seasonal and highly focal to intense widespread and constant transmission as was observed around other water development projects (WHO, 1991; Picequet, *et al.*, 1996). Accurate epidemiological data is very important for effective prevention and control of the disease.

Children are commonly heavily infected, the major contaminators of surface water with urine and faeces and hence the major reservoir of infection in the community (Bundy, *et al.*, 1990; Akogun and Akogun, 1996,). The indirect consequences in children include decrease in school attendance and intellectual development, retard growth, limit physical activity, anemia and disturbing the cognitive function (Savioli, *et al.*, 2004). Therefore, treating urinary schistosomiasis during school age is timely, scientifically sound, cost effective and in line with the current global approach for its control and also the drug cost should not be a factor that determine its proper used ,(WHO, 1985; Noke and Bundy,1993,).

At present, there are no records to show that studies have been carried out with respect to the epidemiological risk factors and effect of Goronyo dam in Sokoto State, Bakalori dam in Zamfara State, and Matan-fada Dam in Kebbi State on the health status of adults and school children residing around the area before or after its construction.

Since current diagnostic methods are time and labour intensive, a new method is needed to ease the burden of diagnosis. In this present research, we try to established LATE-PCR LFA which have not been evaluated for diagnosis of *S. mansoni* and *S. haematobium* infection in human stool and urine samples respectively.

A. Research findings will make tremendous impact on Schistosomiasis control and prevention initiatives

B. Research findings will gather information that may help decrease the prevalence and disease burden especially among the study subjects through optimized control programs.

C. Research findings will be presented in international conferences and published in peer reviewed journals

D. Successful development of a rapid, Simple, Sensitive and Specific diagnostic kit for the detection of *S. haematobium* and *S. mansoni* in urine and stool samples respectively which will enhance the diagnosis of schistosoma.

E. USM will serve as a centre of excellence in Schistosomiasis research initiatives according to World Health Organization ASSURED guidelines.

1.5 Approach to the Study of Schistosomiasis

Strategies to control morbidity due to schistosomiasis may include diagnosis and treatment, health education, promotion of safe water supply and sanitation. Snail control and environmental modification with proper management, these approaches can reduce morbidity and control the transmission of schistosomiasis in endemic countries (World Health Organization, 1993).

1.6 Control of Schistosomiasis

A complex interrelationship was said to exist between people and their environment in the epidemiology of schistosomiasis, implementation of effective control measures requires epidemiological information (Doumenga, *et al.*, 1987& Hunter, *et al*, 1993). These include quality and quantity of data based on local epidemiological pattern, ecology, distribution of intermediate host, state of social services and the overall socioeconomic situation in the affected area. The latter is paramount for its sustainability within the framework of PHC. New drugs and diagnostic techniques, together with other technical advances in the field of chemotherapy, snail control, health education, improved sanitation and water have radically improved its control with varying degree of success.

Factors that determine the choice of control measures are (a).The magnitude of the infection in a given area. (b).Priority rating as a public health problem.(c).The quality and quantity of data on the epidemiological pattern in a given area. (d).Availability and accessibility of resources including health services. (e). Community perception of the problem and the likelihood of their participation towards its control. (f.) The degree of inter-sectoral collaboration.

It is pertinent to note that the schistosomiasis may have been established among the local population of the project area. In such situation, chemotherapy administered during the construction phase of water schemes will be more cost effective than in the post construction phase. However, if the infection is not endemic in the area prior to the construction phase, there is the need for surveillance mechanisms to be put in place to serve as an early warning sign of its introduction (i.e. urinary schistosomiasis). Medical examination should be carried out on all employees and their families before they assume duty. The provision of social services such as potable water, good housing with sanitary facilities will go a long way to limit the spread of schistosomiasis.

Moreover, there is the need for periodic medical examination for all workers and their families. This will further add to the surveillance data for early detection. During the planning phase of water development projects, considerations are made to predict the level and places where migrants are likely to come from. If there is an indication that any of such places is known to be endemic for schistosomiasis, a screening centre(s) could be sited in endemic place or on the site of the project or

both, before the commissioning of the dam. This will prevent the introduction of Schistosomiasis that is not endemic in the project area.

The current approach for the control of Schistosomiasis is directed towards reducing morbidity within the framework of PHC. This approach involves the use of chemotherapy, molluscicide application and improved sanitation.

1.6.1 Snail Control

Snail control is an essential part of Schistosomiasis Control Programme (SCP). This is because snails are necessary for the transmission of Schistosomiasis. In endemic areas, prevention is more difficult. Provision of clean water and proper sewage control are probably the most important means of reducing the burden of schistosomiasis, in addition to improvements in the general socioeconomic status of the population. Molluscicide can be used to reduce snail populations in fresh water and thereby remove the intermediate host from the parasite's life cycle (Conlon et al., 2005).

Biomphalaria and Bulinus specie of aquatic snails which serve as intermediate host of *S.mansoni* and *S. haematobium* which are capable of self or cross fertilization and are capable of laying up to 1000 eggs within a period of one year. This might be the reason why it become difficult to eliminate snail in schistosomiasis endemic settings (NIPD, 2013: Tadesse,2014).

Recently, a heating system of standard cement tanker is developed for the control of snails. The system which has a movable dark canvas covers, that allowed temperature to be controlled between 20^0C to 24^0C. The machine cement tanker

system facilitates mortality of Biomphalaria tenagophilia (Rose *et al.,* 2013: Tedesse, 2014). Snail control is said to be one of the major thrust before the advent of effective and safe anti-helminthic drug in order to reduce infection rate (Robert, *et al.,* 2013).The three methods used in the control of snails are chemical, biological and environmental modification.

1.6.2 Chemical Control

Chemicals in form of molluscicide are known to provide a rapid and effective means of reducing the rate of transmission of Schistosomiasis by causing a fall in the total snail population. An ideal molluscicide should be safe, not toxic to non- target flora and fauna, stable, cheap, available, easy to apply, and cost effective. The effect of molluscicide is more pronounced when the volume of water or area to be sprayed is small. Molluscicide that are widely used are copper sulphate, niclosamide, triphemarp and sodium pentachlorophenate. However, niclosamide is the most widely used, because of its availability, effectiveness and minimal adverse effect to non-target flora and fauna. *S. haematobium* and *S. mansoni* release eggs which hatch to release miracidium and this miracidium has to find a snail intermediate host which may be of the genus Bulinus and Biomphalaria in asexual reproduction respectively. Later Miracidium undergoes multiplication to release thousands of infective cercaria (Walker,2011, El-Ridi & Tallima, 2013).

The methods used in the application of molluscicide are blanket (wide area) and focal. The former is cumbersome, expensive and wasteful. The later (focal method) depends on local epidemiological data on the breeding sites of snails and season for transmission of infection. Focal application is therefore more

scientifically sound, efficient and effective in breaking the chain of schistosomiasis transmission. Biomphalaria and Bulinus snails species which normally live under water care as well as measure should be taken to ensure that plant vegetation, aquatic animals (fish, ducks) and other animal habitat as well as portal water quality for irrigation and drinking are not made harmful by molluscides chemical such as copper sulfate, niclosamide and acrolein (King, 2010, El-Ridi & Tallima, 2013).

However, because of the fact that snail population show seasonal variation and are widely distributed in the endemic areas, the use of molluscicide is therefore limited by seasonality, need for highly skilled and motivated staff, multiple application as well as being capital intensive. Moreover, it has been reported to be toxic to non-target flora and fauna, which may lead to undesirable effects

Recently, molluscicide of plant origin has been accorded greater interest especially in the under developed countries, as an inexpensive technology when compared to their synthetic counterpart. For instance, *Tetrapteura teraptera* locally distributed in West Africa has been shown to be effective against *Bulinus spp* (the intermediate host of urinary schistosomiasis) under laboratory conditions. Examples of other molluscicide of plant origin that are highly effective under laboratory conditions are *Pytolacca dodecandra* (endod) and *Jathrapha curcas*. However, they are not specific to snail only. In general, molluscicide of plant origin have not been field-tested. There is therefore, the need for further studies before it will be recommended for the control of snails on the field.

1.6.3 Biological Control

This is basically a competitive strategy between fresh water snails (intermediate host of schistosomiasis) and their predators. The aim is directed toward displacing and reducing the total snail population, which will ultimately lead to reduction in transmission, morbidity and prevalence. The use of biological agents such as predator snails (*Merisa carnuarities, Helisome dury*ietc), fish and wading birds has been studied under laboratory conditions but has shown limited success .

Currently, the use of micro-parasites that feed on snails is being experimented. Further studies and reports are necessary to highlight the practicability, usefulness and appropriateness of biological methods in the control of schistosomiasis.

1.6.4 Environmental Modification

Environmental modification such as the use of modern irrigation techniques (overhead and trickle type) can reduce water waste leading to an improved water management and decrease in the number of snail breeding sites. Furthermore, it assists in the control of Schistosomiasis by reducing the number of open canals and drains which will reduce human contact with infected water.

Fluctuations in water level was shown to be effective in reducing snail population and snail eggs in lake Volta (Ghana) and Oyam reservoir in Ogun State (Nigeria). Result of the latter study showed that transmission was greatly influenced by the pattern of water discharge during the hot dry season (January – April). A high discharge during this period of rainfall, high temperature and intense sunshine

increased rapid water level fluctuations and lake draw down, which led to significant reduction in all indices of Schistosomiasis transmission: snail density, snail infection rates, water contact pattern and incidence of infection.

Furthermore, the use of concrete lined canals and improving the flow velocity by periodic removal of vegetation along the canals will also be effective in reducing snail population and hence the transmission of Schistosomiasis.

The major drawback of the various technologies employed in environmental management and modification is the fact that their installation and maintenance is beyond the reach of most communities in the developing countries.

1.6.5 Improved Water and Sanitation

The availability of adequate portable water supply and the level of sanitation are closely linked to contamination of surface water and incidence of water related diseases such as schistosomiasis. Several studies have shown that the provision of improved latrine, bathing and washing facilities and potable water supply reduces the transmission of schistosomiasis than in communities without these facilities. For instance in Saint Lucia (USA) , after mass chemotherapy, the establishment of water supply system including laundry and shower facilities, maintained transmission at a low (< 1% in some areas) level. This obviously indicated that, re-infection that commonly follows chemotherapy can be reduced to the minimum if adequate potable water and sanitary facilities are in place. This finding was further strengthened in a community-based study in Southern Nigeria. Report of the study showed that the use of potable water over a period of two years

had reduced the prevalence rates of urinary schistosomiasis from 15.9% to 11.7% and 33.5% to 21.4% among males and females aged 5-14 years respectively.

Sanitation is one basic problem in Sub-saharan countries of Africa. When infected individual urinate or defecate close to a water source, the ova of the parasites can get access to the water easily. Schistosomiasis is easily spread in the communities in the water can be used for different purpose and they do not have access to toilets or sanitation facilities and awareness about transmission patterns (Assefa, *et al.*,2013;:Tedesse, 2014).

Overall, the studies indicated that the rate of contact and usage behaviours of infected water would change only if there is a better, accessible and affordable alternative. Furthermore, maintenance such as dripping taps must be given high priority not only to minimize water wastage but also to prevent the formation of pools that may become snail infested and breeding sites of other vectors of disease such as mosquitoes. However, the installation and maintenance of such facilities is highly expensive and beyond the reach of most communities where Schistosomiasis is endemic. Most importantly however, several studies' indicated clearly for the need for a continuous community awareness campaign regarding the proper utilization of any installed sanitary facilities to achieve optimal benefits. This further underscores the significance of health education in all control measures.

1.6.6 Health Education

Urinary and intestinal schistosomiasis affect humans only and their transmission are greatly influenced by ignorance of their cause and mode of

transmission, false cultural beliefs and poor hygienic practices. The social environment is known to influence our attitudes and behaviours in all spheres of life including health.

Attitudes and behaviours on health during the formative years are adopted based on information from family members and peer group. Such information has no scientific basis and more often than not is mere speculations passed from generation to generation. Thus when children lack factual information, proper and appropriate guidance, they are likely to adopt attitudes and behaviours that could affect not only the individual cases but also his/her family and the community.

However, attitudes are known to be specific and often less enduring. They are therefore likely to change over time. It is also known that the behavior we adopt during childhood will definitely have a profound impact on the health and quality of life of an individual. Behavioral risks are now recognized as important causes of preventable morbidity and avoidable deaths.

Persuading masses and poor uneducated people is potentially very effective but exceedingly difficult to change their tradition and customs. Such effort have achieved dramatic success in some countries while in some only little have been achieved (Roberts *et al.*, 2013).

However, behavioral risks are modifiable and sometimes they do change rapidly as a result of timely and appropriate health information. Health education messages that focus on pollution of water sources, protection against infected water

and significance of early diagnosis and treatment will help people to willingly change their water contact and usage behaviours. This approach has been shown to decrease contact with contaminated water, increase hospital utilization and subsequent reduction in prevalence and intensity of urinary schistosomiasis

Above all, health education brings about individual and community involvement on matters relating to their health. It helps in evolvement of positive attitude and behaviours by taking responsibility of promoting and maintaining their state of health. Such positive participation was reported to be of great measure for sustenance and reduction in the overall cost of control programme. Most of the infected people acquire schistosomiasis through various occupational activities such as farming, fishing, washing, bathing and other recreational activities using water bodies known to be contaminated with snail and cercaria. Health education is compulsory in order to completely eliminate or to some extent reduce the menace as well as the significant global burden of schistosomiasis and other intestinal parasites (Gray *et al.*,2010; El-Ridi & Tallima., 2013).

Without doubt other control measures (chemotherapy, snail control, water and sanitation) are more costly and more difficult to organize but will definitely contribute to the control of urinary schistosomiasis. But perhaps, man being the only reservoir of the parasite will play a more significant role towards a long-term solution through behavioral modification. There is therefore the need for culturally appropriate health education on issues relating to transmission, prevention and control to high-risk groups such as school children. Ultimately, health education intervention will ingrain in them health promotive and preventive attitude and