

ABSTRACT

A total of 784 faecal samples collected during the 7 months period, January to July 2010 from children under 5 years old with diarrhoea who were admitted at the 3 referral hospitals; Parirenyatwa Hospital, Harare Hospital and Chitungwiza hospital, were tested for presence of rotavirus antigen using enzyme immune-assay (EIA). Fifty faecal samples from children without diarrhoea were also tested for rotavirus antigen. Sixteen (32 %) of 50 non-diarrhoeal samples and 515 (65.7 %) diarrhoea samples were rotavirus positive. The association between diarrhoea and detection of rotavirus in faecal samples was statistically significant with an overall odds ratio of diarrhoea patients of 4.08 ($p < 0.0001$). The rotavirus diarrhoea prevalence (59.4 %) was high in children ≤ 18 months old diarrhoea patients. The high prevalence of rotavirus diarrhoea was found during the dry cool season in diarrhoea patients < 59 months of age. Fifty rotavirus positive isolates from diarrhoea patients were genotyped using reverse-transcription polymerase chain reaction. A large proportion of samples could not be genotyped; 28.6 % did not produce G genotype result, and 43.2 % did not produce P genotype result. Of the strains that could be genotyped, G9 (24.3 %) was more predominant, followed by G2 (10.9 %), and P[6] (31 %) was more predominant followed by P[4] (14.3 %). The G and P combinations which were more predominant were G1P[6], G9P[6] and G8/G12P[4]. The RNA electrophoresis of the rotavirus genome was performed on 30 rotavirus positive samples, 90 % produced migration patterns typical of the group A rotavirus: 70.4 % were long electropherotypes and 22.2 % were short electropherotypes. The identification of unusual P and G combinations in Zimbabwe may affect the efficacy of currently available rotavirus vaccine formulations and may contribute to the design and development of a broadly reactive rotavirus vaccine for use in African countries.

DEDICATION

I dedicate this work to my family who allowed me to spend all my spare time on the project.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr P Nnziramasanga of the Department of Medical Microbiology College of Health Sciences, University of Zimbabwe, for his professional supervision; Dr S Zinyowera of the Department of Medical Microbiology College of Health Sciences, University of Zimbabwe and Dr Michael D Bowen of Gastroenteritis and Respiratory Viruses Laboratory Branch Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, United States of America.

I also acknowledge the technical and practical assistance I had from Mrs I Peenze, Dr M Seheri and other staff members of the MRC Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, South Africa.

I also would like to express my thanks to the Department of Medical Microbiology members of staff especially the Virology Laboratory members of staff for their unwavering support.

Last but not least I would like to express my sincere gratitude to the Ministry of Health and Child Welfare granting the authority to use their samples.

TABLE OF CONTENTS

CONTENTS	PAGE
Abstract	i
Dedication	ii
Acknowledgements	iii
Table of contents	iv
List of figures	vii
List of tables	viii
List of appendices	ix
List of abbreviations	x
CHAPTER 1: INTRODUCTION	
1 Introduction	1
1.1 Background	1
1.2 The Rotavirus	2
1.2.1 Epidemiology and Pathogenesis of rotavirus	5
1.2.2 Laboratory diagnosis of rotavirus	9
1.2.2.1 Rotavirus EIA	10
1.2.2.2 Rotavirus dsRNA PAGE	10
1.2.2.3 Rotavirus detection by RT-PCR	10
1.2.2.4 Rotavirus genotyping using RT-PCR	11
1.2.3 Treatment and prevention	12
1.3 Literature review	13
1.3.1 Highlights of the Worldwide Rotavirus Surveillance findings	13

1.3.2	Circulating rotavirus genotypes in America	15
1.3.3	Circulating rotavirus genotypes in some African countries	16
1.3.4	Circulating rotavirus genotypes in Zimbabwe	18
1.4	Project justification	19
1.5	Objectives of the study	20
1.5.1	Main objective	20
1.5.2	Specific objectives	20

CHAPTER 2: MATERIALS AND METHODS

2	Materials and methods	22
2.1	Ethical issues	22
2.2	Study Setting	22
2.3	Specimens	23
2.3.1	Justification of sample size	23
2.3.2	Faecal specimens from gastroenteritis cases	23
2.3.3	Control: faecal specimens from cases without diarrhoea	24
2.4	Bio-safety issues	25
2.5	Methods	25
2.5.1	Rotavirus detection techniques	25
2.5.2	Rotavirus genotyping	28
2.6	Analysis of results	31

CHAPTER 3: RESULTS

3	Results	32
3.1	Detection of rotavirus antigen testing in diarrhoea cases and controls by	

By EIA kit method	33
3.2 Rotavirus genotyping	36
3.2.1 VP7 genotyping	36
3.2.2 VP4 Genotyping	38
3.2.3 G and P combination	39
3.3 Polyacrylamide gel electrophoresis	41
CHAPTER 4: DISCUSSION	
4. Discussion	43
CHAPTER 5: CONCLUSION	
5. Conclusion	49
5.1 Recommendations	51
5.2 Limitation	52
REFERENCES	53
APPENDICES	62

LIST OF FIGURES

FIGURE	TITLE	PAGE
1	Causes of severe diarrhoea requiring hospitalization of infants and young children	2
2	Coding assignments and virion locations of rotavirus proteins and 3D structure of the rotavirus particle	4
3	Rotavirus mortality rate per 100 000 children <5 years of age by country, in 2004	5
4	Schematic illustration of the VP7 genotyping PCR	29
5	Schematic illustration of the VP4 genotyping PCR using Gentsch primers	30
6	Distribution of severe diarrhoea cases by age group in the study	33
7	Distribution of EIA rotavirus positive severe diarrhoea cases by age group in the study	34
8	Distribution of EIA rotavirus positive controls by age group	36

LIST OF TABLES

TABLE	TITLE	PAGE
1	Distribution of EIA rotavirus positive severe diarrhoea by month in 2010	35
2	Distribution of rotavirus G-types by age group	37
3	Distribution of rotavirus P-types by age group	38
4	Distribution of rotavirus G and P type combination by age group	40
5	Association of electropherotypes and genotypes of rotaviruses circulating in Zimbabwe	42

LIST OF APPENDICES

APPENDIX		PAGE
1	Rotavirus diarrhoea case investigation form	62
2	The laboratory safety rules	66
3	The RNA electrophoresis technique	70
4	The primer list and sequences	79
5	The reverse transcriptase-polymerase chain reaction	83
6	Summary of raw data	101
7	Letter granting authority to use MOHCW samples	109

LIST OF ABBREVIATIONS

-	-	negative or minus
%	-	Percent
/	-	and
+	-	positive or plus
>	-	greater than
<	-	less than
≤	-	less than or equal to
≥	-	greater than or equal to
°C	-	degrees Celsius
μl	-	microlitres
AFP	-	Acute Flaccid Paralysis
AFRO	-	Africa
AgNO ₃	-	Silver nitrate
AMP	-	adenine monophosphate pump
BDS	-	Bachelor of Dental Surgery
bp	-	base pair
BSc	-	Bachelor of Science
CDC	-	Centers for Disease Control and Prevention (USA)
cDNA	-	complementary deoxy-ribose nucleic acid
Cl	-	Chloride
Conc	-	concentration
DNA	-	Deoxy-ribose nucleic acid

DPRU	-	Diarrhoeal Pathogens Research Unity
EIA	-	Enzyme immuno-assay
ELISA	-	enzyme-linked immunoassay
et al	-	and colleagues
EPI	-	Expanded Programme on Immunization
H1N1	-	An influenza type A strain (Swine flu)
HBMLS	-	Bachelor of medical laboratory sciences honours
L	-	long electropherotype
Ltd	-	limited
MBChB	-	Bachelor of medicine and bachelor of surgery
mg	-	milligrams
MOHCW	-	Ministry of Health and Child Welfare
MRC	-	Medical Research Council
Msc	-	Master of Science
Na	-	sodium
n	-	number
nm	-	nanometers
No.	-	number
NSP	-	non-structural viral protein
NT	-	non-typeable
OD	-	optical density
PAGE	-	Polyacrylamide gel electrophoresis
QA	-	Quality assurance

QC	-	Quality control
RT-PCR	-	reverse-transcriptase polymerase chain reaction
S	-	short electropherotype
SGLT1	-	Sodium glucose co-transporter 1
SOP	-	Standard operating procedure
TAE	-	Tris-acetic acid-EDTA buffer
TBE	-	Tris-borate EDTA buffer
TM	-	Trade Mark
UK	-	United Kingdom
UV	-	ultra-violet
VP	-	viral protein
VP4	-	P antigenic protein
VP7	-	G antigenic protein
WHO	-	World Health Organization