HAEMATO-BIOCHEMICAL, MOLECULAR AND SEROLOGICALSTUDIES ON *BABESIA* AND *THEILERIA* SPECIES INFECTIONS OF HORSES IN PARTS OF NORTHERN NIGERIA

BY

Idoko Sunday IDOKO

DEPARTMENT OF VETERINARY PATHOLOGY,

FACULTY OF VETERINARY MEDICINE,

AHMADU BELLO UNIVERSITY,

ZARIA

MAY, 2021

HAEMATO-BIOCHEMICAL, MOLECULAR AND SEROLOGICALSTUDIES ON *BABESIA* AND *THEILERIA* SPECIES INFECTIONS OF HORSES IN PARTS OF NORTHERN NIGERIA

BY

Idoko Sunday IDOKO,

DVM (ABU) 2010, MSc. (ABU) 2015

P15VTPT9003

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN VETERINARY PATHOLOGY

DEPARTMENT OF VETERINARY PATHOLOGY,

FACULTY OF VETERINARY MEDICINE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

MAY, 2021

DECLARATION

I declare that the work in this Thesis entitled **"Haemato-Biochemical,Molecular and SerologicalStudies on *Babesia* and *Theileria* Species Infections of Horses in Parts of Northern Nigeria"** has been carried out by me in the Department of Veterinary Pathology under the supervision of Professors S. Adamu, O. O. Okubanjo, and Dr E. O. Balogun. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma at this or any other Institution.

Idoko Sunday IDOKO ---------- ----------

Name of Student Signature Date

CERTIFICATION

This Thesis entitled **"Haemato-Biochemical,Molecular and SerologicalStudies on *Babesia* and *Theileria* Species Infections of Horses in Parts of Northern Nigeria"** by Idoko Sunday IDOKO meets the regulations governing the award of the degree of Doctor of Philosophy (PhD)Veterinary Pathology of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

Prof. S. Adamu	----------	----------
Chairman, Supervisory Committee	Signature	Date
Prof. O. O. Okubanjo	----------	----------
Member, Supervisory Committee	Signature	Date
Dr. E. O. Balogun	----------	----------
Member, Supervisory Committee	Signature	Date
Prof. S. Adamu	----------	----------
Head of Department	Signature	Date
Prof. S. Abdullahi	----------	----------
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This Thesis is dedicated to my lovely parents, Mr Pius Itodo Idoko and Mrs Virginia Ojo Idoko for the endless show of love, unconditional sacrifices, fervent prayers and the constant encouragement and faith in me.

ACKNOWLEDGEMENTS

I sincerely thank and appreciate God Almighty, the King of kings and Lord of lords for His immense faithfulness throughout the duration of my study.

My sincerest appreciation and gratitude goes to members of my Supervisory Committee: Professors S. Adamu, O. O. Okubanjo and Dr. E. O. Balogun. Also, the former Vice Chancellor, University of Abuja, Prof. M. U. Adikwu, the management of University Abuja for the financial support to enable me carry out my bench work in the US and the former Dean, Faculty of Veterinary Medicine, University of Abuja, Prof. C. B. I. Alawa for his strong recommendation to University of Abuja Management.

I wish to specially thank Dr. Chudy Nduaka, the President/Founder, Africa Education Initiative, Lionville, USA for sponsoring my airfare to the US, and to Professors. J. A. Ameh and W. D. Nafarnda for the unique opportunities, support and advice towards attaining academic excellence.

My profound gratitude goes to Dr. Massaro Wilson Ueti and Prof. Donald Patrick Knowles for sponsoring my bench work at the USDA-ARS, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, USA. Also to Lowell Kappmeyer, Carl Johnson, Paul Lacy, Jacob M. Laughery, Carlos Suarez, Richard Bishop, Heba F. Alzan, Hala Elsayed, Rubicca and Heiley for their technical support, guidance and encouragement during my bench work at WSU, Pullman. Worthy of note is Dr. George Wudiri for facilitating the collaboration with USDA-ARS, WSU, Pullman and Dr. Cornelius A. Adewale and his wife, Tosin for their accommodation, generosity and hospitality.

Special thanks to my beautiful, loving and caring wife, Chatramada Yakubu Idoko for her patience, tolerance and encouragement and to my handsome heir ‘King David Oche Wamanyi Idoko’ for putting smiles on my face whenever I am stressed out.

Hearty appreciation to my siblings, nephews, Philip W. Mshelia, Richard E. Edeh, Iniobong Ugochukwu, Samson E. Abalaka, Andrew M. Adamu, Sylvester S. Obeta, Joshua B. Balogun, Godswil O. Agbaji, Bamidele E. Owoeye, Isaac Oluwole, Olumese S. Ehbote and Jibril Y. Jibril for their kind assistance in the field and moral support.

My special gratitude goes to my mentor and friend, Prof. C. O. I. Njoku for his constant advice and support.

I also wish to thank the staff of Biochemistry, Clinical Pathology, Protozoology, Veterinary Pharmacology and Toxicology, and Chemical pathology (ABUTH) Laboratories who tirelessly contributed to the success of this work.

Thank you all.

ABSTRACT

Equine piroplasmosis, caused by *Babesia caballi*, *Theileria equi* and *Theileria haneyi*, is endemic and causes mortality and poor performance of horses in Nigeria, which oftentimes predispose to misdiagnosis and development of carrier status. This study sought to characterize the haemato-biochemical alterations in symptomatic and asymptomatic horses with piroplasmosis, in relation to the parasites' biodiversity in horses. A total of 152 horses, comprising 72 symptomatic and 80 asymptomatic horses were sampled. Ticks were collected from symptomatic horses for morphological identification. Blood samples were collected for parasitological, haematological, biochemical investigations, molecular studies andCompetitive Enzyme Linked Immunosorbent Assay. Complete *Babesia* and *Theileria* 18S rRNA and species-specific primers were used to amplify the hypervariable regions, equine merozoite antigen-1 and the coding region on chromosome 1 of the parasites. The PCR positive samples were sequenced, and those suspected of mixed infections were cloned and sequenced.The ticks identified were *Rhipicephalus evertsi evertsi* (58.1 %), *Rhipicephalus species* (23.1 %), *Amblyomma variegatum* (9.2 %), *Hyalomma dromedarii* (6.4 %), *Hyalomma impeltatum* (0.6 %), *Hyalomma truncatum* (0.2 %), *Hyalomma species* (0.7 %) and *Boophilus decoloratus* (1.6 %).Parasitological examinations of Giemsa-stained thin blood smears were all negative. The 18S rRNA PCR revealed 54 positive samples, comparative genomic analysis of the sequences showed five distinct phylogenetic clades, A, B, C, D and E of *Theileria* species with eight samples having multi-clades infection. Symptomatic and asymptomatic horses demonstrated high anti-*T. equi* antibodies (84.7 % and 90 %, respectively)while anti-*B. caballi*antibodies were 0 % and 3.8 % respectively. Three asymptomatic horses, H44, H67 and H78 with mixed *B. caballi* and *T. equi* infections were seropositive to both parasites using cELISA. The

mean serum BUN and creatinine concentrations (5.87 ± 1.80 mmol/L and 153.0±38.10 µmol/L, respectively) in *B. caballi*- and the values (5.87 ± 1.80mmol/L and 153.0±38.10 µmol/L, respectively) in *B. caballi*-*T. equi*-infectedasymptomatic horses were significantly higher ($P < 0.05$) than *T. equi*-infected (1.98 ± 0.15 mmol/L and 83.13±3.45µmol/L, respectively) and non-infected (2.01 ± 0.42 mmol/L and 67.65±9.31µmol/L, respectively) asymptomatic horses. The mean plateletcrit (0.16 ± 0.07 %) in symptomatic horses with multi-clade infectionwas significantly higher ($P < 0.05$) than the values in*T. equi andT. haneyi*(0.06 ± 0.00 %, 0.07 ± 0.01 %, respectively) infected horses. Also, mean percent platelet distribution width (PDW) in *T. haneyi*-infected horses (14.61 ± 0.10 %) was significantly higher ($P < 0.05$) than the value (12.93 ± 0.40 %) in *T. equi*-infected horses. The mean corpuscular haemoglobin concentration (MCHC) (32.90 ± 0.80 g/dL), RDW (19.04 ± 0.22 %) were significantlylower ($P < 0.05$), while mean lymphocyte counts (6.89 ± 1.08 $\times 10^9$/L), mean platelet volume (8.60 ± 0.40 $\times 10^3$/µL), PDW (14.54 ± 0.11%), serum direct bilirubin (0.56 ± 0.05 mg/dL) were significantly higher ($P < 0.05$) in *T. equi*-infected symptomatic Mares compared to Stallions (35.60 ± 0.46g/dL, 20.41 ± 0.28 %, 4.03 ± 0.41 $\times 10^9$/L, 6.92 ± 0.20 fL, 11.84 ± 0.52 %, 0.34 ± 0.04 mg/dL, respectively). The mean neutrophil counts (4.30 ± 1.20 $\times 10^9$/L) and concentrations of globulin (7.50 ± 1.83 g/dL) and phosphate (7.80 ± 0.50 mmol/L) were significantly higher ($P < 0.05$) but albumin (2.90 ± 0.10 g/dL) was significantly lower ($P < 0.05$) in Geldings compared to the values in Stallions (1.81± 0.26 $\times 10^9$/L, 4.27 ± 0.54 g/dL, 3.08 ± 0.42 mmol/L, 6.54 ± 0.47 g/dL, respectively) and Mares (1.40 ± 0.62 $\times 10^9$/L, 3.20 ± 0.53 g/dL, 4.04 ± 0.97 mmol/L, 7.90 ± 0.99 g/dL, respectively). The mean corpuscular volume (MCV) (47.60 ± 0.80 fL) was significantly lower ($P < 0.05$) but MCHC (35.60 ± 0.46 g/dL), RDW (20.41 ± 0.28 %) and indirect bilirubin (0.73 ± 0.10 mg/dL) in*T. equi*-infected symptomatic West African Barb horses were significantly higher ($P < 0.05$) than the

values (50.25 ± 0.74 fL, 33.23 ± 0.61 g/dL, 19.52 ± 0.30 %, 0.38 ± 0.11 mg/dL, respectively)in Argentine Polo Ponies. The MPV (8.45 ± 0.30 fL), PDW (14.52 ± 0.08 %), serum phosphate (5.06 ± 0.88 mmol/L) and direct bilirubin (0.51 ± 0.05 mg/dL) concentrations were significantly higher ($P < 0.05$) in Argentine Polo Ponies than the values (6.92 ± 0.20 fL, 11.84 ± 0.52 %, 3.08 ± 0.42 mmol/L, 0.34 ± 0.04 mg/dL, respectively) in West African Barb horses. The study showed remarkable haemato-biochemical alterations in symptomatic horses than asymptomatic horses without marked oxidative damage. Heterogeneity in the 18S rRNA gene of *Theileria species*in Nigeria exist. Establishing and domesticating haemato-biochemical reference ranges for horses in Nigeria as guide to clinical laboratory diagnosis and interpretation is essential.There is need to carry out routine treatment of horses against piroplasm infection to prevent development of fulminant disease.

TABLE OF CONTENTS

CONTENT **PAGE**

TITLE PAGE i

DECLARATION ii

CERTIFICATION..... iii

DEDICATION iv

ACKNOWLEDGEMENTS v

ABSTRACT.....vii

TABLE OF CONTENTS x

LIST OF FIGURES.....xvi

LIST OF TABLES..... xxiii

LIST OF PLATES xxv

LIST OF APPENDICES xxvii

LIST OF ABBREVIATIONS xxviii

CHAPTER ONE:INTRODUCTION.....1

1.1 Background of the Study 1

1.2 Statement of Research Problem 3

1.3 Justification 5

1.4 Aim of the Study 7

1.5 Objectives of the Study 7

1.6 Research Questions 9

CHAPTER TWO: LITERATURE REVIEW 10

2.1 Background on Equine Piroplasmosis 10

2.2 Historical Perspective of Equine Piroplasmosis 11

2.3 Epidemiology of Equine Piroplasmosis (EP) in Nigeria 13

2.4. Aetiology, Life Cycle and Transmission of Equine Piroplasmosis 15

2.5 Pathogenesis of Equine Piroplasmosis 20

2.5.1 Horse Erythrocyte 20

2.5.2 Anaemia 20

2.5.3 Biochemical alterations 23

2.5.4 Oxidative stress **..... 24**

2.5.5 Immune mechanisms **..... 26**

2.6 Clinical Signs of Equine Piroplasmosis 28

2.7 Gross Lesions of Equine Piroplasmosis 30

2.8 Diagnosis, Diagnostic Features and Antigenic Targets on the Parasites ... 30

2.8.1 Diagnosis 30

2.8.2 Diagnostic features in giemsa-stained blood smears 35

2.8.3 Antigenic targets on the parasites 40

2.9 Chemotherapeutics used in Treatment of Equine Piroplasmosis 40

2.9.1 Imidocarb dipropionate (ID) 41

2.9.2 Bumped kinase inhibitors 1294 (BKIs 1294) 42

2.9.3 Diminazene aceturate 43

2.9.4 Atovaquone 44

2.10 Prevention and Control 44

CHAPTER THREE:MATERIALS AND METHODS 45

3.1 Study Population and Informed Consent 45

3.2 Ethical Clearance 45

3.3 Inclusion and Exclusion Criteria 46

3.4 Study Design 46

3.4.1 Sampling method 46

3.4.2 Study areas 46

3.4.3 Inclusion and exclusion criteria 49

3.4.4 Study populations 49

3.4.5 Sample collections 49

3.5 Morphological Identification of Tick 50

3.6 Parasitological Examination 50

3.7 Washing of FTA™ classic cards for Polymerase Chain Reaction (PCR) . 50

3.8 Species-specific Nested Polymerase Chain Reaction (nPCR) 51

3.8.1 *Theileriaequi* nPCR 51

3.8.2 *Babesia caballi* nPCR 52

3.8.3 *Theileria haneyi* nPCR 53

3.8.4 18S rRNA PCR 54

3.9 Deoxyribonucleic Acid (DNA) Agarose Gel Electrophoresis 58

3.10 ExoSAP-IT PCR Clean-up and DNA Sequencing 58

3.11 TOPO pCR 2.1 Cloning using M13 Primers 59

3.12 Detection of B. caballi and T. equi Specific Antibodies for Symptomatic and Asymptomatic horses 59

3.13 Determination of Haematological Parameters for Symptomatic and Asymptomatic Horses 60

3.14 Preparation of Haemoglobin-free Erythrocyte Membrane 60

3.15 Biochemical analyses on Erythrocyte Membrane of Symptomatic and Asymptomatic Horses 61

3.15.1 Malondialdehyde (MDA) concentration 61

3.15.2 Superoxide dismutase activity (SOD) 61

3.15.3 Glutathione peroxidase activity (GPX) 62

3.15.4 Catalase activity (CAT) 62

3.15.5 Determination of erythrocyte surface sialic acid concentration 62

3.16 Serum Biochemical Analyses 62

3.17 Determination of Serum Sialic Acid Concentration using the Thiobarbiturate 63

3.18 Data Analysis 63

CHAPTER FOUR: RESULTS 65

4.1 Symptomatic Horses 65

4.1.1 Clinical examination 65

4.1.2 Microscopic examination 65

4.1.3 Morphological identification of ticks 65

4.1.4 Molecular diagnosis using polymerase chain reaction (PCR) 68

4.1.5 Serological analysis of symptomatic horses 83

4.1.6 Mean haematological parameters of symptomatic horses with piroplasmosis. 90

4.1.7 Mean eerythrocyte membrane bound biochemical values of symptomatic horses with piroplasmosis 123

4.1.8 Mean serum biochemical analyses of symptomatic horses with piroplasmosis 140

4.2 Asymptomatic Horses.....185

4.2.1 Serological analysis of asymptomatic horses 185

4.2.2 Haematological analysis 187

4.2.3 Erythrocyte membrane biochemical analyses in asymptomatic horses.....206

4.2.4 Mean serum biochemical analyses in asymptomatic horses 215

4.2.5 Serum antioxidant in asymptomatic horses 230

CHAPTER FIVE:DISCUSSION.....235

CHAPTER SIX:CONCLUSION AND RECOMMENDATION 248

6.1 Conclusion 248

6.2 Recommendation 249

REFERENCES.....251

APPENDICES.....276

LIST OF FIGURES

Figures **Page**

2.1: Developmental stages of *T. equi* within the tick vector (sexual stages) and equine host (asexual stages) adapted from Scoles and Ueti (2015). 18

2.2: Developmental stages of *B. caballi* within the tick vector (sexual stages) and equine host (asexual stages) adapted from Scoles and Ueti (2015). 19

2.3: Blood smear showing pyriform merozoites *ofBabesia caballi* (B) in erythrocytes as indicated by blue arrows. Adapted from Kumar *et al*. (2009) 37

2.4: Intra-erythrocytic (merozoite) forms of *Theileria equi* (A-D) in Giemsa-stained blood film. Arrows indicate forms (merozoites and Maltese crosses) within the infected erythrocytes. Merozoites may be spherical or pyriform (pear shaped) (Mehlhorn and Schein, 1998). Maltese crosses are characterized by an arrangement of four merozoites. Arrows in A-D for *T. equi* indicate different phases of merozoites and Maltese crosses. Adapted from Knowles *et al*. (2018) 38

2.5: Intraerythrocytic forms of *Theileria haneyi* in Giemsa-stained blood film. Arrows indicate forms (merozoites and Maltese crosses) within the infected erythrocytes. Merozoites may be spherical or pyriform (pear shaped) (Mehlhorn and Schein, 1998). Maltese crosses are characterized by an arrangement of four merozoites. Arrows in E-F for *T. haneyi* n. sp. indicate different phases of merozoites and Maltese crosses. Width and length measurements of merozoites were performed on individual masses of Maltese crosses. Adapted from Knowles *et al.* (2018) 39

3.1: Map of the study areas showing Zaria City, DeeBee Farms, 5th.....48

4.1: Maximum likelihood phylogenetic tree of full length nucleotide sequence 18S rRNA of *Theileria* species. Selected sequences were trimmed to be identical length of 1490 base pairs following assembly in order to find common overlap.....82

4.2: The mean ±SEM haematocrit for the infected and non-infected horses. 92

4.3: The mean ±SEM of haemoglobin concentration for the infected and non-infected horses. 93

4.4: The mean ±SEM red blood cell counts for the infected and non-infected horses. 94

4.5: The mean ±SEM corpuscular volume for the infected and non-infected horses. 98

4.6: The mean ±SEM corpuscular haemoglobin for the infected and non-infected horses. 99

4.7: The mean ±SEM corpuscular haemoglobin concentration for the infected and non-infected horses. 100

4.8: The mean ±SEM percent red blood cells redistribution width for the infected and non-infected horses. 101

4.9: The mean ±SEM red blood cells redistribution width for the infectedand non-infected horses. 102

4.10: The mean ±SEM white blood cell counts for the infected and non-infected horses. 107

4.11: The mean ±SEM neutrophil counts for the infected and non-infectedhorses. 108

4.12: The mean ±SEM absolute lymphocyte counts for the infected and non-infected horses. 109

4.13: The mean ±SEM absolute mid-cell counts for the infected and non-infected horses. 110

4.14: The mean ±SEM platelet counts for the infected and non-infected horses. .116

4.15: The mean ±SEM percent plateletcrit count for the infected and non-infected horses. 117

4.16: The mean ±SEM platelet volume for the infected and non-infectedhorses. 118

4.17: The mean ±SEM percent platelets distribution width for the infected and non-infected horses 119

4.18: The mean ± SEM platelets large cell ratio for the infected and non-infectedhorses.....120

4.19: The mean ± SEM erythrocyte membrane bound alanine aminotransferase activities for the infected and non-infected groups 124

4.20: The mean ± SEM erythrocyte membrane bound aspartate aminotransferase activities for the infected and non-infected groups 125

4.21: The mean ± SEM erythrocyte membrane bound catalase activities for the infected and non-infected horses.....130

4.22: The mean ± SEM erythrocyte membrane bound superoxide dismutase activities for the infected and non-infected horses.....131

4.23: The mean ± SEM erythrocyte membrane bound glutathione concentrations for the infected and non-infected horses 132

4.24: The mean ± SEM erythrocyte membrane bound malondialdehydeconcentration for the infected and non-infected horses....133

4.25: The mean ± SEM erythrocyte membrane bound sialic acid concentration for the infected and non-infected horses.....137

4.26: The mean ± SEM serum alanine transferase activity for the infected and non-infected horses.....142

4.27: The mean ± SEM serum aspartate transferase activity for the infected and non-infected horses 143

4.28: The mean ± SEM serum alkaline phosphatase activity for the infected and non-infected horses 144

4.29: The mean serum total bilirubin concentration for the infected and non-infected horses.....149

4.30: The mean direct (conjugated) bilirubin concentration for the infected and non-infected horses 150

4.31: The mean indirect (unconjugated) bilirubin concentration for the infected and non-infected horses 151

4.36: The mean serum total protein concentration for the infected and non-infected horses.....156

4.37: The mean albumin concentration for the infected and non-infected horses 157

4.38: The mean globulin concentration for the infected and non-infected horses 158

4.39: The mean creatinine concentration for the infected and non-infected horses 162

4.40: The mean urea concentration for the infected and non-infected horses.....163

4.41: The mean glucose concentration for the infected and non-infected horses .168

4.42: The mean serum total cholesterol concentration for the infected and non-infectedhorses.....169

4.43: The mean total sialic acid concentration for the infected and non-infected horses.....170

4.44 The mean free sialic acid concentration for the infected and non-infected horses.....171

4.45 The mean serum sodium concentration for the infected and non-infected horses.....177

4.46 The mean serum chloride concentration for the infected and non-infected horses.....178

4.47 The mean serum potassium concentration for the infected and non-infected horses.....179

4.48 The mean serum bicarbonate concentration for the infected and non-infected horses.....180

4.49 The mean serum phosphate concentration for the infected and non-infected horses.....181

4.50 The mean serum calcium concentration for the infected and non-infected horses.....182

4.51: The mean haematocrit for the infected and non-infectedhorses.....189

4.52: The mean haemoglobin concentration for the infected and non-infected horses.....190

4.53: The mean red blood cells count for the infected and non-infected horses...191

4.54: The mean of mean corpuscular volume for the infected and non-infected horses.....192

4.55: The mean of mean corpuscular haemoglobin for the infected and non-infected horses.....193

4.56: The mean of mean corpuscular haemoglobin concentration for the infected and non-infected horses.....194

4.57: The mean red blood cells distribution width for the infected and non-infected horses.....195

4.58: The mean total leukocyte count for the infected and non-infected horses...197

4.59: The mean absolute neutrophil count for the infected and non-infected horses 198

4.60: The mean relative lymphocytes for the infected and non-infected horses...199

4.61 The mean absolute mid cell counts for the infected and non-infected horses 200

4.62: The mean platelet count for the infected and non-infected horses.....202

4.63: The mean plateletcrit for the infected and non-infected horses 203

4.63: The mean platelet volume for the infected and non-infected horses.....204

4.64: The mean platelet distribution width for the infected and non-infected horses 205

4.65: The mean erythrocyte membrane total protein concentration for the infected and non-infected horses.....208

4.66: The mean erythrocyte membrane total sialic acid concentration for the infected and non-infected horses.....209

4.67: The mean erythrocyte membrane bound sialic acid concentration for the infected and non-infected horses.....210

4.68: The mean erythrocyte membrane cholesterol concentration for the infected and non-infected horses.....211

4.69: The mean erythrocyte membrane triglyceride concentration for the infected and non-infected horses.....212

4.70: The mean erythrocyte membrane high density lipoprotein concentration for the infected and non-infected horses.....213

4.71: The mean erythrocyte membrane low density lipoprotein concentration for the infected and non-infected horses.....214

4.72: The mean free sialic acid concentration for the infected and non-infected horses.....216

4.73: The mean serum blood urea nitrogen concentration for the infected and non-infected horses.....217

4.74: The mean serum creatinine concentration for the infected and non-infected horses.....218

4.75: The mean serum Alanine aminotransferase activities for the infected and non-infected horses 220

4.76: The mean serum Aspartate aminotransferase activities for the infected and non-infected horses 221

4.77: The mean serum alkaline phosphatase activities for the infected and non-infected horses.....222

4.78: The mean serum total protein concentration for the infected and non-infected horses.....224

4.79: The mean serum albumin concentration for the infected and non-infected horses.....225

4.80: The mean serum total bilirubin concentrations for the infected and non-infected horses.....227

4.81: The mean serum direct bilirubin concentration for the infected and non-infected horses.....228

4.82: The mean serum indirect bilirubin concentration for the infected and non-infected horses.....229

4.83: The mean serum glutathione concentration for the infected and non-infected horses.....231

4.84: The mean serum catalase activity for the infected and non-infected horses 232

4.85 The mean serum superoxide dismutase activity for the infected and non-infected horses.....233

4.86: The mean serum malondialdehyde concentrations for the infected and non-infected horses.....234

LIST OF TABLES

Tables **Page**

3.1: Target Parasites with respective primer sets, thermocycler conditions and band sizes for nested polymerase chain reaction (nPCR) 56

3.2: 18S rRNA partial and full length polymerase chain reaction (PCR) with primer sets, thermocycler conditions and band sizes 57

4.1. Distribution of various ticks species infesting horses from the sampled areas 67

4.2: Samples 2, 14, z-1, z-2, z-5, z-7, z-9 and z-11 with mixed infections 79

4.3: *T. equi*-unknown samples (*T. equi*-like clades B and E) 81

4.4: *Theileria equi* competitive-ELISA of horse serum 85

4.5: Summary of PCR and c-ELISA results for the seventy samples investigated 87

4.6: Mean Erythrogram in *T. equi*-infected symptomatic horses with means ± SEM based on breeds 103

4.7: Mean Erythrogram in *T. equi*-infected symptomatic horses with means ± SEM based on sex distribution 104

4.8: Leukogram with means ± SEM of the different parameters for West African Barb horses and Argentine Polo Ponies 111

4.9: Leukogram with means ± SEM based on sex distribution 112

4.10: Mean Thrombogram with means ± SEM of the different parameters for West African Barb horses and Argentine Polo Ponies 121

4.11: Mean Thrombogram with means ± SEM based on sex distribution 122

4.12: Means ± SEM of erythrocyte membrane bound enzymatic activities for West African Barb horses and Argentine Polo Ponies 126

4.13: Means ± SEM of erythrocyte membrane bound enzymatic activities based sex distribution 127

4.14: Mean ± SEM of erythrocyte membrane bound oxidative stressbiomarkers in *T. equi*-infected symptomatic horses based on breed distribution 134

4.15: Mean ± SEM of erythrocyte membrane bound oxidative stress biomarkers in *T. equi*-infected symptomatic horses based on sex distribution 135

4.16: Mean ± SEM of erythrocyte membrane bound sialic acid concentrations in *T. equi*-infected symptomatic horses based on breed distribution 138

4.17: Mean ± SEM of erythrocyte membrane bound sialic acid concentrations in *T. equi*-infected symptomatic horses based on sex distribution 139

4.18: Mean ± SEM of serum enzymes activities for *T. equi*-infected symptomatic horses based on breeds distribution 145

4.19: Means ± SEM of serum enzymes activities for *T. equi*-infected symptomatic horses based on sex distribution 146

4.20: Means ± SEM of serum bilirubin concentrations for *T. equi*-infected symptomatic horses based on breeds distribution 152

4.21: Means ± SEM of serum bilirubin concentrations for *T. equi*-infected symptomatic horses based on sex distribution 153

4.22: Means ± SEM of serum total protein, albumin and globulin concentrations for *T. equi*-infected symptomatic horses based on breeds distribution 159

4.23: Means ± SEM of serum total protein, albumin and globulin concentrations for *T. equi*-infected symptomatic horses based on sex distribution 160

4.24: Means ± SEM of serum creatinine and blood urea nitrogen concentrations for *T. equi*-infected symptomatic horses based on breed distribution 164

4.25: Means ± SEM of serum creatinine and blood urea nitrogen concentrations for *T. equi*-infected symptomatic horses based on sex distribution.....165

4.26: Means ± SEM of serum glucose, cholesterol, total sialic acid and free sialic acid concentrations for *T. equi*-infected symptomatic horses based on breed distribution 172

4.27: Means ± SEM of serum glucose, cholesterol, total sialic acid and free sialic acid concentrations for *T. equi*-infected symptomatic horses based on sex distribution 173

4.28: Means ± SEM of serum electrolyte concentrations for *T. equi*-infected symptomatic horses based on breed distribution.....183

4.29: Means ± SEM of serum electrolyte concentrations for *T. equi*-infected symptomatic horses based on breed distribution.....184

4.30: Seroprevalence of *B. caballi* and *T. equi* among asymptomatic horses.....186

LIST OF PLATES

Plate **Page**

I*:* Agarose gel electrophoresis for *B. caballi rap1* gene (Band size 250 bp) 69

II: Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp).....70

III: Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp).....71

IV: Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp).....72

V: Agarose gel electrophoresis for *T. haneyi* hypothetical gene (Band size.....73

VI: Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp).....75

VII: Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp).....76

VIII: Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp).....77

LIST OF APPENDICES

Appendix **Pages**

1: Laboratory data for symptomatic horses 276

2: Leukogram of the study groups 277

3: Thrombogram of the study groups 278

4: Erythrocyte membrane bound enzymatic activities of the study groups.....279

5 Erythrocyte membrane bound oxidative biomarkers of the study groups.....280

6: Serum biochemical parameters of the study groups 279

7: Laboratory data for asymptomatic horses 281

8: Serum biochemical parameters.....282

9: Erythrocyte Membrane Parameters 283

LIST OF ABBREVIATIONS

APP	Argentine Polo Ponies
B. caballi	*Babesia caballi*
Ca	Calcium
cELISA	Competitive Enzyme Linked Immunosorbent Assay
Cl	Chloride
DBiL	Direct bilirubin
ema-1	Equine merozoite antigen-1
FA	Free sialic acid
H	Identification code for asymptomatic horses
HCO_3	Bicarbonate
HCT	Haematocrit
Hgb	Haemoglobin
IBiL	Indirect bilirubin
K	Potassium
LYMPH	Lymphocyte
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
Na	Sodium
nPCR	Nested polymerase chain reaction
NUET	Neutrophil
PCR	Polymerase chain reaction
PCT	Plateletcrit
p-LCR	Platelet large cell ratio

PLT	Platelet
PO_4	Phosphate
rap-1	Rhoptry associated protein-1
RBC	Red blood cell
RDW	Red blood cell distribution width
SA	Sialic acid
T. equi	*Theileria equi*
T. haneyi	*Theileria haneyi*
TBiL	Total bilirubin
TSA	Total sialic acid
WAB	West African Barb Horses
WBC	White blood cell
Z	Identification code for symptomatic horses

CHAPTER ONE

1.0 INTRODUCTION

1.1Background of the Study

Equine piroplasmosis (EP) is an emerging disease that has cosmopolitan distribution, and is mostly endemic in tropical and subtropical regions of the world (Hunfeld *et al.,* 2008; Lobo *et al.,* 2013). The disease is caused by *Babesia caballi*, *Theileriaequi* and *Theileria haneyi,* which are intraerythrocytic protozoans that elicit diverse inflammatory responses (Krause *et al*., 2007; Knowles *et al.,* 2018) in humans and animals. Though biologically different, the three parasites share similar pathologies, life cycles and transmission agents. The major route of transmission is through the saliva of infected Ixodes ticks during bite for blood meal; other routes are transplacental, intrauterine, blood transfusion and organ transplant (Phipps and Otter, 2004). The predominant vectors are a wide-range of tick species of the genus *Amblyomma*, *Dermacentor* and*Rhipicephalus* transmitting pathogens that cause equine piroplasmosis (Alvarez *et al*., 2005; Garba *et al*., 2011).

Infection with the tick-transmitted haemoprotozoan parasites can be clinical or subclinical, but can persist even with resolution of clinical signs (Ueti *et al*., 2005; Ribeiro *et al*., 2013). Incubation period for *B. caballi* is from 10 to 30 days, *T. equi* is 12 to 19 days (CFSPH, 2008) whereas that for *T. haneyi* is unknown, but the prepatent period in splenectomized horses varies from 14 to 30 days (Knowles *et al*., 2018). The synonyms of equine piroplasmosis include Tick fever, Texas fever, Red water and equine malaria.

In Nigeria, equine piroplasmosis (EP) is a clinically important tick-borne disease affecting equine populations. The disease, which is among the World Organization for Animal Health (OIE)-listed notifiable diseases of animals (OIE, 2012), has caused economic losses due to impaired health, cost of treatment, decreased productivity and death of horses.

The clinical signs of EP are variable and non-specific. However, they consist of mainly fever, inapettence, anaemia, haemoglobinuria, icterus, ventral oedema, mild colic and reduced faecal output (Deger *et al*., 2009; Kappmeyer *et al*., 2012; Laus *et al*., 2013; Abedi *et al*., 2014; Hussain *et al*., 2014). Many pathophysiological mechanisms have been adduced to explain the development of the anaemia observed during EP. Excessive intravascular haemolysis along with the phagocytic role of neutrophils and monocyte, which are cellular responses to the parasite antigen adherence to RBCs surface and the toxic haemolytic factor that is released by the parasite, was one of the mechanisms reported (Zygner *et al*., 2007; De Gopegui *et al*., 2007). Another possible cause of anaemia include oxidative stress, as indicated by the high level of malondialdehyde (MDA), which is accompanied by reduction of packed cell volume (PCV), haemoglobin concentration (HB) and red blood cell count (RBC) in infected horses (Nazifi *et al*., 2008; 2009; 2011; Celi *et al*., 2010; Esmaeilnejad *et al*., 2012; Salem and El-Sherif, 2015).

Haematological alterations that occur in equines with piroplasmosis were well described. Further understanding of the serum biochemical alterations and physic-chemical changes in the erythrocyte membrane following infection with these protozoal organisms is vital to fully unravel the mechanisms leading to the disease. Equally

important is the need to understand the biology of the parasites using molecular techniques.

1.2Statement of Research Problem

In countries such as the United State, Japan and Australia,there are strict federal regulations for the elimination of infected horses in order to maintain piroplasmosis-free status in non-endemic countries (Ueti *et al.,* 2012; Committee on Infectious Diseases of Horses, 2014). Nigeria, being an endemic country for EP is a signatory to the Economic Community of West African Countries (ECOWAS) Charter on the free movement of people, livestock, goods and services among member nations (ECOWAS Cross-border Trans-human Agreement, 1998). This policy on the unrestricted transboundary movement of livestock is of infectious disease regulatory and public health concern as it promotes disease dissemination and is a challenge to effective control strategy for transboundary zoonoses (Howell *et al*., 2007; Bowman *et al*., 2009).

Diagnosis of equine piroplasmosis using the conventional thin blood smear is less sensitive especially in sub-chronic, chronic and even acute infections (Posada-Guzman *et al*., 2015). Misdiagnosis due to non-availability of more sensitive and specific diagnostic techniques or over reliance on the less sensitive microscopic examination was thought to be responsible for the development of carrier states of the infection (Kumar *et al*., 2008; Kamyingkird *et al*., 2014). This is a major limitation to the control of the disease in Nigeria. Haemato-biochemical abnormalities are dynamic, non-specific and variable clinical occurrences in EP (Takeet *et al*., 2009; Mahmoud *et al*., 2016), thus, necessitating their evaluation with the view to establishing their profiles in acute and chronic forms of the disease. This is particularly so as transportation of horses

is a stress factor that triggers the transformation of subclinical piroplasmosis to fulminant form of the disease. Stress, in addition to other predisposing factors, stimulates a host of physiological responses that may cause alterations in haemato-biochemical and oxidative biomarkers (NemecSvete *et al*., 2012; Kumar *et al.,* 2013; Cai *et al.,* 2014). These stress-induced inflammatory mediators cause severe immunosuppression leading to clinical form of EP (Hodgson, 2002; Servic *et al*., 2008; Onmaz *et al*., 2011; Bahrami *et al.,* 2014).

Competitive-ELISA (cELISA) is a diagnostic method generally in use but it is inefficient for confirmation of*T. equi* clearance owing to persistence of relatively constant positive antibody titer by cELISA 48 months post treatment with imidocarb (Wise *et al*., 2017). This is worrisome and calls for concerted efforts by scientists in Nigeria to carry out detailed molecular studies of the causative pathogens of EP in the country with a view to developing peculiar indigenous diagnostic tests to effectively control the disease.

Furthermore, reports on haematological changes in *T. equi* and/or *B. caballi*-infected horses are as conflicting (Ike *et al.,* 2005; Alsaad and Al-Mola, 2006; Zobba *et al*., 2008; Pitel *et al*., 2010; Ibrahim *et al*., 2011; Alsaad, 2014; Al-Obaidi *et al.,* 2015; Mahmoud *et al*., 2016) as the numerous cause(s) of these abnormalities (De Gopegui *et al.,* 2007; Zygner *et al*., 2007; Cary *et al*., 2009; Jain *et al*., 2011). It is therefore important to characterize the organisms prevalent in various areas using molecular technique. Also, thorough understanding of the haemato-biochemical abnormalities that occur in clinical and subclinical EP especially with regard to the physicochemical changes on erythrocyte membrane following infection is important, thus, necessitating this study.

1.3 Justification

In Nigeria, tick-borne diseases of horses, especially EP is endemic and well documented (Oduye, 1976; Leeflang and Ilemobade, 1977; Aliu, 1983; Oladosu and Dipeolu, 1983; Lawal *et al*., 1999; Garba *et al*., 2012; Sanusi *et al*., 2014; Turaki *et al*., 2014; Oladipo *et al*., 2015; Mshelia *et al*., 2016). These epidemiological data on EP in Nigeria were based on microscopy and in very few cases, serology. Aside the poor sensitivity associated with microscopic diagnosis (Wong *et al*., 2014), studies have shown lack of concordance or inability of cELISA to detect *B. caballi* infection in horses that were confirmed positive for EP using nPCR and indirect fluorescent antibody test (IFAT) in South Africa (Bhoora *et al*., 2009), Israel (Rapoport *et al*., 2014) and Egypt (Mahmoud *et al*., 2016). The reasons adduced for these failures were diversity in *rap-1* genes repertoire and the remarkable polymorphisms including indel mutations in *B. caballi* isolates in the respective countries (Bhoora *et al*., 2009; Rapoport *et al.*, 2014; Mahmoud *et al*., 2016) when compared to the Bc48/*rap-1* gene of the Florida strain of *B. caballi* that was used to develop the monoclonal antibody (mAB) included in the commercial *B. caballi* ELISA kit (Kappmeyer *et al*., 1999).

Conversely, several years after the first documented evidence of EP in Nigeria (Oduye, 1976), no molecular studies were carried out to characterize the aetiologic agents in Nigeria, except, the recent reports by Idoko *et al.* (2020), Mshelia *et al*. (2020) and Onyiche *et al*. (2020). Therefore, molecular characterization and sequence analyses of the aetiologic agents of EP in Nigeria is vital to predict possible identity or variability with amino acid sequence in genes of the *B. caballi* or *T. equi* strains used to develop this commercial ELISA kits. This is also of regulatory importance to check

misdiagnosis during screening of horses imported into Nigeria, confirmatory diagnosis in Veterinary hospitals/clinics, in addition to research purposes.

Nested Polymerase Chain Reaction was used in this study because of the very high sensitivity to detect parasitaemia as low as 0.000006 (6.0×10^{-6}) (Nicolaiewsky *et al*., 2001; Rampersad *et al*., 2003; Sumbria *et al*., 2016) and its reported importance as a good confirmatory test for successful chemotherapeutical clearance of *T. equi* infection in horses (Ueti *et al.*, 2012; Wise *et al*., 2013).

Several factors such as climate, season, host-specificity, tick's life cycle and the strain(s) of the parasites (Scoles *et al*., 2011) may have peculiar effects on the haemato-biochemical alterations that are often evident in the clinical disease. This partly accounted for this study, with a view to investigating the isolate(s) of *B. caballi* and *T. equi* in the study areas vis-a-vis the peculiarities in their host blood picture.

Infection and inflammation play vital role in activating a cascade of inflammatory cells (neutrophils, eosinophils, mononuclear phagocytes and macrophages) (Belardelli, 1995; Oppenheim *et al*., 2000; Jacobson *et al.*, 2002; Kiral *et al*., 2005; Kocyigit *et al*., 2005; Tajima and Rikihisa, 2005) that play significant roles in the host defense (Djaldetti *et al*., 2002). These cells generate large amounts of highly toxic molecules such as reactive oxygen species ROS (superoxide anion, hydrogen peroxide and hydroxyl radicals) and reactive nitrogen species (nitric oxide NO) as essential effector mechanism of immune control against the immunodominant proteins expressed by *T. equi* and *B. caballi* (Knowles *et al*., 1994).These free radicals have the tendency to degrade numerous biomolecules including DNA, carbohydrates, lipids and proteins (Bogdan *et al*., 2000; Goff *et al*., 2002) when the antioxidant capacity of the host, which

ordinarily should protect these biomolecules and tissues, including the erythrocytes, are overwhelmed (Soares *et al*., 2011).

Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) should not be restricted to being a mere routine test for detection of hepatocellular damage (Sookoian and Pirola, 2012), but also an essential diagnostic tool in the pathophysiology of anaemia development in haemolytic diseases in which hepatic and skeletal/cardiac muscles damage do not occur directly. This is important because these enzymes, particularly ALT, is solely responsible for the synthesis of alanine in erythrocytes and both enzymes also contribute most of the cytosolic glutamate requirement for erythrocyte glutathione biosynthesis (Ellinger *et al*., 2011). Since erythrocyte membrane is impermeable to glutamate (Young *et al*., 1987), the erythrocytes rely on de novo glutamate biosynthesis to maintain their intra-cytosolic amino acids requirements which are furnished by the transferases (Ellinger *et al*., 2011). Variations in the concentrations of these enzymes may have possible effects on erythroid in the absence of hepatocellular damage or steatosis. Therefore, establishing the haemato-biochemical profiles in horses with piroplasmosis may provide useful clinical guide to the diagnosis of the disease.

1.4Aim of the Study

This study sought to characterize the haemato-biochemical alterations in symptomatic and asymptomatic horses with piroplasmosis in relation to the parasites' biodiversity in horses.

1.5 Objectives of the Study

The objectives of this study were to:

1. Detect *T. equi* and/or *B. caballi* infections from the blood of horses suspected of piroplasmosis using microscopy, nested polymerase chain reaction (nPCR), and competitive enzyme-linked Immunosorbent assay (c-ELISA) for both symptomatic and asymptomatic horses.
2. Determine and sequence the 18S rRNA, *ema-1* and *rap-1* genes of the nPCR-positive *T. equi* and/or *B. caballi* with the view to determining the strain(s) of the parasites.
3. Determine the prevalent tick species infesting horses infected with equine piroplasms
4. Determine the activities of serum enzymes (ALT, AST and ALP), concentrations of blood urea nitrogen (BUN), creatinine, bilirubin, total protein, albumin, globulin, glucose and total cholesterol in horses presenting clinical signs suggestive of piroplasmosis with the view to relating changes with development of the disease, and/or changes in relation to the breed and sex of the horses.
5. Determine the haematological parameters and erythrocyte membrane surface and serum sialic acid profile in symptomatic horses with piroplasmosis and asymptomatic horses with the view to possibly relate changes to development of anaemia as well as ascertain possible changes in relation to breed and sex of the horses.
6. Determine the profiles of enzymes (ALT, AST) and antioxidants (SOD, CAT, GSH) and MDA in erythrocyte membrane of horses with symptomatic and asymptomatic piroplasmosis with the view to relating changes to development of the disease, and/or variation in relation to the breed and sex of the horses.

1.6Research Questions

1. Is microscopy more sensitive and specific than competitive enzyme linked immunosorbent assay (cELISA) and polymerase chain reaction (PCR) in detecting *B. caballi* and *T. equi* infections in horses?
2. What are the prevalent strain(s) of *Theileria* or *Babesia* species that naturally infect horses in the study areas?
3. What are the prevalent tick species infesting horses with piroplasms in the study areas?
4. Do the strain(s) of *Theileria* and *Babesia* cause remarkable haemato-biochemicalalteration based on the sex and breed of horses?
5. Are there significant alterations in serum biochemical profile in symptomatic and asymptomatic horses with piroplasmosis?
6. Do the strain(s) of *Theileria* and *Babesia* cause significant physicochemical changes on erythrocyte membrane in the infected horses?
7. Is oxidative stress a major factor in development of the diseases in horses?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background on Equine Piroplasmosis

Equine piroplasmosis (EP) is an emerging zoonosis that has cosmopolitan distribution, and is mostly endemic in tropical and subtropical regions of the world (Hunfeld *et al.*, 2008; Lobo *et al.,* 2013). Emerging infectious diseases constitute serious threat to global health (Jones *et al.,* 2008; Lloyd-Smith *et al*., 2009; Smith *et al*., 2014). Various environmental and man-made factors, particularly, the effect of climate change on mean temperature, seasons, floods and storms, deforestation, land-use conversion, use of pesticides,*etc,* continuously impact tick-pathogen-host relationship (Buegnet and Marie, 2009; Karbowiak, 2014; Mierzejewska *et al*., 2015). Similarly, unrestricted movement of livestock readily translocates ticks during host movement; introduces them into new areas and ultimately exposes naïve livestock to tick infestation (Ikpeze *et al.,* 2015). Understanding the dynamics of tick epidemiology is critical to developing measures to control and prevent tick-borne diseases (Young *et al.,* 2017).

Equine piroplasmosis is caused by *B. caballi*, *T.equi* and now *T. haneyi*, which are intraerythrocytic protozoans that elicit diverse inflammatory responses (Krause *et al*., 2007; Knowles *et al.,* 2018) in humans and animals. Although they are biologically different, infections with the three parasites share similar pathologies, life cycles and

transmission agents. The major route of transmission is through the saliva of infected Ixodes ticks during bite for blood meal; other routes are transplacental, intrauterine, blood transfusion and organ transplant (Phipps and Otter, 2004; Imes *et al*., 2011). The predominant vectors are wide-range tick species of the genus *Amblyomma*, *Dermacentor*, *Boophilus* and *Rhipicephalus* transmitting EP and other diseases (Alvarez *et al*., 2005; Garba *et al*., 2011). Infection with the tick-transmitted haemoprotozoan parasites can be clinical or subclinical, but can persist even with resolution of clinical signs (Ueti *et al*., 2005; Ribeiro *et al*., 2013). Incubation period of infection with*B. caballi* is from 10 to 30 days whereas infection with*T. equi* is 12 to 19 days (CFSPH, 2008).

In Nigeria, EP is a clinically important tick-borne disease affecting the equine population. The disease, which is among the World Organization for Animal Health (OIE)-listed notifiable diseases of animals (OIE, 2012), has caused untold economic losses due to impaired health, cost of treatment, decreased productivity and death of affected horses.

2.2 Historical Perspective of Equine Piroplasmosis

Theileria equi, first called *Piroplasma equi* but later reclassified as *Babesia equi (B. equi)*, was first discovered in South Africa in 1901 (Laveran, 1901 as cited by Mehlhorn and Schein, 1998). A few years later, it was discovered that two biologically distinct parasites could infect the erythrocyte and co-habit within it to cause pathologies associated with EP (Koch, 1906 as cited by Mehlhorn and Schein, 1998). One of the parasites was larger, and so named *Piroplasmacaballi*, but now called *Babesia caballi*, whereas, the second parasite was the earlier reported *B. equi* (Nuttal and Strickland, 1910 as cited by Mehlhorn and Schein, 1998). *Babesia equi* was reclassified to *T. equi*

in 1998 because of extra-erythrocytic evidence of the parasite within peripheral blood mononuclear cells (PBMC), absence of transovarial transmission which occurs in other *Babesia* species, presence of small erythrocytic stages measuring only 1.5-2.5 μm in length instead of the 3-6 μm long erythrocytic stages of *Babesia* species, distinction in the formation and shape of sexual stages of *T. equi* in the tick vector from those of typical *Babesia* species, and the close identity of their surface proteins to those of other *Theileria* species (Mehlhorn and Schein, 1998). Furthermore, phylogenetic and genomic analyses showed that *T. equi* possesses characteristics of both *Babesia* and *Theileria* lineages (Allsopp *et al*., 1994; 2006; Uilenberg, 2006; Allsopp *et al.,* 2007), thus, supporting the concept of a new genus for *T. equi* (Kappmeyer *et al*., 2012).

Theileria haneyi is a serendipitous apicomplexan parasite discovered in a stray horse that was restrained along Eagle Pass at the United States-Mexico border. Like *T. equi*, it has intraerythrocytic merozoite stages but they are morphologically distinct owing to their smaller asexual (merozoite) stages. The mean length and width of each *T. haneyi* merozoite are 1.15 ± 0.16 mm and 0.70 ± 0.07 mm respectively (Knowles *et al*., 2018) while those of *T. equi* are 1.88 ± 0.21 mm and 0.91 ± 0.13 mm respectively (Yokoyama *et al.*, 2003). *Theileria haneyi*is a distinct parasite closely related to the *Theileria spp* isolated from Waterbucks (*Kobus defassa*) and South African mountain Zebras based on phylogenetic analysis of their 18S rDNA. Based on the unique differences in morphology and 18S rDNA between this parasite and other *Theileria* species, the parasite was named*T. haneyi* in honour of the Veterinarian, Dr. D. Haney, who first restrained and clinically examined the stray horse from which the parasite was detected (Knowles *et al.*, 2018).

2.3Epidemiology of Equine Piroplasmosis (EP) in Nigeria

Piroplasmosis is one of the most important parasitic diseases of horses in Nigeria (Mayaki, 2017). Despite the economic losses associated with the disease in Nigeria, it remained under reported at the global stage. This is attested by the conspicuous absence of Nigeria in the list of EP endemic countries in the world (OIE, 2018).

Authors, such as Oduye (1976), and Leeflang and Ilemobade (1979) were the first to report EP in Nigeria. These early reports were followed by those of Aliu (1983), and Oladosu and Dipeolu (1983). As EP continued to devastate the equine industry, further interest to detect and identify the aetiologic agents continued. Study by Lawal *et al*. (1999) reported *T. equi* and *B. caballi* infections in horses and found to occur with prevalence rates of 33% and 2%, respectively, in Zaria, northwest Nigeria. In Abeokuta, Ogun State, south-west Nigeria, *T. equi* infections were identified in horses using Giemsa-stained thin blood smear (Takeet *et al*., 2009). As the morbidity and mortality associated with EP in Nigeria persisted, equids in Niger State, north-central Nigeria were screened for EP. The report indicated an overall point prevalence of 20.1%, with *T. equi* accounting for 80.4% whereas *B. caballi* was 19.6% in the sampled population (Garba *et al*., 2011a, 2011b). Further, Garba *et al*. (2012) screened 87 royal horses from different emirate councils in Northern Nigeria and reported that 29 of the horses were naturally infected with *T. equi* and *B. caballi*. A prevalence of 50% for *T. equi* and 46.7% for *B. caballi* was reported from 83 horses sampled from Jos North and Jos South Local Government Areas (LGAs) respectively in Plateau State, North-central Nigeria (Pam *et al*., 2013). In the neighbouring state of Bauchi, north-east Nigeria, Sanusi *et al*. (2014) reported an overall prevalence of 18% due to *B. caballi* (83.3%) and *T. equi* (11.1%) following random sampling of 400 horses from the three ecological

zones of the state. Similarly, an overall prevalence of 41.7% (100 horses) was reported from the same region but from different states (Borno, Gombe and Taraba) following screening of blood samples collected from 240 West African Barb horses (local horses). *Theileria equi* accounted for 94% (94), whereas, infection due to *B. caballi* was 6% (6). The authors reported a state-wide prevalence of 48.8% (39), 41.3% (33) and 35.05% (28) for Borno, Gombe and Taraba, respectively (Turaki *et al*., 2014). Although, Oladipo *et al*. (2015) did not indicate the species distribution of the parasites they detected from their study in Ibadan, Oyo State, south-west Nigeria, they reported a prevalence of 22% EP in West African Barb horses from the total horses sampled from the study area. Mshelia *et al*. (2016) reviewed 572 cases in horses showing variable non-specific clinical signs of EP in four widely distributed states in Nigeria from September 2007 to June 2014 with the aim of determining the prevalence of piroplasmosis in Polo and pleasure horses, and to clinically correlate the non-specific signs observed with those of EP. The results obtained showed that out of the 156 blood samples tested between years 2007 and 2010, 16 (10.3%) were positive for *T. equi*, while 4 (2.7%) were positive for *B. caballi*. Over all, they documented a total of 20 (12.8%) out of the 156 horses to be EP positive. In 2011, the authors analyzed 252 samples using c-ELISA and observed that 196 (77.8%) were positive for *T. equi*, while 12 (4.8%) were positive for *B. caballi*. A total of 90 sera were assayed in 2013, with 54 (60%) being positive for *T. equi* while 3 (3.3%) were positive for *B. caballi*. In 2014, 74 samples were assayed using c-ELISA and a prevalence of 31.3% (23) due to *T. equi* was observed (Mshelia *et al*., 2016). The only documented report of EP in South-East Nigeria was a case of *B. caballi* infection in a 6-month old colt in Umudike, Abia state (Ememe *et al*., 2018).

Thus, the epidemiological data of EP in Nigeria showed nationwide distribution of the causative pathogens. Exception to this is south-south Nigeria where epidemiological evidence of EP is lacking. Sadly, several years after the first report of EP in Nigeria, no molecular studies have been carried out to characterize the aetiologic agents in Nigeria. All published reports were based on microscopy and in a few cases, serology (cELISA).

2.4. Aetiology, Life Cycle and Transmission of Equine Piroplasmosis

The life cycle of the aetiologic agents of equine piroplasmosis,*B. caballi*, *T. equi* and *T. haneyi*, involves three distinct stages; the asexual transmission stage (sporozoite), asexual blood stage (merozoite), and the sexual blood stage (gametocyte) (Schein *et al*., 1981; Moltmann *et al*., 1983; Scoles and Ueti, 2015; Knowles *et al*., 2018) (Fig.2.1 and2.2). Variations in tick species can impact the development of the parasites within the tick vector (Wise *et al*., 2013). Regardless of variations in the infesting tick species, the infectious sporozoites of *B. caballi*, *T. equi* and *T. haneyi* are transmitted through tick-saliva during blood meal intake (Uilenberg, 2006). The precise duration of the extrinsic acquisition feeding; parasite replication time within the tick vector and transmission feeding (incubation period) is not well defined. But once within the host parasite, the *B. caballi* sporozoites directly invade erythrocytes where they multiply and develop first into trophozoites and later to merozoites. These blood stages (merozoites) are subsequently released and invade the uninfected erythrocytes sequel to erythrocyte rupture. On the other hand, *T. equi* and *T. haneyi* initial invasion is different in that it first enters peripheral blood mononuclear cells (PBMCs) (Mehlhorn and Schein, 1998; Knowles *et al*., 2018). This particular life cycle event for *T. equi* partly accounted for the taxonomic reclassification as *Theileria* (formerly *Babesia equi*) (Kappmeyer *et al*., 2012). Once inside the PBMCs, the sporozoites develop into large schizonts and then

merozoites at about 9 days later. These merozoites are released and invade erythrocytes. Asexual replication results in an expanding population of merozoites and parasitized erythrocytes. Some of the merozoites develop into gametocyte forms within equine peripheral blood and upon ingestion of merozoites and/or gametocytes by a competent tick vector; the parasites undergo sexual reproduction, with the gametocytes developing into gametes, which combine to form zygotes within the tick midgut (Mehlhorn and Schein, 1998; Ueti *et al*., 2003; Uilenberg, 2006). The zygotes develop differently depending on the tick species and the parasite. *Babesia caballi* zygotes multiply and invade numerous tissues and organs of the ticks, including the ovaries, but not, initially, the salivary glands. *Babesia caballi* infection can be passed transovarially to the next tick generation, and development to an infective stage for horses occurs in the salivary glands of immature and adult ticks (Uilenberg, 2006; Rothschild and Knowles, 2007).

In contrast, *T. equi* zygotes develop into kinetes, which invade the ticks' haemolymph and salivary gland cells (Mehlhorn and Schein, 1998; Ueti *et al*., 2003; Uilenberg, 2006; Rothschild and Knowles, 2007). After a period of 6–24 days, continued development results in the presence of sporozoites within the salivary gland of the tick (Moltmann *et al*., 1983; Zapf and Schein, 1994; Guimaraes *et al*., 1998).

The close interaction between the life cycles of ticks and the pathogens they carry determine the mode of transmission of tick-borne pathogens (Scoles and Ueti, 2015). Tick-borne transmission can be biological, when the parasites undergo asexual and sexual developmental stages within the equine host and vectors; mechanical, by contaminated mouthparts; or iatrogenically by contaminated needles or blood transfusion. A successful pathogen transmission is dependent on a host of complex interactions between the tick population dynamics, host density, and closely associated

vector-host behaviours (Ueti *et al*., 2005). The transmission of tick-borne pathogens of EP fall into three unique models; intrastadial transmission, in which case one life stage of the tick acquires the pathogen and transmit it; intersstadial or transtadial transmission, in which one life stage of the tick acquires the pathogen and the subsequent stage transmit it; and transovarial transmission, wherein a female tick acquires the pathogen and her offspring transmit it (Ueti *et al*., 2008). Parasites such as *B. caballi* are transovarially transmitted by one-host ticks, whereas, *T. equi* is intrastadially or transstadially transmitted by only the nymphal or adult ticks (Schein, 1998; Scoles and Ueti, 2015). Persistently infected horses are also reservoirs for transmission aside biological transmission (Ueti *et al*., 2005).

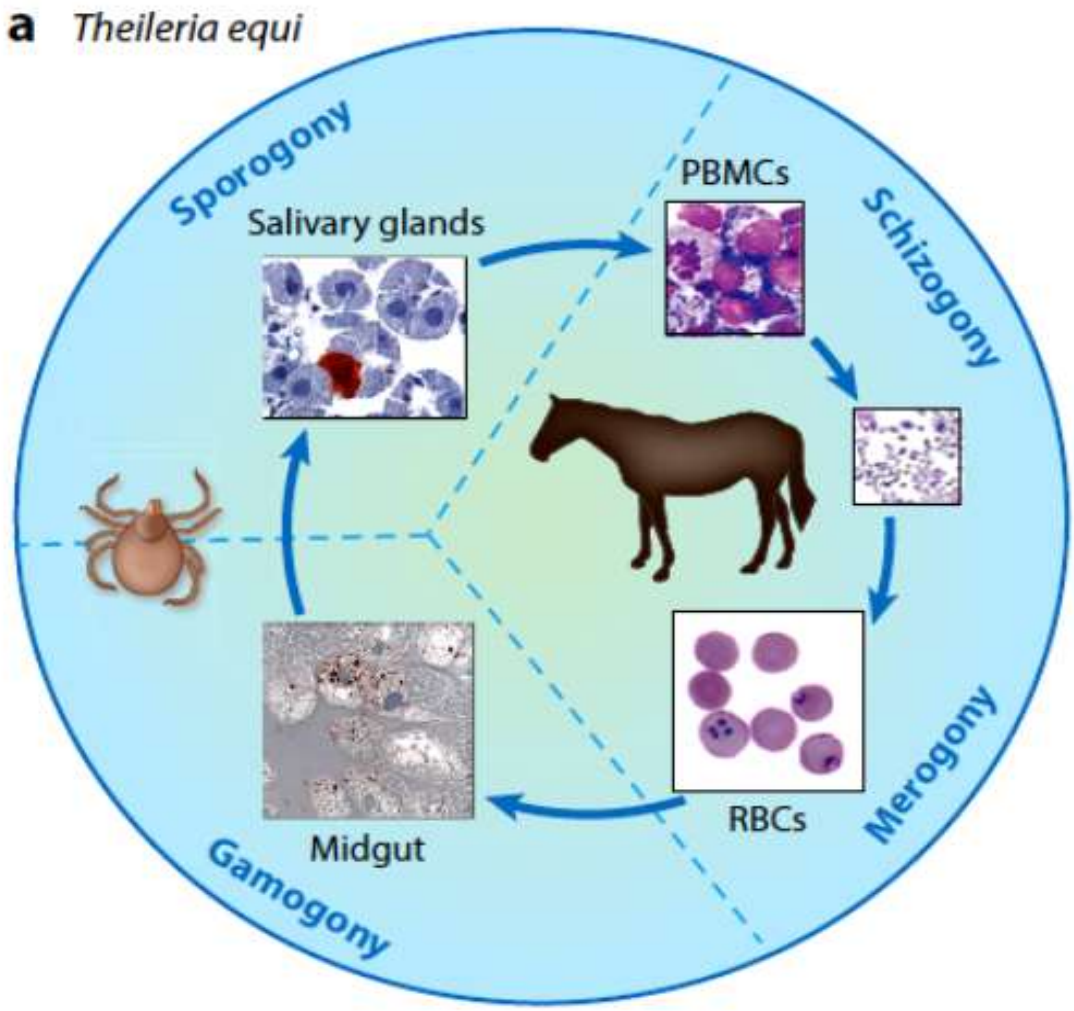


Figure 2.1: Developmental stages of *T. equi* within the tick vector (sexual stages) and equine host (asexual stages) adapted from Scoles and Ueti (2015).

Keys: PBMCs (peripheral blood mononuclear cells); RBCs (red blood cells).

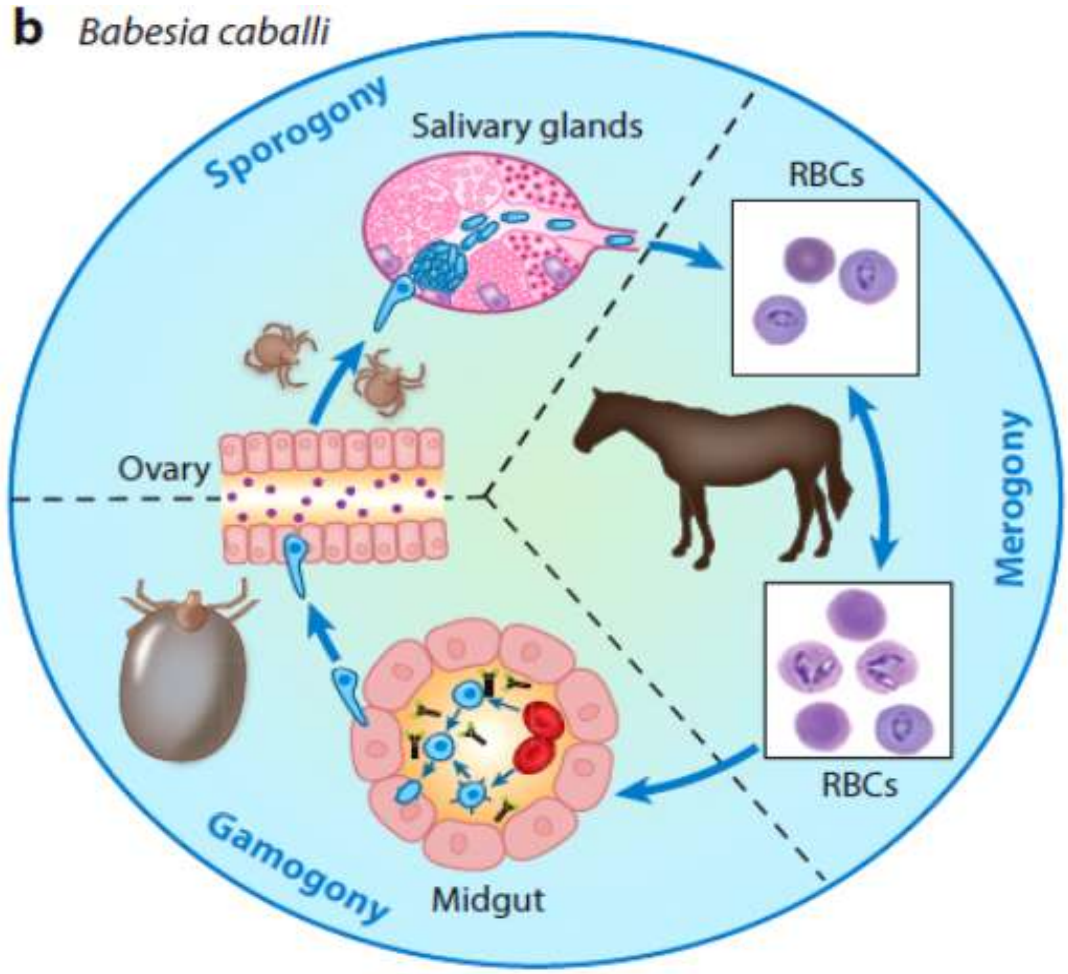


Figure 2.2: Developmental stages of *B. caballi* within the tick vector (sexual stages) and equine host (asexual stages) adapted from Scoles and Ueti (2015).

Keys: PBMCs (peripheral blood mononuclear cells); RBCs (red blood cells).

2.5 Pathogenesis of Equine Piroplasmosis

2.5.1 Horse Erythrocyte

The matured erythrocytes of horses are round, elastic, biconcave with a central pallor. The diameter is 5-6 µm and has no nuclei and organelles, with a lifespan of 140-165 days (Carter *et al*., 1974). Blood film in apparently healthy horses is characterized by rouleaux formation and occasional Howell-Jolly bodies (Kramer, 2000). The equine reticulocytes mature within the bone marrow even in the presence of increased erythropoiesis, thus, accounting for rare occurrence of immature polychromatophilic cells in peripheral blood of horses (Latimer and Rakich, 1992; Grondin and Dewitt, 2010). Therefore, assessing regenerative responses is dependent on measuring parameters such as the red blood cell distribution width (RDW), a coefficient of the degree of anisocytosis of circulating erythrocytes (Jain, 1986). The value of this parameter is significantly altered in microcytic and/or macrocytic anaemias (Kramer, 2000).Evaluation of erythrocytes alteration and anaemia development in EP is central to understanding the mechanism that is vital to successful immune invasion by merozoites and the subsequent erythrocytes colonization and multiplication.

2.5.2 Anaemia

Pathophysiological mechanisms such asdirect mechanical effect of merozoites on erythrocytes; intra-erythrocyte trophozoite and merozoite binary fission; decreased life span due to chelation of sialic acids;alteration of other components of the erythrocyte membrane; suppression of bone marrow erythropoiesis have been adduced to explain the development of the anaemia observed in EP(Dewaal , 1992; Halat *et al*., 1997; Ambawat *et al*., 1999; Al Sa'ad *et al*., 2010); effect of the toxic haemolytic factors produced by the parasites on erythrocyte membrane (De Gopegui *et al*., 2007; Zygner *et*

al., 2007); and immune mediated mechanism by auto antibodies directed against erythrocyte membrane component both in the infected and uninfected cells (Oladosa and Olufemi , 1992; Knowles *et al*., 1994; De Gopegui *et al*., 2007; Zygner *et al*., 2007). The sequalae of the deleterious effect of toxic metabolites produced by the parasites on erythrocyte membrane components are lipid peroxidations evident by elevated MDA levels and oxidative stress (Ambawat *et al*., 1999; Nazifi *et al*., 2008; 2009; 2011; Celi *et al*., 2010; Esmaeilnejad *et al*., 2012; Ali *et al*., 2013; Osama and Gaada, 2013; Salem and El-Sherif, 2015). The development of anaemia may result to tissue hypoxia and consequently anaerobic metabolism and hyperlactaemia ((McMichael *et al*. 2005; Ayers and Warrington, 2008). Tissue hypoxia reflects decreased percent haemoglobin saturation with oxygen and may result to metabolic acidosis. Blood gas parameters such as blood pH, *Pco2*, bicarbonate, base access and oxygen saturation percentage are decreased in such anaemic condition with increased anion gap indicative of titrational metabolic acidosis (Alsaad, 2014). Also, the anaemic hypoxia characterized by decreased tissue oxygenation is responsible for the panting that manifest clinically as tachypnoea and tachycardia (Radostitis *et al*., 2000). Continuous cellular lysis results in the liberation of endogenous pyrogens which ultimately stimulate the thermoregulatory centers of the hypothalamus to cause pyrexia (Svendson and Carter, 1984). The severity of the pyrexia can be affected by the degree of parasitaemia, clinical phase of the disease and systemic involvement (Krause, 2002).

Furthermore, increased plasma ALT and AST due to disruption of erythrocyte membrane and ischaemia-induced hepatocellular damage may occur. Other sequalae of anaemia, especially, haemolytic anaemia in EP is reduction in haptoglobin concentration, an important plasma protein that binds free haemoglobin, sequesters the haem component of haemoglobin and prevents iron utilizing pathogens from benefiting

from the haemolysis (Tecles *et al*. 2005; Cary *et al*. 2009). The clinical significance of the reduction in haptoglobin concentration is excessive breakdown of haemoglobin into haem and globin components. The globin or protein components are usually recycled while the haem component which is cytotoxic, in addition to been utilized by some pathogens, is first converted to a green pigment called biliverdin, and subsequently, to an orange pigment called unconjugated or indirect bilirubin in a reaction catalyzed by biliverdin reductase (Radostitis *et al*., 2008; Cary *et al.*, 2009; Jain *et al.*, 2011; Salib *et al.*, 2013).

Therefore, the development of anaemia is central to the variable, non-specific clinical signs observed in EP (Deger *et al*., 2009; Kappmeyer *et al*., 2012; Laus *et al*., 2013; Abedi *et al*., 2014; Hussain *et al*., 2014). The anaemia could be microcytic hypochromic (Al-Obaidi *et al*., 2015), macrocytic normochromic (Takeet *et al*., 2009), macrocytic hypochromic (Alsaad and Al-Mola, 2006; Zobba *et al*., 2008; Ibrahim *et al*., 2011; Alsaad *et al*., 2014; Mahmoud *et al*., 2016) and normocytic hypochromic (Idoko *et al*., 2018).

Leukocytic alterations associated with EP include leukocytosis due to lymphocytosis (Sandhu *et al*., 1998; Ike *et al*., 2005; Alsaad and Al-Mola, 2006; Zobba *et al*., 2008; Ibrahim *et al*., 2011) caused possibly by the stimulation of bone marrow response, lymphoid depletion and disorganization with massive lymphocytosis (Salem *et al*., 1986; Mohri and Sardari, 2000). Also, leukocytosis due to neutrophilia and eosinophilia has been reported in the disease caused by cellular response to the parasites' antigens and tick infestations (Ibrahim *et al*., 2005; Ibrahim *et al.*, 2011; Al-Obaidi *et al*., 2015).

Thrombocytopaenia is a common clinical sequalae in EP (Radostitis *et al*., 2008; Al-Obaidi *et al*., 2015; Osman, 2017). Sometimes, it is accompanied by an increase in

mean platelet volume (MPV), suggesting that the juvenile platelets are becoming bigger and more active indicating a regenerative response. During platelet activation, their shapes changes from biconcave to spherical discs with resultant pseudopod formation (Ustundag Budak *et al.,* 2016). Clinically, an increase in MPV could suggest a severe infection, systemic inflammatory process (Margetic, 2012; Safak *et al*., 2014) or other chronic disorders (Beyazit *et al*., 2012; Chu *et al*., 2010; Güneş *et al*., 2015) where the consumption and sequestration of platelets in the spleen and/or reticuloendothelial system is evident, or it be could due to small fibrin clots or platelets clumping (Freeman, 2010).The overall clinical sequalae of the haematological alterations associated with EP are systemic inflammatory syndrome, which culminates in multi-systemic dysfunction and hypercoagulopathy (Donnellan and Marais, 2009).

2.5.3 Biochemicalalterations

Liver transferases (ALT and AST) should not only be routine tests that measure hepatocellular damage alone (Sookoian and Pirola, 2012) but as contributors in the pathophysiology of anaemia development in haemolytic diseases. This is important because the enzymes, especially ALT, is solely responsible for the synthesis of alanine in erythrocytes and both enzymes also contribute most of the cytosolic glutamate requirement for erythrocyte glutathione biosynthesis (Ellinger *et al*., 2011). Since erythrocyte membrane is impermeable to glutamate (Young *et al*., 1987), the erythrocytes rely on de novo glutamate biosynthesis to maintain their intracytosolic amino acids requirements which are furnished by the transferases (Ellinger *et al*., 2011). Aside erythrocyte membrane transferases alterations, variations in serum activities of ALT, AST, ALP, gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) also occurs in EP (Camacho *et al*., 2005). These changes

result from centrilobular degeneration and necrosis of hepatocytes (Camacho *et al.*, 2005) and hypoxia-induced hepatobiliary system damage resulting from anaemia and icterus (Sandhu *et al*., 1998; Al-Obaidi *et al*., 2015). Similarly, haemolytic anaemia can predispose to decreased blood osmotic pressure characterized by hypoproteinaemia (Salib *et al*., 2013). Hyperbilirubinaemia, due to high indirect bilirubin manifest clinically as icterus in *B. caballi* and *T. equi* infected horses (OIE, 2008; Radostitis *et al*., 2008; Rothschild, 2013) and is also a net result of haemolytic anaemia. The continuous formation of unconjugated bilirubin can cause elevation in hepatic enzymes (ALT, AST and ALP) (Radostitis *et al*., 2008)

2.5.4 Oxidative stress

Stressors such as transportation, extreme exercise during polo, racing, durbars and even pleasure riding, in addition to intraerythrocytic activities of merozoites trigger a host of behavioural changes and physiological responses that alter heart, respiratory rates as well as stress-related hormones (Jagri *et al.,* 2012; Nemec Svete *et al*., 2012). These changes impact on haematological, biochemical and oxidative stress parameters via generation of reactive oxygen species (Uchino *et al*., 2012; Kumar *et al.,* 2013; *Cai et al.,* 2014; Wessely-Szponder *et al*., 2015). Erythrocyte membranes have high polyunsaturated fatty acid content and are thus very sensitive to oxidative damage evident by haemolysis (Szweda-Lewandowska *et al*., 2003), decreased deformability and impairment of transport via microcirculation (Bellary *et al*., 1995; Chung and Ho, 1999). Horse erythrocytes are particularly more sensitive to oxidative damage and have lower deformability than human erythrocytes (Baskurt *et al*., 1997).

Lipid peroxidation, an important sequalae of oxidative stress (Gibson *et al*., 2000) is implicated in the alterations of erythrocyte membrane structure and function, and causes

increased osmotic fragility and decreased cell fluidity (Hebbel, 1986). Additionally, lipid peroxidation positively correlates with alterations in plasma sialic acid level (Iijima *et al*., 2009; Surapaneni and Priya, 2009).

Desialylation of erythrocyte membrane results in the loss of the very important high molecular weight glycoprotein mucin, a hydroxyl radical scavenger which is an important component of sialic acids. This invariably makes underlying structures to become increasingly susceptible to oxidative damage and also alters the biophysical and biochemical functions of the cells (Ogasawara *et al.,* 2007; Mehdi *et al*., 2012). Similarly, removal of sialic acid by pathogens can be exploited via "molecular mimicry" to evade host immune responses (Vimr *et al.,* 2004).

The deleterious effects of reactive oxygen species and lipid peroxides are protected by an array of endogenous antioxidant defense systems, which serve as potent scavenger of free radicals (Kolanjiappana *et al*., 2002). Alterations in erythrocyte surface membrane molecules caused by invading merozoites of apicomplexan parasites other than *T. equi* have been widely reported (Carruthers and Boothroyd, 2007; Harvey *et al*., 2014; Lamarque *et al.,* 2014). Parasites within the phylum apicomplexan, especially members of the genera *Babesia* and *Theileria* possess important antioxidant systems which they deplore to maintain redox homeostasis as well as survival against host immune responses (Bosch *et al*., 2015). These antioxidant systems comprises of the thioredoxin- and glutaredoxin- systems. These systems control intracellular redox homeostasis by the close synergistic action of thiol/disulfide pairs. The antioxidant capacity of the cell is controlled by the predominant redox pairs, glutathione/glutathione disulfide (GSH/GSSG), while the thioredoxins (Trx_{red}/Trx_{ox}) form a complementing redox system that interacts with a different subset of proteins (Jones and Go, 2010). The

enzymatic antioxidants, broadly classified into primary and secondary antioxidants are also present in addition to the thiol/disulfide pairs. The secondary antioxidants are involved in the regeneration of low molecular weight antioxidant species (Halliwell, 1999), whereas, the primary antioxidants react directly with prooxidants (Bosch *et al*., 2015). Catalase (CAT) and superoxide dismutase (SOD) are examples of primary antioxidant enzymes. The CAT catalyses the reduction of hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$), thereby decreasing its cellular buildup. On the other hand, SODs are metallo- (M-) proteins that catalyse the dismutation of superoxide anions to form molecular oxygen and hydrogen peroxide (Bosch *et al*., 2015).

2.5.5 Immune mechanisms

In equine piroplasmosis, both humoural and cellular immunologic factors are involved as protective immune responses to the aetiologic agents (Mealey *et al*., 2011). At the initial stage, following tick bite, when sporozoites are released into the plasma, immunoglobulin G (IgG), specifically of the subclass IgG3 antibodies, prevents infection by binding and neutralizing the sporozoites before they target the peripheral blood mononuclear cells (PBMCs) (Cunha *et al*., 2006; Lewis *et al*., 2008; Awinda *et al*., 2013). This humoural immune response is therefore critical as the first line of defense between the moment the parasite gains access to the blood stream and the time it invades the target cells (Homer *et al*., 2000). This stage may be preceded by intraerythrocytic infection in immunocompromised horses where the sporozoites overwhelms the humoural immune responses, and transforms to merozoites, thereby invading circulating erythrocytes to cause haemolysis. After lysis has occurred, two major events occur; first, the parasites reach the bloodstream again to initiate a new round of invasion. This repeated cycle of invasion and infection of new erythrocytes

cause the overall parasitaemia level to increase and acute disease develops. On the other hand, haemoglobin (Hb) is released from the lysed infected erythrocytes, thereby scavenging nitric oxide, which is an important regulator of smooth muscle relaxation, endothelial adhesion molecule expression, platelet activation, and platelet aggregation (Rother *et al*., 2005). Free hemoglobin is hydrophobic, thus, it interact with and disrupt lipid membranes and other lipophilic structures (Balla *et al*., 1993). Free Hb, which may act as a secondary modulator of the anti-inflammatory role of Hp can be removed from the circulation by three major mechanisms: high affinity binding of Hb to haptoglobin (Hp); high affinity binding of haem to haemopexin (Hpx); and low affinity binding of Hb to cluster of differentiation 163 (CD163). The mechanisms involving Hpx and CD163 appear to function during massive or localized haemolysis, where Hp concentration is overwhelmed or limited (Quaye, 2008).

The cells of the innate immune system are expected to control the growth rate of the parasites and the extent of parasitaemia (Imes *et al*., 2011; Villoria et *al*., 2012). The inhibition is accomplished by the production of soluble factors such as gamma interferon (IFN-γ), tumour necrosis factor alpha (TNF-α), nitric oxide (NO), and reactive oxygen species (ROSs) by the natural killer cells (NK Cells) and macrophages (Mϕ). The absence of these cellular protective immune responses will result to the development of higher parasitaemia and a cascade of systemic effects (Krause *et al*., 2007; Saleh *et al*., 2010; Woldehiwet *et al*., 2010).

Protective maternal antibodies can be detected in foals up to 5 (Heerden 1996; de Waal *et al.*, 2004) or 6 (Phipps *et al*., 1996) months of age. These foals are thought to be protected by the passively-acquired immunity and non-specific factors during the first 6-9 months of age (de Waal *et al*., 2004). Transmission feeding by competent ticks

during this time usually produces only subclinical disease, but with constant super infections, a stable immunity may develop (Phipps *et al.,* 1996). Cross-immunity in mix infection of *B. caballi* and *T. equi* is unlikely (Schein 1988; Donnellan *et al.,* 2003b; de Waal *et al.,* 2004), but co-infection with both parasites is common (Donnelly *et al.,* 1980). Acquired maternal immunity is transitory, and as it wanes off, foals become more susceptible to neonatal EP (Kumar *et al*., 2008).

The precise function or role of the individual nine members of the *ema* gene family in the *T. equi* life cycle or the host innate response is idiopathic (Kappmeyer *et al*., 2012). High antibody titer to these proteins are mounted within 2-6 weeks following natural infection (Cunha *et al*., 2006) and may persist for life or for a long time with or without chemotherapeutic clearance of the parasite (Cunha *et al*., 2006; Ueti *et al*., 2012). Persistence of antibody titers in the absence of ongoing infection is adduced to be due to "memory" B cells continuous secretion of antibody, particularly IgG, without continued antigenic stimulation (Slifka and Ahmed, 1998; Bernasconi *et al*., 2002; Wise *et al*., 2017).

2.6 Clinical Signs of Equine Piroplasmosis

Equine piroplasmosis should be included in the differential diagnosis for horses with anaemia, icterus, pyrexia, and signs of chronic inflammatory disease. However, the clinical signs in EP are variable and non-specific, and so cannot be diagnosed based on clinical signs alone (Rothschild and Knowles, 2007; Center for Food Security and Public Health, 2008). *Babesia caballi*, *T. equi* and *T. haneyi* infections cannot be distinguished clinically, but differentiation between the three infections using molecular tools may be important for successful treatment and control of the disease (Rothschild and Knowles, 2007; Knowles *et al.,* 2018).

The incubation period, or time from infection to manifestation of clinical signs, ranges from 10 to 30 days for *B. caballi*, 12 to 19 days for *T. equi* (de Waal, 1992) and 14 to 30 days for *T. haneyi* in splenectomized horses (Knowles *et al*., 2018). The course of the disease may be peracute, acute, subacute, or chronic (Rothschild and Knowles, 2007). Peracute and acute signs may include pyrexia, icterus, anaemia, haemoglobinuria, bilirubinuria, splenomegaly, digestive or respiratory signs, and occasionally, death (de Waal, 1992; Ambawat *et al*., 1999; Deger *et al*., 2009; Laus *et al*., 2013; Abedi *et al*., 2014). Horses with subacute piroplasmosis may display anorexia, lethargy, weight loss, anaemia, limb oedema, poor performance, tachycardia and tachypnoea (Takeet *et al*., 2009; Mshelia *et al.*, 2013; Wise *et al*., 2013; Turaki *et al*., 2014). Chronic piroplasmosis is clinically indistinguishable from other chronic inflammatory diseases and generally presents with nonspecific signs, such as inapettence, poor body condition, poor performance and weight loss (Kappmeyer *et al*., 2012; Montes-Cortes *et al*., 2016)**.** Gastrointestinal complications such as colic, impaction and diarrhoea, in addition to pulmonary oedema, pneumonia, cardiac arrhythmias, laminitis, ataxia, myalgia and seizures develop secondary to EP (de Waal *et al*., 2004; Diana *et al.*, 2007; Zobba *et al*., 2008; Hussain *et al*., 2014). Anaemia may be minimal or absent in horses with chronic or persistent infection; these animals are termed carriersand are reservoirs for tick and iatrogenic transmission (via blood transfusion, reuse of needles or intravenous sets, or touching a wound on an infected horse and then handling another horse) (Rothschild and Knowles, 2007).

2.7GrossLesions of Equine Piroplasmosis

At necropsy, there is yellowish discolouration of subcutaneous tissue, congested cardiac lobe of the left lung with presence of multiple solid nodules. Emphysematous pulmonary lesion and atelactetic areas are evident at the cardiac lobe. Distended bladder and filled with dark coloured urine. Haemorrhagic and hypertrophied lymph nodes, especially mesenteric lymph nodes (Al-Obaidi *et al*., 2015), splenomegaly, swollen dark kidneys, liver and myocardial echymosis (de Waal, 1992; Zaugg, 1996) are often associated with Equine piroplasmosis.

2.8 Diagnosis, Diagnostic Features and Antigenic Targets on the Parasites

2.8.1 Diagnosis

The diagnosis of EP is based on clinical examination, microscopic examination of Giemsa stained blood smear, molecular detection based on polymerase chain reaction (PCR) (Vial *et al*., 2006; Knowles *et al*., 2018), serological detection using complement fixation test (CFT), enzyme linked Immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT) (OIE, 2008; Rothschild, 2013) and animal inoculation (OIE, 2008; Schwint *et al.*, 2009). These techniques are valuable tools for accurate diagnosis and epidemiological investigation (Terkawi *et al*., 2012).

2.8.1.1Clinical examination

The mere presence of clinical signs, revealed by anamnesis and clinical examination is insufficient to making an accurate diagnosis of EP. This is because the varied and non-specific clinical signs associated with the disease could also manifest in other diseases such as, equine ehrlichiosis, African horse sickness, equine infectious anaemia, equine viral arteritis, purpura haemorrhagica, immune-mediated haemolytic anaemia, and red

maple leaf toxicity (Alward *et al*., 2006; World Animal Health Information Database, 2012). Therefore, arriving at an accurate diagnosis will involve detailed history, thorough clinical examination and laboratory confirmation of the causative pathogens (Rothschild and Knowles, 2007; Center for Food Security and Public Health, 2008).

2.8.1.2Microscopy

Diagnosis of clinical cases of *B. caballi*, *T. equi* and *T. haneyi* can be made by identifying the parasites within erythrocytes using Giemsa, Leischemann, Wright, or Diff-Quick stains (Donnellan and Marais, 2009; Knowles *et al*., 2018). Blood smears must be thoroughly examined, as the level of parasitaemia, even in acute cases, can be low. *Theileria equi* trophozoites are predominantly oval organisms up to 3 µm. The infective merozoites usually occur as four piriform parasites, measuring 1.5 µm in length, in the characteristic Maltese cross formation, amoeboid, or signet-ring shaped with a halo. The percentage of erythrocytes parasitized is usually 1 to 5 percent in clinically diseased horses, but may exceed 20 percent (Donnellan and Marais, 2009). Parasitaemia with *T. equi* often ranges from 1-7% of erythrocytes, with a maximum of 95% (Rothschild and Knowles, 2007). *Babesia caballi* is larger than *T. equi.* and *T. haneyi. Babesia caballi* trophozoites are oval or elliptical with pear-shaped basophilic-staining merozoites occurring in pairs and measuring about 4 µm in length in a single erythrocyte (Retief, 1964). The percentage of detectable erythrocytes in peripheral blood parasitized by *B. caballi* typically does not exceed 1% and may be lower than 0.1% (Rothschild and Knowles, 2007; Donnellan and Marais, 2009). The sensitivity of detecting parasitized erythrocytes can be increased by using thick blood smears (Rothschild and Knowles, 2007). Additionally, blood samples that are collected and maintained in cold chain prior to slide preparation, and the concentration of parasitized

cells by centrifugation, are some useful steps that optimizes parasitaemia detection ((Theiler, 1902; Sippel *et al*., 1962).

2.8.1.3Molecular diagnosis

The PCR relies on amplification of DNA from the EP pathogens and the detection of specific, unique components of that DNA. There are four different types of PCR test used for detecting *Babesia* and *Theileria* parasites: primary or conventional PCR, which uses one set of primers; real-time PCR that quantifies level of parasite in peripheral blood; nested PCR that uses two sets of primers to increase sensitivity; and nested PCR with hybridization (probe specific for gene target results in enhanced sensitivity and specificity) (Bashiruddin *et al*., 1999; Battsetseg *et al.*, 2001). The specificity in the PCR can be defined by the molecular mass of the band, restriction endonuclease treatment of the band, sequencing of the band, and hybridization with specific probes (Schwint *et al.*, 2009; Bhoora *et al*., 2010; Wise *et al*., 2013). A real-time PCR also allows for quantitative detection of *B.caballi* and *T. equi* (Bhoora, 2010). Quantification of the parasite in blood would allow for studies related to factors that may alter the level of parasitaemia. A nested PCR for *T. equi* based on the sequence of the *ema-1* gene has detected the equivalent calculated parasitaemia of 0.000006 percent (Nicolaiewsky *et al*., 2001). The nested PCR detect 3.6 times more infections than microscopy and 2.2 times more infections than with conventional PCR (Rampersad *et al*., 2003). In other to assess the diagnostic sensitivity and specificity of the PCR, studies should include testing horses in endemic areas, defining the transmission risk posed by a seropositive, but PCR-negative horse in order to define the horse's status from a transmission and regulatory perspective (United States Department of Agriculture APHIS National Center for Risk Analysis, 2011).

2.8.1.4Enzyme linked immunosorbent assay (ELISA)

The competitive inhibition ELISA (cELISA) was developed by Knowles *et al*. (1991), and it uses the *T. equi* immunodominant and highly conserved surface antigen, *ema-1*, as specific monoclonal antibodies for the detection of *T. equi* infection. This was later improved by using recombinant protein instead of the previous culture-derived whole parasites to detect *Theileria* infection in horses (Knowles *et al*., 1992). The use of the recombinant protein facilitated the standardization of cELISA and overcame the need for *in vitro* cultivation of *T. equi* as well as experimental infection of horses for antigen production, thus, making this diagnostic tool the most suitable screening test for both *Babesia* and *Theileria* infections (Rothschild and Knowles, 2007). The recombinant *B. caballi* rhoptry-associated protein (*rap-1*) was developed by Kappmeyer *et al*. (1999) as cELISA diagnostic test for detecting *B. caballi* infection. The development of cELISA was later commercialized by Veterinary Medical Research and Development (VMRD) into two test kits, one for detecting *T. equi* antibodies and the other for detecting *B. caballi* antibodies (Rothschild and Knowles, 2007). The cELISA is licensed by the United State Department of Agriculture-Animal and Plant Health Inspection Service-Veterinary Section (USDA-APHIS-VS) Centers for Veterinary Biologics (CVB) and approved by the World Organization for Animal Health (OIE) as regulatory test for serodiagnosis of Equine Piroplasmosis (OIE, 2004; 2005). This test is very useful in detecting chronic infection in horses that are PCR and Complement Fixation Test (CFT) negative (Brüning, 1996). The test can also detect experimental *T. equi* infection as early as 21 days post infection and five weeks following tick transmission (Knowles *et al*., 1991).

2.8.1.5Complement fixation test (CFT)

Complement fixation test relies on fixation of complement during reaction between specific antigen and antibody (Brüning, 1996; Friedhoff and Soulé, 1996).). A CFT is considered positive when there is a positive reaction at a dilution of 1:5. It is a very specific test for acute infections and can detect antibodies as early as 8 days post infection, but the sensitivity in chronic and inapparent infection is low because some of the antibodies that develop in chronic infections do not fix complement ((Friedhoff and Soulé, 1996). Other demerits of CFT are occurrence of false-negative results, the need to produce large quantities of antigens and cross reactivity between antibodies against *T. equi* and *B. caballi* (Donnelly*et al*., 1982; Brüning, 1996).

2.8.1.6Indirect fluorescent antibody test (IFAT)

Indirect fluorescent antibody test (IFAT) was first described by Ristic and Sibinovic (1964) to detect antibodies against *B. caballi* in horses that were chronically infected. The test is more sensitive than the CFT, and involves binding parasite antigens to a glass slide and allowing it to react with the test sera (Wise *et al*., 2013). The bound antibodies are visible under ultraviolet light following the binding of fluorescein-labeled antiequine sera (Tenter and Friedhoff, 1986). The test sera are considered positive if they show strong fluorescence of the parasites at a dilution of 1:80 or even higher (Mosqueda *et al*., 2012). Antibody responses with the IFAT was detected in horses experimentally infected with *T. equi* and *B. caballi* as early as 3 to 20 days post infection, with significant titers even in the chronic infection (Weiland, 1986). The disadvantages associated with IFAT are time constraint, requires more antigens and difficulty in standardization owing to subjectivity in interpreting fluorescence (Huang *et al*., 2006).

2.8.1.7Western blot or immunoblotting

Western blot or immunoblot is a quantitative and semi-quantitative protein analysis developed by Towbin *et al.* (1979). This technique involves three elements; namely,separation of proteins by size, transferring proteins to a solid support or membrane, and marking proteins by primary and secondary antibodies for visualization (Mahmood and Yang, 2012). The western blot was previously used mainly for research but is now used as adjunct diagnostic aid for the detection of *B. caballi* and *T. equi* (Animal and Plant Health Inspection Service, 2012). This test, like nPCR have proven to successfully confirm chemotherapeutic clearance of *T. equi* by imidocarb diproprionate, and therefore, essential for possible discovery of imidocarb-resistant strains (Hines *et al*., 2015; Wise *et al.,* 2017).

2.8.1.8Animal inoculation

This involve transmission via blood transfusion or direct blood inoculation, or by tick feeding to either demonstrate the presence of *B. caballi, T. equi* or *T. haneyi* infections or clearance of these parasites by antiprotozoal agents (Schwint *et al*., 2009; Knowles *et al*., 2018). Animal inoculation test assists clinicians diagnose suspected horses with piroplasmosis by transfusing their blood to susceptible splenectomized or naïve horses. Alternatively, transmission feeding by tick vector on acutely and chronically infected horses can be done to identify the parasite either in the ticks or in experimentally infected naive horses (OIE, 2008; Ueti *et al*., 2008; Schwint *et al*., 2009).

2.8.2 Diagnostic features in giemsa-stained blood smears

The presence of diagnostic features, such as, large paired pyriform intraerythrocytic forms that are joined at their posterior ends, and measuring 2–5 µm in length and 1.3–

3.0 µm in diameter represent merozoite stages of *B. caballi* (Mehlhorn and Schein, 1984) (Fig.2.3). The arrangement of four pear-shaped merozoites that forms a tetrad known as ‘Maltese cross’ indicate *T. equi* microscopically (Fig. 2.4) (Yokoyama *et al.*, 2003; OIE, 2014). The Maltese cross of *T. haneyi* is smaller in their mean length (1.15 ± 0.16 mm) and width (0.70 ± 0.07 mm) than the mean length (1.88 ± 0.21 mm) and width (0.91 ± 0.13 mm) of *T. equi* (Knowles *et al*., 2018). Also, the minimal and maximal lengths of individual Maltese cross masses for *T. equi* are 1.57 mm and 2.32 mm, while those for *T. haneyi* are 0.87 mm and 1.57 mmrespectfully (Fig. 2.5). Similarly, the minimal and maximal widths of the Maltese crosses for *T. equi* are 0.72 mm and 1.39 mm, whereas, it is 0.56 mm and 0.9 mm, for *T. haneyi* n. sp. (Knowles *et al.*, 2018).

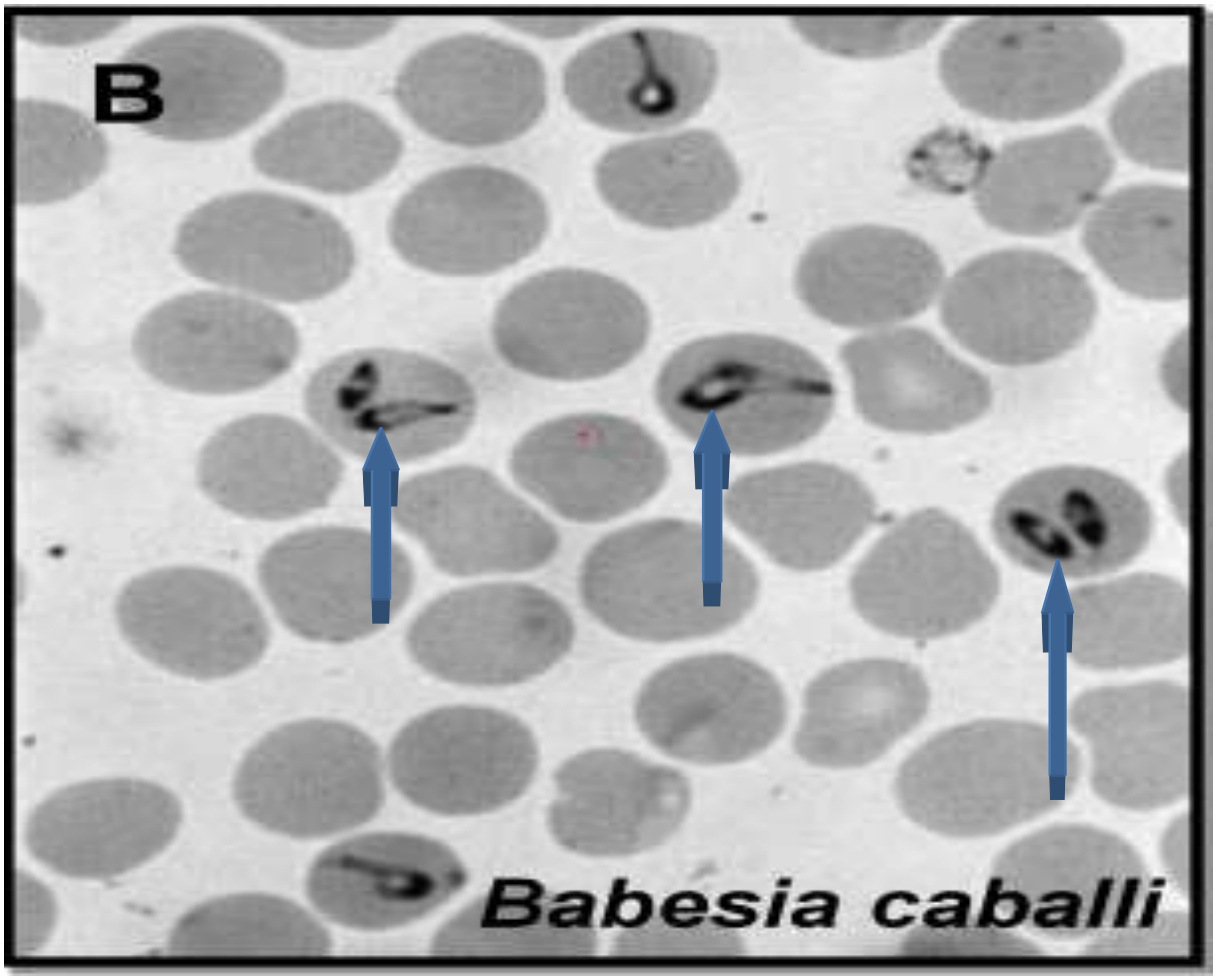


Figure 2.3: Blood smear showing pyriform merozoites *ofBabesia caballi* (B) in erythrocytes as indicated by blue arrows. Adapted from Kumar *et al*. (2009)

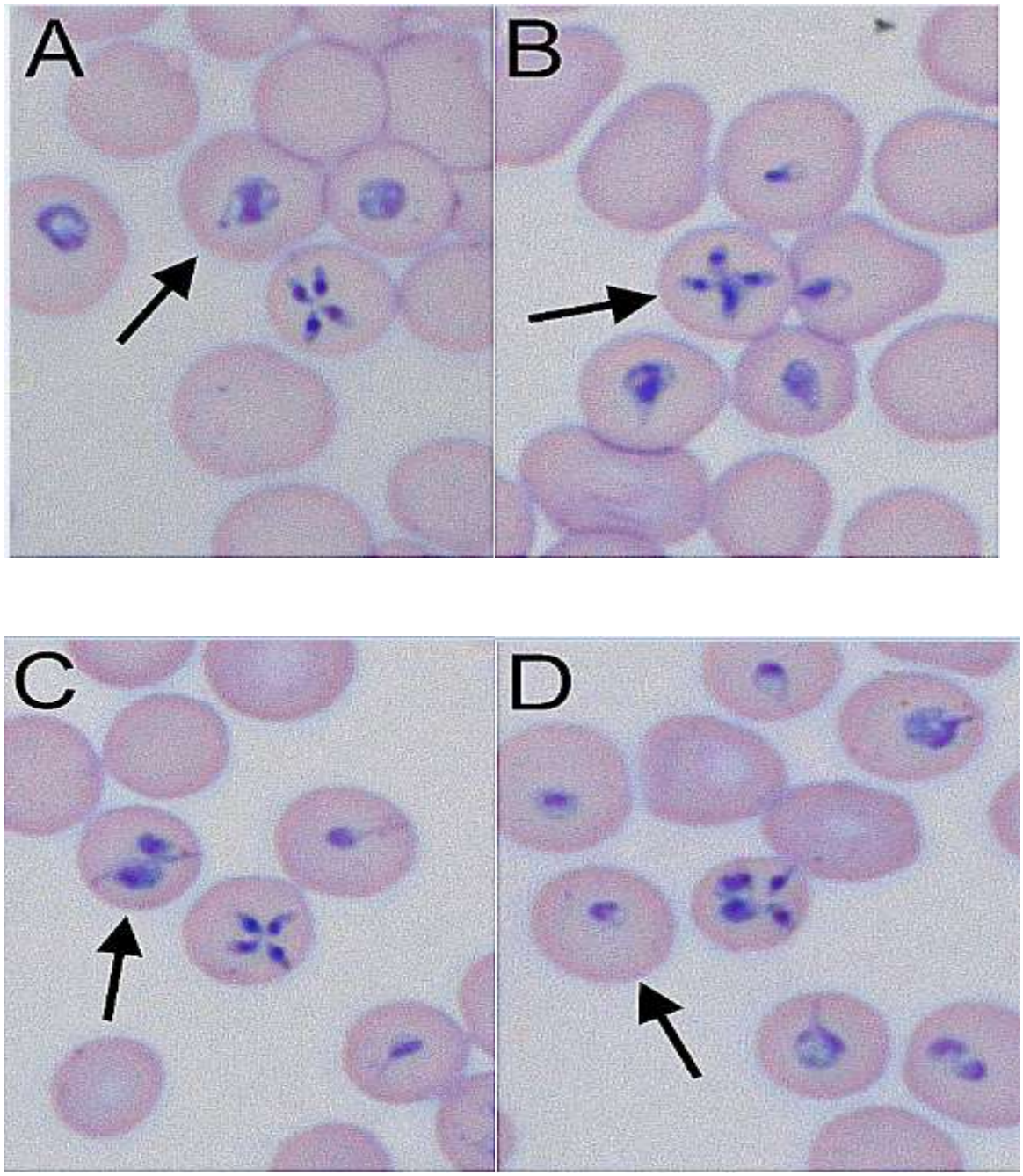


Figure 2.4: Intra-erythrocytic (merozoite) forms of *Theileria equi* (A-D) in Giemsa-stained blood film. Arrows indicate forms (merozoites and Maltese crosses) within the infected erythrocytes. Merozoites may be spherical or pyriform (pear shaped) (Mehlhorn and Schein, 1998). Maltese crosses are characterized by an arrangement of four merozoites. Arrows in A-D for *T. equi* indicate different phases of merozoites and Maltese crosses. Adapted from Knowles *et al*. (2018)

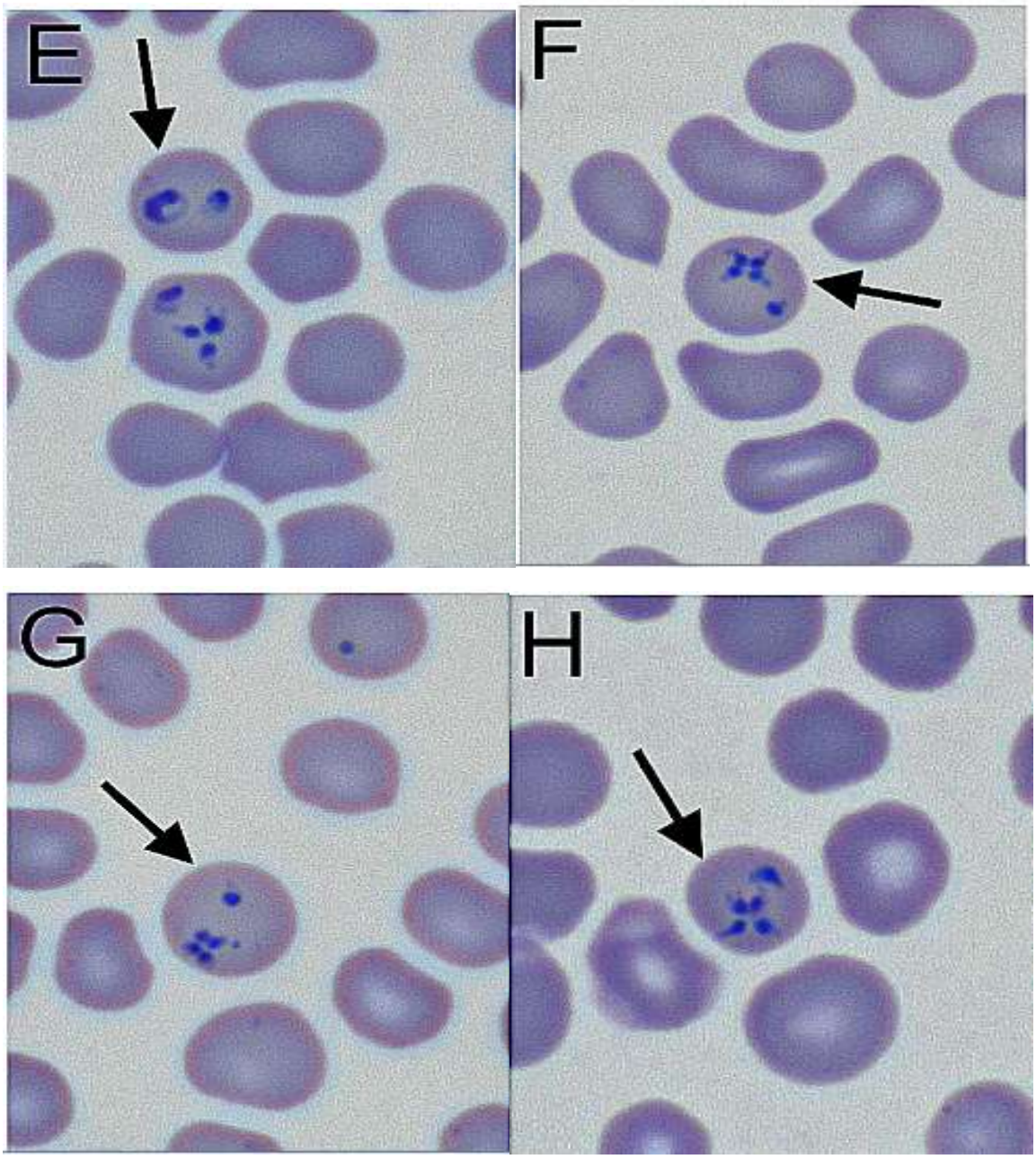


Figure 2.5:Intraerythrocytic forms of *Theileria haneyi* in Giemsa-stained blood film. Arrows indicate forms (merozoites and Maltese crosses) within the infected erythrocytes. Merozoites may be spherical or pyriform (pear shaped) (Mehlhorn and Schein, 1998). Maltese crosses are characterized by an arrangement of four merozoites. Arrows in E-F for *T. haneyi* n. sp. indicate different phases of merozoites and Maltesecrosses. Width and length measurements of merozoites were performed on individual masses of Maltese crosses. Adapted from Knowles *et al.* (2018)

2.8.3 Antigenic targets on the parasites

Several genes such as *ema-1* (Knowles *et al*., 1991; Battsetseg *et al*. 2002; Ueti *et al*., 2008; Knowles *et al*., 2018), *ema-2* (Ueti *et al*., 2008), *rap-1* for (Knowles *et al*., 1992), *β-tubulin* (Cacciò *et al*., 2000), the hypothetical gene (2,118 bp single copy open reading frame on chromosome 1 of *T. haneyi* n. sp., that is unique to this species) (Knowles *et al*., 2018) and the *18S rRNA* (Bashiruddin *et al*., 1999; Alhassan *et al*. 2005; Bhoora *et al*., 2009; Sloboda *et al*., 2011) have been used as antigenic targets for the diagnosis of *B. caballi*, *T. equi* and *T. haneyi*. The expression of *ema-1* is more in blood stages of the parasite while *ema-2* is more highly expressed in the salivary gland of ticks during transmission feeding (Ueti *et al.,* 2008). These proteins in addition to *ema-3* have close interaction with the erythrocyte membrane skeleton (Kumar *et al.,* 2004; Ikadai *et al*., 2006).

2.9Chemotherapeutics used in Treatment of Equine Piroplasmosis

The main goal of chemotherapy in most apicomplexan parasitic diseases is minimizing systemic effects, but, considering the persistent infection that ensues in equine piroplasmosis, successful treatment, particularly in *T. equi* infected horses is challenging (Kerber *et al*., 1999; Ueti *et al.,* 2005). Over emphasis on symptomatic treatment rather than complete elimination of the parasites in endemic countries might be advantageous in that it helps in promoting adaptive immunity, but, such treated horses remain chronically infected and maintain a population of parasites that survived previous drug exposure. This is a risk factor to the development of drug resistance and reservoir for transmission by competent ticks to naïve horses (Ueti *et al*., 2012).

Several antiprotozoal agents such as imidocarb diproprionate (Frerichs *et al*., 1973; Simpson and Neal, 1980; Buttler *et al*., 2008; Ueti *et al*., 2012, Hines *et al*., 2015; Wise

et al., 2017), diminazene aceturate, atovaquone (Hildebrandt *et al*., 2013; Sudan *et al*., 2013), combination of N-acetyl-L-cysteine and diminazene aceturate (Rizk *et al*., 2017), parvaquone (Kuttler *et al*., 1987), buparvaquone (Zaugg and Lane, 1992), Miltefosine (AbouLaila *et al*., 2014), artemisinin derivative and bumped kinase inhibitors 1294 (BKIs 1294) (Hines *et al*., 2015) have been used for the treatment of equine piroplasmosis with variable challenging success rates. Challenges such as inadequate confirmatory tests for successful parasite clearance, extended time between chemotherapeutic clearance of parasites and development of seronegative status, and the variability of these drugs' susceptibility among different strains of the causative pathogens are impediments to successful treatment (Bruning, 1996; Buttler *et al*., 2008; Schwint *et al*., 2009; Ueti *et al*., 2012). Efforts to determine the *in vitro* efficacy of certain drugs against *T. equi* have been undertaken with minimal success (Bork *et al*., 2003; Nagai *et al*., 2003; Aboulaila *et al*., 2010; Munkhjargal *et al*., 2012; Salama *et al*., 2013; Wise *et al*., 2013; Salama *et al*., 2014) due to lack of biological relevance or feasibility for use in horses (Hines et al., 2015).

2.9.1 Imidocarb dipropionate (ID)

Imidocarb diproprionate (ID) is a dicationic diamidine of the carbanilide series [3,3'-bis (2-imidazolin-2- yl)-carbanalidae] of antiprotozoal compounds, commonly used to treat equine piroplasmosis caused by *B. caballi*, *T. equi* and *T. haneyi* (Hines *et al.*, 2015; Knowles *et al*., 2018). The precise mechanism of action of this drug is unknown but causes chromatin clumping, dissolution of nuclear membrane, and cytoplasmic vacuolization of intraerythrocytic stages within 48 hours post administration (Simpson and Neal, 1980; Belloli *et al*., 2002). Other mechanisms such as to interfere with the production and/or utilization of polyamines, or prevent the entry of inositol into

parasitized erythrocytes has been reported (European Medicines Agency, 2001). It is administered as a dihydrochloride or diproprionate salt either subcutaneously or intramuscularly to horses (3.4 mg/kg body weight bw) and cattle (2.1 mg/kg bw) (Donnellan and Marais, 2009). In endemic countries, four doses of the drug at 4 mg/kg bw 72-hourly is recommended with a second dose to be repeated after two weeks (Ueti *et al*., 2012). Despite this established protocol, treatment failures persist (Hines *et al*., 2015). Therefore, due to scarcity of treatment options for piroplasms of horses and the potential for drug resistance with the commonly used ID, an alternative and novel drug, BKIs 1924 was explored for *in vitro* efficacy against *T. equi* (Hines *et al*., 2015).

Anticholinesterase effects such as sweating, diarrhoea, colic and signs of agitation are toxicity signs associated with the drug (Adam, 1981; Meyer *et al*., 2005) and can be mitigated using intravenous injection of 0.3 mg/kg N-butylscopolammonium bromide (Buscopan®) (Ueti *et al*., 2012) and atropine sulphate (Heerden, 1996).

2.9.2 Bumped kinase inhibitors 1294 (BKIs 1294)

Bumped kinase inhibitors (BKIs) are a group of experimental compounds currently used for *in vitro* and *in vivo* clinical trials against toxoplasmosis (Murphy *et al*., 2010; Dogett *et al*., 2014), cryptosporidiosis (Murphy et al., 2010; Castellanos-Gonzalex *et al*., 2013), malaria (Ojo *et al*., 2012; Ojo *et al*., 2014), other protozoal diseases (Keyloun *et al*., 2014) and equine piroplasmosis (Hines *et al*., 2015). The mechanism of action of BKIs is selective inhibition of the apicomplexan calcium-dependent protein kinases (CDPKs) that are critical for multiple parasitic physiologic functions such as parasite motility, invasion, secretory pathways and replication (Ojo *et al*., 2012). The CDPKs are very vital anti-apicomplexan chemotherapeutic candidates because they are absent in vertebrates (Larson *et al*., 2012). Therefore, binding of other mammalian

kinases by BKIs is prevented by gate keeper amino acid residues with large side chains that obstruct access to the ATP-binding pocket (Ojo *et al*., 2012; Castellanos-Gonzalez *et al*., 2013; Ojo *et al*., 2014). The BKI 1294 has demonstrated favourable *in vitro* efficacy against both the Texas isolate and USDA Florida strain of *T. equi* where it completely inhibited the growth of the two isolates (Hines *et al*., 2015). This is consistent with previous reports of its apicomplexan-selective target and favourable pharmacokinetics and safety (Ojo *et al*., 2012; Castellanos-Gonzalez *et al*., 2013; Goggett *et al*., 2014; Keyloun *et al*., 2014; Ojo *et al*., 2014).

2.9.3 Diminazene aceturate

Diminazene aceturate (4,4´(azoamino) dibenzamidine) is an aromatic diamidine, derived from Surfen (*bis*-2-methyl-4-aminoquinolyl-6-carbamide) used to treat piroplasmosis and Trypanosomiasis (Peregrine, 1994). It binds irreversibly to double-stranded DNA through specific interaction with sites rich in adenine-thymine base pairs in the groove between complementary strands (Gresh and Pullman, 1984; Hu *et al*., 1992; Peregrine and Mamman, 1993). This type of binding is non-intercalative and has high affinity for knietoplastid DNA (kDNA), and it impairs kinetoplast replication and function (Preregrine and Mamman, 1993) in addition to inhibiting the mitochondrial topoisomerase II (James and Giles, 1985). Diminazene aceturate is administered at 3.5 mg/kg deep intramuscularly every 48 hours for two treatments against *B. caballi* and *T. equi* (Kuttler, 1981; Rashid *et al*., 2008). Adverse effects associated with intramuscular injection of diminazene aceturate are swelling and necrosis at injection sites, respiratory distress, depression and other signs of intoxication (Kutscha, 2008; Wise *et al.*, 2013).

2.9.4 Atovaquone

Atovaquone (1,4-hydroxynaphthoquinone) is an antiprotozoal compound that has broad-spectrum activity against apicomplexan parasites (Kessl *et al*., 2003) such as *Babesia* spp*, Plasmodium* spp., *Pneumocystis carinii* and *Toxoplasma gondii* (Baggish and Hill, 2002; Weiss, 2002). The mode of action of atovaquone against protozoa parasites is by inhibiting the rate of oxygen consumption (Murphy and Lang-Unnasch, 1999). This drug has been used to treat human Babesiosis (Krause *et al*., 2000) and Plasmodium falciparum infection (Kosinczky *et al*., 2000). Although, this drug is less toxic to host cells compared to other antiprotozoal agents, the development of resistant parasites has limited its use (Guswanto *et al*., 2018).

2.10Prevention and Control

The continuous unrestricted transboundary movement of horses from border towns of Cameroon, Chad and Republic of Niger into Nigeria is worrisome and can aggravate the burden of EP in Nigeria (Turaki *et al.,* 2014), in addition to introduction of newer strain(s) of the parasites. Epidemiological reports confirm nationwide distribution and endemicity of EP in Nigeria (Oduye, 1976; Leeflang and Ilemobade, 1979; Aliu, 1983, Oladosu and Dipeolu, 1983; Lawal *et al*., 1999; Takeet *et al*., 2009; Garba *et al*., 2011, Garba *et al*., 2012; Pam *et al*., 2013; Sanusi *et al*., 2014, Turaki *et al*., 2014; Mshelia *et al*., 2016; Ememe *et al*., 2018; Idoko *et al*., 2018). Therefore, a concerted effort towards preventing the health impacts of EP on equids and effective control of tick vectors is desirable. Vaccination is vital to disease mitigation but unfortunately, vaccines against EP are currently unavailable globally, in both endemic and non-endemic countries (Osman, 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Population and Informed Consent

This study was conducted with a total of 152 horses comprising 72 horses showing varying clinical signs (such as pyrexia, congested, pale, iceteric mucous membranes, inappetence, lethargy, oedema around the fetlock, epiphora, lymphadenopathy, tick infestation, poor body condition) suggestive of piroplasmosis (symptomatic horses), and 80 horses that were apparently healthy (asymptomatic horses).Thirty-four (34) of the symptomatic horses were males, 10 were Geldings and 28 females. Also, 33 of the symptomatic horses were West African Barb horses while 39 were Argentine Polo Ponies. All the asymptomatic horses were West African Barb male horses. Horses presenting clinical signs consistent with piroplasmosis were recommended to be considered potential suspects by OIE (2005). Samples were taken from stables in Zaria and Igabi Local Government Areas of Kaduna State and Keffi Local Government Area of Nasarawa State. The consent of the clients and/or attending veterinarians was sought prior to sample collection. All animals were handled humanely according to established protocol on the care and use of animals.

3.2 Ethical Clearance

This was sought and approved by Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) and given the approval number, ABUCAUC/2020/34.

3.3 Inclusion and Exclusion Criteria

Suspected cases were identified based on clinical signs (such as pyrexia, congested, pale, iceteric mucous membranes, inappetence, lethargy, oedema around the fetlock, epiphora, lymphadenopathy, tick infestation, poor body condition) described in horses with piroplasmosis (OIE, 2005). Horses showing clinical signs of other diseases were excluded from this study. Similarly, horses with evidence of disabilities were excluded from this study.

3.4Study Design

3.4.1 Sampling method

A cross sectional study was carried out from June to November, 2017. The convenient and purposeful sampling technique was used to select horses presenting clinical signs suggestive of piroplasmosis and those that were apparently healthy for this study.

3.4.2 Study areas

Adult horses were sampled from Zaria city (coordinates: 11.0622236N, 7.706379E) (Zaria Local Government Area), DeeBee farms (coordinates: 10.714993N, 7.510049E) and 5th Chukker Polo and Country club (coordinates: 10.7039281N, 7.5328248E) (Igabi Local Government Area) in Kaduna State, and Keffi Polo Club (coordinates: 8.863571N, 7.807865E) (Keffi Local Government Area) in Nasarawa State. Zaria and Igabi lies within the Northern Guinea Savanna Ecological Zone and have an annual average rainfall of 1032 mm and 1200 mm respectively. The highest and lowest average temperatures are 28.9 ˚C and 22.9 ˚C for Zaria, and 35 ˚C and 12.8 ˚C for Igabi (Bose *et al.,* 2015;Abaje *et al.,* 2016).The mean atmospheric relative humidity ranges between 70-90 % and 25-30 % for the rainy and dry seasons respectively with Igabi being sub-

humid while Zaria is dry sub-humid (Umar and Abdulkadir, 2015). The highest amount of evaporation occurs during the dry season (Abaje *et al.,* 2015b).The rainfall in Keffi is bimodal and ranges from 1500 to 2000 mm, which occurs from April to early November yearly (N'Datchoh *et al*., 2012). Relative humidity varies from 80% between July and August to 60% between January and February. The monthly average temperature ranges from 28°C to 29°C in July and August and 34°C to 36°C in February and March (Ifatimehin *et al*., 2013).

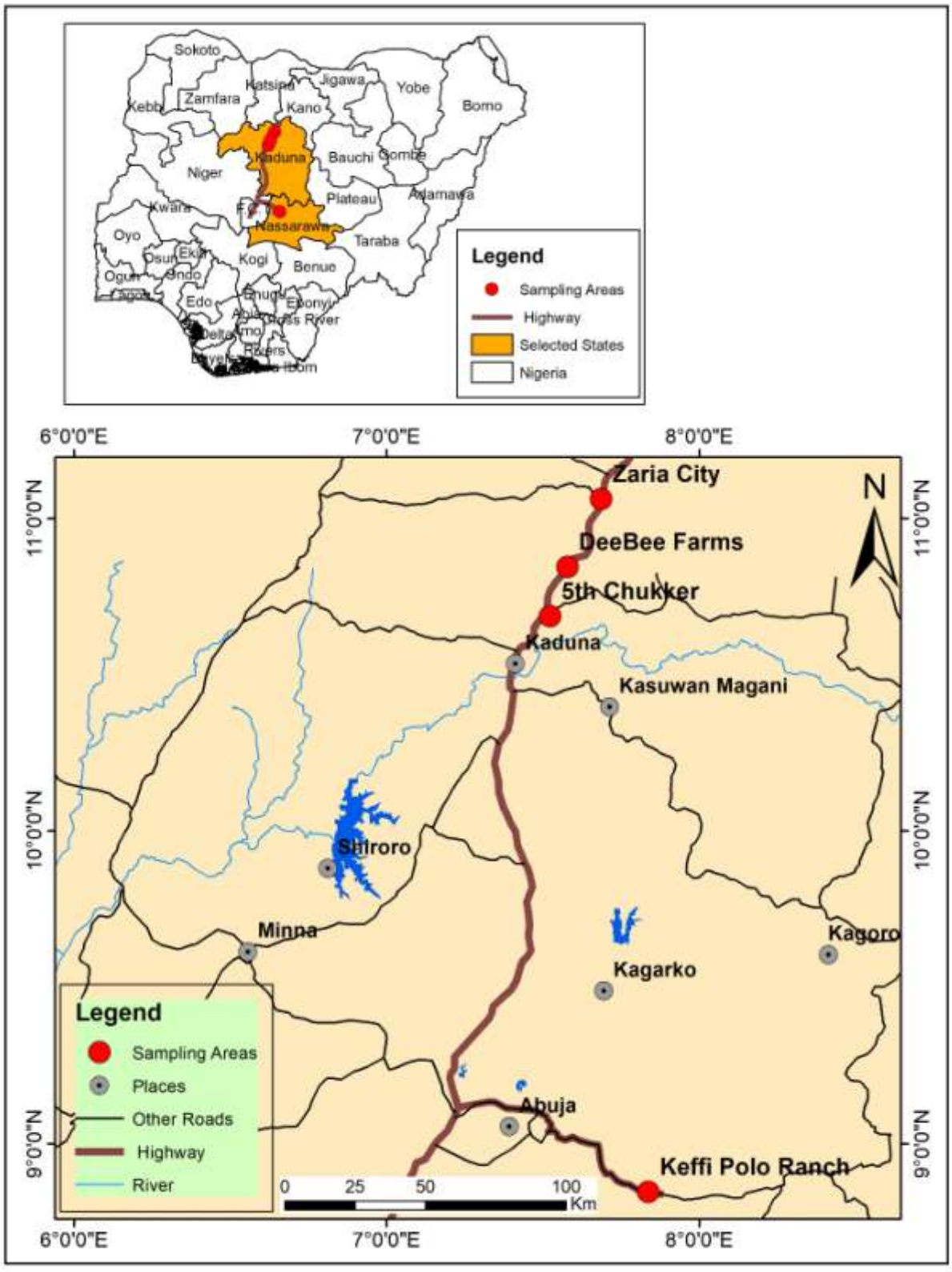


Figure 3.1: Map of the study areas showing Zaria City, DeeBee Farms, 5th Chukker (Kaduna State) and Keffi Polo Ranch (Nasarawa State) highlighted in red colour

3.4.3 Inclusion and exclusion criteria

Suspected cases were identified based on clinical signs described in horses with piroplasmosis (OIE, 2005). Also, horses showing clinical signs of other diseases were excluded from this study. Similarly, horses with evidence of disabilities were excluded from this study.

3.4.4 Study populations

A total of 152 adult horses, comprising 72 presenting various clinical signs of piroplasmosis and infested by ticks (symptomatic horses) and 80 apparently healthy horses (asymptomatic horses without ticks) were used for this study.

3.4.5 Sample collections

Fifteen mililitres (15 mL) of whole blood was collected via jugular venipuncture using 18 gauge needles from each horse. Two mililitres of the blood was dispensed into vacutainer containing acid-citrate-dextrose for erythrocyte ghost preparation, 3 mL of the blood was dispensed into vacutainers containing Ethylenediaminetetraacetic acid (EDTA), out of which 200 µL was spotted on Whatman FTA™ classic cards (Whatman® International Ltd., USA) and allowed to dry under room temperature. A drop of the EDTA blood from each of the horses was used to make Giemsa-stained thin blood smear for parasitological investigation, while the remainder was used for haematological evaluations. The remaining 10 mL of the whole blood was dispensed into plain 10 mL vacutainers and allowed to stand at room temperature for 1 hour, after which sera was harvested and transferred into 5 mL plain vacutainer tubes and stored at -4˚C until used. The skin of the horses covering known predilection sites for ticks including the perineum region, abdomen and thigh, ear and neck were carefully

examined for the presence of ticks. Ticks were collected using tweezers into labeled tubes plugged with cotton. Ticks from each animal were kept in separate tubes.

3.5 Morphological Identification of Tick

All the ticks collected were transferred into Petri dishes, mounted on slides and examined morphologically using a dissecting microscope at a magnification of ×40 and sorted to genus, and sexes. The ticks were morphologically identified using features described by Walker *et al*. (2014).

3.6Parasitological Examination

Demonstration of the presence of *T. equi* (small piroplasmwith Maltese cross form) and/or *B. caballi* (large *Babesia,* pyriform shaped) in infected-erythrocytes was done using Giemsa-stained thin blood smear on glass slides as described previously (Charles, 2007; Elaine and Margi, 2007) and then viewed using light microscopy at ×100 oil immersion magnifications through 60 microscopic fields.

3.7Washing of FTA^{TM} classic cards for Polymerase Chain Reaction (PCR)

One millimeter (1 mm) disposable biopsy punch with plunger (Miltex, Inc. York, USA) was used to punch out disc from each card into labeled PCR amplification tubes. Two hundred microliter (200 µl) of FTA card purification reagent was added into each PCR tube and incubated at room temperature for 5 minutes. The reagent was pipetted off and the steps repeated two more times. Two hundred microliter (200 µl) of Dulbecco's phosphate buffered saline (DPBS 1 X) gibco® (Life Technologies Corporation, Grand Island, NY, USA) was added to each PCR tube and incubated at room temperature for 5 minutes. The DPBS was pipette off and the steps repeated one more time. The lid of

each PCR tube was opened and allowed to dry for 1 hour in a PCR hood at room temperature.

3.8 Species-specific Nested Polymerase Chain Reaction (nPCR)

3.8.1 *Theileriaequi* nPCR

This was performed as described by Schwint *et al*. (2008) but with slight modification. Briefly, reference genomic DNA (gDNA) of *T. equi* and *T. equi* stabilates spotted on FTA^{TM} card served as the positive control. Presence of *T. equi* was detected by *nPCR* targeting the *ema-1* gene. The external primer mix involved 25 µl of *T. equi* external-forward primer 5' –GAGGAGGAGAAACCCAAG -3' (100 mM) paired with 25 µl of *T. equi* reverse primer 5'-GCCATCGCCCTTGTAGAG (100 mM) and 950 µl of nuclease-free water. From the above external primer mix, 100 µl was taken and added to 650 µl of nuclease-free water to make the external reaction mix. The internal primer mix involved 50 µl of *T. equi* internal-forward prime 5' TCAAGGACAACAAGCCATAC -3' along with the 50 µl of *T. equi* reverse primer 5' TTGCCTGGAGCCTTGAAG- 3' in 900 µl of nuclease-free water. One hundred microliter (100 µl) of this was mixed with 250 µl Rediload (Invitrogen®) and 800 µl of nuclease-free water to make the internal reaction mix. The first-round PCR was performed in 25 µl reaction volumes with 12.5 µl of PCR master mix (Roche, Indianapolis, IN, USA), 7.5 µl of external reaction mix and 5 µl of gDNA of *T. equi* (only for positive controls) in labeled PCR amplification tubes containing washed FTA^{TM} card disc. Second-round reactions contained 1 µl of the first-reaction product, 12.5 µl of PCR master mix (Roche, Indianapolis, IN, USA), and 11.5 µl of internal reaction mix. All external and internal reaction mixes were kept on ice for immediate use or refrigerated in +4˚C until use. The amplification of *T. equi* was done using BIO-

RAD 1000™ Thermal cycler (Bio-Rad Laboratories, Inc., USA). Cycling conditions for the external primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 20 s, 60˚C for 20 s, and 72˚C for 20 s, and then a final extension of 72˚C for 5 min, and holding at 4˚C. The cycling conditions for the internal primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 5 s, 60˚C for 5 s, and 72˚C for 5 s, and a 72˚C for 5 min extension, and holding at 4˚C. Description of the *T. equi* nPCR is summarized below in Table3.1.

3.8.2 *Babesia caballi* nPCR

This was performed as described by Schwint *et al*. (2008) but with slight modification. Briefly, reference genomic DNA (gDNA) of *B. caballi* and *B. caballi* tissue culture spotted on FTA™ card served as the positive control. Presence of *B. caballi* was detected by *nPCR* targeting the *rap-1* gene. The external primer mix involved 25 µl of *B. caballi* external-forward primer 5'- GAT TAC TTG TCG GCT GTG TCT -3' (100 mM) paired with 25 µl of *B. caballi* reverse primer 5'- CGC AAA GTT CTC AAT GTC AG -3' (100 mM) and 950 µl of nuclease-free water. From the above external primer mix, 100 µl was taken and added to 650 µl of nuclease-free water to make the external reaction mix. The internal primer mix involved 50 µl of *B. caballi* internal-forward primer 5'- GCT AAG TAC CAA CCG CTG A -3' along with the 50 µl of *B. caballi* reverse primer 5'- CGC AAA GTT CTC AAT GTC AG -3' in 900 µl of nuclease-free water. One hundred microliter (100 µl) of this was mixed with 250 µl Rediload (Invitrogen®) and 800 µl of nuclease-free water to make the internal reaction mix. The first-round PCR was performed in 25 µl reaction volumes with 12.5 µl of PCR master mix (Roche, Indianapolis, IN, USA), 7.5 µl of external reaction mix and 5 µl of gDNA of *B. caballi* (only for positive controls) in labeled PCR amplification tubes containing washed FTA™ card disc. Second-round reactions contained 1 µl of the first-

reaction product, 12.5 µl of PCR master mix (Roche, Indianapolis, IN, USA), and 11.5 µl of internal reaction mix. All external and internal reaction mixes were kept on ice for immediate use or refrigerated in +4˚C until use. The amplification of *B. caballi* was done using BIO-RAD 1000^{TM} Thermal cycler (Bio-Rad Laboratories, Inc., USA), cycling conditions for the external primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 20 s, 60˚C for 20 s, and 72˚C for 20 s, and then a final extension of 72˚C for 5 min, and holding at 4˚C. The cycling conditions for the internal primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 5 s, 60˚C for 5 s, and 72˚C for 5 s, and a 72˚C for 5 min extension, and holding at 4˚C. Description of the *B. caballi* nPCR is summarized in Table 3.1.

3.8.3 *Theileria haneyi* nPCR

This was performed as described by Knowles *et al*. (2018). Briefly, the reference genomic DNA (gDNA) of *T. haneyi* served as the positive control. Presence of *T. haneyi* was detected by *nPCR* targeting a hypothetical gene based on a 2,118 bp single-copy open reading frame (ORF) on chromosome 1 of the parasite. The external primer mix involved 25 µL of *T. haneyi* external-forward primer 5'-CCATACAACCCACTAGAG -3' (100 mM) paired with 25 µl of *T. haneyi* reverse primer 5' CTGTCATTTGGGTTTGATAG -3' (100 mM) and 950 µL of nuclease-free water. From the above external primer mix, 100 µL was taken and added to 650 µL of nuclease-free water to make the external reaction mix. The internal primer mix involved 50 µL of *T. haneyi* internal-forward primer 5'- GACAACAGAGAGGTGATT -3' along with the 50 µL of *T. haneyi* reverse primer 5'- CGTTGAATGTAATGGGAAC -3' in 900 µL of nuclease-free water. One hundred microliter (100 µL) of this was mixed with 250 µL Rediload (Invitrogen®) and 800 µL of nuclease-free water to make the internal

reaction mix. The first-round PCR was performed in 25 µL reaction volumes with 12.5 µL of PCR master mix (Roche, Indianapolis, IN, USA), 7.5 µL of external reaction mix and 5 µL of gDNA of *T. haneyi* (only for positive controls) in labeled PCR amplification tubes containing washed FTA^{TM} card disc. Second-round reactions contained 1 µL of the first-reaction product, 12.5 µL of PCR master mix (Roche, Indianapolis, IN, USA), and 11.5 µL of internal reaction mix. All external and internal reaction mixes were kept on ice for immediate use or refrigerated in +4˚C until use. The amplification of *T. haneyi* was done using BIO-RAD 1000^{TM} Thermal cycler (Bio-Rad Laboratories, Inc., USA), cycling conditions for the external primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 20 s, 60˚C for 20 s, and 72˚C for 20 s, and then a final extension of 72˚C for 5 min, and holding at 4˚C. The cycling conditions for the internal primers are 95˚C for 5 min, followed by 30 cycles of 95˚C for 5 s, 60˚C for 5 s, and 72˚C for 5 s, and a 72˚C for 5 min extension, and holding at 4˚C. Description of the *T. haneyi* nPCR is summarized in Table 3.1.

3.8.4 18S rRNA PCR

3.8.4.1 18S rRNA nPCR (partial length)

The 18S PCR (partial length) was carried out according to established protocol (Bhoora *et al*., 2010). The Oligonucleotide sequence for the respective primers and thermocycler conditions are summarized in Table 3.2.

3.8.4.2 18SrRNAPCR

The full length 18S rRNA PCR was carried out based on the protocol described by Bhoora *et al*. (2010). Briefly, forward primer NBabesia1F (5'-AAGCCATGCATGTCTAAGTATAAGCTTTT -3') and reverse primer 18SRev-TB

(5’- GAATAATTCACCGGATCACTCG -3’) were used in a primary PCR to amplify a fragment of 1600 bp in length. Reactions were performed in a final volume of 25 µL with High Fidelity PCR Master Mix (Roche Diagnostics, Mannheim, Germany), 0.2 mM of each primer and 2.5 µL of gDNA. The cycling conditions were: an initial denaturation of 2 min at 94 ˚C, followed by 40 cycles of 30 s at 94 ˚C, 45 s at 60 ˚C and 1 min at 72 ˚C, and a final extension of 7 min at 72 ˚C. This is summarized in Table 3.2 below.

Table 3.1: Target Parasites with respective primer sets, thermocycler conditions and band sizes for nested polymerase chain reaction (nPCR)

Type of Parasite	External Primer Sequence	Internal Primer Sequence	Band Size
Babesia caballi	forward: 5'- GAT TAC TTG TCG GCT GTG TCT-3' reverse: 5'- CGC AAA GTT CTC AAT GTC AG -3'	forward: 5'- GCT AAG TAC CAA CCG CTG A -3' reverse: 5'- CGC AAA GTT CTC AAT GTC AG -3'	250 (*RAP-1*)
Thermocycler Condition	95 ˚C hold for 5 min 30 cycles: 95 ˚C for 20 s 60 ˚C for 20 s 72 ˚C for 20 s 72 ˚C hold for 5 min Final hold at 4 ˚C	95 ˚C hold for 5 min 30 cycles: 95 ˚C for 5 s 60 ˚C for 5 s 72 ˚C for 5 s 72 ˚C hold for 5 min Final hold at 4 ˚C	
Theileria equi	forward: 5' -GAG GAG GAG AAA CCC AAG -3' reverse: 5'-GCC ATC GCC CTT GTA GAG -3'	forward: 5'-TCA AGG ACA ACA AGC CAT AC -3' reverse 5'-TTG CCT GGA GCC TTG AAG- 3'	250 *(EMA-1)*
Thermocycler Condition	95 ˚C hold for 5 min 30 cycles: 95 ˚C for 20 s 60 ˚C for 20 s 72 ˚C for 20 s 72 ˚C hold for 5 min Final hold at 4 ˚C	95 ˚C hold for 5 min 30 cycles: 95 ˚C for 5 s 60 ˚C for 5 s 72 ˚C for 5 s 72 ˚C hold for 5 min Final hold at 4 ˚C	
Theileria haneyi	forward: 5'- CCA TAC AAC CCA CTA GAG -3' reverse: 5' – CTG TCA TTT GGG TTT GAT AG -3'	forward: 5'- GAC AAC AGA GAG GTG ATT -3' reverse: 5'- CGT TGA ATG TAA TGG GAA C -3'	238 (Hypothetical gene based on a 2,118 bp single-copy open reading frame (ORF) on chromosome 1)
Thermocycler Condition	95 ˚C hold for 4 min 35 cycles: 95 ˚C for 20 s 63.5 ˚C for 30 s 72 ˚C for 20 s 72 ˚C hold for 7 min Final hold at 4 ˚C	95 ˚C hold for 4 min 35 cycles: 95 ˚C for 20 s 58.1 ˚C for 30 s 72 ˚C for 20 s 72 ˚C hold for 7 min Final hold at 4 ˚C	

Table 3.2: 18S rRNA partial and full length polymerase chain reaction (PCR) with primer sets, thermocycler conditions and band sizes

Target gene	External Primer Sequence	Internal Primer Sequence	Band Size
18S Rrna **(Partial length)**	18S 4F Nbab: 5' – AGG CCA TGC ATG TCT AAG TAT - 3' 18S Rev-TB: 5' - GAA TAA TTC ACC GGA TCA CTC - 3'	Bab18S_437-461.nIntF: 5' - AAT CCT GAC ACA GGG AGG TAG TGA C - 3' Bab18S_898-873.nIntR: 5' - CTA AGA ATT TCA CCT CTG ACA GT - 3'	390
Thermocycler Condition	95 ˚C hold for 3 min 35 cycles: 95 ˚C for 30 s 50 ˚C for 30 s 72 ˚C for 1 min 72 ˚C hold for 5 min Final hold at 4 ˚C	95 ˚C hold for 3 min 35 cycles: 95 ˚C for 30 s 65 ˚C for 1 min 72 ˚C for 1 min 72 ˚C hold for 5 min Final hold at 4 ˚C	
18S rRna **(Full length)**	*NBabesia1F* :5' - AAG CCA TGC ATG TCT AAG TAT AAG CTT TT- 3'	*18SRev-TB* :5' – GAA TAA TTC ACC GGA TCA CTC G- 3')	1600
Thermocycler Condition	94 ˚C hold for 2 min 40 cycles: 94 ˚C for 30 s 60 ˚C for 45 s 72 ˚C for 1 min 72 ˚C hold for 7 min Final hold at 4 ˚C		

3.9 Deoxyribonucleic Acid (DNA) Agarose Gel Electrophoresis

Forty five milliliters (45 mL) of 1X Tris acetic EDTA (TAE) buffer (Ambion®, USA) was transferred to a conical flask, 0.45 g (1%) of UltraPure™ agarose gel powder (Invitrogen™ by Life Technologies™, USA) was added and placed inside a microwave for 45 s and then brought out to cool to 52 ˚C. It was then stained with 0.45 µL SYBR® Safe DNA gel stain (Invitrogen™, Molecular Probes™, Eugene, Oregon, USA) and carefully poured into a 7 × 10 cm UV-transparent tray containing two separately placed 1.5 mm thick, fixed-height 15-well combs (Bio-Rad Laboratories Inc., USA) and allowed to stay at room temperature for 30 minutes. The two combs were removed afterwards and the prepared gel carefully dropped into an electrophoresis tank containing 1X Tris acetic EDTA (TAE) buffer fixed at 90 V for 30 min, and the images visualized in Gel Doc™ XR (Bio-Rad Laboratories Inc., USA).

3.10ExoSAP-IT PCR Clean-up and DNA Sequencing

The amplicons obtained from the PCRusing 18S and the species specific primers were cleaned up using ExoSAP-IT (Affmetrix Inc., Santa Clara, USA). Briefly, 10 µL of each of the PCR amplicons were dispensed into newly labeled PCR amplification tubes, 4 µL ExoSAP-IT PCR Product cleanup reagent was added to the respective tubes and run at 37˚C for 15 min, 80˚C for 15 min, and holding at 4˚C. The sequence tubes were labeled accordingly in duplicate, 5 µL each of the internal forward and internal reverse primers were added to the respective duplicate containing the cleaned PCR products, and sent to Eurofins Genomics, USA for sequencing. The obtained sequences were aligned separately for each species with homologues available in the GenBank™ using Kalign (Lassmann and Sonnhammer, 2005) and consequently manually edited in Bioedit (Hall, 1999).

3.11 TOPO pCR 2.1 Cloning using M13 Primers

TheTOPO pCR 2.1 Cloning kit (Thermo Fisher Scientific Inc., USA) was used to clone the *Taq polymerase*-amplified 18S PCR products suspected to have mixed infections. This was performed based on method described by the manufacturers. Briefly, TOPO® Cloning reaction volume of 6 µL, comprising 4 µL of fresh PCR product, 1 µL of salt solution and 1 µL of TOPO® vector. Mix ligation and incubated for 5 minutes at room temperature. Competent *Escherichia coli* (*E. coli*) cells were removed from -80˚C Celsius and immediately placed on ice. Two microliter (2 µL) of the ligation mix was added to the competent cells (Top 10) and placed on ice for 30 minutes. This was then heat shocked in a water bath at 42˚C for 30 seconds and immediately placed on ice for 1 minute. Exactly 250 µL of SOC medium was added to the competent cells and incubated by shaking for 1 hour at 37˚C. The cells are spread on LBA + Xgal agar plate and incubated overnight at 37˚C. White and blue colonies were observed on the plate the following day. The white colonies were screened by PCR using M13 Forward and Reverse primers using the following thermocycler condition, 95˚C for 3 min; 35 cycles of 95˚C for 30 s, 55˚C for 30 s, and 72˚C for 1.5 min; final extension at 72˚C for 5 min; and holding at 12˚C. The blue colonies without the insert were not used for PCR. The M13 PCR positive clones were cleaned up using ExoSAP-IT PCR Clean-up and sent out to Eurofin Genomics, USA for sequencing.

3.12 Detection of *B. caballi* and *T. equi* Specific Antibodies for Symptomatic andAsymptomatic Horses

This was carried out using the competitive enzyme linked Immunosorbent assay (cELISA) kit (VMRD, Pullman, WA, USA) as directed by the manufacturer. Sera from

sampled horses were tested, and the positive result is defined as ≥40% inhibition by the manufacturer.

3.13Determination of Haematological Parameters for Symptomatic andAsymptomatic Horses

Two milliliters (2 mL) of the blood in EDTA bottle was used to determine the Packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count using the Veterinary automated haematology analyzer (HI 2800, Pioway Medical Lab Equipment, China).

3.14 Preparation of Haemoglobin-free Erythrocyte Membrane

Haemoglobin-free erythrocyte membranes (ghosts) were prepared according to the method described by Dodge *et al*. (1963). Briefly, 2 mL of blood was dispensed into vacutainers containing 0.3 mL of acid-citrate-dextrose (ACD) as an anticoagulant. The blood was centrifuged at 8000 ×g for 10 min, after which the plasma was aspirated and discarded. The resultant erythrocytes/sediments was washed by adding 3 mL of isotonic phosphate buffer solution of 30 imOsm followed by gentle agitation and then centrifugation at 8000 ×g for ten min. The supernatant was decanted and the process repeated twice. The washed erythrocytes were haemolysed by suspending them in 3 mL of hypotonic phosphate buffer of about 20 imOsm and agitated for about 5 min. The resulting Haemolysate was centrifuged at 8000 ×g for 20 min. The recovered erythrocyte ghosts were washed in the hypotonic buffer continuously until they became very clear. The erythrocyte ghosts were stored in vials in freezer (-4 ^{o}C) until needed for determination of antioxidants and sialic acid assay.

3.15 Biochemical Analyses on Erythrocyte Membraneof Symptomatic andAsymptomatic Horses

3.15.1 Malondialdehyde concentration

Erythrocyte membrane malondialdehyde levels, an index of lipid peroxidation were measured by the double heating method of Draper and Hadley (1990). The method is based on spectrophotometric measurement of the purple colour generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2.5 mL of trichloroacetic acid solution (10%*w/v*) was added to 0.5 mL erythrocyte ghost solution in each centrifuge tubes, and then placed in boiling water for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000 ×g for 10 min and 2 mL of each sample supernatant was transferred to a test tube containing 1 mL of TBA solution (0.67%*w/v*). Each tube was placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm (using the Shimadzu UV 1601 Spectrophotometer). The concentration of MDA was calculated based on the absorbance coefficient of TBA - MDA complex ($\varepsilon = 1.56 \times 105$ cm M^{-1}).

3.15.2 Superoxide dismutase activity

This was measured using a commercial kit (RANSOD kit, Randox Com, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), to form a red formazan dye. The enzyme activity was determined by the degree of reaction inhibition, where one unit of SOD corresponded to 50 % inhibition of INT reduction under assay condition.

3.15.3 Glutathione peroxidase activity

This was measured using a commercial kit (RANSEL kit, Randox Com, UK) based on the method described by Paglia and Valentine (1967). Glutathione peroxidase catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form, with the resultant oxidation of NADPH to $NADP^+$.

3.15.4 Catalase activity

The activity of catalase was determined using the commercial catalase assay kit (Oxford Biomedical Research, Inc., USA) based on the colorimetric method, described by Slaughter and O'Brien (2000). The analysis was accomplished using the standard autoanalyzer (Cobas-Mira, ABX-Diagnostics, Japan).

3.15.5 Determination of erythrocyte surface sialic acid concentration

Bound sialic acid was determined using 50 μL of the suspension of washed erythrocyte ghosts incubated with 100 μL H_2SO_4 at 80 $^oC$for 1 hour in order to liberate bound sialic acid (Warren, 1963). Sialic acid concentration was subsequently measured in the mixture using thiobarbituric assay (TBA assay) (Aminoff, 1961; Engstler *et al*., 1995).

3.16 Serum Biochemical Analyses

The harvested sera of the symptomatic and asymptomatic horses were also used to determine serum activities of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and concentration of bilirubin, total protein, urea, creatinine, electrolytes (Na, Cl, K, HCO_3^-, Ca, PO_4), glucose and cholesterol using appropriate reagents (RANDOX® and AGAPE®) and

methods described by the manufacturers. The automated Audiocomb analyzer (Bayer Express Plus, Bayer Germany, Serial Number 15950) and Spectrophotometer at the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria were used for the analyses.

3.17 Determination of Serum Sialic Acid Concentration Using the Thiobarbiturate Assay (TBA)

Exactly 50 μL of serum was dispensed into screw-capped test tube. This was followed by the addition of 250 μL of periodic acid and the mixture was incubated in a water bath at 37 ^{o}C for 20 minutes. Exactly 100 μL of sodium arsenite was added to the mixture to destroy excess periodate followed by addition of 1000 μL of thiobarbituric acid. The mixture was heated in a boiling water bath for 7 min. This was followed by cooling in an ice bath. The chromophone was extracted by vigorously shaking the mixture following the addition of 2000 μL of acid-butanol solution (prepared by mixing HCl with Butanol in the ratio 1:19 *v/v*). The mixture was centrifuged (using Heraeus Labofuge 300, Kandro Laboratory Product, Germany) at 1000 ×g for 5 min. After the centrifugation, the clear organic phase was transferred to a cuvette and the optical density (O.D) read on a colorimeter (JENWAY 6051, ESSEX, UK.) at 549 nM against a blank (which contained distilled water). Sialic acid concentrations were extrapolated from sialic acid standard curve.

3.18 Data Analysis

Some of the 18S full length sequences were selected into the Sequencher software program (Seattle, WA, USA) and trimmed to an identical length of 1490 bp following assembly in order to find common overlap. The trimmed sequences were imported into

the program CLC Genomics Workbench, where they were aligned using the program's 'Create Alignments' feature. That alignment was used in a neighbour joining algorithm using the program's 'Create Tree' feature. The distance measure was set for Jukes-Cantor, and 1000 Bootstrap Replicates were done. Sample Z5-16 is a *Babesia caballi* 18S sample used as an out-group. Additional samples were included that represent recognizable equine *Theileria species* clades, for labeling resulting groups in the phylogenetic tree. These additional samples are Ho-79_Fl (A) and Te0002_Tx (A); Sudan_Atbara8 (B); Ho-233_Thaneyi (C); *Theileriaequi* Atbara1 (D); and *T. equi*_Korea (E).

Data generated were presented in tables and analyzed using Microsoft Excel 2007. Statistical differences were evaluated by analysis of variance and student *t* test using the Graph pad prism version 4.0 for windows. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Symptomatic Horses

4.1.1 Clinical examination

Prior to sample collection, the seventy two horses examined showed variable clinical signs of equine piroplasmosis, such as, congested to icteric ocular and/or vaginal mucous membranes, pyrexia, oedema around the fetlock, relaxation of anal sphincter, weight loss, anorexia, unilateral to bilateral epiphora and tick infestation.

4.1.2 Microscopic examination

Microscopic examinations of the Giemsa-stained thin blood smears prepared from all the 72 symptomatic horses were all negative for the piroplasms (*B. caballi* and *T. equi*).

4.1.3 Morphological identification of ticks

In the course of sampling, six hundred and eighty three (683) ticks of different species infesting the horses were collected and morphologically identified. *Rhipicephalus evertsi evertsi* constituted the major tick species identified with a population of 397, out of which 48.9% were males while 51.1% females. Other *Rhipicephalus species* were 158 with 54.4% male while the females were 45.6%. Sixty three (63) of the total ticks were *Amblyomma variegatum*, with male constituting 52.4% while 47.6% were females. Five of the ticks were *Hyalomma species*, out of which 60% were males while 40% were females. *Hyalomma dromedarii* were 44 in numbers with 77.3% males while

22.7% were females. Four (4) out of the total ticks identified were 100% female *Hyalomma impeltatum.* Only one (1) female *Hyalomma truncatum* was identified with the remaining 11 ticks being 100% female *Boophilus decoloratus.* Overall, four (4) genera (*Amblyomma,Boophilus, Hyalomma* and *Rhipicephalus*) and eight (8) species of ticks were identified in this study as shown in Table 4.1 below; there were more males 51.2% (350) than females 333 (48.8%) ticks on the horses sampled.

Table 4.1. Distribution of various ticks species infesting horses from the sampled areas

Tick species	Male	Females	Total
Rhipicephalus evertsi evertsi	194 (48.9%)	203 (51.1%)	397 (58.1%)
Rhipicephalus species	86 (54.4%)	72 (45.6%)	158 (23.1%)
Amblyomma variegatum	33 (52.4%)	30 (47.6%)	63 (9.2%)
Hyalomma species	3 (60%)	2 (40%)	5 (0.7)
Hyalomma dromedarii	34 (77.3%)	10 (22.7%)	44 (6.4%)
Hyalomma impeltatum	0	4 (100%)	4 (0.6%)
Hyalomma truncatum	0	1 (100%)	1 (0.2%)
Boophilus decoloratus	0	11 (100%)	11 (1.6%)
TOTAL	350 (51.2%)	333 (48.8%)	683 (100%)

Ticks identified from symptomatic horses

4.1.4Molecular diagnosis using polymerase chain reaction

4.1.4.1.Babesia caballi rap-1 gene nested polymerase chain reaction (nPCR)

Nested PCR using species specific primers for *B. caballi* amplified the *rap-1 gene* of the parasite at 250 bp in only sample z-1, all other samples were negative (Plate I). The blast outcome of the nucleotide sequence revealed 100% identity to *B. caballi* available at the gene bank (www.ncbi.nlm.nih.gov).

4.1.4.2Theileria equi ema-1 gene nPCR

The species specific primers for *T. equi* amplified the *ema-1* gene in samples 2, 15, 20, 12, 26, 7, 14, 6, 16, and 29 (Plate II), samples z-1, z-2, z-7, z-8, z-9 and z-11 (Plate III), and samples z-18, z-20, z-22 and z-24 (Plate IV) at 250 bp following nPCR. The blast outcome of the respective nucleotides of the nPCR amplicons revealed 100% identity to *T. equi* (Florida and Texas strain) at the gene bank (www.ncbi.nlm.nih.gov).

4.1.4.3Theileria haneyi hypothetical gene (2,118 bp single-copy open reading frame (ORF) on chromosome 1) nPCR

The hypothetical gene of *T. haneyi* was amplified in samples Z-42, Z-46 and Z-54 at 238 bp using species specific primers following nPCR (Plate V). The blast outcome of the respective nucleotides sequences revealed 100% identity to *T. haneyi* available at the gene bank (www.ncbi.nlm.nih.gov).

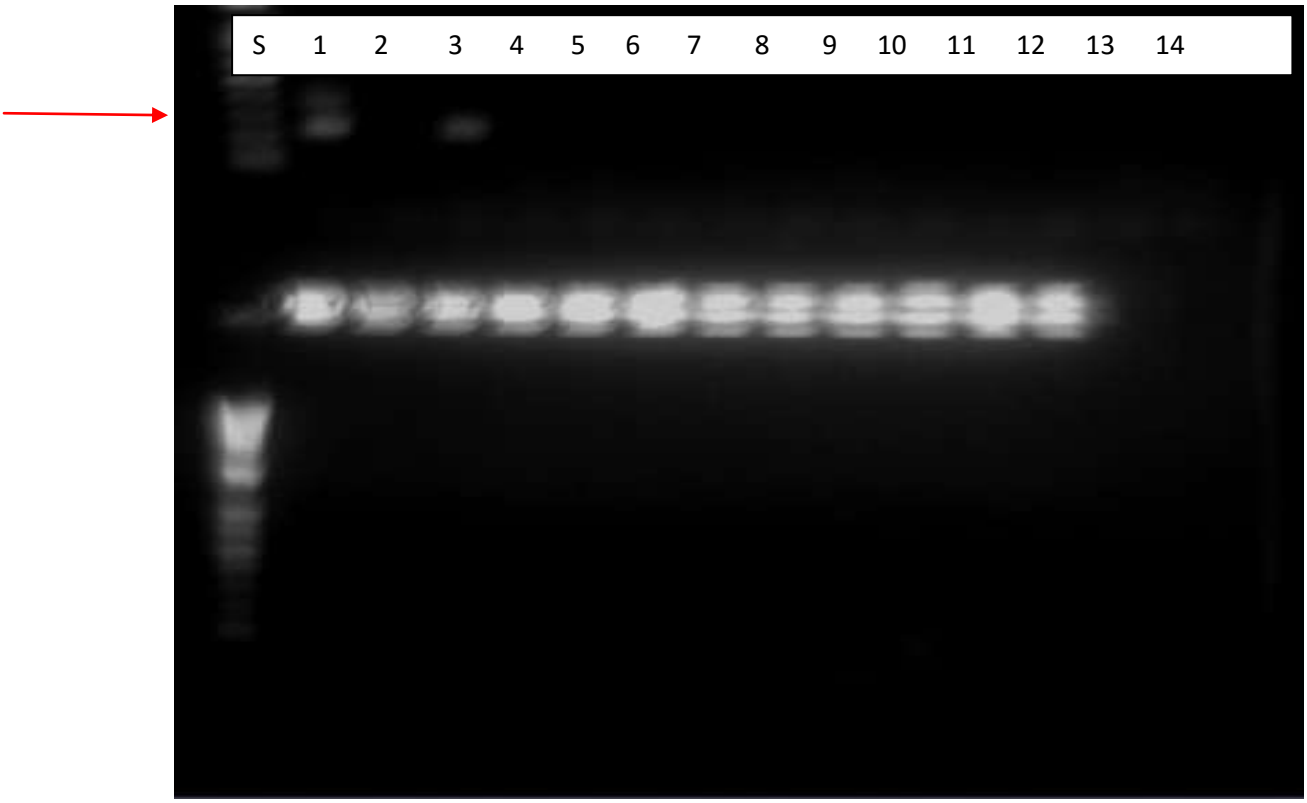


Plate I*:* Agarose gel electrophoresis picture of one*B. caballi rap1* gene positive sample (z-1) (Band size 250 bp)

Electrophoretic pattern of nPCR assay for detection of *B. caballi* using a pair of primers;Lane S reveal 250 ladder, lane (1) contain (+ve) DNA control samples, lane (2) contain (-ve) DNA *B. caballi* , lane 3 was a positive sample while lanes 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 were negative samples

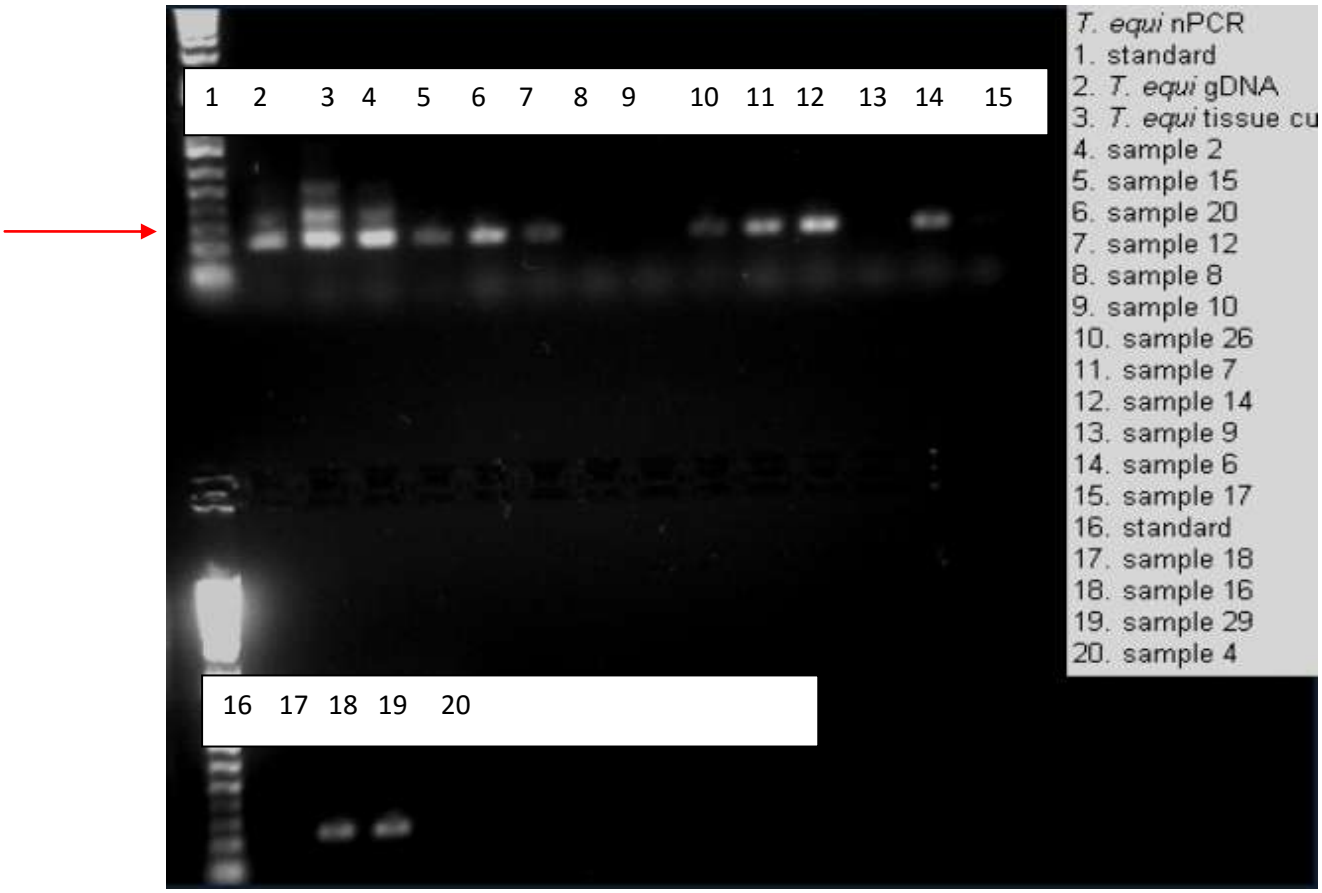


Plate II*:* Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp)

Top lane - Well 1- standard, well 2- *T. equi* gDNA, well 3- *T. equi* tissue culture, wells 4-15 represent samples 2, 15, 20, 12, 8, 10, 26, 7, 14, 9, 6, 17 respectively
Lower lane - well 16- standard, while wells 17-20 represent samples 18, 16, 29 and 4 respectfully

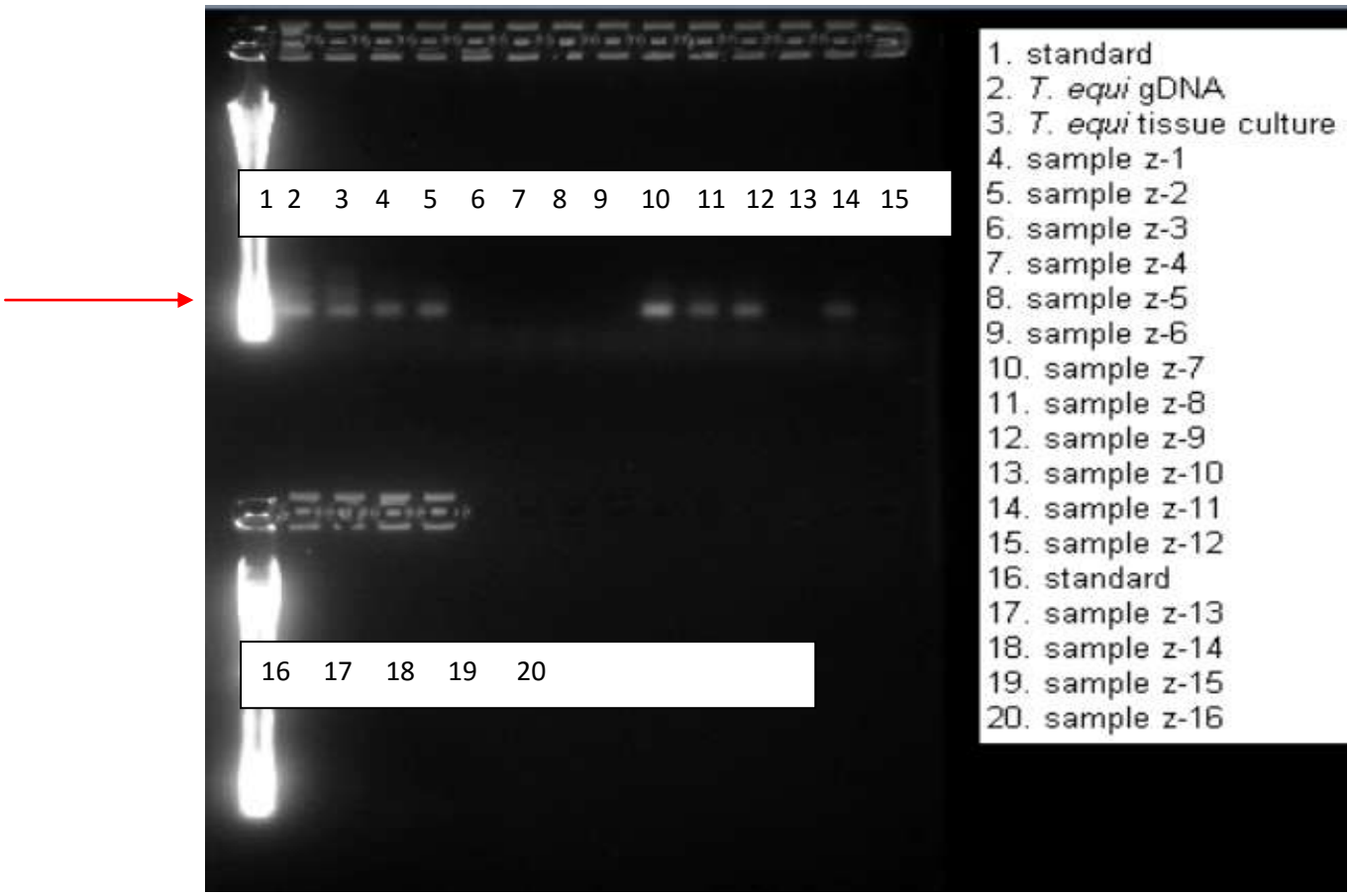


Plate III*:* Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp)

Top lane - Well 1- standard, well 2- *T. equi* gDNA, well 3- *T. equi* tissue culture, wells 4-15 represent samples z-1, z-2, z-3, z-4, z-5, z-6, z-7, z-8, z-9, z-10, z-11 and z-12 respectively
Lower lane - well 16- standard, while wells 17-20 represent samples z-13, z-14, z-15 and z-16 respectively

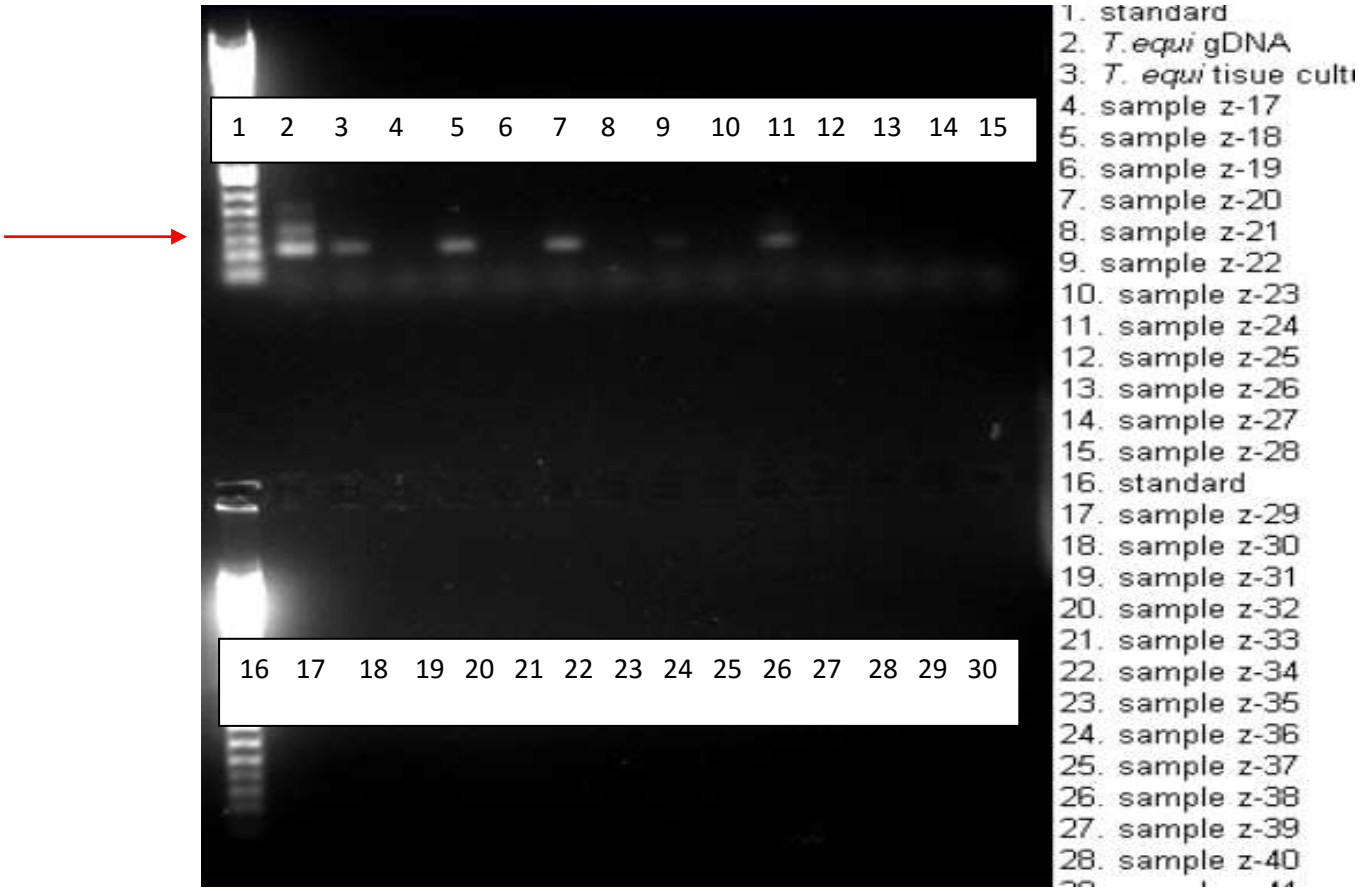


Plate IV*:* Agarose gel electrophoresis for *T. equi ema1* gene (Band size 250 bp)

Top lane - Well 1- standard, well 2- *T. equi* gDNA, well 3- *T. equi* tissue culture, wells 4-15 represent samples z-17, z-18, z-19, z-20, z-21, z-22, z-23, z-24, z-25, z-26, z-27 and z-28 respectively
Lower lane - well 16- standard, while wells 17-30 represent samples z-29, z-30, z-31, z-32, z-33, z-34, z-35, z-36, z-37, z-38, z-39, z-40 and z-41 respectively

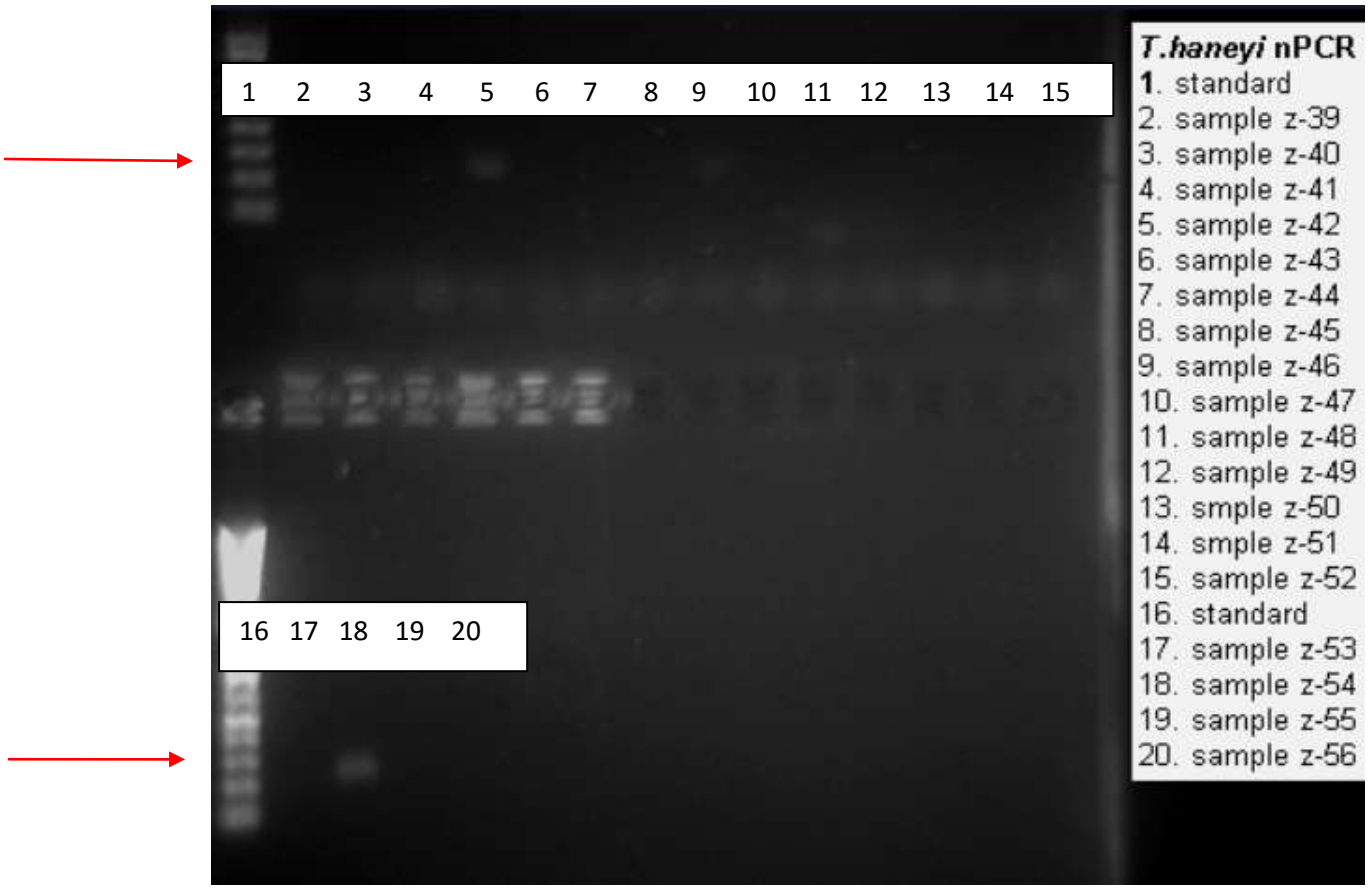


Plate V*:* Agarose gel electrophoresis for *T. haneyi* hypothetical gene (Band size238 bp)

Top lane - Well 1- standard, wells 4-15 represent samples z-39, z-40, z-41, z-42, z-43, z-44, z-45, z-46, z-46, z-47, z-48, z-49, z-50, z-51 and z-52 respectively
Lower lane - well 16- standard, while wells 17-20 represent samples z-53, z-54, z-55 respectively

4.1.4.418s rRNA gene polymerase chain reaction

Polymerase chain reaction for the 72 samples investigated using primers that amplifies the partial (390 bp) and full (1600 bp) lengths of 18S rRNA genes of *Babesia* and *Theileria* species were positive in the following 54 samples; 2, 4, 6, 7, 9, 10, 12, 14, 15, 16, 17, 20, 26, 29, z-2, z-3, z-4, z-5, z-6 and z-7 (Plate VI), z-8, z-9, z-10, z-11, z-13, z-14, z-15, z-16, z-17, z-18, z-19, z-20, z-22, z-24, z-25, z-27, z-28, z-32, z-35, z-38, z-39, z-40, z-41, z-42, z-43, z-44, z-45, z-46, z-49, z-50, z-52 (Plate VII), and z-54, z-55 and z-56 (Plate VIII).

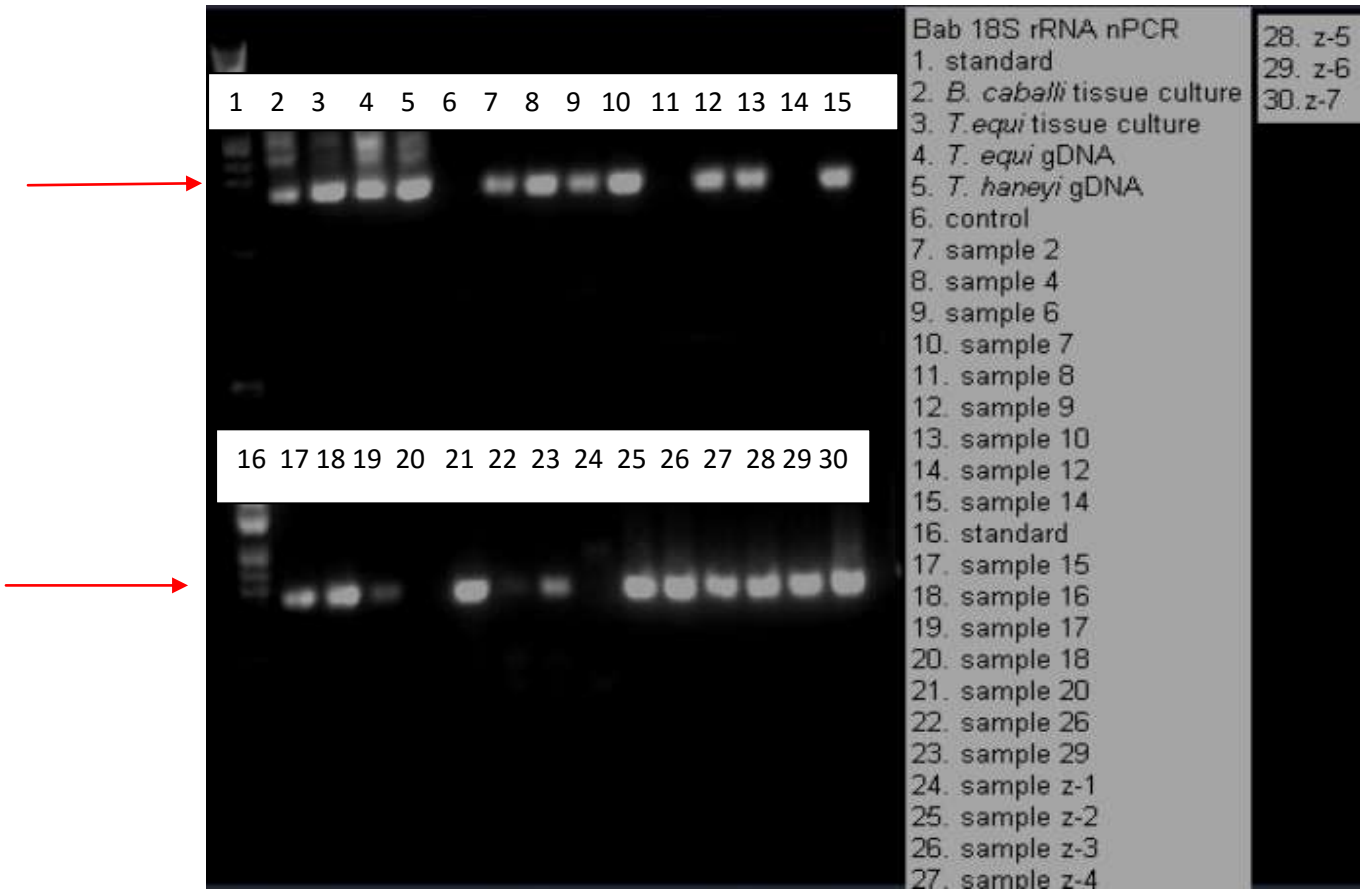


Plate VI*:* Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp)

Top lane - Well 1- standard, well 2- *B. caballi* tissue culture, well 3- *T. equi* tissue culture, well 4- *T. equi* gDNA, well 5- *T. haneyi* gDNA, well 6- control, wells 4-15 represent samples 2, 4, 6, 7, 8, 9, 10, 12 and 14 respectively
Lower lane - well 16- standard, while wells 17-27 represent samples 15, 16, 17, 18, 20, 26, 29, z-1, z-2, z-3, z-4, z-5, z-6 and z-7 respectively

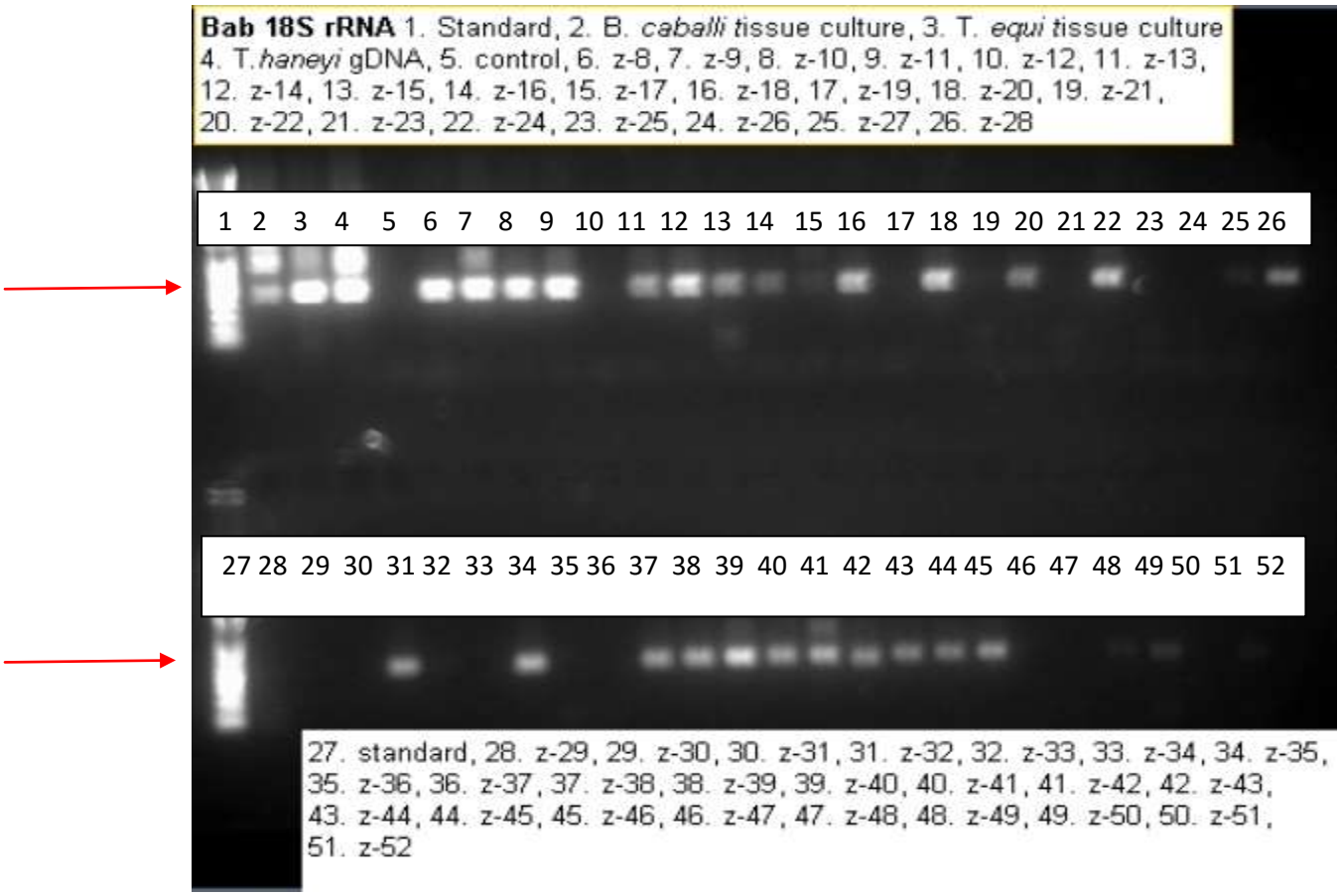


Plate VII: Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp)

Top lane - Well 1- standard, well 2- *B. caballi* tissue culture, well 3- *T. equi* tissue culture, well 4- *T. haneyi* gDNA, well 5- control, wells 4-26 represent samples z-8, z-9, z-10, z-11, z-12, z-13, z-14, z-15, z-16, z-17, z-18, z-19, z-20, z-21, z-22, z-23, z-24, z-25, z-26, z-27 and z-28 respectively

Lower lane - well 27- standard, while wells 28-52 represent samples z-29, z-30, z-31, z-32, z-33, z-34, z-35, z-36, z-37, z-38, z-39, z-40, z-41, z-42, z-43, z-44, z-45, z-46, z-47, z-48, z-49, z-50, z-51 and z-52 respectively

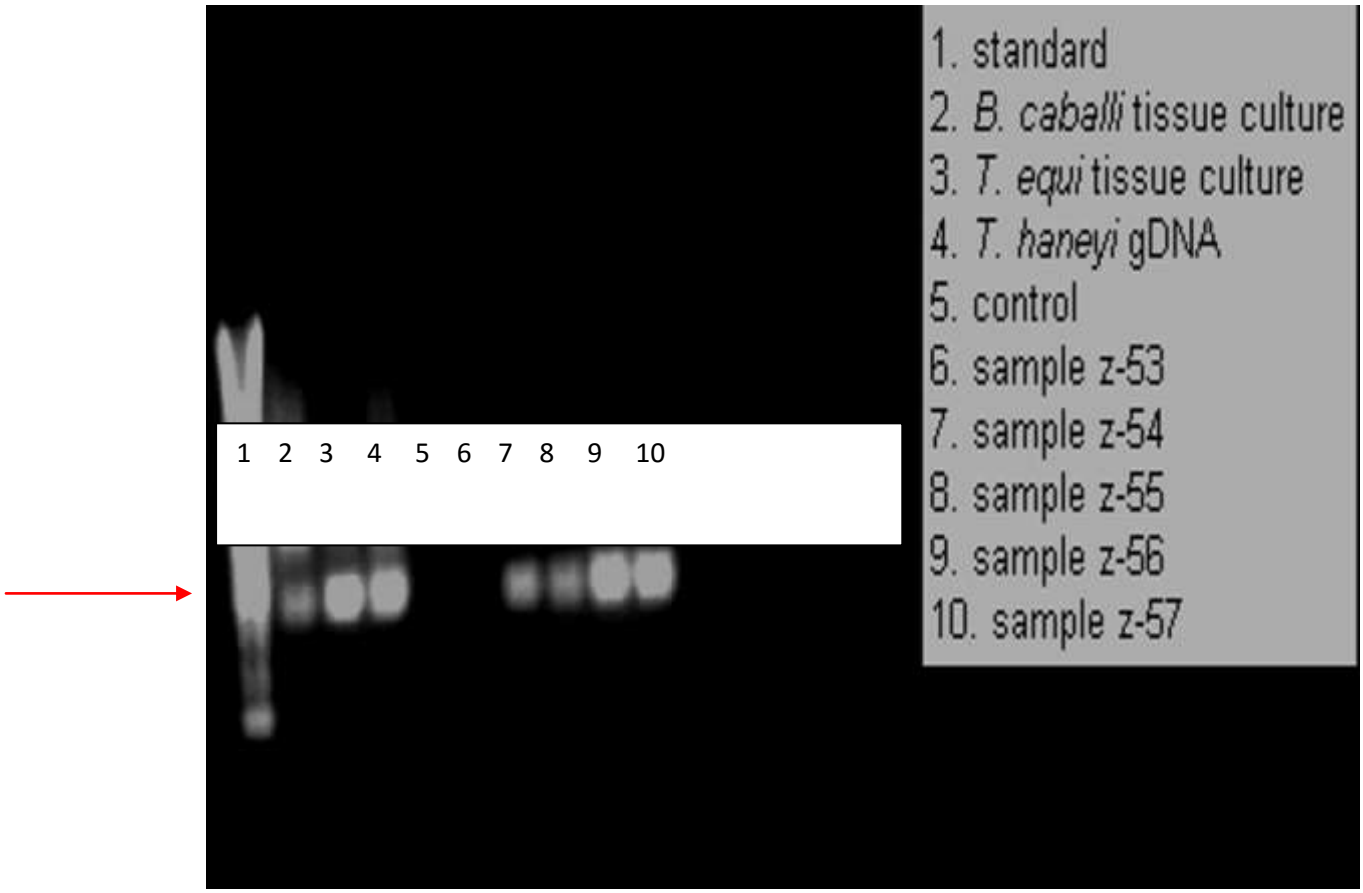


Plate VIII: Agarose gel electrophoresis for Bab 18S rRNA nPCR (Band size 390 bp)

Well 1- standard, well 2- *B. caballi* tissue culture, well 3- *T. equi* tissue culture, well 4- *T. haneyi* gDNA, well 5- control, wells 4-10 represent samples z-53, z-54, z-55, z-56 and z-57 respectively
z-56 and z-57 are from the same sample (z-56)

4.1.4.5Sequence analysis of the 18S rRNA genes

Based on the sequence similarity from the 18S rRNA gene blast outcomes, the parasites in the 55 infected horses were classified into five different clades (A-E), with the exception of z-43 that was *B. caballi* positive (Fig. 4.1). Clade A represents *T. equi* (Flor/Tex strain), clade B is *T. equi*-like unknown parasites, clade C is *T. haneyi*, clade D is *T. equi*-like unknown parasites while clade E is also unknown parasites.

4.1.4.6.TOPOPCR 2.1 cloning and the 18S rRNA gene sequence analysis

Samples 2, 14, z-1, z-2, z-5, z-7, z-9, z-11, z-16, z-18, z-28 and z-52 were selected for TOPO pCR 2.1 cloning based on the presence of multiple bands from the 18S PCR and conflicting 18S nucleotide blast outcomes compared with their respective nPCR results (Table 4.2). For example, the 18S PCR sequence analyses of samples 2, 14, z-7, z-9, z-11 and z-18 indicated *T. haneyi* infections, whereas, the nucleotide blast outcomes of their *ema-1* gene showed *T. equi* (Flor/Tex strain) infection.

4.1.4.7.Mixed infections

Based on the sequence analysis of 18S M13 PCR clones, eight samples comprising 2, 14, z-1, z-2, z-5, z-7, z-9 and z-11were found to have mixed infections (Table 4.2).

Table 4.2: Samples 2, 14, z-1, z-2, z-5, z-7, z-9 and z-11 with mixed infections

Sample I.D.	Parasites detected	Targeted gene
2	*T. equi* (clade A)	*ema-1* (nPCR)
	T. haneyi (clade C)	18S rRNA (PCR)
14	*T. equi* (clade A)	*ema-1* (nPCR)
	T. haneyi (clade C)	18S rRNA (PCR)
z-1	*T. equi* (clade A)	*ema-1* (nPCR)
	B. caballi	rap-1 (nPCR)
z-2	*T. equi* (clade A)	*ema-1* (nPCR)
	T. equi-like (clade D)	18S rRNA PCR of M13 clones
z-5	*B. caballi*	18S rRNA PCR of M13 clones
	T. equi (clade A)	18S rRNA PCR of M13 clones
	T. equi-like (clade B)	18S rRNA PCR of M13 clones
	T. equi-like (clade D)	18S rRNA PCR of M13 clones
	T. equi-like (unknown clade)	18S rRNA PCR of M13 clones
z-7	*T. equi* (clade A)	*ema-1* (nPCR)
	T. equi (clade A)	18S rRNA PCR of M13 clones
	T. haneyi (clade C)	18S rRNA PCR of M13 clones
	T. equi-like (clade D)	18S rRNA PCR of M13 clones
z-9	*T. equi* (clade A)	*ema-1* (nPCR)
	T. equi-like (unknown clade)	18S rRNA PCR of M13 clones
z-11	*T. equi* (clade A)	*ema-1* (nPCR)
	T. haneyi (clade C)	18S rRNA PCR of M13 clones
	T. equi-like (clade D)	18S rRNA PCR of M13 clones

Keys: *T. equi = B. caballi = Babesia caballi*, *Theileria equi*, *T. haneyi = Theileria haneyi*, nPCR = nested polymerase chain reaction, PCR = polymerase chain reaction, *ema-1* = equine

merozoite antigen, *rap-1* = rhoptry associated protein, 18S rRNA = 18S ribosomal ribonucleic acid, z = sample identification code.

4.1.4.8Theileria equi-likeunknown parasites

This category of parasites in clades B, D, E and the unknown clades were classified as unidentified species in this study for the sake of comparative statistical analysis with other groups. Samples z-16, z-28 and z-52 fell within this category while those co-infected with other parasites were categorized in the mixed infection group. The unidentified group is depicted in the Table 4.3.

4.1.4.9Phylogenetic analysis

Phylogenetic analysis of the 18S rRNA locus, as well as genome sequence and organization reveal six distinct clades of parasites designated as *T. equi* clade A, *T. equi*-like clade B, *T. haneyi* clade C, *T. equi*-like clade D, *T. equi*-like clade E and an unknown clade (Fig. 4.1).

Table 4.3: *T. equi*-unknown samples (*T. equi*-like clades B and E)

Sample I.D	Parasite detected
z-16	*T. equi-like* (clade E)
z-28	*T. equi-like* (clade B)
Z-52	*T. equi-like* (clade B)

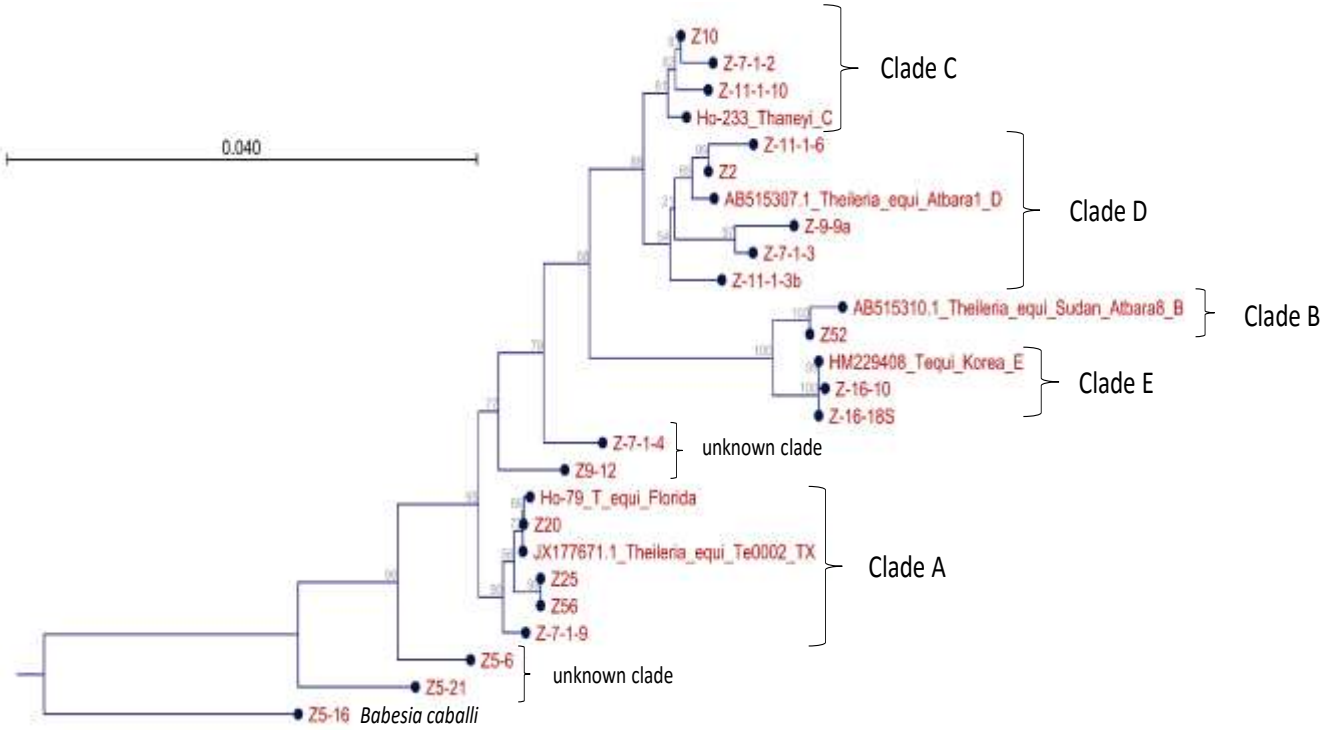


Figure 4.1: Maximum likelihood phylogenetic tree of full length nucleotide sequence 18S rRNA of *Theileria* species. Selected sequences were trimmed to be identical length of 1490 base pairs following assembly in order to find common overlap

4.1.5 Serological analysisof symptomatic horses

4.1.5.1Babesia caballi competitive enzyme linked immunosorbent assay (cELISA)

All the samples investigated were seronegative to anti-*B. caballi* antibodies using the *B. caballi* specific cELISA.

4.1.5.2Theileria equi competitive enzyme linked immunosorbent assay (cELISA)

The cELISA revealed 61 samples with percent inhibition (PI) above 40%, thus, indicating reactivity to *T. equi* antibodies or seropositive (Table 4.4). Twenty three of the 61 seropositive samples were confirmed *T. equi* infections by PCR with one of them been borderline seropositive (sample 4). Four other samples which were*T. equi* positive by PCR were seronegative against *T. equi* antibodies (samples 17, z-14, z-38 and z-39).

Out of the 61 seropositive cases, 12 samples comprising z-10 (46% PI), z-13 (49% PI), z-17 (61% PI), z-18 (76% PI), z-19 (86% PI), z-35 (70% PI), z-42 (82% PI), z-44 (74% PI), z-46 (41% PI), z-49 (73% PI), z-50 (75% PI) and z-55 (81% PI) that were PCR positive for *T. haneyi* had PI above 40%, thus, indicating reactivity to *T. equi* antibodies. Only sample z-54, which was positive with both species specific and 18S primers for *T. haneyi* was seronegative. On the other hand, sample z-43 was 18S PCR positive for *B. caballi*, seronegative against *B. caballi* antibodies (1% PI) (*B. caballi* specific cELISA) but seropositive against *T. equi* antibodies (85% PI) using the *T. equi* specific cELISA.

There were 7 seropositive samples with mixed infections (samples 2, z-1, z-2, z-5, z-7, z-9 and z-11). Only sample 14 with mixed infection was seronegative (36% PI). Three of the sixty one seropositive samples had infection with *T. equi*-like unknown parasites (z-16, z-28 and z-52).

Eleven samples were seronegative with PI below the 40% bench mark for positive samples. Six out of the 11 seronegative samples were PCR positive (samples 14, 17, z-14, z-38, z-39 and z-54) while five were both PCR and cELISA negative (samples 8, z-26, z-31, z-47 and z-53). Table 4.5 summarizes the serology results;

Table 4.4: *Theileria equi* competitive-ELISA of horse serum

S/no	Sample I.D	% Inhibition	Sample outcome
1	2	81	Positive
2	4	43	Borderline positive
3	6	70	Positive
4	7	63	Positive
5	8	1	Negative
6	9	75	Positive
7	10	56	Positive
8	12	70	Positive
9	14	36	Negative
10	15	72	Positive
11	16	81	Positive
12	17	9	Negative
13	18	84	Positive
14	20	81	Positive
15	26	86	Positive
16	29	76	Positive
17	z-1	68	Positive
18	z-2	80	Positive
19	z-3	80	Positive
20	z-4	56	Positive
21	z-5	73	Positive
22	z-6	86	Positive
23	z-7	63	Positive
24	z-8	75	Positive
25	z-9	78	Positive
26	z-10	46	Positive
27	z-11	48	Positive
28	z-12	83	Positive
29	z-13	49	Positive
30	z-14	32	Negative
31	z-15	85	Positive
32	z-16	77	Positive
33	z-17	61	Positive
34	z-18	76	Positive
35	z-19	86	Positive
36	z-20	83	Positive
37	z-21	70	Positive
38	z-22	79	Positive
39	z-23	72	Positive
40	z-24	73	Positive
41	z-25	84	Positive
42	z-26	-1	Negative
43	z-27	84	Positive

44	z-28	77	Positive
45	z-29	62	Positive
46	z-30	49	Positive
47	z-31	-5	Negative
48	z-32	58	Positive
49	z-33	69	Positive
50	z-34	67	Positive
51	z-35	70	Positive
52	z-36	88	Positive
53	z-37	44	Borderline positive
54	z-38	15	Negative
55	z-39	4	Negative
56	z-40	84	Positive
57	z-41	56	Positive
58	z-42	82	Positive
59	z-43	85	Positive
60	z-44	74	Positive
61	z-45	81	Positive
62	z-46	41	Borderline positive
63	z-47	4	Negative
64	z-48	60	Positive
65	z-49	73	Positive
66	z-50	75	Positive
67	z-51	77	Positive
68	z-52	66	Positive
69	z-53	-11	Negative
70	z-54	21	Negative
71	z-55	81	Positive
72	z-56	80	Positive

72 horse samples were analyzed, sixty one horses were positive for *T. equi* while eleven samples were negative.A sample is said to be positive when the percent inhibition is ≥ 40%. It is considered borderline positive when the percent inhibition is within the range 40-44%. A sample is negative if the percent inhibition is < 40%.

Table 4.5: Summary of PCR and c-ELISA results for the seventy samples investigated

Sample I.D	*B. caballi* nPCR	*T. equi* nPCR	*T. haneyi* nPCR	18S rRNA PCR	*B. caballi* cELISA	*T. equi* cELISA	Outcome
2	-	+	-	+	-	+	PCR and cELISA
4	-	-	-	+	-	+	PCR and cELISA
6	-	+	-	+	-	+	PCR and cELISA
7	-	+	-	+	-	+	PCR and cELISA
8	-	-	-	-	-	-	Negative
9	-	-	-	Failed	-	+	cELISA
10	-	-	-	Failed	-	+	cELISA
12	-	-	-	+	-	+	PCR and cELISA
14	-	+	-	+	-	-	PCR
15	-	+	-	+	-	+	PCR and cELISA
16	-	+	-	+	-	+	PCR and cELISA
17	-	+	-	+	-	-	PCR
18	-	-	-	-	-	+	cELISA
20	-	+	-	+	-	+	PCR and cELISA
26	-	+	-	+	-	+	PCR and cELISA
29	-	+	-	+	-	+	PCR and cELISA
z-1	+	+	-	-	-	+	PCR and cELISA
z-2	-	+	-	+	-	+	PCR and cELISA
z-3	-	-	-	+	-	+	PCR and cELISA
z-4	-	-	-	+	-	+	PCR and cELISA
z-5	-	-	-	+	-	+	PCR and cELISA
z-6	-	-	-	+	-	+	PCR and cELISA
z-7	-	+	-	+	-	+	PCR and cELISA

z-8	-	+	-	+	-	+	PCR	and cELISA
z-9	-	+	-	+	-	+	PCR	and cELISA
z-10	-	-	-	+	-	+	PCR	and cELISA
z-11	-	+	-	+	-	+	PCR	and cELISA
z-12	-	-	-	-	-	+	cELISA	
z-13	-	-	-	+	-	+	PCR	and cELISA
z-14	-	-	-	+	-	-	PCR	
z-15	-	-	-	-	Failed	+	cELISA	
z-16	-	-	-	+	-	+	PCR	and cELISA
z-17	-	-	-	+	-	+	PCR	and cELISA
z-18	-	+	-	+	-	+	PCR	and cELISA
z-19	-	-	-	+	-	+	PCR	and cELISA
z-20	-	+	-	+	-	+	PCR	and cELISA
z-21	-	-	-	-	-	+	cELISA	
z-22	-	+	-	+	-	+	PCR	and cELISA
z-23	-	-	-	-	-	+	cELISA	
z-24	-	+	-	+	-	+	PCR	and cELISA
z-25	-	-	-	+	-	+	PCR	and cELISA
z-26	-	-	-	-	-	-	Negative	
z-27	-	-	-	+	-	+	PCR	and cELISA
z-28	-	-	-	+	-	+	PCR	and cELISA
z-29	-	-	-	-	-	+	cELISA	
z-30	-	-	-	-	-	+	cELISA	
z-31	-	-	-	-	-	-	Negative	
z-32	-	-	-	+	-	+	PCR	and cELISA
z-33	-	-	-	-	-	+	cELISA	
z-34	-	-	-	-	-	+	cELISA	
z-35	-	-	-	+	-	+	PCR	and cELISA
z-36	-	-	-	-	-	+	cELISA	

z-37	-	-	-	-	-	+borderline	cELISA	
z-38	-	-	-	+	-	-	PCR	
z-39	-	-	-	+	-	-	PCR	
z-40	-	-	-	+	-	+	PCR and cELISA	
z-41	-	-	-	+	-	+	PCR and cELISA	
z-42	-	-	+	+	-	+	PCR and cELISA	
z-43	-	-	-	+ B.c	-	+	PCR and cELISA	
z-44	-	-	-	+	-	+	PCR and cELISA	
z-45	-	-	-	+	-	_	PCR and cELISA	
z-46	-	-	+	+	-	+borderline	PCR and cELISA	
z-47	-	-	-	-	-	-	Negative	
z-48	-	-	-	-	-	+	cELISA	
z-49	-	-	-	+	-	+	PCR and cELISA	
z-50	-	-	-	+	-	+	PCR and cELISA	
z-51	-	-	-	-	-	+	cELISA	
z-52	-	-	-	+	-	+	PCR and cELISA	
z-53	-	-	-	-	-	-	Negative	
z-54	-	-	+	+	-	-	PCR	
z-55	-	-	-	+	-	+	PCR and cELISA	
z-56	-	-	-	+	-	+	PCR and cELISA	

Keys: + = positive, - = negative

4.1.6 Mean haematological parameters of symptomatic horses with piroplasmosis

4.1.6.1.Mean haematocrit

The mean HCTvalues in horses infected with *T. equi* (32.26±1.39 %), *T. haneyi* (33.11±1.43 %), those with mixed infections (31.80±2.33 %) as well as those infected with *T. equi*-like unknown clade (31.23±1.68 %) were not significantly different (P > 0.05) compared to that of the non-infected group (31.42±2.45 %) (Figure 4.2). Similarly, the mean HCT in the *T. equi*-infected symptomatic West African Barb horses (31.30 ± 2.13 %) was not significantly different (P > 0.05) from the symptomatic Argentine Polo Pony (33.70 ± 1.40 %) with *T. equi* infection (Table 4.6). The mean HCTbased on sex distribution in the *T. equi*-infected symptomatic horses did not reveal significant alteration (P > 0.05) in the Mares (32.44± 1.73 %) compared to the Geldings (36.97± 1.68) and Stallions (31.29± 2.13 %) (Table 4.7).

4.1.6.2 Mean haemoglobin concentration

The horses infected with *T. equi*, *T. haneyi*, horses withmixed infectionsand those with *T. equi*-like unknown cladehad mean Hgb concentrations of 11.11±0.44 g/dL, 11.34±0.61 g/dL, 11.05±0.90 and 10.30±0.40 g/dL respectively. These values of mean Hgb concentrations were not significantly different (P > 0.05) from that of the non-infected group (11.56±0.60 g/dL)(Fig. 4.3). The mean Hgb concentration in the*T. equi*-infected symptomaticWest African Barb horses (11.08 ± 0.68 g/dL) was lower compared to that of the *T. equi*-infected symptomatic Argentine Polo Pony (11.16± 0.47 g/dL) however, the difference was not significant statistically (P > 0.05) (Table 4.6). Also, the mean Hgb was lowest in the symptomaticMares with *T. equi* infection (10.63±0.52 g/dL) compared to the *T. equi*-infected symptomatic Stallions (11.15± 0.65

g/dL) and symptomatic Geldings with *T. equi* infection (12.60± 0.40 g/dL). However, these variations were not significantly different (P > 0.05)(Table 4.7).

4.1.6.3 Mean red blood cell counts

There were variations in the mean RBC in horses infected with *T. equi* (6.61±0.24 $\times 10^{12}$/L), *T. haneyi*-infected (6.76±0.35 $\times 10^{12}$/L), those with mixed infections (6.40±0.28 $\times 10^{12}$/L) and those infected with *T. equi*-like unknown clade (6.25±0.31 $\times 10^{12}$/L) when compared with the non-infected group (6.76 ± 0.31 $\times 10^{12}$/L)(Fig. 4.4). However, these variations were not statistically significant (P > 0.05). The mean RBC count in the *T. equi*-infected symptomatic West African Barb horses (6.54 ± 0.36 $\times 10^{12}$/L)was lower compared to that of the *T. equi*-infected symptomatic Argentine Polo Ponies (6.71± 0.29 $\times 10^{12}$/L) but this variation is not significantly different (P > 0.05) (Table 4.6).The mean RBC counts in the Mares (6.45± 0.35 $\times 10^{12}$/L) was lowest followed by Stallions (6.54± 0.36 $\times 10^{12}$/L) and then that for the Geldings (7.39± 0.14 $\times 10^{12}$/L) but these differences were not statistically significant (P > 0.05) (Table 4.7).

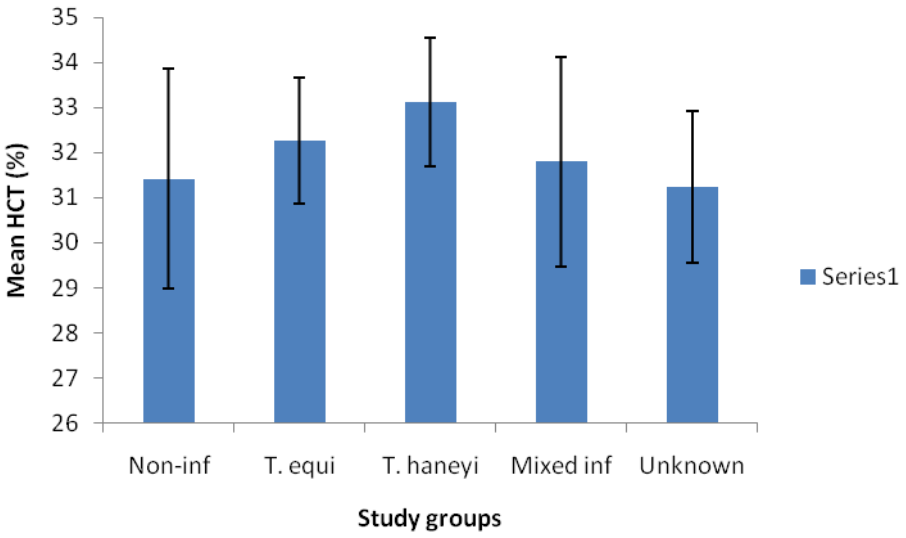


Figure 4.2: The mean ±SEM haematocrit for the infected and non-infected horses.

Keys: HCT = Haematocrit, Non-inf = Non-infected, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

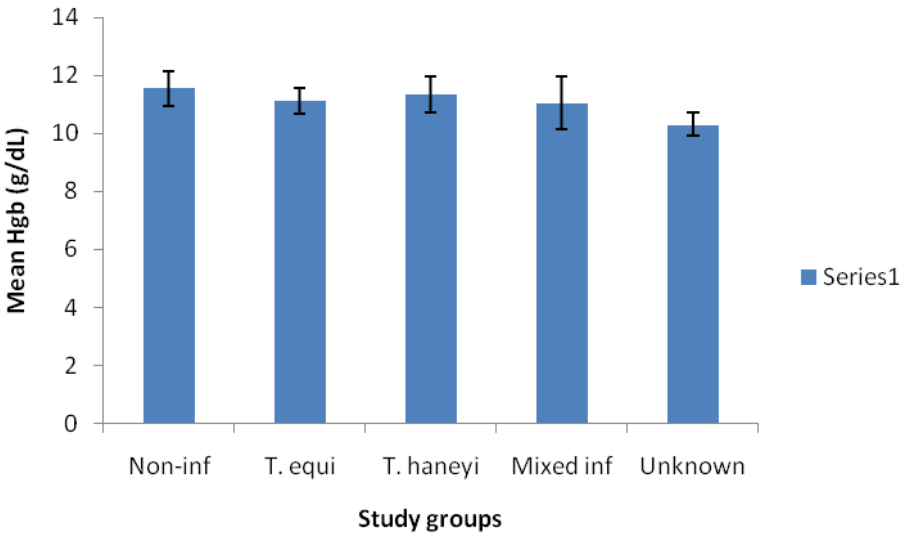


Figure 4.3: The mean ±SEM of haemoglobin concentration for the infectedand non-infected horses.

Keys: Hgb = Haemoglobin concentration,Non-inf = Non-infected,*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

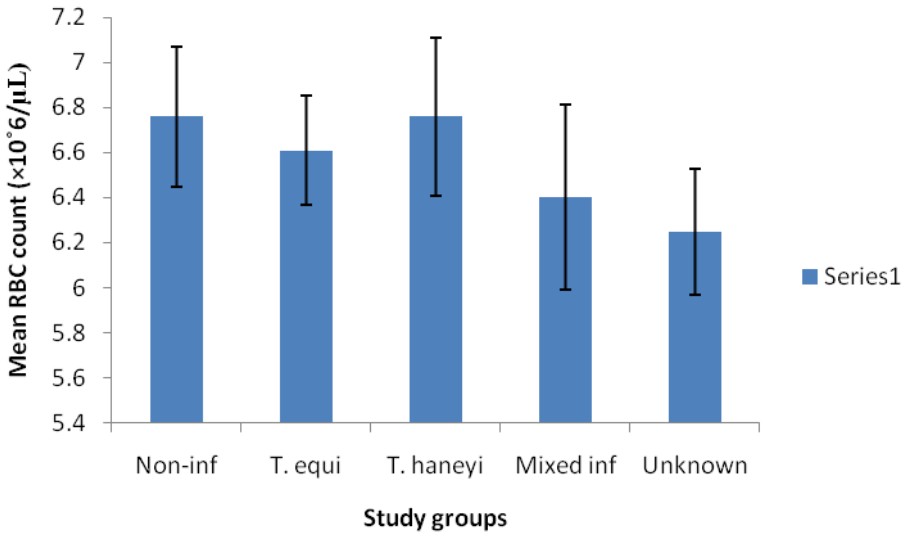


Figure 4.4: The mean ±SEM red blood cell counts for the infected and non-infected horses.

Keys: RBC = Red blood cells count, Non-inf = Non-infected, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

4.1.6.4Mean corpuscular volume

The mean MCV for the *T. equi*-infected horses (49 ± 0.61 fL), *T. haneyi*-infected group (49 ± 0.94 fL), horses with mixed infections (50±1.60 fL) and those with infections caused by *T. equi*-like unknown clade (50±0.55 fL) were statistically non-significant (P > 0.05) when compared with that of the non-infected group (50±1.40 fL) (Fig. 4.5) below. Conversely, the mean MCV in the*T. equi*-infected symptomaticWest African Barb horses (47.60 ± 0.80 fL)was significantly lower (P < 0.05) compared to that of the*T. equi*-infected symptomatic Argentine Polo Ponies (50.25± 0.74 fL) the (Table 4.6) below. Although, the mean MCV was lowest in the *T. equi*-infected symptomatic Stallions (47.60± 0.80 fL), followed by Geldings (50.03± 1.89 fL), these changes were not significantly different (P > 0.05) when compared to the *T. equi*-infected symptomatic Mares (50.34± 0.83 fL) (Table 4.7).

4.1.6.5.Mean corpuscular haemoglobin

The mean MCH of the horses infected with *T. equi* (16.81±0.18 g/dL), *T. haneyi* (16.84±0.19 g/dL), mixed infections (17.20±0.44 g/dL) and those infected with *T. equi*-like unknown clade (16.48±0.21 g/dL) were not significantly changed (P > 0.05) when compared with that of thenon-infected group (17.08±0.19 g/dL) (Fig. 4.6 below). There were no significant changes (P > 0.05) in the mean MCH between the symptomatic West African Barb horses (16.91± 0.27 g/dL) and the Argentine Polo Ponies (16.68± 0.20 g/dL) with *T. equi*infections (Table 4.6). Similarly, there were no significant changes in the mean MCH in the*T. equi*-infected symptomatic Stallions (16.91± 0.27 g/dL) compared to those of the Geldings (17.07± 0.31 g/dL) and Mares (16.54± 0.25 g/dL) (Table 4.7).

4.1.6.6.Mean corpuscular haemoglobin concentration

There were no significant alterations (P > 0.05) in the mean MCHC in the horses infected with *T. equi* (34.63±0.43 g/dL), *T. haneyi* (34.09±0.63 g/dL), mixed infections (34.73±1.07 g/dL) and those with *T. equi*-like unknown clade (33.05±0.63 g/dL) when compared with that of the non-infected group (34.36 ± 1.22 g/dL) (Fig. 4.7). The mean MCHC was significantly higher (P < 0.05) in the *T. equi*-infected symptomatic West African Barb horses (35.60 ± 0.46 g/dL)compared to that of the *T. equi*-infected symptomatic Argentine Polo Ponies (33.23 ± 0.61g/dL) (Table 4.6). The *T. equi*-infected symptomatic Mares (32.90± 0.80 g/dL) had significantly lower mean MCHC (P < 0.05) compared to the*T. equi*-infected symptomatic Stallions (35.60± 0.46 g/dL) but was not significantly changed (P > 0.05) when compared to that of the *T. equi*-infected symptomatic Geldings (34.10 ± 0.67 g/dL) (Table 4.7).

4.1.6.7Mean percent red blood cells redistribution width

There were no significant changes (P > 0.05) in the mean percent RDW of thehorses infected with *T. equi* (20.04±0.22 %), *T. haneyi* (19.41±0.36 %), mixed infections (20.47±0.29 %) and those with *T. equi*-like unknown clades (19.08 ± 0.60 %) when compared to that of the non-infected group (20.06±0.68 %) (Fig. 4.8). The*T. equi*-infected symptomatic West African Barb horses (20.41± 0.28 %) had significantly higher (P < 0.05) mean percent RDW compared to the*T. equi*-infected symptomatic Argentine Polo Ponies (19.52± 0.30 %) (Table 4.6). Similarly, the mean percent RDW in the *T. equi*-infected symptomatic Mares (19.04± 0.22 %) was significantly lower (P < 0.05) compared to those of the Stallions (20.41± 0.28 %) and the Geldings (20.80± 0.32 %) (Table 4.7).

4.1.6.8 *Mean red blood cells redistribution width*

The mean RDW of horses infected with *T. equi* (34.63±0.85 fL), *T. haneyi* (33.29±0.80 fL), horses with mixed infections (35.07±1.12 fL) and those infected with*T. equi-like* unknown clades (31.80±1.25 fL) were not significant altered (P > 0.05) when compared with that of the non-infected group (35.44±1.59 fL) (Fig. 4.9). The mean RDW in the *T. equi*-infected symptomaticWest African Barb horses (35.66± 1.26 fL) was not significantly different (P > 0.05) compared to that of the Argentine Polo Ponies (33.14± 0.89) (Table 4.6). On the other hand, the mean RDW in the *T. equi*-infected symptomatic Mares (31.69± 0.56 fL) was lowest but not significantly changed (P > 0.05) compared to those of the Stallions (35.66± 1.26 fL) and Geldings (37.00 ± 1.27 fL) (Table 4.7).

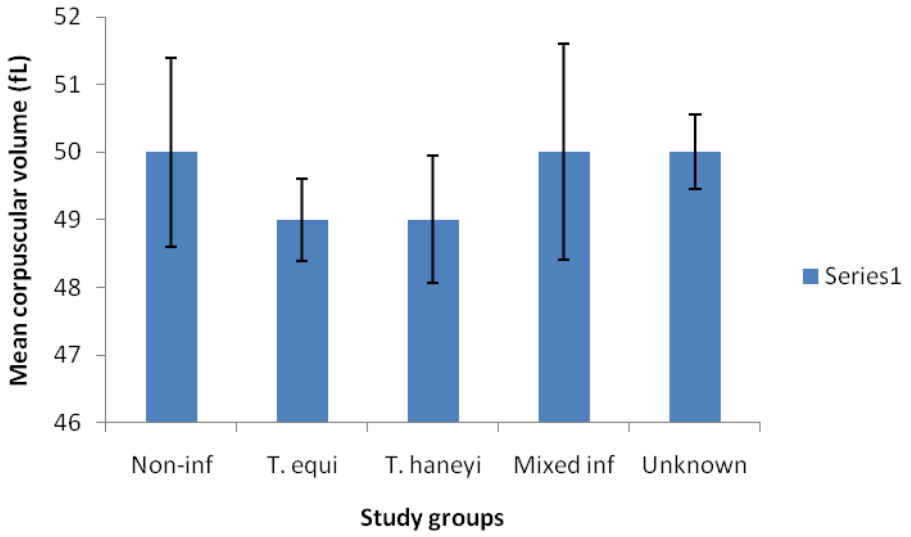


Figure 4.5: The mean ±SEM corpuscular volume for the infected and non-infectedhorses.

Keys: MCV = Mean Corpuscular Volume,Non-inf = Non-infected*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

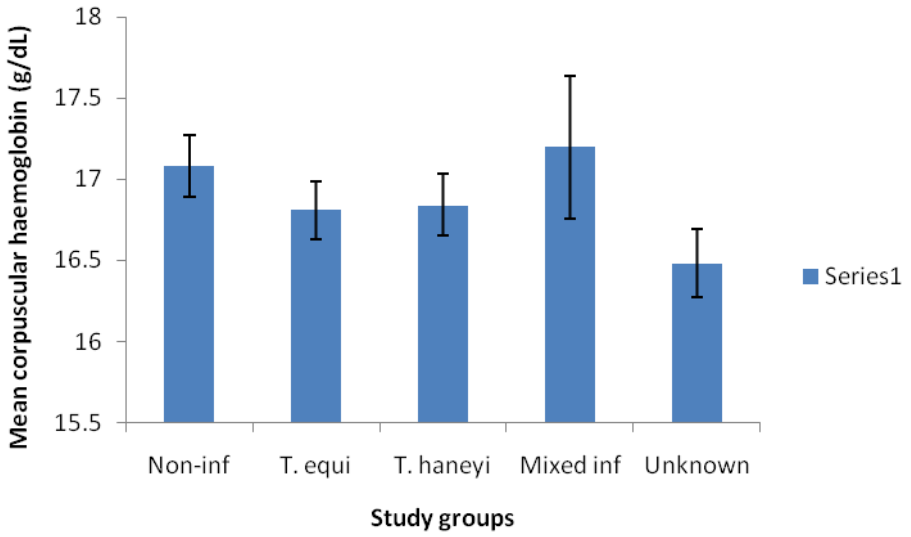


Figure 4.6: The mean ±SEM corpuscular haemoglobin for the infected and non-infectedhorses.

Keys: MCH = Mean Corpuscular Haemoglobin,Non-inf = Non-infected, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

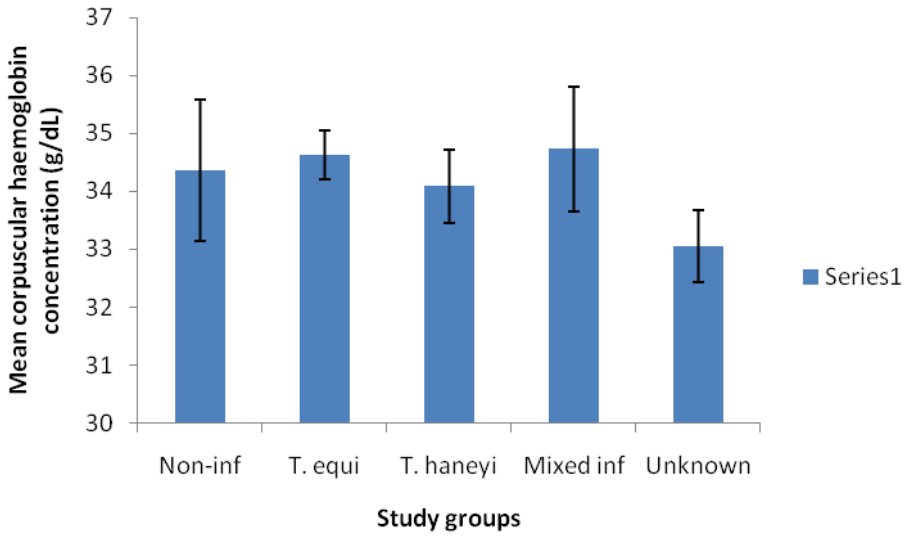


Figure 4.7: The mean ±SEM corpuscular haemoglobin concentration for theinfected and non-infectedhorses.

Keys: MCHC = Mean Corpuscular Haemoglobin Concentration, Non-inf = Non-infected, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

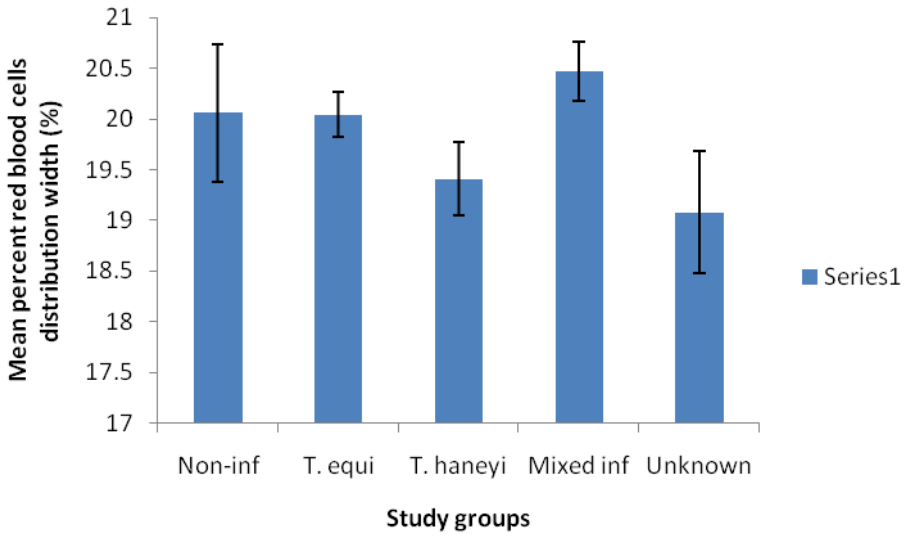


Figure 4.8: The mean ±SEM percent red blood cells redistribution width for the infected and non-infectedhorses.

Keys: RDW = Red Blood Cells Redistribution Width,Non-inf = Non-infected, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

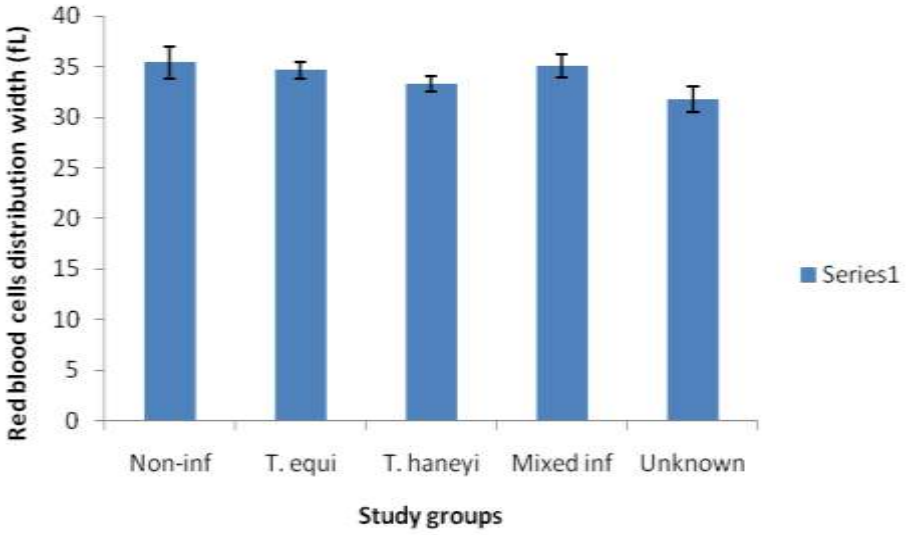


Figure 4.9: The mean ±SEM red blood cells redistribution width for the infectedand non-infectedhorses.

Keys: RDW = Red Blood Cells Redistribution Width, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.6:Mean Erythrogram in*T. equi*-infected symptomatic horses with means ± SEM based on breeds

Parameter	**West African Barb Horses**	**Argentine Polo Ponies**
HCT (%)	31.30± 2.13	33.70 ± 1.40
Hb (g/dL)	11.08 ± 0.68	11.16 ± 0.47
RBC ($\times 10^{12}$/L)	6.54 ± 0.36	6.71 ± 0.29
MCV (fL)	**47.60 ± 0.80[a]**	**50.25 ± 0.74[b]**
MCH (pg)	16.91 ± 0.27	16.68 ± 0.20
MCHC (g/dL)	**35.60 ± 0.46[b]**	**33.23 ± 0.61[a]**
RDW (%)	**20.41 ± 0.28[b]**	**19.52 ± 0.30[a]**
RDW (fL)	35.66 ± 1.26	33.14 ± 0.89

Keys: RBC = red blood cell count, HCT = haematocrit, Hgb = haemoglobin, MCV = mean Corpuscularvolume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width
Means with different superscripts (a and b) are statistically significant ($P < 0.05$)
Means ± SEM with different superscripts (a and b) are statistically significant ($P < 0.05$)

Table 4.7:Mean Erythrogram in *T. equi*-infected symptomatic horses with means ± SEM based on sex distribution

Parameter	Stallions	Gelding	Mares
HCT (%)	31.29 ± 2.13	36.97 ± 1.68	32.44 ± 1.73
Hb (g/dL)	11.15 ± 0.65	12.60 ± 0.40	10.63 ± 0.52
RBC ($\times 10^{12}$/L)	6.54 ± 0.36	7.39 ± 0.41	6.45 ± 0.35
MCV (fL)	47.60 ± 0.80	50.03 ± 1.89	50.34 ± 0.83
MCH (pg)	16.91 ± 0.27	17.07 ± 0.31	16.54 ± 0.25
MCHC (g/dL)	**35.60 ± 0.46^{a}**	34.10 ± 0.67	**32.90 ± 0.80^{b}**
RDW (%)	**20.41 ± 0.28^{a}**	**20.80 ± 0.32^{a}**	**19.04 ± 0.22^{b}**
RDW (fL)	35.66 ± 1.26	37.00 ± 1.27	31.69 ± 0.56

Keys: RBC = red blood cells count, HCT = haematocrit, Hgb = haemoglobin, MCV = mean Corpuscularvolume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width

Means with different superscripts (a and b) are statistically significant ($P < 0.05$) while those with same superscripts are not significantly different ($P > 0.05$)

Means ± SEM with different superscripts (a and b) are statistically significant ($P < 0.05$)

4.1.6. 9Mean white blood cell counts

The mean WBC of horses infected with *T. equi* (8.26±0.59 $\times 10^9$/L), *T. haneyi* (8.09±0.51 $\times 10^9$/L) and those with mixed infections (6.42±1.04 $\times 10^9$/L) as well as horses infected with *T. equi-like* unknown clades (7.68±1.00 $\times 10^9$/L) were statistically non-significant (P > 0.05) when compared with thatin the non-infected group (7.82±0.72 $\times 10^9$/L) (Fig. 4.10). The mean WBC count in the *T. equi*-infectedsymptomatic West African Barb horses (7.85± 0.80 $\times 10^9$/L) was lower compared to that of the Argentine Polo Ponies (8.86± 0.85 $\times 10^9$/L), however, this difference was not statistically significant (P > 0.05) (Table 4.8). The *T. equi*symptomatic Stallions (7.85± 0.80 $\times 10^9$/L) had the least mean WBC count compared to those of the Geldings (8.40± 0.90 $\times 10^9$/L) and Mares (9.04 ± 1.15 $\times 10^9$/L), however, these changes were not significantly different (P > 0.05) (Table 4.9).

4.1.6.10Mean absolute neutrophil counts

The mean absolute Neut of horses infected with *T. equi* (1.95±0.31 $\times 10^9$/L), *T. haneyi* (2.24±0.43 $\times 10^3$/µL), those with mixed infections (1.17±0.51 $\times 10^9$/L) and those with *T. equi*-like unknown clades (1.20 ± 0.77 $\times 10^9$/L) were not significantly different (P > 0.05) (Fig. 4.11) compared to thatin the non-infected group (3.10±0.80 $\times 10^9$/L). The mean absolute Neut was insignificantly lower (P > 0.05) in the *T. equi*-infected symptomaticWest African Barb horses (1.81± 0.26 $\times 10^9$/L) compared to that of the Argentine Polo Ponies (2.20 ± 0.67 $\times 10^9$/L) (Table 4.8). Conversely, the absolute Neut was significantly higher (P < 0.05) in the*T. equi*-infected symptomatic Geldings (4.30± 1.20 $\times 10^9$/L) compared to those of the Stallions (1.81± 0.26 $\times 10^9$/L) and Mares (1.40± 0.62 $\times 10^9$/L) (Table 4.9).

4.1.6.11Mean absolute lymphocyte counts

Horses infected with *T. equi*(4.65±0.50 $\times 10^9$/L), *T. haneyi*(4.19±0.59 $\times 10^9$/L), those with mixed infections (4.17±0.61 ×10/L) and those infected with *T. equi*-like unknown clades (5.55±1.70 $\times 10^9$/L) had their respective mean absolute LYMP counts not significantly altered (P> 0.05) when compared to the non-infected horses (2.98 ± 0.78 $\times 10^9$/L) (Fig. 4.12). There was no significant change (P > 0.05) in the mean absolute LYMP between the*T. equi*-infected symptomatic West African Barb horses (4.03 ± 0.41 $\times 10^9$/L) and Argentine Polo Ponies (5.56 ± 1.00 $\times 10^9$/L) (Table 4.8). On the other hand, the absolute LYMP count was significantly highest in the*T. equi*-infected symptomatic Mares (6.89 ± 1.08$\times 10^9$/L) compared to those of the Geldings (2.03 ± 0.09 $\times 10^9$/L) and Stallions (4.03 ± 0.41 $\times 10^9$/L) (Table 4.9).

4.1.6.12Mean absolute mid-cell counts

There were variations in the mean absolute mid-cells count in horses infected by *T. equi* (1.82±0.21 $\times 10^9$/L), *T. haneyi* (1.66±0.22 $\times 10^9$/L), those with mixed infections (1.08±0.35 $\times 10^9$/L) and those with *T. equi*-like unknown clades (0.93±0.13 $\times 10^9$/L) when compared with the non-infected horses (1.74± 0.50 $\times 10^9$/L). However, these changes were statistically non-significant (P > 0.05) (Fig. 4.13). The mean absolute mid-cell count was not significantly changed (P > 0.05) between the *T. equi*-infected symptomatic West African Barb horses (2.02 ± 0.30 $\times 10^9$/L) and Argentine Polo Ponies (1.53 ± 0.26 $\times 10^9$/L) (Table 4.8). Similarly, the mean mid-cell count were not significantly changed (P > 0.05) in the *T. equi*-infected symptomatic Stallions (2.02 ± 0.31 $\times 10^9$/L), Geldings (2.07 ± 0.40 $\times 10^9$/L) and Mares (1.33 ± 0.32 $\times 10^9$/L) (Table 4.9).

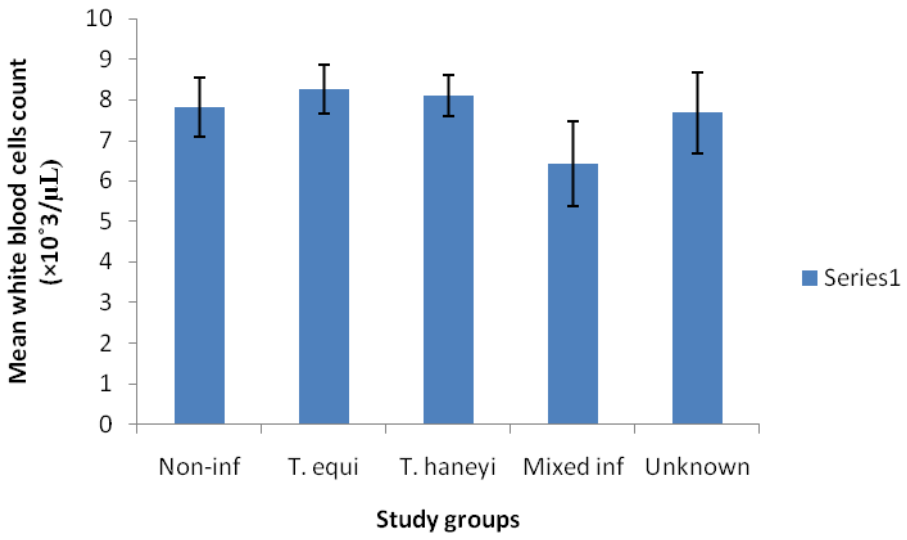


Figure 4.10: The mean ±SEM white blood cell counts for the infected and non-infected horses.

Keys: WBC = White Blood Cells, Non-inf = Non-infected,*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

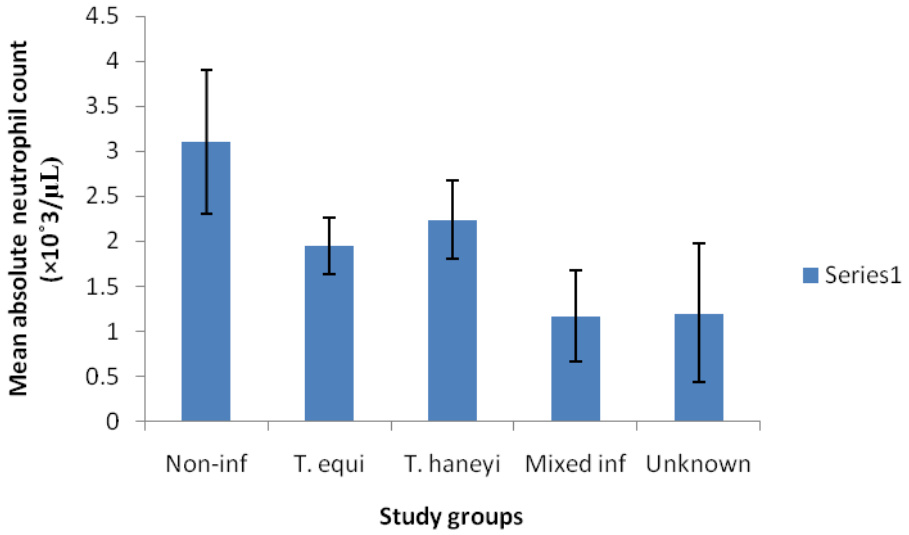


Figure 4.11: The mean ±SEM neutrophil counts for the infected andnon-infectedhorses.

Keys: Neut = Neutrophils,Non-inf = Non-infected,*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

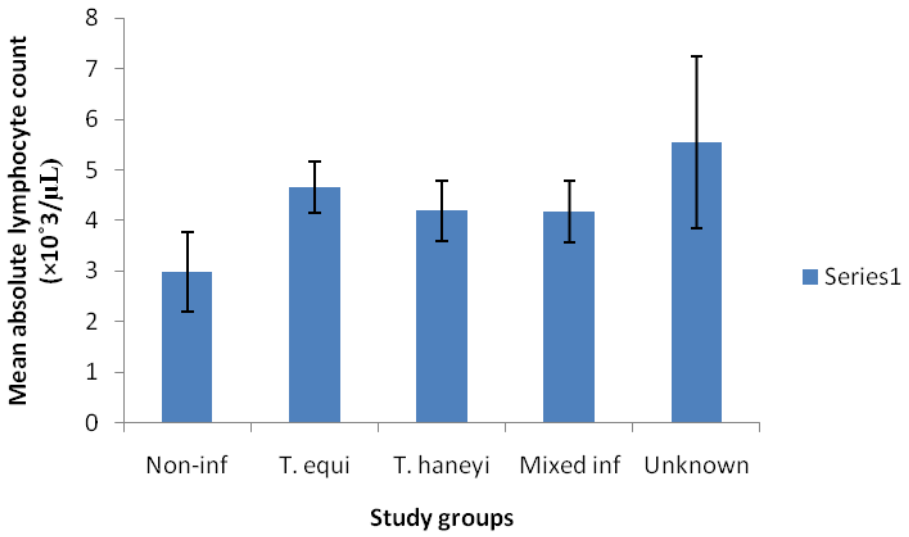


Figure 4.12: The mean ±SEM absolute lymphocyte counts for the infectedand non-infectedhorses.

Keys: LYMP = Lymphocytes, Non-inf = Non-infected,*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

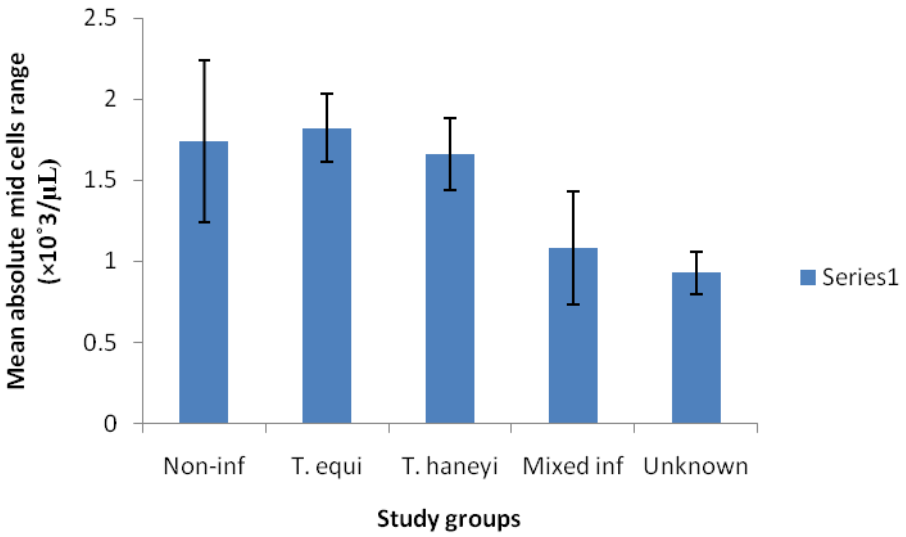


Figure 4.13: The mean ±SEM absolute mid-cell counts for the infected and non-infectedhorses.

Keys: MID = mid-cells representing blood cells of mid range sizes and comprises of monocyte, eosinophil and basophil, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.8: Leukogram with means ± SEM of the different parameters for West African Barb horses and Argentine Polo Ponies

Parameter	West African Barb Horses	Argentine Polo Ponies
WBC ($\times 10^9$/L)	7.85 ± 0.80	8.86 ± 0.85
NEUT ($\times 10^9$/L)	1.81 ± 0.26	2.20 ± 0.67
NEUT (%)	22.53 ± 2.34	24.80 ± 7.46
LYMP($\times 10^9$/L)	4.03 ± 0.41	5.56 ± 1.00
LYMP (%)	53.96 ± 4.48	57.04 ± 8.92
MID ($\times 10^9$/L)	2.02 ± 0.30	1.53 ± 0.26
MID (%)	23.48 ± 2.40	18.10 ± 3.60

Keys: WBC = White blood cell count, NEUT = neutrophils, LYMP = lymphocytes, MID = mid cells representing monocytes, eosinophils and basophils

Table 4.9:Leukogram with means ± SEM based on sex distribution

Parameter	Stallions	Gelding	Mares
WBC ($\times 10^9$/L)	7.85 ± 0.80	8.40 ± 0.90	9.04 ± 1.15
NEUT ($\times 10^9$/L)	**1.81 ± 0.26[a]**	**4.30 ± 1.20[b]**	**1.40 ± 0.62[a]**
NEUT (%)	**22.53 ± 2.34[a]**	**48.97 ± 10.09[b]**	**15.74 ± 7.44[a]**
LYMP($\times 10^9$/L)	**4.03 ± 0.41[a]**	**2.03 ± 0.09[a]**	**6.89 ± 1.08[b]**
LYMP (%)	**53.96 ± 4.50**	**24.63 ± 1.91[b]**	**69.19 ± 8.90[a]**
MID ($\times 10^9$/L)	2.02 ± 0.31	2.07 ± 0.40	1.33 ± 0.32
MID (%)	23.48 ± 2.40	26.17 ± 8.14	15.08 ± 3.73

Keys: WBC = White blood cell count, NEUT = neutrophils, LYMP = lymphocytes, MID = mid cells representing monocytes, eosinophils and basophils

Means ± SEM with different superscripts (a and b) are statistically significant ($P < 0.05$)

4.1.6.13Mean platelet counts

There were no significant (P > 0.05) variations in the mean PLT counts in the uninfected control horses (86.40±12.99×10^9/L) when compared with those infected by *T. equi* (83.11±6.61×10^9/L), *T. haneyi* (98.36±11.98×10^9/L), and horses with mixed infections (120.30 ±26.75×10^9/L) as well as those infected by *T. equi*-like unknown clades (104.30 ±20.06×10^9/L) (Fig. 4.14). The mean PLT was lower in the*T. equi*-infected symptomatic Argentine Polo Ponies (70.91 ± 7.30×10^9/L) but was not statistically different (P > 0.05) compared to that of the West African Barb horses (91.50 ± 9.60×10^9/L) (Table 4.10). The mean PLT was lowest in the *T. equi*-infected symptomatic Mares (68.38 ± 8.53×10^3/µL) followed by those of the Geldings (77.67 ± 16.17×10^9/L) and Stallions (91.50 ± 9.60×10^9/L) but the differences were not significant statistically (P > 0.05) (Table 4.11).

4.1.6.14Mean plateletcrit

The mean PCT of the horses with mixed infections (0.16±0.07 %) was significantly higher (P < 0.05) than those in horses infected with *T. equi* (0.06±0.00 %) and *T. haneyi* infection (0.07±0.01 %). The mean PCT for the respective study groups (Fig. 4.15). The mean PCT in the*T. equi*-infected symptomatic Argentine Polo Ponies (0.06± 0.01 %) was not significantly different (P > 0.05) compared to that of the West African Barb horses (0.06± 0.01 %)(Table 4.10). There were no significant changes (P > 0.05) in the mean PCT of the*T. equi*-infected symptomatic Stallions (0.06± 0.01 %), Geldings (0.06± 0.01 %) and female horses (0.06± 0.01 %) (Table 4.11).

4.1.6.15Mean platelets volume

The mean MPV were variable but not significantly different (P < 0.05) in the horses infected with *T. equi* (7.54±0.22 fL), *T. haneyi* (7.72±0.21 fL), those with mixed infections (7.13±0.31 fL) and those infected by *T. equi*-like unknown clades (7.53±0.29 fL) when compared with non-infected horses (7.04±0.33 fL) (Fig 4.16). The mean MPV in symptomatic Argentine Polo Ponies (8.45± 0.30 fL) infected with *T. equi*was significantly higher compared to the *T. equi*-infected West African Barb horses (6.92± 0.20 fL) (Table 4.10). Similarly, the mean MPV was significantly lower (P < 0.05) in Stallions (6.92± 0.20 fL) compared to that of the Mares (8.60 ± 0.40 fL) but not significantly different (P > 0.05) when compared to the Geldings (8.07 ± 0.40 fL) (Table 4.11).

4.1.6.16Mean percent platelets distribution width

The mean percent PDW was significantly higher (P < 0.05) in the *T. haneyi* (14.61±0.10 %) horses compared to those infected with *T. equi* (12.93±0.40 %). The non-infected horses (13.50±0.85 %), horses with mixed infections (12.98±0.94 %) and those infected by *T. equi*-like unknown clades (14.58±0.05 %) had their mean percent PDW comparatively variable but not significantly changed (P < 0.05). The variations in the respective mean PDW (Fig. 4.17). The mean PDW in the*T. equi*-infected West African Barb horses (11.84±0.52 %) was significantly lower (P < 0.05) compared to that of the Argentine Polo Ponies (14.52± 0.08 %) (Table 4.10). Similarly, the mean PDW was significantly lower (P < 0.05) in the T. equi-infected symptomatic Stallions (11.84± 0.52 %) compared to those of the Geldings (14.47± 0.13 %) and Mares (14.54 ± 0.11 %) (Table 4.11).

4.1.6.17Mean platelets large cell ratio

The mean P-LCR in the *T. equi*-infected horses (0.61±0.10), *T. haneyi*-infected horses (0.99±0.12), horses with mixed infections (1.00±0.37) and those with *T. equi*-like unknown clades (1.08±0.21) were not significantly different (P > 0.05) when compared to that of thenon-infected horses (0.78±0.21) (Fig. 4.18). The mean P-LCR between the symptomatic West African Barb horses (0.50± 0.20) and the Argentine Polo Ponies (0.72± 0.07) with *T. equi* infections were variable but not significantly different (P > 0.05). Also, the mean P-LCR in the *T. equi*-infected symptomatic Stallions (0.53±0.16), Geldings (0.77± 0.15) and the Mares (0.70± 0.09) were variable but not statistically significant (P > 0.05) (Table 4.11).

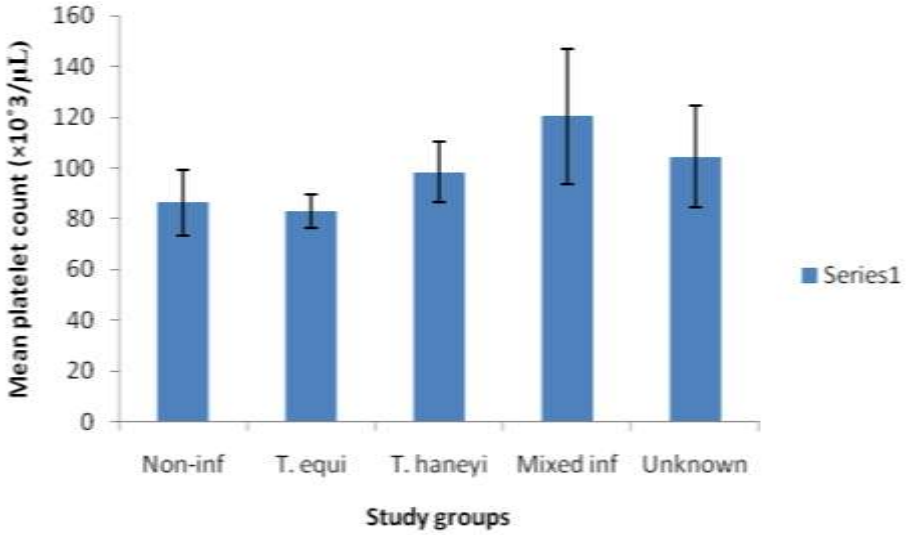


Figure 4.14: The mean ±SEMplatelet counts for the infected and non-infected horses.

Keys: PLT = Platelets, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

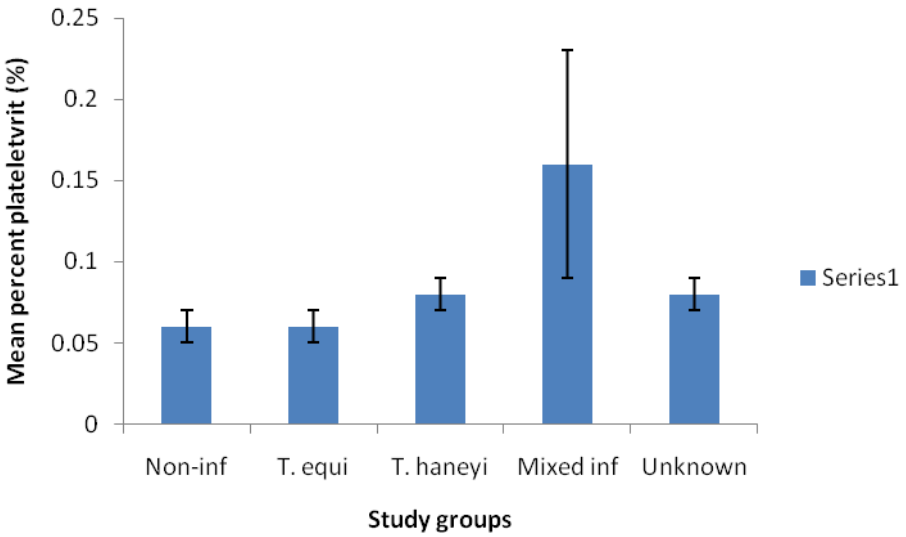


Figure 4.15: The mean ±SEMpercent plateletcrit count for the infected and non-infected horses.

Keys: PCT = Plateletcrit, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

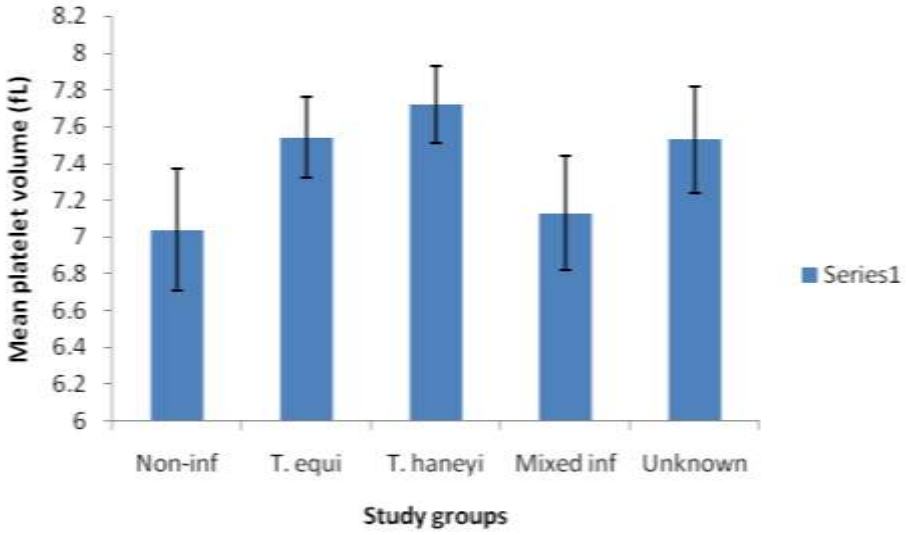


Figure 4.16: The mean ±SEMplatelet volume for the infected and non-infectedhorses.

Keys: MPV = Mean Platelets Volume, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

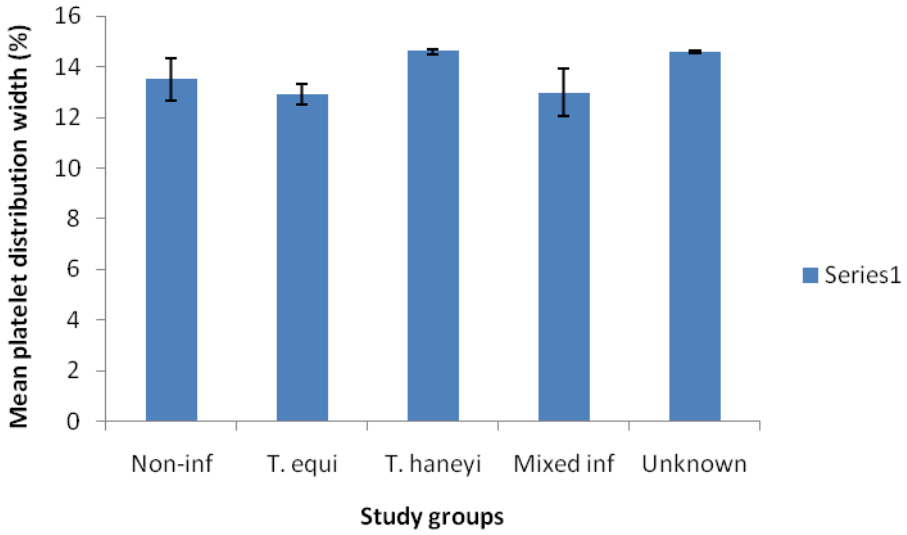


Figure 4.17:The mean ±SEM percent platelets distribution width for the infected and non-infected horses

Keys: PDW = Platelets Distribution Width, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

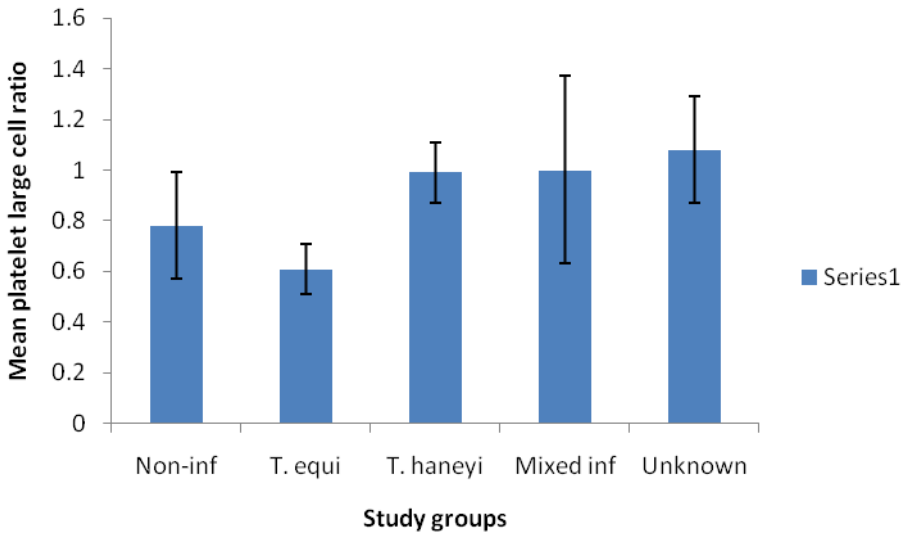


Figure 4.18: The mean± SEM platelets large cell ratio for the infected and non-infectedhorses

Keys: P-LCR = Platelets Large Cell Ratio, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.10:Mean Thrombogram with means ± SEM of the different parameters for West African Barb horses and Argentine Polo Ponies

Parameter	**West African Barb Horses**	**Argentine Polo Ponies**
PLT ($\times 10^9$/L)	91.50 ± 9.60	70.91 ± 7.30
PCT (%)	0.06 ± 0.0	0.06 ± 0.01
MPV (fL)	**6.92 ± 0.20[a]**	**8.45 ± 0.30[b]**
PDW (%)	**11.84 ± 0.52[a]**	**14.52 ± 0.08[b]**
L-PCR	0.50 ± 0.20	0.72 ± 0.07

Keys: PLT = platelets, PCT = plateletcrit, MPV = mean platelet volume, PDW = platelet distribution width

Means ± SEM with different superscripts (a and b) are statistically significant ($P < 0.05$)

Table 4.11:Mean Thrombogram with means ± SEM based on sex distribution

Parameter	Stallions	Gelding	Mares
PLT ($\times 10^9$/L)	91.50 ± 9.60	77.67 ± 16.17	68.38 ± 8.53
PCT (%)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
MPV (fL)	**6.92 ± 0.20**[a]	8.07 ± 0.40	**8.60 ± 0.40**[b]
PDW (%)	**11.84 ± 052**[b]	**14.47 ± 0.13**[a]	**14.54 ± 0.11**[a]
L-PCR	0.53 ± 0.16	0.77 ± 0.15	0.70 ± 0.09

Keys: PLT = platelets, PCT = plateletcrit, MPV = mean platelet volume, PDW = platelet distribution width

Means ± SEM with different superscripts (a and b) are statistically significant ($P < 0.05$)

4.1.7 Mean eerythrocyte membrane bound biochemical values of symptomatic horses with piroplasmosis

4.1.7.1Mean erythrocyte membrane bound alanine aminotransferase (ALT_{mb})activity

There were no statistically significant difference (P > 0.05) in the mean ALT_{mb} of horses infected by *T. equi* (5.30±0.21 U/L), *T. haneyi* (5.43±0.20 U/L), horses with mixed infections (5.17±0.48 U/L) and those infected with *T. equi*-like unknown clades (5.75±0.48 U/L) when compared to the non-infected group (5.40±0.51 U/L) (Fig. 4.19). The mean ALT_{mb} was higher insymptomatic West African Barb horses (5.50 ± 0.50 U/L)when compared to symptomatic Argentine Polo Ponies (5.33± 0.24 U/L) with *T. equi* infections but this was not significantly different (P > 0.05) (Table 4.12). The mean ALT_{mb}were not significantly different in the *T. equi*-infected symptomaticStallion (5.19± 0.28 U/L), Geldings (5.00± 0.58 U/L) and Mares (5.63± 0.42 U/L) (Table 4.13).

4.1.7.2Mean erythrocyte membrane bound aspartate aminotransferase (AST_{mb}) activity

The mean AST_{mb} of the horses infected with *T. equi* (53.81±3.37 U/L), *T. haneyi* (58.07±4.01 U/L), horses with mixed infections (66.00±8.38 U/L) and those infected by *T. equi*-like unknown clades (59.50±2.90 U/L) were not significantly different (P >0.05) from the values of the non-infected horses (59.60±6.41 U/L) (Fig. 4.20). The mean AST_{mb} was significantly higher (P < 0.05) in the*T. equi*-infected symptomatic Argentine Polo Ponies (62.09± 5.40 U/L) compared to West African Barb horses (48.13± 3.80 U/L) as shown in Table 4.12 below. The mean AST_{mb}were not significantly different in the*T. equi*-infected symptomatic Stallions (48.13± 3.80 U/L), Geldings (54.00 ± 3.50 U/L) and Mares (65.13± 7.19 U/L) as shown in Table 4.13 below.

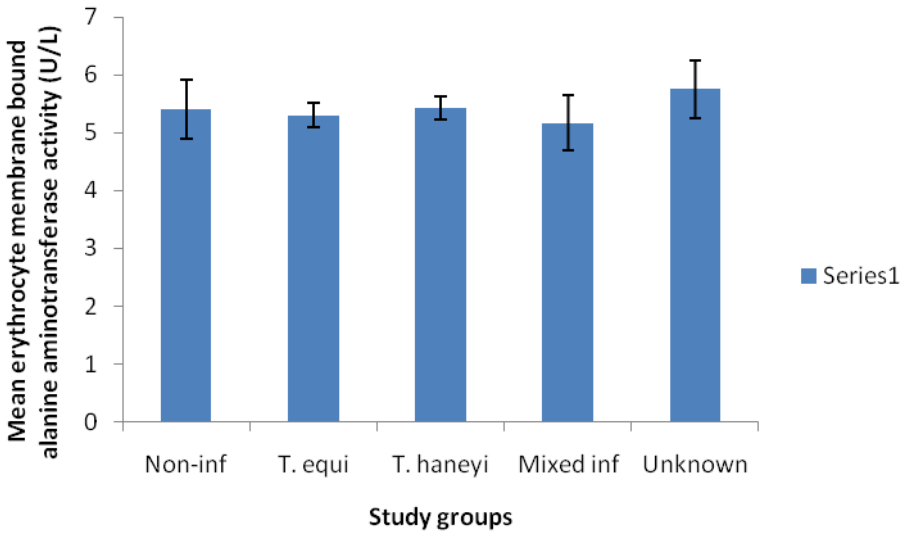


Figure 4.19: The mean ± SEM erythrocyte membrane bound alanine aminotransferase activities for the infected and non-infectedhorses

Keys: ALT = Alanine Transferase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

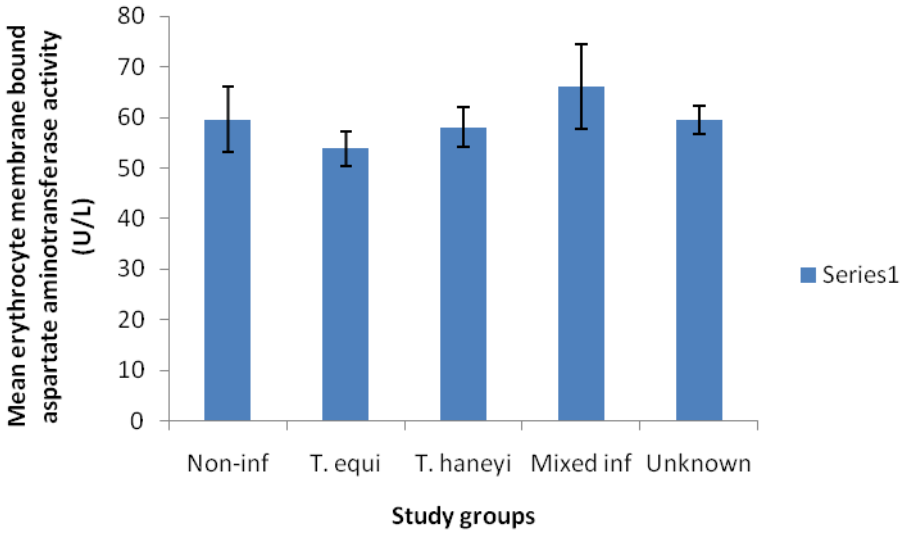


Figure 4.20: The mean ± SEM erythrocyte membrane bound aspartate aminotransferase activities for the infected and non-infectedhorses

Keys: AST = Aspartate Transferase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.12: Means ± SEM of erythrocyte membrane bound enzymatic activities for West African Barb horses and Argentine Polo Ponies

Parameter	West African Barb Horses	Argentine Polo Ponies
ALT (U/L)	5.20± 0.28	5.50± 0.30
AST (U/L)	**48.13 ± 3.80[a]**	**62.09 ± 5.40[b]**

Keys: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

Means ± SEM with different superscripts are statistically significant ($P < 0.05$)

Table 4.13: Means ± SEM of erythrocyte membrane bound enzymatic activities based sex distribution

Parameter	Stallions	Gelding	Mares
ALT (U/L)	5.19 ± 0.28	5.00 ± 0.58	5.63 ± 0.42
AST (U/L)	48.13 ± 3.80	54.00 ± 3.50	65.13 ± 7.19

Keys: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

4.1.7.3Mean erythrocyte membrane catalase (CAT_{mb}) activity

The mean CAT_{mb} in horses infected with *T. equi* (12.97±2.05 u/mL), *T. haneyi* (16.01±3.65 u/mL), horses with mixed infections (15.71±4.34 u/mL) and those infected by *T. equi*-like unknown clades (15.42±4.31 u/mL) were not significantly different (P > 0.05) from the values in non-infected horses (8.16 ± 4.95 u/mL) (Fig. 4.21). The mean CAT_{mb}were not significantly changed between the symptomatic West African Barb horses (11.96± 2.40 u/mL) and the Argentine Polo Ponies (14.43± 3.71 u/mL) with *T. equi* infections (Table 4.14). Similarly, the mean CAT_{mb}were not significantly changed (P > 0.05) between the *T. equi*-infected symptomatic Stallions (11.96± 2.40 u/mL), Geldings (4.30± 1.30 u/mL)and the Mares (18.25± 4.40 u/mL) (Table 4.15).

4.1.7.4Mean erythrocyte membrane superoxide dismutase (SOD_{mb})activity

The mean SOD_{mb} activitywere insignificantly higher (P > 0.05)in horses infected with *T. equi* (10.71±1.31 u/mL), *T. haneyi* (10.26±1.67 u/mL), horses with mixed infections (12.24±1.96 u/mL) and those with *T. equi*-like unknown clades (11.93±4.06 u/mL) than in the non-infected horses (7.58±1.51 u/mL) Fig. 4.22). The mean SOD_{mb} activitieswere not significantly changed between the symptomatic West African Barb horses (10.05± 1.52 u/mL) and the Argentine Polo Ponies (11.67± 2.42 u/mL) with *T. equi* infections (Table 4.14). Similarly, the mean SOD_{mb}in the*T. equi*-infected symptomatic Mares (14.11± 2.90 u/mL)was not significantly higher (P > 0.05) than that of the Geldings (5.10 ± 0.89 u/mL) and Stallions (10.05± 1.52 u/mL)(Table 4.15).

4.1.7.5Mean erythrocyte membrane glutathione (GSH_{mb})concentration

The mean GSH_{mb}varied insignificantly (P > 0.05) in the horses infected with *T. equi* (9.81±1.20 µmol/mL), *T. haneyi* (11.06±1.23 µmol/mL), and horses with mixed

infections (11.68±2.41 µmol/mL), those infected by *T. equi*-like unknown clades (12.74±2.63 µmol/mL) when compared with the non-infected horses (7.02±1.69 µmol/mL) (Fig. 4.23). Similarly, the difference in GSH_{mb} concentrationin the *T. equi*-infected symptomatic West African Barb horses (8.00 ± 1.40 µmol/mL) and the Argentine Polo Ponies (12.39± 1.94 µmol/mL)was not statistically significant ($P > 0.05$) (Table 4.14). The mean $GSH_{mb}$was higher but not significantly altered ($P < 0.05$) in the*T. equi*-infected Mares (14.42± 2.30 µmol/mL) compared to Geldings (7.00± 0.80 u/mL) and Stallions (8.03± 1.40 µmol/mL) (Table 4.15).

4.1.7.6Mean erythrocyte membrane malondialdehyde (MDA_{mb})concentration

The mean MDA_{mb} was significantly higher ($P < 0.05$) in the horses infected with *T. equi*-like unknown clades (41.72±20.22 µmol/mL) than the values among non-infected horses (5.87±4.11 µmol/mL). On the otherhand, the mean MDA_{mb}in horses infected with *T. equi* (16.00±2.88 µmol/mL), *T. haneyi* (16.40±4.22 µmol/mL) as well as in horses with mixed infections (17.60±4.95 µmol/mL) were not significantly different ($P > 0.05$) (Fig. 4.24). The mean MDA_{mb} concentrationvariedinsignificantly ($P > 0.05$) between the*T. equi*-infectedsymptomatic West African Barb horses (12.92± 3.70 µmol/mL) and the Argentine Polo Ponies (20.49± 4.50 µmol/mL) (Table 4.14). Similarly, the mean MDA_{mb}concentrations were not significantly different ($P > 0.05$) in symptomatic Mares (23.88± 5.57 µmol/mL), Geldings (11.45± 5.00 µmol/mL) and Stallions (12.92± 3.67 µmol/mL) (Table 4.15).

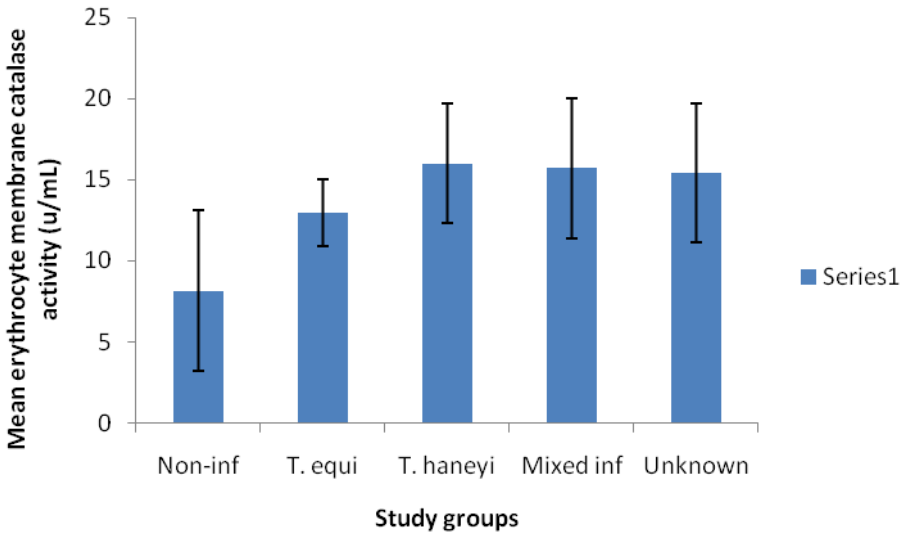


Figure 4.21: The mean ± SEM erythrocyte membrane bound catalase activities for the infected and non-infectedhorses

Keys: CAT = Catalase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,

unknown = *T. equi*-like unknown clade

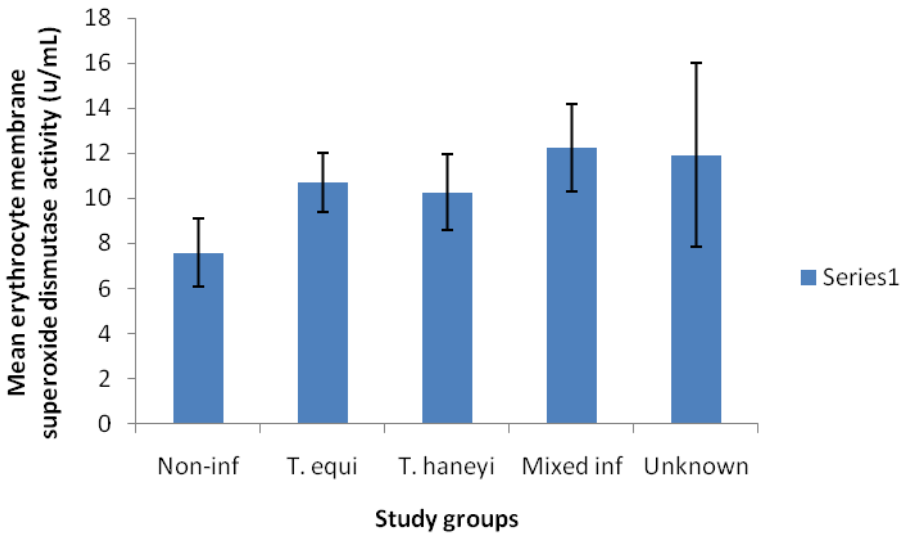


Figure 4.22: The mean ± SEM erythrocyte membrane bound superoxide dismutase activities for the infected and non-infectedhorses

Keys: SOD = Superoxide Dismutase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

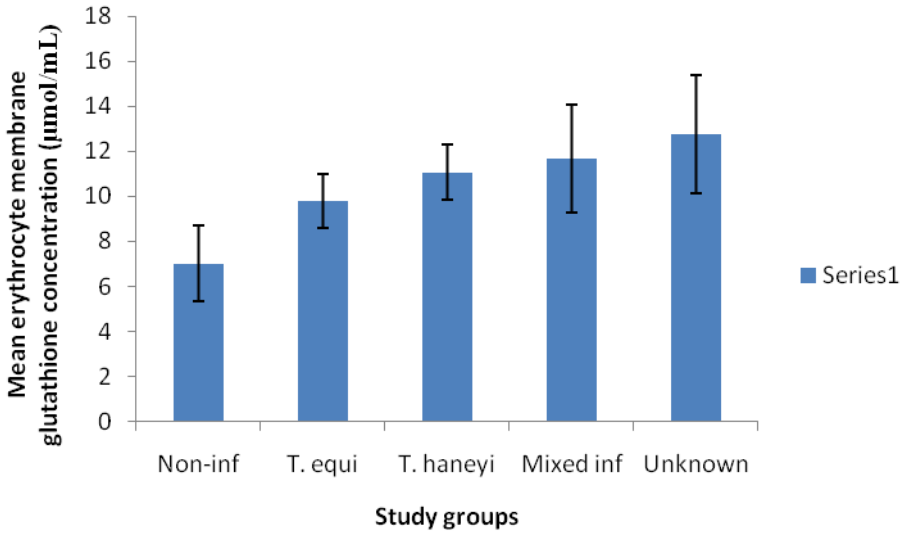


Figure 4.23: The mean ± SEM erythrocyte membrane bound glutathione concentrations for the infected and non-infectedhorses

Keys: GSH = Glutathione, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

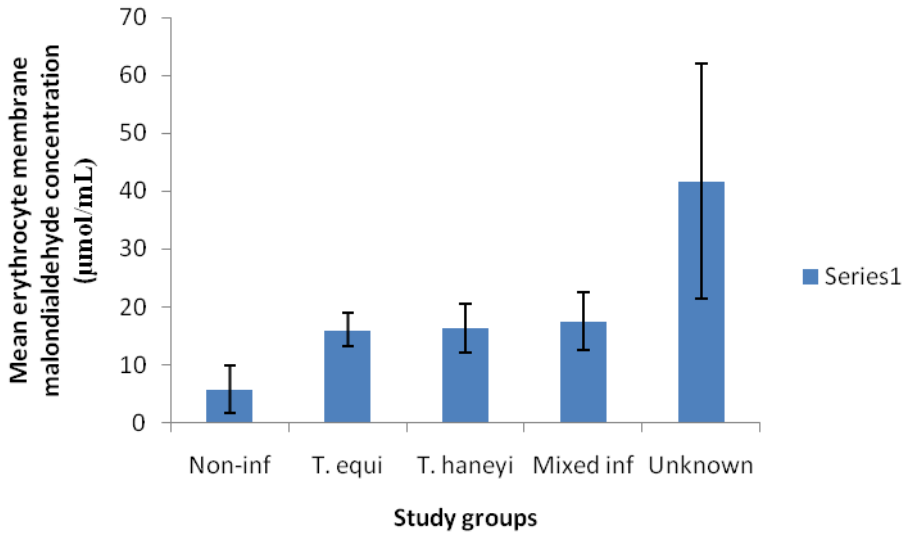


Figure 4.24: The mean ± SEM erythrocyte membrane bound malondialdehydeconcentration for the infected and non-infectedhorses

Keys: MDA = Malondialdehyde, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.14: Mean ± SEM of erythrocyte membrane bound oxidative stress biomarkers in *T. equi*-infected symptomatic horsesbased on breed distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
CAT (u/mL)	11.96± 2.40	14.43± 3.71
SOD (u/mL)	10.05± 1.52	11.67± 2.42
GSH (µmol/mL)	8.00± 1.40	12.39± 1.94
MDA (µmol/mL)	12.92± 3.70	20.49± 4.50

Keys: CAT = Catalase, SOD = Superoxide dismutase, GSH = Glutathione, MDA = Malondialdehyde.

Table 4.15: Mean ± SEM of erythrocyte membrane bound oxidative stress biomarkers in *T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallions	Gelding	Mares
CAT (u/mL)	11.96 ± 2.40	4.30 ± 1.30	18.25 ± 4.40
SOD (u/mL)	10.05 ± 1.52	5.10 ± 0.89	14.11 ± 2.90
GSH (µmol/mL)	8.03 ± 1.40	7.00 ± 0.80	14.42 ± 2.30
MDA (µmol/mL)	12.92 ± 3.67	11.45 ± 5.00	23.88 ± 5.57

Keys: CAT = Catalase, SOD = Superoxide dismutase, GSH = Glutathione, MDA = Malondialdehyde.

4.1.7.8Mean erythrocyte membrane bound sialic acid (SA_{mb})

There were no statistically significant differences (P > 0.05) in the mean values of SA_{mb} of horses infected with *T. equi* (13.38±0.66 µ/L), *T. haneyi* (13.70±0.84 µ/L), mixed infections (12.38±1.07 µ/L) and horses infected with *T. equi*-like unknown clades (11.75±1.96 µ/L)compared to the non-infected horses (15.34±0.92 µ/L) (Fig. 4.25). The mean SA_{mb} in the *T. equi*-infected symptomatic were not significantly different (P > 0.05) between the West African Barb horses (13.04± 0.79 µ/L) and Argentine Polo Ponies (13.86 ± 1.18 µ/L) (Table 4.16). Similarly, the mean SA_{mb} in the *T. equi*-infected symptomatic Stallions (13.04 ± 0.80 µ/L) were not significantly different (P > 0.05) from the values in Geldings (14.27± 1.10 µ/L) and Mares (13.71 ± 1.60 µ/L) (Table 4.17).

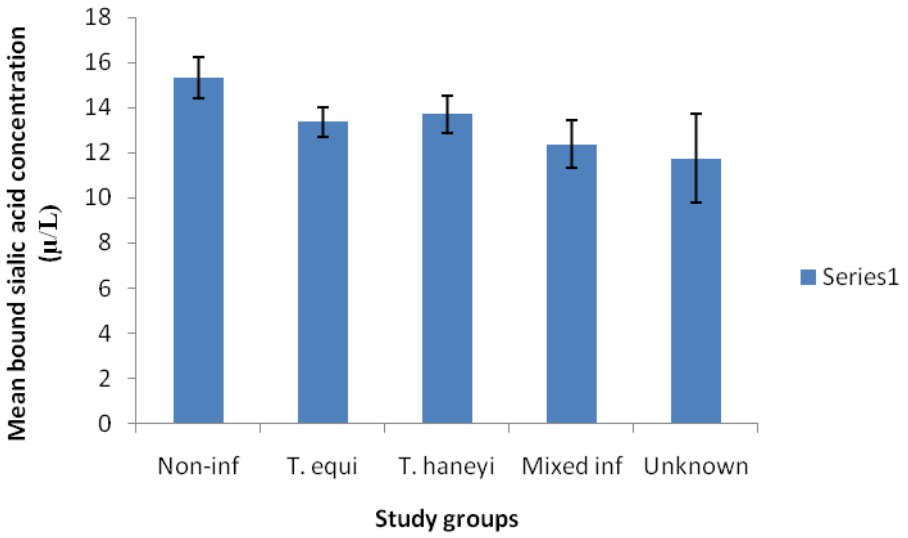


Figure 4.25: The mean ± SEM erythrocyte membrane bound sialic acid concentration for the infected and non-infectedhorses

Keys: SA_{mb} = Erythrocyte Membrane Bound Sialic Acid, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.16: Mean ± SEM of erythrocyte membrane bound sialic acid concentrations in *T. equi*-infected symptomatic horses based on breed distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
SAmb (µ/L)	13.04 ± 0.79	13.86 ± 1.18

Key: SAmb = erythrocyte membrane bound sialic acid

Table 4.17: Mean ± SEM of erythrocyte membrane bound sialic acid concentrations in *T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallions	Gelding	Mares
SAmb (µ/L)	13.04 ± 0.80	14.27 ± 1.10	13.71 ± 1.60

Key: SAmb = erythrocyte membrane bound sialic acid

4.1.8 Mean serum biochemical analyses of symptomatic horses with piroplasmosis

4.1.8.1Mean serum alanine transferase (ALT)activity

The mean serum ALT activity of horses infected with *T. equi* (5.33±0.46 U/L), *T. haneyi* (5.43±0.68 U/L), those with mixed infections (6.00±0.58 U/L) and that of horses infected *T. equi*-like of unknown clades (5.00±1.00 U/L) were not significantly different (P > 0.05) from that of horses in the non-infected group (5.60±0.40 U/L) (Figure 4.26). Similarly, the mean serum ALT in symptomatic West African Barb horses (5.63 ± 0.58 U/L) was not significantly different (P > 0.05) from that of Argentine Polo Ponies (4.91 ± 0.78 U/L) infected with *T. equi*(Table 4.18). Also, the mean serum ALT in the*T. equi*-infected symptomatic Geldings (6.67 ± 0.67 U/L) was insignificantly higher (P > 0.05) than in Stallions (5.63 ± 0.58 U/L) and Mares (4.30 ± 0.96 U/L) (Table 4.19).

4.1.8.2Mean serum aspartate transferase (AST)activity

The mean serum AST activity ofhorses (Fig. 4.27) infected with *T. equi* (20.15±1.12 U/L), *T. haneyi* (18.36±1.57 U/L), with mixed infections (20.17±1.68 U/L) as well as those infected *T. equi*-like unknown parasites (22.25±1.93 U/L)were not significantly different (P > 0.05) from those of non-infected horses (21.60±3.25 U/L).Similarly, the mean serum AST activity in symptomatic West African Barb horses (21.31 ± 1.58 U/L) was not significantly different (P > 0.05) from the values in Argentine Polo Ponies (18.45 ± 1.40 U/L) infected with *T. equi*(Table 4.18) just as the mean serum AST activity in the*T. equi*-infected symptomatic Stallions (21.31 ± 1.60 U/L) was insignificantly higher (P > 0.05) than in Geldings (19.33 ± 4.80 U/L) and Mares (18.13 ± 1.20 U/L) (Table 4.19).

4.1.8.3Mean serum alkaline phosphatase (ALP)activity

The mean serum ALP activity of horses infected with *T. equi* (119.90 ±2.67 U/L), *T. haneyi* (117.10 ±3.95 U/L), horses with mixed infections (123.20 ±4.76 U/L) and those infected with *T. equi*-like unknown clades (111.50 ±9.43 U/L) were not significantly different (P > 0.05) from the values in non-infected horses (114.00 ±5.39 U/L) (Fig. 4.28). Similarly, the mean serum ALP activity in symptomatic West African Barb horses (122.40 ± 3.20 U/L) was not significantly different (P > 0.05) from that of Argentine Polo Ponies (116.10 ± 4.60 U/L) infected with *T. equi*(Table 4.18). There was also no significant difference (P > 0.05) in the mean serum ALP activity in the*T. equi*-infected symptomatic Stallions (122.40 ± 3.20 U/L) than in Geldings (111.70 ± 9.94 U/L) and Mares (117.80 ± 5.40 U/L) (Table 4.19).

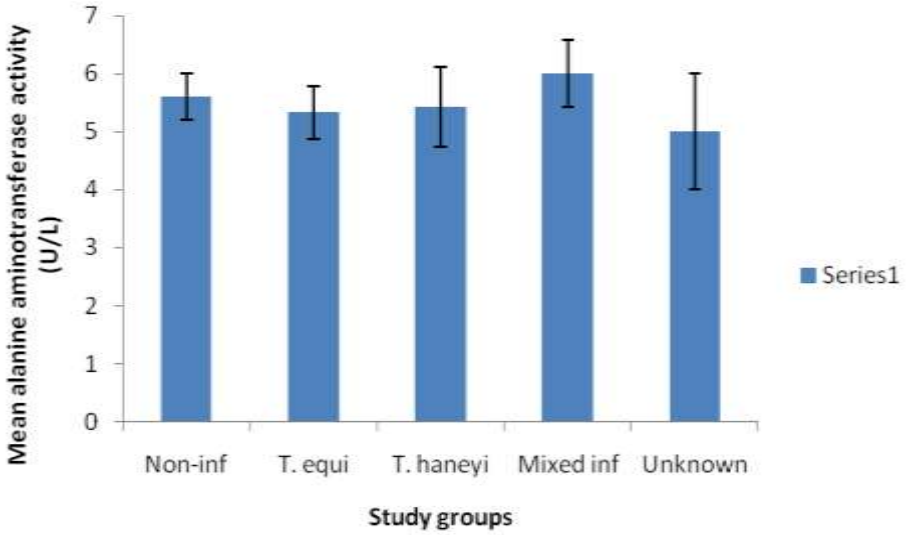


Figure 4.26: The mean ± SEM serum alanine aminotransferase activities for the infectedand non-infectedhorses

Keys: ALT = Alanine transferase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria Haneyi*, unknown = *T. equi*-like unknown clade

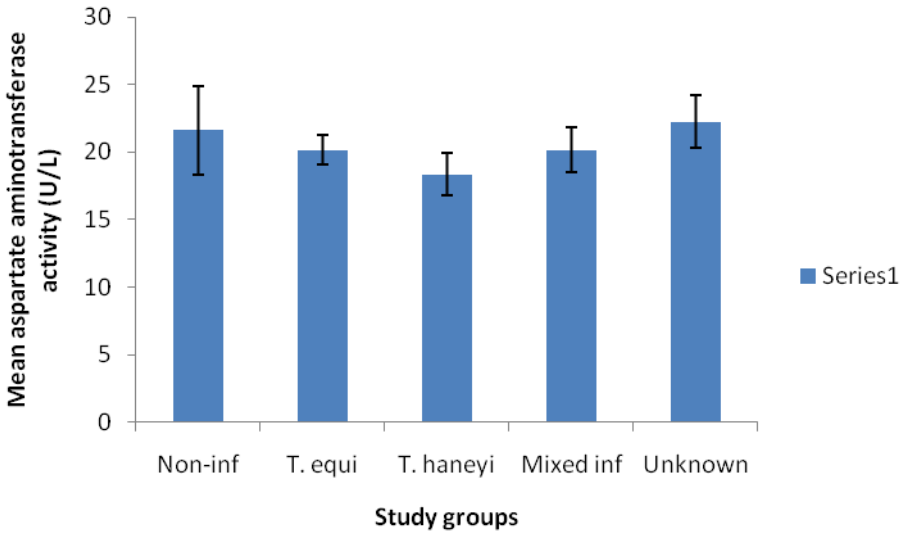


Figure 4.27: The mean ± SEM serum aspartate aminotransferase activities for the infectedand non-infectedhorses

Keys: AST = Aspartate transferase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

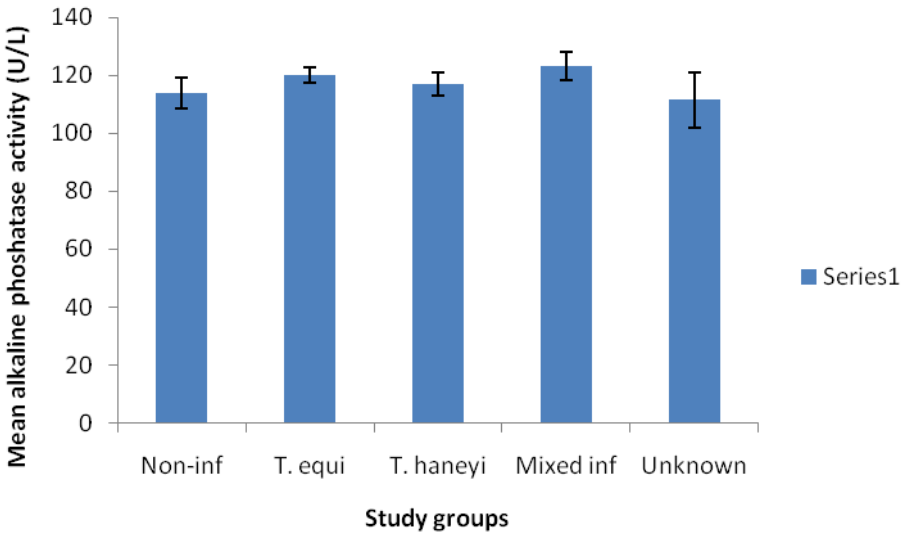


Figure 4.28: The mean ± SEM serum alkaline phosphatase activities for the infected and non-infectedhorses

Keys: ALP = Alkaline Phosphatase, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.18: Mean ± SEM of serum enzymesactivities for*T. equi*-infected symptomatic horses based on breeds distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
ALT (U/L)	5.63± 0.58	4.91± 0.78
AST (U/L)	21.31± 1.58	18.45± 1.40
ALP (U/L)	122.40± 3.20	116.10± 4.60

Keys: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase

Table 4.19: Means ± SEM of serum enzymes activities for*T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallions	Gelding	Mares
ALT (U/L)	5.63 ± 0.58	6.67 ± 0.67	4.30 ± 0.96
AST (U/L)	21.31 ± 1.60	19.33 ± 4.80	18.13 ± 1.20
ALP (U/L)	122.40 ± 3.20	111.70 ± 9.94	117.80 ± 5.40

Keys: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase

4.1.8.4Mean serum total bilirubin concentration (TBil)

The differences in mean serum TBil in the horses infected with *T. equi* (1.14±0.06mmol/L), *T. haneyi* (1.03±0.05mmol/L), with mixed infections (0.93±0.13mmol/L) and horses infected by *T. equi*-like of unknown clades (0.90±0.19mmol/L) and non-infected horses (1.28±0.13mmol/L) were not significant (P > 0.05) (Fig. 4.34). Similarly, the mean serum TBil in symptomatic West African Barb horses (1.12 ± 0.09mmol/L) was not significantly different (P > 0.05) from that of Argentine Polo Ponies (1.17 ± 0.06mmol/L) infected with *T. equi*(Table 4.20). Also, the mean serum TBil in the*T. equi*-infected symptomatic Stallions (1.12 ± 0.08 mmol/L) was not statistically different (P > 0.05) from those of Geldings (1.20 ± 0.09mmol/L) and Mares (1.18± 0.07mmol/L) as shown in Table 4.21).

4.1.8.5Mean direct bilirubin (conjugated bilirubin) concentration

It was observed that the mean conjugated bilirubin concentration in the *T. equi* (0.41±0.04mmol/L), *T. haneyi* (0.41±0.06mmol/L), with mixed infections (0.43±0.06) and those infected by *T. equi*-like of unknown clades (0.35±0.03mmol/L) did not vary significantly (P > 0.05) compared to thenon-infected horses (0.30± 0.05mmol/L) (Fig. 4.35). Conversely, the mean serum conjugated bilirubin concentration in symptomatic Argentine Polo Ponies (0.51 ± 0.05mmol/L) was significantly higher (P < 0.05) than in the West African Barb horses (0.34 ± 0.04mmol/L) with *T. equi* infections (Table 4.20). Also, the mean serum conjugated bilirubin concentration in the*T. equi*-infected symptomatic Mares (0.56± 0.05mmol/L) was statistically higher (P < 0.05) compared to Stallions (0.34± 0.04mmol/L) but not in Geldings (0.37± 0.12mmol/L) (Table 4.21).

4.1.8.6Mean indirect (unconjugated) bilirubin concentration

The means of the unconjugated bilirubin concentration in the horses infected by *T. equi*-like of unknown clades (0.30±0.09mmol/L) and those with *T. haneyi* (0.31±0.10mmol/L) were insignificantly lower (P > 0.05) compared to the means of the unconjugated bilirubin in the non-infected horses (0.42±0.06mmol/L), those infected with *T. equi* (0.59±0.08mmol/L) and in horses with mixed infections (0.53±0.15mmol/L) (Figure 4.36).Conversely, the mean serum unconjugated bilirubin concentration in symptomatic West African Barb horses (0.73± 0.10mmol/L) was significantly higher (P < 0.05) compared to Argentine Polo Ponies (0.38± 0.11mmol/L) with *T. equi* infections (Table 4.20). On the other hand, the mean serum unconjugated bilirubin concentration in the*T. equi*-infected symptomatic Stallions (0.73± 0.10mmol/L) wasinsignificantly higher (P > 0.05) compared to Geldings (0.43± 0.09mmol/L) andMares (0.36± 0.15mmol/L) (Table 4.21).

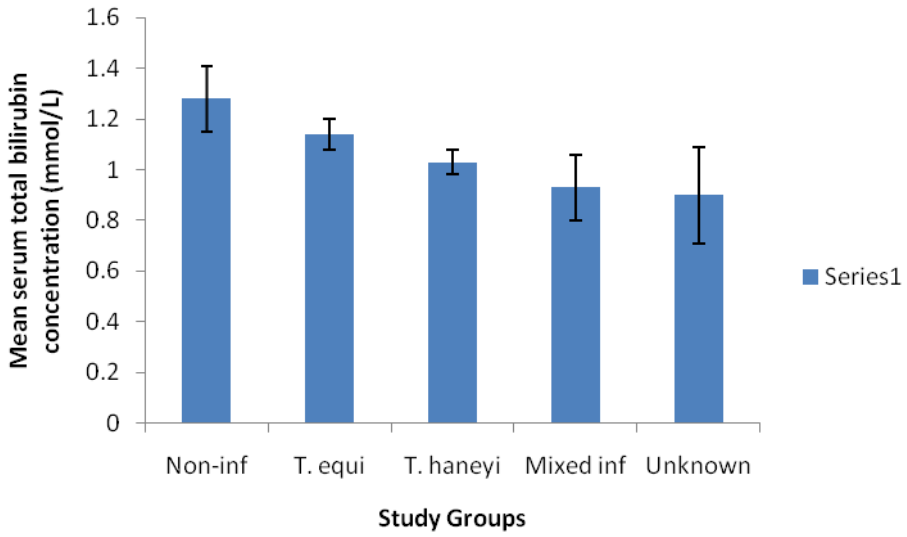


Figure 4.29: The mean serum total bilirubin concentration for the infected and non-infected horses

Keys: TBil = Total Bilirubin, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade

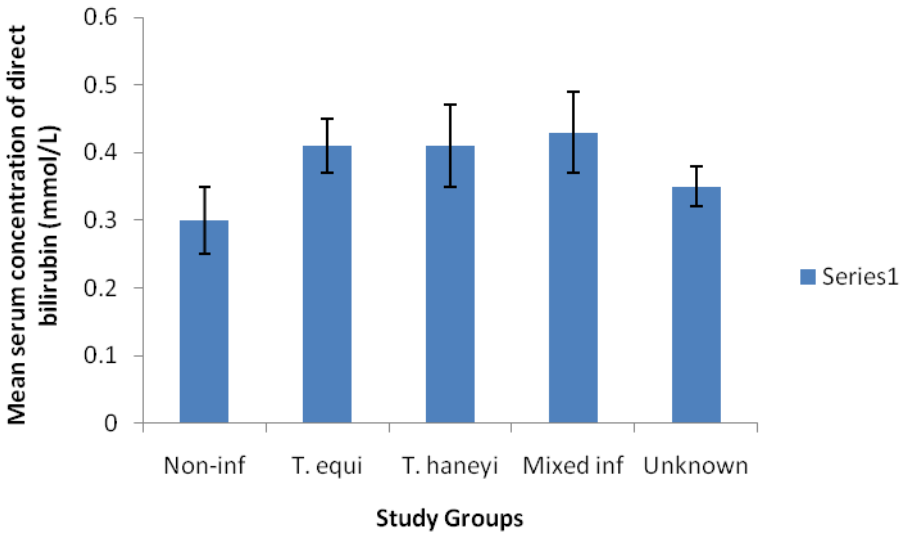


Figure 4.30: The mean direct (conjugated) bilirubin concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

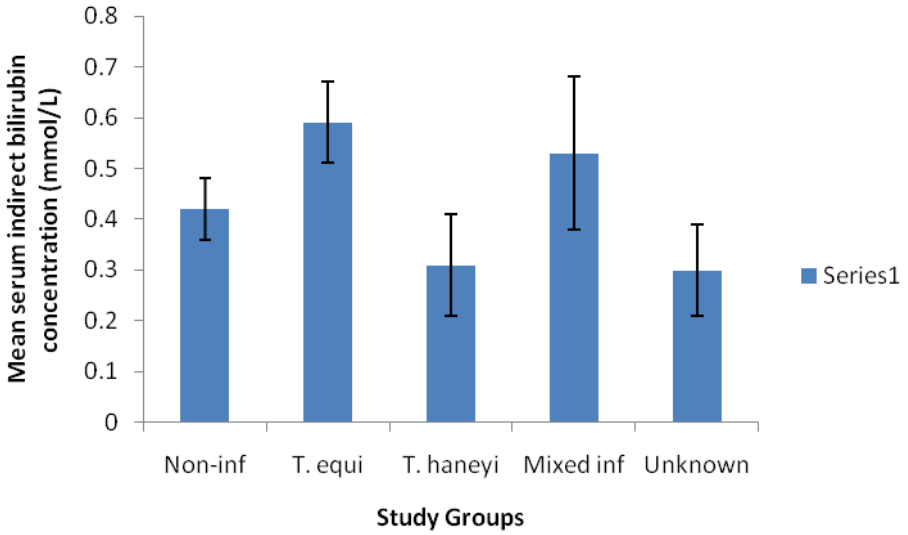


Figure 4.31: The mean indirect (unconjugated) bilirubin concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown Clade

Table 4.20: Means ± SEM of serum bilirubin concentrations for*T. equi*-infected symptomatic horses based on breeds distribution

Parameter	**West African Barb Horses**	**Argentine Polo Ponies**
Total Bilirubin (mmol/L)	1.12± 0.09	1.17± 0.06
Direct Bilirubin (mmol/L)	**0.34 ± 0.04[a]**	**0.51 ± 0.05[b]**
Indirect Bilirubin (mmol/L)	**0.73 ± 0.10[b]**	**0.38 ± 0.11[a]**

Means ± SEM with different superscripts are statistically significant ($P < 0.05$)

Table 4.21: Means ± SEM of serum bilirubin concentrations for *T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallions	Gelding	Mares
Total Bilirubin (mmol/L)	1.12 ± 0.08	1.20 ± 0.09	1.18 ± 0.07
Direct Bilirubin (mmol/L)	**0.34 ± 0.04**[a]	0.37 ± 0.12	**0.56 ± 0.05**[b]
Indirect Bilirubin (mmol/L)	0.73 ± 0.10	0.43 ± 0.09	0.36 ± 0.15

Means ± SEM with different superscripts are statistically significant ($P < 0.05$)

4.1.8.7Mean serum total protein concentration (TP)

There were no significant differences (P > 0.05) in the mean TP concentration ofhorses infected with *T. equi* (10.92±0.49 g/dL), *T. haneyi* (10.11±0.48 g/dL), with mixed infections (10.42±1.27 g/dL) and those infected by *T. equi*-like unknown clades (10.05±1.23 g/dL) when compared to the non-infected horses (11.88±1.65 g/dL) (Fig.4.36). Similarly, the mean serum total protein concentration in symptomatic West African Barb horses (11.09 ± 0.62g/dL) was significantly higher (P < 0.05) compared to Argentine Polo Ponies (10.67 ± 0.80g/dL) with *T. equi* infections (Table 4.22). Also, the mean serum total protein concentration in the*T. equi*-infected symptomatic Stallions (11.09± 0.62g/dL) was insignificantly higher (P > 0.05) than those of Geldings (10.40±1.91g/dL) and Mares (10.78± 1.00g/dL) (Table 4.23).

4.1.8.8Mean serum albumin concentration

The mean serum albumin concentration in horses infected with *T. equi* (6.53±0.48g/dL), *T. haneyi* (6.53±0.73g/dL), with mixed infections (6.42±0.81g/dL) and those infected by unknown parasites (7.70±1.41g/dL) were not significantly different (P > 0.05) from the values in the non-infected horses (5.22±1.75g/dL) (Fig.4.37). The mean serum albumin concentration in symptomatic West African Barb horses (6.54± 0.47g/dL) was not significantly higher (P > 0.05) than that of Argentine Polo Ponies (6.52± 0.99g/dL) with *T. equi* infections (Table 4.22). On the otherhand, the mean serum albumin concentration in the*T. equi*-infected symptomatic Geldings (2.90± 0.10 g/dL) was significantly lower (P < 0.05) than those ofStallions (6.54±0.47 g/dL) and Mares (7.90±0.99 g/dL) (Table 4.23).

4.1.8.9Mean serum globulin concentration

The mean serum globulin concentration in horses infected with *T. equi* (4.30±0.46g/dL), *T. haneyi* (3.40±0.36g/dL), those with mixed infections (4.00±0.65g/dL) and those of horses infected by unknown parasites (4.00±1.02g/dL) varied insignificantly ($P > 0.05$) compared to the non-infected control group (4.20±1.08g/dL) (Fig.4.38).Similarly, the mean serum globulin concentration in symptomatic West African Barb horses (4.27 ± 0.54g/dL) was not significantly different ($P > 0.05$) from that of Argentine Polo Ponies (4.36 ± 0.80g/dL) with *T. equi* infections as (Table 4.22). On the other hand, the mean serum globulin concentration in the*T. equi*-infected symptomatic Geldings (7.50± 1.83 g/dL) was significantly higher ($P < 0.05$) compared to Mares (3.20 ±0.53 g/dL) and theStallions (4.27±0.54 g/dL) (Table 4.23).

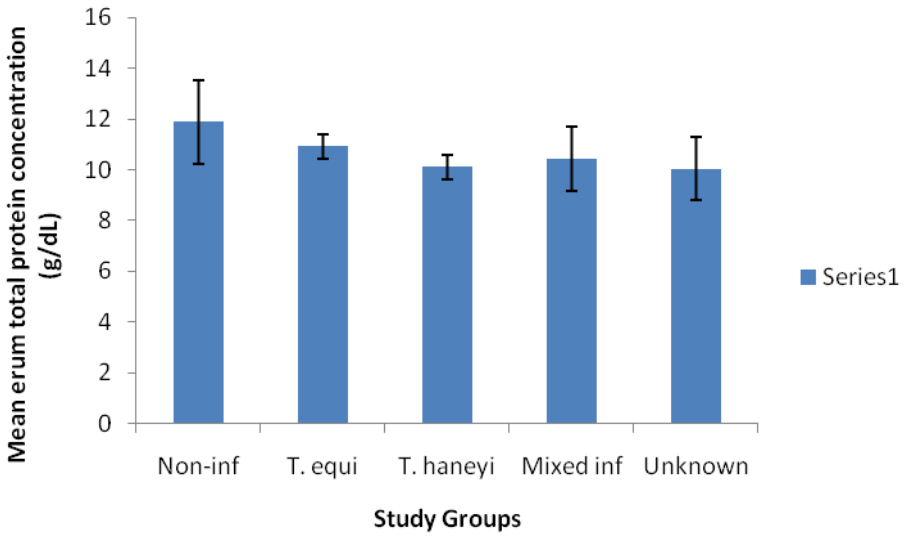


Figure 4.36: The mean serum total protein concentration for the infected and non-infected horses

Keys: TP = Total Protein, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,

unknown = *T. equi*-like unknown clade

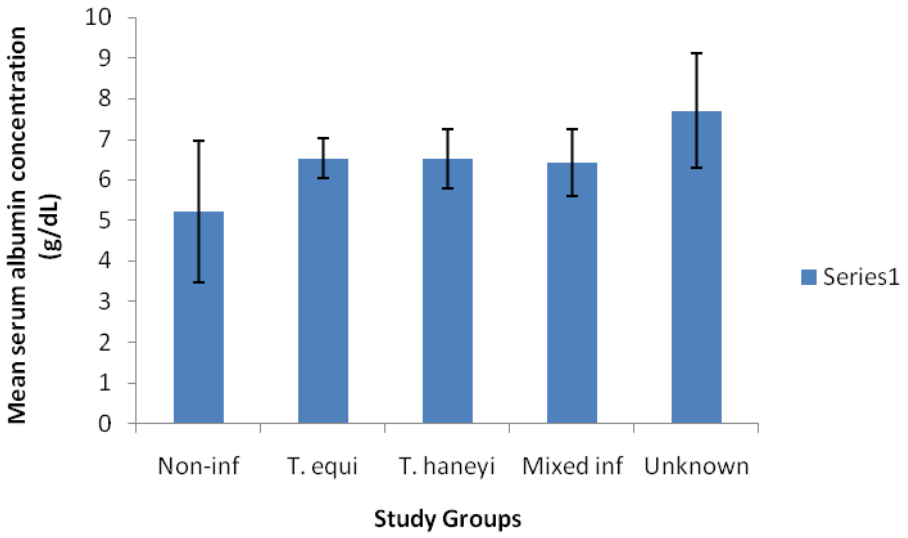


Figure 4.37: The mean albumin concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

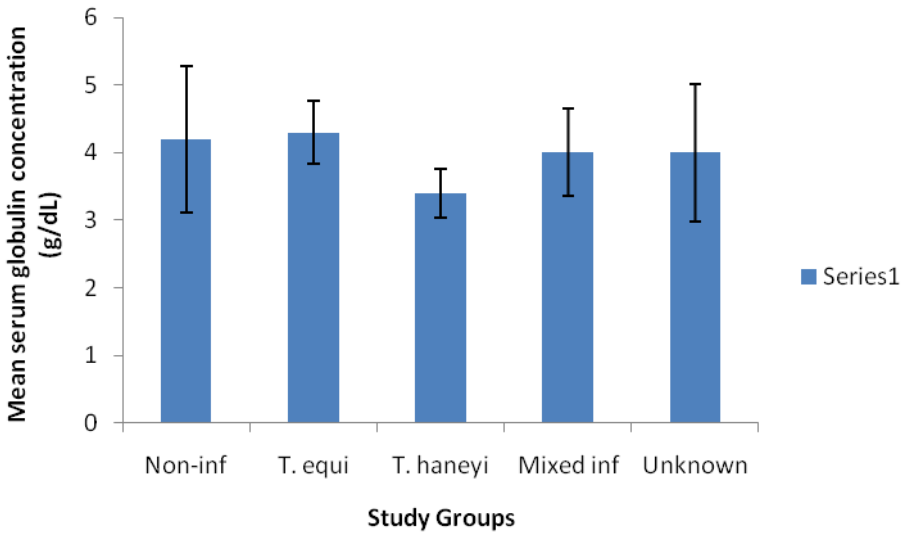


Figure 4.38: The mean globulin concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.22: Means ± SEM of serum total protein, albumin and globulin concentrations for*T. equi*-infected symptomatic horses based on breeds distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
Total protein (g/dL)	11.09 ± 0.62	10.67 ± 0.80
Albumin (g/dL)	6.54 ± 0.47	6.52 ± 0.99
Globulin (g/dL)	4.27 ± 0.54	4.36 ± 0.80

Table 4.23: Means ± SEM of serum total protein, albumin and globulin concentrations for*T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallions	Gelding	Mares
Total protein (g/dL)	11.09 ± 0.62	10.40 ± 1.91	10.78 ± 1.00
Albumin (g/dL)	**6.54 ± 0.47[a]**	**2.90 ± 0.10[b]**	**7.90 ± 0.99[a]**
Globulin (g/dL)	4.27 ± 0.54	**7.50 ± 1.83[b]**	**3.20 ± 0.53[a]**

Means± SEMwith different superscripts are statistically significant ($P < 0.05$)

4.1.8.10Mean serum creatinine concentration

The mean serum creatinine concentrations varied insignificantly (P > 0.05) in horses infected with *T. equi* (1.55±0.16 mg/dL), *T. haneyi* (1.32±0.11 mg/dL), with mixed infections (1.75±0.22 mg/dL) and those infected by *T. equi*-like unknown clades (0.83 ± 0.14 mg/dL) when compared with the non-infected horses (1.20±0.11 mg/dL) (Fig. 4.39). It was also observed that the mean serum creatinine concentration was not significantly different (P > 0.05) in the *T. equi*-infected symptomatic West African Barb horses (1.48 ± 0.14 mg/dL) and Argentine Polo Ponies (1.66 ± 0.40 mg/dL) (Table 4.24). Similarly, the mean serum creatinine concentration in the *T. equi*-infected symptomatic Stallions (1.50 ± 0.14 ×mg/dL), Geldings (1.17 ± 0.13mg/dL) and Mares (1.84 ± 0.48mg/dL) did not vary significantly (P > 0.05) (Table 4.25).

4.1.8.11Mean serum urea concentration

There were no significant alterations (P > 0.05) in the mean serum urea concentration in horses infected with *T. equi* (20.11±0.74 mg/dL), *T. haneyi* (23.71±2.44 mg/dL), horses with mixed infections (27.67±5.77 mg/dL) and those infected with *T. equi*-like unknown clades (21.00±1.35 mg/dL) in comparison to the non-infected horses (19.20±1.39 mg/dL) (Fig. 4.40). The difference in mean serum urea concentration in*T. equi*-infected symptomatic West African Barb horses (20.44 ± 1.17 mg/dL) and Argentine Polo Ponies (19.64 ± 0.70 mg/dL) (Table 4.24) was also not significant (P > 0.05). Similarly, the mean serum urea concentration were not significantly different (P > 0.05) in the *T. equi*-infected symptomatic Stallions (20.44 ± 1.17 mg/dL), Geldings (21.67 ± 1.50 mg/dL) and Mares (18.88 ± 0.67 mg/dL) (Table 4.25).

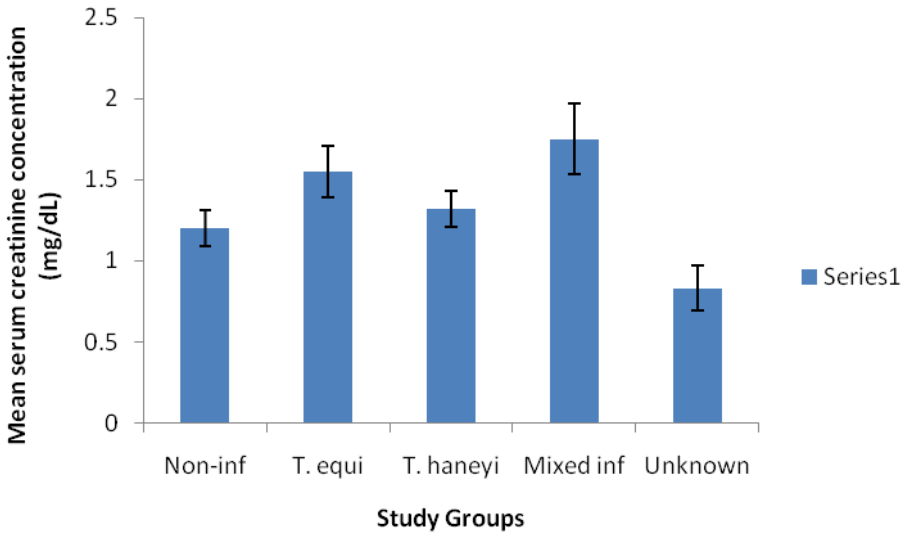


Figure 4.39: The mean creatinine concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

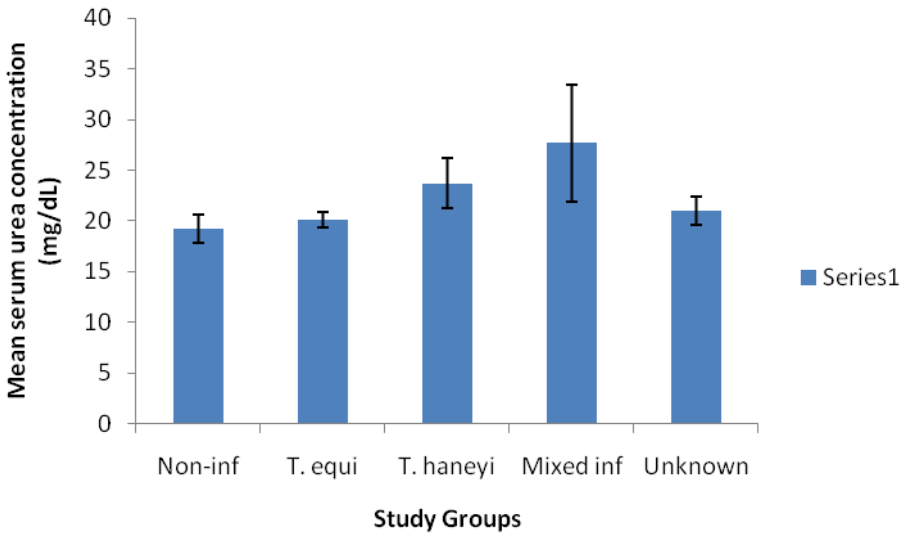


Figure 4.40: The mean urea concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

Table 4.24: Means ± SEM of serum creatinine and blood urea nitrogen concentrations for*T. equi*-infected symptomatic horses based on breed distribution

Parameter	**West African Barb Horses**	**Argentine Polo Ponies**
Creatinine (mg/dL)	1.48 ± 0.14	1.66 ± 0.40
Blood Urea Nitrogen (mg/dL)	20.44 ± 1.17	19.64 ± 0.70

Table 4.25: Means ± SEM of serum creatinine and blood urea nitrogen concentrations for*T. equi*-infected symptomatic horses based on sex distribution

Parameters	Stallion	Gelding	Mare
Creatinine (mg/dL)	1.50 ± 0.14	1.17 ± 0.13	1.84 ± 0.48
Blood Urea Nitrogen (mg/dL)	20.44 ± 1.17	21.67 ± 1.50	18.88 ± 0.67

4.1.8.12Mean serum glucose concentration

The mean serum glucose levels varied insignificantly (P > 0.05) in horses parasitized by *T. equi* (94.43±5.30 mg/dL), *T. haneyi* (86.90±4.56 mg/dL), with mixed infections (88.85±13.54) as well as those infected by *T. equi*-like unknown clades (87.98±7.86 mg/dL) compared to the non-infected horses (110.6±14.11 mg/dL) (Fig.4.41). The difference in mean serum glucose concentration between the *T. equi*-infected symptomatic West African Barb horses (102.90 ± 7.12 mg/dL) and Argentine Polo Ponies (82.12 ± 6.58 mg/dL) was not significant (P > 0.05)(Table 4.26). Similarly, the mean serum glucose concentration were not significantly different (P > 0.05) among the *T. equi*-infected symptomatic Stallions (102.90 ± 7.12 mg/dL), Geldings (73.12 ± 6.70 mg/dL) and Mares (85.50 ± 8.62 mg/dL) (Table 4.27).

4.1.8.13Mean serum total cholesterol concentration

Although, the mean serum total cholesterol concentration (Fig. 4.42) in the horses infected with *T. haneyi* (62.70±4.87 mg/dL) was lowest compared to those in the *T. equi*-infected horses (68.76±3.70 mg/dL), with mixed infections (81.25±15.04 mg/dL), with *T. equi*-like unknown parasites (81.23±21.08 mg/dL) and the non-infected horses (74.72±11.30), the differences were not statistically significant (P > 0.05). The mean serum total cholesterol concentration of*T. equi*-infected symptomatic West African Barb horses (71.29 ± 5.75 mg/dL) and Argentine Polo Ponies (65.08 ± 3.59 mg/dL) were not statistically different (P > 0.05) (Table 4.26). Similarly, the mean serum total cholesterol concentration did not vary significantly (P > 0.05) among the *T. equi*-infected symptomatic Stallions (71.29 ± 5.75 mg/dL), Geldings (78.90 ± 3.01 mg/dL) and Mares (59.90 ± 3.20 mg/dL) (Table 4.27).

4.1.8.14Mean total sialic acid concentration

The mean TSA concentration were variable in horses infected with *T. equi* (24.37±0.68µg/mL), *T. haneyi* (24.37±0.95µg/mL), horses with mixed infections (23.92±1.25µg/mL), those infected by parasites of unknown origin (23.25±2.64µg/mL) and in the non-infected horses (26.82±0.95µg/mL). These variations, however, were comparatively insignificant (P > 0.05) (Fig. 4.43). There was no statistically significant difference (P > 0.05) in the mean serum total sialic acid concentration of the *T. equi*-infected symptomatic West African Barb horses (24.16 ± 0.90µg/mL) and Argentine Polo Ponies (24.67 ± 1.10 µg/mL) (Table 4.26). Similarly, the mean serum total sialic acid concentration were not significantly different (P > 0.05) in the *T. equi*-infected symptomatic Stallions (24.16 ± 0.90µg/mL), Geldings (25.40 ± 1.40 mg/dL) and Mares (24.40 ± 1.45µg/mL) (Table 4.27).

4.1.8.15 Mean free sialic acid concentration

The mean FSA concentration in the horses infected with *T. equi* (10.92±0.21µg/mL), *T. haneyi* (10.46±0.38µg/mL), with mixed infections (11.53±1.10µg/mL) and those infected by *T. equi*-like of unknown clades (11.50±1.10µg/mL) were not significantly different (P > 0.05) from that of thenon-infected horses (11.48±0.89µg/mL) (Fig.4.44).Between the *T. equi*-infected symptomatic West African Barb horses (10.99 ± 0.30µg/mL) and Argentine Polo Ponies (10.81 ± 0.31 µg/mL), the difference in mean serum FSA concentrations was not significant (P > 0.05)(Table 4.26). Similarly, the mean serum free sialic acid concentrations variedinsignificantly (P > 0.05) in the *T. equi*-infected symptomatic Stallions (10.99 ± 0.30µg/mL), Geldings (11.13 ± 0.55 mg/dL) and Mares (10.69 ± 0.39µg/mL) (Table 4.27).

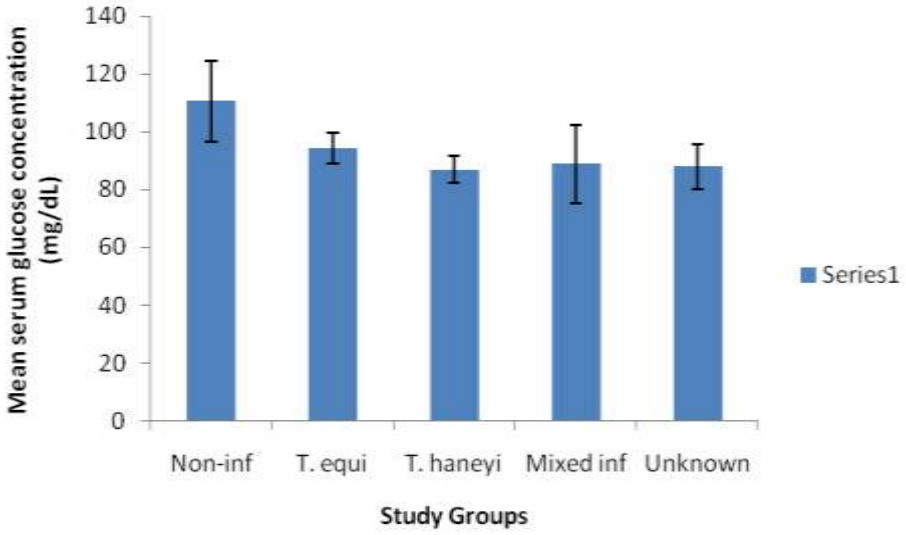


Figure 4.41: The mean glucose concentration for the infected and non-infected horses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

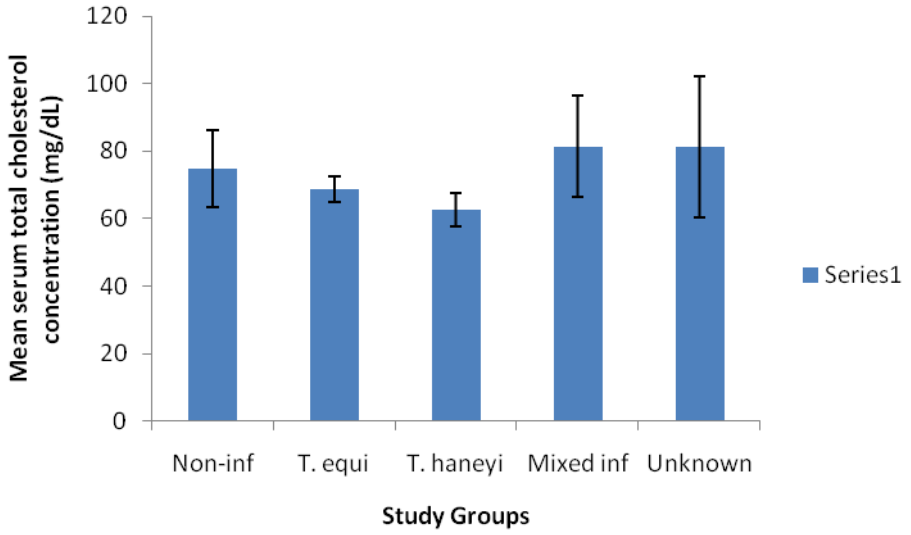


Figure 4.42: The mean serum total cholesterol concentration for the infected and non-infectedhorses

Keys: *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*, unknown = *T. equi*-like unknown clade

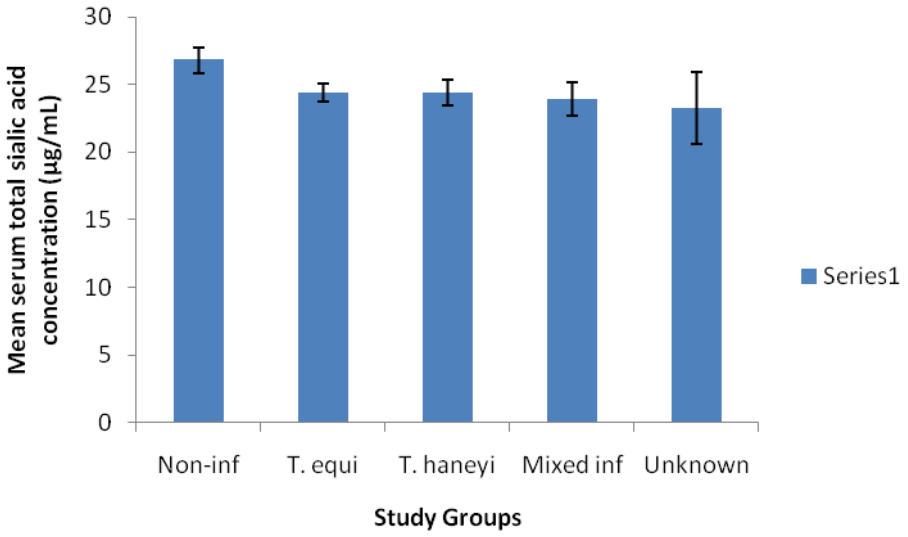


Figure 4.43: The mean total sialic acid concentration for the infected and non-infected horses

Keys: TSA = Total Sialic Acid, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria Haneyi*, unknown = *T. equi*-like unknown clade

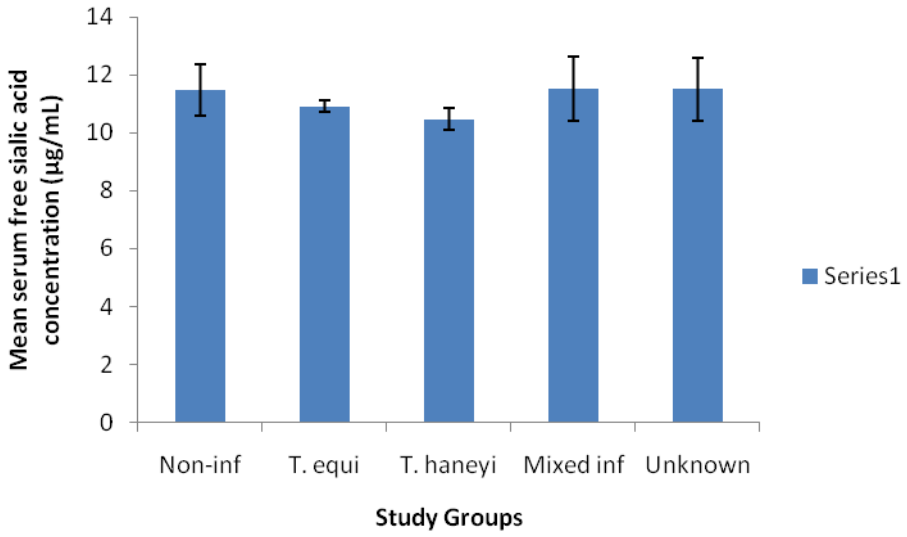


Figure 4.44 The mean free sialic acid concentration for the infected and non-infected horses

Keys: FSA = Total Sialic Acid, *T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade

Table 4.26: Means ± SEM of serum glucose, cholesterol, total sialic acid and free sialic acid concentrations for*T. equi*-infected symptomatic horses based on breed distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
Glucose (mg/dL)	102.90 ± 7.12	82.12 ± 6.58
Cholesterol (mg/dL)	71.29 ± 5.75	65.08 ± 3.59
Total Sialic Acid (µg/dL)	24.16 ± 0.90	24.67 ± 1.10
Free Sialic Acid (µg/dL)	10.99 ± 0.30	10.81 ± 0.31

Table 4.27: Means ± SEM of serum glucose, cholesterol, total sialic acid and free sialic acid concentrations for*T. equi*-infected symptomatic horses based on sex distribution

Parameter	Stallion	Gelding	Mare
Glucose (mg/dL)	102.90 ± 7.12	73.12 ± 6.70	85.50 ± 8.62
Cholesterol (mg/dL)	71/29 ± 5.75	78.90 ± 3.01	59.90 ± 3.20
Total Sialic Acid (µg/dL)	24.16 ± 0.90	25.40 ± 1.40	24.40 ± 1.45
Free Sialic Acid (µg/dL)	10.99 ± 0.30	11.13 ± 0.55	10.69 ± 0.39

4.1.8.16 Mean serum sodium concentration

The mean Na concentration in the horses infected with *T. equi* (67.57 ± 1.77mmol/L), *T. haneyi* (72.19 ± 3.22mmol/L), horses with mixed infections (65.05 ± 6.78mmol/L) and those infected by *T. equi*-like of unknown clades (72.25 ± 3.51mmol/L) were not significantly different (P > 0.05) from that of thenon-infected horses (67.66 ± 6.54mmol/L) (Fig.4.45).Similarly, the mean serum Na concentration was not significantly different (P > 0.05) among the *T. equi*-infected symptomatic West African Barb horses (65.53 ± 1.88mmol/L) and Argentine Polo Ponies (70.55 ± 3.30 mmol/L) (Table 4.28) just as the values among the *T. equi*-infected symptomatic Stallions (65.53 ± 1.90mmol/L), Geldings (78.00 ± 3.90 mmol/L) and Mares (67.75 ± 3.90mmol/L) (Table 4.29).

4.1.8.17Mean serum chloride concentration

The mean Cl concentration in the horses infected with *T. equi* (77.93 ± 1.41mmol/L), *T. haneyi* (77.07 ± 1.90mmol/L), horses with mixed infections (75.17 ± 3.35mmol/L) and those infected by *T. equi*-like of unknown clades (69.25 ± 2.14mmol/L) were not significantly different (P > 0.05) from that ofnon-infected horses (75.20 ± 3.18mmol/L) (Fig.4.46).The mean serum Cl concentration of*T. equi*-infected symptomatic West African Barb horses (78.06 ± 2.02mmol/L) and Argentine Polo Ponies (77.73 ± 1.90 mmol/L) were not significantly different (P > 0.05) (Table 4.28)just as the values among among*T. equi*-infected symptomatic Stallions (78.06 ± 2.02mmol/L), Geldings (80.00 ± 3.06 mmol/L) and Mares (76.88 ± 2.50mmol/L) varied insignificantly (Table 4.29).

4.1.8.18Mean serum potassium concentration

The mean K concentration in the horses infected with *T. equi* (4.82 ± 0.21mmol/L), *T. haneyi* (4.99 ± 0.28mmol/L), horses with mixed infections (4.37 ± 0.36mmol/L) and those infected by *T. equi*-like of unknown clades (4.60 ± 0.25mmol/L) were not significantly different (P > 0.05) from that ofnon-infected horses (4.04 ± 0.32mmol/L) (Fig.4.47).The mean serum K concentration variedinsignificantly (P > 0.05) between the *T. equi*-infected symptomatic West African Barb horses (4.63 ± 0.30mmol/L) and Argentine Polo Ponies (5.10 ± 0.38 mmol/L) (Table 4.30). Similarly, the mean serum K concentration were not significantly different (P > 0.05) among*T. equi*-infected symptomatic Stallions (4.63 ± 0.24mmol/L), Geldings (5.50 ± 0.36 mmol/L) and Mares (4.94 ± 0.50mmol/L) (Table 4.31).

4.1.8.19Mean serum bicarbonate concentration

The differences in mean HCO_3 concentration of the horses infected with *T. equi* (22.00 ± 0.56mmol/L), *T. haneyi* (21.86 ± 0.69mmol/L), with mixed infections (20.33 ± 0.80mmol/L) and those with*T. equi*-like of unknown clades (21.75 ± 1.65mmol/L) wereinsignificantly different (P > 0.05) fromnon-infected horses (20.20 ± 1.28mmol/L) (Fig.4.48).The mean serum HCO_3 concentration was also not significantly different (P > 0.05) among the *T. equi*-infected symptomatic West African Barb horses (22.19 ± 0.70mmol/L) and Argentine Polo Ponies (21.73 ± 0.97 mmol/L) (Table 4.28). The values were not significantly different (P > 0.05) among the *T. equi*-infected symptomatic Stallions (22.19 ± 0.70mmol/L), Geldings (22.69 ± 1.80 mmol/L) and Mares (21.38 ± 1.21mmol/L) (Table 4.29).

4.1.8.20Mean serum phosphate concentration

The mean PO_4 concentration in the horses infected with *T. equi* (3.89 ± 0.47mmol/L), *T. haneyi* (4.54 ± 0.70mmol/L), horses with mixed infections (2.48 ± 0.20mmol/L) and those infected by *T. equi*-like of unknown clades (2.83 ± 0.24mmol/L) were not significantly higher ($P > 0.05$) than the values in non-infected horses (2.46 ± 0.23mmol/L) (Fig.4.49).Conversely, the mean serum PO_4 concentration was significantly higher ($P < 0.05$)in the *T. equi*-infected symptomatic Argentine Polo Ponies (5.06 ± 0.88 mmol/L) than in the West African Barb horses (3.08 ± 0.42 mmol/L) as (Table 4.28). Similarly, the mean serum PO_4 concentration was significantly higher ($P < 0.05$) in the *T. equi*-infected symptomatic Geldings (7.80 ± 0.50mmol/L) than in the Stallions (3.08 ± 0.42 mmol/L) and Mares (4.04 ± 0.97mmol/L) (Table 4.29).

4.1.8.20Mean serum calcium concentration

The mean Ca concentration in the horses infected with *T. equi* (12.13 ± 0.35mmol/L), *T. haneyi* (11.30 ± 0.51mmol/L), horses with mixed infections (10.92 ± 0.87mmol/L) and those infected by *T. equi*-like of unknown clades (11.53 ± 0.69mmol/L) variedinsignificantly ($P > 0.05$) from that ofnon-infected horses (11.62 ± 0.93mmol/L) (Fig.4.50).The seemingly higher mean serum Ca concentration among the *T. equi*-infected symptomatic Argentine Polo Ponies (12.40 ± 0.53 mmol/L) was not significantly different ($P > 0.05$) compared to the West African Barb horses (11.94 ± 0.48 mmol/L) (Table 4.28). Similarly, the mean serum Ca concentration was higher but not significantly changed ($P > 0.05$) in the *T. equi*-infected symptomatic Geldings (13.77 ± 0.27mmol/L) compared to Stallions (11.94 ± 0.48 mmol/L) and Mares (11.89 ± 0.64mmol/L) (Table 4.29).

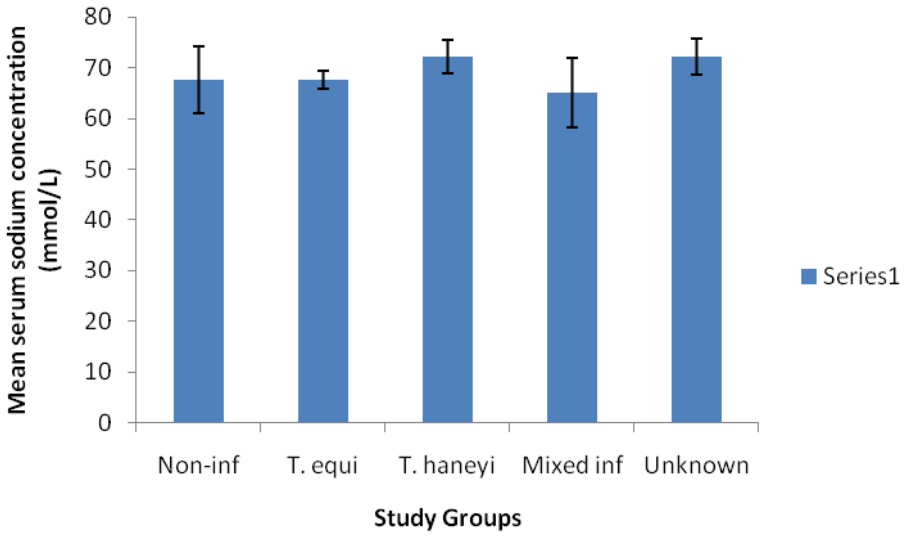


Figure 4.45 The mean serum sodium concentration for the infected and non-infectedhorses

Keys:*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade, Non-inf = non-infected

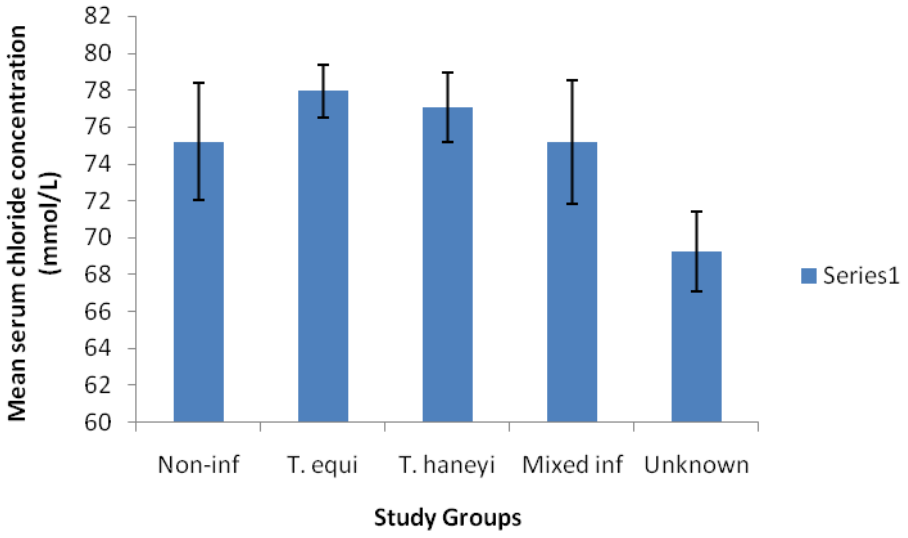


Figure 4.46 The mean serum chloride concentration for the infected and non-infectedhorses

Keys:*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade, Non-inf = non-infected

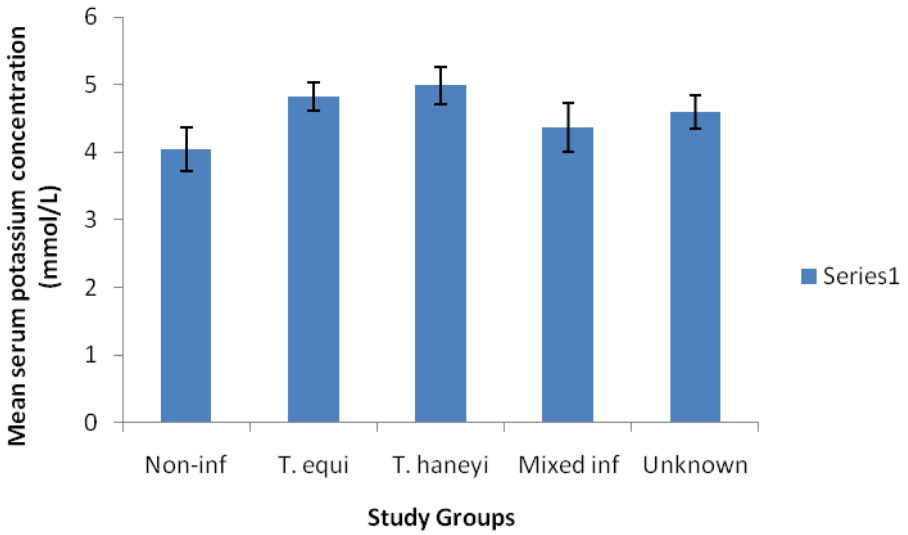


Figure 4.47 The mean serum potassium concentration for the infected and non-infectedhorses

Keys:*T. equi = Theileria equi*, *T. haneyi = Theileria haneyi*,unknown = *T. equi*-like unknown clade, Non-inf = non-infected

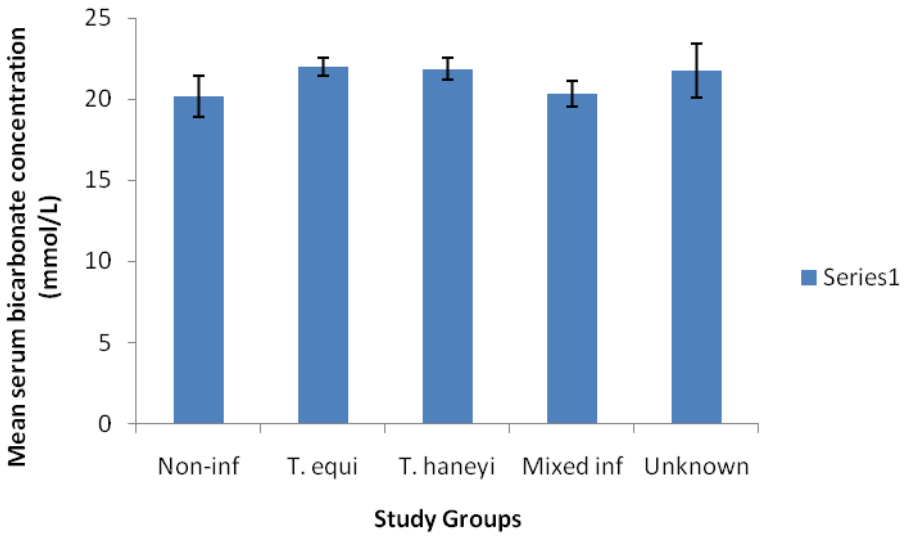


Figure 4.48 The mean serum bicarbonate concentration for the infected and non-infectedhorses

Keys:*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknownclade, Non-inf = non-infected

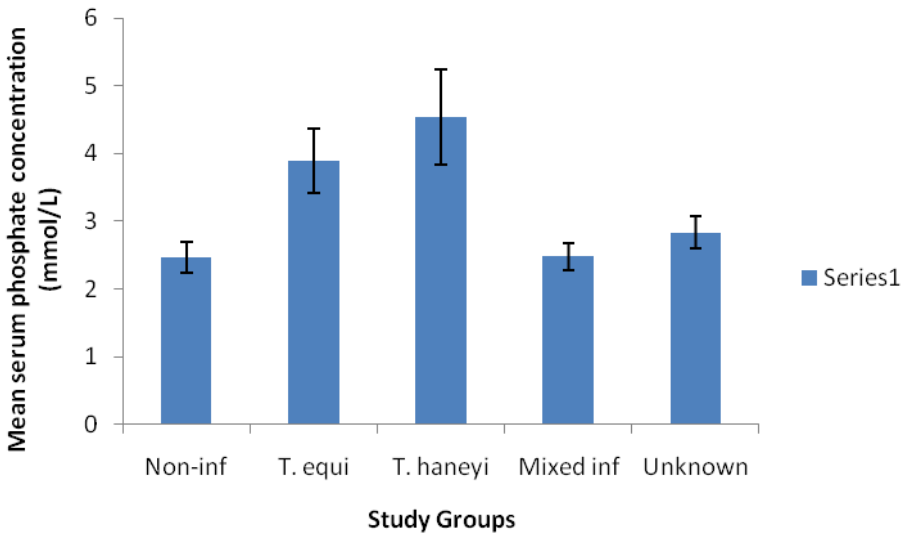


Figure 4.49 The mean serum phosphate concentration for the infected and non-infectedhorses

Keys:*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade, Non-inf = non-infected

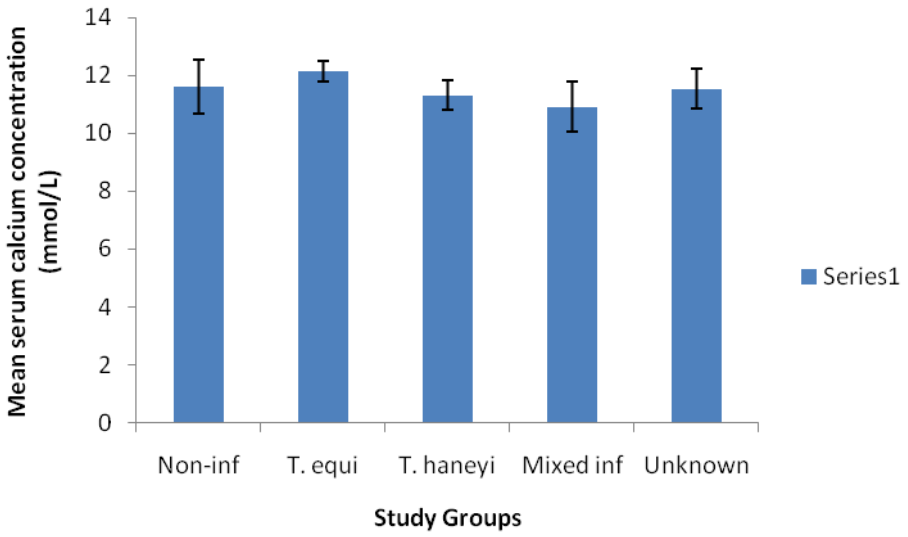


Figure 4.50 The mean serum calcium concentration for the infected and non-infectedhorses

Keys:*T. equi* = *Theileria equi*, *T. haneyi* = *Theileria haneyi*,unknown = *T. equi*-like unknown clade, Non-inf = non-infected

Table 4.28:Means ± SEM of serum electrolyte concentrations for *T. equi*-infected symptomatic horses based on breed distribution

Parameter	West African Barb Horses	Argentine Polo Ponies
Na (mmol/L)	65.53 ± 1.88	70.55 ± 3.30
Cl (mmol/L)	78.06 ± 2.02	77.73 ± 1.90
K (mmol/L)	4.63 ± 0.30	5.10 ± 0.38
HCO_3 (mmol/L)	22.19 ± 0.70	21.73 ± 0.97
PO_4 (mmol/L)	**3.08 ± 0.42[a]**	**5.06 ± 0.88[b]**
Ca (mmol/L)	11.94 ± 0.48	12.40 ± 0.53

Keys: Na = sodium, Cl = chloride, K = potassium, HCO_3 = bicarbonate, PO_4 = phosphate, Ca = calcium

Means± SEMwith different superscripts are statistically significant ($P < 0.05$)

Table 4.29:Means ± SEM of serum electrolyte concentrations for *T. equi*-infected symptomatic horses based on breed distribution

Parameter	Stallions	Geldings	Mares
Na (mmol/L)	65.53 ± 1.90	78.00 ± 3.90	67.75 ± 3.90
Cl (mmol/L)	78.06 ± 2.02	80.00 ± 3.06	76.88 ± 2.50
K (mmol/L)	4.63 ± 0.24	5.50 ± 0.36	4.94 ± 0.50
HCO_3 (mmol/L)	22.19 ± 0.70	22.67 ± 1.80	21.38 ± 1.21
PO_4 (mmol/L)	**3.08 ± 0.42^a**	**7.80 ± 0.50^b**	**4.04 ± 0.97^a**
Ca (mmol/L)	11,94 ± 0.48	13.77 ± 0.27	11.89 ± 0.64

Keys: Na = sodium, Cl = chloride, K = potassium, HCO_3 = bicarbonate,

PO_4 = phosphate, Ca = calcium

Means ± SEM with different superscripts are statistically significant ($P < 0.05$)

4.2 Asymptomatic Horses

4.2.1 Serological analysis of asymptomatic horses

4.2.1.1Babesia caballi competitive enzyme linked immunosorbent assay

Three samples H44 (93.9% PI), H67 (96.11% PI) and H78 (92% PI) were seropositive to anti-*B. caballi* antibodies using the *B. caballi* specific cELISA. The remaining seventy seven samples had percent inhibition below 40% which implies seronegativity to anti-*B. caballi* antibodies (Table 4.30).

4.1.2.2Theileria equi competitive enzyme linked immunosorbent assay

Seventy two sampleshad percent inhibition (PI) above 40%, thus, indicating reactivity to anti-*T. equi* antibodies or seropositive. Eight sampleshad percent inhibition (PI) below 40%, indicating non reactivity to anti-*T. equi* antibodies or seronegative (Table 4.30).

Table 4.30: Seroprevalence of *B. caballi* and *T. equi* among asymptomatic horses

Target Parasites	Number of Positive	Number of Negative
Babesia caballi	3 (3.8 %)	77 (96.2 %)
Theileria equi	72 (90 %)	8 (10 %)

Horses with percent inhibition (PI) ≥ 40 % = positive, whereas those with PI ≤ 40 % = negative. Horses with PI between 40 – 44% = border-line positive

4.2.2 Haematological analyses

4.2.2.1Mean haematocrit

The mean haematocrit for the horses infected with *B. caballi* (28.93±4.95 %), *T. equi* (26.91±0.94 %), with mixed infections (28.93±4.95 %) were not significantly different (P > 0.05) than that of the non-infected horses (25.50±2.17 %)(Fig.4.51).

4.2.2.2Mean haemoglobin concentration

The mean Hgb concentration in horses infected with *B. caballi*(9.73±1.73 g/dL), *T. equi*(9.11±0.36 g/dL), with mixed infections was (9.73±1.73 g/dL)were not significantly different (P > 0.05) than the non-infected horses (9.03±0.81 g/dL) (Fig.4.52).

4.2.2.3Mean red blood cells count

There were no significant variations (P > 0.05) in the mean RBC in horses infected with *B. caballi* (5.76 ±1.04 $\times 10^6$/μL), *T. equi* (5.67 ± 0.19$\times 10^6$/μL) and those with mixed infections (5.76 ±1.04$\times 10^6$/μL) when compared with the non-infected horses (5.50 ± 0.51$\times 10^6$/μL) (Fig.4.53).

4.2.2.4Mean corpuscular volume

The mean MCV for the *B. caballi*-infected horses (50.43± 1.88 fL), *T. equi*-infected (47.79±0.45 fL) and horses with mixed infections (50.43±1.88 fL) were statistically insignificant (P > 0.05) than that of the non-infected horses (46.65±0.51 fL) (Fig.4.54).

4.2.2.5Mean corpuscular haemoglobin

The mean MCH concentration in horses infected with *B. caballi* (16.90±0.21 pg), *T. equi* (15.97±0.29 pg) and with mixed infections (16.90± 0.21 pg) were insignificantly varied (P > 0.05) compared to the non-infected horses (16.45±0.15 pg) (Fig.4.55).

4.2.2.6Mean corpuscular haemoglobin concentration

There were no significant alterations (P > 0.05) in the mean MCHC in the horses infected with *B. caballi* (33.60±0.84 g/dL), *T. equi* (33.44±0.64 g/dL), mixed infections (33.60±0.84 g/dL) compared to the non-infected horses (35.29±0.32 g/dL) (Fig.4.55).

4.2.2.7Mean red blood cells redistribution width

The mean RDW of horses infected with *B. caballi* (21.33±0.72 fL), *T. equi* (19.22±0.33) and with mixed infections (21.33±0.72 fL) were not significant different (P > 0.05) compared to the non-infected horses (19.83±0.15 fL) (Fig.4.55).

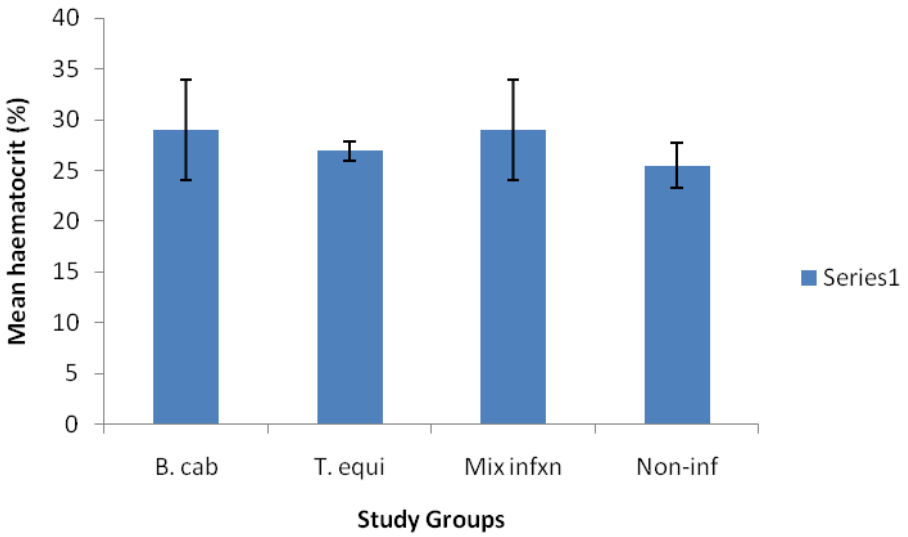


Figure 4.51: The mean haematocrit for the infected and non-infectedhorses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

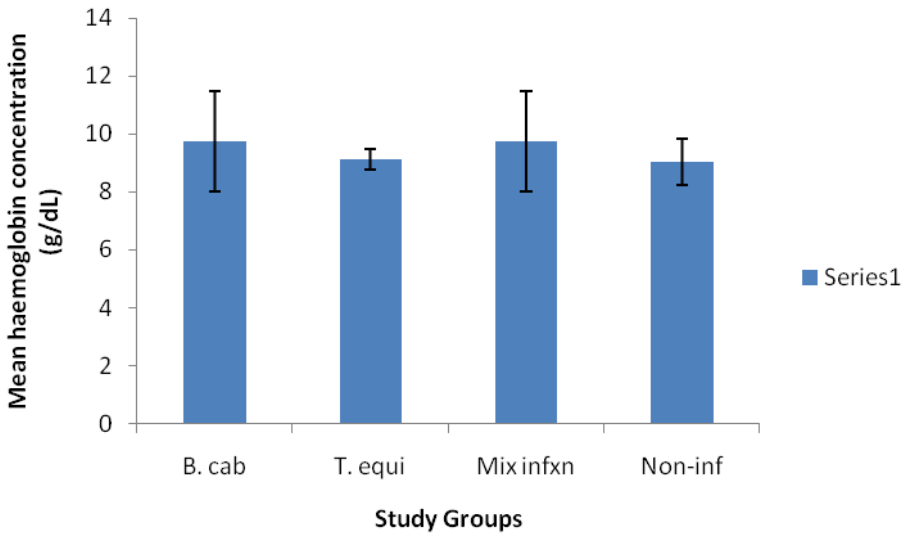


Figure 4.52: The mean haemoglobin concentration for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

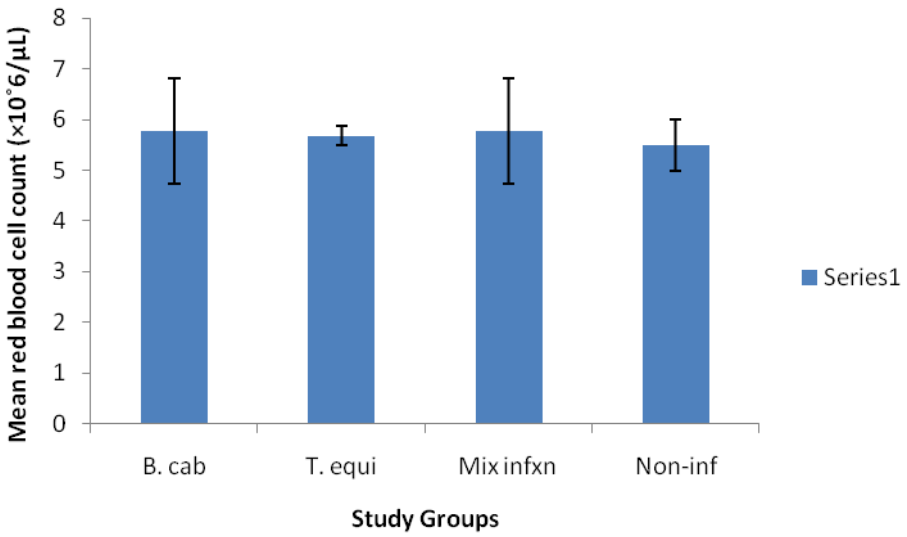


Figure 4.53: The mean red blood cell counts for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = non-infected

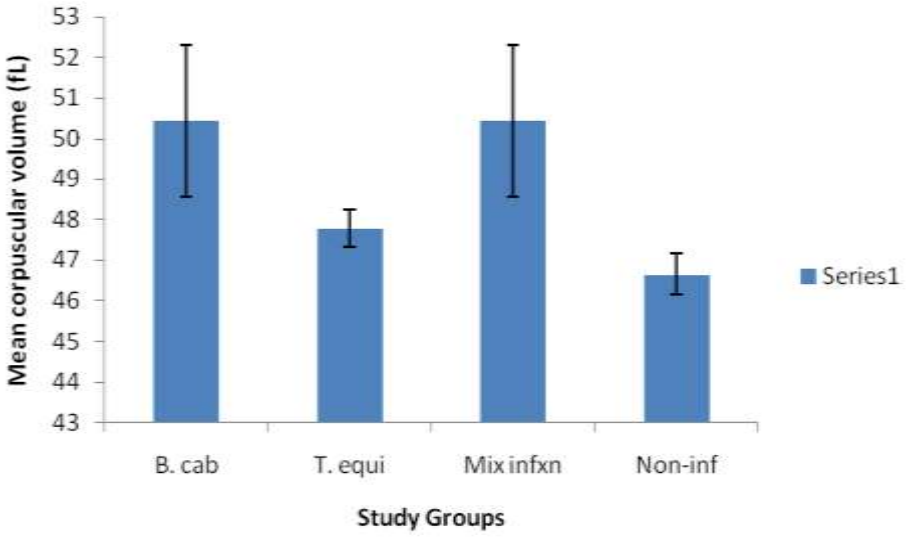


Figure 4.54: The mean of mean corpuscular volume for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

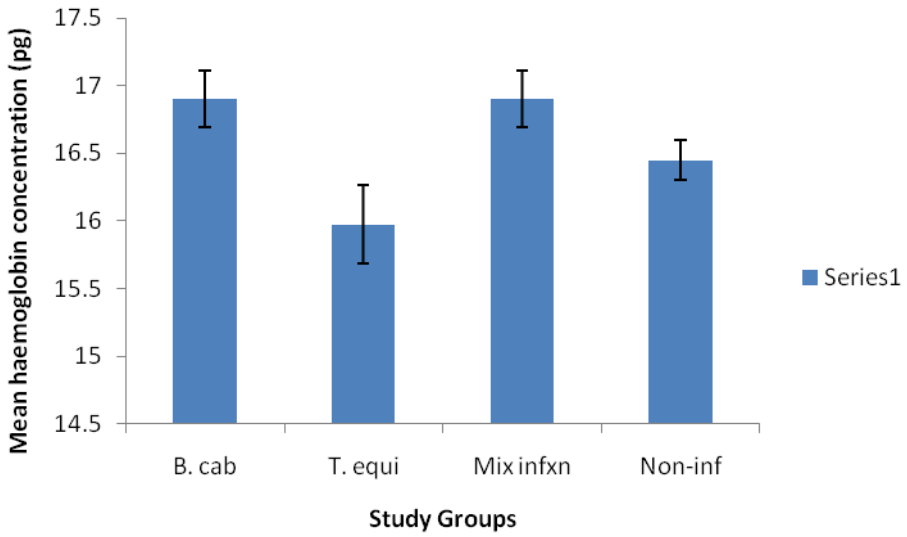


Figure 4.55: The mean of mean corpuscular haemoglobin for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

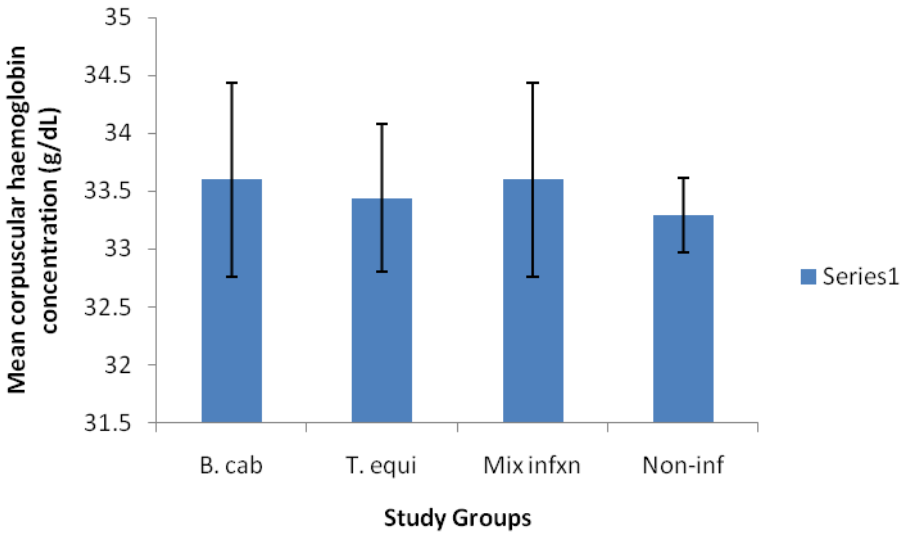


Figure 4.56: The mean of mean corpuscular haemoglobin concentration for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

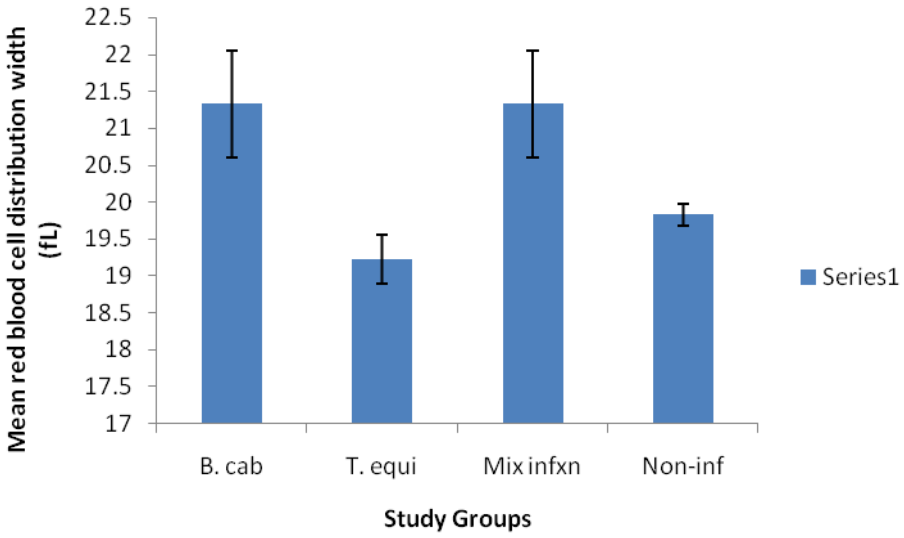


Figure 4.57: The mean red blood cells distribution width for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

4.2.2.8Mean total leukocyte counts

The mean TLC of horses infected with *B. caballi* (5.60±2.36 $\times 10^9$/L), *T. equi* (5.92±0.46 $\times 10^9$/L) and with mixed infections (5.60 ± 2.36 $\times 10^9$/L) were insignificantly lower (P > 0.05) than the non-infected horses (6.01±1.29 $\times 10^9$/L)(Fig.4.58).

4.2.2.9Mean absolute neutrophil counts

The mean absolute Neut of horses infected with *B. caballi* (2.37±1.48 $\times 10^9$/L), *T. equi* (2.33±0.21 $\times 10^9$/L) and with mixed infections (2.37 ± 1.48 $\times 10^9$/L) were not significantly different (P > 0.05) than in non-infected horses (2.24±0.51 $\times 10^9$/L) (Fig.4.59).

4.2.2.10Mean absolute lymphocyte counts

Horses infected with *B. caballi*, *T. equi* and with mixed infections had their respective mean absolute LYMP values(2.13±1.00$\times 10^9$/L), (2.15±0.17$\times 10^9$/L), (2.13±1.00$\times 10^9$/L) insignificantly different (P > 0.05) compared to the non-infected (2.41±0.52$\times 10^9$/L) (Fig.4.60).

4.2.2.11Mean absolute mid-cell counts

There were insignificant variations (P > 0.05) in the mean absolute mid-cell counts in horses infected by *B. caballi* (1.13±0.52$\times 10^9$/L), *T. equi* (1.43±0.13$\times 10^9$/L), with mixed infections (1.13±0.52$\times 10^9$/L) than in non-infected horses (1.38±0.33$\times 10^9$/L) (Fig.4.51).

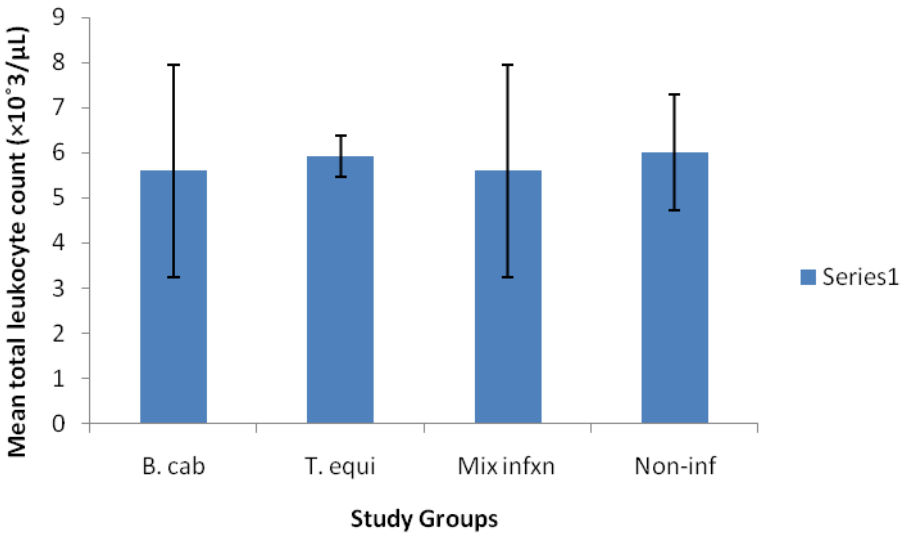


Figure 4.58: The mean total leukocyte count for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = non-infected

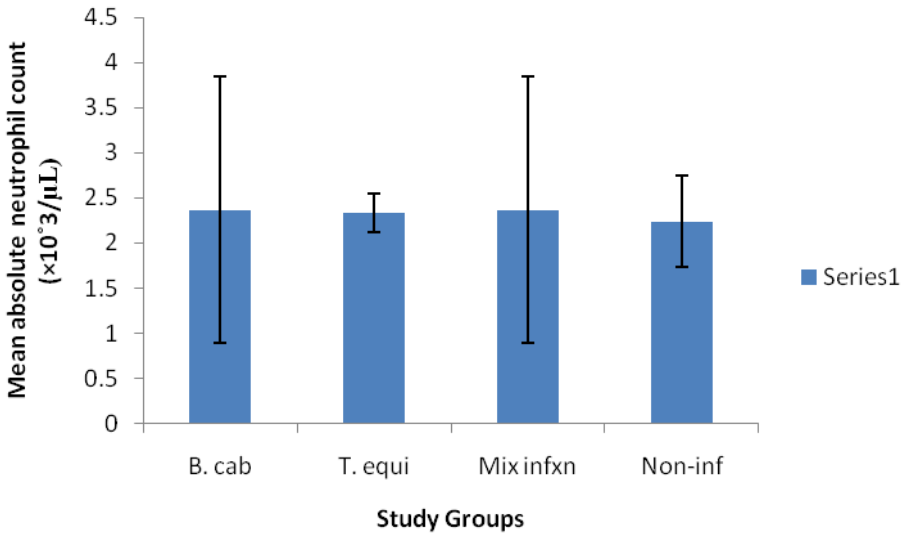


Figure 4.59: The mean absolute neutrophil counts for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

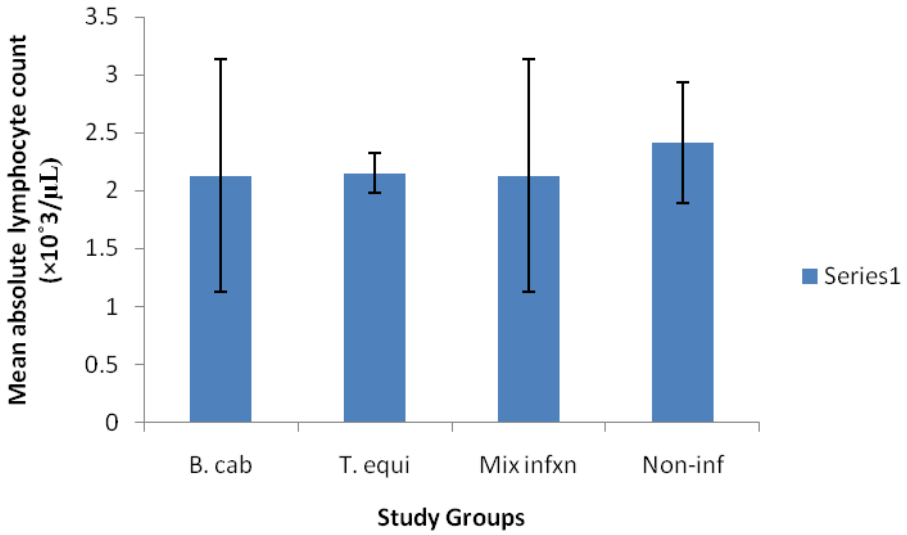


Figure 4.60: The mean lymphocyte counts for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

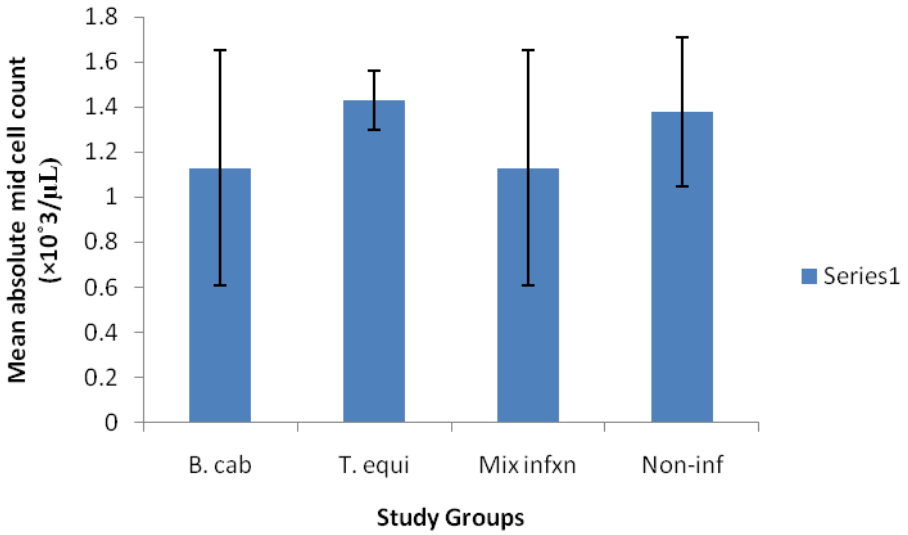


Figure 4.61 The mean absolute mid cell counts for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

4.2.2.12Mean platelets count

Themean PLT counts were insignificantly lower (P > 0.05) inhorses infected by *B. caballi* (65.33±25.22 $\times 10^9$/L), *T. equi* (103.3±6.14 $\times 10^9$/L), and with mixed infections (65.33±25.22 $\times 10^9$/L) than the non-infected control horses (116.1±16.36 $\times 10^9$/L) (Fig.4.62).

4.2.2.13Mean plateletcrit

The mean PCT of the horses infected by *B. caballi* (0.05±0.02 %) and those with mixed infections (0.05±0.02 %) were lower than those infected by *T. equi* (0.07±0.00 %) but was not significantly higher (P > 0.05) compared to the non-infected control horses (0.08±0.01 %). The mean PCT for the respective study groups (Fig.4.63).

4.2.2.17Mean platelets volume

The mean MPV were variable but not significantly different (P < 0.05) in horses infected with *B. caballi* (8.67±0.94 fL), *T. equi* (7.99±0.15) and those with mixed infections (8.67±0.94 fL) compared to the non-infected horses (7.53±0.45 fL) (Fig. 4.64).

4.2.2.18Mean percent platelets distribution width

The mean percent PDW were insignificantly different (P > 0.05) in the *B. caballi*-infected horses (14.43±0.18 %), those with *T. equi* (14.37±0.03 %) and mixed infections (14.43±0.18 %) when compared to the non-infected horses (14.25±0.10 %) (Fig.4.65).

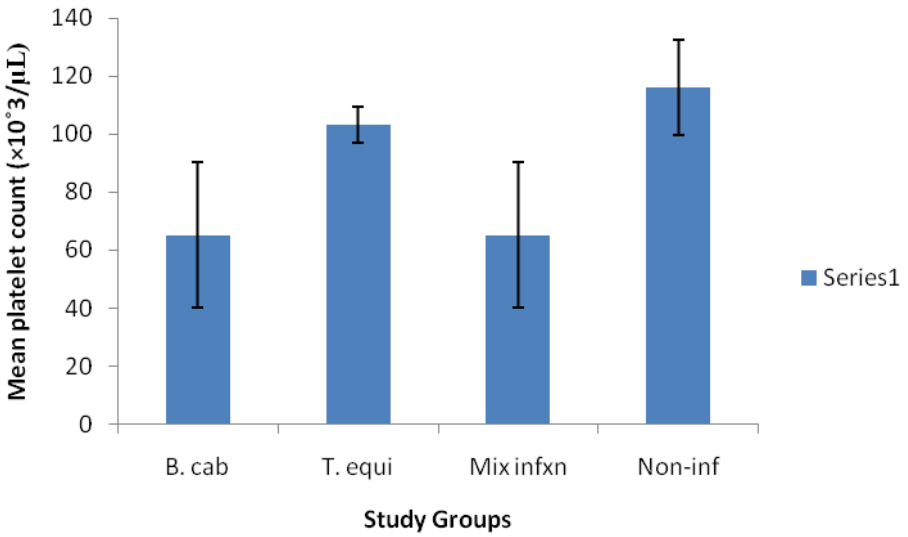


Figure 4.62: The mean platelet counts for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

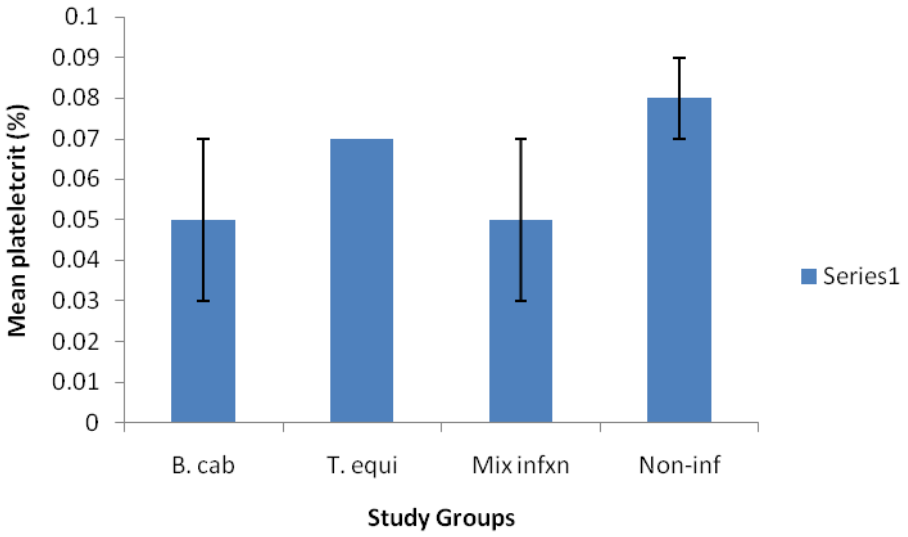


Figure 4.63: The mean plateletcrit for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

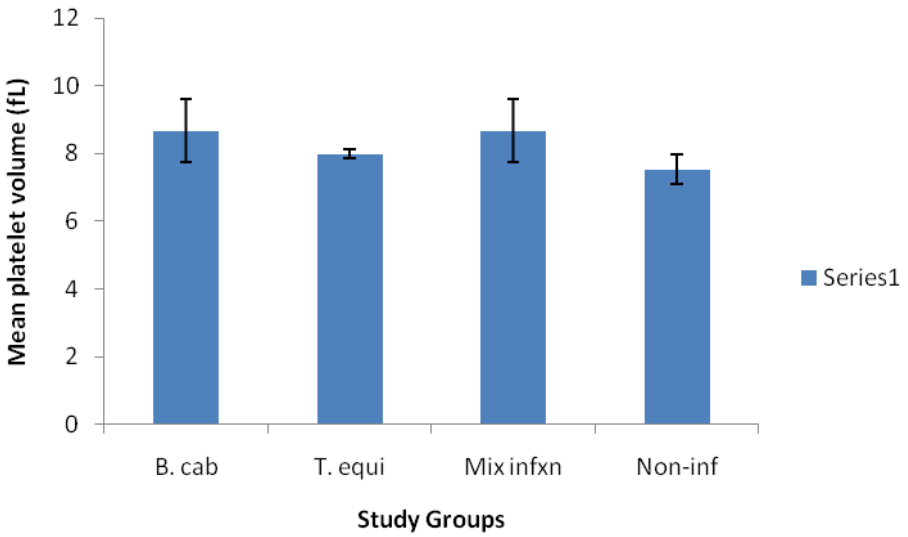


Figure 4.63: The mean platelet volume for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

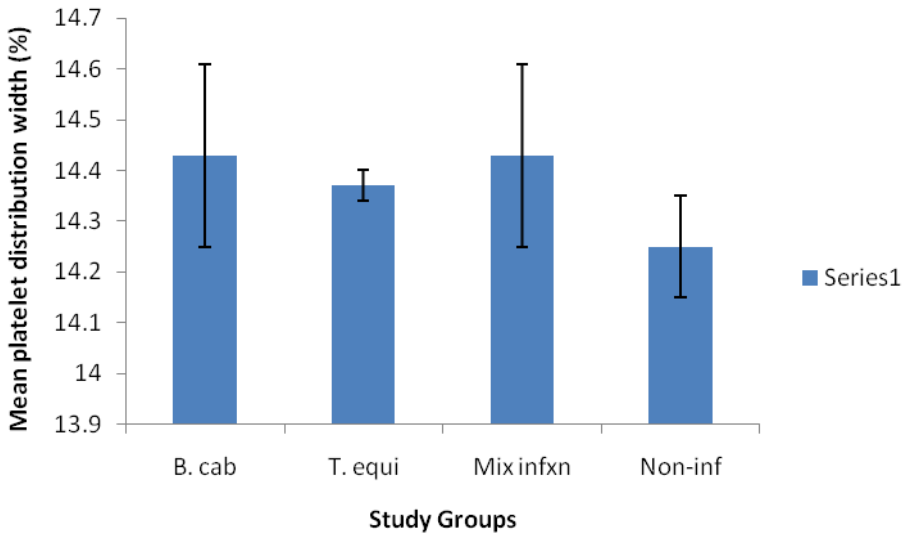


Figure 4.64: The mean platelet distribution width for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Non-inf = uninfected

4.2.3 Erythrocyte membrane biochemical analyses in asymptomatic horses

4.2.3.1Mean erythrocyte membrane total protein

The mean erythrocyte membrane total protein concentration for the *B. caballi*-infected horses (0.55±0.04 g/dL) and horses with mixed infection (0.55±0.04 g/dL) were insignificantly lower (P > 0.05) than those with *T. equi* (0.68±0.03 g/dL) and the non-infected horses (0.79±0.14 g/dL) (Fig.4.65).

4.2.3.2Mean erythrocyte membrane total sialic acid

The mean erythrocyte membrane total sialic acid for the *B. caballi*-infected horses (98.50±2.68µg/mL) and horses with mixed infection (98.50±2.68µg/mL) were insignificantly higher (P > 0.05) than those infected with *T. equi* (94.53±1.28µg/mL) and the uninfected horses (95.86±4.01µg/mL) (Fig.4.66).

4.2.3.3Mean erythrocyte membrane bound sialic acid

The mean erythrocyte membrane bound sialic acid for *B. caballi*-infected horses (35.90±4.31µg/mL), horses infected by *T. equi* (37.32±2.16µg/mL) and those with mixed infection (35.90±4.31µg/mL) had means that were statistically not significant (P > 0.05) (Fig.4.67).

4.2.3.4Mean erythrocyte membrane cholesterol concentration

The *B. caballi*-infected horses (48.33±22.18 mg/dL) have and horses with mixed infection (48.33±22.18 mg/dL) had mean erythrocyte membrane cholesterol values that were insignificantly varied (P > 0.05) than horses infected by *T. equi* (40.73±2.33 and among non-infected horses (27.65 ± 7.53 mg/dL) (Fig.4.68).

4.2.3.5Mean erythrocyte membrane triglyceride concentration

The *B. caballi*-infected horses (18.57±5.60 mg/dL) have and horses with mixed infection (18.57±5.60 mg/dL) had mean erythrocyte membrane triglyceride values that were higher than horses infected by *T. equi* (21.33±1.32 mg/dL) but not significantly different ($P > 0.05$) when compared to the non-infected horses (23.96 ± 2.67 mg/dL) (Fig4.69).

4.2.3.6Mean erythrocyte membrane high density lipoprotein concentration

The *B. caballi*-infected horses (6.62±2.34 mg/dL) and horses with mixed infection (6.62±2.34 mg/dL) had mean erythrocyte membrane high density lipoprotein values that were higher than horses infected by *T. equi* (4.16±0.53 mg/dL) but not significantly changed ($P > 0.05$) when compared to the control group (4.13±0.88 mg/dL) (Fig.4.70).

4.2.3.7Mean erythrocyte membrane low density lipoprotein concentration

The *B. caballi*-infected horses (40.03±22.00 mg/dL) and horses with mixed infection (40.03±22.00 mg/dL) had mean erythrocyte membrane low density lipoprotein values that were higher than horses infected by *T. equi* (33.57±2.24 mg/dL) but not significantly different ($P > 0.05$) compared to the non-infected horses (29.88 ± 8.44 mg/dL)(Fig.4.71).

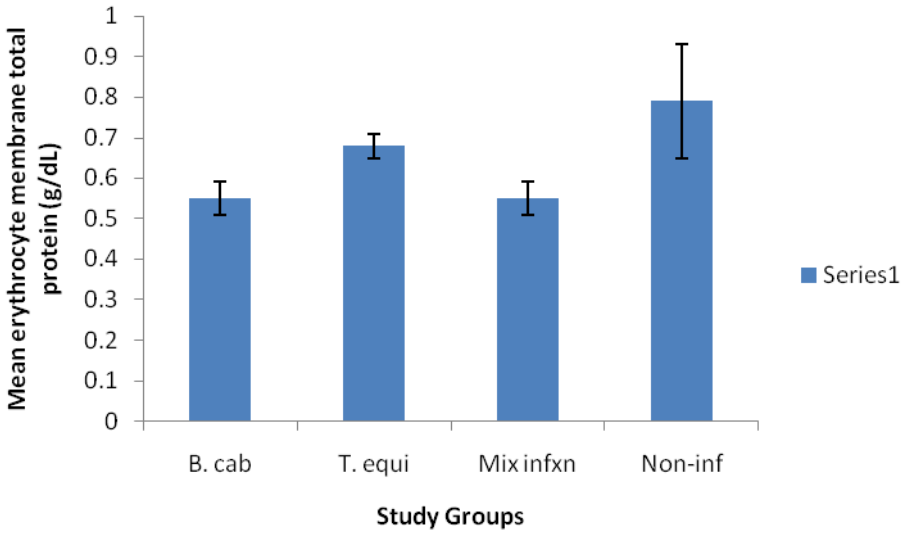


Figure 4.65: The mean erythrocyte membrane total protein concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

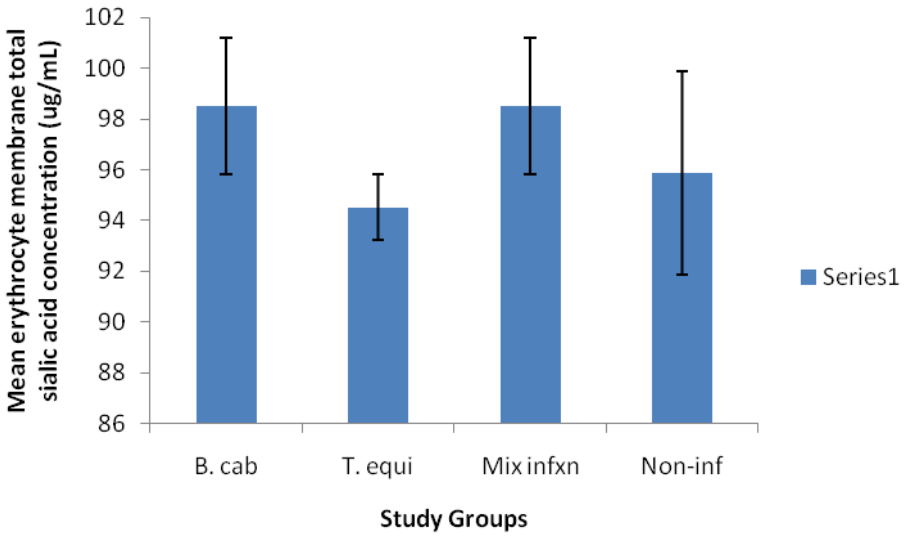


Figure 4.66: The mean erythrocyte membrane total sialic acid concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

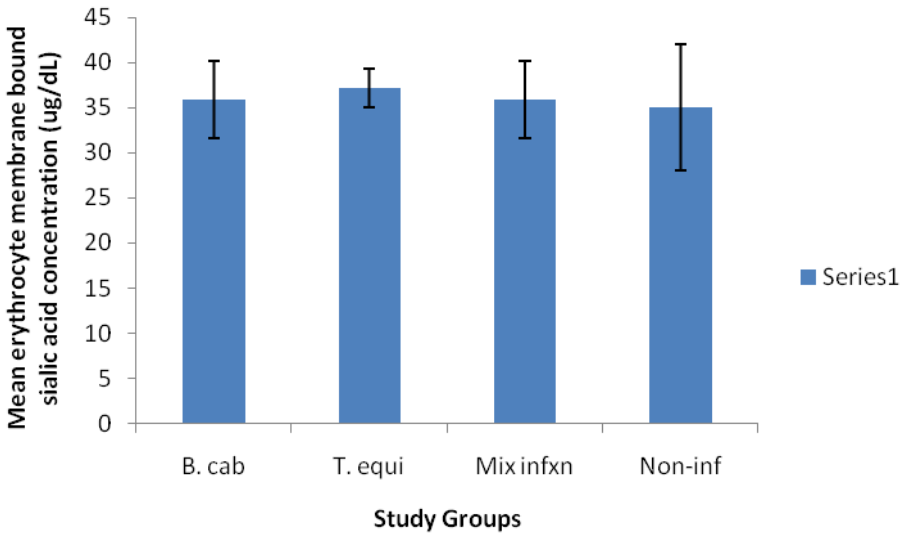


Figure 4.67: The mean erythrocyte membrane bound sialic acid concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

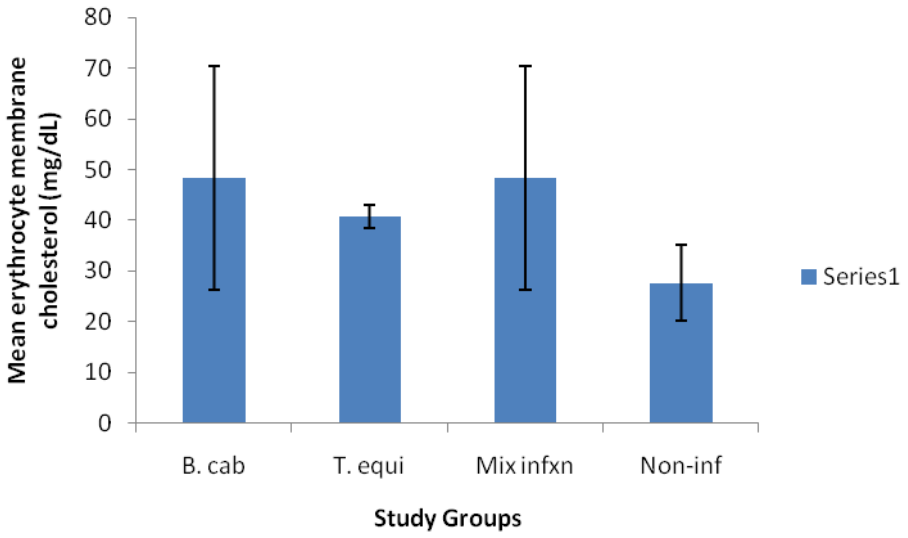


Figure 4.68: The mean erythrocyte membrane cholesterol concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

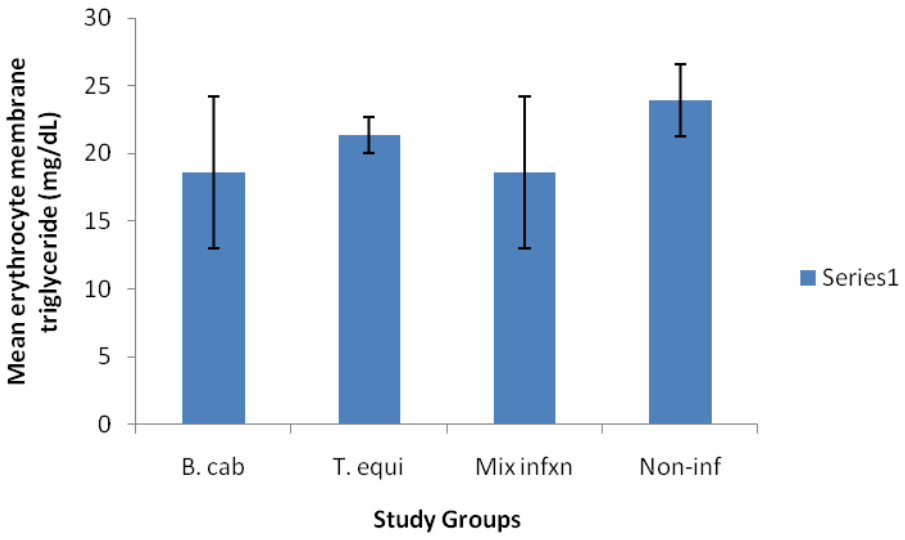


Figure 4.69: The mean erythrocyte membrane triglyceride concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

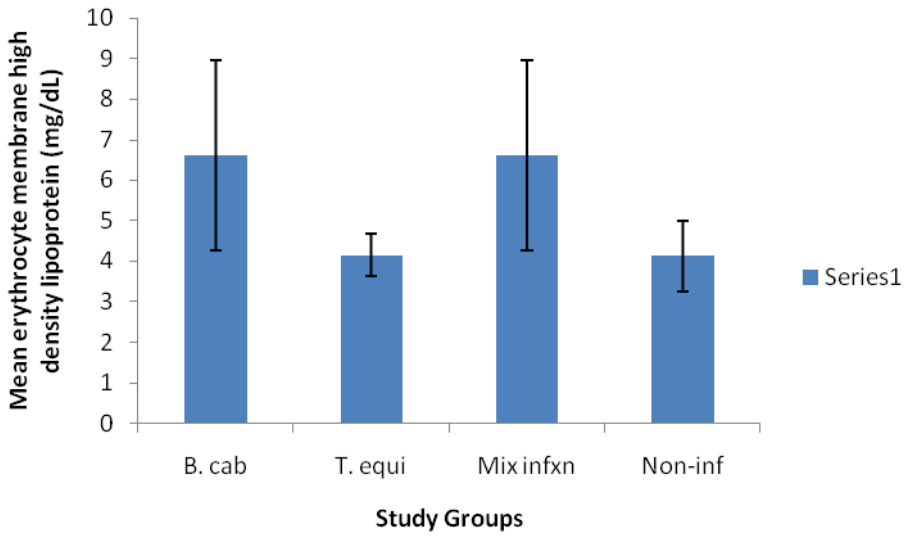


Figure 4.70: The mean erythrocyte membrane high density lipoprotein concentration for the infected and non-infected horses

Keys: HDL = High density lipoprotein, *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection, Non-inf = non-infected

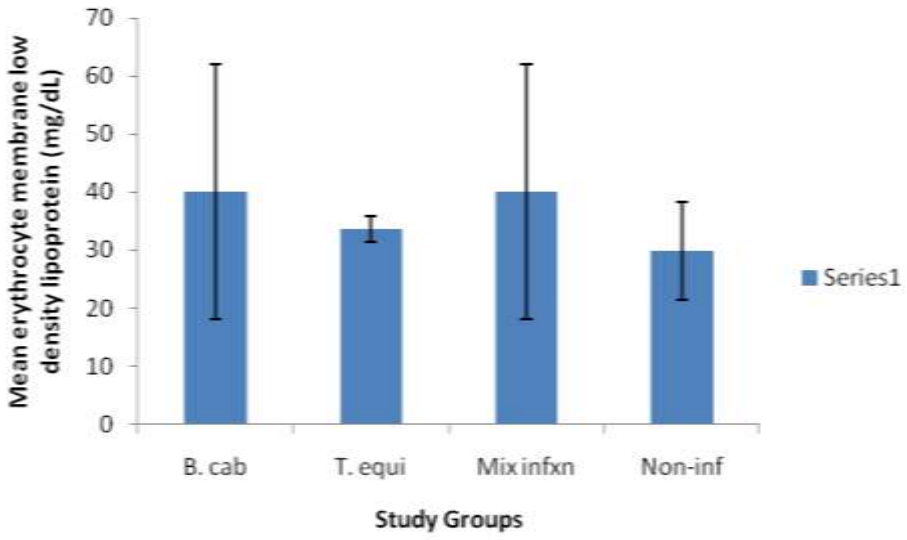


Figure 4.71: The mean erythrocyte membrane low density lipoprotein concentration for the infected and non-infected horses

Keys: LDL = Low density lipoprotein, *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mixinfxn = Mixed infection, Non-inf = non-infected

4.2.4 Mean serum biochemical analyses in asymptomatic horses

4.2.4.1Mean serum free sialic acid concentration

The mean value for serum free sialic acid concentration in *T. equi-infected horses* (57.19±1.65 µg/mL) was insignificantly lower (P > 0.05) than those in*B. caballi*-infected horses (62.57±5.20µg/mL), horses with mixed infection (62.57±5.20µg/mL) and the non-infected (60.79±3.95µg/mL) (Fig. 4.72).

4.2.4.2Mean serum blood urea nitrogen concentration

The mean serum blood urea nitrogen concentration in the *B. caballi*-infected (5.87±1.80 mg/dL) horses and those with mixed infections (5.87±1.80 mg/dL) were significantly higher (P < 0.05) compared to the *T. equi*-infected (1.98 ± 0.15 mg/dL) and non-infected (2.01±0.42 mg/dL) horses. The mean value of BUN for the *T. equi*-infected horses (1.98±0.15 mg/dL) is lower compared to the non-infected horses (2.01±0.42 mg/dL) (Fig. 4.73).

4.2.4.3Mean serum creatinine concentration

The mean serum creatinine concentration in the *B. caballi*-infected (153.0±38.10 mg/dL) horses and those with mixed infections (153.0±38.10 mg/dL) were significantly higher (P < 0.05) compared to the*T. equi*-infected (83.13±3.45 mg/dL) and non-infected (67.65±9.31 mg/dL) horses (Fig. 4.74).

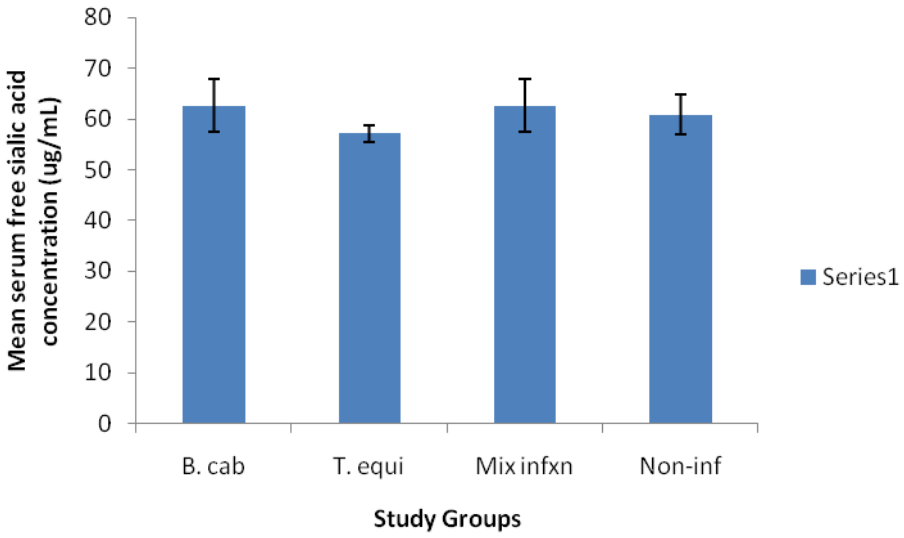


Figure 4.72: The mean free sialic acid concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

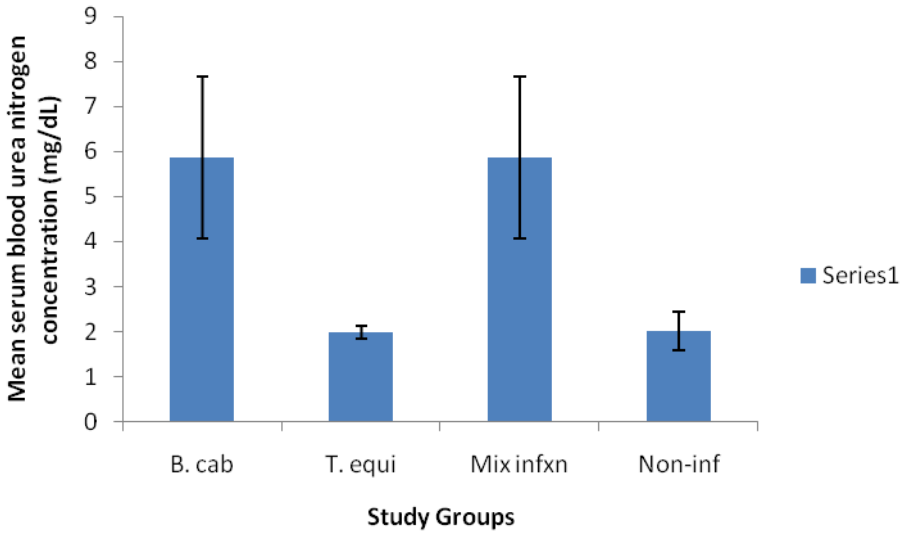


Figure 4.73: The mean serum blood urea nitrogen concentration for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Mix inf = mixed infection

Non-inf = non-infected

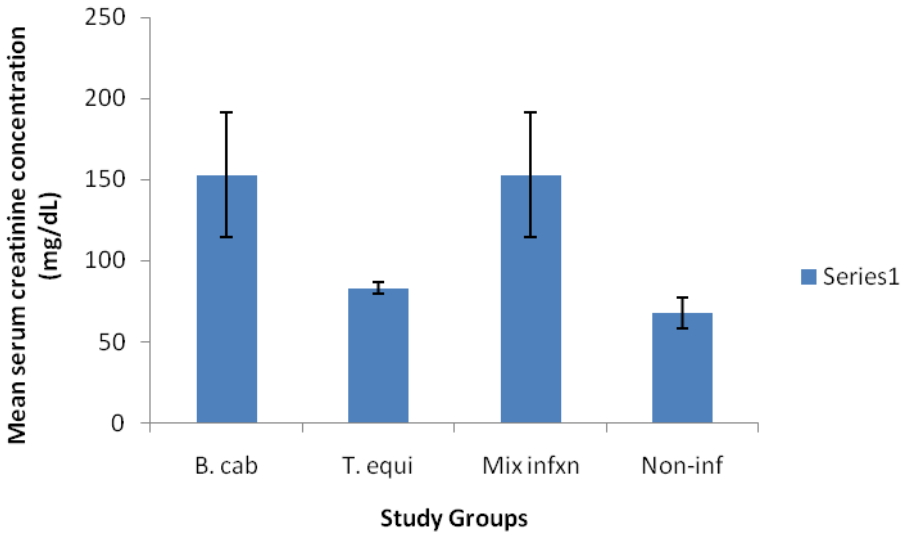


Figure 4.74: The mean serum creatinine concentration for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Mixed infxn = mixed infectionNon-inf = non-infected

4.2.4.4Mean serum alanine aminotransferase activity

Horses infected with *B. caballi* (18.30±1.45 U/L) and with mixed infections (18.30±1.45 U/L) had insignificantly lower (P > 0.05) mean ALT activities than those infected with *T. equi* (25.11±1.30 U/L) and non-infected control (31.09±4.70 U/L) (Fig.4.75).

4.2.4.5Mean serum aspartate transferase activity

Horses infected with *B. caballi* (22.87±4.22 U/L) and those with mixed infections (22.87±4.22 U/L) had lower mean ALT activities but the values were not significantly different (P > 0.05) than those infected with *T. equi* (27.97±1.54 U/L) non-infected horses (29.74±5.62 U/L) (Fig.4.76).

4.2.4.6Mean serum alkaline phosphatase activity

The mean serum ALP activity of the *T. equi* (640.1±19.97 U/L) was the insignificantly higher (P > 0.05) than those infected with *B. caballi* (529.7±60.44 U/L), with mixed infections (529.7±60.44 U/L) and among the non-infected horses (620.7 ± 78.24 U/L) (Fig.4.77).

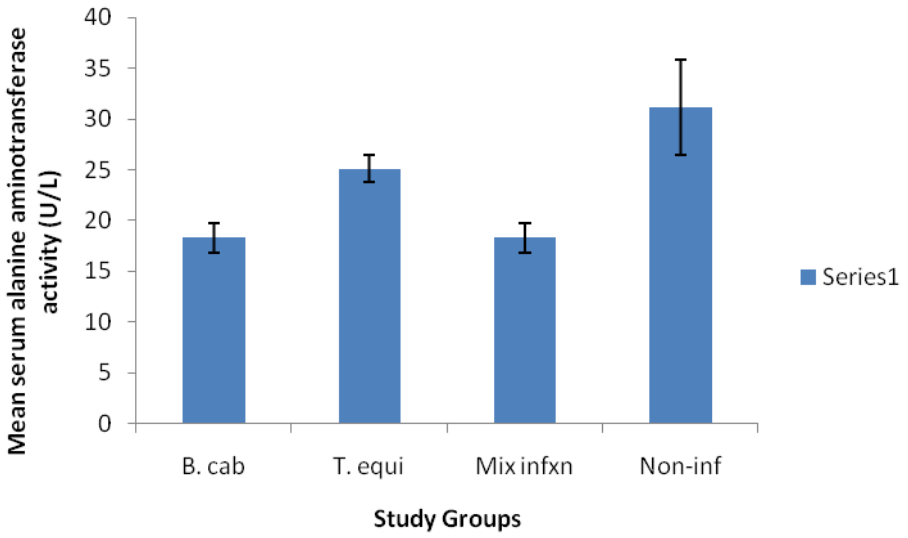


Figure 4.75: The mean serum Alanine aminotransferase activities for the infected and non-infected horses

Keys: ALT = Alanine aminotransferase, *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mixinfxn = Mixed infection, Non-inf = non-infected

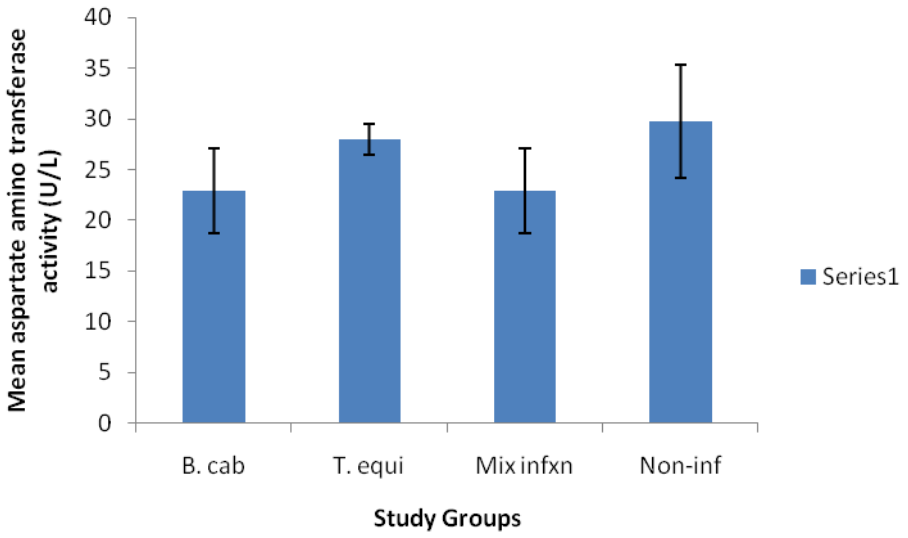


Figure 4.76: The mean serum Aspartate aminotransferase activities for the infected and non-infected horses

Keys: AST = Aspartate aminotransferase, *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection, Non-inf = non-infection

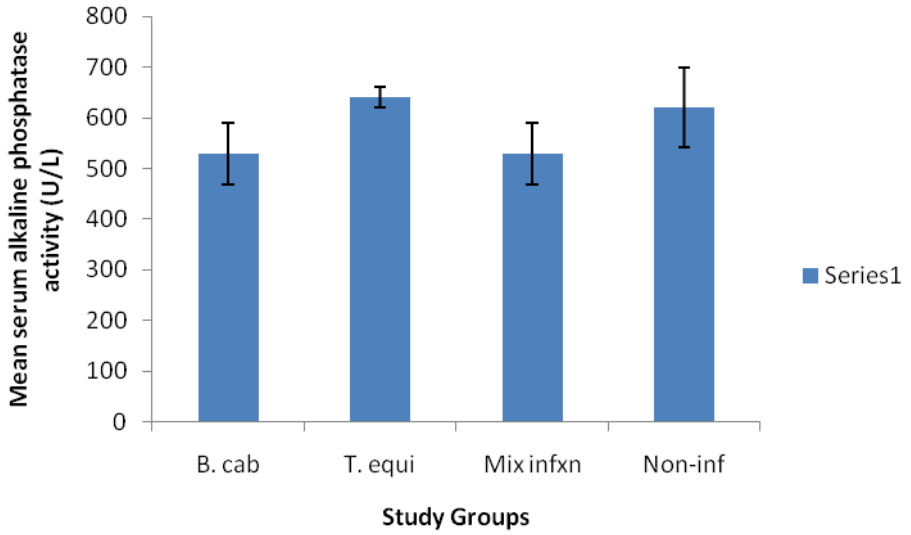


Figure 4.77: The mean serum alkaline phosphatase activities for the infected and non-infected horses

Keys: ALP = Alkaline phosphatase, *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection, Non-inf = non-infection

4.2.4.7Mean serum total protein concentration

The mean serum TP concentration for*T. equi*-infected horses (7.83±0.13 g/dL) was insignificantly higher (P > 0.05)than those with*B. caballi* (8.70±0.42 g/dL), with mixed infections (8.70±0.42 g/dL) and non-infected (7.90±0.28 g/dL) (Fig.4.78).

4.2.4.8Mean serum albumin concentration

The mean serum AL concentration for *T. equi*-infected horses (4.05±0.09 g/dL), *B. caballi* (4.57±0.52 g/dL) and those with mixed infections (4.57±0.52 g/dL) wereinsignificantly higher (P > 0.05) than in non-infected horses (3.83±0.22 g/dL) (Fig.4.79).

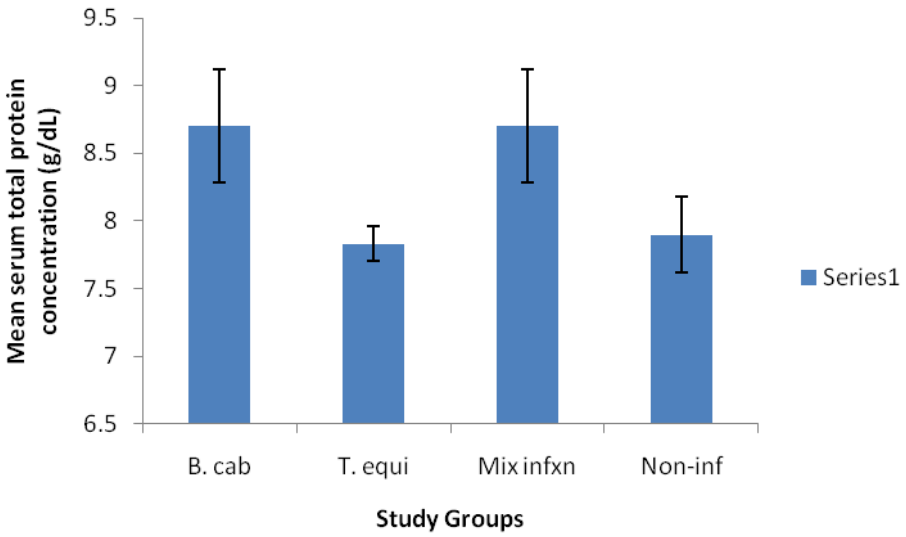


Figure4.78: The mean serum total protein concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi, T. equi = Theileria equi,* Mix infxn = Mixed infection,Non-inf = non-infected

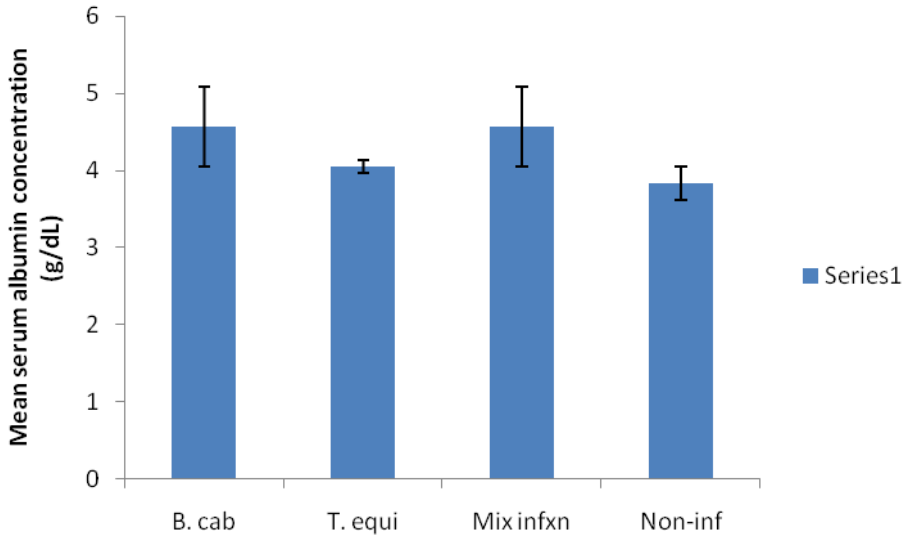


Figure 4.79: The mean serum albumin concentration for the infected and non-infected horses

Keys: *B. cab* =*Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

4.2.4.9Mean serum total bilirubin concentration

The mean serum TBiL concentration for *T. equi*-infected horses (11.71±0.99mmol/L) is the highest compared to the*B. caballi*-infected horses (3.57±1.68mmol/L) and those with mixed infections (3.57±1.68mmol/L). They were not statistically significant (P > 0.05) when compared with the non-infected horses (6.65±1.37mmol/L) (Fig. 4.80).

4.2.4.10Mean serum direct bilirubin concentration

The mean serum DBiL concentrations in*T. equi*-infected horses (5.20±0.44mmol/L), *B. caballi*-infected (2.67±1.18mmol/L) and horses with mixed infections (2.67±1.18mmol/L) were not significantly varied (P > 0.05) than those in uninfected horses (2.50±0.42mmol/L) (Fig. 4.81).

4.2.4.11Mean serum indirect bilirubin concentration

The mean serum IBiL concentrations in*T. equi*-infected horses (6.57±0.66mmol/L), *B. caballi*-infected horses (0.90±0.50mmol/L) and those with mixed infections (0.90±0.50mmol/L) were not statistically significant (P > 0.05) compared to the non-infected horses (4.15±1.07mmol/L) (Fig. 4.82).

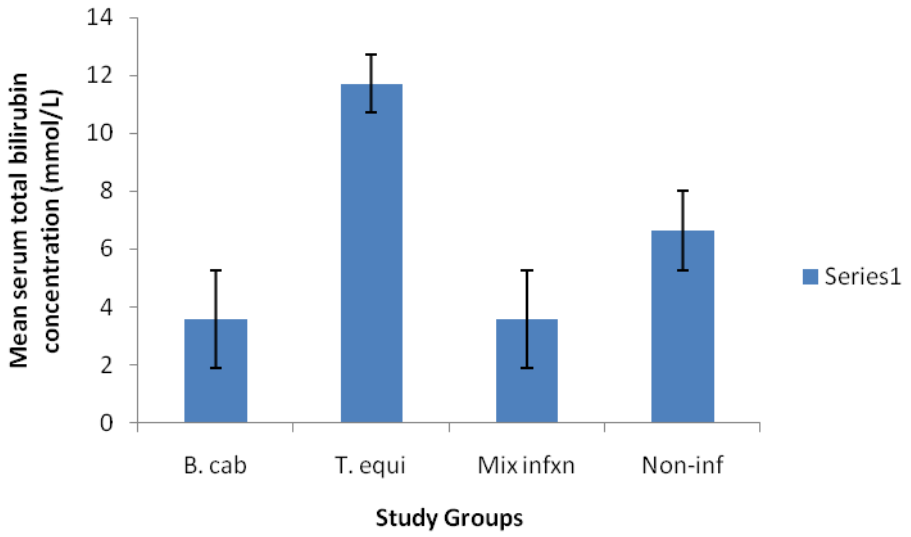


Figure 4.80: The mean serum total bilirubin concentrations for the infected and non-infected horses

Keys: Conc. = Concentration, *B. cab =Babesia caballi, T. equi = Theileria equi,*

Mix infxn =Mixed infection, Non-inf = non-infected

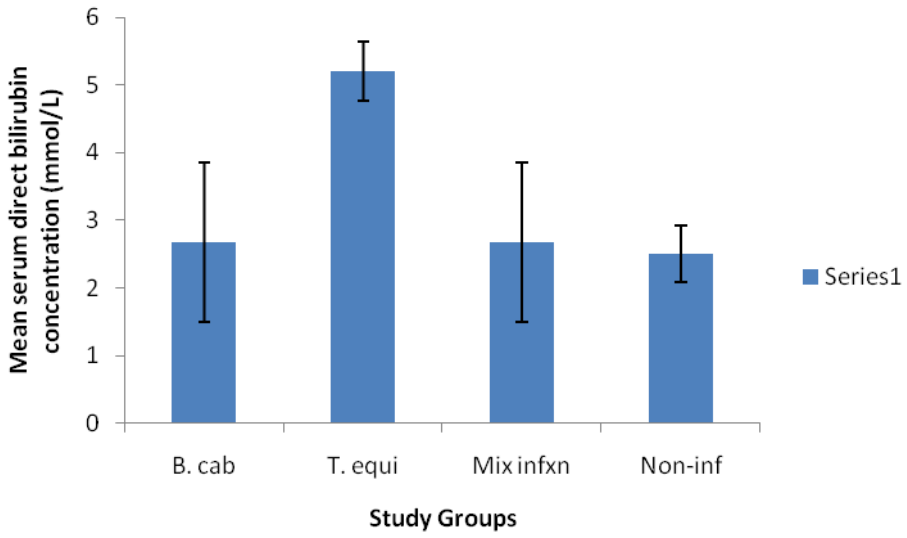


Figure 4.81: The mean serum direct bilirubin concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

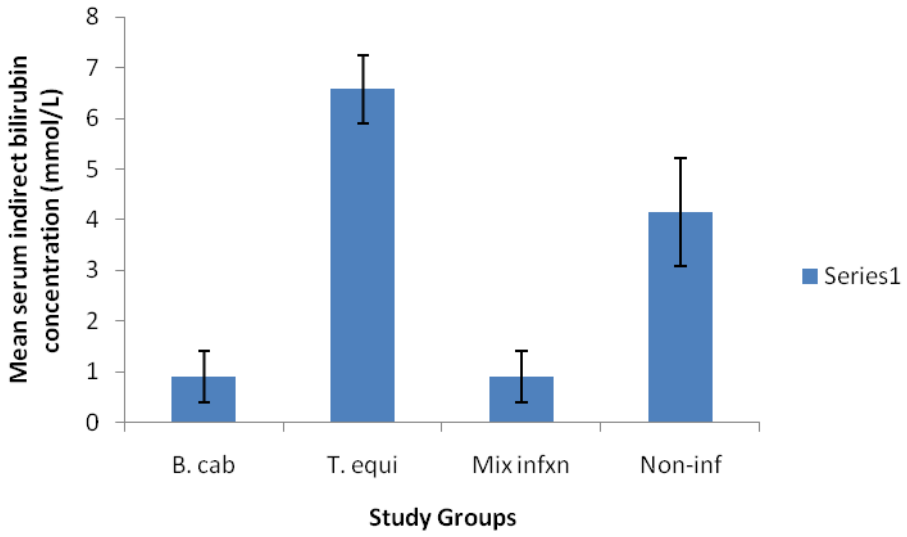


Figure4.82: The mean serum indirect bilirubin concentration for the infected and non-infected horses

Keys: *B. cab =Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = Mixed infection,Non-inf = non-infected

4.2.5 Serum antioxidant in asymptomatic horses

4.2.5.1Mean serum glutathione concentration

The mean serum GSH concentration for *T. equi*-infected horses (13.41±0.36µmol/mL) is the highest while those for *B. caballi*-infected horses (10.55±1.66 µmol/mL) and horses with mixed infections (10.55±1.66µmol/mL) were lowest compared to the uninfected horses (12.44±1.39 µmol/mL). These variable means were not statistically significant (P > 0.05) (Fig. 4.83).

4.2.5.2Mean serum catalase activity

The mean serum CAT activity for *T. equi*-infected horses (24.74±2.21 u/mL) is the least when compared to those for *B. caballi*-infected horses (30.21±19.19 u/mL) and horses with mixed infections (30.21±19.19 u/mL). They were not statistically different (P > 0.05) when compared to the control horses (33.79±7.42 u/mL)(Fig. 4.84).

4.2.5.3Mean serum superoxide dismutase activity

The mean serum SOD activity for the *B. caballi*-infected horses (19.42±0.62 u/mL) and horses with mixed infections (19.42±0.62 u/mL) were higher than that for *T. equi*-infected horses (14.81±0.59 u/mL) but were not statistically different (P > 0.05) when compared to the control horses (13.56±1.04 u/mL) (Fig. 4.85).

4.2.5.4Mean serum malondialdehyde concentration

The mean serum MDA concentration for the *B. caballi*-infected horses (1.60±0.18µmol/mL) and horses with mixed infections (1.60±0.18µmol/mL) were the least followed by that of the control horses (3.38±1.01µmol/mL). The *T. equi*-infected horses (3.65±0.30µmol/mL) has the highest MDA concentration but these mean values

were not statistically different (P > 0.05) when compared between the groups (Fig. 4.86).

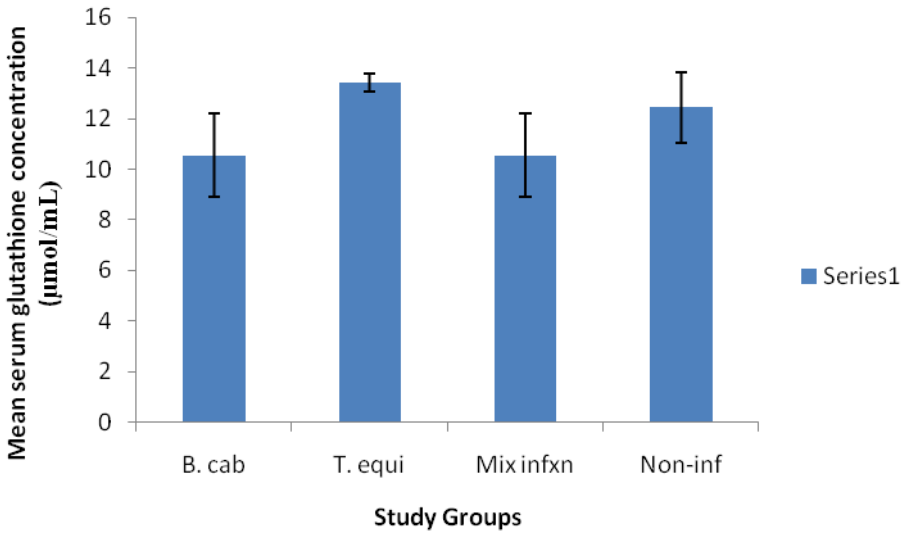


Figure 4.83: The mean serum glutathione concentration for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = mixed infection, Non-inf = non-infected

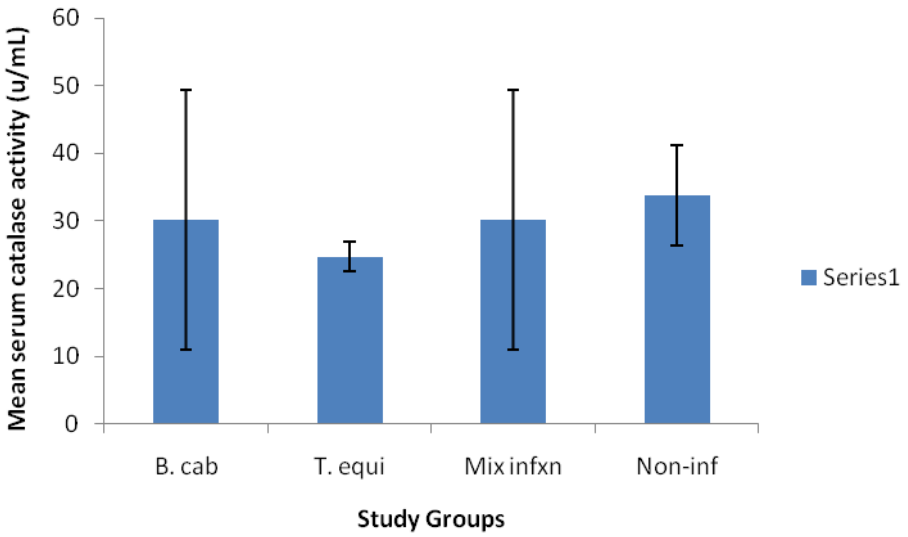


Figure 4.84: The mean serum catalase activities for the infected and non-infected horses

Keys: *B. cab* = *Babesia caballi*, *T. equi* = *Theileria equi*, Mix infxn = mixed infection,Non-inf = non-infected

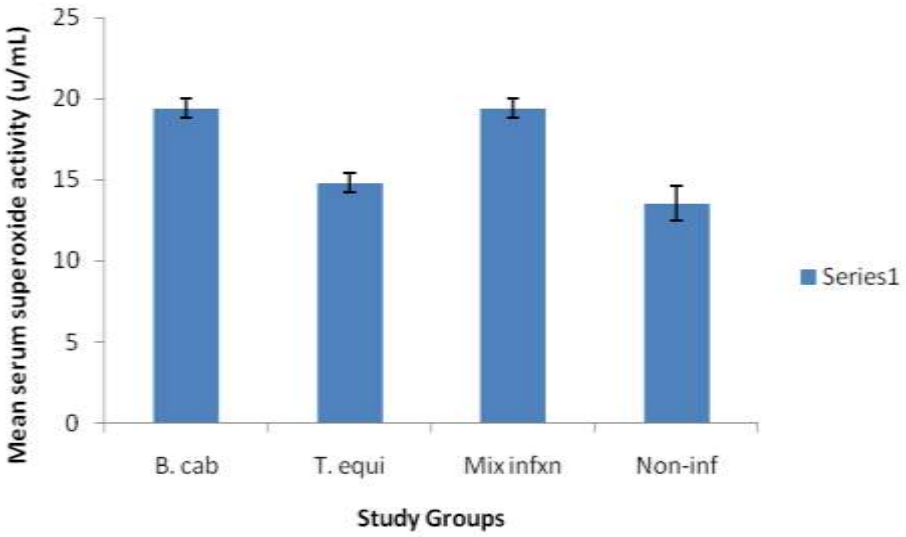


Figure 4.85 The mean serum superoxide dismutase activities for the infected and non-infected horses

Keys: *B. cab = Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = mixed infection, Non-inf= non-infected

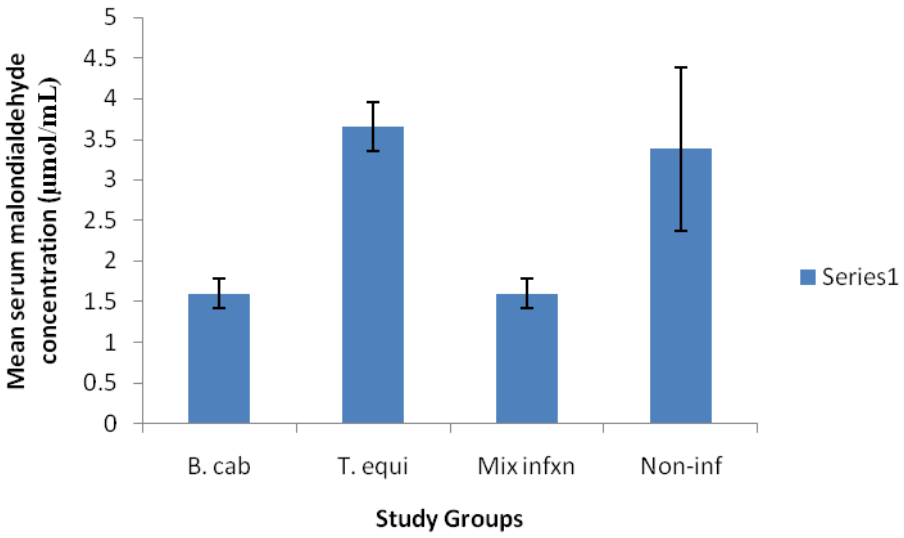


Figure 4.86: The mean serum malondialdehyde concentrations for the infected and non-infected horses

Keys: *B. cab = Babesia caballi*, *T. equi = Theileria equi*, Mix infxn = mixed infection, Non-inf= non-infected

CHAPTER FIVE

5.0 DISCUSSION

The symptomatic horses investigated had pyrexia, anorexia, and pale, congested to icteric mucous membranes, epiphora (unilateral and bilateral), and oedema of the fetlock, poor body condition, and tick infestation as the predominant clinical signs. Although, most of the horses sampled presented variable clinical signs that were suggestive of piroplasmosis, parasitaemia was below the detection limit by microscopic examination of Giemsa-stained thin blood films. This result is in concordance with that reported in naïve horse infected with 2 mL of 12% parasitized erythrocytes (PPE) of *T. haneyi* stabilates derived from an infected splenectomized horse (Knowles *et al*., 2018). Similarly, Motloang *et al*. (2008) did not detect *B. caballi* and *T. equi* in the entire blood smear from suspected horses but recorded high seroprevalence of the parasites using IFAT. Also, Moloi *et al*. (2010) did not detect any parasite from 543 horses screened for EP using thin blood smears. The mere absence of positive result by microscopic examination does not preclude infection (Ali *et al*., 1996) but suggests difficulty in detecting *B. caballi* and *T. equi* in the latent phase of the infection or onset of acute disease and/or in carrier animals with low parasitaemia (Nagore *et al.,* 2004; Qablan *et al*., 2013). The level of parasitaemia can also be very low in the acute disease (Donnellan and Marais, 2009). This lends credence to use of molecular diagnosis using PCR to overcome the constraint of poor sensitivity associated with microscopic diagnosis. The asymptomatic horses were apparently healthy without clinical signs of a disease process.

The predominant tick species that infested the horses in this study were *Rhipicephalus evertsi evertsi,* followed by *Rhipicephalus spp, Amblyomma variegatum, Hyalomma*

dromedarii, Boophilus decoloratus, Hyalomma species, Hyalomma impeltatum and *Hyalomma truncatum.* Although, transmission study was not conducted in the current research to confirm tick-vector competence to transmit the detected parasites, it is reasonable to speculate that the identified ticks are epidemiologically responsible for the transmission of the detected pathogens. Recently, Onyiche *et al*. (2020) detected *B. caballi* in *Rhipicephalus evertsi evertsi*only while *T. equi* was detected in *Rhipicephalus evertsi evertsi,Hyalomma dromedarii*and *Hyalomma truncatum* in Jigawa state, Nigeria. This study further confirms the persistence of competent tick vectors of equine piroplasmosis in Nigeria.

The current study documented evidence of anti-*T. equi* antibodies in both symptomatic and asymptomatic horses to the epitope defined by the mAb36/133.97 (Knowles *et al*., 1991a) in pathogens other than *T. equi* detected in horses in Nigeria. This finding is contrary to the reported inability of the *T. equi* specific cELISA to detect *T. haneyi* infection (Knowles *et al*., 2018). Eleven symptomatic horses with *T. haneyi* infection by both 18S and species specific nPCR were seropositive with cut-off above 40% inhibition. The three symptomatic horses infected by *T. equi*-like pathogens were seropositive to *T. equi* antibodies by cELISA. Since *ema-1* is the immunodominant antigenic target for the detection of *T. equi* and together with *ema-2* share an epitope defined by mAb36/133.97 (Knowles *et al*., 1991a), the likely reason to the presence of anti- *T. equi* antibodies in the *T. haneyi* Nigerian isolates is that the coding sequence/region (CDS) of the gene from which the *T. haneyi* specific primers were derived and/or the conserved region of their 18S rRNA gene, have a homolog in *T. equi*. This is because the CDS of the gene of *T. haneyi* is situated in the same genomic location occupied by *ema-1* within the *T. equi* genome (Knowles *et al*., 2018). The possibility of antigenic cross-reactivity among the EMAs cannot be overruled, since

both *T. equi* and *T. haneyi* contain nine *ema* copies, with six being orthologous between the two species (Knowles *et al*., 2018). Similarly, we hypothesize that *ema-1* gene may be present in the *T. equi*-like parasites that naturally infect horses in Nigeria.

Although, both PCR and c-ELISA are highly sensitive to *B. caballi* and *T. equi*, the specificity varies and tends to be much higher and accurate with molecular than serological diagnosis (Ibrahim *et al*., 2011). The prevalence of *T. equi* in the present study was higher using c-ELISA than PCR and zero for microscopy. This finding corroborate that of Ibrahim *et al*. (2011) where prevalence of *T. equi* infection using c-ELISA was higher followed by PCR and then microscopy. Also, Seroprevalence of *T. equi* (76.4%) was higher than *B. caballi* (0%) for symptomatic horses; likewise, the asymptomatic horses had higher seroprevalence of 90% for *T. equi* and 3.8% for *B. caballi*. These findings agree with previous reports (Sellon and Long 2007; Posada, 2012; Jimenez *et al*., 2014; Kamyingkird *et al*., 2014; Turaki *et al*., 2014). The likely reason for this could be that horses infected with *T. equi* remain lifelong carriers whereas, those infected with *B. caballi* are only seropositive for several years, and infections are usually transient (Sellon and Long, 2007; Onyiche *et al*., 2019; Tirosh-Levy *et al.,* 2020). Additionally, the likelihood of *T. equi* resistance to most of the conventional antiprotozoal agents that are probably in use in the study area may not be overruled. Reports of *T. equi* persistence due to resistance to babesicidal drugs have been documented (Rothschild and Knowles, 2007; Sigg *et al*., 2010; Wise *et al*., 2013). The current study documents persistent anti- *T. equi* antibody titers without concurrent active or ongoing infection in the symptomatic horses. Similar finding was reported in humans, and the reason adduced was continuous secretion of antibody, predominantly, IgG, by "memory" B cells without continued antigenic stimulation (Slifka and Ahmed, 1998; Bernasconi *et al*., 2002). Genetic diversity between the *rap*-1 genes of *B. caballi*

Nigerian isolate and that which was used to develop the currently available *B. caballi* c-ELISA may account for the 100% seronegative status in the symptomatic and low seroprevalence in asymptomatic horses. Recent epidemiological evidence reported discordance in the sequence conservation between the *B. caballi* isolates from Egypt (Mahmoud *et al*., 2016), Israel (Rapoport *et al*., 2014), South Africa (Bhoora *et al*., 2010) and Spain (Montes Cortes *et al*., 2017), and the epitope used as antibody recognition site in the OIE-approved competitive inhibition antibody assay (cELISA) for *B. caballirap-1*. Also, sequence heterogeneity in the *ema-1* epitope exists based on data obtained following comparative detection of *T. equi* antibodies between IFAT and cELISA (Bhoora *et al*., 2010b). Regional differences may impact accurate molecular and serological diagnosis of haemoparasitic infections, thus, the need to use antigens from autochthonous strains and/or isolates is desirable. The possibility that majority of the ticks that infested the symptomatic horses are appropriate for transmission of *Theileria species* than *B. caballi* cannot be completely ruled out.

The serendipitous detection of *T. haneyi* and other unknown *T. equi*-like parasites in horses in Nigeria highlights the role of genetic diversity in limiting detection of apicomplexan parasites of livestock. Peculiar life-long persistent infection in EP through asexual reproduction in mammalian domestic and wildlife hosts, as well as sexual reproduction in the tick vector, promotes dispersal among different mammalian hosts. This in turn provides an environment that facilitates continuous evolution of the parasites, which are exposed to a distinct set of selective pressures from hosts and vectors, thereby leading to potential changes in virulence, transmissibility, detectability and immunogenicity (Knowles *et al*., 2018). Favourable climatic factors such as temperature, humidity and rainfall favour tick population and questing behaviour, and these promote pathogen transmission from infected to naïve horses. These, in addition

to poor management and non-implementable regulatory policies compound the endemicity of EP in Nigeria.

Although, the aetiologic agents of EP cause haematological and biochemical changes, several factors such as strain(s) of the parasites, innate immunity, presence or absence of stressors, nutritional status, management practices, endemic stability, and other peculiar regional and environmental conditions may impact their clinical manifestation (Biu *et al*., 2012; Ehizibolo *et al*., 2012; Turaki *et al*., 2014). Data from this study showed that *T. equi* caused more significant haemato-biochemical alterations than *B. caballi, T. haneyi, T. equi*-like parasites, and horses with multi-clade infections in the symptomatic horses. An exception to this finding was the significantly higher mean plateletcrit in symptomatic horses with multiple-clade infections (mixed infections) compared to those with *T. equi* and *T. haneyi* infections. Similarly, the mean percent platelet distribution width was remarkably higher in *T. haneyi*-infected compared to the *T. equi*-infected symptomatic horses. Also, the erythrocyte membrane malondialdehyde concentration was higher in symptomatic horses infected with *T. equi*-like unknown clade. The remarkable increase in plateletcrit in symptomatic horses with multi-clade infections reflects increased platelet mass probably as a favourable regenerative response to ongoing platelet destruction or consumption.Although, the mean platelet count was not significantly altered in this study, thrombocytopaenia is a common sequalae in horses infected with *T. equi, B. caballi* as well as horses with co-infection of piroplasms (Zobba *et al*., 2008;Laus *et al*., 2015; Mahmoud *et al*., 2016). Likewise, the mean percent platelet distribution width in *T. haneyi*-infected horses was remarkably higher, thus, indicating marked variations in platelets size, changes with platelet activation, and an indication of heterogeneity in platelet morphology. The exact mechanism of platelet alterations in equine piroplasmosis is poorly understood and yet

to be elucidated, but several hypotheses suggest immune-mediated destruction, splenic sequestration and/or excess consumption as is observed in disseminated intravascular coagulation (Wise *et al*., 2013). Moreso, *T. haneyi* was only characterized in the year 2018 (Knowles *et al*., 2018), and so very little is known about the virulence and clinicopathological alterations associated with the parasite. Clinically, results of the platelet indices reflect platelet anisocytosis, a finding corroborated by other authors (Osselaer *et al*., 1997; Wiwanitkit, 2004; Vagdatli *et al*., 2010). Platelets play important roles in the pathophysiology of diseases (Budak *et al*., 2016), and are principally involved in haemostasis and thrombosis (Golebiewska and Poole, 2015). Their granules contain small molecules and proteins (interleukin-1β, CD40 ligands, β-thromboglobulin, growth factors etc) which influence endothelial wall and circulating immune cells (Margetic, 2012; Mariani *et al*., 2014; Frelinger *et al*., 2015).

The high erythrocyte membrane malondialdehyde concentration in symptomatic horses with *T. equi*-like unknown clade infection indicates lipid peroxidation that suggests accumulation of oxidative ions which can cause haemolysis. Since the red blood cell parameters were not significantly altered in this group of horses, It is likely that there is adequate antioxidant response or capacity to the activities of the *T. equi*-like parasites on the erythrocyte membrane of the infected horses. This is further buttressed by the in-significant alterations in the antioxidant enzymes activities (catalase and superoxide dismutase) and glutathioneconcentration that were investigated in the study. Ambawat *et al*. (1999) and Radwan *et al*.(2013) had reported increased malondialdehyde concentration in the erythrocyte membrane of donkeys and cattle infected with *T. equi* and *B. bigemina* respectively.

Evaluation of the haemato-biochemical parameters in the *T. equi*-infected symptomatic horses based on sex distribution showed that symptomatic mares with *T. equi* infections had lower MCHC and percent RDW respectively as the only significant alteration in the erythrogram. This connotes presence of uniformly sized erythrocytes with low haemoglobin concentration in the peripheral blood of symptomatic mares with *T. equi* infection. Clinically, this may be responsible for the dyspnoea and weakness because decrease haemoglobin concentration will predispose to tissue hypoxia. The presence of hypochromic erythrocytes in *T. equi*-infected equids have been reported by other authors (Zobba *et al*., 2008; Mahoud *et al*., 2016; Idoko *et al*., 2018). The low MCHC could be associated with hypovitaminosis B6 secondary to iron deficiency. The leukogram showed higher values in absolute neutrophil counts in symptomatic geldings as well as absolute increase in lymphocyte counts in symptomatic mares with *T. equi* infection. These increases may be due to the destructive effects of *T. equi*sporozoites in the peripheral blood mononuclear cells, specifically, the lymphocytes where they undergo schizogonic stages to form microschizonts and macroschizonts. An acute, favourable cellular inflammatory response possibly accounted for the higher values of neutrophils and lymphocytes to the parasites' antigens in both geldings and mares. Similar findings in *T. equi*-infected horses have been reported (Ibrahim *et al*., 2005; Ibrahim *et al*., 2011; Al-Obaidi *et al*., 2015). The thrombogram revealed higher values in MPV and percent PDW in the *T. equi*-infected symptomatic mares, thus, suggesting juvenile platelets are becoming bigger and more active. This may connote regenerative response to *T. equi* infection, systemic inflammatory process where the consumption of platelets in the spleen and/or reticuloendothelial system is evident, or it could be due to small fibrin clots or platelets clumping. Similar findings have been reported (de Waal *et al*., 1987; Donnelan and Marais, 2009).

The only significant changes in serum biochemical profiles were concurrent lowalbumin, high globulin, and high phosphate concentrations in the *T. equi*-infected symptomatic geldings, as well as, high direct bilirubin concentration in the symptomatic mares.*Theileria equi* causes splenomegaly and hepatomegaly. The later may compromise liver function to cause hypoalbuminaemia and hyperglobulinaemia (de Waal and van Heerden, 2004; Onyiche *et al*., 2019). Therefore, the hypoalbuminaemia and hyperglobulinaemia may indicate chronic inflammatory disease of the liver. Although, low serum phosphate concentration have been associated with infecting piroplasms of horses due to altered erythrocyte metabolism (de Waal and van Heerden, 2004; Camacho *et al*., 2005), finding from this study showed higher levels of serum phosphate in *T. equi*-infected symptomatic geldingswhich contradicted the report of Garba *et al*. (2012). This increase may be associated with renal insufficiency that resulted in decreased renal excretion of phosphate. Acute renal failure in equine piroplasmosis has been associated with hemoglobin-induced pigment nephropathy and systemic responses to severe inflammation (de Waal, 1992).The concurrent low serum albumin and high globulin concentration observed in this study corroborate but contradicts the low serum phosphate concentration reported in horses with predominant *T. equi* infection(Zobba *et al*., 2008; Al-Obaidi *et al*., 2015).The low serum albumin concentration may be associated with the oedema around the fetlock that was observed clinically in symptomatic horses (Alsaad *et al*., 2010).

The high conjugated bilirubin concentration in the *T. equi*-infected symptomatic mares may be due to hepatic damage or cholestasisfollowing hypoxia-induced hepatobiliary system damage resulting from the decreased haemoglobin concentration observed in the erythrogram of symptomatic mares.Although, the liver enzymes in this study were not

significantly altered, liver damage in equine piroplasmosis has been reported (de Waal and Heerden, 2004; Al-Obaidi *et al*., 2015).

On the basis of breed distribution of the haemato-biochemical alterations in the *T. equi*-infected symptomatic horses, a low MCV, high MCHC, and high percent RDW were observed in West African Barb horses as remarkable changes in the erythrogram.These alterations suggest marked increase in the number of small sized erythrocytes that contain more haemoglobin than normal. Although, a low MCV and high MCHC is rarely reported, it is observed in congenital spherocytosis where erythrocytes do not form correctly, thus, are removed by erythrophagocytosis in the spleen. Similar reports of increased MCHC values in *T. equi*-infected donkeys (Laus *et al*., 2015) and horses (Osman *et al.,* 2017)have been documented.

The leukogram was comparatively normal but the thrombogram showed high MPV and percent PDW in symptomatic Argentine Polo Ponies with *T. equi* infection. This finding indicates marked increase in large size platelets and adequate bone marrow response to ongoing platelet destruction or consumption. As stated previously, the exact mechanism of platelet alterations in equine piroplasmosis is unknown but thrombocytopaenia is a common laboratory finding (Alsaad, 2014; Osman *et al*., 2017), even though the platelet counts were comparativelyunaltered in this study.

The significantly higher serum phosphate and direct bilirubin concentrations in the *T. equi*-infected symptomatic Argentine Polo Ponies and higher indirect bilirubin concentration in the symptomatic West African Barb horses with *T. equi* infection were significant alterations in the biochemical profiles investigated based on breed distribution. The high serum phosphate concentration may be due to renal disease that

impairs urinary excretion of phosphate. The conjugated hyperbilirubinaemia may suggest liver failure with compromised hepatic excretion of bilirubin and/or hepatic bile obstruction. However, there was no corresponding increase in unconjugated bilirubin in the symptomatic Argentine Polo Ponies. Unconjugated hyperbilirubinaemia in horses is often associated with haemolytic diseases, anorexia or liver failure. Therefore, the high values in the *T. equi*-infected West African Barb horses may be due to increased breakdown of haemoglobin which was significantly higher from the erythrogram and/or decreased hepatocellular uptake due to hepatic disease. Interestingly, the erythroid indices were not significantly altered, thus, excluding the possibility of haemolysis. This may partly account for the icterus observed during physical examination of the ocular mucous membrane, which corroborates the reports of Zobba *et al*. (2008) and Salib *et al.* (2013).

Among the asymptomatic horses, therewere higher levels of blood urea nitrogen and creatinine in both *B. caballi*-infected and horses with co-infection of *B. caballi* and *T. equi*.The azotaemia may be associated with renal damage with impaired glomerular filtration. Similar finding in donkeys with piroplasmosis was reported by Dede *et al*. (2008) and Takeet *et al*. (2009).

Although, active infections in horses with piroplasmosis usually coincide with anaemia that often result in acute disease, and sometimes, death (Knowles *et al*., 1996), results of the haematological parameters in horses with ongoing active infection in this study did not reveal anaemia but showed favourable inflammatory responses in symptomatic horses compared to the non-infected horses. Similarly, there were no remarkable alterations in the haematological parameters between the infected asymptomatic horses and those that were non-infected. Premunition due to endemicity of EP in Nigeria and

the presence of intact spleen could impact a favourable innate response. It has been shown that clinico-pathological changes in horses possessing an intact spleen is quite minimal compared to those in splenectomized horses (Knowles *et al*., 2018). Also, horses develop persistent but low-level infection with mild clinical signs in endemic areas (García-Bocanegra *et al*., 2012; Muñoz *et al*., 2013).

Assessment of biomarkers for changes in the liver and kidney functions did not reveal significant alterations within and between the *Theileria* species-infected symptomatic and the non-infected horses. Also, the mean serum total cholesterol and glucose concentrations within the infected symptomatic horses, and the uninfected horses showed non-significant pathologies (risk factors for cardiovascular disease). Cholesterol is essential for normal cellular function in animals, and is a vital component of cell membranes (Cox *et al*., 1990). Conversely, significant increase in serum blood urea nitrogen and creatinine concentrations were observed in asymptomatic horses that were seropositive to *B. caballi* infection as well as asymptomatic horses with mixed *B. caballi* and *T. equi* infections.Although *B. caballi* is less pathogenic than *T. equi* (Radostitis *et al*., 2000), the high antibody titre in the *B. caballi* seropositive asymptomatic horses as well as those with mixed infections may account for the renal pathology, attested by the elevated serum BUN and creatinine concentrations.

The high levels of erythrocyte membrane bound antioxidant markers in the *Theileria*-infected symptomatic horses were not statistically significant compared to the non-infected horses. However, marked increase in the erythrocyte membrane malondialdehyde concentration; an indicator of lipid peroxidation was observed in the *T. equi*-like unknown infected symptomatic horses. The absence of significant oxidative stress in both symptomatic and asymptomatic horses in this study may connote

adequate antioxidant capacity by the host animals or effective regulation of free radicals production by antioxidants, thus, mitigating tissue damage. This possibly explains the in-significant biochemical changes in most parameters investigated for both the infected symptomatic and asymptomatic horses.

There were no remarkable losses of erythrocyte surface sialic acids, attested by the non-significant changes in the serum free sialic acid concentrations in symptomatic and asymptomatic horses infected by the different *Theileria* species and *B. caballi*in this study. This may partly explain the non-anaemic state observed in the infected horses. Loss of erythrocyte surface sialic acids is associated with accelerated ageing process, hence, shortened erythrocyte life-span and destruction by erythrophagocytosis (Esievo *et al.*, 1982). Based on these findings, it is possible to posit that the *Theileria* species and *B. caballi*did not significantly impact the ageing process of the parasitized erythrocytes in the infected horses, as such, the erythrocytes life-span were likely unaltered.

The current study documents more significant haemato-biochemical alterations in symptomatic geldings and mares with *T. equi* infections compared to the *T. equi*-infected symptomatic stallions. These variations may be attributed to the level of sex hormones. Susceptibilities to protozoan infections based on sex distribution have been reported by Robert *et al.* (2001). Oestrogen and progesterone-induced suppression of cells of innate immune system, such as mast cells, macrophages, dendritic cells and natural killer (NK) cells had been postulated in females (Baley and Schacter, 1985; Szekeres-Bartho *et al*., 1997; Laskarin *et al*., 1999). These cells not only form the first line of defense against many organisms, but also play an important role in directing the developing adaptive immune response (Robert *et al*., 2001). Studies by Rüegg*et al*.

(2007), Moretti *et al*. (2010) and Oladipo *et al*. (2015) showed higher infectivity to *T. equi* in female horses compared to the males.

The likelihood of endemic stability as justification for the mild haemato-biochemical alterations among the infected symptomatic horses may not suffice since they all presented variable but non-specific clinical signs of piroplasmosis. Therefore, it is hereby perceived that effective host protective immune and regenerative responses may have possibly contributed to mitigating the systemic or clinico-pathologic effects of the identified parasites. However, the serologic evidence of *B. caballi* and *T. equi* infections in the asymptomatic horses reflects endemic stability but this did not cause remarkable haemato-biochemical alterations.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study did not detect any parasite by microscopy, thus, justifying the superior specificity and sensitivity of molecular diagnosis used in this study over conventional Giemsa-stained blood smear examination.

The current study revealed heterogeneity in the 18S rRNA of *Theileria* species naturally infecting horses in Nigeria. This study to the best of our knowledge is the first to document the presence of five distinct clades of *Theileria* species, clade A (*Theileria equi* Florida/Texas strain), clade B (*Theileria equi*-like), clade C (*Theileria haneyi*), clade D (*Theileria equi*-like), clade E (*Theileria equi*-like), and two unknown clades that naturally infect horses in Nigeria. Similarly, the study is the first to document multi-clade genotype infection within a single equine host in a naturally infected population.

The present study revealed high seroprevalence of *T. equi* with considerable variation in their genotypes in Nigeria. Based on the seropositivity of *T. haneyi* and *T. equi*-like parasites to the anti- *T. equi* antibodies observed in this study, we hypothesize that *ema1* is present in the genome of the Nigerian isolates.

Ticks belonging to the genera, *Amblyomma*, *Hyalomma* and *Rhipicephalus* are the possible vectors of the detected parasites, with *Rhipicephalus evertsi evertsi* being the

predominant species. Seropositivity to anti- *T. equi* antibodies was remarkably demonstrated, unlike the seronegative *B. caballi* for symptomatic horses, and high antibody titre for *B. caballi* in three asymptomatic horses.

Overall, there were evidences of haemato-biochemical alterations in symptomatic than asymptomatic horses. The symptomatic horses with *T. equi* infection had more significant alteration in the haemato-biochemical profile compared to symptomatic horses with *T. haneyi*, multi-clade or mixed infections and horses infected by *T. equi*-like unknown parasites.

There were more haemato-biochemical alterations in symptomatic geldings and mares with *T. equi* infection compared with symptomatic stallions infected by *T. equi*. The symptomatic Argentine Polo Ponies with *T. equi* infection showed more remarkable haemato-biochemical alteration compared to the *T. equi*-infected symptomatic West African Barb horses.

Overall, there were no significant physicochemical changes in the erythrocyte membrane parameters investigated in both symptomatic and asymptomatic horses, except for the lipid peroxidation evident by high malondialdehyde concentration in symptomatic horses infected with *T. equi*-like unknown clade. Therefore, no marked oxidative stress was observed in symptomatic and asymptomatic horses with piroplasmosis from this study.

6.2Recommendations

It is therefore recommended that further studies should be carried out to:

i. Investigatethe virulence of the Nigerian isolates of *Theileria* species in clades A, B, C, D and E in an experimental study.

ii. Determine detailed comparative genomic analysis of the five distinct clades of the Nigeria isolates of *Theileria* species that naturally infect horses in order to determine the presence or absence of equine merozoite antigen-1 (*ema*1).

iii. Sequence more samples in order to determine the phylogeographical structure of the parasites in Nigeria.

iv. Explore the use of antigens from autochthonous strain(s) to develop peculiar diagnostics and/or chemoprophylaxis in Nigeria.

v. Determine tick vector competence to transmit the detected pathogens.

vi. Experimentally compare the haemato-biochemical profiles of the Nigerian isolates of *Theileria* species infections in splenectomized and spleen-intact horses.

vii. Establishing and domesticating of haemato-biochemical reference ranges for horses in Nigeria as guide to clinical laboratory diagnosis and interpretation.

viii. Routine treatment of horses to prevent development of fulminant disease, especially in Geldings and Mares that are of exotic breeds.

REFERENCES

Abaje, I. B., Sawa, B. A., Iguisi, E. O. and Ibrahim, A. A. (2015b). A Quantitative approach to vulnerability assessment of rural communities to climate change in Kaduna State, Nigeria. *Nigerian Geographical Journal New Series*, 10 (2): 180-195.

Abaje, I. B., Sawa, B. A., Iguisi, E. O. and Ibrahim, A. A. (2016). Impacts of climate change and adaptation strategies in rural communities of Kaduna State, Nigeria. *Ethiopian Journal of Environmental Studies and Management*, 9 (1): 97 – 108.

Abedi, V., Razmi, G., Seifi, H. and Naghibi, A. (2014): Molecular and serological detection of Theileria equi and Babe-sia caballi infection in horses and ixodid ticks in Iran. *Ticks and Tick Borne Diseases*, 5: 239–244.

Aboulaila, M., Batadoj, D., Salama, A., Munkhjargal, T., Ichikawa-Seki, M. A., Terkawi, M., Yokoyama, N. and Igarashi, I. (2014). Evaluation of the inhibitory effects of miltefosine on the growth of *Babesia* and *Theileria* parasites. *Veterinary Parasitology*, 204 (3-4):104-110.

Adams, L. G. (1981). Clinicopathological aspects of imidocarb dipropionate toxicity in horses. *Research in Veterinary Science*, 31: 54–61.

Alhassan, A., Pumidonming, W., Okamura, M., Hirata, H., Battsetseg, B., Fujisaki, K., Yokoyama, N. and Igarashi, I. (2005). Development of a single-round and multiplex PCR method for the simultaneous detection of *Babesia caballi* and *Babesia equi* in horse blood. *Veterinary Parasitology*, 129: 43–49.

Ali, S., Sugimoto, C. and Onuma, M. (1996). Equine Piroplasmosis. *Journal of EquineScience.* **7** (4): 67-77.

Aliu, Y. O. (1983). Tick-borne diseases of domestic animals in Nigeria: Current treatment procedure. *Veterinary Bulletin*. 53: 233- 251.

Allsopp, M. T. and Allsopp, B. A. (2006). Molecular sequence evidence for the reclassification of some Babesia species. *Annals of New York Academy of Science,* 1081: 509–517.

Allsopp, M. T., Cavalier-Smith, T. and De Waal, D. T. (1994). Phylogeny and evolution of the piroplasms. *Parasitology*, 108 (Pt 2):147–152.

Allsopp, M. T., Lewis, B. D. and Penzhorn, B. L. (2007). Molecular evidence for transplacental transmission of Theileria equi from carrier mares to their apparently healthy foals. *Veterinary Parasitology*, 148:130–136.

Al-Obaidi, Q. T., Al-Sultan, I. I., Arshad, M. M., Mohd-Azam, K. G. K. and Mimi, A. M. (2015). Clinical case of acute equine piroplasmosis in a Malaysian Mare. *Research Opinion in Animal and Veterinary Science*, 5(6): 270-274.

Alsaad, K. M. and AL-Mola, G. M. (2006). Clinical and pathological study of equine babesiosis in drought horses in Mosul. *Iraqi Journal of Veterinary Science*, 20 (1): 89-101.

Alsaad, K. M., Alsaad, E. A. and Al-Derawie, H. A. (2010). Clinical and diagnostic study of equine babesiosis in drought horses in some areas of Basrah Province. *Research Journal of Animal Science*, 4 (1):16-22.

Alsaad, K. M. (2014). Evaluation of haemogram, acute phase response, acid base balance and blood gas analysis in newborn foals infected with Babesiosis. Journal of Animal and Plant Science, 24: 738-742.

Alvarez, D. E., Ezcurra, A. L. D., Fucito, S. and Gamarnik, A. V. (2005). Role of RNA structures present at the 3 ' UTR of dengue virus on translation, RNA synthesis, and viral replication. *Virology*, 339: 200–212.

Alward, A., Corriher, C. A. and Barton, M. H. (2006). Red maple (Acer rubrum) leaf toxicosis in horses: A retrospective study of 32 cases. *Journal of Veterinary Internal Medicine*, 20: 1197–1201.

Ambawat, H. K., Malhotra, D. V. and Kumar, S. (1999). Erythrocyte associated haemato-biochemical changes in *Babesia equi* infection experimentally produced in donkeys. *Veterinary Parasitology*, 85:319–324.

Animal and Plant Health Inspection Service. (2012). *Animal Health Laboratory Information and Services*. Retrieved from: http://www.aphis.usda.gov/ animal_health/lab_info_services.

Aminoff, D. (1961). Methods for the Quantitative Estimation of N-Acetylneuraminic Acid and their Application to Hydolysates of Sialomucoids. *Biochemistry Journal*, 81: 384-392.

Awinda, P. O., Mealey, R. H., Williams, L. B., Conrad, P. A., Packham, A. E and Reif, K. E. (2013). Serum antibodies from a subset of horses positive for *Babesia caballi* by competitive enzyme-linked immunosorbent assay demonstrate a proteinrecognition pattern that is not consistent with infection. *Clinical Vaccine and Immunology*, 20:1752–1757.

Ayers, P. and Warrington, L. (2008). Diagnosis and treatment of simple acid-base disorders. *Nutrition and Clinical Practice,* 23 (2): 122-127.

Baggish, A. L. and Hill, D. R. (2002). Antiparasitic agent atovaquone. *Antimicrobial Agents andChemotherapeutics, 46* (5): 1163-1173.

Bahrami, S., Ghadrdan, A. R., Mirabdollahi, S. M. and Fayed, M. R. (2014). Diagnosis of subclinical equine theileriosis in center of Iran using parasitological and molecular methods. *Tropical Biomedicine,* 31: 110–117.

Baley, J. E., and Schacter, B. Z. (1985). Mechanisms of diminished natural killer cell activity in pregnant women and neonates. *Journal of Immunology,* 134:3042–3048.

Balla, J., Jacob, H. S., Balla, G., Nath, K., Eaton, J. W. and Vercellotti, G. M. (1993). Endothelial-cell heme uptake from heme proteins: Induction of sensitization and desensitization to oxidant damage. *Proceeding of National Academy of Science,* 90: 9285–9289.

Bashiruddin, J. B., Camma, C. and Rebelo, E. (1999). Molecular detection of *Babesia equi* and *Babesia caballi* in horse blood by PCR amplification of part of the 16S rRNA gene. *Veterinary Parasitology*, 84: 75–83.

Baskurt, O. K., Farley, R. A. and Meiselman, H. J. (1997). Erythrocyte aggregation tendency and cellular properties in horse, human and rat: a comparative study. *American Journal of Physiology*, 273: H2604–2612.

Battsetseg, B., Xuan, X. and Ikadai, H. (2001). Detection of *Babesia caballi* and *Babesia equi* in *Dermacentor nuttalli* adult ticks. *International Journal of Parasitology,* 31: 384–386.

Bellary, S.S., Anderson, K.W., Arden, W. A. and Butterfield, D.A. (1995). Effect of lipopolysaccharide on the physical conformation of erythrocyte cytoskeletal proteins. *Life Science*, **56**: 91–98.

Belloli, C., Crescenzo, G., Lai, O., Carofiglio, V., Marang, O. and Ormas, P. (2002). Pharmacokinetics of imidocarb dipropionate in horses after intramuscular injection. *Equine Veterinary Journal*, 34 (6):625-629.

Beyazit, Y., Sayilir, A., Torun, S., Suvak, B., Yesil, Y., Purnak, T., Oztas, E., Kurt, Kekilli, M. and Ibis, M. (2012). Mean platelet volume as an indicator of disease severity in patients with acute pancreatitis. *Clinical Research in Hepatology and Gastroenterology,* 36 (2):162-168.

Bernasconi, N., Traggiai, E. and Lanzavecchia, A. (2002). Maintenance of serological memory by polyclonal activation of human memory B cells. *Science*, 298: 2199–2202.

Beugnet, F. and Marié, J. L. (2009). Emerging arthropod-borne diseases of companion animals in Europe. *Veterinary Parasitology*, 163: 298–305.

Bhoora, R., Franssen, L., Oosthuizen, M. C., Guthrie, A. J., Zweygarth, E., Penzhorn, B. L., Jongejan, F. and Collins, N. E. (2009). Sequence heterogeneity in the *18S rRNA* gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. *Veterinary Parasitology,* 159: 112–120.

Bhoora, R., Quan, M., Matjila, P. T., Zweygarth, E., Guthrie, A. J. and Collins, N. E. (2010). Sequence heterogeneity in the equi merozoite antigen gene (*ema-1*) of *Theileria equi* and development of an ema-1-specific TaqMan MGB assay for the detection of *T. equi*. *Veterinary Parasitology,*172: 33–45.

Bishop, R., Musoke, A., Morzaria, S., Sohanpal, B. and Gobright, E. (1997). Concerted evolution at a multicopy locus in the protozoan parasite *Theileria parva*: extreme divergence of potential protein-coding sequences. *Molecular and Cellular Biology*, 17: 1666–1673.

Biu, A. A., Ahmed M. I. and Yunusa, A. I. (2006). Prevalence of equine babesiosis in Maiduguri, Nigeria. *International Journal of Biomedical and Health Science*, 2 (2): 81-83.

Bogdan, C., Röllinghoff, M. and Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion in Immunology,* 12:64–76.

Bork, S., Yokoyama, N., Matsuo, T., Claveria, F. G., Fujisaki, K. and Igarashi, I. (2003). Growth inhibitory effect of triclosan on equine and bovine Babesia parasites. *American Journal of Tropical Medicine and Hygiene*, 68: 334-340.

Bosch, S. S., Kronenberger, T., Meissner, K. A., Zimbres, F. M., Stegehake, D., Izui, N. M., Schettert, I., Liebau, E. and Wrenger, C. (2015). "Oxidative Stress Control by Apicomplexan Parasites", *BioMedResearchInternational*, ID 351289: 1-10.

Bose, R., Jorgensen, W. K., Dalgliesh, R. J., Friedhoff, K. T. and De Vos, A. J. (1995). Current state and future trends in the diagnosis of babesiosis. *Veterinary Parasitology,* 57:61–74.

Bose, A. A., Abba, I. Y., Madaki, M. J. and Obute, O. L. (2015). Analysis of Poultry (Layers) Enterprises in IGABI Local Government Area, Kaduna State Nigeria. *Journal of Agriculture and Veterinary Science*, 8 (4-1): 43-49.

Bowman, D., Little, S. E., Lorentzen, L., Shields, J., Sullivan, M. P., Carlin, E. P. (2009). Prevalence and geographic distribution of *Dirofilaria immitis*, Borrelia burgdorferi, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Veterinary Parasitology,* 160: 138-148.

Bruning, A. (1996). Equine piroplasmosis an update on diagnosis, € treatment and prevention. *Brazilian Veterinary Journal*, 152: 139–151.

Budak, Y.U., Polat, M. and Huysal, K. (2016). The Use of Platelet Indices, Plateletcrit, Mean Platelet Volume and Platelet Distribution Width in Emergency Non-Traumatic Abdominal Surgery: A Systematic Review. *Biochemia Medica*, 26 (2): 178-193.

Bukar, M. M., Sadiq, M. A. and Geidam, Y. A. (2007). A survey of cutaneous neoplasms among horses used for cultural festivals in Borno state, Nigeria. *Nigerian Veterinary Journal,*28: 27–33.

Cacciò, S., Cammà, C., Onuma, M. and Severini, C. (2000). The β-tubulin gene of Babesia and Theileria parasites is an informative marker for species discrimination. *International Journal for Parasitology*, 30: 1181–1185.

Cai, L., Yi, F., Dai, Z., Huang, X., Zhao, Y. D., Mirza, M. K., Xu, J., Vogel, S. M. and Zhao, Y. Y. (2014). Loss of caveolin-1 and adiponectin induces severe inflammatory lung injury following LPS challenge through excessive oxidative/nitrative stress. *American Journal of Physiology, Lung Cell and Molecular Physiology*, 306: 566-573.

Camacho, A. T., Guitian, F. J., Pallas, E., Gestal, J. J., Olmeda, A. S., Habela, M. A., Telford, S. R. and Spielman, A. (2005). *Theileria* (*Babesia*) *equi* and *Babesia caballi* infections in horses in Galicia, Spain. *Tropical and Animal Health Production*, 37: 293–302.

Carruthers, V. and Boothroyd, J. C. (2007). Pulling together: an integrated model of Toxoplasma cell invasion. *Current Opinion in Microbiology*, 10: 83–89.

Carter, E. I., Valli, V. E., McSherry, B. J., Milne, F. J., Robinson, G. A., and Lumsden. J. H. (1974). The kinetics of hematopoiesis in the light horse I. the lifespan of peripheral blood cells in the normal horse. *Canadian Journal of Comparative Medicine*, 38: 303-313.

Cary, C., Zaias, J. and Altman, N. H. (2009). Acute Phase Response in Animals: A Review. *Comparative Medicine*, 59 (6):517-526.

Castellanos-Gonzalez, A., White Jr, A. C., Ojo, K. K., Vidadala, R. S., Zhang, Z. and Reid, M. C. (2013).A novel calcium-dependent protein kinase inhibitor as a lead compound for treating cryptosporidiosis. *Journal of Infectious Disease*, 208:1342–1348.

Celi, P., DI Trana, A. and Claps, S. (2010). Effects of plane of nutrition on oxidative stress in goats during the peripartum period. *The Veterinary Journal*, 184(1): 95 – 99.

Center for Food Security and Public Health (CFSPH). (2008). Equine Piroplasmosis. Institute for International Cooperation in Animal Biologics, Iowa State University. www.cfsph@iastate.edu

Charles, M. H. (2007). *Internal parasites, External paraspites*. In: Charles M.H, Margi S, eds. Laboratory procedures for veterinary technicians. 5th ed. Louis Missouri: Mosby Elsevier Inc; p.187-252.

Chu, S. G., Becker, R. C., Berger, P. B., Bhatt, D. L., Eikelboom, J. W., Konkle, B., Mohler, E. R., Reilly, M. P. and Berger, J. S. (2010). Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. *Journal of Thrombosis and Haemostasis*. 8 (1):148-156.

Chung, T. W. and Ho, C. P. (1999). Changes in viscosity of low shear rates and viscoelastic properties of oxidative erythrocyte suspensions. *Clinical Hemorheol and Microcirculation,* 21 (2): 99-103.

Committee on Infectious Diseases of Horses. (2014). Resolution #21: Equine Piroplasmosis – Release of Test Negative Treated Horses. United States Animal Health Association 115th Annual Meeting.

Cox, R. A. and Garcia-Palmieri, M. R. (1990). *In: Walker, H. K., Hall, W. D., Hurst, J. W. Editors. Clinical Methods: The History, Physical and Laboratory Examinations.* 3rd Edition. Boston: Butterworths, chapter 31.

Cunha, C. W., McGuire, T. C. and Kappmeyer, L. S. (2006). Development of specific immunoglobulin Ga (IgGa) and IgGb antibodies correlates with control of parasitemia in Babesia equi infection. *Clinical Vaccine and Immunology*, 13:297–300.

Dede, S., Deger, Y., Deger, S. and Tanritanir, P. (2008). Plasma levels of zinc, copper, copper/zinc ratio, and activity of carbonic anhydrase in equine piroplasmosis. *Biological Trace Element Research,* 125: 41-45.

Deepachandi, B., Weerasinghe, S., Soysa, P., Karunaweera, N. and Siriwardana, Y. (2019). A highly sensitive modified nested PCR to enhance case detection in leishmaniasis. *Infectious Diseases*, 19 (623): 1-10.

Deger, S., Deger, Y. and Bicek, K. (2009). Status of lipid peroxidation, antioxidant and oxidation products of nitric oxide equine babesiosis: status of antioxidant and oxidant in equine babesiosis. *Journal of Equine Veterinary Science,* 29: 743-747.

De Gopegui, R. R., Penalba, B., Goicoa, A., Espada, Y., Fidalgo, L. E. and Espino, L. (2007). Clinicopathological findings and coagulation disorders in 45 cases of canine babesiosis in Spain. *Veterinary Journal*, 174: 129–132.

Djaldetti, M., Salman, H., Bergman, M., Djaldetti, R., Bessler, H., (2002). Phagocytosis? The mighty weapon of the silent warriors. *Microscopy Research and Techniques*, 57 (6): 421-431.

de Waal, D. T., van Heerden, J. and Potgieter, F. T. (1987). An investigation into the clinical pathological changes and serological response in horses experimentally

infected with Babesia equi and Babesia caballi. *OnderstepoortJournal of Veterinary Research*, 54: 561–568.

de Waal, D. T. (1992). Equine piroplasmosis: A review. *Brazilian Veterinary Journal*, 148:6–14.

de Waal, D. T. and Van Heerden, J. (2004). *Equine piroplasmosis*. In: Coetzer, J. A. W and R. C. Tustin, Editors, Infectious Diseases of Livestock, Oxford, Southern Africa. 425–434.

Diana, A., Guglielmini, C., Candini, D., Pietra, M. and Cipone, M. (2007). Cardiac arrhythmias associated with piroplasmosis in the horse: A case report. *Veterinary Journal*, 174: 193-195.

Doggett, J. S., Ojo, K. K., Fan, E., Maly, D. J. and Van Voorhis, W. C. (2014). Bumped Kinase Inhibitor 1294 Treats Established Toxoplasmosis gondii Infection. *Antimicrobial Agents and Chemotherapy*, 58: 3547-3549.

Donnellan, C., Page, P., Nurton, P., van den Berg, J. S. and Guthrie, A. (2003). *Piroplasmosis – current trends*. Conference Proceedings of the Equine Practioners Group of South Africa, 86-87.

Donnellan, C. M. and Marais, H. J. (2009). Equine Piroplasmosis. *In: Mair TS, Hutchinson RE, editors: Infectious Disease of Horses, UK,EVJ Ltd*, pp 333-340.

Donnelly, J., Joyner, L. P., Graham-Jones, O. and Ellis, C. P. (1980). A Comparison of the complement fixation and immunofluorescent antibody tests in a survey of the prevalence of Babesia equi and Babesia caballi in horses in the Sultanate of Oman. *Tropical Animal Health and Production*, 12 (1): 50-60.

Donnelly, J., Phipps, L. P. and Watkins, K. L. (1982). Evidence of maternal antibodies to Babesia equi and B caballi in foals of seropositive mares. *Equine Veterinary Journal*, 14: 126–128.

Economic Community of West Africa (ECOWAS). (1998). ECOWAS protocol on cross-border and trans-human agreement.

Ehizibolo, D. O., Kamani, J., Ehizibolo, P. O., Egwu, K. O., Dogo, G. I. and Salami-Shinaba, J. O. (2012). Prevalence and Significance of Parasites of Horses in Some States of Northern Nigeria. *Journal of Equine Science*, 23 (1): 1 – 4.

Elaine, A. and Margi, S. (2007). *Hematology and hemostasis*. In: Charles MH, Margi S eds. Laboratory procedures for veterinary technicians, 5th edition. St Louis Missouri: Mosby Elsevier Inc. pp. 27-73.

Ellinger, J. J., Lewis, I. A. and Markley, J. L. (2011). Role of aminotransferase in glutamate metabolism of human erythrocytes. *Journal of Biomolecular Nuclear Magnetic Resonance*, 49: 221-229.

Ememe, M. U., Onyegbula, O., Egwuogu, F. C., Nlebedum, C. U. and Ogbu, C. C. (2018). *Babesia caballi* infection in a 6-month-old colt. *Sokoto Journal of Veterinary Sciences*, 16 (1): 102-106.

Engstler, M., Schauei, R. and Brun, R. (1995). Distribution of developmentally regulated trans-sialidase activity in the Kinetoplastida and characterization of the trans-sialidase activity from procyclic Trypanosoma congolense. *Acta Tropica*, 59: 117-129.

Esmaeilnajed, N., Tavassoli, M., Asri-Rezaei, S. and Dalir-Naghadeh, B. (2012). Evaluation of antioxidant status and oxidative stress in sheep naturally infected with *Babesia ovis*. *Veterinary Parasitology*, 185: 124-130.

European Medicine Agency for the Evaluation of Medicinal Products. (2001). Seventh Annual Report 2001.

Freedman, J. E. (2008). Oxidative Stress and Platelets. *Atherosclerosis, Thrombosis, and Vascular Biology*, 28:s11-s16.

Frelinger, A. L., Torres, A. S., Caiafa, A., Morton, C. A., Berny-Lang,M. A. and Gerrits, A. (2015). Platelet-rich plasma stimulated by pulse electric fields: platelet activation, procoagulant markers, growth factor release and cell proliferation. *Platelets*,19: 1-8.

Frerichs, W. M., Allen, P. C. and Holbrook, A. A. (1973). Equine piroplasmosis (*Babesia equi*): Therapeutic trials of imidocarb dihydrochloride in horses and donkeys. *Veterinary Records*, 93: 73–75.

Friedhoff, K. T. and Soule, C. (1996). An account on equine babesioses. *Revue Scientifique et Technique,* 15 (3): 1191-1201.

Garba, U. M., Sackey, A. K. B., Tekdek, L. B., Agbede, R. I. S and Bisalla, M. (2011). Clinical manifestatious and prevalence of piroplasmosis in Nigerian royal horses. *Journal of Veterinary advances*, 1 (1): 11 – 15.

Garba, U. M., Sackey, A. K. B., Agbede, R. I. S., Tekdek, L. B. and Bisalla, M. (2011a). Serum urea and creatinine levels in Nigerian local horses naturally infected with *Babesia*. *Pakistan Veterinary Journal*, 31 (2): 163-165.

Garba, U. M., Sackey, A. K. B., Tekdek, L. B., Agbede, R. I. S. and Bisalla, M. (2011b). Clinical manifestations and prevalence of piroplasmosis in Nigerian royal horses. *Journal of Veterinary Advances*, 1 (1): 11-15.

Garba, U. M., Sackey, A. K. B., Agbede, R. I. S., Tekdek, L. B. and Bisalla, M. (2012). Plasma Total Protein, Serum Calcium and InorganicPhosphate Levels in Nigerian Horses with NaturalPiroplasmosis. *Journal of Physiology and Pharmacology Advances*, 2 (2): 117 121.

García-Bocanegra, I., Arenas-Montes, A., Hernández, E., Adaszek, L., Carbonero, A., Almería, S., Jaén-Téllez, J. A., Gutiérrez-Palomino, P. and Arenas, A. (2012). Seroprevalence and risk factors associated with *Babesia caballi* and *Theileria equi* infection in equids. *The Veterinary Journal,* 195: 172-178.

Georges, K. C., Ezeokoli, C. D., Sparagano, O., Pargass, I., Campbell, M., D'Abadie, R. and Yabsley, M. J. (2011). A case of transplacental transmission of *Theileria equi* in a foal in Trinidad. *Veterinary Parasitology*, 175 (3-4): 363-366.

Goff, W. L., Johnson, W.C., Parish, S. M., Barrington, G. M., Elsasser, T. H., Davis, W. C. and Valsez, R. A. (2002). IL-4 and IL-10 inhibition of IFN-γ- and TNF-αdependent nitric oxide production from bovine mononuclear phagocytes exposed to *Babesia bovis* merozoites. *Veterinary Immunology and Immunopatholo*gy,84: 237-251.

Golebiewska, E. M. and Poole, A. W. (2015). Platelet secretion: From haemostasis to wound healing and beyond. *Blood Review*, 29: 153- 162.

Gresh, N. and Pullman, B. (1984). A theoretical study of the nonintercalative binding of berenil and stilbamidine to double-stranded (dA-dT)n oligomers. *Molecularpharmacology, 25* (3): 452-458.

Grondin, T. M. and Dewitt, S. F. (2010). Normal Hematology of the Horse and Donkey. *In: Weiss DJ, Wardrop KJ editors. Schalm's Veterinary hHematology, 6th edition. Wiley-Blackwell Publishing Ltd, Ames, Iowa, USA,* pp. 821-828.

Guimaraes, A. M., Lima, J. D. and Ribeiro, M. F. (1998). Ultrastructure of sporogony in Babesia equi in salivary glands of adult female Boophilus microplus ticks. *Parasitology Research*, 84: 69–74.

Gunes,S., Al-Sadaan, M. and Agarwal, A. (2015).Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. *Reproductive BioMedicine Online*, 31: 309–319.

Guswanto, A., Nugraha, A. B., Tuvshintulga, B., Tayebwa, D. S., Rizk, M. A., Batiha, G. E., Gantuya, S., Sivakumar, T., Yokoyama, N. and Igarashi, I. (2018). 17-DMAG inhibits the multiplication of several Babesia species and Theileria equi on in vitro cultures, and Babesia microti in mice. *Drugs and Drugs Resistance*, 8: 104-111.

Hailat, N. Q., Lafi, S. Q., Al-Darraji, A. M. and Al-Ani, F. K. (1997). Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. *Veterinary Parasitology*, 69: 1–8.

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and AnalysisProgram for Windows 95/98/NT/ *Nucleic Acids Symposium Series*, 41: 95-98.

Hamsho, A., Tesfamarym, G., Megersa, G. and Megersa, M. (2015). A Cross-Sectional Study of Bovine Babesiosis in Teltele District, Borena Zone, Southern Ethiopia. *Journal of Veterinary Science and Technology*, 6 (3): 1-4.

Harvey, K. L., Yap, A., Gilson, P. R., Cowman, A. F. and Crabb, B. S. (2014). Insights and controversies into the role of the key Apicomplexan invasion ligand, Apical Membrane Antigen 1. *International Journal of Parasitology*, 44(12): 853-857.

Hebbel, R. P. (1986). Erythrocyte antioxidants and membrane vulnerability. *Journal of Laboratory and Clinical Medicine*, 107: 401–405.

Heerden, J. V. (1996). Equine babesiosis in South Africa: a report of two cases. *Equine Veterinary Education*, 8, 3-5.

Hines, S., Ramsay, J., Kappmeyer, L., Lau, A., Ojo, K., Van Voorhis, W., Knowles, D. andMealey, R. (2015). *Theileria equi* isolates vary in susceptibility to imidocarb dipropionate but demonstrate uniform in vitro susceptibility to a bumped kinase inhibitor. *Parasites and Vectors*, 8: 33.

Hu, S. H., Weisz, K., James, T. L. and Shafer, R. H. (1992). H-NMR studies on d(GCTTAAGC)2 and its complex with berenil. *European journal ofbiochemistry / FEBS*, *204* (1): 31-38.

Huang, X., Xuan, X., Yokoyama, N., Katayama, Y., Anzai, T. and Igarashi, I. (2006). Evaluation of enzyme-linked immunosorbent assays with recombinant antigens for the serodiagnosis of equine Babesia infections. *Veterinary Parasitology*, 140: 158-161.

Hunfeld, K.P., Hildebrandt, A. and Gray, J. S.(2008). Babesiosis: recent insights into an ancient disease. *International Journal of Parasitology*, 38: 1219–1237.

Hussain, M. H., Saqib, M., Raza, F., Muhammad, G., Asi, M. H., Mansoor, M. K., Saleem, M. and Jabber, A. (2014). Seroprevalence of *Babesia caballi*, and *Theileria equi*in five draught equine populated metropolis of Punjab, Pakistan. *Veterinary Parasitology*, 202: 248-256.

Ibrahim, A.K, W.S. Awad, N.M. Ezz El-Din, N.A. Hegazy and M.M. Fahmy, 2005. Molecular and conventional diagnostic studies of equine babesiosis caused by *Babesia equi* in Egypt. *Egyptian Veterinary Medical Society of Pathology Journal*, 2: 232-249.

Ibrahim, A. K., Gamil, I. S., Abdelbaky, A. A., Hussein, M. M. and Tohamy, A. A. (2011). Comparative molecular and conventional detection methods of *Babesia equi* (*B.equi*) in Egyptian Equine. *Global Veterinaria*, 7:201-210.

Idoko S. I., Abalaka S. E., Mshelia P. W., Edeh R. E., Kolo H. N. and Adeyanju K. Y. (2018). Ureamic Syndrome in *Theileria equi*-infected Argentine Polo Pony: A Case Report. *Abuja Journal of Veterinary and Biomedical Sciences*, 1 (1): 74 – 79.

Idoko Sunday Idoko, Sharon Tirosh-Levy, Monica Leszkowicz Mazuz,Babagana Mohammed Adam, Bello Sikiti Garba,Daniel Wesley Nafarnda and Amir

Steinman. (2020). Genetic Characterization of Piroplasms in Donkeysand Horses from Nigeria. *Animal*, 10 (324): 1-9.

Ifatimehin, O. O., Adeyemi, J. O. and Saliu, O. A. (2013). The Impact of urban micro-climate change on human comfort in Lokoja, Nigeria. *Katsina Journal of Natural and Applied Sciences*, 3: 93-104.

Iijima, H., Patrzyc, H. B., Budzinski, E. E., Freund, H. G., Dawidzik, J. B., Rodabaugh, K. J. and Box, H. C. (2009). A study of pyrimidine base damage in relation to oxidative stress and cancer. *British Journal of Cancer*, 101: 452 – 456.

Ikadai H, Ishida H, Sasaki M, Taniguchi K, Miyata N, Koda M, Igarashi I, Oyamada T: (2006). Molecular cloning and partial characterization of *Babesia equi* EMA-3. *Molecular Biochemistry and Parasitology*, 150(2):371–373.

Ike, K., Takeuchi, K., Uchida, Y. and Imai, S.(2005). Hematological findings and antibody responses in Syrian hamster (Mesocricetus auratus) infected with Babesia microti. *Journal of Veterinary Medical Science*, 67: 457-460.

`Ikpeze, O.O., Eneanya, C.I., and Onyido, A.E. (2015). "Burden, Seasonality, Sex Ratio and Preferred Sites of Ticks of Public Health Importance on Cattle found at Amansea, Anambra State, Nigeria". *International Journal of Research – Granthaalayah*, 3 (12): 61-71.

Jagri, M. S., Nemec Svete, A., Zrimek, P., Kramari, P., Kos, K. V., Vovk, T. and Kobal, Silvestra (2012). Plasma Malondialdehyde, Biochemical and Haematological Parameters in Standardbred Horses during a selected field Exercise Test. *Acta Veterinaria (Beograd)*, 62 (1): 53-65.

Jain, S., Gautam., V. and Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. *Journal ofPharmaceutical and Bioallied Science*, 3 (1):118–127.

James, D. M. and Giles, H. M. (1985). In *Pharmacology and usage*. Sons, J.W., Ed.: New York.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. and Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451: 990–993.

Kamani, J., Chung, P., Lee, C. and Chung, Y. (2018). In search of the vector(s) of *Babesia rossi* in Nigeria: molecular detection of B. rossi DNA in Rhipicephalus sanguineus sensu lato (Acari: Ixodidae) ticks collected from the dogs, circumstantial evidence worth exploring. *Experimental and Applied Acarology*, 76 (2): 243-248.

Kamyingkird, K., Yangtara, S., Desquesnes, M., Cao, S., Adjou Moumouni, P.K., Jittapalapong, S., Nimsupan, B., Terkawi, M.A., Masatani, T., Nishikawa, Y., Igarashi, I. and Xuan, X. (2014). Seroprevalence of *Babesia caballi* and

Theileria equi in horses and mules from NorthernThailand.*Journal of Protozoology Research*, 24: 11-17.

Kappmeyer, L. S., Perryman, L. E., Hines, S. A., Baszler, T. V., Katz, J. B., Hennager, S. G. and Knowles, D.P. (1999). Detection of equine antibodies to Babesia caballi by recombinant B. caballi rhoptry-associated protein 1 in a competitive-inhibition enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 37: 2285–2290.

Kappmeyer, L. S., Thiagarajan, M., Herndon, D. R., Ramsay, J. D., Caler, E., Djikeng, A., Gillespie, J. J., Lau, A. O. T., Roalson, E. H., Silva, J. C., Silva, M. G., Suarez, C. E., Ueti, M. W., Nene, V. M., Mealey, R. H., Knowles, D. P. and Brayton, K. A. (2012). Comparative genomic analysis and phylogenetic position of *Theileria equi*. *Genomics*, 13:603.

Katzer, F., Ngugi, D., Oura, C., Bishop, R.P., Taracha, E.L., Walker, A. R., and McKeever, D.J. (2006). Extensive genotypic diversity in a recombining population of theapicomplexan parasite *Theileria parva*. *Infection and Immunity*, 74, 5456–5464.

Kerber, C. E. Ferreira, F.and Pereira, M. C. (1999). Control of equine piroplasmosis in Brazil.*Onderstepoort Journal of Veterinary Research*, 66:123–127.

Kessl, J. J., Lange, B. B., Merbitz-Zahradnik, T., Zwicker, K., Hill, P., Meunier, B., Palsdottir, H., Hunte, C., Meshnick, S. and Trumpower, B. L. (2003). Molecular basis for atovaquone binding to the cytochrome bc1 complex. *Journal ofBiological Chemistry*,*278* (33): 31312-31318.

Keyloun, K. R., Reid, M. C., Choi, R., Song, Y., Fox, A. M. and Hillesland, H. K. (2014). The gatekeeper residue and beyond: homologous calcium-dependent protein kinases as drug development targets for veterinarian Apicomplexa parasites. *Parasitology*, 141:1499–1509.

Knowles, D. P., Perryman, L. E., Goff, W. L., Miller, C. D., Harrington, R. D. and Gorham, J. R. (1991). A monoclonal antibody defines a geographically conserved surface protein epitope of Babesia equi merozoites. *Infection and Immunity*, 59: 2412–2417.

Knowles Jr., D. P., Kappmeyer, L. S., Stiller, D., Hennager, S. G., Perryman, L. E. (1992). Antibody to a recombinant merozoite protein epitope identifies horses infected with *Babesia equi*. *Journal of Clinical Microbiology*, 30: 3122–3126.

Knowles, D. P. Jr., Kappmeyer, L. S. and Perryman, L. E. (1994). Specific immune responses are required to control parasitemia in *Babesia equi* infection. *Infection andImmunology*, 62:1909 –1913.

Knowles, D. P., Kappmeyer, L. S., Haney, D., Herndon, D. R., Fry, L. M., Munro, J. B., Sears, K., Ueti, M. W., Wise, L. N. and Silva, M. (2018). Discovery of a novel species, *Theileria haneyi* n. sp., infective to equids, highlights exceptional

genomic diversity within the genus Theileria: Implications for apicomplexan parasite surveillance. *International Journal of Parasitology*, 48: 679–690.

Kocyigit A., Keles, H., Selek, S., Guzel, S., Celik, H. and Erel, O. (2005). Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. *Mutation Research*,585: 71-78.

Kolanjiappana, K., Manoharana, S. and Kayalvizhi, M. (2002). Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. *Clinica Chimica Acta*, 326: 143–149.

Korsinczky, M., Chen, N., Kotecka, B., Saul, A., Rieckmann, K. and Cheng, Q.(2000). Mutations in Plasmodium falciparum cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. *Antimicrobial Agents and Chemotherapy*, 44: 2100–2108.

Kramer, J. W. (2000). Normal Hematology of the Horse. *In: Feldman BF, Zinkl JG, Jain NC editors. Schalm's Veterinary Hematology, 5th edition. Lippincott, Williams and Wilkins, Philadelphia, USA*,pp. 1069-1074.

Krause, P. J., Lepore, T. and Sikand, V. K. (2000). Atovaquone and azithromycin for the treatment of human babesiosis. *New England Journal of Medicine*, 343:1454–1458.

Krause, P. J. (2002). Equine Babesiosis. *Medical Clinics of North America*, 86: 361-373.

Krause, P. J., Daily, J. and Telford, S. R. (2007). Shared features in the pathobiology of babesiosis and malaria. *Trends Parasitology*, 23, 605-610.

Kumar, K. J., Yang, H. L., Tsai, Y. C., Hung, P. C., Chang, S. H., Lo, H. W., Shen, P. C., Chen, S. C., Wang, H. M., Wang, S. Y., Chou, C. W. and Hseu, Y. C. (**2013**). Lucidone protects human skin keratinocytes against free radical-induced oxidative damage and inflammation through the up-regulation of HO-1/Nrf2 antioxidant genes and down-regulation of NF-κB signaling pathway *Food and Chemical Toxicology*, 59: 55-66.

Kumar, S., Kumar, R., Gupta, A. K. and Dwivedi, S. K. (2008). Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. *Veterinary Parasitology*, 151: 80-85.

Kutscha, J. (2008). *Effects of Specific Equine Babesiosis Treatments on Equine Oro-caecal Transit Time as measured by the Lactose C-Ureide Breath Test.* PhD thesis, the Faculty of Veterinary Medicine of the Ludwig Maximilians-University Munich. Pp: 27-32.

Lamarque, M. H., Roques, M., Kong-Hap, M., Tonkin, M. L. and Rugarabamu, G. (2014). Plasticity and redundancy among AMA-RON pairs ensure host cell entry of Toxoplasma parasites. *Nature Communication*, 5: 4098.

Larson, E. T., Ojo, K. K., Murphy, R. C., Johnson, S. M., Zhang, Z. and Kim, J. E. (2012). Multiple determinants for selective inhibition of apicomplexan calcium-dependent protein kinase CDPK1. *Journal of Medicinal Chemistry*, 55:2803–2810.

Laskarin, G., N. Strbo, V. Sotosek, D. Rukavina, Z. Faust, J. SzekeresBartho, and Podack, E. R. (1999). Progesterone directly and indirectly affects perforin expression in cytolytic cells. *American Journal of Reproductive Immunology*, 42:312–320.

Lassmann, T. and Sonnhammer, E. L. L. (2005). Automatic assessment of alignment quality. *Nucleic Acids Research*, 33 (22): 7120-7128.

Latimer, K. S. and Rakich, P. M. (1992). Peripheral Blood Smears. *In: Cowell RL, Tyler RD editors. Cytology and Hematology of the Horse, 1st edition. American Veterinary Publications, Goleta, California, USA*, pp. 191-207.

Laus, F., Veronesi, F., Passamonti, F., Paggi, E., Cerquetella, M., Hyatt, D., Tesei, B. and Piergili Fioretti, D. (2013). Prevalence of tick borne pathogens in horses from Italy. *Journal of Veterinary Medicine and Science*, 75: 715-720.

Laus, F., Spaterna, A., Faillace, V., Veronesi, F., Ravagnan, S., Beribé, F. and Tesei, B. (2015). Clinical investigation on *Theileria equi* and *Babesia caballi* infections in Italian donkeys. *Veterinary Research*,11: 100.

Laveran, A. (1901). Contribution al'etude du *Piroplasma equi*. *CR Seances Society of Biology Paris*, 53: 2.

Lawal, A. I., Kwanashie, G., Okubanjo, O. O., Natala, A. J. and Idoko, R. (1999). The prevalence of piroplasmosis and microflaria infections in local and exotic horses in Zaria. *Zaria Veterineriya*, 6: 16-22.

Leeflang, P. and Ilemobade, A. A. (1977). Tick-borne diseases of domestic animals in northern Nigeria. *Tropical Animal Health and Production*, 9 (4): 211-218.

Lobo, C.A., Cursino-Santos, J. R., Alhassan, A. and Rodrigues, M. (2013). Babesia: an emerging infectious threat in transfusion medicine. *PLoS Pathogen*, 9(7):1003387

Mahmood, T. and Yang, P. C. (2012). Western blot: technique, theory, and trouble shooting. *North American Journal of Medical Sciences*, 4 (9): 429.

Mahmoud, M. S., El-Ezz, N. T., Abdel-Shafy, S., Nassar, S. A., El Namaky, A. H., Khalil, W. K., Knowles, D., Kappmeyer, L., Silva, M. G. and Suarez, C. E. (2016). Assessment of Theileria equi and Babesia caballi infections in equine populations in Egypt by molecular, serological and haematological approaches. *Parasites and Vectors*, 9 (1): 260.

Margetic, S. (2012). Inflammation and haemostasis. *BiochemiaMedica (Zagreb)*, 22: 49–62.

Mariani, E., Filardo, G., Canella, V., Berlingeri, A., Bielli, A. and Cattini,L. (2014). Platelet-rich plasma affects bacterial growth in vitro.*Cytotherapy*, 16: 1294-304.

McMichael, M. A., Lees, G. E., Hennessey, J., Sanders, M. and Boggess, M. (2005). Serial plasma lactate concentrations in 68 puppies aged 4 to 80 days. *Journal of Veterinary Emergency and Critical Care*, 15 (1): 17–21.

Mealey, R. H., Kappmeyer, L. S., Ueti, M .W., Wagner, B. and Donald P. Knowles, D. P. (2011). Protective Effects of Passively Transferred Merozoite-Specific Antibodies against *Theileria equi* in Horses with Severe Combined Immunodeficiency. *Clinical and Vaccine Immunology*, 100 –104.

Mehdi, M. M., Singh, P. and Rizvi, S. I. (2012). Erythrocyte sialic acid content during aging in humans: Correlation with markers of oxidative stress. *Disease Markers*, 32: 179–186.

Mehlhorn, H. and Schein, E. (1984). The piroplasms: Life cycle and sexual stages. *Advances in Parasitol*ogy, 23: 37–103.

Mehlhorn, H. and Schein, E. (1998). Redescription of Babesia equi Laveran, 1901 as *Theileria equi* Mehlhorn, Schein. *Parasitology Research*, 84: 467–475.

Meyer, C., Guthrie, A. J. and Stevens, K. B. (2005). Clinical and clinicopathological changes in 6 healthy ponies following intramuscular administration of multiple doses of imidocarb dipropionate. *Journal of South African Veterinary Association*, 76: 26–32.

Mierzejewska, E. J., Alsarraf, M., Behnke, J. M. and Bajer, A. (2015). The effect of changes inagricultural practices on the density of Dermacentor reticulatus ticks. *Veterinary Parasitology*,211: 259–265.

Mohri, M. and Sardari,K. (2000). Clinical and laboratory findings in equine piroplasmosis. *Journal of Equine Veterinary Science*, 20: 857-859.

Moloi, T. P. (2010). Serology, Molecular Epidemiology and Strain Diversity of Equine Piroplasms in South Africa. *MSc. Thesis Submitted to the Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Free State, Gwagwa, South Africa*.

Moltmann, U. G., Mehlhorn, H. and Schein, E. (1983). Fine structure of Babesia equi Laveran, 1901 within lymphocytes and erythrocytes of horses: An in vivo and in vitro study. *Journal of Parasitology*, 111–120.

Montes Cortés, M. G., Fernández-García, J. L., and Martínez-Estéllez, M. A. H. (2017). Seroprevalence of *Theileria equi* and *Babesia caballi* in horses in Spain. *Parasite*, 24 (14): 1-11.

Moretti, A., Mangili, V., Salvatori, R., Maresca, C., Scoccia, E., Torina, A. and Pietrobelli, M. (2010). Prevalence and diagnosis of *Babesia* and *Theileria*

infections in horses in Italy: A preliminary study. *Veterinary Journal*,184: 346–350.

Mosqueda, J., Olvera-Ramírez, A., Aguilar-Tipacamú, G. and Cantó, G. J. (2012). Current Advances in Detection and Treatment of Babesiosis. *Current Medicinal Chemistry*,19:1504-1518.

Motloang, M. Y., Thekisoe, O. M. M., Alhassan, A., Bakheit, M., Motheo, M. P., Masangane, F. E. S, Thibedi, M. L., Inoue, N., Igarash, I., Sugimoto, C. and Mbati, P. A. (2008). Prevalence of *Theileria equi* and *Babesia caballi* Infections in Horses Belonging to Resource-Poor Farmers in the North-Eastern Free State Province, South Africa. *Onderstepoort Journal of Veterinary Research*. 75: 141-146.

Mshelia, W. P., Sambo, W. K., Adamu, S. Edeh, E. R. and Onoja, I. (2016). Persistence of equine piroplasmosis in horses in Nigeria. *Journal of Equine Veterinary Science*, 39: 104-105.

Mshelia, P. W., Kappmeyer, L., Johnson, W. C., Kudi, C. A., Oluyinka, O. O., Balogun, E. O., Richard, E. E., Onoja, E., Sears, K. P. and Ueti, M.W. (2020). Molecular detection of *Theileria species* and *Babesia caballi* from horses in Nigeria. *Parasitology Research*, 119: 2955–2963.

Munkhjargal, T., Aboulaila, M., Terkawi, M. A., Sivakumar, T., Ichikawa, M. and Davaasuren, B. (2012). Inhibitory effects of pepstatin A and mefloquine on the growth of Babesia parasites. *American Journal of Tropical Medicine and Hygiene*, 87:681–688.

Muñoz, A., Rodríguez, R. G. M., Riber, C., Trigo, P., Gómez-Díez, M. and Castejon, F. (2013). Subclinical *Theileria equi* infection and rhabdomyolysis in three endurance horses. *Pakistan Veterinary Journal*, 33 (2): 257-259.

Murphy, A. D. and Lang-Unnasch, N. (1999). Alternative oxidase inhibitors potentiate the activity of atovaquone against Plasmodium falciparum. *AntimicrobialAgents and Chemotherapeutics*, 43: (3), 651-654.

Nagai, A., Yokoyama, N., Matsuo, T., Bork, S., Hirata, H. and Xuan. X. (2003). Growth-inhibitory effects of artesunate, pyrimethamine, and pamaquine against Babesia equi and Babesia caballi in in vitro cultures. *Antimicrobial Agents and Chemotherapy*,47:800–803.

Nagore, D., Garcia-Sammartin, J., Garcia-Perez, A. L., Juste, R. A. and Hurtado, A. (2004). Detection and Identification of Equine *Theileria* and *Babesia* Species by Reverse Line Blotting: Epidemiological Survey and Phylogenetic Analysis. *Veterinary Parasitology*, 123: 41-45.

Nazifi, S., Razavi, S. M., Mansourian, M., Nikahval, B. and Moghaddam, M.(2008). Studies on correlation among parasitaemia and some hemolytic indices in two

tropical diseases (Theileriosis and Anaplasmosis) in Fars province of Iran. *Tropical Animal Health and Production*, 40: 47-53.

Nazifi, S., Razavis,.M., Hasanshahi, F. and Esmailnezhad, Z. (2009). Effect of the severity of Theileria annulata infection on some hematological parameters and antioxidant enzymes in naturally infected cattle. *Bulgarian Journal of Veterinary Medicine*, 12: 63-71.

N'Datchoh, E. T., Konaré, A., Diedhiou, A. and Assamoi, P. (2012). Effects of climate variability on Savannah fire regimes in West Africa. *Earth System Dynamics Discussion*, 3: 1021-1053.

Nemec Svete, A., Ćebulj-Kadunc, N., Frangeź, R. and Kruljc, P. (2012). Serum cortisol and haematological, biochemical and antioxidant enzyme variables in horse blood sampled in a slaughterhouse lairage, immediately before stunning and during exsanguination. *Animal*, 6: 1300-1306.

Nicolaiewsky, T. B., Richter, M. F. and Lunge, V. R. (2001). Detection of *Babesia equi* (Laveran, 1901) by nested polymerase chain reaction. *Veterinary Parasitology*, 101: 9–21.

Nuttal, G. H. and Strickland, C. (1910). Die parasiten der pferdepiroplasmose response der biliary fever. *Zentralbl Bakteriol*, 1: 2.

Oduye, O. O. (1976). Survey of blood parasites of horses in Ibadan, Western Nigeria. *Wiad Parazytology*, 22: 155-159.

Ogasawara, Y., Namai, T., Yoshino, F., Lee, M. C. and Ishii, K. (2007). Sialic acid is an essentialmoiety ofmucin as a hydroxyl radical scavenger. *Federation of European Biochemical Societies Letters*, 581**:** 2473–2477.

Ojo, K. K., Pfander, C., Mueller, N. R., Burstroem, C., Larson, E. T. and Bryan, C. M. (2012). Transmission of malaria to mosquitoes blocked by bumped kinase inhibitors. *Journal of Clinical Investigation*, 122:2301–2315.

Ojo, K. K., Eastman, R. T., Vidadala, R., Zhang, Z., Rivas, K. L. and Choi, R. (2014). A specific inhibitor of PfCDPK4 blocks malaria transmission: chemical-genetic validation. *Journal of Infectious Diseases*, 209:275–284.

Oladipo, T.A., Adekunle, O.F., Okuneye, O.J., Adebayo, M.D., Adeoye, A.T. and Banjoko, O.J. (2015).Pre and Post Polo Competition Prevalence of Equine Babesiosis inStable Horses in Ibadan, Oyo state, Nigeria. *International Journal of Research in Agriculture and Forestry*,2 (8): 6-9.

Oladosa, L. A. and OluFemi, B. E. (1992). Hematology of experimental babesiosis and enrlichiosis in steroid immuno-suppresscal horse. *Journal of Veterinary Medicine*, 39: 345-352.

Oladosu, L. A. and Dipeolu, O. O. (1983). The clinico-pathological features and control strategies of equine Babesiosis in Nigeria. *Proceedings of National Symposium on control of ticks and tick-borne diseases in Nigeria*.

Olusa, T. A. O., Adegunwa, A. K., Aderonmu, A. A. and Adeyefa, C. A. O. (2010). Serologic Evidence of Equine H7 InfluenzaVirus in Polo Horses in Nigeria. *Science World Journal,* 5 (2): 17-19.

Onyiche, T. E., Taioe, M. O., Molefe, N. I., Biu, A. A., Luka, J., Omeh, I. J., Yokoyama, N. and Thekisoe, O. (2019). Equine piroplasmosis: An insight into global exposure of equids from 1990 to 2019 by systematic review and metaanalysis. *Parasitology,* 147: 1411-1424.

Onyiche, T. E., Taioe, M. O., Ogo, N. I., Sivakumar, T., Biu, A. A., Mbaya, A. W., Xuan, X., Yokoyama, N. and Thekisoe, O. (2020). Molecular evidence of *Babesia caballi* and *Theileria equi* in equines and ticks in Nigeria: Prevalence and risk factors analysis. *Parasitology,* 147 (11): 1238-1248.

Osman, S. A. (2017). Clinical, haematological and therapeutic studies on babesiosis in Arabian horses in the Qassim region, central of Saudi Arabia. *Journal of Applied Animal Research,* 45 (1): 118-121.

Osselaer, J. C., Jamart, J. and Scheiff, J. M. (1997). Platelet distribution widthfor differential diagnosis of thrombocytosis. *Clinical Chemistry,* 43:1072–1076.

Peregrine, A. S. (1994). Chemotherapy and delivery systems: haemoparasites. *VeterinaryParasitology, 54* (1-3): 223-248.

Peregrine, A. S. and Mamman, M. (1993). Pharmacology of diminazene: a review. *ActaTropical, 54* (3-4): 185-203.

Phipps, L.P and A. Otter (2004). Transplacental transmission of *Theileria equi* in two foals bornand reared in the United Kingdom. *Veterinary Records,* 154: 406-408.

Pitel, P. H., Pronost, S. and Scrive, T. (2010). Molecular detection of Theileria equi and Babesia caballi in the bone marrow of asymptomatic horses. *Veterinary Parasitology,* 170:182–184.

Posada, M. F. (2012). *Babesia caballi* y *Theileria equi* en equinos de comunidades indígenas de Costa Rica. (*Babesia caballi* and *Theileria equi* in horses from indigenous communities of Costa Rica). M.Sc. thesis, Universidad Nacional, Heredia, Costa Rica.

Posada-Guzmán, M. F., Dolz, G.,Romero-Zúñiga, J. J. and Jiménez-Rocha, A. E. (2015). Detection of *Babesia caballi* and *Theileria equi* in Blood fromEquines from Four Indigenous Communities in Costa Rica. *Veterinary Medicine International,* 1-6.

Qablan, M. A., Obornik, M., Petrzelkova, K. J., Sloboda, M., Shudiefat, M. F., Horin, P., Lukes, J. and Modry, D. (2013). Infections by *Babesia caballi* and *Theileria equi* in Jordanian equids: epidemiology and genetic diversity. *Parasitology*, 1-8.

Quaye, I. K. (2008). Haptoglobin, inflammation and disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102:735–742.

Radostitis, O.M., Gay, C. C., Blood, D. C. and Hinchliff, K. W.(2000). Veterinary Medicine: *A text Book of the diseases of cattle, sheep, Goats and horses.* 9 Ed. WB Saunders Co. London, New York, Philadelephia, pp: 1289-1296.

Radostitis, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P. D. (2008). *Textbook of Veterinary Medicine, 10 edition, 2008 print.; printed by El-sevier Spain,* ISBN: 978-0-7020-2777-2, pp.1421-1423.

Radwan, M. E. I., Hamied, O. A. and Ali, A. F. (2013). Evaluation of Erythrocytes Antioxidant Mechanisms inBovine Babesiosis and Current Advances Treatment inKaliobea Governorate, *American Journal of Infectious Diseases and Microbiology,* 1 (4): 59-63

Rampersad, J., Cesar, E., Campbell, M. D., Samlal, M. and Ammons, D. A. (2003). A field evaluation of PCR for the routine detection of *Babesia equi* in horses. *Veterinary Parasitology*, 114: 81-87.

Rapoport, A., Aharonson-Raz, K., Berlin, D., Tal, S., Gottlieb, Y., Klement, E. and Steinman, A. (2014). Molecular characterization of the *Babesia caballi* rap-1 gene and epidemiological survey in horses in Israel. *Infection, Genetics and Evolution*, 23: 115–120.

Rashid, H., Chaudhry, M. and Rashid, H. (2008). Comparative efficacy of diminazene diaceturate and diminazene aceturate for the treatment of babesiosis in horses. *Tropical Animal Health and Production*, 40: 463–467.

Ribeiro, I. B., Câmara, A.C. L., Bittencourt, M. V., Marçola, T. G., Paludo, G. R. and Soto–Blanco, B. (2013). Detection of Theileriaequi in spleen and blood of asymptomatic piroplasm carrier horses. *Acta Parasitology*, 5: 218–222.

Roberts, C. W., Walker, W. and Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. *Clinical Microbiology Review*, 14: 476–488. +

Rothschild, C. M. (2013). Equine piroplasmosis. *Journal of Equine Veterinary Science*, 33: 497–508.

Rothschild, C. and Knowles, D. (2007). Equine piroplasmosis. In: Sellon, D.C. and Long, M.T. (eds.).*Equine InfectiousDiseases*. Elsevier, St. Louis, MO, p.465-473.

Rüegg, S. R., Torgerson, P., Deplazes, P. and Mathis, A. (2007). Age-dependent dynamics of *Theileria equi* and *Babesia caballi* infections in southwest

Mongolia based on IFAT and/or PCR prevalence data from domestic horses and ticks. *Parasitology*, *134*, 939–947.

Salama, A. A., Aboulaila, M., Moussa, A. A., Nayel, M. A., El-Sify, A. and Terkawi, M. A. (2013). Evaluation of in vitro and in vivo inhibitory effects of fusidic acid on Babesia and Theileria parasites. *Veterinary Parasitology*, 191:1–10.

Salama, A.A., Aboulaila, M., Terkawi, M. A., Mousa, A., El-Sify, A. and Allaam, M. (2014).Inhibitory effect of allicin on the growth of Babesia and *Theileria equi*parasites. *Parasitology Research*, 113:275–283.

Salem, N. Y. and El-Sherif, M. A.(2015). Malondialdehyde status, trace minerals and hematologic results of anemic - *T. equi* infected Egyptian horses. *International Journal of Veterinary Science*, 4(3): 118-122.

Salib, F. A., Youssef, R. R., Rizk, L. G. and Said, S. F. (2013). Epidemiology, diagnosis and therapy of *Theileria equi* infection in Giza, Egypt. *Veterinary World*, 6 (2): 76-82.

Sandhu, G. S.(1998). *Histopathological, biochemical and hematological studies in cross-bred calves and hematological studies in cross bred calves*, M.V.Sc. Thesis, Punjab Agricultural University, Ludhiana.

Sanusi, M., Ahmed, I. A., Tahir, I., Mai, H. M., Kalla, D. J. U. and Shuaibu, I. (2014). Survey of equine piroplasmosis in savanna areas, Bauchi State, North-east Nigeria. *Ippologia-Cremona*, 25 (1): 3-8.

Schein, E., Rehbein, G. and Voigt, W. P. (1981). Babesia equi (Laveran 1901) 1. Development in horses and in lymphocyte culture. *Tropical Entomology and Medical Parasitology*, 32:223–227.

Schein, E. (1988). Equine babesiosis. In: *Babesiosis of Domestic Animals andMan*, Ed.: M. Ristic, CRC Press, Boca Raton, Florida, pp:197-208.

Schwint, O. N., Ueti, M. W. and Palmer, G. H. (2009). Imidocarb dipropionate clears persistent *Babesia caballi* infection with elimination of transmission potential. *Antimicrobial Agents and Chemotherapeutics*, 53: 4327–4332.

Scoles, G. A., Hutcheson, H. J., Schlater, J. L., Hennager, S. G., Pelzel, A. M. and Knowles, D. P. (2011). Equine piroplasmosis associated with Amblyomma cajennense Ticks, Texas, USA. *Emerging Infectious Disease*, 17:1903–1905.

Scoles, G.A. and Ueti, M. W. (2015). Vector ecology of equine piroplasmosis. *Annual Review of Entomology*, 60: 561–580.

Sellon, D. C. and Long, M. (2007). *Equine infectious diseases*. Saunders Elsevier, St. Louis Missouri.

Svendson, P. and Carter, A. M. (1984). An Introduction to Animal Physiology. 2nd Edn, MTP Press, Boston, USA, pp:156-159.

Servinc, F., Maden, M., Kumas, C., Sevinc, M., and Ekici, O. D. (2008). A comparative study on the prevalence of *Theileria equi* and *Babesia caballi* infections in horse sub-populations in Turkey. *Veterinary Parasitology*, *156*: 173–177.

Sigg, L., Gerber, V., Gottstein, B., Doherr, M. G. and Frey, C. F. (2010). Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population. *Parasitology International*, 59 (3): 313-317.

Sivakumar, T., Tattiyapong, M., Okubo, K., Suganuma, K., Hayashida, K., Igarashi, I., Zakimi, S., Matsumoto, K., Inokuma, H. and Yokoyama, N. (2014). PCR detection of Babesia ovata from questing ticks in Japan. *Ticks and Tick Borne Diseases*, 5: 305– 310.

Slifka, M. and Ahmed, R. (1998). Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Current Opinion in Immunology*, 10: 252–258.

Sloboda, M., Jirků, M., Lukešová, D., Qablan, M. A., Batsukh, Z., Fiala, I., Hořín, P., Modrý, M. and Lukeš, J. (2011). A survey of piroplasmids in horses and Bactrian camels in North-Eastern Mongolia. *Veterinary Parasitology*, 179: 246–249.

Smith, K. F., Goldberg, M., Rosenthal, S., Carlson, L., Chen, J., Chen, C. and Ramachandran, S. (2014). Global rise in human infectious disease outbreaks. *Journal of Royal Society Interface*, 11.

Sookoian, S. and Pirola, C. J. (2012). Alanine and aspartate aminotransferase and glutamine-cycling pathway: Their roles in pathogenesis of metabolic syndrome. *World Journal of Gastroenterology*, 18 (29): 3775-3781.

Sumbria, D., Singla, L. D., Kumar, S., Sharma, A., Dahiya, R. K. and Setia, R. (2016). Spatial distribution, risk factors and haematobiochemical alterations associated with Theileria equi infected equids of Punjab (India) diagnosed by indirect ELISA and nested PCR. *Acta Tropica*, 155: 104–112.

Szekeres-Bartho, J., G. Par, L. Szereday, Smart, C. Y. and Achtaz, I. (1997). Progesterone and non-specific immunologic mechanisms in pregnancy. *American Journal of Reproductive Immunology*, 38:176–182**.**

Takeet, M., Adeleye, A., Adebayo, O. and Akande, F. (2009). Haematology and serum biochemical alteration in stress induced equine theileriosis. A case report. *Science World Journal*, 4: 19–21.

Tecles, F., Spiranelli, E., Bonfanti, U., Ceron, J. J. and Paltrileiri, S. (2005). Preliminary studies of serum acute phase protein concentrations in hematologic and neoplastic diseases in dogs. *Journal of Veterinary Internal Medicine*, 19: 865–870.

Tenter, A. M. and Friedhoff, K. T. (1986). Serodiagnosis of experimental and natural *Babesia equi* and *B. caballi* infections. *Veterinary Parasitology*, 20: 49–61.

Terkawi, M. A., Alhasan, H., Huyen, N. X., Sabagh, A., Awier, K., Cao, S., Goo, Y. K., Aboge, G., Yokoyama, N., Nishikawa, Y., Kalb-Allouz, A. K., Tabbaa, D., Igarashi, I. and Xuan, X. (2012). Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in cattle from central region of Syria. *Veterinary Parasitology*, 187: 307-311.

Tirosh-Levy, S., Gottlieb, Y., Fry, L. M., Knowles, D. P. and Steinman, A. (2020). Twenty years of equine piroplasmosis research: Global distribution; molecular diagnosis; and phylogeny. *Pathogens*, 9: 926.

Turaki, U. A., Kumsha, H. A., Biu, A. A. and Bokko, P. B. (2014). Prevalence of Piroplasmosis amongst local horses in NortheasternNigeria. *Journal of Agriculture and Veterinary Science*, 7 (12; 4):04-07.

Uchino, Y., Kawakita, T., Miyazawa, M., Ishii, T., Onouchi, H., Yasuda, K., Ogawa, Y., Shimmura, S., Ishii, N. and Tsubota, K. **(2012).** Oxidative stress induced inflammation initiates functional decline of tear production. *PLoS One*, 7.

Ueti, M. W., Palmer, G. H., Kappmeyer, L. S., Scoles, G. A. and Knowles, D.P. (2003). Expression of Equi Merozoite Antigen 2 during Development of Babesia equi in the Midgut and Salivary Gland of the Vector Tick Boophilus microplus. *Journal of Clinical Microbiology,* 41: 5803–5809.

Ueti, M.W., Palmer, G.H., Kappmeyer, L.S., Statdfield, M., Scoles, G. A.and Knowles, D. P. (2005). Ability of the vector tick Boophilus microplus to acquire and transmit *Babesia equi* following feeding on chronically infected horses with low-level parasitemia. *Journal of Clinical Microbiology*, 43: 3755–3759.

Ueti, M. W., Palmer, G. H., Scoles, G. A., Kappmeyer, L. S. and Knowles, D. P. (2008). Persistently infected horses are reservoirs for intrastadial tick-borne transmission of the apicomplexan parasite *Babesia equi*. *Infection and Immunity*,76 (8): 3525–3529.

Ueti, M. W., Mealey, R. H., Kappmeyer, L. S., White, S. N., Kumpula-McWhirter, N. andPelzel, A. M. (2012). Re-emergence of the apicomplexan *Theileria equi* in the United States: elimination of persistent infection and transmission risk. *PLoS One*,7:e44713.

Uilenberg, G. (2006). Babesia-A historical overview. *Veterinary Parasitology*, 138:3–10.

Umar, A. S. S. and Abdulkadir, M. B. (2015). Analysis of Resource-use Efficiency and Productivity of Residual Soil Moisture Tomato Production in Kaduna State, Nigeria. *International Letters of Social and Humanistic Sciences*, 51: 152-157

United States Department of Agriculture.–APHIS National Center (USDA-APHIS). (2009). Seroprevalence of Equine Piroplasmosis Disease Agents in the United States,*APHIS Info.sheet.*

United States Department of Agriculture APHIS National Center for Risk Analysis (USDA-APHIS). (2011). Equine piroplasmosis domestic pathways assessment: Pathways assessment for the spread of the causative agents of equine piroplasmosis from the movement of a horse from quarantined premises within the contiguous United States Updated December 2011. Retrieved from: http://www.aphis.usda.gov/animal_health/emergingissues/downloads/epdomesticpathwaysfinalapril11.pdf.

Useh, N. M., Oladele, S. B., Ibrahim, N. D. G., Nok, A. J. and Esievo, K. A. N. (2005). Prevalence of Equine Diseases in the Northern Guinea Savannah of Zaria, Nigeria. *Journal of Equine Science*, 16 (1): 27-28.

Vagdatli, E., Gounari, E., Lazaridou, E., Katsibourlia, E., Tsikopoulou, F. and Labrianou, I. (2010). Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia*, 14: 28-32.

Vial, H. J. and Gurenflot, A. (2006). Chemotherapy against babesiosis. *Veterinary Parasitology.* 138:147-160.

Villoria, D., Andrea, J., Leal, L., García, F. and Morales, A.(2012). Severed Hemoparasitosis Complicated in Thoroughbred Horses from Venezuela. *Analecta Veterinaria*, 32 (1): 29-32.

Vimr, E. R., Kalivoda, K. A., Deszo, E. L. and Steenbergen, S. M. (2004). Diversity of microbial sialic acid metabolism.*Microbiologyand Molecular Biology Reviews,* 68: 132–153.

Walker, A., Bouattour, A., Camicas, J., Estrada-Pena, A., Horak, I., Latif, A., Pegran, R. and Preston, P. (2014). *Ticks of Domestic Animals in Africa: A Guide to Identification of Species.* Bioscience Reports, Edinburg, Scotland, U.K.

Weiland, G. (1986). Species-specific serodiagnosis of equine piroplasma infections by mean of complement fixation test (CFT), immunofluorescence (IF), and enzymelinked immunosorbent assay (ELISA). *Veterinary Parasitology*, 20: 43-48.

Weir, W., Ben-Miled, L., Karagenc, T., Katzer, F., Darghouth, M., Shiels, B. and Tait, A. (2007). Genetic exchange and sub-structuring inTheileria annulatapopulations.*Molecular and Biochemical Parasitology*, 154: 170–180.

Weir W., Karagenc T., Baird M., Tait A. and Shiels B.R. (2010). Evolution and diversity of secretome genes in the apicomplexan parasite *Theileria annulata*. *BMC Genomics,* 11:42.

Weiss, L. M. (2002). Babesiosis in humans: a treatment review. *Expert Opinion onPharmacotherapy, 3* (8): 1109-1115.

Wessely-Szponder, J., Bełkot, Z., Bobowiec, R., Kosior-Korzecka, U. andWójcik, M. (2015).Transport induced inflammatoryresponses in horses. *Polish Journal of Veterinary Sciences,* 18 (2): 407–413.

Wise, L. N., Kappmeyer, L. S., Mealey, R. H., and Knowles, D. P. (2013). Review of equine piroplasmosis.*Journal of Veterinary Internal Medicine,*27:1334–1346.

Wise, L. N., Kappmeyer, L. S., Silva, M. G., White, S. N., Grause, J. F. and Knowles, D. P. (2017). Verification of post-chemotherapeutic clearance of Theileria equi through concordance of nested PCR and immunoblot. *Ticks and Tick-borne Diseases*, 1-6.

Wiwanitkit, V. (2004). Plateletcrit, mean platelet volume, platelet distribution width: its expected values and correlation with parallel red blood cell parameters.*Clinical and Applied Thrombosis/Hemostasis,* 10: 175–178.

World Animal Health Information Database (WAHID). (2012).

World Organization for Animal Health (OIE). (2005). Laboratory Criteria: OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals 2008, Chapter 2.5.8 (OIE, 2005).

World Organisation for Animal Health (OIE). (2008). Equine Piroplasmosis (Equine Babesiosis, Equine Theileriosis, Biliary Fever). August 29, 2008., Pp:1-5.

World Organisation for Animal Health (OIE). (2014) World Animal Health Information Database(WAHID). Retrieved 2014 from: www.oie.int/wahis_2/public/wahid.php

World Organization for Animal Health (OIE). (2018). OIE-Listed diseases, infections and infestations in force in 2018.

Yokoyama, N., Bork, S., Nishisaka, M., Hirata, H., Matsuo, T., Inoue, N., Xuan, X., Suzuki, H., Sugimoto, C. and Igarashi, I. (2003). Roles of the Maltese cross form in the development of parasitemia and protection against *Babesia microti* infection in mice. *Infection and Immunity*, 71: 411–417.

Young, J. D., Wolowyk, M. W., Fincham, D. A., Cheeseman, C. I., Rabenstein, D. L. and Ellory, J. C. (1987) Conflicting evidence regarding the transport of alpha-glutamyl-dipeptides by human erythrocytes. *Biochemistry Journal*, 242:309–311.

Young, H. S., McCauley, D. J., Dirzo, R., Nunn, C. L., Campana, M. G., Agwanda, B., Otarola-Castillo, E. R., Castillo, E. R., Pringle, R. M., Veblen, K. E., Salkeld, D. J., Stewardson, K., Fleischer, R., Lambin, E. F., Palmer, T. M. and Helgen, K. M. (2017). Interacting effects of land use and climate on rodent-borne pathogens in central Kenya. *Philosophical Transactions of the Royal Society*, 372: 1-11.

Zapf, F., and Schein, E. (1994). The development of *Babesia (Theileria) equi* (Laveran, 1901) in the gut and the haemolymph of the vector ticks. *Hyalomma species*. *Parasitology Research*, 80: 297–302.

Zaugg, J. L. and Lane, V. M. (1992). Efficacy of buparvaquone as a therapeutic and clearing agent of Babesia equi of European origin in horses. *American Journal of Veterinary Research*, 53(8):1396-1399.

Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., Cocco, R. and Pinna Parpaglia, M.L. (2008). Clinical and laboratory findings in equine piroplasmosis. *Journal of Equine Veterinary Science,* 28: 301–308.

Zygner, W., Gojska, O., Rapacka, G., Jaros, D. and Wędrychowicz, H. (2007). "Hematological changes during the course of canine babesiosis caused by large Babesia in domestic dogs in Warsaw (Poland)," *Veterinary Parasitology*, 145 (1-2):146–151.

APPENDICES

Appendix 1: Laboratory data for symptomatic horses

Table 1. Erythrocyte parameters of the infected and uninfected horses

Parameters	Control (Uninfected)	*T. equi*	*T. haneyi*	Mixed Infections	Unknown infections
RBC ($\times 10^{12}$/L)	6.76±0.69	6.61±1.25	6.76±1.29	6.40±1.01	6.25±0.55
HCT (%)	31.42±2.45	32.26±1.39	33.11±1.43	31.80±2.33	31.23±1.68
Hgb (g/dL)	11.56±0.60	11.11±0.44	11.34±0.61	11.05±0.90	10.30±0.40
MCV (fl)	50.00±1.40	49.00±0.61	49.00±0.94	50.00±1.60	50.00±0.55
MCH (pg)	17.08±0.19	16.81±0.18	16.84±0.20	17.20±0.44	16.48±0.21
MCHC g/dL	34.36±1.22	34.63±0.43	34.09±0.63	34.73±1.07	33.05±0.63
RDW (fl)	35.44±1.59	34.63±0.85	33.29±0.80	35.07±1.12	31.80±1.25
RDW (%)	20.06±0.68	20.04±0.22	19.41±0.36	20.47±0.29	19.08±0.60

Keys: RBC = red blood cells count, HCT = haematocrit, Hgb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width

Appendix 2: Leukogram of the study groups

Table 2. Leukocyte parameters of the infected and uninfected horses

Parameters	**Control**	***T. equi***	***T. haneyi***	**Mixed Infections**	**Unknown infections**
WBC ($\times 10^9$/L)	7.82±0.72	8.26±0.59	8.09±0.51	6.42±1.04	7.68±1.00
NEU (%)	38.74±9.88	23.46±3.26	28.56±5.43	15.78±5.3	19.18±13.16
NEU ($\times 10^9$/L)	3.10±0.80	1.95±0.31	2.24±0.43	1.17±0.51	1.20±0.77
LYM (%)	40.64±13.10	55.21±4.41	50.70±5.64	69.38±8.12	67.90±16.01
LYM ($\times 10^9$/L)	2.98±0.78	4.65±0.50	4.19±0.59	4.17±0.61	5.55±1.70
MID (%)	20.58±4.62	21.29±2.07	20.69±2.60	14.87±2.89	12.93±2.96
MID ($\times 10^9$/L)	1.74±0.50	1.82±0.21	1.66±0.22	1.08±0.35	0.93±0.13

Keys: WBC = white blood cells count, NEU = neutrophil, LYM = lymphocytes, MID = mid cells (monocytes, eosinophils and basophils).

Appendix 3: Thrombogram of the study groups

Table 3: Mean platelet parameters of the infected and uninfected horses. Superscripts with similar alphabets (aa) or (bb) are not significantly different but those with different alphabets are significantly different (ab).

Parameters	**Control**	***T. equi***	***T. haneyi***	**Mixed Infections**	**Unknown infections**
PLT ($\times 10^9$/L)	86.40±12.99	83.11±6.61	98.36±11.98	120.30±26.75	104.30±20.06
PCT (%)	0.062±0.01	0.060±0.01[a]	0.076±0.01[a]	0.163±0.07[b]	0.078±0.01
MPV (fL)	7.04±0.33	7.54±0.22	7.72±0.22	7.13±0.31	7.53±0.29
PDW (%)	13.50±0.85	12.93±0.40[a]	14.61±0.10[b]	12.98±0.94	14.58±0.05
P-LCR	0.78±0.21	0.61±0.10	0.99±0.12	1.00±0.37	1.08±0.21

Keys: PLT = platelets count, PCT = plateletcrit, MPV = mean platelets volume, PDW = platelet distribution width, P-LCR = platelet large cell ratio.

Appendix 4:Erythrocyte membrane bound enzymatic activities of the studygroups

Table 4. Erythrocyte membrane bound alanine amino transferase, aspartate amino transferase and alkaline phosphatase activities of the infected and uninfected horses

Parameters	**Control**	***T. equi***	***T. haneyi***	**Mixed Infections**	**Unknown infections**
ALT (U/L)	5.40±0.51	5.30±0.21	5.43±0.20	5.17±0.48	5.75±0.48
AST (U/L)	59.60±6.41	53.81±3.37	58.07±4.01	66.00±8.38	59.50±2.90

Keys: ALT = Alanine amino transferase, AST = aspartate amino transferase

Appendix 5 Erythrocyte membrane bound oxidative biomarkers of the study groups

Table 5: Mean erythrocyte membrane bound oxidative biomarkers of the infected and uninfected horses. Superscripts with similar alphabets are not significantly different but those with different alphabets are significantly different.

Parameters	**Control**	***T. equi***	***T. haneyi***	**Mixed Infections**	**Unknown infections**
CAT (u/mL)	8.16±4.95	12.97±2.05	16.01±3.65	15.71±4.34	15.42±4.31
SOD (u/mL)	7.58±1.51	10.71±1.31	10.26±1.67	12.24±1.96	11.93±4.06
GSH (µmol/mL)	7.02±1.69	9.81±1.20	11.06±1.23	11.68±2.41	12.74±2.63
MDA (µmol/mL)	5.87±4.11[a]	16.00±2.88	16.40±4.22	17.60±4.95	41.72±20.22[b]

Keys: CAT = Catalase, SOD = Superoxide dismutase, GSH = Glutathione, MDA = Malondialdehyde.

Appendix 6: Serum biochemical parameters of the study groups

Table 6. Serum biochemical parameters of the infected and non-infected horses

Parameters	**Non-infected**	***T. equi***	***T. haneyi***	**Mixed Infections**	**Unknown infections**
ALT (U/L)	5.60±0.40	5.33±0.46	5.43±0.68	6.00±0.58	5.00±1.00
AST (U/L)	21.60±3.25	20.15±1.12	18.36±1.57	20.17±1.68	22.25±1.93
ALP (U/L)	114.00±5.39	119.90±2.67	117.10±3.95	123.20±4.76	111.50±9.43
T. BiL (mg/dL)	1.28±0.14	1.14±0.06	1.03±0.06	0.93±0.13	0.90±1.19
D. BiL(mg/dL)	0.30±0.05	0.41±0.04	0.41±0.06	0.43±0.06	0.35±0.03
Indirect BiL(mg/dL)	0.42±0.06	0.59±0.08	0.31±0.10	0.53±0.15	0.30±0.09
Total protein (g/dL)	11.88±1.65	10.92±0.49	10.11±0.48	10.42±1.27	10.05±1.23
Albumin (g/dL)	5.22±1.75	6.53±0.48	6.53±0.73	6.42±0.81	7.70±1.41
Globulin (g/dL)	4.20± 1.08	4.30± 0.46	3.40± 0.36	4.00± 0.65	4.00± 1.02
Creatinine (mg/dL)	1.20± 0.11	1.55± 0.16	1.32± 0.11	1.75± 0.22	0.83± 0.14
BUN (mg/dL)	19.20± 1.39	20.11± 0.74	23.71± 2.44	27.67± 5.77	21.00± 1.35
T. Chol (mg/dL)	74.72±11.30	68.76±3.70	62.70±4.87	81.25±15.04	81.23±21.08
Glucose (mg/dL)	110.60±14.11	94.43±5.30	86.90±4.56	88.85±13.54	87.98±7.86
TSA (µ/L)	26.82±0.95	24.37±0.68	24.37±0.95	23.92±1.25	23.25±2.64
FSA (µ/L)	11.48±0.89	10.92±0.21	10.46±0.38	11.53±1.10	11.50±1.10
BSA (µ/L)	15.34±0.92	13.38±0.66	13.70±0.84	12.38±1.07	11.75±1.96

Keys: ALT = alanine aminotransferase, AST = aspartate transferase, ALP = alkaline phosphatase, TBiL = total bilirubin, Direct BiL = direct bilirubin, Indirect BiL = indirect bilirubin, BUN = blood urea nitrogen, TChol = total cholesterol, TSA = total sialic acid, FSA = free sialic acid, BSA = bound sialic acid

Table 7. Serum electrolyte concentration of the infected and non-infected horses

Parameter	**Non-infected**	***T. equi***	***T. haneyi***	**Mixed infection**	***T. equi*-like Unknown clade**
Na (mmol/L)	67.66 ± 6.54	67.57 ± 1.77	72.19 ± 3.22	65.05 ± 6.78	72.25 ± 3.51
Cl (mmol/L)	75.20 ± 3.18	77.93 ± 1.41	77.07 ± 1.90	75.17 ± 3.35	69.25 ± 2.14
K (mmol/L)	4.04 ± 0.32	4.82 ± 0.21	4.99 ± 0.28	4.37 ± 0.36	4.60 ± 0.25
HCO_3 (mmol/L)	20.20 ± 1.28	22.00 ± 0.56	21.86 ± 0.69	20.33 ± 0.80	21.75 ± 1.65
PO_4 (mmol/L)	2.46 ± 0.23	3.89 ± 0.47	4.54 ± 0.70	2.48 ± 0.20	2.83 ± 0.24
Ca (mmol/L)	11.62 ± 0.93	12.13 ± 0.35	11.30 ± 0.51	10.92 ± 0.87	11.53 ± 0.69

Key: Na = sodium, Cl = chloride, K = potassium, HCO_3= bicarbonate, PO_4 = phosphate

Ca = calcium

Appendix 7: Laboratory data for asymptomatic horses

6.4.2.1 *Haemogram*

Table 7: Mean haematological parameters of the infected and uninfected horses

Parameters	*Babesia caballi*	*Theileria equi*	Mixed Infection	Uninfected (control)
RBC ($\times 10^{12}$/L)	5.76 ±1.04	5.67 ± 0.19	5.76 ±1.04	5.50 ± 0.51
HCT (%)	28.93 ± 4.95	26.91 ± 0.94	28.93 ± 4.95	25.50 ± 2.17
Hb (g/dL)	9.73 ± 1.73	9.11 ± 0.36	9.73 ± 1.73	9.03 ± 0.81
MCV (fL)	50.43 ± 1.88	47.79 ± 0.45	50.43 ± 1.88	46.65 ± 0.51
MCH (pg)	16.90 ± 0.21	15.97 ± 0.29	16.90 ± 0.21	16.45 ± 0.15
MCHC (g/dL)	33.60 ± 0.84	33.44 ± 0.64	33.60 ± 0.84	35.29 ± 0.32
RDW (fL)	21.33 ± 0.72	19.22 ± 0.33	21.33 ± 0.72	19.83 ± 0.15
TLC ($\times 10^{9}$/L)	5.60 ± 2.36	5.92 ± 0.46	5.60 ± 2.36	6.01 ± 1.29
NEUT ($\times 10^{9}$/L)	2.37 ± 1.48	2.33 ± 0.21	2.37 ± 1.48	2.24 ± 0.51
NEUT (%)	34.63 ± 13.79	38.41 ± 1.47	34.63 ± 13.79	36.05 ±2.71
LYMP($\times 10^{9}$/L)	2.13 ± 1.00	2.15 ± 0.17	2.13 ± 1.00	2.41 ± 0.52
LYMP (%)	45.57 ± 12.61	38.13 ± 1.51	45.57 ± 12.61	43.51 ± 5.18
MID ($\times 10^{9}$/L)	1.13 ± 0.52	1.43 ± 0.13	1.13 ± 0.52	1.38 ± 0.33
MID (%)	19.80 ± 3.00	23.45 ± 1.13	19.80 ± 3.00	20.44 ± 2.85
PLT ($\times 10^{9}$/L)	65.33 ± 25.22	103.3 ± 6.14	63.33 ± 25.22	116.1 ± 16.36
PCT (%)	0.05 ± 0.02	0.07 ± 0.00	0.05 ± 0.02	0.08 ± 0.01
MPV (fL)	8.67 ± 0.94	7.99 ± 0.15	8.67 ± 0.94	7.53 ± 0.45
PDW (%)	14.43 ± 0.18	14.37 ± 0.03	14.43 ± 0.18	14.25 ± 0.10

Keys: RBC = red blood cells count, HCT = haematocrit, Hgb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width, TLC = total leukocyte count, NEUT = neutrophils, LYMP = lymphocytes, MID = mid cells representing monocytes, eosinophils and basophils, PLT = platelets, PCT = plateletcrit, MPV = mean platelet volume, PDW = platelet distribution width

Appendix 8:Serum biochemical parameters

Table 8. Mean serum biochemical parameters of the infected and uninfected horses

Parameters	***Babesia caballi***	***Theileria equi***	**Mix Infection**	**Uninfected**
ALT (U/L)	18.30 ± 1.45	25.11 ± 1.30	18.30 ± 1.45	31.09 ± 4.70
AST (U/L)	22.87 ± 4.22	27.97 ± 1.54	22.87 ± 4.22	29.74 ± 5.62
ALP (U/L)	529.7 ± 60.44	640.1 ± 19.97	529.7 ± 60.44	620.7 ± 78.24
T.BiL (mmol/L)	3.57 ± 1.68	11.71 ± 0.99	3.57 ± 1.68	6.65 ± 1.37
D.BiL (mmol/L)	2.67 ± 1.18	5.20 ± 0.44	2.67 ± 1.18	2.50 ± 0.42
I. BiL (mmol/L)	0.90 ± 0.50	6.57 ± 0.66	0.90 ± 0.50	4.15 ± 1.07
Creatinine (µmol/L)	153.0± 38.10[a]	83.13± 3.45[b]	153.0 ± 38.10[a]	67.65 ± 9.31[b]
BUN (mmol/L)	5.87 ± 1.80[a]	1.98 ± 0.15[b]	153.0 ± 38.10[a]	2.01 ± 0.42[b]
Total protein(g/dL)	8.70 ± 0.42	7.83 ± 0.13	8.70 ± 0.42	7.90 ± 0.28
Albumin (g/dL)	4.57±0.52	4.05±0.09	4.57±0.52	3.83±0.22
MDA (umol.mL)	1.60 ± 0.18	3.65 ± 0.30	1.60 ± 0.18	3.38 ± 1.01
SOD (U/mL)	19.42 ± 0.62	14.81 ± 0.59	19.42 ± 0.62	13.56 ± 1.04
CAT (U/mL)	30.21 ± 19.19	24.74 ± 2.21	30.21 ± 19.19	33.79 ± 7.42
GSH (umol/mL)	10.55 ± 1.66	13.41 ± 0.36	10.55 ± 1.66	12.44 ± 1.39

Keys: ALT = alanine aminotransferase, AST = aspartate transferase, ALP = alkaline phosphatase, TBiL = total bilirubin, Direct BiL = direct bilirubin, Indirect BiL = indirect bilirubin, BUN = blood urea nitrogen, MDA = malondialdehydel, SOD = superoxide dismutase, CAT = catalase, GSH = glutathione

Appendix 10:Competitive Enzyme Linked Immunosorbent Assay for *T. equi* in Asymptomatic horses

Seventy two samples that comprised of H2 (71.8% PI), H3 (80.7% PI), H5 (42% PI), H6 (47.9% PI), H7 (70.6% PI), H8 (70.7% PI), H9 (81.7% PI), H10 (79% PI), H11 (72% PI), H12 (70.2% PI), H13 (66.6% PI), H14 (72% PI), H15 (54.6% PI), H17 (46% PI), H18 (71.4% PI), H19 (52.5% PI), H21 (51.1% PI), H22 (78.3% PI), H23 (85.1% PI), H24 (78.2% PI), H25 (68.2% PI), H26 (69.8% PI), H27 (63.6% PI), H28 (76.3% PI), H29 (61.7% PI), H30 (61.9% PI), H31 (73.3% PI), H32 (62.4% PI), H33 (72.2% PI), H34 (62.2% PI), H35 (62.7% PI), H37 (81.3% PI), H38 (81.9% PI), H39 (83.7% PI), H40 (82.9% PI), H41 (50.5% PI), H42 (64.9% PI), H43 (83.3% PI), H44 (62.7% PI), H45 (80.7% PI), H46 (76.4% PI), H47 (56% PI), H48 (72.8%), H49 (55.2% PI), H50 (79.1% PI), H51 (73.3% PI), H52 (84.3% PI), H53 (80.4% PI), H54 (77.6% PI), H55 (63% PI), H56 (56.8% PI), H57 (61.7% PI), H58 (77.3% PI), H59 (75.2% PI), H60 (58.8% PI), H61 (73% PI), H62 (51.1% PI), H63 (59.3% PI), H65 (76.2% PI), H66 (68% PI), H67 (68.2% PI), H68 (77.4% PI), H69 (82.3% PI), H70 (66.1% PI), H73 (83.1% PI), H74 (55.8% PI), H75 (79.2% PI), H76 (76.5% PI), H77 (77.2% PI), H78 (85% PI), H79 (79.4% PI) and H80 (53.9% PI) had percent inhibition (PI) above 40%, thus, indicating reactivity to anti-*T. equi* antibodies or seropositive. Eight samples, H1 (16.8% PI), H4 (0.6% PI), H16 (-5.9% PI), H20 (-5.2% PI), H36 (25% PI), H64 (12.9% PI), H71 (33.5% PI) and H72 (26.5% PI) had percent inhibition (PI) below 40%, indicating non reactivity to anti-*T. equi* antibodies or seronegative.

Appendix 9:Erythrocyte Membrane Biochemical Parameters

Table 9: Mean erythrocyte membrane biochemical parameters for the infected and uninfected horses. Superscripts with similar alphabets are not significantly different but those with different alphabets are significantly different.

Parameters	***Babesia caballi***	***Theileria equi***	**Mix Infection**	**Uninfected**
Total Protein (µg/mL)	0.55 ± 0.04	0.68 ± 0.03	0.55 ± 0.04	0.79 ± 0.14
Total Sialic Acid (µg/mL)	98.50 ± 2.68	94.53 ± 1.28	98.50 ± 2.68	95.86 ± 4.01
Free Sialic Acid (µg/mL)	62.57 ± 5.20	57.19 ± 1.65	62.57 ± 5.20	60.79 ± 3.95
Bound Sialic Acid (µg/mL)	35.90 ± 4.31	37.32 ± 2.16	35.90 ± 4.31	35.05 ± 6.96
Cholesterol (mg/dL)	48.33 ± 22.18	40.73 ± 2.33	48.33 ± 22.18	27.65 ± 7.53
TG (mg/dL)	18.57 ± 5.60	21.33 ± 1.32	18.57 ± 5.60	23.96 ± 2.67
HDL (mg/dL)	6.62 ± 2.34	4.16 ± 0.53	6.62 ± 2.34	4.13 ± 0.88
LDL (mg/dL)	40.03 ± 22.00	33.57 ± 2.24	40.03 ± 22.00	29.88 ± 8.44

Keys: TG = triglyceride, HDL = high density lipoprotein, LDL = low density lipoprotein

Appendix 10: Molecular and Serological Detection of Piroplasms in Horses from Nigeria