EFFECTS OF HIV AND INTESTINAL PARASITES CO-INFECTION ON HEMATOLOGICAL PARAMETERS AMONG PREGNANT WOMEN ATTENDING SELECTED HEALTH FACILITIES IN NYERI COUNTY, KENYA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PUBLIC HEALTH (EPIDEMIOLOGY AND DISEASE CONTROL) IN THE SCHOOL OF PUBLIC HEALTH OF KENYATTA UNIVERSITY

NOVEMBER 2017
DECLARATION

This thesis is my original work and has not been presented for degree or other awards in any other university.

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DEDICATION

I dedicate this thesis to my family: my wife Teresiah Njoki, our daughter Margaret Nyambura, our son Dominic Wang’ombe, my siblings, our late mother Margaret Nyambura and above all to God for his provision.
ACKNOWLEDGEMENTS

I thank God for giving me the grace to undertake this research. I am grateful to my supervisors Prof. Ephantus W. Kabiru and Prof. Michael M. Gicheru both of Kenyatta University for their professional guidance and mentorship. Many thanks to Prime-K project for financial support during this study. Appreciation to NACOSTI for permission to conduct this study and Nyeri County Government, Kieni, Nyeri Central, Mukurweini and Mathira Sub-Counties for the clearance to conduct the study. I am grateful to all heads and staff of the health facilities where the study was conducted. My appreciation to all women who consented to participate in the study. I thank Mr Francis Gitonga the Laboratory Manager, Mr Mwangi Muthee, the deputy Laboratory Manager and Mr Gerald Gachomo, Laboratory Technologist for assistance in processing of blood and stool samples in the laboratory in Nyeri level 5 hospital. Many thanks to all my colleagues and all who supported me in different ways during this study. I am grateful to School of Public Health and Department of Community Health for facilitating the study. I thank Graduate School for facilitating examination of thesis. I thank Kenyatta University for provision of training position. May God bless you.
# TABLE OF CONTENTS

DECLARATION.................................................................................................................. iv

ACKNOWLEDGEMENTS .................................................................................................... iv

TABLE OF CONTENTS ...................................................................................................... v

LIST OF TABLES ................................................................................................................ xii

LIST OF FIGURES ............................................................................................................ xv

LIST OF APPENDICES ........................................................................................................ xviii

DEFINITION OF OPERATIONAL TERMS ....................................................................... xx

ABSTRACT ............................................................................................................................. xx

CHAPTER ONE: INTRODUCTION......................................................................................... 1

1.1 Background information ............................................................................................ 1

1.2 Statement of the problem ......................................................................................... 2

1.3 Justification ................................................................................................................ 3

1.4 Research questions .................................................................................................. 3

1.5 Null Hypothesis ....................................................................................................... 4

1.6 Broad objective ........................................................................................................ 4

1.6.1 Specific objectives ............................................................................................. 4

1.7 Significance of the study ........................................................................................ 4

1.8 Limitation and delimitation ..................................................................................... 5

1.9 Conceptual Frame Work ........................................................................................ 5

CHAPTER TWO: LITERATURE REVIEW .......................................................................... 6

2.1 Intestinal parasites infection .................................................................................... 6

2.1.1 Global distribution ............................................................................................. 6

2.1.2 Distribution in Kenya ......................................................................................... 10

2.1.3 Transmission of intestinal parasites .................................................................... 11

2.1.4 Risk factors to intestinal parasites infection ...................................................... 12

2.1.5 Life cycles of intestinal helminths ..................................................................... 12
2.2.8 Management of HIV infection

2.2.8.1 Antiretroviral treatment

2.2.8.2 Nutritional management

2.2.9 HIV prevention and control

2.2.9.1 Testing and counselling

2.2.9.2 Prevention of mother to child transmission using ARV prophylaxis

2.2.9.3 Nutrition

2.2.9.4 Public health education

2.3 Haematological changes in women during pregnancy

2.3.1 Red blood cells changes during pregnancy

2.3.2 Haemoglobin changes during pregnancy

2.3.3 Haematocrit changes during pregnancy

2.3.4 Platelets changes during pregnancy

2.3.4 White Blood Cells changes during pregnancy

2.4 Haematological changes in HIV infection

2.5 Haematological changes in intestinal parasites infection

2.6 Co-infection with HIV and intestinal parasites

2.7 Gaps in knowledge from literature review

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

3.2 Variables

3.2.1 Dependent variable

3.2.2 Intervening variable

3.2.3 Independent variables

3.3 Study area

3.4 Study population

3.4.1 Inclusion criteria
3.4.2 Exclusion criteria ............................................................................................................ 36
3.5 Sampling techniques ........................................................................................................ 37
3.6 Sample size determination ............................................................................................... 37
3.7 Data collection techniques .............................................................................................. 39
3.7.1 Pre-testing of research tools ....................................................................................... 39
3.7.2 Exit Interviews ............................................................................................................ 39
3.7.3 Laboratory Investigations .......................................................................................... 40
3.7.3.1 Blood specimen collection and processing .......................................................... 40
3.7.3.2 Stool specimen collection and processing ............................................................ 40
3.8 Validity ............................................................................................................................. 40
3.9 Reliability ........................................................................................................................ 41
3.10 Data analysis .................................................................................................................. 41
3.11 Ethical considerations .................................................................................................... 41

CHAPTER FOUR: RESULTS ................................................................................................. 43

4.1 Socio-Demographic Characteristics of the Study Participants ......................................... 43
4.1.1 Age of the respondents .............................................................................................. 43
4.1.2 Marital status of the respondents .............................................................................. 43
4.2 Socio-Economic Characteristics .................................................................................... 44
4.2.1 Level of education of the respondents ..................................................................... 44
4.2.2 Occupation of the respondents ............................................................................... 45
4.2.3 Income level of the respondents .............................................................................. 46
4.3 Prevalence of intestinal parasites infection ....................................................................... 47
4.3.1 Prevalence of intestinal helminth parasites among respondents ............................... 47
4.3.2 Prevalence of intestinal protozoan parasites among respondents ........................... 47
4.3.3 Prevalence of specific intestinal protozoan parasites among respondents ............... 48
4.3.4 Prevalence of intestinal parasites among respondents by health facilities .......... 49
4.4 Practices that would predispose respondents to intestinal parasites infection .............. 50
4.5 Management of pregnant women with HIV and intestinal parasite infections .................................. 51
4.6 Factors associated with intestinal protozoan infection ................................................................. 52
4.6.1 Socio-demographic characteristics of respondents ................................................................. 52
4.6.2 Socio-economic characteristics of respondents ................................................................. 53
4.6.3 HIV infection ......................................................................................................................... 53
4.6.4 Practices of the respondents .................................................................................................. 54
4.6.6 Multivariate analysis of factors associated with intestinal protozoan parasites infection among respondents ................................................................. 55
4.7 Haematological Parameters ..................................................................................................... 56
4.7.1 White blood cells count for HIV positive and HIV negative respondents ......................... 56
4.7.2 Red blood cells (RBC) count for HIV positive and HIV negative respondents ................... 57
4.7.3 Haemoglobin concentration for HIV positive and HIV negative respondents ................. 57
4.7.4 Haematocrit for HIV positive and HIV negative respondents .............................................. 58
4.7.5 Platelets count for HIV positive and HIV negative respondents .......................................... 59
4.8 Effects of HIV and intestinal parasites co-infection on haematological parameters .............. 60
4.8.1 Effect on white blood cells of respondents ............................................................................ 60
4.8.1.1 Mean white blood cells of respondents .............................................................................. 60
4.8.1.2 Analysis of variance (ANOVA) for white blood cells among respondents .................... 61
4.8.1.3 Multiple comparison test of mean white blood cells among four groups of respondents based on HIV and intestinal parasites infection status ........................................... 62
4.8.2 Effect on red blood cells of respondents ................................................................................. 65
4.8.2.1 Mean red blood cells of respondents ................................................................................. 65
4.8.2.2 Analysis of variance (ANOVA) of mean red blood cells among respondents .......... 65
4.8.2.4 Multiple comparison test of mean red blood cells among five age groups of respondents .. 69
4.8.3 Effect on haemoglobin concentration of respondents ............................................................ 71
4.8.3.1 Mean haemoglobin concentration of respondents ............................................................. 71
4.8.3.2 Analysis of variance (ANOVA) of mean haemoglobin among respondents ................. 72
4.8.3.3 Multiple comparison test of mean haemoglobin concentration among four groups of respondents based on HIV and intestinal parasites infection status ........................................ 73

4.8.4 Effect on haematocrit (Packed Cell Volume) of respondents ........................................... 75

4.8.4.1 Mean haematocrit of respondents ................................................................................. 75

4.8.4.2 Analysis of variance (ANOVA) of mean haematocrit of respondents ......................... 76

4.8.4.3 Multiple comparison test of mean haematocrit among four groups of respondents based on HIV and intestinal parasites infection status ........................................ 77

4.8.5 Effect on platelets of respondents ..................................................................................... 80

4.8.5.1 Mean platelets of respondents....................................................................................... 80

4.8.5.2 Analysis of variance (ANOVA) of mean platelets among respondents ....................... 80

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS ............... 82

5.1 Discussion ................................................................................................................................. 82

5.1.1 Socio-demographic and economic characteristics of respondents ................................. 82

5.1.2 Prevalence of co-infection of HIV and intestinal parasites among respondents ............. 83

5.1.3 Practices that would predispose respondents to intestinal parasites infection ............... 84

5.1.4 Factors associated with intestinal parasites infection ..................................................... 85

5.1.5 Effects of HIV and intestinal parasite co-infection on white blood cells, red blood cells, haemoglobin, haematocrit and platelets of respondents ........................................ 87

5.1.6 Summary of discussion ....................................................................................................... 90

5.2 Conclusions ............................................................................................................................ 90

5.3 Recommendations .................................................................................................................. 91

5.4 Suggestion for further research .............................................................................................. 91

REFERENCES ............................................................................................................................... 92

APPENDICES .................................................................................................................................. 108

Appendix I: Ethical Clearance, Kenyatta University 108
Appendix II: Graduate School Approval, Kenyatta University 109
Appendix III: Graduate School Research Authorization 110
Appendix IV: NACOSTI Research Permit 111
Appendix V: NACOSTI Research Authority 112
Appendix VI: Nyeri County Research Clearance 113
Appendix VII: Map of the Study Area 114
Appendix VIIIA: Consent Form (English) 115
Appendix VIIIB: Translated Consent Form (Kikuyu) 117
Appendix IX: Questionnaire 119
Appendix X: Blood Sample Analysis Result Sheet 122
Appendix XI: Stool Sample Analysis Result Sheet 123
Appendix XII: Standard Operation Procedure: Analysing Samples Using Medonic M-series 3-Part Haematology Auto-analyser by Boule Medical AB, Stockholm Sweden 124
Appendix XIV: Guide on how to collect stool sample 128
Appendix XV: Formol-ether Concentration Technique for Faecal Specimen Processing (WHO, 1991) 129
Appendix XVI: Tukey Kramer Test Formula 131
LIST OF TABLES

Table 3.1: Distribution of HIV positive pregnant women in selected health facilities 35

Table 4.1: Distribution of intestinal protozoan parasites infected respondents by health facility 45

Table 4.2: Distribution of respondents by practices related to intestinal protozoan parasites infection 46

Table 4.3: Socio-demographic factors associated with intestinal protozoan parasites infection – bivariate analysis 47

Table 4.4: Socio-economic factors associated with intestinal protozoan parasites infection – multivariate analysis 48

Table 4.5: Practices associated with intestinal protozoan parasites infection among respondents – bivariate analysis 49

Table 4.6: Factors associated with intestinal protozoan parasites infection among respondents – multivariate analysis 50

Table 4.7: Distribution of mean white blood by age, HIV and intestinal protozoan parasites infection status 56

Table 4.8: Relationship between white blood cells and age, HIV and intestinal protozoan parasites infection among respondents 57

Table 4.9: Pair wise critical values and difference between true white blood cells averages among respondents 58

Table 4.10: Comparison of Wij with (Xi – Xj) 59

Table 4.11: Comparison of Wij with (Xi – Xj) 59

Table 4.12: Distribution of mean red blood cells by age, HIV and intestinal protozoan parasites infection among respondents 60

Table 4.13: Relationship between red blood cells and age, HIV and intestinal parasites infection
protozoan parasites infection

Table 4.14: Pairwise critical values and differences between true red blood cells averages among respondents

Table 4.15: Comparison of Wij with (Xi – Xj)

Table 4.16: Comparison of Wij with (Xi – Xj)

Table 4.17: Pairwise critical values and differences between true red blood cells averages among age groups of respondents

Table 4.18: Comparison of Wij with (Xi – Xj)

Table 4.19: Comparison of Wij with (Xi – Xj)

Table 4.20: Distribution of mean haemoglobin by age, HIV and intestinal protozoan parasites infection among respondents

Table 4.21: Relationship between haemoglobin and age, HIV and intestinal protozoan parasites infection among respondents

Table 4.22: Pairwise critical values and differences between true haemoglobin averages among respondents

Table 4.23: Comparison of Wij with (Xi – Xj)

Table 4.24: Comparison of Wij with (Xi – Xj)

Table 4.25: Distribution of mean haematocrit by age, HIV and intestinal protozoan parasites infection among respondents

Table 4.26: Relationship between haematocrit and age, HIV and intestinal protozoan parasites infection among respondents

Table 4.27: Pairwise critical values and differences between true haematocrit averages among respondents

Table 4.28: Comparison of Wij with (Xi – Xj)

Table 4.29: Comparison of Wij with (Xi – Xj)
Table 4.30: Distribution of mean platelets by age, HIV and intestinal protozoan parasites infection among respondents

Table 4.31: Relationship between platelets and age, HIV and intestinal protozoan parasites infection among pregnant women
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Conceptual framework</td>
<td>5</td>
</tr>
<tr>
<td>4.1</td>
<td>Distribution of respondents by age group</td>
<td>38</td>
</tr>
<tr>
<td>4.2</td>
<td>Distribution of respondents by marital status</td>
<td>39</td>
</tr>
<tr>
<td>4.3</td>
<td>Distribution of respondents by education</td>
<td>40</td>
</tr>
<tr>
<td>4.4</td>
<td>Distribution of respondents by occupation</td>
<td>41</td>
</tr>
<tr>
<td>4.5</td>
<td>Distribution of respondents by monthly income in Kenya shillings</td>
<td>42</td>
</tr>
<tr>
<td>4.6</td>
<td>Distribution of respondents by intestinal parasites infection</td>
<td>43</td>
</tr>
<tr>
<td>4.7</td>
<td>Distribution of respondents by intestinal protozoan parasites infection</td>
<td>44</td>
</tr>
<tr>
<td>4.8</td>
<td>Distribution of respondents by white blood cells count</td>
<td>51</td>
</tr>
<tr>
<td>4.9</td>
<td>Distribution of respondents by red blood cells count</td>
<td>52</td>
</tr>
<tr>
<td>4.10</td>
<td>Distribution of respondents by haemoglobin concentration</td>
<td>53</td>
</tr>
<tr>
<td>4.11</td>
<td>Distribution of respondents by packed cell volume (haematocrit)</td>
<td>54</td>
</tr>
<tr>
<td>4.12</td>
<td>Distribution of respondents by platelets count</td>
<td>55</td>
</tr>
</tbody>
</table>
# Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td>AIDS Defining Illness</td>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ART</td>
<td>Antiretroviral Therapy</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra Acetic Acid</td>
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<tr>
<td>GIT</td>
<td>Gastrointestinal Tract</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>KDHS</td>
<td>Kenya Demographic Health Survey</td>
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<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
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<tr>
<td>LBW</td>
<td>Low Birth Weight</td>
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<tr>
<td>NACC</td>
<td>National AIDS Control Council</td>
</tr>
<tr>
<td>NACOSTI</td>
<td>National Commission for Science, Technology and Innovation</td>
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<tr>
<td>NASCOP</td>
<td>National Sexually Transmitted Infections Control Program</td>
</tr>
<tr>
<td>NTD</td>
<td>Neglected Tropical Diseases</td>
</tr>
<tr>
<td>PLT</td>
<td>Platelet</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of Mother to Child Transmission</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>STH</td>
<td>Soil Transmitted Helminths</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infections</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations Childrens’ Fund</td>
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<tr>
<td>UNAID</td>
<td>United Nations Agency for International Development</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>-----------</td>
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<tr>
<td>WBC</td>
<td>White Blood Cell</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
**DEFINITION OF OPERATIONAL TERMS**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite:</td>
<td>A living organism that depends on host metabolically for existence.</td>
</tr>
<tr>
<td>Intestinal parasite:</td>
<td>A living organism that inhabits gastrointestinal tract of the host.</td>
</tr>
<tr>
<td>Helminth:</td>
<td>A multicellular organism that is not able to multiply while in the human body.</td>
</tr>
<tr>
<td>Protozoa:</td>
<td>A single celled microorganism and is able to multiply while in the human body.</td>
</tr>
<tr>
<td>Co-infection:</td>
<td>Simultaneous infection of the human body by two or more pathogens.</td>
</tr>
<tr>
<td>White blood cells:</td>
<td>Cells in blood that are part of the body’s defense system against infections and also play a role in allergies and inflammations.</td>
</tr>
<tr>
<td>Red blood cells:</td>
<td>Cells in blood that transport oxygen throughout the body.</td>
</tr>
<tr>
<td>Hemoglobin:</td>
<td>The total amount of the oxygen carrying protein in blood.</td>
</tr>
<tr>
<td>Hematocrit:</td>
<td>The percentage of a person’s total blood volume that consists of red blood cells.</td>
</tr>
<tr>
<td>Platelets:</td>
<td>Cells in blood that play important role for normal blood clotting.</td>
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Coagulopathic properties: Tendency to increase development of blood clots or thrombosis.

Haemolysis: The breakage of red blood cell’s membrane, causing the release of haemoglobin and other internal components.

Haemogram: (also known as complete blood count) is a profile of tests that examines different parts of the blood: white blood cells count, red blood cells count, haemoglobin, haematocrit and platelets count.

Cytopenia: A disorder characterized by low or decreased levels of one or more blood cellular components in the circulation.

Bivariate analysis: Allows for efficient estimation of measures of association about risk factors for a given outcome.

Multivariate analysis: Allows for the efficient estimation of measures of association while controlling for a number of confounding factors simultaneously, even in situations where stratification would fail due to insufficient numbers.

Gestation period: The time in which the foetus develops beginning at conception when the sperm from man fertilizes the egg or ovum from the woman and ends at birth.

Autoimmune disorder: A malfunction of the body’s immune system that makes the body to attack its own tissues.
ABSTRACT
Pregnancy is associated with higher demand for haemoglobin; intestinal parasitic infections and HIV infection have also independently been associated with anaemia. A woman with the three conditions together is likely to face a challenge. Prevalence of HIV among women in Nyeri County has increased from 2.5% in 2007 to 5.5% in 2009 and 6.3% in 2013. In Nyeri County, there is limited data on prevalence of co-infection of HIV and intestinal parasites among pregnant women and the effects of the co-infection on haematological parameters. Therefore, the aim objective of this study was to determine the effects of the co-infection of HIV and intestinal parasites on selected haematological parameters among pregnant women attending selected health facilities in Nyeri County. A comparative cross sectional and analytical study was conducted where 130 pregnant women participated. Questionnaire was used to collect data. Stool and blood samples were collected and processed in the laboratory using standard procedures. Data was analysed using SPSS software. Results show that among 130 respondents, 34% were infected with intestinal protozoa. Among 65 HIV positive respondents, 25% were infected with *Entamoeba coli* and 2% were infected with *Iodamoeba butschlii*. Among 65 HIV negative respondents, 38% were infected with *Entamoeba coli*, and 6% *Iodamoeba butschlii*. One HIV negative respondent was infected with *Hymenolepis nana*. Practices such as eating soil, walking barefoot, treating drinking water and use of latrine were not significantly different between HIV positive and HIV negative pregnant women (p>0.05). Factors associated with infection with intestinal protozoans were education (OR = 2.379, 95% CI 1.07-5.288, p= 0.031), employment (OR = 0.4, 95% CI 0.187 – 0.855, p = 0.017) and access to latrine (OR = 0.033, 95% CI 0.009 – 0.12, p= 0.0001). Access to a latrine was a predictor of intestinal protozoan infection in pregnancy (AOR = 0.037, 95% CI 0.01- 0.136 < 0.05). Co-infection of HIV and intestinal parasites lowered WBC (F<sub>0.95</sub> (3, 11) = 5.56, p < 0.05), RBC (F<sub>0.95</sub> (3, 11) = 43, p < 0.05), Haemoglobin (F<sub>0.95</sub> (3, 11) = 11.62, p < 0.05) and haematocrit (F<sub>0.95</sub> (3, 11) = 15.23, p < 0.05). Though not statistically significant, co-infection increased platelets count. The researcher concluded that (i) there is high infection with intestinal protozoan parasites among pregnant women and low prevalence of helminths infection (ii) majority of pregnant women used latrine for defecation and did not eat soil or walk barefoot while almost a half drunk untreated water (iii) low education, unemployment and sharing latrine were associated with intestinal parasite infection among pregnant women and (iv) co-infection of HIV and intestinal protozoan parasites decreased white blood cells, red blood cells, haemoglobin and haematocrit in pregnant women. The researcher recommends that in antenatal care, in addition to HIV treatment, management of intestinal parasite should be considered to minimize co-infection that impact negatively by reducing haematological parameters and possibly growth of foetus.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Over two billion people are chronically infected with one or more Neglected Tropical Diseases (NTD) and more than half a million people die yearly from their infections (Hotez, 2008; Hotez 2009; Molyneux, 2010). Women of reproductive age are among 450 million people with clinical disease due to intestinal parasite infection in developing world (Quihui et al., 2006). Infection with soil-transmitted helmithes leads to malnutrition, anaemia, and thrombocytopenia, attributed to effects of long term inflammation, poor nutrients absorption and loss of blood (Fuseini et al., 2013; Pullan et al., 2014). The common diseases caused by intestinal protozoans are amoebiasis, giardiasis, cryptosporidiosis, and cyclosporiasis. They are associated with diarrhoea and anaemia (Davis et al., 2002; Alzain and Sharma, 2006). Intestinal parasite infections are associated with negative effects on haematological parameters and therefore co-infection with HIV is likely to cause more reduction (Shapira-Nahor et al., 1998; Gallagher et al., 2005; Borkow and Bentwich, 2006)

HIV infection may affect the bone marrow resulting to reduced haematopoises and observed cytopenias (Klatt, 2016). Anaemia, low count of thrombocytes, neutrophils, lymphocytes, and monocytes can happen in more than 90% of AIDS patients (Klatt, 2016). Haematological indices of pregnant women largely reflect their general health (Shaw et al., 2010; Osonuga et al., 2011). In normal pregnancy there is decrease in haematocrit, haemoglobin and platelets (Ichipi-Ifukor et al., 2013, Kadir et al., 2011). White blood cells increases during normal pregnancy (Ichipi-Ifukor et al., 2013). Pregnant women are susceptible to intestinal parasites infection due to reduced cell mediated immunity. In pregnant women, *Ascaris lumbricoides* coagulopathic properties can contribute to bleeding after birth (Zapardiel et al., 2010). Intestinal parasitic infections have consistently been associated with anaemia in pregnant
women (Fuseini et al., 2013). Anaemic and pregnant women are 3.5 times more likely to die than non-anaemic women (Brooker et al., 2008).

HIV mainly affects women where in sub-Saharan Africa about 1.5 million pregnant women had HIV infection in 2010 (WHO and UNICEF, 2013) and this number remained unchanged up to 2013 (UNAIDS, 2014). In Rwanda, among HIV positive pregnant women some had co-infection of intestinal helminth (Ivan et al., 2012). In Kenya, maternal mortality rate is high at 362 for every 100,000 live births (Kenya National Bureau of Statistics and ICF Macro, 2014). HIV is among the leading causes of death in Kenya (CDC, 2015). Women are more vulnerable to HIV infection (NACC, 2014), and among pregnant women 79,000 were in need of prevention of mother to child transmission services (NACC, 2014). In coast region, some pregnant women had HIV and Necator americanus and Ancylostoma duodenale co-infection (Gallagher et al., 2005).

1.2 Statement of the problem

In Nyeri County, maternal mortality rate is high at 318 per 100,000 live births and HIV infection contributes to these deaths (Republic of Kenya, 2013). It has been observed that HIV prevalence among women in Nyeri County has been on increase from 2.5% in 2007, 5.5% in 2009 and 6.3% in 2013 (MOH, 2014). Intestinal parasite infection is an area of public health research that has been neglected in Kenya and Nyeri County in particular. Therefore, there is limited data on prevalence of intestinal parasites among pregnant women in Nyeri County. Both pregnancy and HIV infection are known to lower the immunity. Therefore, a HIV positive pregnant woman is vulnerable to infections such as intestinal parasite. Co-infection of HIV and intestinal parasite is likely to reduce haematological parameters during pregnancy and that can lead to low birth weight or death. However, there
are scanty documented reports of epidemiological data on intestinal parasite and HIV co-infection among pregnant women in Nyeri County.

1.3 Justification

Because of the reported increasing HIV prevalence among women in Nyeri County and limited data on prevalence of intestinal parasites among women in Nyeri County; it is important to investigate the possible prevalence of co-infection of HIV and intestinal parasites. The study will also investigate effects of co-infection of HIV and intestinal parasites on haematological parameters. Any harmful effects on haematological parameters could lead to poor health of pregnant women with adverse pregnancy outcomes. Therefore, findings from this study may provide the much needed evidence for management of co-infection of HIV and intestinal parasites during pregnancy for better pregnancy outcomes. Findings from this study could provide useful information for the prevention of intestinal parasites infection in Nyeri County and the country.

1.4 Research questions

i. What is the prevalence of HIV-1-intestinal parasite co-infection among pregnant women attending selected health facilities in Nyeri County?

ii. What practices would predispose pregnant women to intestinal parasites infection in selected health facilities in Nyeri County?

iii. What factors are associated with intestinal parasite infection among pregnant women attending selected health facilities in Nyeri County?

iv. What are the effects of HIV and intestinal parasite co-infection on white blood cells, red blood cells, haemoglobin, haematocrit and platelets in pregnant women attending selected health facilities in Nyeri County?
1.5 Null Hypothesis
There is no significant relationship between co-infection of HIV and intestinal parasites and haematological parameters among pregnant women attending selected health facilities in Nyeri County.

1.6 Broad objective
To determine effects of the co-infection of HIV and intestinal parasites on selected haematological parameters among pregnant women attending selected health facilities in Nyeri County.

1.6.1 Specific objectives
i. To establish the prevalence HIV-1- intestinal parasite co-infection among pregnant women attending selected health facilities in Nyeri County.

ii. To establish practices that predispose to intestinal parasites infection among pregnant women attending selected health facilities in Nyeri County.

iii. To identify factors associated with intestinal parasite infection among pregnant women attending selected health facilities in Nyeri County.

iv. To determine the effects of HIV-1- intestinal parasite co-infection on white blood cells, red blood cells, haemoglobin, haematocrit and platelets in pregnant women attending selected health facilities in Nyeri County.

1.7 Significance of the study
The study is expected to provide information that will be useful for management of co-infection of HIV and intestinal parasites among pregnant women during antenatal care. This is important specifically because diagnosis of intestinal parasites is not a routine practice in
antenatal care. Therefore, management will be evidence based. Provision of information that can be used to prevent intestinal parasites infection is also a benefit to women of child bearing age in Kenya.

1.8 Limitation and delimitation

It was assumed that HIV positive pregnant women recruited in the study were not co-infected with other pathogens other than intestinal parasites. To reduce the effect of this limitation, pregnant women were asked whether they were on other medications other than ART and if the answer was yes they were excluded from the study.

1.9 Conceptual Frame Work

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<tr>
<th>Independent Variables</th>
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<td>Socio-demographic and Socio-economic characteristics</td>
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<td>Individual practices predisposing intestinal parasite infection</td>
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<td>Abnormal Haematological Parameters</td>
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<td>HIV status of an individual</td>
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Figure 1.1 Conceptual framework showing the co-infection of HIV and intestinal parasites, associated factors, practices and effects on haematological parameters.

Source: Developed from literature review.
CHAPTER TWO: LITERATURE REVIEW

2.1 Intestinal parasites infection

2.1.1 Global distribution

Infections with intestinal parasites are a global public health challenge and particularly in tropical and sub-tropical developing countries where safe and adequate water and sanitation are lacking due to poverty (Ngui et al., 2011). They include intestinal worms such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Necator americanus* and *Ancylostoma duodenale*. They are also referred to as geohelminths and soil-transmitted helminths because they are transmitted through contaminated soil. Other intestinal helminths are *Enterobius vermicularis* and cestodes such as *Taenia saginata*, *Taenia solium*, *Diphyllobothrium latum* and *Hymenolepis nana* (Haque, 2007; Bogitsh et al., 2013; Eleni et al., 2014). Approximately over 2.5 billion persons have intestinal helminth infections (Balcioglu et al., 2007; Kurt et al., 2007; Haque, 2007). Globally, about 819 million persons had Ascariasis, 464 million had Trichuriasis and 438 million had *Necator americanus* and *Ancylostoma duodenale* infections in 2010 (Pullan et al., 2014).

Intestinal protozoan parasite infections that commonly infect humans include *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* (Haque, 2007; Eleni et al., 2014). Globally around 50 million people are infected with pathogenic amoeba per year (Petri et al., 2000). Prevalence of *Cryptosporidium parvum* infection has increased among AIDS patients including under five years old in both developing and developed world (Haque, 2007). Several diarrhoeal disease outbreaks caused by *Cyclospora cayetanensis* have also been documented (Herwaldt, 2000; Haque, 2007). In
2001, it was estimated that 40 million pregnant women were infected with soil transmitted helminths globally (Hotez, 2001).

Soil transmitted helminths such as hook worm have been reported in United States of America in Tennessee in 1955 and in Georgia in 1972. *Trichuris trichiura* infection was reported in Kentucky in 1965 and continued to be prevalent among rural school children in Kentucky up to 1982. *Ascaris lumbricoides* was prevalent in Cherokee Native American school children and it was also reported among school children in Kentucky in early 1980s. *Strongyloides stercoralis* was also reported among school children in endemic area of the United States in 1982 (Starr and Montgomery, 2011). There is a wide distribution *Taenia solium* infection in Latin America (Hotez, 2014). Cryptosporidiosis and giardiasis are the common intestinal protozoan parasites reported in United States although they are associated with poverty or neglect (Hotez, 2014).

*Ascaris lumbricoides* and *Trichuris trichiura* are two prevalent intestinal helminth infections among adults and children living in Southeastern European nations of Albania (Spinelli *et al*., 2006) and Armenia (Avetisian, 2004) and among children in Poland (Malafiej and Spiewak, 2001; Biadun *et al*., 2001; Bitkowska, 2004). *Enterobius vermicularis* is prevalent in Turkey, Estern Europe and Italy (Avetisian, 2004; Malafiej and Spiewak, 2001; Biadun *et al*., 2001; Bitkowska, 2004; Remm and Remm, 2008; Muge *et al*., 2008; Crotti and D’Annibale, 2006). *Strongyloides stercoralis* has been reported in southwestern France and Spain (Magnaval *et al*., 2000; Roman Sanchez *et al*., 2001; Roman Sanchez *et al*., 2003). *Taenia saginata* is common in Poland (Waloch, 2005) Eastern Europe (Steele, 2000) and Western Europe (Craig and Ito, 2007). *Taenia solium* has been eliminated in Poland, however, its overall prevalence in Europe is unknown (Pawlowski, 2008). *Hymenolepis nana* infection is prevalent in Albania (Spinelli *et al*., 2006) and probably it also occurs in Eastern Europe. *Giardia lamblia*
is prevalent in Eastern Europe (Spinelli et al., 2006; Biadun et al., 2001; Bitkowska, 2004) and Turkey (Aksoy et al., 2007). *Entamoeba histolytica* is prevalent in Turkey (Koltas et al., 2007).

A study by Sehgal et al. (2010) in India shows that pregnant women were infected with intestinal parasites. A study among rural women of Terai belt of Bihar India reported that out of 404 stool samples 73% had helminths infection and 15% had protozoan infections. Helminths reported in India included *Ascaris lumbricoides* (46.25%), *Enterobius vermicularis* (15%), *Ancylostoma duodenale* (8.25%) and *Hymenolepis nana* (2.75%) (Pandey et al., 2013). Another study in India has indentified six states with more than 20% prevalence and another six states with more than 50% prevalence of soil transmitted helminths. More children are affected more than women by these infections in these states (Salam and Azam, 2017).

In China the prevalence of soil transmitted helminths are been decreasing over the years. In 1992 the prevalence of ascariasis, trichuriasis and hookworm was 47.0 %, 18.8% and 17.2 % respectively. In 2004, prevalence rates were 12.7 %, 4.6 % and 6.1 % respectively preventing approximately 407 million infections (Yu et al., 1994, Wang et al., 2016). In 2010, further reduction of ascariasis (6.8%), trichuriasis (1.8%) and hookworm (3.7%) was recorded (Lai et al., 2013). Nevertheless, in 2010, some provinces still had high prevalence of soil transmitted infection such as 30.6% in Sichuan, 34.6% in Guizhou, and 40.8% in Hainan (Lai et al., 2103). Soil transmitted infections continue to be major health problem in China for both adults and children living in poor rural areas (Wang et al., 2016).

In Nigeria the prevalent intestinal protozoan were *Giardia lamblia*, *Entamoeba coli*, and *Entamoeba histolytica* (Mohammed et al., 2015). Entamoeba histolytica was also prevalent in Gwagwalada area in Abuja (Gimba and Dawam, 2015). Intestinal helminths reported in
Nigeria include Ancyclostoma duodenale and *Necator americanus, Ascaris lumbricoides* among school children and adults in communities in North-Western Nigeria (Mohammed *et al.*, 2015). *Ascaris lumbricoides* and *Strongyloides stercoralis* were also reported among children in Gwagwalada Area in Abuja Nigeria (Gimba and Dawam, 2015. In Accra Ghana prevalent intestinal helminths among food vendors include *Ascaris lumbricoides, Strongyloides stercoralis, Enterobius vermicularis* and *Ancyclostoma duodenale* (Ayeh-Kumi *et al.*, 2009). Intestinal protozoan parasites reported among food vendors in Accra Ghana include *Giardia lamblia, Cryptosporidium parvum* and *Entamoeba histolytica* (Ayeh-Kumi *et al.*, 2009)

In South Africa the prevalent intestinal helminths were *Ascaris lumbricoides* in Port Shepstone, Empangeni and Jozini; *Trichuris trichiura* in Jozini, Port Shepstone and Durban regions; *Ancyclostoma duodenale* and *Necator americanus* in Jozini region (Kwitshana *et al.*, 2008). Intestinal protozoan parasites prevalent in South Africa were *Endolimax nana, Entamoeba coli*, and *Cryptosporidium* (Kwishana *et al.*, 2008). *Entamoeba histolytica, Cryptosporidium spp* and *Giardia lamblia* were prevalent among children and adults in Vhembe South Africa (Samie *et al.*, 2009)

In 2001, the soil transmitted helminths reported among school children in Uganda were *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Ancyclostoma duodenale* and *Necator americanus* (Kambatereine *et al.*, 2001). Other reported helminths were *Enterobius vermicularis, Hymenolepis nana* and *Taenia spp* (Kambatereine *et al.*, 2001). In 2016, the prevalent soil transmitted helminths in Uganda were *Ancyclostoma duodenale, Necator americanus, Trichuris trichiura, and Ascaris lumbricoides* (Fuhrimann *et al.*, 2016). Intestinal protozoan prevalent in Uganda was *Entamoeba histolytica* (Fuhrimann *et al.*, 2016).
Intestinal helminths reported in Tanzania were *Ancylostoma duodenale*, *Necator americanus*, *Trichuris trichiura*, *Enterobius vermicularis* and *Taenia* spp (Mazigo et al., 2010). Intestinal protozoan parasites prevalent in Tanzania were *Giardia lamblia* and *Entamoeba histolytica* (Mazigo et al., 2010). *Entamoeba histolytica*, *Giardia lamblia*, *Blastocyst hominis*, *Entamoeba coli*, *Entamoeba hartmanni*, *Iodamoeba butschlii* and *Endolimax nana* were reported among school children on Pemba Island Tanzania (Speich et al., 2010).

More than 24 million women get pregnant every year in sub-Saharan Africa and due to behavioural and immune changes, majority of these women are vulnerable to parasitic infections with poor pregnancy outcomes (Beck, 2001; WHO, 2002; van Eijk 2009). About 38 million women between 15 to 49 years old had *Necator americanus* and *Ancylostoma duodenale* in 2005 and around 7 million of them were pregnant and therefore in danger of being anaemic (Brooker et al., 2008).

### 2.1.2 Distribution in Kenya

In western Kenya, 76.2% of pregnant women who ate different foods were infected with at least one of the soil transmitted helminths (van Eijk et al., 2009). Ascariasis (52.3%) was most prevalent, followed by hookworm (39.5%) and Trichuriasis (29.0%). In Kwale District, nearly 35 % of pregnant women attending antenatal clinic were infected with intestinal parasites (Hopkins, 2013). In Nairobi intestinal helminth *Ascaris lumbricoides* and intestinal protozoan parasites *Giardia lamblia* and *Entamoeba histolytica* were reported among certified food vendors (Kamau et al., 2012). Intestinal helminths *Ascaris lumbricoides* and *Hymenolepis nana* and protozoans *Cryptosporidium* spp., *Giardia lamblia*, and *Entamoeba histolytica* were reported among children with diarrhoea disease in inpatient and outpatient settings in low income settlement of Nairobi (Mbae et al., 2013). In central Kenya the
prevalence of hookworm disease was 5.1% (MoPHS, 2011) and the prevalence of helminths infection among primary school children was 42% in Thika (Ngonjo et al., 2012). There is limited data on prevalence of intestinal parasites infection among pregnant women in Nyeri County.

2.1.3 Transmission of intestinal parasites

Nematodes such as *Ascaris lumbricoides*, and *Trichuris trichiura* are mainly transmitted through faecal-oral route by consumption of infective eggs from human faecal matter that pollute the food, drinking water, hands and living environment. Intestinal protozoan parasites such as *Entamoeba histolytica*, *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts are also transmitted through faecal-oral route by ingesting contaminated water or food (Chan et al., 1994; Murray et al., 2005; Basuni et al., 2012; Bogitsh et al., 2013). Both *Necator americanus* and *Ancyclostoma duodenale* gain entry in the susceptible host by infective larvae penetrating the skin of bare hands or feet that have come into contact with polluted soil. *Strongyloides stercoralis* is also transmitted through the larvae penetrating the skin of the feet or bare hands (Murray et al., 2005; Woodburn et al., 2009; Getachew et al., 2013).

Cestodes such as *Taenia saginata* and *Taenia solium* are spread when humans consume undercooked beef (*Taenia saginata*) or pork (*Taenia solium*) infected with cysticercii, the larval stage. Humans get accidentally infected by *Hymenolepis nana* by ingesting the ova excreted in the stool of the rodents such as mice and rat (Murray et al., 2005; Balcioglu et al., 2007; Mahsol et al., 2008; Woodburn et al., 2009; Akinbo et al., 2010; Getachew et al., 2013).
2.1.4 Risk factors to intestinal parasites infection

Individual hygiene practices such as washing hands after visiting toilet, before serving food; before eating and after changing baby’s napkins. Food preparation practices such as cooking and covering of food during and after cooking. Other practices such as drinking untreated water, walking barefoot, lack of using latrine for defecation predispose human to intestinal parasite infection. Socio-demographic and socio-economic factors such as age, low education, low economic standards are also associated with intestinal helminth and protozoan parasites infections (Balcioglu et al., 2007; Mahsol et al., 2008; Woodburn et al., 2009; Akinbo et al., 2010; Getachew et al., 2013).

2.1.5 Life cycles of intestinal helminths

2.1.5.1 Direct Life Cycles

Direct lifecycles occurs in many nematodes of public health importance. Direct life cycle means that there is no intermediate host involved. In these life cycles the parasites live and grow only in a mammalian or avian definitive host, in which they undergo sexual reproduction. The parasite is transmitted to the host by free living stages in the environment. Examples of nematodes that exhibit direct life cycle are *Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Necator americanus* (Murray et al., 2005; Polley and Thompson, 2009).

2.1.5.2 Indirect life cycle

Indirect life cycles are found in other nematodes and in all cestodes and trematodes. In these life cycles; a definitive host is involved and environment and one or more intermediate hosts in which immature parasites undergo important growth and may reproduce asexually but not sexually. *Strongyloides stercoralis* also exhibit indirect life cycle where the larvae in the soil
develops into free living adults that produces eggs and larvae that eventually become skin penetrating. Examples of cestodes that exhibit indirect life cycle are *Taenia solium, Taenia saginata,* and *Hymenolepis nana* (Murray et al., 2005; Polley and Thompson, 2009).

2.1.6 Life cycles of intestinal protozoan parasites

Intestinal protozoan parasites demonstrate similar life cycles that consists of a cyst stage and a trophozoite. For example *Entamoeba histolytica* cyst is infectious and trophozoite is non-infectious. Therefore, once a susceptible host ingests food or water contaminated with cysts it becomes infected. The cysts excyst into trophozoites which shows active metabolism and motility. After taking nutrients the parasite undergoes asexual replication during the trophic phase. Instead of undergoing replication some trophozoites undergo encystation and become cysts and are excreted with the faeces. The cyst has a resistant wall that protects the parasite from dehydration while in the external environment and the organism goes through a fairly inactive period waiting to be ingested by the next susceptible host. *Entamoeba polecki* cyst is also infectious and trophozoite non-infectious. Other amoeba that can parasitize human intestines are *Entamoeba coli, Entamoeba hartmanni, Entamoeba nana, Iodamoeba butschlii* (Stanley, 2003, Murray et al., 2005).

2.1.7 Clinical manifestation of intestinal parasites infection

Intestinal protozoan parasites such *Giardia lamblia, Cryptosporidium spp., Cyclospora spp.,* and *Entamoeba histolytica* cause diseases known as giardiasis, cryptosporidiosis, cyclosporiasis and amoebiasis respectively which are associated with diarrhoea in man (Davis et al., 2002; Haque, 2007). *Giardia lamblia* is also associated with severe malabsorption syndrome (Murray et al., 2005). *Balantidium coli* is associated with intestinal haemorrhage and ulceration. *B. coli* has also been associated with fatal myocarditis in Russia (Bogtish et al., 2013)
Nematode such as *Trichuris trichiura* is associated with diarrhoea in man (Murray *et al*., 2005; Hotez, 2009; Pullan *et al*., 2014). Heavy intensity of *Trichuris trichiura* in presence of other intestinal helminths has been linked to anaemia (Murray *et al*., 2005; Ezeamama *et al*., 2008). *Trichuris trichiura* is also associated with rectal prolapse in children, wastage and weight loss in adults (Murray *et al*., 2005; Hotez, 2009). The link between *Ascaris lumbricoides* and anaemia is not clear (Murray *et al*., 2005; Ezeamama *et al*., 2008). *Ascaris lumbricoides* is associated with intestinal obstruction (Murray *et al*., 2005; Hotez, 2009). *Necator americanus* and *Ancyclostoma duodenale* cause anaemia through sucking blood in the intestines (Crompton and Whitehead, 1993; Olsen *et al*., 1998; Dreyfuss *et al*., 2000; Hall *et al*., 2001; Ezeamama *et al*., 2005). Chronic infection of *Necator americanus* and *Ancyclostoma duodenale* also cause malabsorption of nutrients due to intestinal bleeding, wastage and weight loss in human (Murray *et al*., 2005; WHO 2006; Varkey *et al*., 2007; Amuta *et al*., 2009; Hotez, 2009). *Strongyloides stercoralis* causes malabsorption (Murray *et al*.; 2005)

Cestodes such as *Taenia solium* cause irritation of intestinal mucosa, peritonitis and human cysticercosis. *Taenia saginata* causes abdominal pain, loss of appetite and weight loss. In high numbers *Hymenolepis nana* destroys the intestinal mucosa causing enteritis (Bogtish *et al*., 2013). In children with moderate *H. nana* burden, there is loss of appetite, diarrhoea, abdominal pain and dizziness. *Diphyllobothrium latum* infection causes abdominal pain, weight loss, weakness and nervous disorders. *D. latum* also competes with the host for ingested vitamin B₁₂ causing megaloblastic anaemia. *Hymenolepis diminuta* cause abdominal pain, diarrhoea, insomnia and convulsions (Bogtish *et al*., 2013)

Trematodes such as *Fasciolopsis buski* cause intestinal mucosa inflammation, ulceration and abscesses at the sites of attachment. Intestinal pain, diarrhoea and nausea are common in the
morning hours. Severe infections can lead to intestinal edema and large number of worms can cause intestinal obstruction. Reactions to the worms’ metabolites can cause leucocytosis, eosinophilia and anaemia (Bogtish et al., 2013). *Echinostoma trivolvis* infection in human causes diarrhoea. Severe infection may cause ulceration of the intestinal mucosa and children sometimes experience diarrhoea, abdominal pain, edema and anaemia (Bogtish et al., 2013).

**2.1.8 Diagnosis of intestinal parasites**

Macroscopic examination involves examination of faecal specimen for consistency and for the presence of mucus, blood, worms, and proglottids (Murray et al., 2005). Microscopic examination involves: (i) direct wet mount method; fresh stools are examined under microscope using saline or iodine wet-mount technique to identify motile trophozoites or larvae such as *Strongyloides* species. The saline and iodine wet mounts are used to identify protozoan cysts and helminth eggs. (ii) Concentration method, faecal specimens are placed in 10% formalin to preserve parasite morphology and concentrated using a procedure such as formalin – ethyl acetate or formol ether sedimentation or zinc sulfate floatation. These methods separate helminth eggs and protozoan cysts from the bulk of faecal material and therefore make it easy to identify small number of organisms commonly missed by the use of direct smear alone (WHO, 1991; Murray et al., 2005).

(iii) Permanently stained slide; to detect and correctly identify intestinal protozoan parasites. It is important to examine permanently stained smear. The slides offer a permanent record of the protozoan organisms that are detected. The cytologic details exposed by one of the permanent staining method are important for correct identification and most identification are considered uncertain until established by the permanently stained slide. The commonly used permanent stains are iron hematoxylin, phosphotungstic acid-hematoxylin and trichome.
Slides are prepared by making smears of fresh faecal material and placing them in Schaudinn’s fixative solution (Murray et al., 2005).

2.1.9 Treatment of intestinal parasites infection

The WHO recommended antihelminthic drugs for use in prevention and therapy of *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura* are albendazole and mebendazole. For *Strongyloides stercoralis* recommended drugs albendazole and ivermectin while for *Enterobius vermicularis* they are albendazole, mebendazole and pyrantel have been recommended (WHO, 2004). Pregnant women should get treatment for intestinal helminths at the end of first trimester (WHO, 2002). Metronidazole has been recommended for treatment of intestinal protozoans including for amoebiasis. The drug crosses the placenta, a fact that restricts its use in pregnant women (WHO, 1981).

Pregnant women in Sierra Leon were treated with albendazole with no pregnancy adverse outcome reported. Pregnant women in Cote d’Ivoire were treated for hookworm with pyrantel pamoate (Welfens-Ekra et al., 1990). Pregnant women, in India were treated using mebendazole (Abel et al., 2000). Pregnant women in Western Kenya did not report use of anthelmintic medication although some of them were infected with geohelminth (van Eijk et al., 2009). Pregnant women infected with intestinal parasites in Kwale district in Kenya reported albendazole was the treatment of choice (Hopkins, 2013).

2.1.10 Prevention and control

2.1.10.1 Use of drugs

Soil-transmitted helminth infection is controlled by controlling morbidity through periodic treatment of people at risk living in endemic areas. The people at risk include preschool children, school age children, women of child bearing age including pregnant women in
second and third trimesters two and three and breastfeeding women. The adults in specific high risk occupations such as tea pickers and miners are also included. It is recommended that people living in endemic areas be given medicinal treatment for deworming regardless of previous contact(s). People living in the community where prevalence of soil-transmitted helminth is above 20% should be treated once in a year while those in the community with above 50% should be treated twice in a year. The recommended medicines include mebendazole (500mg) and albendazole (400mg) that are inexpensive, effective and easy to administer by people who are not medical professionals such as teachers (WHO, 2016).

2.1.10.2 Early diagnosis

Conducting surveys in schools provides an opportunity for early diagnosis and good entry for deworming activities. They offer easy opportunity to provide health and hygiene education components for instance promotion of improved sanitation and hand washing practices in schools (Ngonjo et al., 2012; WHO, 2016). Surveys in schools have shown that there is high prevalence of soil-transmitted helminths and intestinal protozoan parasites among children in both urban and rural areas. The school-going children are among people who require mass treatment for deworming and eradication of intestinal protozoan parasites infection (Ngonjo et al., 2012).

2.1.10.3 Public Health Education

Public health and personal hygiene education such as (i) building and using sanitary latrine for safe disposal of human faeces (ii) washing hands with soap before eating or feeding children and after defecation; and (iii) washing and cooking vegetables thoroughly reduces transmission and reinfection with soil transmitted helminths (WHO, 2016). The main protection against infection by *Necator americanus* and *Ancylostoma duodenale* is wearing appropriate foot-ware for protecting the skin of the feet, ankle and legs from coming into
contact with infective larvae (UNICEF, 2002; Stanley, 2003). Intestinal protozoan parasites infection are eradicated through breaking their transmission routes and life cycles especially by: (i) improvement of environmental sanitation (ii) provision and use of safe and adequate water supply (UNICEF, 2002; Stanley, 2003).

2.2 HIV Infection

2.2.1 Types of HIV

HIV is a retrovirus that weakens the immune system making the body vulnerable to opportunistic infections. HIV is a ribo nucleic acid (RNA) virus (Montagnier, 1985) and is the etiologic agent of acquired immunodeficiency syndrome (AIDS). It is now known that HIV type 1 which is most abundant originated from chimpanzees (Klatt, 2016). The HIV type 1 subtypes include group M, N, O, and P, and every group seems to have spread differently (Sharp and Hahn, 2011). HIV type 1 Group M has infected millions of people globally hence it is the pandemic form. Molecular epidemiologic information shows that from 1910-1930 SIVcpz spread widely across species to humans. This could have happened through skin coming into contact with body fluids or blood of infected primates during hunting. HIV type 1 subtypes and HIV type 2 are reported to have originated in Africa (Sharp and Hahn, 2011; Klatt, 2016). An individual is considered a HIV infection case if laboratory diagnosis confirms presence of HIV regardless of the clinical stage of the disease (WHO, 2007).

2.2.2 HIV Distribution

2.2.2.1 Global distribution

Globally, about 36.7 million people were living with HIV in 2015 with 5.1 million in Asia and Pacific, 2 million in Latin America and the Caribbean, 2.4 million in Western and Central Europe and North America and 1.5 million in Eastern Europe (UNAIDS,2016). In 2015,
approximately 2.1 million people were newly infected with HIV globally and 1.1 million deaths were from AIDS related illness (UNAIDS, 2016). By end of 2015, 17 million HIV positive people were receiving anti-retroviral therapy (UNAIDS, 2016). In 2008, men who have sex with men (MSM) had HIV prevalence of 19% in North America, and 44% of them did not know that they were infected (Lansky, 2010). A report by Patterson (2011) indicated that everyday 3000 women were infected with HIV worldwide.

2.2.2.2 Sub-Saharan Africa

Among the developing countries, Sub-Saharan Africa is hardest hit by HIV disease with 25.5 million people being estimated to be living with HIV in 2015 (UNAIDS, 2016). In Eastern and Southern Africa, 19 million people were living with HIV by 2015, and half of these people were women. Around 960,000 people were newly infected with HIV and 56,000 were children, 10.3 million were receiving antiretroviral therapy and 470,000 died of AIDS related illness in Eastern and Southern Africa (UNAIDS, 2016). In Western and Central Africa, there were 6.5 million HIV positive people by 2015 and 410,000 of them were newly infected. Around 1.8 million HIV positive people were receiving antiretroviral therapy while 330,000 people died of AIDS related sickness in these regions (UNAIDS, 2016). Women are the most affected by HIV in Sub-Saharan Africa. Ninety two per cent of HIV infected pregnant women live here (UNAIDS, 2012). Recent reports have shown that pregnant women are more likely to be infected with HIV compared to those breast feeding or not lactating (Klatt, 2016).

2.2.2.3 Kenya

In 2013, approximately 1.6 million Kenyans were living with HIV and women were the most affected at 57% and men 43% (NACC and NASCOP, 2014). The prevalence of HIV among women in Homa Bay County was higher (27.4%) compared to that of men (23.7%).
Approximately 9,674 pregnant women were living with HIV in this county (MOH, 2014). In 2013, Kakamega County had around 2,754 HIV pregnant women living with HIV. The prevalence of HIV among women in Nyeri County was higher (6.3%) compared to that of men (2.3%) and approximately 982 pregnant women were living with HIV in Nyeri County by 2013 (MOH, 2014). Around 66% of pregnant women living with HIV in Nyeri County avoid delivering in a health facility and only 41% of pregnant women seek antenatal care four times as recommended (MOH, 2014).

2.2.3 Transmission of HIV

2.2.3.1 Sexual transmission

The mode of transmission of HIV is very crucial. Specific groups are at higher risk of infection considering that the main mode of HIV transmission is through sexual contact. Heterosexual transmission poses greatest risk in developing countries compared to developed countries where homosexual transmission is the highest. Men who have sex with men are at highest risk of infection in high income countries of Western Europe and North America compared to Africa, Asia and parts of the Caribbean where this practice is less common. However, homosexual transmission is increasing over time in developing countries as heterosexual transmission is increasing in developed countries (Hader 2001; Morison 2001, Klatt, 2016). Infection with HIV may occur between men who have sex with men, but rarely occurs between women who have sex with women unless they are bisexual (Bevier, 1995; Klatt, 2016). HIV is less likely to be transmitted through oral sex due to factors found in saliva that inhibit HIV (Klatt, 2016). However, oral injury or ulcers can increase the likelihood of HIV infection (Campo et al., 2006). In Kenya, heterosexual contact contributed to 64.4% of new HIV infections making it the main mode of transmission. Commercial sex
workers and their clients contributed 14.1% while sex between men who have sex with men contributed 15.2% of new HIV infections (NACC, 2009).

2.2.3.2 Mother to child transmission (MTCT)

Pregnant woman infected with HIV can transfer the virus to her baby through placenta, during birth canal delivery, and during breast feeding (Branson, 2006; Cavarelli et al., 2011). About 5-10% of all HIV infections worldwide occur in children and mother to child transmission accounts for 90% of these infections (MOH, 2005). Generally, the rates of HIV transmission are about 15-40% worldwide. In Kenya, per year, 79,000 pregnant women need prevention of mother to child transmission of HIV. This shows that high number of infants is at risk of HIV infection if no control measures are implemented (NACC, 2014).

2.2.3.3 Other modes of transmission

Infection with HIV more likely happens when an injured skin or mucous membrane comes into contact with infected blood, genital fluids and breast milk (Kreiss, 1997; Quinn, 2000; Sierra, 2005). Nevertheless, it is less likely for HIV to be transmitted through sweat, saliva, urine and tears due to the low viral load in these fluids (Shepard et al., 2000). Although it is a very rare occurrence, coming into contact with infected cerebrospinal fluid can spread HIV (Chaillon et al., 2014). Ear wax (cerumen) does not carry identifiable HIV (Hanege et al., 2015). In Kenya, 2.5% of new HIV infections were health facility related and 2.3% were due to injection drug use (IDU) (NACC, 2009). Health facility-related transmission occurred when a health care provider was accidentally injured and got into contact with body fluids or blood of a HIV positive patient (NACC, 2009).
2.2.4 Risk factors to HIV transmission

The risk factors to HIV infection include having been diagnosed with sexually transmitted vaginal infection, alcohol consumption, and being unmarried. Other risk factors include: low education level, multiple marriages, multiple sex partners, exposure to a partner who is HIV positive (Woodburn et al., 2009). Factors that contribute to HIV transmission from mother to child are (i) maternal factors such as micronutrient deficiencies, breast health and chorioamnionitis or vaginal infections, low maternal CD4 count and disease stage that influences transmission risk irrespective of viral load and maternal viral load which is linearly correlated with risk of transmission during pregnancy and breast feeding, (ii) obstetric factors such as duration of membrane rupture and mode of delivery,(iii) infant factors such as delivery before 34 weeks of gestation and breast feeding that contributes more than 30% of mother to child transmission and reduces the efficacy of antiretroviral prophylaxis provided for prevention of mother to child transmission (MOH,2005)

2.2.5 Life Cycle of HIV

HIV successfully enters into the host cells when HIV envelope glycoprotein GP 120 binds to the host receptor CD4 molecules. Through mediation by gp41, the viral binding to the host cell causes fusion of the viral and host cell membrane allowing entry of virus core into the host cell cytoplasm. The core protein is dissolved by host enzymes and releases viral RNA and enzymes. The enzyme reverse transcriptase converts the viral RNA into a DNA molecule and the DNA enters into the host cell nucleus. The process of integrating the viral DNA into the host’s cell DNA is catalysed by integrase enzyme. The integrated viral DNA makes the host cell to produce more viruses. The viral proteins are produced as single multi-protein molecules and are cleaved by protease enzyme. Together with RNA the viral proteins assemble at the cell membrane of CD4+ cells. Viral particles are formed and bud off from the
cell and enter into the blood stream. Because of HIV infection and replication, the CD4 cells are destroyed causing immune suppression of the host (MOH, 2005)

2.2.6 Clinical manifestations of HIV infection

World Health Organization (WHO) has outlined the stages that the HIV infected person goes through as follows: Primary HIV infection which is characterized with unrecognized severe retroviral syndrome and severe febrile sickness for two to four weeks after exposure frequently with lymphadenopathy and dermatological signs (MOH, 2005; WHO, 2007). Stage I is asymptomatic and there is persistent generalized lymphadenopathy. Stage II is characterized with moderate unexplained loss of weight (less than 10% of measured or presumed body weight); recurring upper respiratory tract infections such as bronchitis, pharyngitis, sinusitis and otitis media; herpes zoster (current or past episodes during the last two years); angular cheilitis; frequent oral ulcerations (two or more episodes in six months); seborrhoeic dermatitis; fungal nail infections of fingers; and papular pruritic eruptions (MOH, 2005; WHO, 2007)

Stage III in HIV infection is characterized with conditions where a presumptive diagnosis can be made using simple investigation or clinical manifestations such as severe necrotizing ulcerative stomatitis, periodontitis or gingivitis; acute presumed bacterial infections such as empyema, pneumonia, meningitis, bacterimia, pyomyositis and bone or joint infections; diagnosed pulmonary tuberculosis in the last two years; oral hairy leucoplakia; oral candidiasis; unexplained recurrent fever for one month; unexplained chronic diarrhoea for one month; and acute loss of more than 10% of measured or presumed body weight. Conditions that require confirmation through diagnostic testing are unexplained thrombocytopenia (less than 30,000/mm$^3$), neutropenia (less than 1,000/mm$^3$), and anaemia (less than 8 gm / dl) (Levin et al., 2001; MOH, 2005; WHO, 2007)
Stage IV in HIV infection is characterized with conditions where a presumptive diagnosis can be made using simple investigations or clinical manifestations such as isosporiasis; cryptosporidiosis with diarrhoea for more than one month; extra pulmonary tuberculosis, HIV encephalopathy; Kaposi’s sarcoma; prolonged genital or orolabial or anorectal herpes simplex infection for more than one month; brain toxoplasmosis; cryptococcal meningitis; persistent acute bacterial pneumonia; pneumocystis carinii pneumonia; and HIV wasting syndrome. Conditions that require confirmatory diagnostic testing are any disseminated endemic mycosis such as histoplasmosis; progressive multifocal leukoencephalopathy; disseminated non tuberculous mycobacterial infection; cryptococcosis (extrapulmonary); visceral leishmaniasis; invasive cervical carcinoma; lymphoma cerebral; non-typhoid salmonella septicaemia; cytomegalovirus retinitis or disease of organs other than liver, spleen or lymph nodes; and candidiasis of the oesophagus (MOH,2005; WHO, 2007). Haemolysis can also be due to various medications for HIV (Soriano et al., 2002). For instance antiretroviral drug Zidovudine (AZT) causes suppression of the bone marrow thus increasing the likelihood of becoming anaemic (Areechokchai et al., 2009).

2.2.7 Diagnosis of HIV infection

2.2.7.1 Counselling before HIV testing

HIV testing and counselling can be started by the client, patient, or health care provider in any setting. In client-initiated, the individual, couple or group seeks out HIV testing and counselling in places where these services are offered (NASCOP, 2008). In provider initiated HIV testing and counselling, the HIV counselling and testing provider offers HIV test to a client or patient not considering the reason why he or she attended the facility. This is different from directed testing and counselling (DTC) which targets patients presenting with HIV associated signs and symptoms (NASCOP, 2008). During the pre-test session the patient
is provided with basic information on HIV. The individual, couple or group should be given time for asking questions and receiving personalized information and be allowed to give consent for getting tested. As the minimum, the information provided during pre-test should include: the importance of knowing one’s HIV status, importance of a couple knowing their status together, full explanation of the HIV testing procedure and the necessity for consenting to HIV test, risk assessment, referral to care, treatment and support and the need of disclosing to family members and partners (NASCOP, 2008). After recommendation by health care provider and the consent by the patient, he or she should be tested and get results regardless of the system used (NASCOP, 2008).

2.2.7.2 HIV testing

HIV testing can be done using serological methods, that is, antibody/antigen based tests such as (i) enzyme linked immunosorbent assay (ELISA) which has over 40 different kits available, applies a relatively simple methodology, has high specificity of over 99.7% and high sensitivity of 99.3-99.7% (the assay is suitable for large sample population and is able to detect both HIV 1 and HIV 2), (ii) Rapid tests which can be done easily without complex instruments in less than 20 minutes. A positive result is shown by the formation of a coloured line or dot. Determine unigold and oraquick are examples of simple or rapid assays. All individuals who test positive need to undergo a confirmatory test using western blot method (MOH, 2005; NASCOP, 2008).

2.2.7.3 Counselling after HIV test

Based on the results the health care provider must provide counselling to the patient or client. Information on how to reduce risk and emotional support should be given considering one’s risk factors and be referred to correct follow up services (NASCOP, 2008). For HIV positive adults they should be provided with psychosocial support and HIV literacy, clinical
assessment that includes WHO staging, treatment of common opportunistic infections, and provision of antiretroviral treatment. Patients are also counselled on importance of adherence to care through entering into and going on in a program, attending appointments and taking tests as planned, taking medications as recommended, changing lifestyle as required and avoiding risk behaviour (MOH, 2005; NASCOP, 2008).

2.2.8 Management of HIV infection

2.2.8.1 Antiretroviral treatment

From 1984, various drugs for treatment of HIV patients have been developed. These include reverse transcriptase inhibitors, nucleoside analogue drugs and protease inhibitors. The purpose of antiretroviral treatment is to reduce HIV viral load and suppression of the immunity which can lead to AIDS. All the different antiretroviral drugs developed so far cannot cure HIV patients (Warnke et al., 2007). Zidovudine which is a reverse transcriptase inhibitor prolongs the life of HIV patients and improves the immune system by preventing the increase of HIV viral load and increasing CD4 cells count. This treatment prevents opportunistic infections. (Hirsch and D’Aquila, 1993). Nucleoside analogue drugs include didanosine (ddl), zalcitaine (ddc), stavudine (d4T), lamivudine (3TC), abacavir and tenofovir disoproxil fumarate (TDF). The name of these drugs is nucleosides reverse transcriptase inhibitors.

Other important drugs are protease inhibitors that also prevent the increase of HIV viral load (Wensing et al., 2010). Protease inhibitors act by blocking metabolism mediated by cytochrome P450. Protease inhibitors are well tolerated and are effective in preventing viral replication and improving CD4 cells count. However, protease inhibitors are made less effective by HIV resistance (Flexner, 1998; Wensing et al., 2010). It is now recommended
that all HIV patients be treated with antiretroviral drugs without considering CD4 cell count. Antiretroviral treatment reduces the chances of transmitting HIV by 96% and almost 100% when viral load is reduced to levels that cannot be detected (Hoffmann et al., 2014).

According to WHO (2013) guidelines on antiretroviral therapy HIV positive pregnant women should be treated using a combination: Tenofovir Disoproxil Fumerate (TDF) + Lamivudine (3TC) + Efavirenz (EFV) or Nevirapine (NVP). This is important because it is simple, can be used in all pregnant women and can be continued after delivery. The treatment can easily be harmonized with treatment for adult women who are not pregnant. The treatment ensures that immunosuppressed women who cannot get CD4 cells count test are treated promptly. The ART delays disease progression over the course of treatment and prevent HIV transmission to the children and sexual partners. The ARV combination has received community preference and acceptability (WHO, 2013).

**2.2.8.2 Nutritional management**

Nutrition is very important during the management of HIV infection. Nutrients such as carbohydrates can be obtained from whole grain cereals, roots and tubers. Proteins can be obtained from milk, meat, fish, eggs and legumes while lipids can be obtained from visible lipids and invisible lipids. Minerals can be obtained from milk, nuts, fish, meat, vegetables and table salt. Vitamins are obtained from vegetable and fresh fruits and fiber is obtained from whole grain cereals, seeds, legumes and fruits. Water can be obtained from all cooked food, beverages and water. HIV infected persons require 10- 15% more calories per day and 50 -100% more protein per day (MOH, 2005). To assess nutritional status of HIV patient, the patient should be weighed during every clinic appointment. The recommended nutritional management for HIV infected adults is applicable to pregnant women living with HIV. There is no need for more fat consumption as a result of HIV infection. However, people on anti-
retroviral therapy and experiencing persistent diarrhoea require special attention. Supplements such as B-complex vitamins, and vitamins E and C can boost immune status, improve pregnancy outcomes including healthier maternal prenatal weight gain. Several studies show that micronutrients such as vitamin A, iron and zinc, can produce adverse outcomes in HIV infected individuals. For prevention of anaemia during pregnancy and in lactating women, it is recommended daily 60mg of iron and 400μg folate supplementation for six months and twice daily supplement for treatment of severe anaemia (WHO, 2003).

2.2.9 HIV prevention and control

2.2.9.1 Testing and counselling

Testing and counselling is important for early diagnosis and it offers a good opportunity for the person who tests negative to make informed decision on ways to protect himself or herself from HIV infection. For the person who test positive he or she is able to make informed decision on how to protect others from HIV infection through disclosure and use of protection every time they have sex. HIV infected person is able to be enrolled in treatment which helps to control the infection from progressing to AIDS by reducing viral load. Low viral load also reduces the likelihood of transmitting the HIV (NASCOP, 2008 NACC, 2014). HIV positive persons are screened for tuberculosis and referred appropriately (NASCOP, 2008; NACC, 2014).

2.2.9.2 Prevention of mother to child transmission using ARV prophylaxis

When HIV positive pregnant woman takes antiretroviral therapy with optimal adherence it reduces the risk of HIV transmission from the mother to the child by reducing the viral load (MOH, 2005; NACC, 2014; WHO and UNAIDS, 2015). In Kenya, since 2009 on average 58,000 out of 79,000 pregnant women living with HIV have received ARV prophylaxis
annually. The coverage has reduced from 86% in 2010 to 70% in 2013 due to increased demand (NACC, 2014). Most of male partners are not involved in prevention of mother to child transmission of HIV. There is need to involve males in PMTCT and ensure all women who attend ANC are tested and those who test positive are treated with ARVs (NACC, 2014).

2.2.9.3 Nutrition

HIV positive patients are assessed to establish those who require extensive nutrition management and individualized nutrition care plans are established (MOH, 2005; NASCOP, 2008). In areas with limited alternatives for infant feeding, breast feeding is important for infant health. This is because breast milk provides optimal nutrition, protective immune factors, emotional and psychosocial benefits and allows birth spacing. Exclusive breast feeding for first six months by women living with HIV reduces the risk of mother to child transmission. In areas where replacement feeding is feasible, acceptable, affordable, safe and sustainable, it is recommended that women living with HIV avoid breast feeding at all from birth. Replacement feeding protects the infant from HIV infection, however, where it is not safe and sustainable it increases the likelihood of diarrheal diseases and malnutrition (MOH, 2005; NASCOP, 2008).

2.2.9.4 Public health education

HIV infected individuals are provided with education on how to protect others through disclosure and use of condoms whenever they have sex. Men and women are also provided with information on importance of family planning. They are also educated on prevention of malaria and treatment, palliative care and management of symptoms, importance of consuming safe food and water and maintaining safe personal hygiene and sanitation (NASCOP, 2008). HIV negative persons are provided with information on how to protect themselves against HIV infection through use of condoms every time they have sex, having
only one sexual partner, and health care providers are informed on importance of wearing personal protective equipment like gloves when attending HIV patient, taking post exposure prophylaxis and going for testing regularly (NASCOP, 2008; Klatt, 2016).

2.3 Haematological changes in women during pregnancy

2.3.1 Red blood cells changes during pregnancy

In pregnant woman plasma can increase from 25% to 80% within the 6th and 24th week of pregnancy (Lund and Donovan, 1967). Nevertheless, it has been found that quantity of red blood cells increases by about 30% when a pregnant woman gets folate and iron supplements (Taylor and Lind, 1979). Physiologic haemodilution can cause reduction of red blood cells (physiologic anaemia) in pregnant woman (Kelton and Cruickshank, 1988). Towards the end of gestation period, slow rate in plasma volume increase slightly raises haematocrit level. It is difficult to set normal haematological ranges for pregnant woman due to these physiological changes (Shen et al., 2010).

2.3.2 Haemoglobin changes during pregnancy

A pregnant woman is considered anaemic when haemoglobin level is less than 11.0g/dl during trimester one and three and less than 10.5 g/dl during trimester two (CDC, 1998). A pregnant woman can become anaemic due lack of adequate iron, folic acid, vitamin B12, long term bleeding, malignant conditions and weakened bone marrow (Reveiz, 2007; Johnston, 2010). During pregnancy, anaemia due to autoimmune haemolysis is likely to occur four times (Bryant and Larsen, 2009). Iron absorption in the gut varies from 0.8mg daily during trimester one to 7.5 mg daily in trimester two, hence, the mean is about 4mg daily in pregnancy (Pavord et al., 2012). Iron demand is increased in trimester two and trimester three because of foetus growth, but, iron absorbed from the gut is not adequate for the increased
requirement. Hence, maternal iron storage during pregnancy regulates the iron balance (Johnston, 2010).

2.3.3 Haematocrit changes during pregnancy

Even though physiological hemodilution related with pregnancy leads to the drop in haematocrit in trimester one and two, towards the end of pregnancy, plasma volume increases slowly, causing a small increase in Packed Cell Volume that may explain the small increase in haematocrit in trimester three (Shen et al., 2010; Akinbami et al., 2013). Normal level of haematocrit has been measured from 36 to 48 percent for women in child bearing age. Haematocrit decrease during pregnancy has been associated with anaemia while its large increase is associated with chronic obstructive pulmonary disease, myeloproliferative disorders and other hypoxic lung disorders (Khoigani et al., 2012).

2.3.4 Platelets changes during pregnancy

Platelets count in pregnant women is marginally lower than that in women who are not pregnant (Abbassi-Ghanavati et al., 2006). Physiological hemodilution during pregnancy partially contributes to reduced platelets count (Kelton and Cruickshank, 1988). Even though, van Buul et al. (1995) stated that there is increase in platelets during pregnancy; it is estimated that there is 10% reduction in platelets count at end of pregnancy compared to that before pregnancy (Boehlen et al., 2000; Jensen et al., 2011). Platelets count is likely to be low in women with twin pregnancy compared to singleton pregnancy, possibly as result of increased thrombin production. Generally, in pregnant women around 75% of those with changed platelets count is as a result of pregnancy related thrombocytopenia, 15% to 20% due to hypertension disease, 3% to 4% from immune processes and 1% to 2% as a result of occasional malignancy, diseases and reduction in thrombocytes (Burrows and Kelton, 1990).
2.3.4 White Blood Cells changes during pregnancy

Increase in white blood cells observed during pregnancy is as a result of the physiologic changes during pregnancy (Pitkin and White, 1979). Neutrophils are the main type of leucocytosis that is observed during gestation period. Lympocyte count reduces in gestation period through the first and second trimesters and increases during trimester three. Monocytes increase during trimester one and decrease as gestation progresses while there is no significant change in eosinophil and basophil counts (Edlestam et al., 2001; Chandra et al., 2012).

2.4 Haematological changes in HIV infection

Production of blood cells from pluripotent stem cells in the bone marrow changes in HIV infected individuals where all the three cell lines, that is, white blood cells, red blood cells and platelets are affected. As a result, HIV positive individuals are likely to suffer from lowered levels of red blood cells (anaemia), lowered levels of neutrophils (neutropenia) and lowered levels of platelets (thrombocytopenia) or any combination of the three situations. The cause of these defects is the HIV infection of the haematopoietic stem cells that changes their normal function. Abnormal functioning of haematopoietic stem cells cause inadequate production of haematopoietic growth factors hence reduced production of blood cells. Antiretroviral treatment for HIV infection, treatment of HIV related malignant conditions, opportunistic infections and their treatment can change haematopoiesis resulting to poor production of blood cells (Consolin et al., 2007; Mwinga et al., 2009).

2.5 Haematological changes in intestinal parasites infection

_Ancylostoma duodenale, Trichuris trichiura_ infections in human are associated with anaemia (Chaudhary and Chandra, 2012). Infection with intestinal helminths such as _Ancylostoma duodenale_ cause an increase in eosinophil count where as _Ascaris_
*lumbricoides* does not affect the eosinophil count in the peripheral blood of children (Wasilewska et al., 2011). *Entamoeba histolytica* and *Giardia intestinalis* infections have independently been associated with anaemia in human (Alzain and Sharma, 2006). Neutrophil level and activity is not affected by intestinal parasite infection (White et al., 1986). Infection with *Ancylostoma duodenale* and *Necator americanus* in human reduced the mean platelet volume and lowered platelets count (Wiwanitkit et al., 2003).

### 2.6 Co-infection with HIV and intestinal parasites

Some of intestinal protozoans that have been reported among HIV patients include: *Isospora belli, Cryptosporidium parvum, Entamoeba spp., Microsporida spp.,* and *Giardia lamblia.* Intestinal helminths that have been reported among HIV positive pregnant women include: *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Ancylostoma duodenale* and *Necator americanus* (Lucas, 1990; Gallagher et al., 2005; Ivan et al., 2012; Tian et al., 2012). HIV-positive women are more likely to be anaemic than HIV-negative women. Women infected with HIV also suffer high burden of moderate and severe anaemia. Co-infection with HIV and intestinal helminths complicates the immune system (Gallagher et al., 2005) considerably increasing the probability of becoming anaemic (Muhangi et al., 2007; Fuesin et al., 2013).

Hookworms are known to be heavy feeders on blood of the host causing anaemia (Brooker et al., 2008). Mature *Necator americanus* can suck about 0.05 mls of blood while mature *Ancylostoma duodenale* can suck about 0.25 mls of blood (MacLeod, 1988). Gut bleeding, poor absorption, and poor appetite (Cline et al., 1984) can worsen zinc, protein energy and iron deficits and anaemia in pregnant women. Studies have demonstrated that even infection with very few hookworms can cause poor increase in weight and foetal development in pregnant women (Rodriguez-Morales et al., 2006). Intestinal worms are associated with
micronutrients deficits hence increasing the risk of anaemia, retarded growth and development. Infection with intestinal worms increases the rate of HIV-replication and disease progression, through immune activation of cellular mechanisms rendering the CD4 T lymphocyte cells susceptible to HIV-infection and resulting increased viral load (Shapira-Nahor et al., 1998). Increased HIV replication is associated with rapid CD4 T lymphocyte cells death and disease progression to AIDS (Borkow and Bentwich, 2006).

Haematological modifications are the most revealed in HIV infected individuals (Massawe, 2002). Haematological parameters are crucial in monitoring health status of HIV infected population. Low haematocrit level and high proportion of microcytosis were observed among pregnant women infected with HIV (Bleyere et al., 2013). HIV-positive pregnant women with helminthic infection are more likely to transmit HIV to their offspring (Gallagher et al., 2005). Infection with Entamoeba histolytica in pregnant women can cause anaemia and poor foetal development (Weigel, 1996).

2.7 Gaps in knowledge from literature review

Many studies done locally and in other parts of the world have reported the prevalence of intestinal parasites among pregnant women (Phuanukoonnon et al., 2013; van Eijk et al., 2009). Prevalence of HIV among women in Nyeri County has also been reported (MoH, 2014). However, in Nyeri County there is limited data on: (i) prevalence of co-infection of HIV and intestinal parasites among pregnant women (ii) practices that would predispose pregnant women to intestinal parasites infection, (iii) factors associated with intestinal parasites infection among pregnant women, and (iv) effects of HIV and intestinal parasites co-infection on haematological parameters among pregnant women.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

This was a comparative cross sectional analytical study. Initially two groups of respondents were included in the study, that is, HIV positive and HIV negative respondents. The two groups resulted to four groups after intestinal parasites diagnosis. The design was appropriate because it allowed comparison of data collected at one point in time for four groups of respondents, that is, respondents infected with both HIV and intestinal parasites, infected with HIV and not intestinal parasites, infected with intestinal parasites and not HIV and not infected with either HIV or intestinal parasites.

3.2 Variables

3.2.1 Dependent variable

The dependent variable was haematological parameters, that is, normal haematological parameters or abnormal haematological parameters.

3.2.2 Intervening variable

The intervening variable was co-infection with HIV and intestinal parasites.

3.2.3 Independent variables

The dependent variables were age, marital status, level of education, occupation, income, access to latrine for defecation, treatment of drinking water, soil eating and HIV status.

3.3 Study area

The study was conducted in Nyeri County. Nyeri County has an estimated population of 723,392 and a surface area of 3,337 km². It lies within Longitudes 36° 57’ and east within the equator and Latitude 0° 38’ south. The county bounders, Kirinyaga County to east, Meru
County to the north east, Nyandarua County to the west, Laikipia County to the north, Murang’a County to the south (Appendix VI). Nyeri County has one level 5 hospital, four level 4 hospitals and twenty level 3 hospitals owned by ministry of health (Republic of Kenya, 2013; Nyeri County Information System, 2015).

3.4 Study population

Pregnant women attending antenatal clinic and Prevention of Mother-To-Child Transmission (PMTCT) units in selected health facilities in Nyeri County.

3.4.1 Inclusion criteria

i. Pregnant women who were resident in the study area for at least 12 months before the study.

ii. Pregnant women who consented to participate in the study.

iii. Both HIV positive and HIV negative pregnant women

iv. Pregnant women who were able to provide both blood and stool samples.

3.4.2 Exclusion criteria

i. Pregnant women who had resided in the study area for less than 12 months by the start of the study.

ii. Pregnant women who did not consent to participate in the study.

iii. Pregnant women who were not able to provide both stool and blood sample.

iv. Pregnant women who were in labour and those who presented with symptoms of lung infection including coughing.
3.5 Sampling techniques

Stratified random sampling technique was used to select health facilities. Levels of the health facilities represented various strata. At level 5, Nyeri level 5 hospital was selected because it is the only level 5 hospital in Nyeri County. At level 4 and level 3, health facilities were randomly selected. At level 4, Karatina, Mukurweini, and Mt Kenya level 4 hospitals were selected and at Level 3, Nyeri Town, Kiganjo, Narumoru and Mweiga level 3 hospitals were selected. Study participants were selected using systematic random sampling. According to health records, on average, the number of new pregnant women attending selected health facilities in a month was 450. The sample size required was 130 pregnant women, therefore, the sampling interval was $450/130 = 3.46$ approximately 3. For every pregnant woman infected with HIV, a pregnant woman not infected with HIV was recruited in the study.

3.6 Sample size determination

Formula by Rosner (2010) was used

$$n = \left( \frac{\sigma_1^2 + \sigma_2^2}{\Delta^2} \right) \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\mu_1 - \mu_2} \right)^2$$

Where

- $n$ = appropriate sample size
- $1-\beta$ = probability of finding a significant difference based on a two sided test
- $\alpha$ = level of significance
- $\mu_1$ = mean for population 1
- $\sigma_1^2$ = variance for population 1
- $\mu_2$ = mean for population 2
\( \sigma^2 = \) variance for population 2

\( \Delta = \mu_1 - \mu_2, \) the absolute value of true difference in means between the two groups.

Sample data \((X_1, S_1^2, X_2, S_2^2)\) were used as estimates of the population parameters \((\mu_1, \sigma_1^2, \mu_2, \sigma_2^2)\), then ensuring an 80% chance of finding a significant difference using a two-sided significance test with \(\alpha = 0.05\) required a sample of

\[
n = \frac{(S_1^2 + S_2^2) (Z_{1-\alpha/2} + Z_{1-\beta})}{\Delta^2 (X_1 - X_2)}
\]

where

\(S_1^2 = \) variance of sample 1

\(X_1 = \) mean of sample 1

\(S_2^2 = \) variance of sample 2

\(X_2 = \) mean of sample 2

therefore,

\[
n = (1.1^2 + 1.17^2) (1.96 + 0.84)^2 / (12.3 - 11.9)^2 = 126
\]

Minimum sample size = 126.

A sample size of 130 (65 HIV positive and 65 HIV negative) was used in the study.

The sample of HIV positive pregnant women was distributed proportionate to the number enrolled in selected health care facilities (Table 3.1).

The formula by Rosner (2010) is used when determining the minimum sample size required for comparing two groups that normally distributed and equal in size. Therefore, using means and variances of haemoglobin of HIV positive pregnant women co-infected with intestinal helminths and those not co-infected from a similar study conducted in Rwanda the sample size for this study was calculated.
Table 3.1: Distribution of HIV positive pregnant women by selected health facilities

<table>
<thead>
<tr>
<th>Health facility</th>
<th>Number of enrolled HIV positive pregnant women</th>
<th>Proportionate sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyeri Level 5</td>
<td>20</td>
<td>20/90*65 = 15</td>
</tr>
<tr>
<td>Karatina Level 4</td>
<td>22</td>
<td>22/90*65 = 16</td>
</tr>
<tr>
<td>Mukurweini Level 4</td>
<td>8</td>
<td>8/90*65 = 6</td>
</tr>
<tr>
<td>Mt Kenya Level 4</td>
<td>10</td>
<td>10/90*65 = 7</td>
</tr>
<tr>
<td>Nyeri Town Level 4</td>
<td>8</td>
<td>8/90*65 = 6</td>
</tr>
<tr>
<td>Mweiga Level 3</td>
<td>6</td>
<td>6/90*65 = 4</td>
</tr>
<tr>
<td>Kiganjo Level 3</td>
<td>6</td>
<td>6/90*65 = 4</td>
</tr>
<tr>
<td>Naromoru Level 3</td>
<td>10</td>
<td>10/90*65 = 7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>90</strong></td>
<td></td>
</tr>
</tbody>
</table>

3.7 Data collection techniques

3.7.1 Pre-testing of research tools

The questionnaires were pre-tested at Kiambu level 4 hospital to ensure they were comprehensive enough to collect data on socio demographic, socio economic and practices associated with intestinal parasites infection among pregnant women.

3.7.2 Exit Interviews

Exit interviews were conducted among pregnant women using interviewer administered questionnaires (Appendix VIII). Data was collected on socio demographic, socio-economic characteristics, and practices that would predispose respondents to intestinal parasites infections.
3.7.3 Laboratory Investigations

3.7.3.1 Blood specimen collection and processing

Five (5) mls of venous blood was aseptically collected from the participants by a qualified Medical Technologist. Blood for complete blood counts (CBCs) was collected using ethylene diamine tetra acetic acid (EDTA) vacutainers. The blood specimens collected from other health facilities apart from Nyeri level 5 hospital, were packed in a cool box and transported to Nyeri level 5 hospital laboratory where they were processed by a certified Medical Laboratory Technologist on the same day. Blood specimen processing was done using Medonic M-Series 3-part haematology autoanalyser, Boule Medical AB. Stockholm Sweden (Appendix XII).

3.7.3.2 Stool specimen collection and processing

Stool samples were collected in universal stool containers. Respondents were guided on how to collect stool sample (Appendix XIII). Stool samples collected from other health facilities apart from Nyeri level 5 hospital, were packed in cool box and transported to Nyeri level 5 hospital laboratory for processing the same day they were collected. Stool specimens were processed using formol-ether concentration technique and examined under microscope for intestinal parasites according to WHO (1991) procedure manual (Appendix XIV).

3.8 Validity

Questionnaires were reviewed by the supervisor to ensure they were valid. For quality assurance the performance of the medonic m-series auto analyser was checked daily by running and passing at least 2 levels of certified blood control (authorized by boule) before and after patients samples were analysed.
3.9 Reliability

To ascertain reliability of the questionnaires, research assistants were trained on data collection before participating in the study. The data collection was supervised on daily basis during the entire study. The data collected was scrutinized on daily basis to ensure accuracy, consistency and completeness during the entire data collection process. Data that did not meet this criteria was discarded and another interview was conducted the following day for replacement.

3.10 Data analysis

The data collected was analysed using SPSS software (version 20). Descriptive statistics such as mean, frequency and percentages were used to summarize the data. Bivariate analysis was done using cross tabulations and odds ratio statistic was used to determine the association between variables. Multivariate analysis using binary logistic regression was done to determine factors associated intestinal parasites infection. ANOVA test was used to compare means and Tukey Kramer post ANOVA test was done to determine where the difference between means was (Appendix XV). P-value ≤ 0.05 was considered to be statistically significant. Data was presented using tables, and charts.

3.11 Ethical considerations

Approval to conduct the study was sought from Kenyatta University Research Ethics Committee (Appendix I). Authority was sought from National Commission for Science, Technology and Innovation (NACOSTI) (Appendix IV). Permission to conduct the study was obtained from Department of Health Services Nyeri County (Appendix V). Informed consent was sought from pregnant women before participating in the study. They signed a consent form, English version (Appendix VIIA) and Kukuyu translated version (Appendix
VIIB). This was done after explaining the objectives, possible outcomes and benefits of the research. Confidentiality and privacy were guaranteed throughout the study.
CHAPTER FOUR: RESULTS

4.1 Socio-Demographic Characteristics of the Study Participants

4.1.1. Age of the respondents

Age varied from 16 to 40 years among 65 HIV negative respondents and from 17 to 40 years among 65 HIV positive respondents. The age bracket of 26-30 years had the highest proportions 21 (32%) and 18 (28%) for HIV negative and HIV positive respondents respectively. Age group of 36-40 years had the lowest proportion 2 (3%) among HIV negative respondents and age group of 15-20 years had the lowest proportion 6 (9%) among HIV positive respondents (Figure 4.1).

Figure 4.1: Distribution of respondents by age group

4.1.2 Marital status of the respondents

Marital status varied from highest proportion 47 (73%) among the married to lowest proportion 5 (8%) among the separated for 65 HIV positive respondents. Among 65 HIV
negative respondents marital status varied from highest proportion 83% (n=55) among the married to 5% (n=3) who were separated (Figure 4.2).

**Figure 4.2: Distribution of respondents by marital status**

### 4.2 Socio-Economic Characteristics

#### 4.2.1 Level of education of the respondents

Education level of 65 HIV positive respondents varied from 49% (n= 32) with secondary education to 3% (n=2) with no formal education and among 65 HIV negative respondents education level varied from 48% (n=31) with secondary education to 22% (n=14) with primary education (Figure 4.3).
4.2.2 Occupation of the respondents

Occupation among 65 HIV positive respondents varied from 52% (n=34) self employed to 12% (n=1) student while occupation among 65 HIV negative respondents varied from 35% (n=23) self employed to 11% (n=7) students (Figure 4.4).
4.2.3 Income level of the respondents

Monthly income among 65 HIV positive respondents varied from 38% (n=25) earning less than Kenya shillings (Kshs) 1000 and 38% (n=25) earning between Kshs 1001-5000 to 2% (n= 1) earning between Kshs 15001- 20000 monthly (Figure 4.5). Among 65 HIV negative respondents, monthly income varied from 35% (n=23) earning between Kshs 1001-5000 to 2% (n=1) earning between Kshs 15001– 20000 per month (Figure 4.5).
4.3 Prevalence of intestinal parasites infection

4.3.1 Prevalence of intestinal helminth parasites among respondents

Only one case of helminth (*Hymenolepis nana*) was identified among HIV negative respondents from Kiganjo level 3 hospital.

4.3.2 Prevalence of intestinal protozoan parasites among respondents

Among 65 HIV negative respondents 41.5% (n=27) were infected with intestinal protozoan parasites and 58.5% (n=38) were not infected while 26.2% (n=17) of 65 HIV positive respondents were infected with intestinal protozoan parasites and 73.8% (n=48) were not infected (Figure 4.6).
Figure 4.6: Distribution of respondents by intestinal parasites infection

4.3.3 Prevalence of specific intestinal protozoan parasites among respondents

Thirty eight percent (n=25) of HIV negative respondents and 25% (n=16) of HIV positive respondents were infected with *Entamoeba coli* while 6% (n=4) of HIV negative respondents and 2% (n=1) of HIV positive respondents were infected with *Iodamoeba butschlii* (Figure 4.7)
4.3.4 Prevalence of intestinal parasites among respondents by health facilities

One HIV negative respondent from Kiganjo level 3 hospital was infected with *Hymenolepis nana*. Cases of *Entamoeba coli* infection varied from 3 in both Mt Kenya level 4 and Nyeri level 3 hospitals to 8 in Nyeri level 5 (Table 4.1). *Iodamoeba butschlii* was isolated from respondents at Karatina level 4, Mt Kenya level 4, Nyeri town level 3 and Kiganjo level 3 hospitals (Table 4.1)
Table 4.1: Distribution of intestinal parasites infected respondents by health facility

<table>
<thead>
<tr>
<th>Health Facility</th>
<th>No of respondents</th>
<th>Number of respondents infected with Intestinal Protozoan Parasites</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (130)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Entamoeba coli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyeri level 5</td>
<td>30</td>
<td>8 (6.1%)</td>
<td>8 (6.1%)</td>
</tr>
<tr>
<td>Karatina level 4</td>
<td>32</td>
<td>7 (5.3%)</td>
<td>8 (6.1%)</td>
</tr>
<tr>
<td>Mukurweini level 4</td>
<td>12</td>
<td>4 (3.1%)</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td>Mt Kenya level 4</td>
<td>14</td>
<td>3 (2.3%)</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td>Nyeri Town level 3</td>
<td>12</td>
<td>3 (2.3%)</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td>Mweiga level 3</td>
<td>8</td>
<td>5 (3.8%)</td>
<td>5 (3.8%)</td>
</tr>
<tr>
<td>Kiganjo level 3</td>
<td>8</td>
<td>6 (4.6%)</td>
<td>8 (6.1%)</td>
</tr>
<tr>
<td>Naromoru level 3</td>
<td>14</td>
<td>5 (3.8%)</td>
<td>5 (3.8%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>130</td>
<td>41 (31.54%)</td>
<td>46 (35.38%)</td>
</tr>
</tbody>
</table>

| Iodamoeba butschlii   |                   |                                                                  |       |
|                       |                   |                                                                  |       |

4.4 Practices that would predispose respondents to intestinal parasites infection

The respondents’ practices during pregnancy that would predispose them to intestinal parasitic infection included: eating soil, walking barefoot, drinking untreated water and sharing of latrine (Table 4.2).
Table 4.2: Distribution of respondents by practices that predispose to intestinal parasite infection

<table>
<thead>
<tr>
<th>Practice during pregnancy</th>
<th>HIV Status</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n (%)</td>
<td>n (%)</td>
<td>Negative n (%)</td>
<td>Total</td>
</tr>
<tr>
<td>Eating soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 (42%)</td>
<td>19 (29%)</td>
<td>46 (35.4%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38 (58%)</td>
<td>46 (71%)</td>
<td>84 (64.6%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65 (100%)</td>
<td>65 (100%)</td>
<td>130 (100%)</td>
<td></td>
</tr>
<tr>
<td>Walking barefoot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (28%)</td>
<td>18 (28%)</td>
<td>36 (28%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>47 (72%)</td>
<td>47 (72%)</td>
<td>94 (72%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65 (100%)</td>
<td>65 (100%)</td>
<td>130 (100%)</td>
<td></td>
</tr>
<tr>
<td>Drink untreated water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (49%)</td>
<td>29 (45%)</td>
<td>61 (47%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33 (51%)</td>
<td>36 (55%)</td>
<td>69 (53%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65 (100%)</td>
<td>65 (100%)</td>
<td>130 (100%)</td>
<td></td>
</tr>
<tr>
<td>Share latrine with other households</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55 (85%)</td>
<td>49 (75%)</td>
<td>104 (80%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (15%)</td>
<td>16 (25%)</td>
<td>26 (20%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65 (100%)</td>
<td>65 (100%)</td>
<td>130 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

4.5 Management of pregnant women with HIV and intestinal parasite infections

It was observed that pregnant women who were diagnosed with intestinal helminths and were in second trimester were treated with mebendazole and those infected with intestinal
protozoans were treated with metronidazole and hygiene education. Management of pregnant women who were HIV positive included: (i) counselling before and after HIV diagnosis, (ii) nutritional education, (iii) treatment using Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine (NVP) or Evafirenz (EFV), (iv) CD4 monitoring, and (iv) viral load testing.

### 4.6 Factors associated with intestinal protozoan infection

#### 4.6.1 Socio-demographic characteristics of respondents

Intestinal protozoan infection was higher among respondents who were in the age 36 and above years at 42% (n= 5) compared to 33% (n=39) of those in the age 35 and below years (Table 4.3). There was no significant association between age and intestinal protozoan infection (OR = 1, CI 0.206 – 2.318, p > 0.05) (Table 4.3). Intestinal protozoan prevalence was higher among respondents who were married or living with a partner at 34% (n= 34) compared to 33% (n= 10) of those who were single or separated. There was no significant association between marital status and intestinal protozoan infection (OR = 1, CI 0.434 – 2.446, p > 0.05) (Table 4.3).

**Table 4.3: Socio-demographic factors associated with intestinal protozoan parasites infection-bivariate analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intestinal protozoan infection</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes n (%)</td>
<td>No n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 35 years</td>
<td>39 (33.1)</td>
<td>79 (66.9)</td>
<td>1</td>
<td>0.206 – 2.318</td>
</tr>
<tr>
<td>≥36 years</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married and cohabiting</td>
<td>34 (34)</td>
<td>66 (66)</td>
<td>1</td>
<td>0.434 -2.446</td>
</tr>
<tr>
<td>Single, divorced and</td>
<td>10 (33.3)</td>
<td>20 (66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>widowed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.6.2 Socio-economic characteristics of respondents

Intestinal protozoan prevalence was higher among respondents who had no formal and primary education at 49% compared to 28% of those who had secondary and tertiary education (Table 4.4). There was significant association between low education and intestinal protozoan infection (OR = 2.379, 95% CI 1.070–5.288, p < 0.05) (Table 4.4). Intestinal protozoan prevalence was higher in respondents who were unemployed at 48% compared to 27% of those employed. There was significant association between unemployment and intestinal protozoan infection (OR = 0.4, 95% CI 0.187–0.855, p < 0.05) (Table 4.4).

Table 4.4: Socio-economic factors associated with intestinal protozoan parasites infection-bivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intestinal protozoan infection</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes n (%)</td>
<td>No n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal and primary</td>
<td>17 (48.6)</td>
<td>18 (51.4)</td>
<td>2.379</td>
<td>1.07–5.288</td>
</tr>
<tr>
<td>Secondary and tertiary</td>
<td>27 (28.4)</td>
<td>68 (71.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>23 (26.7)</td>
<td>63 (73.3)</td>
<td>0.4</td>
<td>0.187–0.855</td>
</tr>
<tr>
<td>Unemployed</td>
<td>21 (47.7)</td>
<td>23 (52.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* indicate significant difference between the two categories

4.6.3 HIV infection

Intestinal protozoan infection was higher among HIV negative respondents at 42% compared to 26% of HIV positive respondents. There was no significant association between HIV infection and intestinal protozoan infection (OR = 0.498, 95% CI 0.238–1.046, p > 0.05).
4.6.4 Practices of the respondents

Intestinal protozoan prevalence was higher among respondents who did not treat their drinking water at 36% compared to 32% of those who treated (Table 4.5). However, there was no significant association between drinking untreated water and intestinal protozoan infection (OR = 1.205, 95% CI 0.582 – 2.495, p > 0.05) (Table 4.5). Intestinal protozoan infection was higher among respondents who did not eat soil at 35.7 % compared to 30.4 % of those who ate soil. There was no significant association between eating soil and intestinal protozoan infection in pregnancy (OR = 0.788, CI 0.364 – 1.702, p > 0.05) (Table 4.5). Intestinal protozoan parasites infection was higher among respondents who shared latrine with their neighbours at 89% compared to 20% of those who used their own latrine. There was significant association between sharing latrine and intestinal protozoan parasites infection (OR = 0.033, 95% CI 0.009 - 0.120, p < 0.05) (Table 4.5)

Table 4.5: Practices associated with intestinal protozoan parasites infection among respondents-bivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intestinal protozoan infection</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yeal (%)</td>
<td>No (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drink untreated water</td>
<td>No</td>
<td>22 (36.1)</td>
<td>39 (63.9)</td>
<td>1.205</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>22 (31.9)</td>
<td>47 (68.1)</td>
<td></td>
</tr>
<tr>
<td>Eat soil during pregnancy</td>
<td>Yes</td>
<td>14 (30.4)</td>
<td>32 (69.6)</td>
<td>0.788</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30 (35.7)</td>
<td>54 (64.3)</td>
<td></td>
</tr>
<tr>
<td>Share latrine with other households</td>
<td>No</td>
<td>21 (20.2)</td>
<td>83 (79.8)</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>23 (88.5)</td>
<td>3 (11.5)</td>
<td></td>
</tr>
</tbody>
</table>

*indicate significant difference between categories
4.6.6 Multivariate analysis of factors associated with intestinal protozoan parasites infection among respondents

Multivariate analysis showed that, there was no significant association between education and intestinal protozoan parasites infection (AOR = 1.87, 95 % CI 0.74 – 4.967, p > 0.05) (Table 4.6). There was no significant association between employment and intestinal protozoan parasites infection (AOR = 0.483, 95 % CI 0.192 – 1.214, p > 0.05) (Table 4.6). There was significant association between sharing latrine with other households and intestinal protozoan parasites infection (AOR = 0.037, CI 95% 0.01 – 0.136, p < 0.05); an indicator that sharing of latrine was an independent factor (predictor) that contributed to intestinal protozoan parasites infection among pregnant women (Table 4.6).

Table 4.6: Factors associated with intestinal protozoan parasites infection-multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intestinal protozoan infection</th>
<th>AOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes n (%)</td>
<td>No n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal and primary</td>
<td>17 (48.6)</td>
<td>18 (51.4)</td>
<td>1.87</td>
<td>0.704 – 4.967</td>
</tr>
<tr>
<td>Secondary and tertiary</td>
<td>27 (28.4)</td>
<td>68 (71.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>23 (26.7)</td>
<td>63 (73.3)</td>
<td>0.483</td>
<td>0.192 – 1.214</td>
</tr>
<tr>
<td>Unemployed</td>
<td>21 (47.7)</td>
<td>23 (52.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Share latrine with other household</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>21 (20.2)</td>
<td>83 (79.8)</td>
<td>0.037</td>
<td>0.01 – 0.136</td>
</tr>
<tr>
<td>Yes</td>
<td>23 (88.5)</td>
<td>3 (11.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*indicate significant difference between categories
4.7 Haematological Parameters

4.7.1 White blood cells count for HIV positive and HIV negative respondents

Eighty five percent (n=55) of HIV positive respondents and 75% (n= 49) of HIV negative respondents had normal white blood cells count while 3% (n=2) of HIV positive respondents had below normal white blood cells count. None of HIV negative respondents had below normal white blood cells count (Figure 4.8). Cross tabulation indicated significant difference in white blood cells between HIV positive and HIV negative respondents where HIV positive respondents had decreased white blood cells ($\chi^2 = 5.8$, df = 2, p < 0.05).

![Distribution of respondents by white blood cells count](image)

**Figure 4.8: Distribution of respondents by white blood cells count**

Normal range for white blood cells is $4 \text{–} 10 \times 10^9$ cells / L of blood.
4.7.2 Red blood cells (RBC) count for HIV positive and HIV negative respondents

Sixty nine percent (n= 45) of HIV positive respondents and 95% (n=62) of HIV negative respondents had normal red blood cells count while 3% (n=2) of HIV positive respondents and 2% (n= 1) HIV negative respondents had blood cells count above normal range (Figure 4.9). Cross tabulation indicated significant difference in red blood cells between HIV positive and HIV negative respondents where HIV positive respondents had decreased red blood cells ($\chi^2 = 15.8$, df = 2, p < 0.001).

![Figure 4.9: Distribution of respondents by red blood cells count](image)

Normal range for red blood cells is $3.85 - 5.20 \times 10^{12}$ cells / L of blood.

4.7.3 Haemoglobin concentration for HIV positive and HIV negative respondents

Fifty three (81%) of HIV negative and 43 (66%) of HIV positive respondents had normal haemoglobin while 12 (19%) of HIV negative and 22 (34%) of HIV positive respondents...
were anaemic (Figure 4.10). Cross tabulation showed significant difference in haemoglobin between HIV positive and HIV negative respondents where HIV positive respondents had decreased haemoglobin ($\chi^2 = 3.98$, df = 1, p < 0.046).

![Figure 4.10: Distribution of respondents by haemoglobin concentration](image)

Normal range for haemoglobin is 11.0 – 16 g/dl of blood sample

### 4.7.4 Haematocrit for HIV positive and HIV negative respondents

Sixty one (94%) of HIV negative and 33 (51%) of HIV positive respondents had haematocrit (Packed Cell Volume) in the normal range while 4 (6%) of HIV negative and 32 (49%) HIV positive respondents had haematocrit below normal range (Figure 4.11). Cross tabulation indicated significant difference in haematocrit between HIV positive and HIV negative respondents where HIV positive respondents had decreased haematocrit ($\chi^2 = 30.18$, df = 1, p < 0.001).
Normal range for haematocrit is 34.7 – 46 % of blood sample

4.7.5 Platelets count for HIV positive and HIV negative respondents

Sixty four (98%) of HIV positive and 64 (98%) of HIV negative respondents had platelets in the normal range while 1(2%) of HIV positive and 1 (2%) of HIV negative had platelets below normal range (Figure 4.12). Cross tabulation indicated no significant difference in platelets between HIV positive and HIV negative respondents ($\chi^2 = 5$, df = 1, p > 0.05)
Figure 4.12: Distribution respondents by platelets count

Normal range for platelets is $140 - 440 \times 10^9$ cells / L of blood sample

4.8 Effects of HIV and intestinal parasites co-infection on haematological parameters

4.8.1 Effect on white blood cells of respondents

4.8.1.1 Mean white blood cells of respondents

The mean white blood cells of respondents were distributed by co-infection of HIV and intestinal parasites and age group (Table 4.7)
Table 4.7: Distribution of mean white blood cells by age, HIV and intestinal protozoan parasites infection status

<table>
<thead>
<tr>
<th>Age group</th>
<th>HIV –ve</th>
<th>HIV –ve</th>
<th>HIV +ve</th>
<th>HIV +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protozoan +ve</td>
<td>Protozoan –ve</td>
<td>Protozoan +ve</td>
<td>Protozoan –ve</td>
</tr>
<tr>
<td></td>
<td>WBC Mean</td>
<td>SD</td>
<td>WBC Mean</td>
<td>SD</td>
</tr>
<tr>
<td>15 –20 years</td>
<td>9.743 ± 2.66</td>
<td>10.225 ± 3.24</td>
<td><strong>8.1 ± 0.8</strong></td>
<td>8.825 ± 3.32</td>
</tr>
<tr>
<td>21 -25 years</td>
<td>7.8 ± 1.59</td>
<td>7.585 ± 1.33</td>
<td><strong>7.5 ± 1.72</strong></td>
<td>8.545 ± 2.57</td>
</tr>
<tr>
<td>26-30 years</td>
<td>9.788 ± 3.00</td>
<td>9.633 ± 2.74</td>
<td><strong>5.367 ± 0.92</strong></td>
<td>7.413 ± 2.09</td>
</tr>
<tr>
<td>31-35 years</td>
<td>9.2 ± 2.65</td>
<td>9.078 ± 2.22</td>
<td><strong>8.425 ± 2.41</strong></td>
<td>7.255 ± 2.25</td>
</tr>
<tr>
<td>36-40 years</td>
<td>8.35 ± 2.55</td>
<td><strong>6.433 ± 0.82</strong></td>
<td>7.086 ± 1.51</td>
<td></td>
</tr>
</tbody>
</table>

-ve indicates no infection of HIV or intestinal protozoan parasite (infection status)
+ve indicates infection of HIV or intestinal protozoan parasite (infection status)

4.8.1.2 Analysis of variance (ANOVA) for white blood cells among respondents

The mean white blood cells of respondents co-infected with HIV and intestinal protozoan parasites was compared with that of respondents infected with HIV alone, infected with intestinal protozoan parasites alone and that of respondents with no infection. The means of white blood cells were also compared between age groups of respondents. Analysis of variance showed that infection status had significant effect on white blood cells among pregnant women, $F_{\text{calculated}} = 5.56$, is greater than $F_{0.95} (3, 11) = 3.59$; $p < 0.05$ while age had no significant effect, $F_{\text{calculated}} = 2.515$, is less than $F_{0.95} (4, 11) = 3.36$; $p > 0.05$ (Table 4.8).
Tukey Kramer post ANOVA test was done to show which infection produced significant effect on white blood cells (Table 4.9, Table 4.10, and Table 4.11)

**Table 4.8: Relationship between white blood cells and age, HIV and intestinal protozoan parasites infections among respondents**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F&lt;sub&gt;calculated&lt;/sub&gt;</th>
<th>F&lt;sub&gt;table&lt;/sub&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV and intestinal parasite co-infection status</td>
<td>3</td>
<td>12.52</td>
<td>4.173</td>
<td>5.56</td>
<td>3.59</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td>4</td>
<td>7.552</td>
<td>1.888</td>
<td>2.515</td>
<td>3.36</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>8.258</td>
<td>0.7507</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>28.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**4.8.1.3 Multiple comparison test of mean white blood cells among four groups of respondents based on HIV and intestinal parasites infection status**

Tukey Kramer test (Appendix XV) was used to determine where the difference was between four groups of pregnant women

Group infected with intestinal protozoan parasites and not HIV was named X<sub>1</sub>

Group not infected with either HIV or intestinal protozoan was named X<sub>2</sub>

Group infected with both HIV and intestinal protozoan parasites was named X<sub>3</sub>

Group infected with HIV and not intestinal protozoan parasites was named X<sub>4</sub>
Group means were $X_1 = 8.9762$, $X_2 = 9.130$, $X_3 = 7.165$, $X_4 = 7.825$

Since there were four groups, six possible pairwise comparisons were done (Table 4.9)

**Table 4.9: Pair wise critical values and difference between true white blood cells averages among respondents**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$W_{ij}$</th>
<th>$(X_i - X_j)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>1.676</td>
<td>0.1538</td>
</tr>
<tr>
<td>1 and 3</td>
<td>1.581</td>
<td>1.8112</td>
</tr>
<tr>
<td>1 and 4</td>
<td>1.581</td>
<td>1.1512</td>
</tr>
<tr>
<td>2 and 3</td>
<td>1.676</td>
<td>1.96</td>
</tr>
<tr>
<td>2 and 4</td>
<td>1.676</td>
<td>1.305</td>
</tr>
<tr>
<td>3 and 4</td>
<td>1.581</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Comparing $W_{ij}$ with $(X_i - X_j)$

where

$W = \text{critical value}$

$i = \text{population i}$

$j = \text{population j}$

**Null Hypothesis**

$H_0 \; \mu_i = \mu_j$

**Alternative hypothesis**

$H_1 \; \mu_i \neq \mu_j$

where i and j are two different populations
Reject null hypothesis if \((X_i - X_j) > W_{ij}\)

Group means were arranged in increasing order: \(X_3 < X_4 < X_1 < X_2\)

The smallest mean \(X_3\) was compared first (Table 4.10)

**Table 4.10: Comparison of \(W_{ij}\) with \((X_i - X_j)\)**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>((X_i - X_j))</th>
<th>(W_{ij})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_3 - X_4)</td>
<td>0.66</td>
<td>1.581</td>
</tr>
<tr>
<td>(X_3 - X_2)</td>
<td>1.96</td>
<td>1.676</td>
</tr>
<tr>
<td>(X_3 - X_1)</td>
<td>1.8112</td>
<td>1.581</td>
</tr>
</tbody>
</table>

\(X_3 \leq X_4 < X_1 < X_2\)  Sample means were arranged in increasing order and pairs that differed by less than \(W_{ij}\) (critical value) were underlined

Then mean \(X_4\) was compared (Table 4.11)

**Table 4.11: Comparison of \(W_{ij}\) with \((X_i - X_j)\)**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>((X_i - X_j))</th>
<th>(W_{ij})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_4 - X_2)</td>
<td>1.305</td>
<td>1.676</td>
</tr>
<tr>
<td>(X_4 - X_1)</td>
<td>1.1512</td>
<td>1.581</td>
</tr>
</tbody>
</table>

\(X_3 \leq X_4 \leq X_1 \leq X_2\)  Sample means were arranged in increasing order and pairs that differed by less than \(W_{ij}\) (critical value) were underlined. Any pair of sample mean not connected by an underscore (that is, differing more than \(W_{ij}\) ) implies a difference in the corresponding means. Therefore, Tukey Kramer method indicates that the difference between the true average white blood cells in pregnant women co-infected with HIV and intestinal parasites (\(X_3\)) and those not co-infected (\(X_2\)) is significant. Showing that pregnant women with co-infection of HIV and intestinal protozoan parasites had reduced white blood cells.
4.8.2 Effect on red blood cells of respondents

4.8.2.1 Mean red blood cells of respondents

The mean red blood cells of respondents were distributed according to co-infection of HIV and intestinal protozoan parasites and age group (Table 4.12)

Table 4.12: Distribution of mean red blood cells by age, HIV and intestinal protozoan parasites infection among respondents

<table>
<thead>
<tr>
<th>Age group</th>
<th>HIV-ve</th>
<th>HIV-ve</th>
<th>HIV+ve</th>
<th>HIV+ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protozoan +ve</td>
<td>RBC Mean</td>
<td>SD</td>
<td>RBC Mean</td>
</tr>
<tr>
<td></td>
<td>Protozoan –ve</td>
<td>RBC Mean</td>
<td>SD</td>
<td>RBC Mean</td>
</tr>
<tr>
<td>15 –20 years</td>
<td>4.55 ± 0.44</td>
<td>4.605 ± 0.15</td>
<td>3.93 ± 0.19</td>
<td>3.938 ± 0.36</td>
</tr>
<tr>
<td>21 -25 years</td>
<td>4.284 ± 0.39</td>
<td>4.455 ± 0.26</td>
<td>3.932 ± 0.44</td>
<td>3.873 ± 0.56</td>
</tr>
<tr>
<td>26-30 years</td>
<td>4.328 ± 0.30</td>
<td>4.535 ± 0.28</td>
<td>3.467 ± 0.19</td>
<td>4.129 ± 0.46</td>
</tr>
<tr>
<td>31-35 years</td>
<td>4.493 ± 0.27</td>
<td>4.598 ± 0.31</td>
<td>4.108 ± 0.45</td>
<td>4.193 ± 0.48</td>
</tr>
<tr>
<td>36-40 years</td>
<td>4.37 ± 0.25</td>
<td>3.637 ± 0.50</td>
<td>3.963 ± 0.71</td>
<td></td>
</tr>
</tbody>
</table>

-ve indicates no infection of HIV or intestinal protozoan parasite (infection status)

+ve indicates infection of HIV or intestinal protozoan parasite (infection status)

4.8.2.2 Analysis of variance (ANOVA) of mean red cells blood cells among respondents

The mean red blood cells of respondents co-infected with HIV and intestinal protozoan parasites was compared with that of respondents infected with HIV alone, infected with intestinal protozoan parasites alone and that of respondents with no infection. The means of red blood cells were also compared between age groups of respondents. Analysis of variance
showed that infection status had significant effect on red blood cells among pregnant women, $F_{\text{calculated}} = 43$, is greater than $F_{0.95} (3, 11) = 3.59$; $p < 0.05$ (Table 4.13). The two-way ANOVA also showed that age had significant effect on red blood cells, $F_{\text{calculated}} = 5.46$, is greater than $F_{0.95} (4, 11) = 3.36$; $p < 0.05$ (Table 4.13). Tukey Kramer post ANOVA test was done to show which infection status and which age group produced significant effect on red blood cells (Table 4.14, Table 4.15, Table 4.16, Table 4.17, Table 4.18, and Table 4.19).

Table 4.13: Relationship between red blood cells and age, HIV and intestinal protozoan parasites infection among respondents

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>$F_{\text{calculated}}$</th>
<th>$F_{\text{table}}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV and intestinal parasites co-infection status</td>
<td>3</td>
<td>1.5907</td>
<td>0.5302</td>
<td>43</td>
<td>3.59</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td>4</td>
<td>0.2689</td>
<td>0.0672</td>
<td>5.46</td>
<td>3.36</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>0.1354</td>
<td>0.0123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>1.995</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.8.2.3 Multiple comparison test of mean red blood cells among four groups of respondents based on intestinal parasites and HIV infection status

Tukey Kramer test (Appendix XIV) was used to determine where the difference was between four groups of pregnant women.

Group infected with intestinal protozoan parasites and not HIV was named $X_1$.

Group not infected with either HIV or intestinal protozoan was named $X_2$.

Group infected with both HIV and intestinal protozoan parasites was named $X_3$.

Group infected with HIV and not intestinal protozoan parasites was named $X_4$.

Group means were $X_1 = 4.405$, $X_2 = 4.548$, $X_3 = 3.815$, $X_4 = 4.0192$.

Since there were four groups, six possible pairwise comparisons were done (Table 4.14).

**Table 4.14: Pair wise critical values and difference between true red blood cells averages among respondents**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$W_{ij}$</th>
<th>$(X_i - X_j)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>0.2146</td>
<td>0.143</td>
</tr>
<tr>
<td>1 and 3</td>
<td>0.2024</td>
<td>0.59</td>
</tr>
<tr>
<td>1 and 4</td>
<td>0.2024</td>
<td>0.3858</td>
</tr>
<tr>
<td>2 and 3</td>
<td>0.2146</td>
<td>0.733</td>
</tr>
<tr>
<td>2 and 4</td>
<td>0.2146</td>
<td>0.5288</td>
</tr>
<tr>
<td>3 and 4</td>
<td>0.2024</td>
<td>0.2042</td>
</tr>
</tbody>
</table>

Comparing $W_{ij}$ with $(X_i - X_j)$

Null Hypothesis

$H_0 \; \mu_i = \mu_j$

Alternative hypothesis

$H_1 \; \mu_i \neq \mu_j$
where i and j are two different populations

Reject null hypothesis if \( (X_i - X_j) > W_{ij} \)

Group means were arranged in increasing order: \( X_3 < X_4 < X_1 < X_2 \)

The smallest mean \( X_3 \) was compared first (Table 4.15)

**Table 4.15: Comparison of \( W_{ij} \) with \((X_i - X_j)\)**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>((X_i - X_j))</th>
<th>(W_{ij})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_3 - X_4)</td>
<td>0.2042</td>
<td>0.2024</td>
</tr>
<tr>
<td>(X_3 - X_2)</td>
<td><strong>0.733</strong></td>
<td><strong>0.2146</strong></td>
</tr>
<tr>
<td>(X_3 - X_1)</td>
<td>0.59</td>
<td>0.2024</td>
</tr>
</tbody>
</table>

\(X_3 < X_4 < X_1 < X_2\) Sample means were arranged in increasing order and pairs that differed by less than \( W_{ij} \) (critical value) were underlined

Then mean \( X_4 \) was compared (Table 4.16)

**Table 4.16: Comparison of \( W_{ij} \) with \((X_i - X_j)\)**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>((X_i - X_j))</th>
<th>(W_{ij})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_4 - X_2)</td>
<td>0.5288</td>
<td>0.2146</td>
</tr>
<tr>
<td>(X_4 - X_1)</td>
<td>0.3858</td>
<td>0.2024</td>
</tr>
</tbody>
</table>

\(X_3 < X_4 < X_1 < X_2\) Sample means were arranged in increasing order and pairs that differed by less than \( W_{ij} \) (critical value) were underlined. Any pair of sample mean not connected by an underscore (that is, differing more than \( W_{ij} \)) implies a difference in the corresponding means. Therefore, Tukey Kramer method indicates that the difference between the true average red blood cells in pregnant women co-infected with HIV and intestinal
parasites (X₃) and those not co-infected (X₂) is significant. Showing that pregnant women with co-infection of HIV and intestinal protozoan parasites had reduced red blood cells.

4.8.2.4 Multiple comparison test of mean red blood cells among five age groups of respondents

Tukey Kramer test (Appendix XV) was used to determine where the difference was between five age groups of pregnant women.

Respondents in age group 15-20 years was named X₁

Respondents in age group 21-25 years was named X₂

Respondents in age group 26-30 years was named X₃

Respondents in age group 31-35 years was named X₄

Respondents in age group 35-40 years was named X₅

Group means were X₁ = 4.256, X₂ = 4.136, X₃ = 4.115, X₄ = 4.348, X₅ = 3.99

Since there were five groups, ten possible pairwise comparisons were done (Table 4.17)

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>Wᵢⱼ</th>
<th>(Xᵢ - Xⱼ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>0.244</td>
<td>0.12</td>
</tr>
<tr>
<td>1 and 3</td>
<td>0.244</td>
<td>0.141</td>
</tr>
<tr>
<td>1 and 4</td>
<td>0.244</td>
<td>0.092</td>
</tr>
<tr>
<td>1 and 5</td>
<td>0.264</td>
<td>0.266</td>
</tr>
<tr>
<td>2 and 3</td>
<td>0.244</td>
<td>0.021</td>
</tr>
<tr>
<td>2 and 4</td>
<td>0.244</td>
<td>0.212</td>
</tr>
<tr>
<td>2 and 5</td>
<td>0.264</td>
<td>0.146</td>
</tr>
<tr>
<td>3 and 4</td>
<td>0.244</td>
<td>0.233</td>
</tr>
<tr>
<td>3 and 5</td>
<td>0.264</td>
<td>0.125</td>
</tr>
<tr>
<td>4 and 5</td>
<td>0.264</td>
<td>0.358</td>
</tr>
</tbody>
</table>

Comparing Wᵢⱼ with (Xᵢ - Xⱼ)
Null Hypothesis

\[ H_0: \mu_i = \mu_j \]

Alternative hypothesis

\[ H_1: \mu_i \neq \mu_j \]

where \( i \) and \( j \) are two different populations

Reject null hypothesis if \((X_i - X_j) > W_{ij}\)

Group means were arranged in increasing order: \( X_5 < X_3 < X_2 < X_1 < X_4 \)

The smallest mean \( X_5 \) was compared first (Table 4.18)

**Table 4.18: Comparison of \( W_{ij} \) with \((X_i - X_j)\)**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>((X_i - X_j))</th>
<th>( W_{ij} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( X_5 - X_4 )</td>
<td>0.358</td>
<td>0.264</td>
</tr>
<tr>
<td>( X_5 - X_3 )</td>
<td>0.125</td>
<td>0.264</td>
</tr>
<tr>
<td>( X_5 - X_2 )</td>
<td>0.146</td>
<td>0.264</td>
</tr>
<tr>
<td>( X_5 - X_1 )</td>
<td>0.266</td>
<td>0.264</td>
</tr>
</tbody>
</table>

\( X_5 < X_3 < X_2 < X_1 < X_4 \) Sample means were arranged in increasing order and pairs that differed by less than \( W_{ij} \) (critical value) were underlined

Then mean \( X_3 \) was compared (Table 4.19)
Table 4.19: Comparison of $W_{ij}$ with $(X_i - X_j)$

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$(X_i - X_j)$</th>
<th>$W_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_3 - X_2$</td>
<td>0.021</td>
<td>0.244</td>
</tr>
<tr>
<td>$X_3 - X_1$</td>
<td>0.141</td>
<td>0.244</td>
</tr>
<tr>
<td>$X_3 - X_4$</td>
<td>0.233</td>
<td>0.244</td>
</tr>
</tbody>
</table>

$X_5 < X_3 < X_2 < X_1 < X_4$ Sample means were arranged in increasing order and pairs that differed by less than $W_{ij}$ (critical value) were underlined. Any pair of sample mean not connected by an underscore (that is, differing more than $W_{ij}$) implies a difference in the corresponding means. Therefore, Tukey Kramer method indicates that the difference between the true average red blood cells in pregnant women in age group five ($X_5$) and those in age group four ($X_4$) is significant. Showing that, pregnant women in age group 5 ($X_5$) had reduced red blood cells.

4.8.3 Effect on haemoglobin concentration of respondents

4.8.3.1 Mean haemoglobin concentration of respondents

The mean haemoglobin of respondents were distributed by co-infection of HIV and intestinal parasites and age group (Table 4.20)
Table 4.20: Distribution of mean haemoglobin concentration by age, HIV and intestinal protozoan parasites infection among respondents

<table>
<thead>
<tr>
<th>Age group</th>
<th>HIV –ve Protozoan +ve</th>
<th>HIV –ve Protozoan –ve</th>
<th>HIV +ve Protozoan +ve</th>
<th>HIV +ve Protozoan –ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb Mean SD</td>
<td>Hb Mean SD</td>
<td>Hb Mean SD</td>
<td>Hb Mean SD</td>
</tr>
<tr>
<td>15–20 years</td>
<td>12.514 ± 0.80</td>
<td>12.7 ± 1.19</td>
<td>12.05 ± 0.95</td>
<td>10.975 ± 0.13</td>
</tr>
<tr>
<td>21–25 years</td>
<td>12.686 ± 1.00</td>
<td>12.33 ± 1.28</td>
<td>10.3 ± 0.48</td>
<td>11.181 ± 1.02</td>
</tr>
<tr>
<td>26-30 years</td>
<td>12.775 ± 0.80</td>
<td>12.61 ± 1.10</td>
<td>11.2 ± 0.86</td>
<td>11.66 ± 1.30</td>
</tr>
<tr>
<td>31-35 years</td>
<td>12.833 ± 0.76</td>
<td>13.12 ± 1.02</td>
<td>10.88 ± 1.60</td>
<td>12.818 ± 1.48</td>
</tr>
<tr>
<td>36-40 years</td>
<td>13.4 ± 0.70</td>
<td>11.13 ± 0.60</td>
<td>11.443 ± 1.85</td>
<td></td>
</tr>
</tbody>
</table>

-ve indicates no infection of HIV or intestinal protozoan parasite (infection status)
+ve indicates infection of HIV or intestinal protozoan parasite (infection status)

4.8.3.2 Analysis of variance (ANOVA) of mean haemoglobin among respondents

The mean haemoglobin of respondents co-infected with HIV and intestinal protozoan parasites was compared with that of respondents infected with HIV alone, infected with intestinal protozoan parasites alone and that of respondents with no infection. The means of haemoglobin were also compared between age groups of respondents. Analysis of variance showed that infection status had significant effect on haemoglobin among pregnant women, $F_{\text{calculated}} = 11.6186$, is greater than $F_{0.95}(3, 11) = 3.59; p < 0.05$ while age had no significant effect, $F_{\text{calculated}} = 1.0831$, is less than $F_{0.95}(4, 11) = 3.36; p > 0.05$ (Table 4.21). Tukey Kramer post ANOVA test was done to show which infection produced significant effect on haemoglobin (Table 4.22, Table 4.23, and Table 4.24)
Table 4.21: Relationship between haemoglobin and age, HIV and intestinal protozoan parasites infections among respondents

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F_calculated</th>
<th>F_table</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV and intestinal parasites co-infection status</td>
<td>3</td>
<td>10.10889</td>
<td>3.36963</td>
<td>11.6186</td>
<td>3.59</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td>4</td>
<td>1.25646</td>
<td>0.314115</td>
<td>1.0831</td>
<td>3.36</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>3.19025</td>
<td>0.29002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.3.3 Multiple comparison test of mean haemoglobin concentration among four groups of respondents based on HIV and intestinal parasites infection status

Tukey Kramer test (Appendix XIV) was used to determine where the difference was between four groups of pregnant women

Group infected with intestinal protozoan parasites and not HIV was named $X_1$

Group not infected with either HIV or intestinal protozoan was named $X_2$

Group infected with both HIV and intestinal protozoan parasites was named $X_3$

Group infected with HIV and not intestinal protozoan parasites was named $X_4$

Group means were $X_1 = 12.84$, $X_2 = 12.69$, $X_3 = 11.112$, $X_4 = 11.62$

Since there were four groups, six possible pairwise comparisons were done (Table 4.22)
Table 4.22: Pair wise critical values and difference between true haemoglobin averages among respondents

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>( W_{ij} )</th>
<th>(( X_i - X_j ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>1.0422</td>
<td>0.15</td>
</tr>
<tr>
<td>1 and 3</td>
<td>0.9826</td>
<td>1.728</td>
</tr>
<tr>
<td>1 and 4</td>
<td>0.9826</td>
<td>1.22</td>
</tr>
<tr>
<td>2 and 3</td>
<td>1.0422</td>
<td>1.578</td>
</tr>
<tr>
<td>2 and 4</td>
<td>1.0422</td>
<td>1.07</td>
</tr>
<tr>
<td>3 and 4</td>
<td>0.9826</td>
<td>0.508</td>
</tr>
</tbody>
</table>

Comparing \( W_{ij} \) with (\( X_i - X_j \))

Null Hypothesis

\( H_0 \ \mu_i = \mu_j \)

Alternative hypothesis

\( H_1 \ \mu_i \neq \mu_j \)

where \( i \) and \( j \) are two different populations

Reject null hypothesis if (\( X_i - X_j \)) > \( W_{ij} \)

Group means were arranged in increasing order: \( X_3 < X_4 < X_2 < X_1 \)

The smallest mean \( X_3 \) was compared first (Table 4.23)

Table 4.23: Comparison of \( W_{ij} \) with (\( X_i - X_j \))

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>(( X_i - X_j ))</th>
<th>( W_{ij} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( X_3 - X_4 )</td>
<td>0.508</td>
<td>0.9826</td>
</tr>
<tr>
<td>( X_3 - X_2 )</td>
<td>\textbf{1.578}</td>
<td>\textbf{1.0422}</td>
</tr>
<tr>
<td>( X_3 - X_1 )</td>
<td>1.728</td>
<td>0.9826</td>
</tr>
</tbody>
</table>
Sample means were arranged in increasing order and pairs that differed by less than $W_{ij}$ (critical value) were underlined.

Then mean $X_4$ was compared (Table 4.24)

**Table 4.24: Comparison of $W_{ij}$ with $(X_i - X_j)$**

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$(X_i - X_j)$</th>
<th>$W_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_4 - X_2$</td>
<td>1.07</td>
<td>1.04</td>
</tr>
<tr>
<td>$X_4 - X_1$</td>
<td>1.22</td>
<td>0.9826</td>
</tr>
</tbody>
</table>

Sample means were arranged in increasing order and pairs that differed by less than $W_{ij}$ (critical value) were underlined. Any pair of sample mean not connected by an underscore (that is, differing more than $W_{ij}$) implies a difference in the corresponding means. Therefore, Tukey Kramer method indicates that the difference between the true average haemoglobin in pregnant women co-infected with HIV and intestinal parasites ($X_3$) and those not co-infected ($X_2$) is significant. Showing that, pregnant women with co-infection of HIV and intestinal protozoan parasites had reduced haemoglobin.

**4.8.4 Effect on haematocrit (Packed Cell Volume) of respondents**

**4.8.4.1 Mean haematocrit of respondents**

The mean haematocrit of respondents was distributed by co-infection of HIV and intestinal protozoan parasites and age group (Table 4.25)
Table 4.25: Distribution of mean haematocrit by age, HIV and intestinal protozoan parasites infection among respondents

<table>
<thead>
<tr>
<th>Age group</th>
<th>HIV-ve Protozoan +ve</th>
<th>HIV-ve Protozoan –ve</th>
<th>HIV+ve Protozoan +ve</th>
<th>HIV +ve P –ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCV Mean SD</td>
<td>PCV Mean SD</td>
<td>PCV Mean SD</td>
<td>PCV Mean SD</td>
</tr>
<tr>
<td>15–20 years</td>
<td>38.071 ± 1.46</td>
<td>39.8 ± 1.25</td>
<td><strong>35.95 ± 2.25</strong></td>
<td>33.425 ± 0.94</td>
</tr>
<tr>
<td>21–25 years</td>
<td>38.071 ± 2.38</td>
<td>37.76 ± 2.53</td>
<td><strong>31.78 ± 2.05</strong></td>
<td>33.655 ± 2.80</td>
</tr>
<tr>
<td>26–30 years</td>
<td>38.188 ± 2.36</td>
<td>38.53 ± 2.76</td>
<td><strong>32.9 ± 1.74</strong></td>
<td>35.353 ± 3.37</td>
</tr>
<tr>
<td>31–35 years</td>
<td>37.933 ± 1.56</td>
<td>40.12 ± 2.98</td>
<td><strong>34.03 ± 4.17</strong></td>
<td>38.264 ± 4.29</td>
</tr>
<tr>
<td>36–40 years</td>
<td>40.95 ± 2.15</td>
<td></td>
<td><strong>33.6 ± 1.44</strong></td>
<td>34.9 ± 4.68</td>
</tr>
</tbody>
</table>

-ve  indicates no infection of HIV or intestinal protozoan parasite (infection status)
+ve  indicates infection of HIV or intestinal protozoan parasite (infection status)

4.8.4.2 Analysis of variance (ANOVA) of mean haematocrit of respondents

The mean white blood cells of respondents co-infected with HIV and intestinal protozoan parasites was compared with that of respondents infected with HIV alone, infected with intestinal protozoan parasites alone and that of respondents with no infection. The means of haematocrit were also compared between age groups of respondents. Analysis of variance showed that infection status had significant effect on haematocrit among pregnant women, \( F_{\text{calculated}} = 15.23168 \), is greater than \( F_{0.95} (3, 11) = 3.59 \); \( p < 0.05 \) while age had no significant effect, \( F_{\text{calculated}} = 1.2639 \), is less than \( F_{0.95} (4, 11) = 3.36 \); \( p > 0.05 \) (Table 4.26). Tukey
Kramer post ANOVA test was done to show which infection produced significant effect on haematocrit (Table 4.27, Table 4.28, and Table 4.29).

**Table 4.26: Relationship between haematocrit and age, HIV and intestinal protozoan parasites infection among respondents**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F&lt;sub&gt;calculated&lt;/sub&gt;</th>
<th>F&lt;sub&gt;table&lt;/sub&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV and intestinal parasites co-infection status</td>
<td>3</td>
<td>99.1263</td>
<td>33.0421</td>
<td>15.23168</td>
<td>3.59</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td>4</td>
<td>10.9669</td>
<td>2.7417</td>
<td>1.2639</td>
<td>3.36</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>23.8619</td>
<td>2.1693</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>133.9551</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.4.3 Multiple comparison test of mean haematocrit among four groups of respondents based on HIV and intestinal parasites infection status

Tukey Kramer test (Appendix XIV) was used to determine where the difference was between four groups of pregnant women

Group infected with intestinal protozoan parasites and not HIV was named X<sub>1</sub>

Group not infected with either HIV or intestinal protozoan was named X<sub>2</sub>

Group infected with both HIV and intestinal protozoan parasites was named X<sub>3</sub>

Group infected with HIV and not intestinal protozoan parasites was named X<sub>4</sub>

Group means were X<sub>1</sub> = 38.64, X<sub>2</sub> = 39.05, X<sub>3</sub> = 33.651, X<sub>4</sub> = 35.12

Since there were four groups, six possible pairwise comparisons were done (Table 4.27)
Comparing $W_{ij}$ with $(X_i - X_j)$

**Null Hypothesis**

$H_0 \ \mu_i = \mu_j$

**Alternative hypothesis**

$H_1 \ \mu_i \neq \mu_j$

where $i$ and $j$ are two different populations

Reject null hypothesis if $(X_i - X_j) > W_{ij}$

Group means were arranged in increasing order: $X_3 < X_4 < X_1 < X_2$

The smallest mean $X_3$ was compared first (Table 4.28)
Table 4.28: Comparison of $W_{ij}$ with $(X_i - X_j)$

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$(X_i - X_j)$</th>
<th>$W_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_3 - X_4$</td>
<td>1.469</td>
<td>2.6874</td>
</tr>
<tr>
<td>$X_3 - X_2$</td>
<td>5.399</td>
<td>2.85</td>
</tr>
<tr>
<td>$X_3 - X_1$</td>
<td>4.989</td>
<td>2.6874</td>
</tr>
</tbody>
</table>

$X_3 < X_4 < X_1 < X_2$  Sample means were arranged in increasing order and pairs that differed by less than $W_{ij}$ (critical value) were underlined.

Then mean $X_4$ was compared (Table 4.29).

Table 4.29: Comparison of $W_{ij}$ with $(X_i - X_j)$

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>$(X_i - X_j)$</th>
<th>$W_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_4 - X_2$</td>
<td>3.93</td>
<td>2.85</td>
</tr>
<tr>
<td>$X_4 - X_1$</td>
<td>3.52</td>
<td>2.6874</td>
</tr>
</tbody>
</table>

$X_3 < X_4 < X_1 < X_2$  Sample means were arranged in increasing order and pairs that differed by less than $W_{ij}$ (critical value) were underlined. Any pair of sample mean not connected by an underscore (that is, differing more than $W_{ij}$ ) implies a difference in the corresponding means. Therefore, Tukey Kramer method indicates that the difference between the true average haematocrit in pregnant women co-infected with HIV and intestinal parasites ($X_3$ ) and those not co-infected ($X_2$ ) is significant. Showing that, pregnant women with co-infection of HIV and intestinal protozoan parasites had reduced haematocrit.
4.8.5 Effect on platelets of respondents

4.8.5.1 Mean platelets of respondents

The mean platelets among pregnant women were distributed according to co-infection of HIV and intestinal parasites and age group (Table 4.30)

**Table 4.30: Distribution of mean platelets by age, HIV and intestinal protozoan parasite infection status among respondents**

<table>
<thead>
<tr>
<th>Age group</th>
<th>HIV-ve</th>
<th>HIV-ve</th>
<th>HIV +ve</th>
<th>HIV +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLT</td>
<td>PLT</td>
<td>PLT</td>
<td>PLT</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>15 –20 years</td>
<td>258.43 ± 38.06</td>
<td>275.25 ± 9.55</td>
<td><strong>299.5 ± 20.50</strong></td>
<td>230.25 ± 16.78</td>
</tr>
<tr>
<td>21 -25 years</td>
<td>243.29 ± 41.58</td>
<td>248.92 ± 40.06</td>
<td><strong>238.8 ± 31.50</strong></td>
<td>273.82 ± 71.93</td>
</tr>
<tr>
<td>26-30 years</td>
<td>216.5 ± 34.91</td>
<td>272.92 ± 57.16</td>
<td><strong>292 ± 58.70</strong></td>
<td>260.47 ± 74.24</td>
</tr>
<tr>
<td>31-35 years</td>
<td>257.33 ± 42.09</td>
<td>213.11 ± 52.64</td>
<td><strong>270.75 ± 48.15</strong></td>
<td>218.73 ± 33.84</td>
</tr>
<tr>
<td>36-40 years</td>
<td>273.5 ± 78.5</td>
<td></td>
<td><strong>246.67 ± 19.60</strong></td>
<td>226.86 ± 54.59</td>
</tr>
</tbody>
</table>

-ve indicates no infection of HIV or intestinal protozoan parasite (infection status)
+ve indicates infection of HIV or intestinal protozoan parasite (infection status)

**4.8.5.2 Analysis of variance (ANOVA) of mean platelets among respondents**

The mean platelets of respondents co-infected with HIV and intestinal protozoan parasites was compared with that of respondents infected with HIV alone, infected with intestinal protozoan parasites alone and that of respondents with no infection. The means of platelets
were also compared between age groups of respondents. Analysis of variance showed that infection status had no significant effect on platelets among pregnant women, $F_{\text{calculated}} = 0.941$, is less than $F_{0.95} (3, 11) = 3.59$; $p > 0.05$ and age had no significant effect, $F_{\text{calculated}} = 0.566$, is less than $F_{0.95} (4, 11) = 3.36$; $p > 0.05$ (Table 4.31).

Table 4.31: Relationship between platelets and age, HIV and intestinal protozoan parasites infection among respondents

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>$F_{\text{calculated}}$</th>
<th>$F_{\text{table}}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV and intestinal parasites co-infection status</td>
<td>3</td>
<td>2016.9846</td>
<td>672.3282</td>
<td>0.941</td>
<td>3.59</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td>4</td>
<td>1617.9393</td>
<td>404.4848</td>
<td>0.566</td>
<td>3.36</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>7859.0421</td>
<td>714.4583</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>11,493.966</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic and economic characteristics of respondents

The average age of the respondents in this study was 27 years. This result is slightly higher than that reported in Malawi, Tanzania and Zambia where the mean age of respondents was 25 years (Mwinga et al., 2009). The distribution of respondents by age group shows that majority (57%) were in 20 – 30 years age group. This indicates that most women in the study area become pregnant during this period when they are most active sexually. This finding is similar to those reported in Nigeria where the highest proportion of women infected with HIV was in the 20-29 years age group (Ekwempu et. al., 2012). The distribution of respondents by marital status shows that majority (79%) were married. This means that majority of the population of pregnant women in the study area were married. The proportion of pregnant women who were married in the study area is low compared to 91.5% of those in Malawi, Tanzania and Zambia (Mwinga et al., 2009). None of the respondents reported a widow which is contrary to that reported in the Kenya AIDS Indicator Survey (NASCOP, 2014) where the majority of people living with HIV reported to have ever been widowed. HIV respondents who reported their marital status as single did not state whether their being single was as a result of death of their spouse or not. However, the reason for not disclosing was not established in this study.

Majority 67 % (n=44) of HIV positive respondents and 78% (n=51) of HIV negative respondents in this study had attained secondary and tertiary education. This means that majority of the population of pregnant women in the study area have attained secondary education. But it was interesting to note that 3% (n=2) of HIV positive respondents had no
formal education. This result is similar to that stated in Kenya AIDS Indicator Survey (NASCOP, 2014) that HIV prevalence among women was highest among those reporting secondary or higher education and lowest among women reporting no primary education. The proportion of respondents in this study with no formal education among pregnant women found in this study is lower compared to 21% previously reported in coastal Kenya (McClure et al., 2014). The proportion of respondents who had completed secondary and above level of education in this study is higher than 52% reported in Papua New Guinea (Phuanukoonnon et al., 2013). Majority 66% (n= 86) of respondents were employed indicating that most of women who were pregnant in the study area were employed. This result is comparable to that described in Kenya AIDS Indicator Survey (NASCOP, 2014) that HIV prevalence was higher among women who were currently employed. However, majority (73%) of respondents earned monthly income of Kshs 5,000 or less meaning that, majority of the population of pregnant women in the study area though employed, were low income earners. This could be due to the fact that most of the respondents were in self-employment that earned low income.

5.1.2 Prevalence of co-infection of HIV and intestinal parasites among respondents

The over all intestinal parasites prevalence 35.38% (n=46) reported in this study is low compared to that found in other developing countries such as Papua New Guinea (81%), Ecuador (93%), Venezuela (74%) and Indonesia (70%) (Weigel, 1996; Rodriguez-Morales, 2006; Meloni et al., 2011; Phuanukoonnon et al., 2013). Among the 130 respondents, one respondent was infected with Hymenolepis nana. The number of Hymenolepis nana cases found in this study varies with 4 cases reported previously in Uganda by Woodburn et al. (2009). The prevalence of helminths, 0.8%, found in this study indicates that majority of the population of pregnant women in the study area were not infected with intestinal helminths. This prevalence is low compared to 31% reported in Papua New Guinea. Intestinal helminths
reported in Guinea included *Necator americanus, Ascaris lumbricoides, Strongyloides stercoralis* and *Trichuris trichiura* (Phuanukoonnon *et al.*, 2013). The variation in intestinal helminths prevalence could be attributed to difference in geographical areas of the study, where Nyeri County is in highlands with cold climate.

Colonization by the intestinal protozoan parasites *Entamoeba coli* and *Iodamoeba butschlii* were the most common in this study compared to *Entamoeba histolytica* and *Giardia lamblia* which were most common in Papua New Guinea. Prevalence of intestinal protozoan parasites found in this study was higher than that of intestinal helminths. This finding is similar to that reported in Papua New Guinea that infections with intestinal protozoan among pregnant women were more prevalent than infections with intestinal helminths (Asma *et al.*, 2011; Phuanukoonnon *et al.*, 2013). In this study more HIV negative pregnant women 27(41.5%) were infected with intestinal protozoan compared to 26.2% (n=17) of pregnant women infected with HIV. This is contrary to what is known that people infected with HIV are at high risk of intestinal parasite infection. This observation may be attributed to the likelihood that HIV positive pregnant women being aware of their health status were more careful about their health. The prevalence of protozoan infection among HIV positive respondents found in this study is higher than 10% reported by Dibua *et al.* (2013). This may be attributed to the fact that majority 51% (n=33) of HIV positive and HIV negative respondents 55% (n=36) in this study did not boil their drinking water. They reported that they did not see the point of boiling water because they believed that the tap water distributed from Nyeri Water and Sewerage Company was treated.

### 5.1.3 Practices that would predispose respondents to intestinal parasites infection

The distribution of respondents by eating soil during pregnancy showed that 35% had eaten soil during the pregnancy. This implies that soil eating practice was high among women who
were pregnant in the study area. However, the prevalence of soil eating practice found in this study is lower compared to 46.9% reported previously in Western Kenya by van Eijk et al. (2009). In this study 28% of pregnant women reported having walked barefoot during the pregnancy. This implies that high proportion of the pregnant women in the study area walk barefoot during pregnancy. The percentage of pregnant women walking barefoot found in this study is lower compared to 69% reported in Ethiopia by Getachew et al. (2012).

In this study nearly half of women did not treat their drinking water. This finding is comparable to that described by Amuta et al. (2010) where 50.8% of women used unsafe water for drinking. However, in Western Kenya a higher proportion (63.3%) of pregnant women reported drinking untreated water (van Eijk et al., 2009). It was observed that majority (80%) of respondents in this study could easily access latrine for defecation since they had their own latrine. This implies that majority of pregnant women in the study area own a latrine and therefore to a large extent there is proper disposal of human faecal matter. This finding is similar to that reported by Akinbo et al. (2010) where 80% of respondents reported having a toilet facility. It is important to note that 20% reported that they shared a latrine with their relatives or fellow tenants. This could not ensure high standards of sanitary conditions. This may be due to lack of cleanliness of the latrine when the latrine is shared by more than one household. This may have contributed to high proportion 34.6% (n=45) of respondents who had intestinal protozoan parasites infection in this study.

5.1.4 Factors associated with intestinal parasites infection

In this study age was not significantly associated with intestinal parasites infection among pregnant women. This finding is comparable to that reported in Papua New Guinea by Phuanucoonnon et al. (2013) that age was not associated with intestinal parasite infection in
pregnant women. However, this finding is not similar to those reported in Ghana by Yatich et al. (2009) and in Nigeria by Akinbo et al. (2010) that young age is associated with intestinal parasites infections. Marital status was not significantly associated with intestinal parasite infection in pregnant women. In this study low education was significantly associated with intestinal parasites infection in pregnant women. This finding is comparable to that reported by Akinbo et al. (2010) that low education is associated with intestinal parasites infection. In this study unemployment was associated with intestinal parasites infection in pregnant women. This finding is similar to that reported in Nigeria by Akinbo et al. (2010) and Dibua et al. (2013) that occupation is associated with intestinal parasite infections among HIV patients. Eating soil was not associated with intestinal protozoan parasites infection indicating that soil transmission is not an important way of transmission of intestinal protozoan parasites. Eating soil during pregnancy in this study was not associated with intestinal helminths infection. This finding is similar to that reported in Western Kenya by van Eijk et al. (2009) and in Zanzibar by Young et al. (2007). The reason why there was no geohelminths reported in the study may be due to self-medication using anti-helminths and cold climate of the study area.

In this study HIV infection was marginally not significantly associated with intestinal parasites infection. This finding is not similar to those previously reported in Nigeria (Akinbo et al., 2010; Dibua et al., 2013). This may be attributed to small sample size compared to that reported by other studies. In this study women who lacked safe drinking water were infected with intestinal protozoan. Similarly in this study respondents who shared latrine with relatives or fellow tenants were infected with intestinal protozoans. These results are comparable to those previously reported by Akinbo et al. (2010) and Dibua et al. (2013) that lack of safe drinking water, poor hygienic and sanitary conditions were associated with
intestinal parasite infection. Unemployed pregnant women were more likely to drink unsafe water and being exposed to low sanitary conditions. This may explain the observed high prevalence of intestinal protozoans in this group.

5.1.5 Effects of HIV and intestinal parasite co-infection on white blood cells, red blood cells, haemoglobin, haematocrit and platelets of respondents

Pregnancy is associated with leucocytosis which begins in the second month of pregnancy through third trimester when the total white blood cells may range from $9 \text{ – } 15 \times 10^9$ cells / L (Kuvin and Brecher, 1962, Paidas and Hossain, 2010). The distribution of respondents in this study by white blood cells count shows that majority (51.5%) had a count in the range $4 - 7.9 \times 10^9$ cells /L. This means that majority of the population of pregnant women in the study area had normal white blood cells count. The white blood cells count was within the ranges of those described in Malawi, Tanzania and Zambia (Beers et al., 2006; Mwinga et al., 2009).

Majority (76.9%) of respondents in this study had normal red blood cells count and 28.4 % had packed cell volume (haematocrit) below normal range (< 34%). Twenty six per cent had anaemia (Hb < 11 g/dl); among them, 24% had mild anaemia (9 – 10.9 g/dl) and 2% had moderate anaemia (7 – 8.9 g/dl). The prevalence of anaemia found in this study is low compared to 56% mild anaemia and 6% moderate anaemia reported in Papua New Guniea (Phuanukoonnnon et al., 2013). The distribution of respondents by platelets count shows that majority (72%) had a count in the range $140 - 279 \times 10^9$ cells /L. This indicates that most women in the study area who were pregnant had normal platelets count. Nevertheless, it is important to note that 2% of the respondents had thrombocytopenia. The prevalence of Thrombocytopenia found in this study is low compared that described by Burrows and Kelton in about 8% of pregnancies (Burrows and Kelton 1993; Burrows and Kelton,1998). This thrombocytopenia can be attributed to pregnancy because, pregnancy is a relatively
hypercoagulable state with an increased platelet activity and consumption (Valera et al., 2010). This increased platelet activity and consumption combined with the hemodilution state, leads to mean platelet count that is slightly lower than in the non-pregnant state (Giles and Inglis, 1981; Mathews et al., 1990; Paidis and Hossain, 2010)

White blood cells count results of pregnant women co-infected with HIV and intestinal protozoan parasites compared to those of pregnant women not infected with HIV were significantly different. This finding is like to that described in Malawi, Tanzania and Zambia by Mwinga et al. (2009) that HIV infection lowers white blood cells count in pregnant women. The results of this study show that co-infection of HIV and intestinal protozoan was associated with decreased red blood cells among pregnant women. This finding is similar to those reported by Davis and Zaul (1995) and Mwinga et al. (2009) that HIV infection lowers red blood cells count in pregnant women.

In this study, co-infection of HIV and intestinal parasites was significantly associated with anaemia in pregnancy. This finding is similar to that reported in Tanzania, Zambia and Malawi by Massawe et al. (1999) and Mwinga et al. (2009). The likely cause of anaemia in these respondents is suppression of haematopoiesis and destruction of red blood cells. The other reason could be the treatment for HIV, since those pregnant women who were HIV positive were under ARV regimen: Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine (NVP) or Efavirenz (EFV). Zidovudine has been associated with anaemia in HIV patients and may have contributed to anaemia observed in HIV positive respondents in this study. The mean maternal haemoglobin level in women not infected with HIV in this study was higher than values reported elsewhere for pregnant women in Africa (mean = 12.1g/dL) while that of HIV infected pregnant women was lower (Beers et al., 2006). However, the mean maternal haemoglobin level in HIV infected pregnant women in this study was higher than that
reported in Malawi, Tanzania, Zambia and India (11.1 ± 1.6 g/dl) (Devi et al., 1989; Mwinga et al., 2009). There was no association between intestinal parasites infection and anaemia in pregnancy. This result is similar to that found in Papua New Guinea (Phuanukoonnon et al., 2013). This result differs with that reported in Rwanda that helminth infection was associated with anaemia in pregnancy (Ivan et al., 2012). The reason for this finding may be due to low prevalence of helminth found in the study area where only one case of intestinal helminth was found.

In this study, co-infection with HIV and intestinal protozoan parasites was associated with haematocrit or packed cell volume in pregnancy. This finding is similar to that of Mwinga et al. (2009), and Ekwempu et al., (2012) that haematocrit level is low in people infected with HIV. The results are also similar to those reported in Bailere and in Nigeria that HIV infection in pregnant women causes reduction in packed cell volume and hence predisposes them to anaemia (Davis and Zauli, 1995; Nneli and Egene, 2007). In this study, intestinal parasite infection alone was not associated with haematocrit in pregnancy. Mean platelets count of HIV positive pregnant women was higher at 251 compared to 248 of HIV negative pregnant women. This result is similar to the finding reported in Tanzania, Malawi and Zambia by Mwinga et al. (2009) that HIV infection increased the mean platelets count in pregnant women. Infection with intestinal protozoan parasites alone was not associated with platelet count in pregnancy. Although intestinal protozoan parasite infection alone was not significantly associated with haematological parameters during pregnancy in this study; the fact that co-infection with HIV was significantly associated with haematological parameters apart from platelets; is an indicator that Entamoeba coli could be pathogenic. This finding is similar to results reported by Kaya et al. (2005) that Entamoeba coli could be pathogenic. In
this study, there was no control for the effects of antiretroviral therapy on haematological parameters among HIV positive pregnant women thus making it a limitation of this study.

5.1.6 Summary of discussion

The specific objectives of this study were to (i) establish the prevalence of co-infection with HIV and intestinal parasites among pregnant women, (ii) establish practices that would predispose pregnant women to intestinal parasites infection, (iii) identify factors associated with intestinal parasite infection among pregnant women, and (iv) determine the effects of HIV-Intestinal parasite co-infection on white blood cells, red blood cells, haemoglobin, haematocrit and platelets, among pregnant women. The results of this study show that the specific objectives were achieved where the prevalence of co-infection of HIV and intestinal parasites infection among pregnant women was found to be 26.2%. Some pregnant women ate soil, walked barefoot, did not boil drinking water, and did not own a latrine but shared with relatives and fellow tenants. Co-infection of HIV and intestinal parasite caused reduction of white blood cells, red blood cells, haemoglobin, and haematocrit. However, the co-infection did not have significant effect on platelets during pregnancy. These findings are comparable to the results reported in other countries in Africa such as Malawi, Tanzania, Zambia and Nigeria.

5.2 Conclusions

i. Co-infection of HIV-1 and intestinal parasites among pregnant women was 26%

ii. Among practices that predisposed pregnant women to intestinal parasites infection was sharing of latrine among others such as eating soil, drinking untreated water and walking barefoot that were not significantly associated with intestinal parasite infection.
iii. Among factors that were significantly associated with higher prevalence of intestinal parasites among pregnant women was low education and unemployment.

iv. Co-infection of HIV-1 and intestinal protozoan parasites lowered white blood cells, red blood cells, haemoglobin, and haematocrit during pregnancy. Co-infection of HIV and intestinal protozoan parasites did not significantly lower platelets during pregnancy.

5.3 Recommendations

Based on this study it is recommended that:

i. Ministry of health should make diagnosis of intestinal parasites a routine practice in antenatal healthcare services provision.

ii. Every household in Kenya should be encouraged to own a latrine

iii. Communities should be encouraged to embrace education to minimize the number of women with no formal education and devolved governments should empower women by providing opportunities for business

iv. At antenatal clinic, in addition to HIV, management of intestinal parasites should be considered to reduce co-infection among pregnant women which impact negatively on haematological parameters and possibly growth of the foetus.

5.4 Suggestion for further research

Considering this study was facility based there is need to determine the burden of intestinal parasites through community based study that will include general population including pregnant women.
REFERENCES


UNICEF (2002). Human Helminth Infections in Southeast Asia, a report to UNICEF East Asia and pacific Regional Office by Dr Simon Brooker of London School of Hygiene and Tropical Medicine. London, UK.


APPENDICES

Appendix I: Ethical Approval Kenyatta University

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@kun.ac.ke
secretary.kuerc@kun.ac.ke
ercha2008@gmail.com
Website: www.kun.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
Fax: 87112428711575

Our Ref: KU/R/COMM/51/381

Date: 19th November, 2014

Anthony Wanjohi Nyambura
Kenyatta University,
P.O Box 43844,
Nairobi.

Dear Wanjohi,

RE APPLICATION NUMBER PKU/261/I 237—“EFFECTS OF HIV-INTESTINAL PARASITES CO-INFECTION ON SELECTED HEMATOLOGICAL PARAMETERS AMONG PREGNANT WOMEN IN SELECTED HEALTH FACILITIES IN NYERI COUNTY, KENYA” –VERSION 2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic “Effects of HIV-intestinal parasites co-infection on selected hematological parameters among pregnant women in selected health facilities in Nyeri County, Kenya” Version 2 received on 19th November, 2014.

2. APPLICANT

Anthony Wanjohi Nyambura, Community Health.

3. STUDY SITE

Nyeri County, Kenya, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 19th November, 2014.

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return the copy of this letter.

[NICOLAS K. GIKONYO]
CHAIRMAN ETHICS REVIEW COMMITTEE

I hereby accept the advice given and will fulfill the conditions therein.

Signature: __________________________ Dated this day: __________________________ 2014.

cc. Vice-Chancellor
Appendix II: Graduate School Approval Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
      dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

FROM: Dean, Graduate School
TO: Mr. Anthony Wanjohi Nyambura
     C/o Community Health Dept.
     Kenyatta University

DATE: 16th September, 2014
REF: P97/25465/11

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting of 8th May 2013 approved your Ph.D. Research Proposal entitled “Effects of HIV-Intestinal Parasites Co-Infection on Selected Hematological Parameters among Pregnant Women in Selected Health Facilities in Nyeri County, Kenya”.

You may now proceed with your Data collection, subject to clearance with the Principal Secretary, Higher Education, Science and Technology.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University’s Website under Graduate School webpage downloads.

Thank you.

JOSEPHINE KENDI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Community Health Dept.

Supervisors:

1. Prof. Ephraim W. Kabiru
   C/o School of Public Health
   KENYATTA UNIVERSITY

2. Dr. Michael Gicheru
   C/o Zoological Sciences Dept.
   KENYATTA UNIVERSITY

JK/cao
E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

KENYATTA UNIVERSITY
GRADUATE SCHOOL

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

OUR REF: P97/25465/11

Date: 22nd September, 2014

The Principal Secretary,
Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. ANTHONY W. NYAMBURAREG.NO. P97/25465/11

I write to introduce Mr. Nyambura who is a Postgraduate Student of this University. He is registered for Ph.D. Degree programme in the Department of Community Health in the School of Public Health.

Mr. Nyambura intends to conduct research for a proposal entitled, “Effects of HIV-Intestinal Parasites CO-Infection on Selected Hematological Parameters among Pregnant Women in Selected Health Facilities in Nyeri County, Kenya”.

Any assistance given will be highly appreciated.

Yours faithfully,

[Signature]

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

JK/cao
Appendix IV: National Commission for Science Technology and Innovation Research Permit

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two (2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice.

THIS IS TO CERTIFY THAT:

MR. ANTHONY WANJIOHI NYAMBURA

of KENYATTA UNIVERSITY, 43844-100

naairobi, has been permitted to conduct research in Nyeri County

on the topic: EFFECTS OF HIV-INTESTINAL PARASITES

CO-INFECTION ON SELECTED HAEMATOLOGICAL PARAMETERS AMONG PREGNANT WOMEN IN SELECTED HEALTH FACILITIES IN NYERI COUNTY, KENYA

for the period ending: 31st December, 2016

Applicant’s Signature

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Serial No. A 4356

RESEARCH CLEARANCE PERMIT

Republic of Kenya

National Commission for Science, Technology and Innovation

Permit No: NACOSTI/P/15/8548/4613

Date of Issue: 6th March, 2015

Fee Received: Ksh 2,000

National Commission for Science, Technology and Innovation

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NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471, 2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacost.go.ke
Website: www.nacost.go.ke
When replying please quote
Ref: No.

Date: 6th March, 2015

NACOSTI/P/15/8548/4613

Anthony Wanjohi Nyambura
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Effects of HIV–Intestinal parasites co-infection on selected haematological parameters among pregnant women in selected health facilities in Nyeri County, Kenya,” I am pleased to inform you that you have been authorized to undertake research in Nyeri County for a period ending 31st December, 2016.

You are advised to report to the County Commissioner, the County Director of Education and the County Coordinator of Health, Nyeri County before embarking on the research project.

On completion of the research, you are required to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

Said Hussein
FOR: DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Nyeri County.

The County Director of Education
Nyeri County.
Appendix VI: Nyeri County Research Clearance

COUNTY GOVERNMENT OF NYERI

P.O. BOX 110-10100
Telephone
Fax No.
NYERI

Email: nyericountyhealth@yahoo.com

DEPARTMENT OF HEALTH SERVICES

OUR REF: CP/CIRC/20/39
Date: 25th March 2015

- THE MEDICAL SUPERINTENDENTS’
- HOSPITAL DIRECTORS
- SUB COUNTY MEDICAL OFFICERS’ OF HEALTH
- FACILITY INCCHARGES

RE: RESEARCH AUTHORIZATION

This is to inform you that the bearer of this letter Mr. Anthony Wanjohi Nyambura is a student at Kenyatta University and is hereby authorized by this office to conduct Research on “Effects of HIV-Intestinal parasites co-infection on selected haematological parameters among pregnant women in selected health facilities in Nyeri County” for a period ending 31st December 2016.

The purpose of this letter is therefore to request you to accord him the necessary assistance.

The researcher MUST deposit a copy of final research findings with the department following completion of the same.

Date: 25.03.15

DR. NELSON MURUJU
COUNTY EPIDEMIOLOGIST
For: COUNTY DIRECTOR OF HEALTH SERVICES
NYERI
Map of Nyeri County Showing the location of the study Hospitals. (Source: MFL: Master Facility List MOH (2015) and Nyeri County Government 2010))
Appendix VIIIA: Consent Form (English Version)

Informed Consent

My name is Anthony Wanjohi Nyambura. I am a PhD student from Kenyatta University. I am conducting a study on “Effects of HIV-intestinal parasite co-infection on selected haematological parameters among pregnant women in selected health facilities in Nyeri County, Kenya”

Procedures to be followed

Participation in this study will require that I ask you some questions and also examine you in order to screen for HIV infection and Intestinal parasite infection. Some specimen will be taken from you for further test. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in the future.

Benefits

If you participate in this study you will help us to learn how to provide effective screening services that can improve the health of women and reduce the risk of HIV and Intestinal parasite infection. You will also benefit from being screened for HIV and Intestinal parasite infection and if you are found to have a problem, you will be advised on treatment.

Reward

If you agree to participate in this study, lunch will be provided and transport expenses will be reimbursed.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. The questionnaires will be kept in a locked cabinet for safe keeping at Kenyatta University. Everything will be kept private.
Contact information
If you have any questions you may contact Prof. Ephantus W. Kabiru Supervisor 1 on 0721998558/0733805863 or Dr Michael Gicheru Supervisor 2 on 0722609765 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke ercku2008@gmail.com

Participant’s statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of participant ………………………………………………………………………………

_________________________________                       ____________________
Signature or Thumbprint                    Date

Investigator’s Statement

I, the undersigned, have explained to the volunteer in a language s/he understands, the procedures to be followed in the study and the risks and benefits involved

Name of interviewer ………………………………………………………………………………

_________________________________                       ____________________
Interviewer signature                    Date
Appendix VIIIIB: Translated Consent Form (Kikuyu)

Gwitikira kumanite na umenyo


Mutaratara uri ukarumiriruo

Kunyitanira thiini wa uthuthuria uyu kurabatara nii ndimurie ciuria na guthurima kana wina murimu wa HIV na tugunyu twa nda(minyoo). Thakame nini, na kioro giaku ni ikubatarania nigo gutuithiriria gwika utuiria uyu. Maumirira ma utuiria uyu ni ngumandika iratathiini ria uthuthuria.

Wina kihoto gia kurega kunyitanira thiini wa uthuthuria uyu. Niuguthii nambere kwamukira urigitani ota uria umukagira hau mbere wona wetikira kana warega kunyitanira uthuthuriani uyu. Itua riaku ritiguchenjia urigitani uria ukumwakira thiini wa thibitari ino kana uria ukamukira thibitari ingi yothe o ihinda riothe.

Ririkana ati kunyitanira thiini wa uthururia uyu ni kwiruturwa gwaku. No urie kiuria o gioethe gikonanie na uthuthuria uyu o ihinda riothe.

No urege gucokia kiuria o giothe na no urugamie uthururia uyu o ihinda riothe. Ningi no urugamie gukoruo uthuthuriaini uyu ihinda riothe gutari thiina kuri urigitani uria wamukagira kuma thibitariini ino kana thibitari ingi yothe riu kana matuku maguka.

Mauguni ma uthuthuria uyu

Angikoruo niukunyitanira uthuthuriani uyu ni ugututeithiria guthoma uria tungiheana urigitani mukinyaniru uria unghothithia kwagiriria ugima wa mwiri wa atumia na kunyihia ugwati wa kugwatu o ni murimu wa HIV na minyoo ya nda. Ningi niukugunika na njira ya guthurimwo murimu wa HIV na minyoo ya nda na ungimenyeka ati wina thiina, niukuheo mataro makonie urigitani.

Kiheo

Ingikoruo niugwitikira kunyitanira uthuthuriani uyu, niukuheo irio cia mutheya na ucokerio mahuthiro ma tigiti.

Thiri

Uthuthuria uyu ugwikiruo handu keheriini thiini wa thibitari ino. Ritwa riaku ritikwandikuo iritathiini ria uthuthuria. Iratathi ria uthuthuria rikugwao thiini wa kabati ihingituo na kuburi handu hari na ugitiri muiganu shukuruini wa Kenyatta University. Maundu mothe makuiguo mari thiri.
Njira ya kwaraniria

Ungikoruo na kiuria o giothe no waranirie na Prof. Ephantus W. Kabiru kuhetukira 0721998558/ 0733805863 kana waranirie na Dr Michael Gicheru kuhetukira 0722609765 kana waranirie na mwandiki wa kamitii ikonie maundu ma uthuthuria ya Kenyatta University kuhetukira chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com

Ciugo cia munyaianiria uthuthuriani


Ritwa……………………………………………………………………………………………………..

________________________________                         ________________________

Kirore                                                                        Date

Ciugo cia muthuthuria

Nii,ndekira kirore haha, nindatariria mutumia uyu werutira kunyitanira uthuthuriani uyu, mutaratarata, ugwati na mauguni ma uthuthuria uyu na ruthiomi akunyita.

Ritwa……………………………………………………………………………………………………..

________________________________                         ________________________

Kirore                                                                        Mweri
Appendix X: Questionnaire

(A) Basic Information

1. Date of interview_________________________
2. Study site_____________________________
3. Code of the interview____________________

(B) Socio-Demographic Information

4. Age in years______________
5. What is your current marital status?
   (1) Married [ ]
   (2) Separated/Divorced [ ]
   (3) Widowed/widower [ ]
   (4) Single [ ]
6. If married, state if it is?
   (1) First [ ]
   (2) Second [ ]
   (3) Third [ ]

(C) Socio-economic

8. What is your level of education?
   (1) None [ ]
   (2) Primary [ ]
   (3) Secondary [ ]
   (4) College/University
9. What is your occupation?
   (1) Student [ ]
   (2) Employed [ ]
   (3) Self-employed [ ]
   (4) Unemployed [ ]
10. What is your level of income in a month? Kshs______________

D) Antenatal care services

11. What is the duration of pregnancy (No of weeks)?______________

12. How many times have you attended clinic so far?______________

13. Weight at previous visit _______________Current Weight _______________

14. What is your height?_____________________

15. What is your Mid Upper Arm Circumference?_____________________

16. What is your BMI? _________________________________

17. Other than this clinic do you seek health services elsewhere?

➢ Other clinics___________Traditional Birth attendants_____________

18. Do you experience any of the following?

➢ Tiredness ____ Rapid Heart Beat ____Blood in urine ____Abdominal Pain_______

19. Blood pressure at current visit_____________________

(E) HIV related factors

20. Have you been tested for HIV during this pregnancy? (1) Yes [ ] (2) No [ ]

21. If yes, what is your HIV status?

(1) Positive [ ]

(2) Negative [ ]

(3) Not received results yet [ ]

22. Were you counselled before undergoing HIV test Yes [ ] No [ ]

(F) Intestinal parasites related factors

23. What is your source of water (1) Tap water [ ] (2) River [ ] (3) Bore hole [ ]

24. Do you treat your drinking water? (1) Yes [ ] (2) No [ ]

25. How do you treat your drinking water? (1) Boiling [ ] (2) Using chemicals [ ]

26. Do you eat soil/stones? (1) Yes [ ] (2) No [ ]

27. Does your household have a toilet/latrine? (1) Yes [ ] (2) No [ ]

28. If No, where do you go to answer long call of nature? (1) Bushes [ ] (2) Open field [ ]

29. Do you use human faeces as fertilizer? (1) Yes [ ] (2) No [ ]

30. Do you walk bare foot? (1) Yes [ ] (2) No [ ]
31. Have you been exposed to stagnant water near your house? (1) Yes [ ] (2) No [ ]
32. Have you ever been tested for intestinal parasites before? (1) Yes [ ] (2) No [ ]
33. If yes, what were the results (1) Positive [ ] (2) Negative [ ]
34. If the results were positive, did you receive any treatment? (1) Yes [ ] (2) No [ ]
35. Have ever received any health information about intestinal parasites? (1) Yes (2) No [ ]
36. If yes, where did you get this information from? (1) Health care provider [ ]
      (2) Radio [ ] (3) TV [ ]
37. Are you satisfied with the services you have received in this clinic Yes [ ] Non [ ]

END OF INTERVIEW. THANK YOU
Appendix XI: Blood Sample Analysis Result Sheet

1. Haemoglobin ______________________________

2. Red blood cells(erythrocytes) count ________________________

3. Haematocrit ______________________________

4. White blood cells(leukocytes) count__________________________

5. Platelets (thrombocytes) count ______________________________
## Appendix XI: Stool Sample Analysis Result Sheet

<table>
<thead>
<tr>
<th>Intestinal (helminths)</th>
<th>Macroscopic diagnosis (Adult worm present)</th>
<th>Microscopic diagnosis (Egg/larva present)</th>
<th>Infection intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>eggs per gram of faeces (epg)</td>
</tr>
<tr>
<td>Hook worm(A. duodenale)</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hook worm(N. americanus)</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Strogyloides stercoralis</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Trichostrongylus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestinal protozoans</th>
<th>Microscopic diagnosis (protozoa present)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td></td>
</tr>
<tr>
<td>Entamoebes histolytica</td>
<td></td>
</tr>
<tr>
<td>Entamoeba cola</td>
<td></td>
</tr>
<tr>
<td>Iodamoeba butschlii</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td></td>
</tr>
<tr>
<td>Microsporidium spp</td>
<td></td>
</tr>
<tr>
<td>Isospora belli</td>
<td></td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td></td>
</tr>
</tbody>
</table>
Appendix XII: Standard Operation Procedure: Analysing Samples Using Medonic M-Serie 3-Part Haematology Auto Analyser by Boule Medical AB. Stockholm Sweden

1 Purpose: To provide guidelines for sample analysis using Medonic M-series analyser

2 Responsibilities

2.1 All laboratory staff were responsible for compliance with these SOP and to notify the quality assurance officer or laboratory in charge when changes in practice occurred that warranted review

2.2 Lab Director or Designee is responsible for approval of all documents

3 Terms and Abbreviations

3.1 NPGHL-Nyeri Provincial General Hospital Lab

3.2 QC- Quality Control

4 Materials and Equipment

4.1 Medonic M-series analyser

4.2 Printer

4.3 Blood mixer

4.4 EDTA whole blood

4.5 Printing papers

4.6 Controls

4.7 Reagents

5 Procedures

5.1 Principle: the measuring principle is based on impedance and spectrophotometric principle. In medonic M-series the number of cells for determining the RBC and WBC values is counted from suspension of 1:40,000 for RBC and 1:400 for the WBC ratio of whole blood. For WBC differential, the medonic M-series uses a floating discriminator technology which performs a mathematical calculation to estimate the best separation between three populations of WBCs (lymphocytes, granulocytes and mid-cell fractions)

5.2 Analyzer start-up

5.2.1 Check the waste container if it needs to be emptied

5.2.2 Verify that the printer is ready and has paper

5.2.3 Verify the reagent levels manually

5.2.4 Turn on the machine at the back
5.2.5 Press (“exit stand –by”) or (pwrup), depending on how the analyser was shut down previously to wake up the analyser

5.2.6 When wake up cycle is complete, press start plate to begin the first step of the start-up sequence. When complete the background count results are displayed that is background check (acceptable)

5.2.7 Results: WBC ≤ 0.01(10^9L)
RBC ≤ 0.01(10^12L)
HGB ≤ 0.2 (g/dL)
PLT ≤ 0.01(10^9L)

5.2.8 Check values for system information messages to make sure the system is performing to specification with clear back ground measurements

5.2.9 If the background count results have high indicator, press the re-run and follow the scree instructions to analyse background count again

5.2.9.1 If the results are acceptable, run the controls by selecting samples, then con/cal when complete, the control results are displayed. If control results are acceptable, press (re-run) to run next level of control

5.2.9.2 The start-up sequence is complete when all control results are acceptable

5.2.9.3 Press analyse samples to go to the main screen to analyse the samples

5.3 Sample analysis

5.3.1 Human samples should be collected in an EDTA (K₃ or K₂) tube in sufficient quantity and gently mixed

5.3.2 Capillary blood samples should be collected in BD microtainer (K₂ EDTA)

5.3.3 Samples should be analysed between 15 minutes of collection and six hours for most accurate results. The blood should be allowed to adapt to the EDTA for 10- 15 minutes after sampling

4.3.4 The sample should be thoroughly and gently mixed before analysis. It is recommended to use a mixer (i.e. 10 -15 minutes)

5.3.5 All samples for analysis should be kept at room temperature. Excessive cold or heat could cause erroneous results. Always wear protective gloves

5.3.6 All samples should bear a unique identifier

5.3.7 From the main screen press (new sample)

5.3.8 Enter sample special number, press OK

5.3.9 Open the sample cup; introduce the sample into aspiration nozzle and start.

5.3.10 A message is displayed when the aspiration is complete. Remove the sample and wait.

5.3.11 The analyser analysis the sample and automatically prints the results. The results are also displayed on the screen
5.3.12 Verify the validity of the results.
5.3.13 Repeat the procedure for all other samples
5.3.14 Review the results for any flagged paying attention to critical results
5.3.15 All reports should be reviewed by a second person prior to dispatch
5.3.16 At the end of the day clean the sample probe and the probe rinse cup using a paper tissue moistened with a 70% alcohol solution to remove any residual blood and salt crystals

5.4 **Calibration:** The instrument has been calibrated prior to shipment. The analyser however requires regular checks and calibration of the measured parameters. (Refer to manual for calibration procedure)

5.5 **Quality control:**

5.5.1 It is advisable that the performance of the Medonic m-series analyser be checked daily with a certified blood control (authorized by boule). **At least 2 levels of controls must be run and pass before patients samples are analysed**

5.5.2 Controls may also be used for trouble shooting purposes
5.5.3 Also used when changing to new lot of reagent, to check damage during transport
5.5.4 Never use controls beyond expiry date
5.5.5 Construct a Levy Jennings chart for WBC, RBC, HB and Platelets for all the control levels at the end each month. This is used to monitor trends, bias and shift.

5.6 **Maintenance:**

5.6.1 Daily cleaning: clean the aspiration and pre-dilute needles using a paper tissue with 70% alcohol solution. Remove possible traces of salt crystals or blood at the top of aspiration and predilute needles, probe rinse cup, using a paper tissue moistened in a disinfecting solution.

5.6.2 Monthly cleaning: As indicated in operation manual

5.6.3 Preventive maintenance: The maintenance should be performed at the following interval by local distributor annually or 20,000 samples

6. **References**

6.1 Medonic m-series user’s manual

6.2 NPGHL QA manual NPGH/LAB/PAM-QM001
Appendix XIII: Haematological Parameters Ranges According to Medonic M-series 3-Part Haematology Autoanalyser by Boule Medical AB. Stockholm Sweden

Normal range for white blood cells is between $4 \times 10^9$ / L of blood sample
Upper limit for white blood cells is $10 \times 10^9$ / L of blood sample
Lower limit for white blood cells is $4 \times 10^9$ / L of blood sample

Normal range for red blood cells is between $3.85 \times 10^{12}$ / L of blood sample
Upper limit for red blood cells is $5.20 \times 10^{12}$ / L of blood sample
Lower limit for red blood cells is $3.85 \times 10^{12}$ / L of blood sample

Normal range for haemoglobin is $11.5 – 16$ g/dl of blood sample
Upper limit for haemoglobin is $16$ g/dl of blood sample
Lower limit for haemoglobin is $11.5$ g/dl of blood sample

Normal range for haematocrit is $34.7 - 46\%$ of blood sample
Upper limit for haematocrit is $46\%$ of blood sample
Lower limit for haematocrit is $34.7\%$ of blood sample

Normal range for platelets is $140 – 440 \times 10^9$ / L of blood sample
Upper limit for platelets is $440 \times 10^9$ / L of blood sample
Lower limit for platelets is $140 \times 10^9$ / L of blood sample
Appendix XIV: Guide on how to collect stool sample

Study participants were provided with universal stool container and scoop. They were also provided with a piece of toilet paper. To avoid contamination of the stool sample the participants were advised to pass stool on the toilet paper and use the scoop provided to scoop about 10 grams of stool and place it into the universal stool container and then cover and tighten the cock. This was done to avoid contamination of the stool sample.
Appendix XV: Formal Ether Concentration Technique for Faecal Specimen Processing (WHO, 1991)

Concentration technique

If the number of organisms in the stool specimen is low, examination of a direct wet mount may not detect parasites parasites. Thus, whenever possible, the tool should be concentrated. Worm eggs, larvae and protozoan cysts may be recovered by concentration but protozoan trophozoites will NOT be seen as they are usually destroyed during the concentration procedure. This makes direct wet mount examination obligatory as the initial phase of microscopic examination. The concentration procedure is indicated when the initial wet mount examination is negative despite the clinical symptoms indicating parasitic infection of a patient and for the detection of Schistosoma and Taenia. The concentration procedure recommended is the formalin – ether (or formalin-ethyl acetate) method. All types of worm eggs (roundworms, tapeworms, schistosomes, and other fluke eggs), larvae, and protozoan cysts may be recovered by this method.

Materials and reagents

1. Applicators sticks, wooden
2. Bottles, dispensing or plastic “squeeze”, 250 ml or 500 ml. The bottles are convenient for adding formalin to the centrifuge tubes. However, any small bottles or flasks may be used
3. Centrifuge, with head and cups to hold 15 ml conical tubes. Sealed buckets must be used
4. Centrifuge tubes, 15 ml, conical (make a graduation at 7 ml and 10 ml with a grease pencil)
5. Cotton swabs
6. Coverslips
7. Funnel
8. Surgical gauze
9. Microscope slides
10. Pipettes, Pasteur, with rubber bulbs
11. Rack or support for tubes
12. Formalin, 10% (reagent no.10).
13. Ether or ethyl acetate
14. Lugol’s iodine, 1% solution – in a dispensing battle with a pipette (reagent no. 18)
15. Saline solution, isotonic (reagent no. 24)

Technique

1. Add 10 ml of 10% formalin to approximately 1 g of faeces and stir using an applicator stick, until you get a slightly cloudy suspension
2. Fit a gauze filter into a funnel and place the funnel on top of the centrifuge tube
3. Pass the faecal suspension through the filter into the centrifuge tube until the 7 ml mark is reached
4. Remove the filter and discard the filter with the lumpy residue
5. Add 3 ml of ether or ethyl acetate and mix well for one minute
6. Transfer back to the centrifuge tube and centrifuge for 1 minute. The tube should have four layers. The top layer is the ether, second layer from top is debris, third layer from top is formalin and fourth layer from top is the sediment
7. Loosen the fatty plug (debris) with applicator stick, and pour away the supernatant by quickly inverting the tube
8. Replace the tube in its rack and allow the fluid on the sides of the tube to drain down to the sediment. Mix well and transfer a drop to a slide for examination under a coverslip. Also make iodine--stained preparation

9. Use the X 10 and X 40 objectives to examine the whole area under the coverslip for ova, cysts, and larvae.

**Examination of sediment**

Mounts of concentrated material should be examined in the same way as described for direct wet mounts. The saline (or unstained) mount should be examined systematically, looking for eggs, larvae, and cysts. If cysts, or structures resembling cysts, are seen, you should examine the iodine mount to see more details. Organism will look the same as described for direct wet mounts. In saline mounts of formalin-ether (or ethyl acetate) concentrate, the nuclei of cysts are fixed and may be visible. However, iodine wet mounts should still be examined for more reliable identification.
Appendix XVI: Tukey Kramer Test Formula

Tukey Kramer test is used when groups being compared are of different sizes.

Tukey Kramer uses another probability distribution called studentized range distribution and critical value \( W_{ij} \) is calculated using the following formula:

\[
W_{ij} = q_{\alpha (k, N-K)} - \text{Upper } - \text{tail } \alpha \text{ critical value of the studentized range distribution where } k \text{ is the number of groups and } N \text{ total number of observations}
\]

\[
W_{ij} = q_{\alpha (k, N-K)} \sqrt{\text{MSE}/2} \left(1/n_i + 1/n_j\right). \text{ Where}
\]

\( k \) = number of groups

\( N \) = total number of observations

\( n_i \) and \( n_j \) are number of observations in population \( i \) and \( j \)

MSE – mean square within